DROSOPHILA INFORMATION SERVICE

70

July 1991

Material contributed by
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and arranged by
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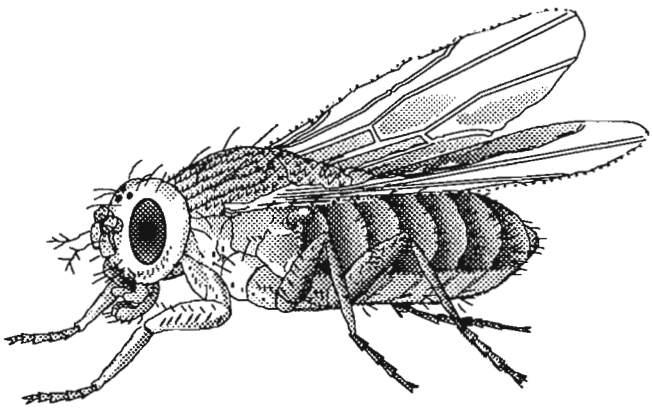
Drosophila Information Service



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Preface

Dear Colleagues,

I have learned a great deal and corresponded with many creative and knowledgeable people since assuming the editorship of Drosophila Information Service last fall. It is hard to follow the outstanding editorial work of people like Edward Novitski and Philip Hedrick, but I hope to live up to the example they have set.

As you are probably aware, the last regular issue was DIS 67 (1988), and we have devoted this issue to printing the large number of research reports, technique notes, and new mutant descriptions that have accumulated since then. I hope you find many valuable pieces of information in these pages, and I welcome your advice and ideas about ways to improve future issues.

Several initiatives are underway. In this issue, I include a form to solicit directory information, and an updated directory will be included in the next issue. A DIS editorial board of experienced *Drosophila* researchers is being formed to help recommend and evaluate material for DIS. Their enthusiasm and complementary areas of expertise will help keep DIS a timely source of informal communication for *Drosophila* researchers in all fields and countries. In addition, we are interested in eventually producing a computer diskette version of the DIS text. One goal is to be able to distribute a diskette version, as well as the printed copy of each issue, to interested individuals. A computerized subject index to DIS research and technical notes is also under consideration. Future issues will also reprint the material distributed by way of the electronic Drosophila Information Newsletter (DIN), which is described elsewhere in this issue. I strongly believe, however, that the traditional printed copy of DIS, with its illustrations and ready accessibility, remains an important product.

I appreciate the support shown by the Drosophila Board for this project. The current composition of the Board is listed separately. The production of DIS 70 could not have been completed without the generous efforts of many people, most working in a voluntary capacity. Jean Ware, Caroline Tawes, Carolee Frank, and Sue Rollins did the complex word processing of manuscripts and mailing lists; Laura Jackson, April Sholl, and James Rutter helped organize lists and proof articles; Coral McCallister assisted in preparing the illustrations and working with the University of Oklahoma printing services; and Jenna Hellack worked diligently to prepare the tables, paste up the artwork, and help me insure that the final version was as accurately prepared as possible. We now realize that we did many things the "hard way", and the next issue will be easier. We appreciate the patience of those who have corresponded with us in this transition, and I apologize for any errors I have made in putting this issue together. I invite you to let me know what can be done to improve the usefulness of DIS as an informal source of communication among *Drosophila* researchers.

James N. Thompson, jr.
July, 1991

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The Drosophila Board

The Drosophila Board represents *Drosophila* researchers by deciding upon locations for the annual meeting and contracting for meeting sites, making recommendations about "Drosophila Information Service", and overseeing similar activities that affect the Drosophila community. Terms are for three years, and one-third of the Board is replaced each year. A more detailed description of the Drosophila Board will be included in the next issue.

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Request for Directory Information

The next issue of DIS will include an updated directory of *Drosophila* researchers. Please provide the following information as soon as possible.

(I recommend that you submit your information on a photocopy of this page so that the original remains available to you for updating your directory listing.)

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Please mail to:
James N. Thompson, jr., Department of Zoology,
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Standing Orders

Several years ago, formal standing orders were discontinued, due to the need to obtain prepayment for issues. "Standing Orders" are now handled through a mailing list of active subscribers. All individuals on the active subscriber list will receive notices of forthcoming regular and special issues and an order form, requests for directory information, and similar material. If you would like to be added to the Drosophila Information Service mailing list, please write to James Thompson or make a special note on the directory list above.

List of Contributions

Announcements

Research Notes

Technical Notes

New Mutants: D. melanogaster

New Mutants: Other Species

Research Notes

Abdelhay, E. and V.S.F. Braga. Instituto de Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro, Brasil. **Internal calcium variation in *Drosophila* embryos during initial embryogenesis.**

Drosophila initial morphogenesis has been exhaustively studied and during the past five years lots of genes involved in developing pattern were cloned and analysed. Nevertheless the idea of morphogenetic signals distributed in gradients generating positional information fields, and triggering the developmental process, is each day more evident.

In *Drosophila* as well as in other organisms the existence of maternal mRNA restricted in embryo regions shows that the development at least at the beginning is in some way determined by the topological distribution of macromolecules and perhaps by smaller molecules. In this category the ions are probably the most important, calcium being the best candidate. Local variation of calcium concentration has been observed as mediator of important events in cell life. In this work we studied the internal calcium variation during development as well as the compartmentalization of this free calcium. For this purpose embryos with zero to three hours of development were collected from Oregon-R flies. After being dechorionated with 50% clorox the eggs were exhaustively rinsed in deionized water, mounted as described by Adbelhay et al. (1987) and cut into 40u slices. Four consecutive slices still frozen were dissolved in 1 ml of deionized water. In this way, four blocks containing 100 embryos gives four samples corresponding to head, thorax, abdomen I and abdomen II. The samples were analysed by atomic absorption spectroscopy calibrated to read free calcium. To calculate the amount of free calcium in each embryo region at different stages of development, 100 samples containing the fragments of 400 embryos at each stage were analysed. The media and variation obtained are presented in Table 1.

Table 1. Free Calcium Level (µg/embryo)

Embryo Fraction	Hours of Development 0 hr	1hr	2 hr	3hr
C	0.00049 ± 0.00003	0.00025 ± 0.00001	0.00042 ± 0.00002	0.00045 ± 0.00002
T	0.00011 ± 0.00001	0.00000 ± 0.00000	0.00060 ± 0.00002	0.00048 ± 0.00002
A_1	0.00072 ± 0.00003	0.00037 ± 0.00004	0.00054 ± 0.00003	0.00052 ± 0.00003
A_2	0.00061 ± 0.00002	0.00050 ± 0.00003	0.00075 ± 0.00003	0.00068 ± 0.00001

Table 2. Total Calcium Level (µg/ embryo)

Embryo Fraction	Hours of Development 0 hr	1 hr	2 hr	3hr
C	0.00052 ± 0.00002	0.00031 ± 0.00002	0.00050 ± 0.00002	0.00051 ± 0.00001
T	0.00058 ± 0.00002	0.00073 ± 0.00002	0.00063 ± 0.00003	0.00054 ± 0.00003
A_1	0.00069 ± 0.00003	0.00040 ± 0.00001	0.00061 ± 0.00003	0.00060 ± 0.00002
A_2	0.00065 ± 0.00002	0.00059 ± 0.00003	0.00073 ± 0.00002	0.00072 ± 0.00003

These data shows that an internal calcium variation occurs during *Drosophila* initial embryogenesis. It is important to note the low level of free calcium in the thorax region during the first hour of development. To confirm the hypothesis that all calcium present in this region is involved in the maintenance of the nuclear membranes that are being formed, we treated our samples with lantanium to liberate the bound calcium. After this treatment the thorax samples showed a relevant increase in calcium level while the other samples presented a relatively small increment (Table 2).

These results indicate that while less than 10% of total calcium is bound in the thorax in stages 2 and 3, more than 80% is bound in stages 0 and 1. Although the differences appear to be small they are statistically significant and can be considered inducers of differential transcriptional activity.

References: Abdelhay, E. et al. D.I.S 66: 155-156, 1987.

Aizenzon, M.G.[1] and M.R. Stolina[2]. [1]Ukrainian Academy of Sciences, Plant Physiology and Genetics Institute, Vasilkovskaya 31/17, Kiev, USSR. [2]Ukrainian Academy of Sciences, Molecular Biology and Genetics Institute, Zabolotnogo 150, Kiev, USSR. Sequences of adeno-associated virus genome and non-mutagenic for *Drosophila*.

It is known that not only DNA preparations but even certain short sequences are mutagenic for a number of organisms. It was demonstrated, in particular, that although pBR322 plasmid is not mutagenic for *Drosophila* other plasmids designed on its basis and containing various inserts have specific mutagenic effects and produce different spectra of mutations, depending on the insert's nature (Gazarian, *et al.*, 1987). Research into such genetic engineering designs is of interest both from the point of view of further study of the mutagenic role of exogenous DNAs, and because of the fact that in the case of absence of mutagenicity the plasmid under consideration can be used as a vector for purposes of genetic therapy. It is in view of the above that present research was undertaken and carried out.

Table 1. Obtaining mutations.

Experiment No	DNA in	F_1 N	F_1 n	F_2 N	F_2 n	F_3 N	F_3 n	F_4 N	F_4 n	Summary N	Summary n
1	pBR322 with inserted antisense sequence	922	0	16.361	3 (1.9×10^{-4})	30.523	0	30.446	2 (6.6×10^{-5}	78.322	5 (6.4×10^{-5})
2	pBR322	916	0	6.360	0	22.758	1 (4.4×10^{-5})	21.526	3 (1.4×10^{-4})	51.560	4 7.7×10^{-5})
3	TE buffer	486	0	14.605	2 (1.4×10^{-4})	24.106	1 (4.0×10^{-5})	15.266	0	54.463	3 (5.4×10^{-5})

N - number of analyzed males
n - number of mutations; mutation rate in brackets

Adult males of *Drosophila melanogaster* of the *v f* strain were used as recipients. In experiment No. 1 (Table 1) pBR322 plasmid with antisense to DNA-binding protein class 2 of AAV was injected. This sequence was flanked by promotors and long terminal repeats of human adeno-associated virus. The injected DNA concentration was 1.7 mg/ml. In experiment No. 2 (Table 1) pBR322 plasmid with 1.8 mg/ml concentration was injected. In experiment No. 3 (Table 1) standard TE buffer, that served as solvent in all previous experiments, was injected. In all the experiments each fly was injected with 0.4 ul.

Males that received injection (F_0) were mated to *C(1)DX,y f* females. To examine the possibility of a continuous mutagenic effect of the exogenous DNA the males produced in F_1, F_2, and F_3 were mated to virgin females taken from *C(1)DX, y f* strain. Mating to this female makes it

Table 2. List of mutations

Experiment No.	Injected DNA	Strain No.	Generation in which mutation was found	Location	Phenotype
1	pBR322 with inserted anti-sense sequence	7-13 d,g,j	F_2	3-67.1	Wing veins have delta-like thickenings.There is cluster, containing 3 dominant alleles.
		5-8-1	F_4	3,67.1	Wing veins have delta-like thickenings.This mutation is dominant.
		3-13-4	F_4	1-32.8	This new-appeared mutation (*ras)* together with marker *v* gives orange-eyed phenotype.
2	pBR322	2-9-2	F_3	1-58.7	Little eyes, wings extended uniformly at 90° from body axis. May be iallelic to *os.*
		3-6-3	F_4	3-67.1	Wing veins have delta-like thickenings. This mutation is dominant.
		3-6-4	F_4	3-67.1	Wing veins have delta-like thickenings. This mutation is dominant.
		3-2-3	F_4	3-?	Wings reduced by marginal excision. This mutation was lost.
3	TE Buffer	2-5	F_2	3-?	Wing veins have delta-like thickenings.This dominant mutation is not allelic to 7-13 and 5-8-1.
		5-4	F_2	1-?	Wing veins have delta-like thickenings.This is recessive mutation with low penetrance and expressivitiy.
		1-4-2	F_3	3-67.1	Wing veins have delta-like thickenings. This mutation is dominant.

possible to prevent the accumulation of modifier-genes (Golubovsky, *et al.* 1975). In each of the four generations *v f* males were examined for visible mutations on the X chromosome and dominant visible mutations on the autosomes.

In all three experiments, mutations were not found in the first generations owing, most likely, to small sample sizes (Table 1). In the following generations mutations are to be found at a rate of 10^{-4} through 10^{-5} (Table 1) even after buffer injection. Observed high level of mutability is, apparently, caused not by the alien DNA but by crossing two laboratory strains. Similar phenomena, differing from hybrid dysgenesis, were discovered earlier (Georgiev, *et al.*, 1988).

The list of resulting strains is shown in Table 2. All of them possess *v f* markers inherited from F_0 male. It is interesting to note, that in all three experiments mutually allelic mutations (position: 3 - 76.1) resulting in the formation of an excrescence on the wing veins were found. Similarity of resulting mutation spectra also shows that their origin is not dependent on injected DNA. It should be concluded that pBR322 plasmid DNA (as was shown earlier, Gazarian, *et al.* 1987) as well as the plasmid with antisense sequence inserted are non-mutagenic for *Drosophila* under the conditions analyzed.

The authors are grateful to A.D. Shved and S.B. Zolotukhin for kindly providing them with pBR322 plasmid with antisense sequence insert DNA and to T.I. Gerasimova and M. Ashburner for discussion.

References: Gazarian, K.G., S.D. Nabirochkin, E.N. Nabirochkina, *et al.* 1989. Genetica 23(2):214-227; Golubovsky, M.D. and I.K. Zakharov 1975, Genetica 15(9):1599-1609; Georgiev, P.G., O.B. Simonova, and T.I. Gerasimova 1988, Genetica 24:867-877.

Alexandrova, M.V. and I.D. Alexandrov, Joint Inst. for Nuclear Research, P.O. Box 79, Moscow, USSR. **Deletions of the coding sequences flank the inversion, but not the translocation, breakpoints at the white locus of *Drosophila melanogaster*.**

Complementation analysis of the chromosome rearrangements with the *black* phenotype induced by ionizing radiation reveals that inversions are regularly accompanied by mutations of the linked genes associated with the breakpoints, and such mutations have been interpreted as small deletions (Alexandrov and Alexandrova, this issue). The possibility that the "position effect" rather than the physical loss of the genetic material results in the simultaneous change of the linked genes can, however, not be dismissed. To test this assumption and to further refine upon localization of the breakpoints around or within the locus involved in the radiation-induced chromosome changes, in situ hybridization of the cloned fragments of the locus of interest to the rearrangements, one end of which is close to this locus, was performed. For these purposes, a chance set of the inversions and translocations with breakpoints at the w region (3C2) on the X-chromosome recovered simultaneously with the *black, cinnabar* and *vestigial* mutations in the same experiments (e.g., Alexandrov 1986) was used (Table 1). As a probe, the ^{3}H-labeled EcoR1-BamH1 DNA fragment from -5.5 to +2 (w^{L+}) and BamH1-EcoR1 DNA fragment from +2 to +9.2 (w^{R+}) of the *white* locus from an Or^R genome (Levis et al., 1982) were chosen. Both fragments have a BamH1 restriction site in common localized in the large proximal intron. The standard protocols were used for in situ hybridization (Pardue and Gall, 1975) and to obtain the labeled probes by nick translation (Maniatis et al. 1982).

Table 1. Genetics, cytogenetics and in situ hybridization analysis of the radiation-induced inversions and translocations involving the *w* locus of *Drosophila melanogaster*.

Designation of mutation	Phenotype	Complementation	Cytology	Position ** of		Origin of mutations	
				w^{L+}	w^{R+}	Radiation used	Genotypes
79b3	w	-	In(1)3C2;16D	+	+	neutrons	D-32
79d5	w	-	In(1)3C2;4B	+	+@	neutrons	D-32
79g	w	-	In(1)3C2;3F	+	+@	γ-rays	c(3)G
83c19	w^m	-	In(1)3C2;20B	+@	+@	γ-rays	D-32
72b	w	-	In(1)3C2;8F	+	-	γ-rays	D-32
88c87	w	-	In(1)3C2;19F	+	-	neutrons	D-32
81a	w	-	In(1)2F;3C2	-	+@	γ-rays	c(3)G
83d13	lethal	-	In(1)3C2;3C11	-	-	^{252}Cf	D-32
88l64	w	-	In(1)3C2;4F	-	-	neutrons	D-32
88c60	w^m	-	In(1)3C2;9A	-	-	neutrons	D-32
88e73	lethal	-	In(1)3C2;4A	-	-	neutrons+ γ-rays	D-32
88f83	lethal	-	In(1)3C2;20B	-	-	-"-	D-32
74c	w	-	T(1;3)3C2;65B	+@	+	γ-rays	D-32
78h	w	-	T(1;3)3C2;91E	+@	+	γ-rays	c(3)G
85e1	w	-	T(1;3)3C2;65E	+@	+	γ-rays	c(3)G
88c34b	w	-	T(1;3)3C2;92C	+@	+	neutrons	D-32
88d107	w	-	T(1;3)3C2;63E	+@	+	γ-rays	D-32
88d39	w^a	+	T(1;2)3C2;58E	+@	+@	γ-rays	D-32
81k11	w^{ec}	-	T(1;3)3C2;87F	+@	+@	γ-rays	D-32
88d80	lethal	-	T(1;3)3C2;81F	+	+	γ-rays	D-32

*(-) No complementation in the w^X/w^{spA} heterozygotes; ** the position of the w^{L+} or w^{R+} is left intact (+) or changed by the rearrangement (+@) whereas (-) denotes the loss of the fragment.

As in situ hybridization analyses show (Table 1) 5 out of 20 rearrangements examined (namely, In(1)w79b3, In(1)w83c19, T(1;2)w88d39, T(1;3)w81k11, T(1;3)w88d80) have the breakpoints which are found to be located outside the locus in question since both w^{L+} and w^{R+} fragments are completely safe and the behavior of these "twins" is the same for each rearrangement named. Nevertheless, none of the *w* mutations associated with these breakpoints (excluding the w88d39) were complemented with the w^{spA} in heterozygotes (Table 1). Further, all the translocations and two inversions (79d5, 79g) of twelve examined also display the safety of both clones. One of these, however, is carried over from the 3C subregion to another place in the genome, providing confirmation that their breakpoints are located within *w* locus and split it in two through intron named. As a result an aberrant *w* product appears which does not complement the w^{spA}. Finally, 8 out of 10 inversions studies do not hybridize with one or both fragments cloned, indicating that a part or the entire *w* region is deleted under processing of the initial lesions leading to the inversion formation.

Acknowledgments: We are grateful to Prof. V.A. Gvozdev, Inst. of Molecular Genetics, Acad. Sci. USSR, for providing laboratory facilities for the molecular-genetic research. We are indebted to Drs. G.L. Kogan and M.D. Balakireva for technical assistance. We also thank Dr. S.G. Georgieva (Inst. of Molecular Biology, Acad. Sci. USSR) for kindly supplying the cloned fragments of the *w* locus employed in the present study.

References: Alexandrov, I.D., 1986, DIS 63:19-21; 159-161; Levis, R., P.M. Bingham and G.M. Rubin, 1982, PNAS USA 79:564-568; Maniatis, T., E.F. Fritsch and J. Sambrook, 1982, Molecular Cloning: A laboratory manual. CSHL Press, CSHL N-Y.

Alexandrov, I.D. and M.V. Alexandrova, Joint Inst. for Nuclear Research, Moscow, USSR.

Cytogenetics of the *cinnabar* mutations induced by different quality radiations.

The following list (Table I) contains the complete information on the 205 transmissible *cinnabar* mutations which were recovered simultaneously with the *black* and *vestigial* ones in the experiments described previously (Alexandrov 1986; 1987; for details). As earlier, the mutations are named (first column) by the accepted alphanumeric code, and the contents of the other list columns are essentially self-explanatory. Only the conditions of experiments with the phr^- mutant appear to require some additional comments. At this point the "dark" means that the phr^- males irradiated were mated with tester females in red light (dark control) whereas in the "experimental light" series the males mated with females in natural light.

As seen from the list, a great many of the transmissible *cinnabar* mutations (122 out of 205 preserved) induced by low- or/and high-LET radiations have a normal 43E region 2R chromosome (the putative location of the gene in question). Among them 45 being non-viable in homozygotes have a separable lethal phenotype (so-called "twin" *cinnabar* mutants). They arise more frequently after action of photon than neutron irradiation and this picture is analogous to that for "twin" mutants at the *black* and *vestigial* loci (loc. cit.). Further, 83 out of 205 mutations studied proved to be associated with either chromosome alterations among which deletions were found to be predominant changes (66 out of 83). This peculiarity of the radiation induced mutagenesis at the *cinnabar* locus (cf. other loci, loc. cit.) seems to be conditioned by a unique feature of the locus itself and/ or of the topology of the chromosome region 43E as a whole.

References: Alexandrov, I.D., 1986, DIS 63:19-21, 159-161; Alexandrov, I.D., 1987, DIS 66:10-11; 185-187; Lindsley, D.L. and E.H. Grell, 1968, Carnegie Inst. Wash. Publ. 627.

Please see table on facing pages.

Alexandrov, I.D. and M.V. Alexandrova, Joint Inst. for Nuclear Research, P.O. Box 79, Moscow, USSR. The genetic and cytogenetic boundaries of the radiation-induced rearrangements scored as lethal *black* mutations in *D. melanogaster*.

To further refine the genetic breakpoints for the radiation -induced chromosome *black* mutants (Table 1), each of them was tested for allelism with the 42 reference lethals and visibles in divisions 34 and 35 of *D. melanogaster* 2L chromosome. The results of the complementation analysis are shown in Figure 1 and are essentially self-explantory. However, some findings require additional comments. First, the "hotspot" in 34D2-4 subregion, lying just distal to *b* and cytologically detected as one point (Table 1), consists of four hotspots on the genetic map. Second, a great many of the cytologically simple rearrangements induced by low- or high-LET radiations are basically complex and consist of two, three, or more genetic lessions (point mutations and/or deletions), simultaneously. Third, the boundaries

Table 1. Origin and cytology of the cinnabar mutations of Drosophila melanogaster. Notes:-* See Lindsley & Grell1968; **C - caffeine and AD - actinomycin - D; *** - MS - mature sperm, L, M, and E - late, middle and early spermatids (Sd), respectively; @ - the "twin" cinnabar mutations with independent lethal separated by crossing over; c - lost mutants; o - the equal contribution of the neutrons and gamma-rays to the total dose of the consecutive irradiation.

Designation of mutation	Phenotype*	Cytology	Modifier** used, radiation, dose	Genotype, germ cells irradiated
1	2	3	4	5
67d	cn@	Normal	-γ-rays, 40Gy	D-18,E Sd***
72a2	cn@	Normal	γ-rays 40Gy	D-18,E Sd
74b1	lethal	Normal	γ-rays, 40Gy	D-18,E Sd
74b2	cn(vg)@	Normal	γ-rays, 40Gy	D-18,E Sd
74b3	lethal	Normal	γ-rays, 40Gy	D-18,E Sd
74b4	cn@	Normal	C+ γ-rays, 40Gy	D-32,MS
74c2c	cn	Normal	C+ γ-rays, 40Gy	D-32,L Sd
74c3	lethal	Df(2R)43D5-E1;43F6-44A1.2	C+ γ-rays, 40Gy	D-32,L Sd
74c5	cn@	Normal	C+ γ-rays, 40Gy	D-32,M Sd
74c6	lethal	Df(2R)43D5-E1;43F6-44A1.2	C+ γ-rays, 40Gy	D-32,M Sd
74c7	cn	T(2;3)43E12-15;98A12-B1	C+ γ-rays, 40Gy	D-32,L MS
74c8	cn(vg)@	Normal	C+ γ-rays, 40Gy	D-32,L MS
74c9	cn2@	Normal	γ-rays, 40Gy	D-32,MS
74d1	cn2@	Normal	C+ γ-rays,40Gy	D-32,M Sd
74d4	cn	Normal	C+ γ-rays,40Gy	D-32,M Sd
74d5	cn@	In(2LR)24C4-D1;43E12-15;	C+ γ-rays,40Gy	D-32,M Sd
74d7	cn	Normal	C+ γ-rays,40Gy	D-32,M Sd
74d8	cn2@	Normal	C+ γ-rays, 40Gy	D-32,M Sd
75a2c	cn	Normal	-γ-rays, 40Gy	c(3)G/D-32,MS
76b	cn@	Normal	C+ γ-rays,40Gy	c(3)G,MS
76e2	cn@	Normal	γ-rays,40Gy	c(3)G,MS
76i	lethal	Normal	AD+ γ-rays,40Gy	D-32,MS
76k1	cn@sterile	Normal	AD+ γ-rays,40Gy	D-32,MS
76k2	cn2@	Normal	AD+ γ-rays,40Gy	D-32,L Sd
76k3	lethal	Df(2R)43A2-B1;44B7-C1	AD+ -γ-rays,40Gy	D-32,M Sd
77a2	cn@	Normal	AD+ γ-rays,40Gy	D-32,MS
77a3	cn2@	Normal	AD+ γ-rays,40Gy	D-32,MS
77a4	cn2@	Normal	AD+ γ-rays,40Gy	D-32,MS
77a6	lethal	Df(2R)43D7-E1;43F6-8	AD+ γ-rays,40Gy	D-32,M Sd
77a9	cn	Normal	AD+ γ-rays,40Gy	D-32,MS
77a10	cn@	Normal	AD+ γ-rays,40Gy	D-32,E Sd
77c2	cn	Normal	γ-rays,40Gy	D-32, M Sd
78a	cn	Normal	γ-rays,40Gy	c(3)G,M Sd
78b1	cn	Normal	γ-rays,40Gy	c(3)G, M Sd
78ec	lethal	Df(2R)43C6-D1;44C6-D1	γ-rays,40Gy	c(3)G,L Sd
78g	cn	Normal	NaF+ γ-rays,40Gy	D-32,M Sd
78j1c	lethal	Df(2R)43C1-2;44C1-2	γ-rays,40Gy	c(3)G,E Sd
78j2	cn	Normal	γ-rays,40Gy	c(3)G,E Sd
78f	cn(vg)@	Normal	γ-rays,40Gy	c(3)G,E Sd
78k1	cn sterile	Normal	NaF+ -γ-rays,40Gy	D-32,M Sd
78k5	lethal	Df(2R)43E5-7;44B2-5	NaF+ γ-rays,40Gy	D-32,M Sd
78l	cn@	Normal	γ-rays,40Gy	c(3)G,MS
79b2	lethal	Df(2R)43B3-C1;44B9-C1; + T(2L;Y) 30C5-7	γ-rays,40Gy	D-32,MS
79b5	cn	Normal	γ-rays,40Gy	D-32,M Sd
79b7	cn(vg)@	Normal	γ-rays,40Gy	D-32,L Sd
79b8	lethal	In(2R)43E10-15;56D2-E1	0.35MeV n,10Gy	D-32,MS
79b9	lethal	Df(2R)43E5-7;43E16-F1	0.85MeV n,20Gy	D-32,MS
79b10	cn(vg)@	Normal	0.85MeV n,20Gy	D-32,MS
79b11	cn+lethal@	Df(2L)22A2-3;22D-E1	0.35MeV n,10Gy	D-32,MS
79b13	lethal	Df(2R)43E2-16	0.35MeV n,10Gy	D-32,MS
79b14c	lethal	Df(2R)43E5-7;43E14-17	0.85MeV n,10Gy	D-32,MS
79b15	cn2@	Normal	0.85MeV n,10Gy	D-32,MS
79b18	lethal	Df(2R)43C6-D1;43E14-15	0.85MeV n,10Gy	D-32,MS
79c	cn	Normal	NaF+ γ-rays,40Gy	D-32,MS
79d1	cn@	Normal	γ-rays,20Gy	D-32,MS
79d2c	cn@	Normal	γ-rays,20Gy	D-32,MS
79d3c	cn2	Normal	0.85MeV n,5Gy	D-32,MS
79d6	cn	Normal	0.85MeV n,10Gy	D-32,MS
79d9	lethal	Tp(2R)41A;43F1;60A;41B 43E15-F1;60B1,2	0.35MeV n,20Gy	D-32,MS
79d10c	lethal	Df(2R)43D5-E1;43E16	0.35MeV n,20Gy	D-32,MS
79d12	cn2@	Normal	0.85MeVn+ γ-rays,20Gyo	D-32,MS
79d18	cn2@	Normal	0.85MeV n,10Gy	D-32,MS
79h1	cn@	Normal	γ-rays,40Gy	c(3)G,MS
79h2	cn sterile	Normal	γ-rays,40Gy	c(3)G,MS
79h3	cn	Normal	γ-rays,40Gy	c(3)G,L Sd
79h5	lethal	Normal	γ-rays,40Gy	c(3)G,L Sd
79h6c	cn@	cn Normal + In(2R)41D-E;58F2-4	γ-rays,40Gy	c(3)G,L Sd
79h7	cn sterile	Normal	γ-rays,40Gy	c(3)G,L Sd
80j	cn@	Normal	γ-rays,40Gy	c(3)G,M Sd
81a1	cn	Normal	γ-rays,60Gy	D-32,MS
81a2	lethal	cn Normal + In(2L)22D-E; 36C-D	γ-rays,60Gy	D-32,MS
81c21ac	cn(vg)@	Normal	γ-rays,40Gy	D-32,MS
81c32	cn(vg)	Normal	γ-rays,40Gy	D-32,M Sd
81c51	cn@	Normal	γ-rays,40Gy	D-32,M Sd
81f1	cn	In(2R)41B; 43E16-F1	0.1MeV n,0.75Gy	D-32,MS
81f3	lethal	Normal	0.1MeV n,2 Gy	D-32,MS
81f55	lethal	Df(2R)42A4-6;42A19-B1; + T(2L;Y) 37D4-6	0.1MeV n,2 Gy	D-32,MS
81f6c	lethal	Df(2R)42B2-3;43E15-F1	0.1MeV n,2 Gy	D-32,MS
81k1	cn	Normal	γ-rays,10Gy	D-32,MS
81k2	cn	Normal	γ-rays,10Gy	D-32,MS
81k3	lethal	Df(2R)43C7-D1;43F8-44A1	γ-rays,10Gy	D-32,MS
81k4	lethal	Df(2R)43D5-6;43E15-F1	γ-rays,10Gy	D-32,MS
81k5	cn	Normal	-γ-rays,10Gy	D-32,MS
81l	cn sterile	In(2R)43E1±; 43E17±	γ-rays,10Gy	D-32,MS
81l1	lethal	Df(2R)43E5-8;43F8-44A1	γ-rays,20Gy	D-32,MS
81l2	lethal	Df(2R)43E5-8;43E15-F1	γ-rays,20Gy	D-32,MS
81l4	cn	Normal	γ-rays,30Gy	D-32,MS
81l5c	cn sterile@	Normal	γ-rays,50Gy	D-32,MS
82c1	cn@	Normal	^{252}Cf,14Gy	D-32,MS
82c33	lethal	cn Normal + In(2LR) 28B-C; 45B-C1	^{252}Cf,28Gy	D-32,MS
82c41	cn(vg)	Normal	252 Cf,28Gy	D-32,MS
82c63	cn(vg)	Normal	^{252}Cf,14Gy	D-32,L Sd
83b27	lethal	cn Normal + In(2L) 22C-D1; 36C-D1	γ-rays,40Gy	D-32,M Sd
83b31	cn	Normal	γ-rays,40Gy	D-32,M Sd
83b33	lethal	Df(2R)43C5-D1; 44B2-5	γ-rays,40Gy	D-32,M Sd
83c17	cn sterile	In(2R)41A-C; 42E-43A1	γ-rays,40Gy	D-32,MS
83c23	lethal	Df(2R)43C4-D1;44B6-C1	γ-rays,40Gy	D-32,M Sd
83c58	cn@	Normal	γ-rays,40Gy	D-32,L Sd
83d15	cn	Normal	^{252}Cf,14Gy	D-32,MS
83d21	lethal	Df(2R)43D5-6;43F8-44A1	^{252}Cf,14Gy	D-32,MS
83f16	cn	Normal	γ-rays,40Gy	D-32,MS
83f17	cn	Normal	γ-rays,40Gy	D-32,M Sd

83f50	cn@	Normal	γ-rays,40Gy	D-32,MS
83f51	cn	Normal	X-rays,40Gy	c(3)G,L Sd
83l	lethal	Df(2R)43C2-4;43E15-F1	0.7MeV n,10Gy	D-32,MS
84f37	cn@	Normal	X-rays,40Gy	c(3)G,M Sd
84f76	cn	Normal	X-rays,40Gy	c(3)G,MS
84g	cn@	Normal	X-rays,40Gy	c(3)G,E Sd
84h	lethal	Df(2R)43B3-C1;44A6-B1	X-rays,20Gy	c(3)G,MS
84h27	cn	Normal	X-rays,20Gy	c(3)G,MS
84h29[c]	lethal	Df(2R)43B3-C1;44B9-C1 +T(2R;Y) 56F16-57A1	X-rays,40Gy	c(3)G,MS
85b1	cn	Normal	0.85MeV n,5Gy	c(3)G,MS
85b2	cn(vg)@	Normal	0.85MeV n,5Gy	c(3)G,MS
85b3	cn	In(2LR)29F8-30A1; 43E15-F1	γ-rays,10Gy	C(3)G,MS
85c1	lethal	Df(2R)43D2-4; 43E15-F1	0.85MeV n,10Gy	c(3)G,MS
85c2	lethal	Df(2R)43D5-6;43F2-4	0.85MeV n,15Gy	c(3)G,MS
85d1[c]	lethal	In(2LR)25A4-6;43E15+T(2;3) 43E15; 75C7-D1+T(3L;Y) 75C7; Y	0.85MeV n,5Gy	c(3)G,MS
85d2[c]	lethal	Df(2R)42B6-C1;43F6-44A1	0.85MeV n,10Gy	c(3)G,MS
85d3	lethal	Df(2R)43E5-6; 43E18-F1	γ-rays,10Gy	c(3)G,MS
85d4	lethal	Normal	0.85MeV n,10Gy	LA, MS
85d5	cn	Normal	0.85MeV n,10Gy	LA, MS
85e1	lethal	Df(2R) 42E4-F1; 43E16-F1	0.35MeV n,10Gy	c(3)G,MS
85e2	cn	Normal	γ-rays, 20Gy	c(3)G,MS
85e3	lethal	Df(2R)43D4-6; 43E16-F1	γ-rays, 20Gy	c(3)G,MS
85f1[c]	lethal	In(2R)41A; 43E15±	0.35MeV n,10Gy	c(3)G,MS
85f2	lethal	Df(2R)43C7-D1; 43E16-F1	γ-rays, 10Gy	c(3)G,MS
85f3[c]	lethal	Df(2R)42B5-C1; 44A8-B1	γ-rays, 20Gy	c(3)G,MS
85f4	lethal	Df(2R)43D4-6; 43E16-F1	γ-rays, 20Gy	c(3)G,MS
87e61	lethal	Df(2R)42B4-C1; 44A1	γ-rays, 10Gy	D-32,MS
87e62	cn@	Normal	γ-rays, 10Gy	D-32,MS
87e80	lethal	Normal	γ-rays, 20Gy	D-32,MS
87e94	lethal	Df(2R)43D7-E1; 43E18-F1	γ-rays, 10Gy	D-32,MS
87e130	cn	Normal	γ-rays, 10Gy	D-32,MS
87e150	cn	Normal	γ-rays, 10Gy	D-32,MS
87e158	cn	Normal	γ-rays, 20Gy	D-32,MS
87f65	lethal	Df(2R)42B4-C1; 43F8-44A1	γ-rays, 10Gy	D-32,MS
87f73[c]	cn	Normal	γ-rays, 5Gy	D-32,MS
87f99	cn@	Normal	γ-rays, 5Gy	D-32,MS
87f147	cn sterile	Normal	γ-rays, 10Gy	D-32,MS
87f149	cn sterile	Normal	γ-rays, 10Gy	D-32,MS
87f165	cn	Normal	γ-rays, 5Gy	D-32,MS
87g12	cn@	Normal	γ-rays 10Gy	D-32,MS
87g18	lethal	Df(2R)43D7-E1;44B7-C1	γ-rays, 10Gy	D-32,MS
87g54	cn	Normal	γ-rays, 10Gy	D-32,MS
87h32	cn sterile	Normal	γ-rays, 5Gy	D-32,MS
88b37	cn	Normal	0.85MeV n,2,5Gy	D-32,MS
88b44	lethal	cn Normal+Df(2R)42A-E	0.85MeV n,5Gy	D-32,MS
88c3	cn@	Normal	0.85MeV n,10Gy	D-32,MS
88c25	cn	In(2R)43E14-F1;49B11-C1	0.85MeV n,2,5Gy	D-32,MS
88c36	lethal	Df(2R)42B5-7;44B4-5	0.85MeV n,5Gy	D-32,MS
88c63	lethal	Normal	0.85MeV n,10Gy	D-32,MS
88c81	cn	Normal	0.85MeV n,2,5Gy	D-32,MS
88c83	cn	cn Normal+In(2R)50B-C1; 56B-C1	0.85MeV n,5Gy	D-32,MS
88c84	cn@	Normal	0.85MeV n,2,5Gy	D-32,MS
88c90	lethal	Df(2R)43C3-5;43F-44A1	0.85MeV n,2,5Gy	D-32,MS
88c99	cn	In(2R)41A-B;43E10-15	0.85MeV n,2Gy	D-32,MS
88c102	lethal	Normal	0.85MeV n,2Gy	D-32,MS
88d6	lethal	Df(2R)43C4-5; 44B8-C1	γ-rays,60Gy	D-32,MS
88d7	cn sterile	Normal	γ-rays,60Gy	D-32,MS
88d29	cn sterile	Normal	γ-rays,40Gy	D-32,MS
88d33	cn(vg)	Normal	γ-rays,40Gy	D-32,MS
88d64	lethal	Df(2R)43E2-4; 43F4-5	γ-rays,60Gy	D-32,MS
88d78	cn	Normal	γ-rays,40Gy	D-32,MS
88e7	cn	Normal	0.85MeV n+ γ-rays,20Gy	D-32,MS
88e30	lethal	In(2LR)40E;43E17-F1	0.85MeV n+ γ-rays,30Gy	D-32,MS
88e43	lethal	T(2;3)42F2-43A1;93B11-C1 +Df(2R)42F2-43A1;44B3-5	0.85MeV n+ γ-rays,20Gy	D-32,MS
88e59	cn@	Normal	0.85MeV n+ γ-rays, 20Gy	D-32,MS
88e69	lethal	Df(2R)42B3-C1; 43E18-F1	0.85MeV n+ γ-rays,20Gy	D-32,MS
88f5	lethal	Df(2R)43D7-E1; 43E18-F1	0.85MeV n+ γ-rays,15Gy	D-32,MS
88f29	lethal	T(2;3)43E17-F1; 70F-71A	0.85MeV n+ γ-rays,15Gy	D-32,MS
88f34a	lethal	Df(2R)43A3-B1; 44B9-C1	0.85MeV n+ γ-rays,15Gy	D-32,MS
88f34b	lethal	Df(2R)43E10-12; 44A2-4	0.85MeV n+ γ-rays,15Gy	D-32,MS
88f74	cn	Normal	0.85MeV n+ γ-rays,30Gy	D-32,MS
88f84	lethal	In(2R)41A; 43E17-F1	0.85MeV n+ γ-rays15Gy	D-32,MS
88f87	lethal	Df(2R)43C7-D1;44A7-B3	0.85MeV n+ γ-rays, 20Gy	D-32,MS
88f93	lethal (vg)	Df(2R)42C9-D1; 44B4-5	0.85MeV n+ γ-rays,30Gy	D-32,MS
88g42a	cn	cn Normal + In (2L) 22B2-4; 36F10-37A1	0.85MeV n+ γ-rays, 20Gy	D-32,MS
88g42b	cn(vg)	Normal	0.85MeV n+ γ-rays,15Gy	D-32,MS
88g43	lethal	Df(2R)43E5-6;43E18-F1	0.85MeV n+ γ-rays,15Gy	D-32,MS
88g53	lethal	cn Normal + In(2L) 23A6-B1; 35D4-E1	0.85MeV n+ γ-rays,20Gy	D-32,MS
88g69	cn sterile@	Normal	0.85MeV n+ γ-rays,15Gy	D-32,MS
88g74	cn	Normal	0.85MeV n+ γ-rays,15Gy	D-32,MS
88g76	lethal	Df(2R)43D7-E1; 43E18-F1	0.85MeV n+ γ-rays,20Gy	D-32,MS
88g77	cn	Normal	0.85MeV n+ γ-rays,15Gy	D-32,MS
88g88	lethal	Df(2R)43C7-D1; 43E18-F1	0.85MeV n+ γ-rays,15Gy	D-32,MS
88g91	lethal	In(2R)41D; 43E12-16	0.85MeV n+ γ-rays,20Gy	D-32,MS
88h2	lethal	Df(2R)43D7-E1; 43F7-44A1	0.85MeV n+ γ-rays,20Gy	D-32,MS
89d4	lethal	Df(2R)42B3-5; 44B7-C1	γ-rays 40Gy, Dark	phr⁻,MS
89d64	lethal	Df(2R)43B3-C1; 44B7-C1	γ-rays, 40Gy, Darkp	phr⁻,MS
89d88	cn(vg)	Normal	γ-rays, 40Gy, Dark	phr⁻,MS
89d99	lethal	Df(2R)42B4-C1; 43F-44A1	γ-rays, 40Gy, Dark	phr⁻,MS
89e8	lethal	Df(2R)42E5-F1; 43E-15-F1	γ-rays, 40Gy, Dark	phr⁻,MS
89e12	lethal	Df(2R)43E5-8; 44B5-C1	γ-rays, 40Gy, Dark	phr⁻,MS
89e15	cn	Normal	γ-rays, 40Gy, Light	phr⁻,MS
89e16	lethal	Df(2R)43D7-E1; 43E15-F1	γ-rays, 40Gy, Dark	phr⁻,MS
89e20	lethal	Df(2R)43B3-C1; 44B5-C1	γ-rays, 40Gy, Light	phr⁻,MS
89e44	lethal	Df(2R)42E2-5; 44B7-C1	γ-rays, 40Gy, Light	phr⁻,MS
89e48	lethal	T(2;3)43E; 62B; 64A + In(2R) 43F; 55F + T(2;3)43F; 62A + T(2;3)56A; 64B	γ-rays, 40Gy, Light	phr⁻,MS
89e52	lethal	Df(2R)42E7-F1; 44B5-C1	γ-rays, 40Gy, Dark	phr⁻,MS
89e72a	lethal	Normal	γ-rays, 40Gy, Dark	phr⁻,MS
89e72b	lethal	Normal	γ-rays, 40Gy, Light	phr⁻,MS
89e75	lethal	Df(2R)43B3-C1; 44C2-4 + T(2;3)41A-B; 64A5-B1	γ-rays, 40Gy, Light	phr⁻,MS
89e76	lethal	Df(2R)43E10-14; 44B8-C1	γ-rays, 40Gy, Dark	phr⁻,MS
89e84	lethal	Df(2R)42F3-43A1; 43E15-F1	γ-rays, 40Gy, Lightp	phr⁻,MS
89e104	lethal	Df(2R)43C2-4; 44B5-C1	γ-rays, 40Gy, Dark	phr⁻,MS

of the inversions are regularly flanked by the deletions as well. Admittedly, a loss of the two genes linked (e.g., br5-*b*) under inversions may be alternatively explained by the "position effect". However, the results of the in situ hybridization experiments with the coding DNA sequences obtained for the similar genetic system (inversions affecting the *w+* locus of the X chromosome) do not appear to support this assumption (see our other Note in this issue). Fourth, the size and position of the radiation-induced multilocus deletions relative to each other change multiply to one, two, three or more of the complementation groups. Their sizes are widely varied from deletions involving the single gene to ones larger than numbered divisions on the polytene map. These findings seem to be very well explained within the framework of the rosette-loopy model for higher level of the chromatin organization and of the notions about the illegitimate recombinations as a fundamental mechanism involved in deletion formation (Alexandrova and Alexandrov, 1989).

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References: Alexandrova, M.V. and I.D. Alexandrov, 1989, In: Proc. Workshop on Genetic Effects of Charged Particles, Dubna, p. 259-289.

Table 1. Origin and cytology of the radiation-induced chromosome rearrangements recovered as a lethal black mutations.

No. of mutations	Designation of mutation	Cytology	Mutagen	Genotype, germ cells irradiated
1	Df(2L)74c6	34D2-4; 34E1.2	γ-rays,40Gy	D-32,M Sd*
2	Df(2L)77c	34D2-4; 34F1.2	γ-rays,40Gy	c(3)G, MS
3	Df(2L)78j	34D2-4; 34F2-4	γ-rays,40Gy	c(3)G, E Sd
4	Df(2L)79b3	34C7-D2;34F1.2	n, 10Gy	D-32, MS
5	Df(2L)79b4	34D2-4; 34F1.2	n, 10Gy	D-32, MS
6	Df(2L)80k	34D2-4; 35C1-2	γ-rays,40Gy	c(3)G,L Sd
7	Df(2L)801	34D2-4; 34E1.2	n, 12Gy	D-32, MS
8	Df(2L)81f2	34D2-4; 34F1.2	n, 8Gy	D-32, MS
9	Df(2L)81142	34D3-8.	γ-rays,10Gy	D-32, MS
10	Df(2L)8311	34D2-4; 34E1.2	n, 10Gy	D-32, MS
11	Df(2L)8312	34D2-4; 34E1.2	n, 10Gy	D-32, MS
12	Df(2L)84h14	34D1-2; 34F1.2	γ-rays,20Gy	c(3)G, MS
13	Df(2L)84h50	34D2-4; 35C1-2	γ-rays,20Gy	c(3)G,L Sd
14	Df(2L)85b1	34D2-4; 34E1.2	γ-rays,10Gy	c(3)G, MS
15	Df(2L)85b2	34D2-8.	γ-rays,20Gy	c(3)G, MS
16	Df(2L)85c1	34D2-4; 34F1.2	n, 10Gy	c(3)G, MS
17	Df(2L)85f1	34D2-4; 34E4.5	γ-rays,20Gy	c(3)G, MS
18	Df(2L)85f2+In	34D1-2; 34F4.5 + In 33A; 35E3	n, 10Gy	c(3)G, MS
19	Df(2L)87e25	34C1; 35C1-2	γ-rays,10Gy	D-32, MS
20	Df(2L)87g23	34D2-4; 34F1.2	γ-rays20Gy	D-32, MS
21	Df(2L)88b15	34D1-2; 35E2-5	n, 2.5Gy	D-32, MS
22	Df(2L)88b42	not visible	n, 2.5Gy	D-32, MS
23	Df(2L)88c25	34B12-C1; 35B2-4	n, 2.5Gy	D-432, MS
24	Df(2L)88c58	34D2-4; 35C2-3	n, 5Gy	D-32, MS
25	Df(2L)88c75	34C5-7; 35E2-5	n, 5Gy	D-32, MS
26	Df(2L)88f32	34D2-4; 34E2-4	n+ γ-rays,20Gy**	D-32, MS
27	Df(2L)88c40	34D2-4; 34F1.2	n+ g-rays,30Gy	D-32, MS
28	Df(2L)88f43	34D2-4; 34F1.2	n+ g-rays,15Gy	D-32, MS
29	Df(2L)88g26+ T(2;Y)	34D2-4; 34F4	n+ g-rays,15Gy	D-32, MS
30	Df(2L)88g83	34D2-4; 34E1.2	n+ γ-rays,30Gy	D-32, MS
31	Df(2L)88g96	34D2-4; 34F1.2	n+ γ-rays,15Gy	D-32, MS
32	Df(2L)88g98	not visible	n+ γ-rays,30Gy	D-32, MS
33	Df(2L)88h49	34C5-7; 35A2	γ-rays + n,20Gy	D-32, MS
34	Df(2L)89e8	34D2-4; 34F1.2	γ-rays, 40Gy	phr⁻, MS
35	Df(2L)89e64b+ T(2,3)	34C5-7; 34E4.5 47C4-D1; 69C5-D1	γ-rays, 40Gy	phr⁻, MS
36	Df(2L)89e68+ T(2,Y)	34D2-4; 35B1.2	γ-rays, 40Gy	phr⁺, MS
37	Df(2L)89e72	34D1-2; 34F1.2	γ-rays, 40Gy	phr⁺, MS
38	Df(2L)89e80a	34C2-4; 35D2-4	γ-rays, 40Gy	phr⁻, MS
39	Df(2L)89e80b	34D2-4; 35B1.2	γ-rays, 40Gy	phr⁻, MS
40	Df(2L)89e88a	34C5-7; 35B8-10	γ-rays, 40Gy	phr⁻, MS
41	Df(2L)89e88b	34C2-4; 35B1.2	γ-rays, 40Gy	phr⁻, MS
42	Df(2L)89e96	34D1-2; 34F1.2	γ-rays, 40Gy	phr⁻, MS
43	In(2LR)71k1	34D4 ± 43C2	γ-rays, 40Gy	D-18, MS
44	In(2L)79d5	34D4±; 35B10±	n, 10Gy	D-32, MS
45	In(2L)79h1	34D4±; 40E	γ-rays, 40Gy	D-32, M Sd
46	In(2LR)81a	34D4±; 41	γ-rays, 60Gy	D-32, MS
47	In(2L)8117	34D4±; 40	γ-rays, 20Gy	D-32, MS
48	In(2L)83b22	34D4±; 35B10±	γ-rays, 40Gy	D-32, MS
49	In(2LR)87e152	34D4±; 41A	γ-rays, 20Gy	D-32, MS
50	In(2L)88c24	34D4±; 34F4-35A1	n, 5Gy	D-32, MS
51	In(2L)88e16	34D2-4; 36C10-D1	n + γ-rays, 20Gy	D-32, MS
52	In(2L)88e45	34D2-4; 40E-F	n + γ-rays, 30Gy	D-32, MS
53	In(2L)89e100	34D2-4; 40E-F	γ-rays, 40Gy	phr⁻, MS
54	T(2;Y)88e1 + In(2LR)	34D4±; Y 34E1.2; 43E18-F1	n+ γ-rays, 30Gy	D-32, MS
55	Tp(2)88e32	34D4±;51D11-50C14;34E1	n + γ-rays,30Gy	D-32, MS
56	T(2;3)88e88	34D8; 72A; 34D2;	n + γ-rays,30Gy 80D; 40D; 71F	D-32, MS
57	T(2;Y)88g22	34D2-4+T(2;3) 34D2-4; 87F8-12;+In(2R)41; 52A14-B1+Tp(3)69D2-3; 92A14;93A6	n + γ-rays, 30Gy	D-32, MS
58	T(2;3)88g68	34D2-4;76A6-B1+Df(2L) 34D2;35B1.2	n + γ-rays, 15Gy	D-32,MS
59	T(2;3)89e12	34D2-4;79C3-D1	γ-rays,40Gy	phr⁻,MS

*MS - mature sperm, L, M and E - late, middle and early spermatids (Sd), respectiviey
** - The equal contribution of the neutrons and gammag-rays to the total dose of the consecutive irradiation.

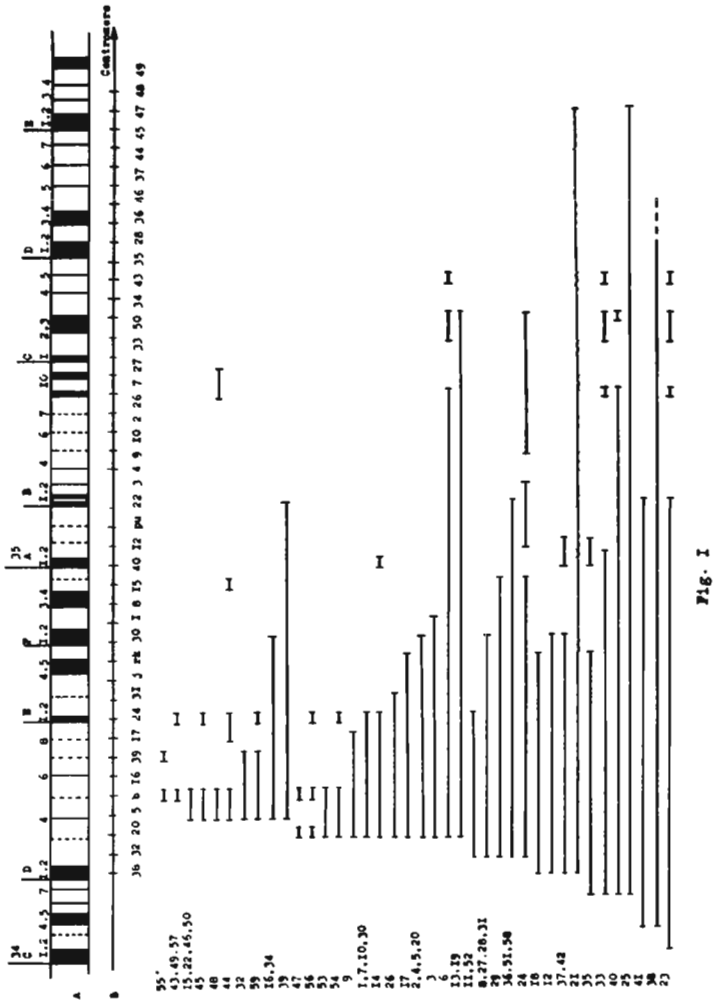

Figure 1. Complementation map of the radiation-induced chromosome *b* mutations the numbers of which (*) listed in Table 1. Deletions are ranged according to their complementation behavior with respect to the point mutations (br38, br32, etc.) on the genetic map (B). Dashed line for Df(2L)89e80a (No. 38) means uncertainty in assignment of the breakpoint. Wavy line for Df(2L) 88b15 (No. 21) indicates the fragment translocated to Y-chromosome.

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Alterations of methyl methanesulfonate induced mutation frequencies by treatment with actinomycin-D in *D. melanogaster*.

Methyl methanesulfonate (MMS) is one of the potent monofunctional alkylating agents which is mutagenic in higher eukaryotic systems (Vogel *et al.*, 1985). The mutagenic action of MMS in *Drosophila* on the induction of dominant lethals (Bateman and Chandley, 1964; Vogel and Leigh, 1975; Ryo *et al.*, 1981) and recessive lethal mutations (Fahmy and Fahmy, 1957; Zimmering, 1982; Vogel *et al.*, 1982; Cooper *et al.*, 1983; Eeken and Sobels, 1983; Vogel *et al.*, 1985) has been studied.

The genetic effect of actinomycin-D(ACM-D) is contrasting on different parameters. One one hand it causes chromosome aberrations, but, on the other, inhibits the occurrence of spontaneous mutations (Puglisi, 1968).

The present work was undertaken with a view to find out whether ACM-D would be able to alter the MMS induced dominant and sex-linked recessive lethal mutations.

Oregon-K and Muller-5(M-5) strains were used for the experiment. Before treatment with chemicals tester flies were starved for 6 hours to assure immediate uptake to test solution. Duration of treatment was restricted for 24 hours at 25°C. MMS treatment was given to flies by supplying a piece of filter paper moistened with 1ml of 1.0 mM MMS solution. For the treatment with ACM-D, a properly drenched filter paper in ACM-D solution (1.5 mg ACM-D +2 mg sugar + 100 ml distilled water) was kept in the vials of flies. The amount of genetic damage induced in cells of various spermatogenic stages was tested by transferring the treated males every three days to fresh virgin females. Each transfer constituted a brood. For the purpose of the experiment four such broods were studied. Dominant lethal mutations were measured by comparing the percentage of unhatched eggs in a control line with the treated series (Abrahamson and Lewis, 1971). For the detection of sex-linked recessive lethal mutation, Muller-5 (M-5) method was used.

Results pooled for experiments are tabulated in Tables 1 and 2.

Effects of MMS and ACM-D on successive stages of spermatogenesis showed that brood I and brood II followed by spermatozoa and spermatids were more sensitive to the induction of dominant and sex-linked recessive lethal mutations in comparison to the brood III and IV followed by spermatocytes and spermatogonia. The effects of ACM-D on the frequency of MMS induced mutations revealed that ACM-D increased MMS induced dominant lethal mutations and reduced sex-linked recessive lethal mutations recovered from all spermatogenic stages.

Acknowledgment: Authors are grateful to Dr. Maksudul Alam, Max-Plank Institute of Biochemistry, Munchen, West Germany for supplying required chemicals and articles relevant to the work; to the Bangladesh University Grants Commission, Dhaka for financial assistance.

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Please see tables on next page.

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Effects of caffeine and methyl methanesulfonate on the frequency of dominant and sex-linked recessive lethal mutations in *Drosophila melanogaster*.

Caffeine is a purine, 1,3,7-trimethyl xanthine. Since it is consumed in great quantities by man, the question concerning whether it is a mutagen for man is of great significance. Although there is now good evidence that caffeine is mutagenic in many animals, but negative and ambiguous results are also reported. In *Drosophila*, it was reported that caffeine is mutagenic (Andrew, 1959; Shakarnis, 1970; Fillipposa and Jurkov, 1973), but neither of these findings have found confirmation in subsequent experiments with *Drosophila* by other workers (Yanders and Seaton, 1962; Clark and Clark, 1968; Mittler and Callaghan, 1969).

The present work was undertaken with a view to find out whether caffeine would be able to induce dominant and sex-linked recessive lethal mutations and whether it could modify the methyl methanesulfonate (MMS) induced mutations.

Oregon-K and Muller-5 strains were used for the experiments. Before treatment all the tester flies were starved for 6 hours. Treatment with MMS was made by placing a piece of moistened filter paper with 1ml MMS solution of 1.0 mM

Table 1. Dominant lethal mutations (DLM) following treatment with methyl methanesulfonate (MMS) (1.0 mM) and actinomycin-D (ACM-D) in D. melanogaster.

Cross*		Brood	No. of eggs	No. of unhatched eggs	% of unhatched eggs ± S.E	% of DLM **
Control	♂	I	3047	431	14.09 ± 0.71	-
		II	2435	367	15.00 ± 0.71	-
X		III	2789	372	13.21 ± 1.39	-
		IV	2548	383	15.01 ± 0.64	-
Control	♀	I-IV	10819	1553	14.33 ± 0.43	-
MMS	♂	I	2978	1355	45.46 ± 0.79	36.51^{l}
		II	2784	1170	42.02 ± 0.25	31.79^{l}
X		III	3195	1328	41.54 ± 0.63	32.64^{l}
		IV	2266	815	35.85 ± 4.12	24.52^{m}
Control	♀	I-IV	11223	4668	41.22 ± 1.99	31.36 ± 2.50^{a}
ACM-D	♂	I	2867	584	20.35 ± 0.39	7.29^{l}
		II	3170	607	19.05 ± 0.20	4.76^{l}
X		III	2593	449	17.25 ± 0.81	4.65^{l}
		IV	2790	475	17.02 ± 0.60	2.35^{l}
Control	♀	I-IV	11420	2115	18.41 ± 0.79	4.76 ± 1.01^{b}
MMS + ACM-D	♂	I	2802	1546	55.17 ± 1.36	47.82^{l}
		II	2219	1178	53.16 ± 0.71	44.89^{lm}
X		III	3124	1595	51.01 ± 0.89	43.55^{lm}
		IV	2428	1162	47.80 ± 0.96	38.58^{m}
Control	♀	I-IV	10573	5481	51.78 ± 1.58	43.71 ± 1.93^{c}
MMS	♂	I	2735	1444	52.72 ± 1.78	44.96^{l}
		II	2467	1259	51.01 ± 0.75	42.36^{lm}
X		III	1990	901	45.24 ± 0.43	36.90^{m}
		IV	2228	1003	44.98 ± 0.83	35.26^{m}
ACM-D	♀	I-IV	9420	4607	48.49 ± 1.98	39.87 ± 2.28^{c}
ACM-D	♂	I	1897	971	51.17 ± 1.25	43.16^{l}
		II	2025	997	49.20 ± 0.67	40.23^{l}
X		III	2972	1341	45.06 ± 0.80	31.85^{m}
		IV	3013	1350	44.73 ± 1.22	34.97^{m}
MMS	♀	I-IV	9907	4659	47.54 ± 1.58	37.55 ± 2.55^{c}

* Brood means indicated by the same letter do not show significant difference among crosses. **% of DLM indicated by the same letter do not elicit significant difference within broods of the same cross.

Table 2. Sex-linked recessive lethal mutations (SLRLM) following treatment with methyl methanesulfonate (MMS) (1.0mM) and actinomycin-D (ACM-D) in *D. melanogaster.*

Cross*		Brood**	No. of chromosomes tested	No. of lethals	% of SLRLM ± S.E
Control	♂	I	839	1	0.09 ± 0.09
		II	854	1	0.10 ± 0.10
X		III	871	2	0.21 ± 0.10
		IV	862	0	0
Control	♀	I-IV	3426	4	0.10 ± 0.04^{a}
MMS	♂	I	818	63	7.70 ± 0.11^{l}
		II	852	45	5.28 ± 0.03^{l}
X		III	812	18	2.21 ± 0.08^{m}
		IV	836	6	0.70 ± 0.03^{m}
Control	♀	I-IV	3318	132	3.97 ± 1.57^{b}
ACM-D	♂	I	871	5	0.56 ± 0.07^{l}
		II	843	4	0.46 ± 0.06^{l}
X		III	839	3	0.36 ± 0.01^{l}
		IV	864	2	0.21 ± 0.10^{l}
Control	♀	I-IV	3417	14	0.40 ± 0.07^{bc}
MMS + ACM-D	♂	I	857	35	4.09 ± 0.08
		II	872	31	3.56 ± 0.08^{lm}
X		III	839	11	1.31 ± 0.04^{mn}
		IV	879	4	0.44 ± 0.06^{n}
Control	♀	I-IV	3447	81	2.35 ± 0.88^{ac}
MMS	♂	I	748	34	4.56 ± 0.10^{l}
		II	817	31	3.79 ± 0.04^{l}
X		III	828	15	1.80 ± 0.05^{m}
		IV	813	5	0.60 ± 0.07^{m}
ACM-D	♀	I-IV	3206	85	2.69 ± 0.91^{b}
ACM-D	♂	I	837	40	4.78 ± 0.12^{l}
		II	846	32	3.78 ± 0.02^{l}
X		III	827	14	1.69 ± 0.06^{m}
		IV	858	4	0.45 ± 0.06^{m}
MMS	♀	I-IV	3368	90	2.67 ± 0.98^{b}

* Brood means indicated by the same letter do not show significant difference among crosses. ** Means indicated by the same letter do not elicit significant difference within broods of the same cross.

Table 1. Dominant lethal mutations (DLM) following treatment with methyl methanesulfonate (MMS) (1.0 mM) and caffeine in D. melanogaster.

Cross *		Brood	No. of eggs	No. of unhatched eggs	% of unhatched eggs ± S.E.	% of DLM **
Control	♂	I	3211	418	13.03 ± 0.32	-
		II	2830	453	15.97 ± 0.44	-
X		III	1945	281	14.37 ± 0.58	-
		IV	2655	351	13.16 ± 0.86	-
Control	♀	I-IV	10641	1503	14.13 ± 0.68	-
MMS	♂	I	3190	1446	45.30 ± 0.84	37.10^{l}
		II	3117	1389	44.25 ± 0.66	33.65^{m}
X		III	2248	953	42.29 ± 0.96	33.460^{m}
		IV	2259	1184	39.93 ± 1.65	30.83^{m}
Control	♀	I-IV	11514	4972	42.94 ± 1.18	33.54 ± 1.32^{a}
Caffeine	♂	I	2379	417	17.49 ± 0.74	5.13^{l}
		II	2558	435	16.94 ± 0.68	1.15^{m}
X		III	2701	444	16.43 ± 0.13	2.40^{lm}
		IV	2693	412	15.28 ± 0.29	2.44^{lm}
Control	♀	I-IV	10331	1708	16.53 ± 0.47	2.78 ± 0.84^{b}
MMS +Caffeine	♂	I	2587	1218	47.08 ± 0.07	39.15^{l}
		II	2844	1327	46.64 ± 0.33	36.50^{ln}
X		III	2273	968	42.48 ± 1.73	32.83^{mn}
		IV	2818	1146	40.63 ± 1.11	31.63^{m}
Control	♀	I-IV	10522	4659	42.21 ± 1.58	35.03 ± 1.72^{a}
MMS	♂	I	2951	1382	46.83 ± 0.03	38.86^{l}
		II	2014	924	45.93 ± 0.54	35.65^{lm}
X		III	2565	1091	42.48 ± 0.58	32.83^{mn}
		IV	2648	1067	40.25 ± 0.47	31.19^{n}
Caffeine	♀	I-IV	10178	4464	43.87 ± 1.53	34.63 ± 1.68^{a}
Caffeine	♂	I	3027	1400	46.23 ± 0.30	38.86^{l}
		II	2851	1305	45.79 ± 0.47	35.49^{lm}
X		III	3121	1312	42.03 ± 0.17	32.30^{mn}
		IV	2605	1046	40.13 ± 0.20	31.06^{n}
MMS	♀	I-IV	11604	5063	43.54 ± 1.48	34.25 ± 1.60^{a}

* Brood means indicated by the same letter do not show significant difference among crosses.
** % of DLM indicated by the same letter do not elicit significant difference within broods of the same cross.

Table 2. Sex-linked recessive lethal mutations (SLRLM) following treatment with methyl methanesulfonate (MMS) (1.0 mM) and caffeine in *D. melanogaster*.

Cross *		Brood **	No. of chromosomes tested	No. of lethals	% of SLRLM ± S.E.
Control	♂	I	752	2	0.24 ± 0.12
		II	768	1	0.10 ± 0.10
X		III	832	1	0.09 ± 0.09
		IV	82	1	0.12 ± 0.12
Control	♀	I-IV	3134	5	0.14 ± 0.03^{a}
MMS	♂	I	973	78	8.02 ± 0.06^{l}
		II	867	52	6.00 ± 0.13^{l}
X		III	865	20	2.31 ± 0.07^{lm}
		IV	832	5	0.59 ± 0.05^{m}
Control	♀	I-IV	3537	155	4.23 ± 1.69^{b}
Caffeine	♂	I	843	4	0.45 ± 0.05^{l}
		II	897	3	0.34 ± 0.01^{l}
X		III	932	3	0.32 ± 0.01^{l}
		IV	837	2	0.21 ± 0.10^{l}
Control	♀	I-IV	3509	12	0.33 ± 0.05^{a}
MMS +Caffeine	♂	I	852	66	7.74 ± 0.12^{ll}
		II	866	50	5.77 ± 0.03^{lm}
X		III	876	18	2.05 ± 0.03^{mn}
		IV	847	4	0.46 ± 0.06^{n}
Control	♀	I-IV	3441	138	4.00 ± 1.67^{b}
MMS	♂	I	819	65	7.93 ± 0.09^{l}
		II	832	49	5.89 ± 0.06^{lm}
X		III	764	16	2.09 ± 0.02^{mn}
		IV	52	5	0.51 ± 0.06^{n}
Caffeine	♀	I-IV	3367	135	4.10 ± 1.70^{b}
Caffeine	♂	I	878	70	7.97 ± 0.07^{l}
		II	964	55	5.70 ± 0.08^{lm}
X		III	832	17	2.02 ± 0.10^{mn}
		IV	812	5	0.60 ± 0.09^{n}
MMS	♀	I-IV	3486	147	4.07 ± 1.68^{b}

* Brood means indicated by the same letter do not show significant difference among crosses. ** Means indicated by the same letter do not elicit significant difference within broods of the same cross.

concentration in the starved flies containing vials. For the treatment with caffeine a piece of filter paper soaked with 1ml solution of 0.2% caffeine in 10% sucrose solution was kept in the starved flies containing vials. Duration of treatment was restricted for 24 hours for both MMS and caffeine. Brood pattern analysis has been made with four 3-day broods for the detection of induced mutations at stage specific times of spermatogenesis (Fahmy and Fahmy, 1961).

Results are presented in tables 1 and 2.

The effects of caffeine on the yield of dominant and sex-linked recessive lethal mutations revealed that although caffeine induced mutations (significant or not) but it showed its weak effect in the induction. The effects of caffeine was found something higher in the post-meiotic stages.

It was also observed that caffeine at the present experimental dose in no way influenced or modified the effects of MMS induced mutations and it also did not influence any brood.

Acknowledgments: The financial support from the Bangladesh University Grants Commission, Dhaka, is thankfully acknowledged.

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Allemand, R. Universite C. Bernard, 69622, Villeurbanne, France. Chromosomal analysis of the circadian oviposition behavior in selected lines of *D. melanogaster*.

In *D. melanogaster*, several traits of the ovipositional behavior can show a genetic divergence in response to selection: the oviposition site preference (OSP), the choice between two media (Bird and Semeonoff, 1986) or the tendency to insert eggs (Takamura and Fuyama, 1980). Recently, the oviposition blocking capacity and the circadian oviposition rhythm that both are related to egg retention show a successful response to selection (Allemand and Bouletreau-Merle, 1989).

For the daily oviposition rhythm, the European populations patterns present high laying rate during photophase and a peak at dusk. After 9 generations of selection, two different lines obtained were one with an only maximum laying during photophase without further peak during scotophase, and the other with a high peak at the beginning of the scotophase (figure, selected lines sh and sb).

Technique of chromosome transfers with balancer stocks was used to assay the genetic role of the three major chromosomes on the daily oviposition rhythm. Chromosomes 2 and 3 were marked respectively with Cy (In 2 LR) L/Pm and In (3LR) TM3 Ser/Sb. For the X chromosome, no marker was used and the wild chromosome was manipulated by using its hemizygous state in males.

The chromosome substitution experiment began at generation 7, when selection was becoming ineffective (extreme phenotypes). After series of crosses, the chromosome transfers gave rise to 8 different lines carrying homozygous chromosomes in various combinations. The initial genotypes containing three pairs of chromosomes issued from the initial selected lines were reconstituted after the crosses. No attempt was made to study the effects of chromosomes in heterozygous condition. Results for the eight phenotypes are given in figure (part B). The reconstructed parental lines (sh2 and sb2) were almost identical to the initial selected lines (sh and sb), thus demonstrating the validity of the method.

Concerning the peak at the beginning of darkness, lines with two chromosomes from the high line had a higher peak at dusk than those with one high line's chromosome only, what suggested an additive effect of those chromosomes. Lines with chromosomes 1-3 and 2-3 (figure b,c) from the high line that were almost identical to this parent line revealed the main role of chromosome 3. These results were confirmed by the patterns of lines f and g (figure). Both lines that carried 2 chromosomes from the low selected line (one of which was the chromosome 3) were very similar to it.

Overall statistical analysis was done on the 8 curves transformed by the multivariate method of reciprocal averaging (see Allemand and David, 1984). Anovas on the values of the first axis (which explains 75% of the total variation) confirmed the conclusions drawn from the figure, i.e. the additive effect of the main chromosomes and the major role of chromosome 3 ($p < .05$). Genetic analysis by crosses and also by chromosome substitution in old laboratory strains which had similar pattern to those of selected lines had led to the same conclusions (Allemand and David, 1984). This result may be explained by few genes having broad and mainly additive effects, involved in regulation of the egg-laying physiology.

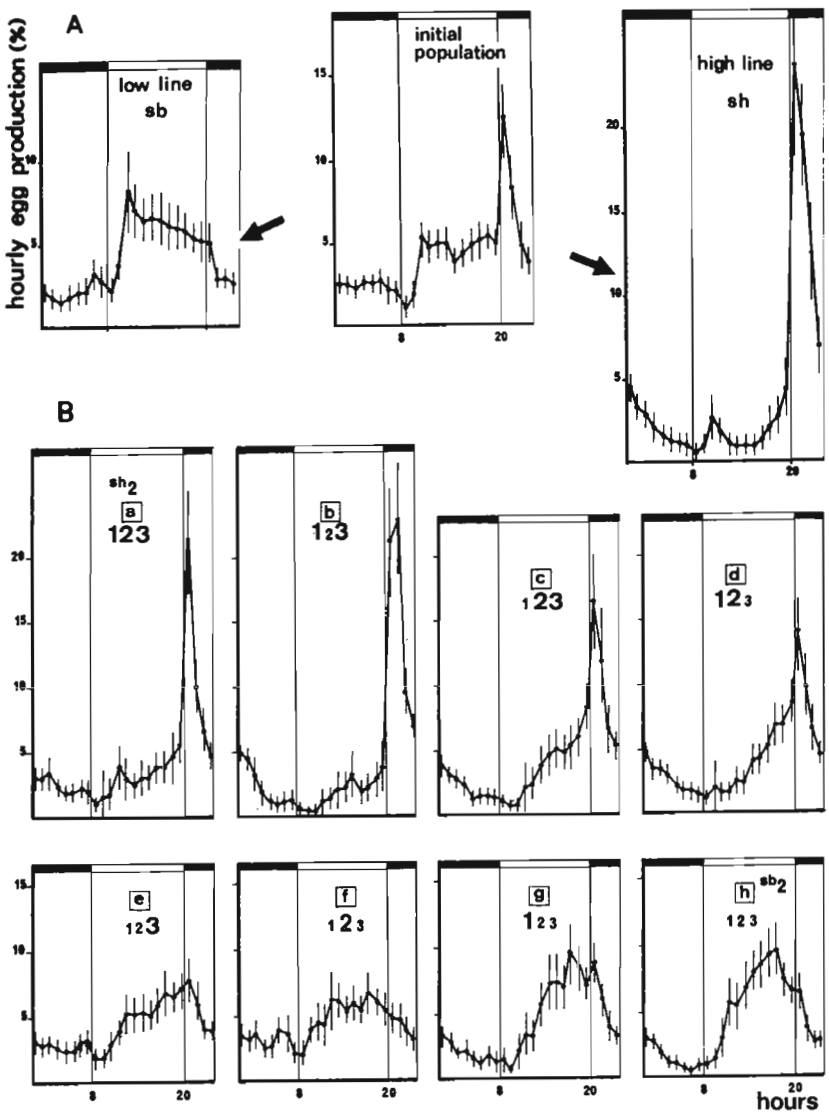

Figure. A: Selection for the circadian oviposition rhythm of *D. melanogaster* under LD 12:12 photoperiod. Initial population (France) and selected patterns (sh and sb) at generation 8. B: Results of chromosome transfers between the two selected lines. Genotypes of the 8 lines (chromosomes in homozygous condition) are indicated by figures. Large figures refer to the major chromosomes issued from the high line (high peak in dark phase) and small figures from the low line (no peak).

Under experimental conditions, the female are young, well fed with a constant supply of food and of laying substrate. The photoperiod induces a cyclical acitivity of the ovarian physiology and somes eggs are regularly retained in the ovarian tubes for the end of the photophase till the light-off signal results in the laying peak. By selection, rhythm patterns are strongly modified without significant change of the daily production. Genetic changes seem to concern the capacity of egg retention and the mechanisms which control the oviposition at the uterus level (Allemand and Bouletreau-Merle, 1989). This mechanisms allow the females to adjust their reproductive response to environmental events, in particular by laying numerous eggs when conditions are favorable. This trait which seems to be selected by natural factors (Bouletreau-Merle et al., 1987), might be particularly important for the population when oviposition sites are scattered, as should be the case in temperate regions.

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Arbona, M.[1], R. de Frutos[1], and J.L. Diez[2].
[1]Departmento de Genetica, Universitat de Valencia, Spain; [2]CSIC, Madrid, Spain. Localization of DNA-RNA hybrids in polytene chromosomes of *Drosophila subobscura* by indirect immunofluorescence.

The method of immunoflourescent localization of DNA-RNA hybrids has been extensively used to study the sites of transcriptional activity in polytene chromosomes. The technique used was indirect immunofluorescence by means of specific antibodies directed against DNA-RNA hybrids.

Several methods to detect the existence of DNA-RNA hybrids on polytene chromosomes have been described (Rudkin and Stollar, 1977; Büsen et al., 1982; Alcover et al., 1982). Depending on the method followed the results may be interpreted in different ways.

There were differences in the experimental procedures followed by these authors. Whereas Rudkin and Stollar (1977) and Büsen et al. (1982) carefully avoided air-drying of the slides, Alcover et al. (1982) air-dried the preparations and then subjected them to conditions promoting denaturation/renaturation prior to staining with the antibody.

In this work, we have studied the sites of transcriptional activity in polytene chromosomes of *Drosophila subobscura* using the immunofluorescence technique. We also show data on the distribution of DNA-RNA hybrids on polytene chromosomes subjected to heat shock treatment.

Third instar larvae of *Drosophila subobscura* (H271 strain) cultured at 19°C were subjected to heat shock (31°C) for 30 m. DNA on squashed preparations of the salivary glands chromosomes was denatured, reannealed, incubated with goat antihybrid serum and stained with fluorescein labelled rabbit IG as described by Alcover et al. (1982).

1) **DNA-RNA hybrids in *Drosophila* polytene chromosomes.** Following the procedure described by Alcover et al. (1982) indirect immunofluorescence of *Drosophila subobscura* polytene chromosomes led to the patterns shown in Figure 1a. Fluorescent bands can be detected in the whole genoma. Chromosome E show well-defined banding patterns. Width and intensity of fluorescence varied considerably from one band to another.

2) **Distribution of DNA-RNA hybrids in polytene chromosomes of heat-shocked *Drosophila subobscura* larvae.**

In *Drosophila subobscura*, the heat-shock response has been well characterized. In response to heat shock, or recovery from anoxia, the expression of several loci placed at different points of the genome is induced. These loci are: 2C (A chromosome) 18C, 27A (J chromosome) 85AB, 89A and 94A (O chromosome) (Pascual et al., 1986; Arbona et al., 1987). The majority of puffs active during normal development decrease in their activity. As a consequence, transcription in these polytene chromosomes is almost exclusively restricted to the t-puffs. As can be seen in the Figure 1b, (Staining procedure according to Alcover et al., 1982), the fluorescence was not restricted to the t-puffs. However, the highest level of fluorescence intensity at the heat shock puffs has been detected.

Acknowledgments: This work was supported by a grant from Conselleria de Cultura, Educacio i Ciencia de la Generalitat Valenciana.

References: Arbona, M. and R. de Frutos 1987, Biology of the Cell 60:173-182; Alcover A., M. Izquierdo, B.D. Stollar, Y. Kitagawa, M. Miranda, and C. Alonso 1982, Chromosoma 87:263-277; Büsen, W., J.M. Amabis, O. Leoncini, B.D. Stollar, and F.J.S. Lara 1982, Chromosoma 87:263-277; Pascual, L. and R. de Frutos 1986, Biology of the Cell 57:127-133; Rudkin, G.T. and B.D. Stollar 1977, Nature 265:472-473.

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Reduction of the frequency of the reaction mass formation by successive matings in Drosophila.

Reaction mass formation in *Drosophila* was first reported by Patterson (1946). Reaction mass formation has seemed to play important roles in fecundity and self defense in two related species of *Drosophila nasuta* and *D. pallidifrons*, belonging to the *D. nasuta* subgroup, *D. immigrans* species group (Asada and Kitagawa, 1988). In cross experiments and observation, the relationship between the duration of copulation and the frequency of reaction mass formation was investigated. Results showed that the duration of copulation presented longer times in the "high strain" and shorter times in the "low strain" in the ability of reaction mass formation, respectively. These results suggested that the duration of copulation played an important role on the ability of reaction mass formation and possibly existed the threshold for quantity of secretions from male's internal reproductive organs to form the reaction mass (Asada, 1988). No sperm was required for the reaction mass formation (Asada and Kitagawa, 1988).

Successive successful mating was performed. A single male was made to mate with virgin females successively. The copulated male was replaced to the vial containing approximately 20 virgin females and fresh medium after the termination of copulation. Copulated females were dissected within two hours after the termination of copulation in order to clarify whether reaction mass was formed or not in the same manner as the previous paper (Asada and Kitagawa, 1988). Matings were performed in less than six hours, and 100 copulated pairs were used for the first matings in all crosses with the exception of one intraspecific cross of *D. pallidifrons*. The interspecific crosses between *D. nasuta* females and *D. pallidifrons* males were not recorded because no copulation occurred.

Results are shown in Fig. 1. The frequency of reaction mass formation and its size became linearly lower and smaller, respectively, as the number of successive matings increased in all crosses. Additionally, the size of male's internal reproductive organs, testis and accessory gland, became smaller with an increasing number of successive matings (data not shown). The reduction values were statistically significant as measured by the Chi-Square test. In all crosses except for the interspecific crosses between *D. pallidifrons* females and *D. nasuta* males, reaction mass formation were not observed in females copulated by the second-mated males in intraspecific crosses of *D. pallidifrons* to the fifth-mated males in intraspecific crosses of *D. nasuta*. When *D. pallidifrons* females were copulated with *D. nasuta* males, the size of reaction mass became smaller as the number of successive matings increased although the frequency did not clearly decrease. Almost the same tendencies were represented when females of both of the species were copulated with the XO sterile males, that is, the third-mated males could not induce the reaction mass formation. These

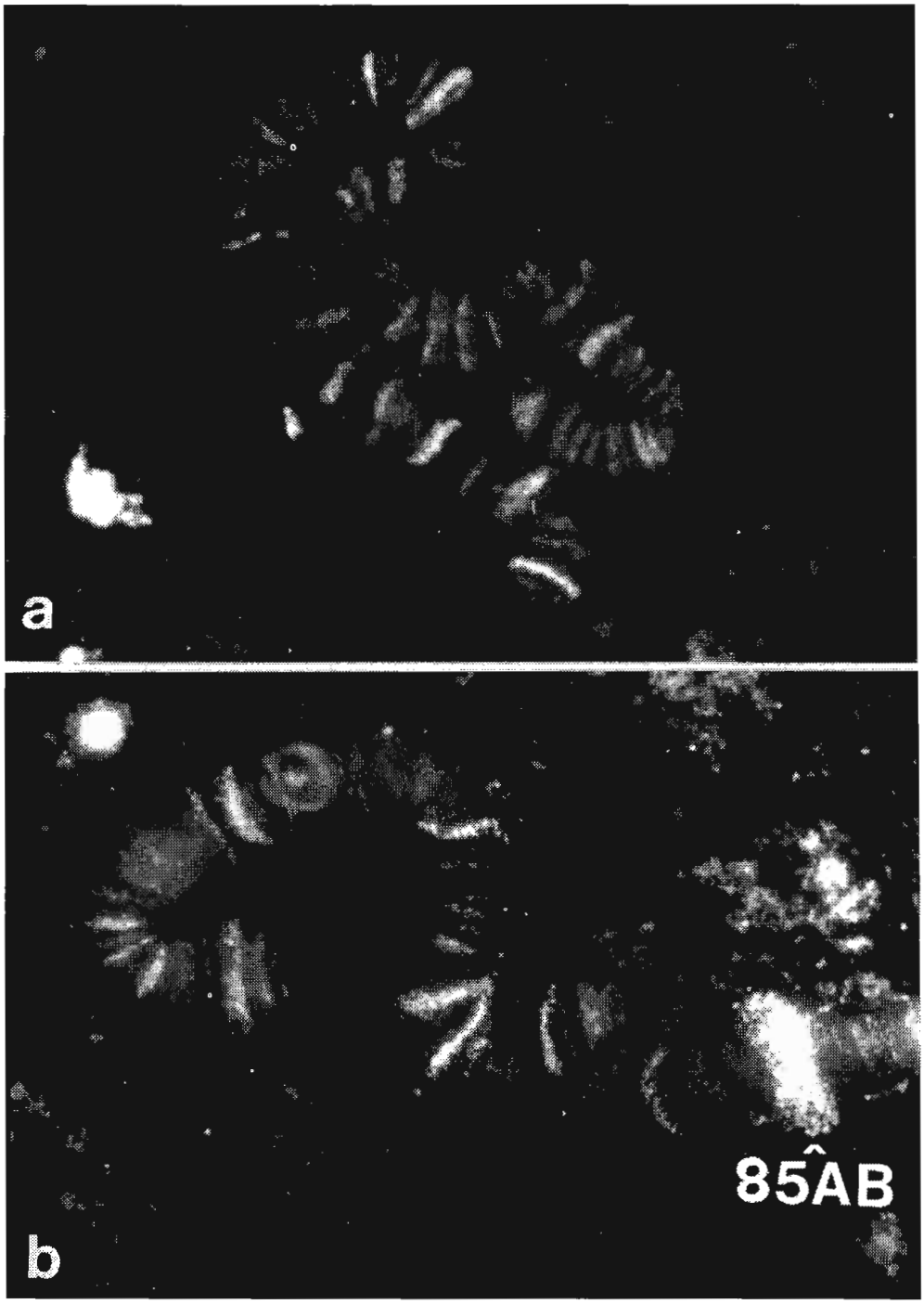

Figure 1. Immunofluorescent localization of DNA-RNA hybrids on polytene chromosomes of *Drosophila subobscura*. Staining procedure according to Alcover et al. (1982). (a) Fluorescence pattern of E chromosome. (b) Fluorescence pattern of O chromosome after experimental treatment: heat shock (31°C). A strong fluorescence at the heat shock puff (85AB) has been detected.

results suggested that the minimum quantity, such as threshold, of secretions from male's internal reproductive glands other than sperm for the formation of reaction mass was required.

Acknowledgment: I wish to express my hearty thanks for reading the manuscript to Dr. Kimble, D. M., Okayama University of Science.

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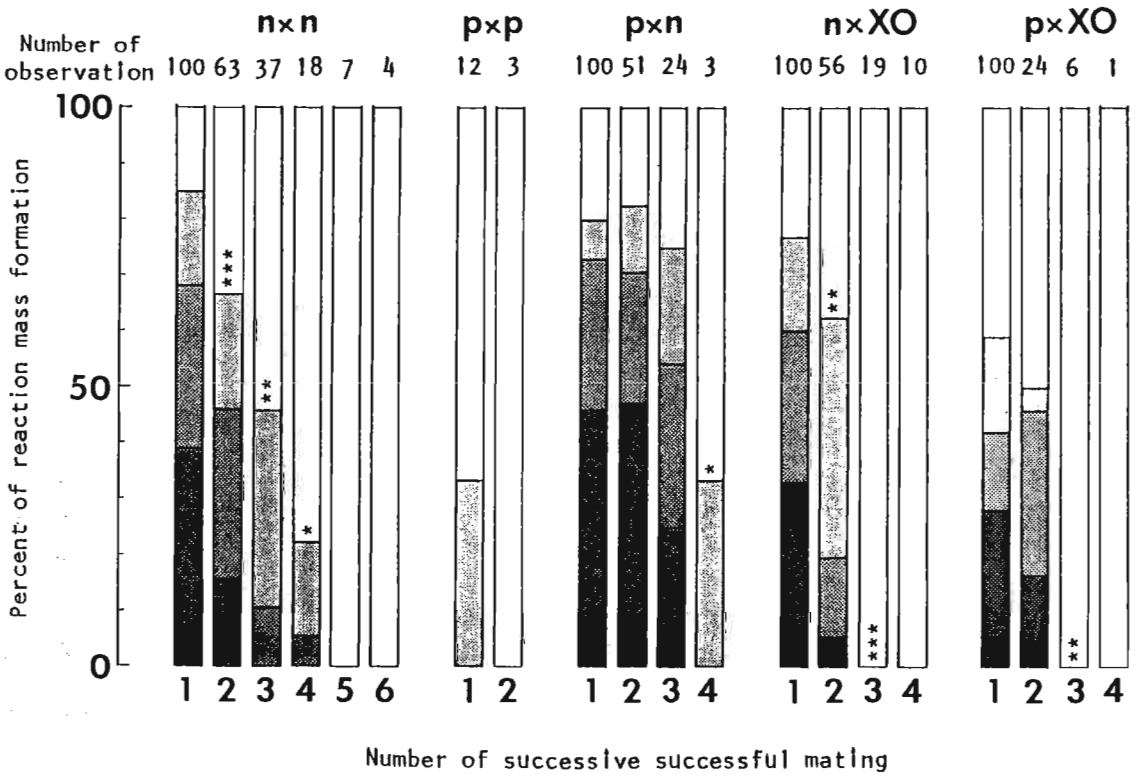

Figure 1. Frequency of reaction mass formation after successive matings. Species: n: *D. nasuta*, p: *D. pallidifrons*, XO: XO sterile male. Dark hatchmarks, large; medium hatchmarks, middle; light hatchmarks, small.
*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

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The *vasa* protein is not required for germ-line specific splicing.

The expression of *vasa* is limited to the germ-line in *Drosophila melanogaster* (Hay et al. 1989 Development 103: 625-640; Lasko and Ashburner, 1989 Nature 335: 611-617; Hay et al. 1990 Development 4: 905-921). Its product is required both for germ-line determination and for the development of the oocyte. *vasa* is expressed in the testes, but this is apparently not required, since males homozygously deleted for *vasa* are fertile. The protein product of *vasa* shows strong amino-acid sequence similarity to a number of proteins, from *E. coli* to man, that have been named the DEAD-family of proteins (Linder et al. 1989 Nature 337: 121-122). There is strong biochemical evidence that the common functional feature of these proteins is that they are RNA-dependent ATPases (e.g. Nishi et al. 1988 Nature 336: 496-498; Iggo et al. 1990 Nucleic Acids Res. 18: 5413-5417; Iggo and Lane 1989 EMBO J. 8: 1827-1831). Several members of this protein family are apparently involved in various aspects of RNA metabolism, including splicing (see, for example, Chang et al. 1990 Proc. nat. Acad. Sci. USA 87: 1571-1575 and Wassarman and Steitz 1991 Nature 349: 463-464). At the Schloss Ringberg meeting in January 1990 Allan Spradling and Peter Gergen independently suggested to me that *vasa* might be required for germ-line specific splicing. An obvious test of this hypothesis was to see whether or not *vasa* was required for the splicing of the third intron of the P-element. Laski and Rubin (1989 Genes and Development 3: 720-728) had already showed that the expression of lacZ could be made

germ-line specific if this gene's coding region included this intron in frame.

Frank Laski gave me several lines transformed with either P[hsp70-1911 Pst-2183 Bam-lacZ], where the hsp70 promoter drives a lacZ into which the third intron of the P-element had been engineered, or with a control plasmid, P[hsp70-1911 Pst-(delta2-3)-2183 Bam-lacZ]. I took two lines, each with a single insertion on the X-chromosome: 4011 with the experimental construct and 4018 with the control. Each of these chromosomes were then crossed into flies mutant for *vasa*. Two series of experiments were done, one with an EMS-induced allele D1 of *vasa* that lacks immunologically detectable protein, vasa, and the other with two deletions that, when heterozygous with each other delete about 6-kb of the *vasa* gene, Df(2L)A267 and Df(2L)TE116(R)GW18. As controls for both series all of these mutants were studied when heterozygous with *CyO*.

Virgin adult flies (2-3 days old) were heat-shocked, according to Frank Laski's protocol, i.e. 60 min at 37°C, 90 min at room temperature and then 60 min at 37°C, and within the next 30 min roughly dissected in *Drosophila* saline and the entire animals transferred to X-gal solution (as Laski and Rubin, loc cit.). After staining over night the gonads were dissected and mounted for study.

The patterns of staining in the controls were very similar to those reported by Laski and Rubin: i.e. in flies with the 4018 construct there was extensive and heavy staining of somatic tissues, including the follicular epithelium of the ovary; in flies carrying the 4011 construct there was only weak staining in somatic tissues (especially the gut) but intense staining in the ovarian germ-line. I did not detect any significant staining in the apex of the testes in these flies. In females homozygous for $vasa^{D1}$ or that were Df(2L)A267/Df(2L)TE116(R)GW18 the staining patterns were identical to those in their *CyO* sibs. The ovaries of these mutant flies were, as expected, much smaller than those of the controls and lacked all late stages of oogenesis.

From these data I conclude that the *vasa* protein is not required for the germ-line specific splicing of the third P-element intron.

Acknowledgments: This study was supported by an MRC Programme Grant. I thank John Roote and, especially, Glynys Johnson, for constructing the necessary strains and my students Bruce Reed and Mark Metzstein for some of the boring bits. I also thank Allan and Peter for giving me the idea for the experiment, Don Rio for encouragement (although, of course, he knew what the answer would be) and Frank Laski for the transformed lines that made it possible. Finally I thank Janni Nusslein-Volhard for organizing the meeting in Schloss Ringberg and giving us all the chance to see Michael Levine at his best.

Announcements

Drosophila Information Newsletter (DIN)

In addition to the hard copy versions of DIS, Kathy Matthews and Carl Thummel have started the Drosophila Information Newsletter (DIN). It is hoped that this publication will complement the traditional role of DIS in encouraging informal communication among *Drosophila* workers. DIN is intended to provide a forum for rapid communication of short, technically oriented information and announcements/requests of general interest to the *Drosophila* community. (Research reports will not be included.) In order of preference, material for DIN publication may be submitted as e-mail, on disk (text/ASCII file) or as high resolution hard copy. No figures or graphs can be accepted. DIN will be published quarterly and distributed electronically, free of charge. Information appearing in DIN will be reprinted in DIS for those without access to e-mail.

Requests for Assistance

Bruce Wallace and Bruce J. Turner would appreciate correspondence with any worker who has stocks or can collect *Drosophila mangabeirai* Malogolowkin or who is otherwise familiar with it. This parthenogenetic species is known from Bahia (Brazil), Costa Rica, El Salvador, Honduras, Nicaragua, Panama, Puerto Rico and Trinidad. It was studied in H.L. Carson's laboratory in the 1950's and 1960's, but stocks are apparently no longer available. Please write to Bruce Wallace and Bruce Turner, Department of Biology, VPI & SU, Blacksburg, VA 24061. (703) 231-744.

Band, H.T. Michigan State University, E. Lansing. A correction on unidentified *Chymomyza* species no. 1 in Virginia.

Band (1986) reported the existence of 2 unidentified *Chymomyza* species in Giles Co. in Virginia's Alleghany Mountains. Both species belong to a new species group; flies have a black frons and yellowish-brown mesoscutum rather than matching colors, either black or yellowish-brown, in these two areas (Okada, 1976). However, species #1 is larger and has dark forelegs and tarsi in contrast to smaller species #2 in which second and fifth tarsal joints and tarsi are yellowish.

Band also reported one pair captured among the first unidentified species in this group in mid-July, 1985. She described the female which escaped following too light etherization, as having a 'shovel-like ovipositor.' This is in error. Retrospectively, the 'female' probably was a *Chymomyza caudatula* male, previously unreported in Virginia until this species was collected with *C. procnemoides* and *C. aldrichii* in 1986 when interspecies male aggression involving *C. caudatula* and the smaller males was also observed (Band, 1988). A sp. no. 1 female was collected in 1987 (Band, ibid.). There was no "shovel-like ovipositor."

Fresh cut wood and freshly damaged trees at the 3900 foot elevation that have acted as lek sites for sympatric chymomyzid species provide no evidence they are also used as oviposition sites. Therefore where forests and the chymomyzids in Giles Co., VA. are breeding and what types of substrates the diverse species use remain unknown. Enomoto (1981) reported that *C. costata* in Japan bred in tree stumps.

References: Band, H.T. 1986, DIS 63:26-27; Band, H.T. 1988, Inter. J. Comp. Psychol. 2:3-26; Enomoto, O. 1981, Low Temp. Sci., Ser. B. 39:31-39; Okada, T. 1976, Kontyu, Tokyo 44:496-511.

Band, H.T. Michigan State University, E. Lansing. More on *Drosophila* overwintering.

Plough (1939) was the first to argue that *Drosophila* populations in the north successfully overwintered. This provided the foundation for Ives' important on-going research on the South Amherst (M.A.) *D. melanogaster* population. Population overwintering was never seriously challenged until McCoy (1962) and led to distinctions between protected sites (Ives 1970) and unprotected sites (Band and Band 1980).

Nearly ten years' work on cold hardy *Chymomyza amoena* in Michigan has turned up only one other species, an otitid, emerging from overwintered dropped apples in orchards (Band 1988). By contrast, as shown in Table 1, *D. affinis* group adults emerged from overwintered apples collected among wood chips that enlarge the back patio at the author's residence. Emergence of other species as diastatids and *Lonchaea* which have emerged from Virginia apples in summer (Band 1988 and unpublished) strengthen the protected site concept. In insect coldhardiness work, wood is recognized as a good insulator.

Table 1. Species emerging from overwintered apples collected from a protected site in spring, 1988 in Michigan.

Month, day	Species	No.	Emergence date at 22°C
April 11	*Chymomyza amoena*	1	21
	Drosophila affinis group	3	22
	Diastatids	2	21,22
May 8	*Drosophila duncani*	1	10
	Drosophila algonquin	4	15-20
	Lonchaea	1	18
	Anicipoda	2	19
	unidentified midge	1	18

From the emergence date of the first *Drosophila* group, overwintering in the egg stage is postulated. This is also the stage most likely to escape detection from repeated visits by birds in autumn, spring and snow-free days in winter.

Emerging *Drosophila* are transferred without etherization to glass population bottles and supplied with food. In the process of checking for eggs in the food dish, the male in the first group escaped before sex combs could be inspected. Sex combs on the male in the second group (3 females, 1 male) identified it as *D. algonquian*. Females also never produced progeny in the second group despite addition of more yeast to the food. Males and females of different species in both groups might account for the sterility; *D. affinis* has also been collected in E. Lansing. Otherwise, failure of adults to become fertile in laboratory conditions of constant temperature and random lighting after a previous stage has successfully survived subzero conditions may have important implications for the *Drosophila* cryobiology project.

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Band, H.T. Michigan State University, East Lansing, Michigan. Literature on drosophilid cold survival.

Cryobiologists maintain an active file of papers on plant and arthropod cold tolerance. Despite the *Drosophila* cryobiology project, no survey of the literature exists on work on the effects of chilling or subzero temperatures on this insect group. The following list, though incomplete, demonstrates the range of studies that have been carried out: cold shock effects, chromosomal effects, mutagenesis, laboratory studies on survival at chilling or subzero temperatures and natural population work.

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Beckman, C. and C. Gryllis. Department of Biology, Concordia University, Montreal, Quebec. Competition between wild type and shaker males for a single virgin female.

Inexperienced males and virgin females were collected and kept in the same sexed group of 20 to 30 flies in shell vials with food until they were 3 days old. Then the males were reetherized and placed in pairs in a shell vial with medium. The males were allowed to recover from the etherization. After this a single virgin female was placed in each vial. In the table the genotypes of the males and females are shown. The table shows the number of trios in which each type of male was able to mate first with the female. Sh^3/FM7A females and Sh^5/FM7A females were first generation individuals from the mating of the Sh^C/FM7A females to Sh^3 or Sh^5 stock males. Sh^C is a new shaker mutation which is lethal in homozygous and hemizygous form. Wild type, Sh^3 and Sh^5 males are from the stock bottles and hence can be expected to be somewhat inbred.

In the two cases where the shaker mutation in the male and the female is the same, wild type and shaker males are roughly equal in ability to mate first. Neither case shows a significant deviation from a 1 to 1 ratio by Chi-square goodness of fit tests. In all other cases the wild type male is clearly superior. A Chi-square contingency test for all the samples in which the wild type male predominates show no significant differences.

The data suggest that Sh^3 and Sh^5 stocks have behavioral adaptations that make the males and females more compatible. These could not be due to recessive genes in the females because 1st generation females which share half their chromosomes with the Sh^C/FM7A stock were used. The fact that no particular advantage is shown for cases where the shaker mutation is not the same indicates that each stock may have different adaptations. The data suggests that the males have behavioral mutations that aid them with their own females.

	Males			
Female	+	Sh^3	+	Sh^5
$\frac{Sh^C}{FM7A}$	79	18	80	20
$\frac{Sh^3}{FM7A}$	40	54	78	16
$\frac{Sh^5}{FM7A}$	80	18	38	53
$\frac{+}{+}$	99	12	90	10

Bhakta, R.K. and A.S. Mukherjee. Department of Zoology, Calcutta University, India. Replication cytology of polytene chromosomes in a sex linked recessive lethal (l^4) in *Drosophila melanogaster*.

A number of point mutation lethals were generated at the 3A2-8 map region of the X chromosome which are located near the tip of the polytene X chromosome. These were arbitrarily named from l^1 to l^{19}. These lethals were genetically mapped by complementation mapping (Bhakta and Mukherjee, 1984). Cytological characterization of one of these mutations is presented here.

The lethal larvae (without cover) from l^4 stock were grown in the culture medium and the samples were collected for the dissection of the salivary gland at various intervals (viz. 6th day, 13th day, 20th day and 27th day after hatching) as they have long larval life. The polytene chromosomal preparations reveal that on the 6th day after hatching the chromosomes (Fig. 1a) appear very small and indistinct with respect to normal band-interband-puff configuration and a similar chromosomal structure is observed in 13 day old larvae which have little larger chromosomal complements (Fig. 1b). A relatively better polytene morphology has been attained when the larvae become 20 days old (Fig 1c) and finally near the lethal phase on the 27th day the salivary gland chromosome becomes thickened and shortened with a stumpy structure (Fig. 1d).

In order to find out the relative rate of polytenization, the DNA content was measured cytophotometrically on the chromosomes of the l^4 mutant and compared with that in Oregon R^+. The mitotic chromosomes were measured to obtain the polytenic value of the chromosomes in the l^4 and wild type polytene chromosome. The transformed absorbance of the polytene nuclei and neural ganglia nuclei of both lethal larvae (at 27th day after hatching) and the late 3rd instar larvae of Oregon R^+ has been measured by "one wavelength two area method" (Garcia and Iorio, 1966) using the 547 nm interference band filter in the Leitz MPV II cytophotometer with HBO 200 UV lamp. The data (Table 1) reveals that the mean absorbance of polytene nuclei of lethal mutant is about half of that of the salivary gland nuclei of Oregon R^+. The polytenic to mitotic absorbance ratio shows that while wild type reveals nearly a 20 fold increase of DNA content, the mutant has an increase of about 10

Table 1. Cytophotometric Measurement of DNA (transformed absorbance ± s.e.) for the salivary gland nuclei and mitotic nuclei of the lethal mutant l^4 (at 27th day after hatching) and control.

Salivary gland nuclei		Mitotic nuclei	
Control	Lethal	Control	Lethal
569.50	229.08	33.13	27.58
±5.76	±2.64	±0.60	±0.43

fold. Therefore, it could be argued that in the lethal salivary gland chromosomes the endoreplication occurs for one cycle less which could be attained by slow rate of DNA synthesis.

Acknowledgments. The authors are grateful to UGC for providing the Teacher Fellowship to RKB.

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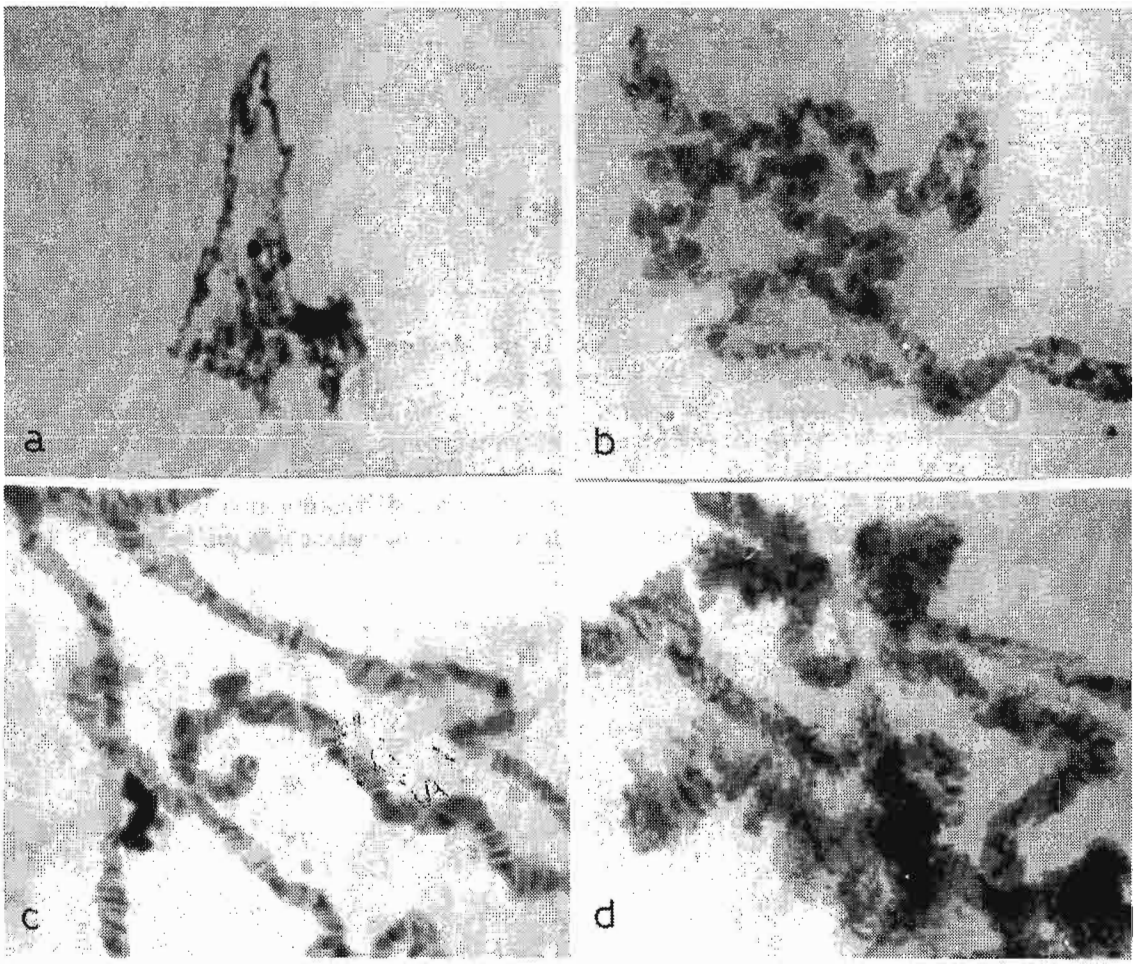

Figure 1. The photomicrographs showing the morphology of salivary gland chromosomes of lethal larvae (l^4/Y) at different larval ages; a) 6th day after hatching, b) 13th day after hatching, c) 20th day after hatching, and d) 27th day after hatching.

Bigler, D. and E. Hauschteck-Jungen. Zoologisches Institut der Universitat, Zurich, Switzerland. No uptake of injected arginine in sperm nuclei of *Drosophila*.

According to Das *et al.* (1964), Hauschteck-Jungen and Hartl (1982) and Hauschteck-Jungen and Rutz (1983), mature sperm of *Drosophila* have a high amount of arginine in its basic nucleoproteins, higher than nuclei of spermatocytes or somatic nuclei. We hoped to label the nuclei of mature sperm of *Drosophila subobscura* by injecting tritiated lysine L-(4,5-^{3}H(N)) and arginine L-(2,3-^{3}H) (specific activity 88,7 Ci/mmol resp. 25,0 Ci/mmol) into adult males of *D. subobscura*. As a control we used insect ringer solution.

Different times after injection we disected their testes, spread and dried the content onto a slide and prepared autoradiographs. For staining we used Toluidine blue.

The first label from lysine was found after 2 h over spermatogonia, spermatocytes (Fig. 1a) and extended over the whole length of elongated cysts (Fig. 1b) as described from the translation product of the mst(3)gl-9 gene (Kuhn *et al.* 1988) in *D. melanogaster*.

Individualized cysts were not labelled even 5 h after injection (Fig. 1c) and coiled cysts showed incorporation not before 36 h after injection. We suppose therefore that individualized and coiled cysts did not translate any more.

The table shows that lysine was incorporated faster into cells than arginine which appeared after 6-7 h.

Table 1. No label (-) or label (+) after injection of ^{3}H-arginine or ^{3}H-lysine into adult males of *D. subobscura*. -; + = some males did not, some showed incorporation of amino acids. In most of the testes some cells or cysts were not labelled, and the density of the silver grains varied.

h after injection	Spermatogonia and Spermatocytes		Spermatids						n Males
			Elongation		Individuallisation		Coling		
	arg	lys	arg	lys	arg	lys	arg	lys	
2-5	-	+	-	+	-	-	-	-	3
6-7	-;+	+	-;+	+	-;+	+	-	-	2;7
13-24	+	+	+	+	+	+	-	-	22
36	+	+	+	+	+	+	+	-	4
48-72	+	+	+	+	+	+	+	+	17
96-144	+	+	+	+	+	+	+	+	13

Four days after injection spermatogonia, spermatocytes, spermatids and sperm (Fig. 1d) were labelled. From our data we can calculate that the transfer of incorporated arginine from elongated cysts to mature sperm lasts minimal four days in *D. subobscura*, when the flies are kept in 18°C. The expected result, the strong labelling of the head region because of more arginine in the sperm nucleus than in any other nucleus, did not occur. The weak label in the head region of mature sperm are supposed not to originate exclusively from the nucleus because of the following reason.

In *D. subobscura* the head region of the sperm consists out of the nucleus, the axonema and one of the mitochondria derivatives (Hauschteck-Jungen and Maurer 1976; Dubler-Hanggi 1977). In all cases mature sperm were homogenously labelled, as we would assume if the nucleus did not incorporate arginine or lysine to a greater extent. We assume that the weak labelling of the head region represent mainly the incorporation of the aminoacids in those structures which run parallel the nucleus in *D. subobscura*, different from for instance *D. melanogaster.*

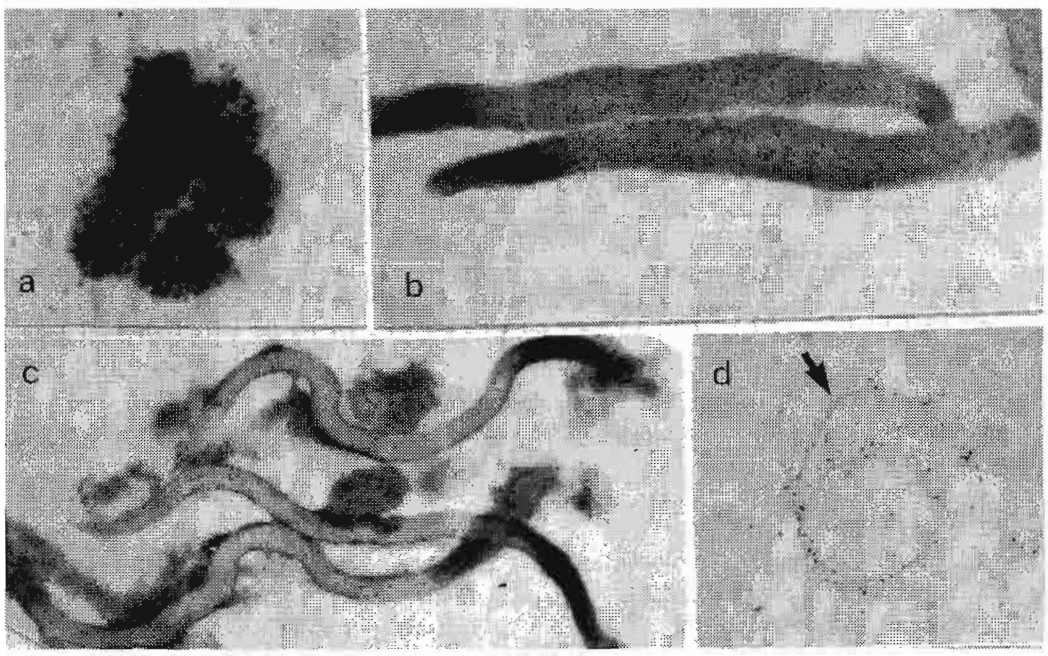

Figure 1a-d. Autoradiographs from different stages of spermatogenesis of *D. subobscura* after injection of ^{3}H-arginine and ^{3}H-lysine into adult males. a) Spermatocytes 4 days after injection of ^{3}H-arginine. b) Two short, elongated cysts 13 h after injection of ^{3}H-lysine Homogenous label over the whole cysts. The tissue was treated with H_2O_2, eliminated weak latent silver image specks. c) Three unlabelled short cysts after individualization 13 hours after injection of ^{3}H-lysine. They had been entrapped in the therminal epithelium. Labelled pieces of epithelial cells are scattered around the cysts. d) A labelled short sperm from the seminal vesicle, 11 days after injection of ^{3}H-arginine. The arrow points to the posterior end of the nucleus. a) and d) 880x, b) and c) 560x.

Why is it impossible to find tritiated arginine and lysine in the nucleus of the mature sperm? We can not discriminate between the following possibilities: 1) There is not newly incorporated arginine and lysine in the sperm, not more than in spermatogonia. 2) The amount of tritiated arginine and lysine in the arg/lys pool in the cells were too low, so that most of the incorporated arginine and lysine were "cold". 3) The amount of arginine and lysine in the sperm nucleus is too low to be detected with the applied method. 4) We lost the proteins in question by our microscopical techniques. 5) Sperm proteins were labelled originally but modified when incorporated into chromatin, loosing the tritium atom. Other techniques are necessary to determine which of the five above mentioned possibilities or a sixth, so far not mentioned, is correct.

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Bijlsma, R. and Bijlsma-Meeles, E. Department of Genetics, University of Groningen, Haren, The Netherlands. Induction of tolerance for ethanol in eggs of *Adh* null mutants of *Drosophila melanogaster.*

Recent evidence has suggested that tolerance to ethanol is not solely a function of the alcohol dehydrogenase (ADH) activity levels in *Drosophila melanogaster* (Barbancho et al., 1987; Bijlsma-Meeles and Bijlsma, 1988). Support for this fact is the observation made by one of us (Bijlsma-Meeles, 1979) of phenotypic adaptation to ethanol in the egg stage. This observation showed that eggs laid directly on medium containing 12% ethanol had a significantly better survival than eggs that were not brought into contact with ethanol before they were 14 hours old. This occurred in all three genotypes at the *Adh* locus and resulted in an almost optimal egg-to-adult survival in the presence of 12% ethanol. This finding has recently been confirmed by Kerver and Rotman (1987) and was shown to occur also in the presence of other primary alcohols. In both papers the change in tolerance was accompanied by a relative increase in ADH activity in the eggs exposed to ethanol compared to eggs on normal food. This increase in ADH activity, however, was in no way sufficient to explain the observed increase in ethanol tolerance, so the question remains whether the induced tolerance has anything to do with the presence of ADH. In this paper we therefore have studied this phenomenon of phenotypic adaptation to ethanol in the egg stage in *D. melanogaster* flies homozygous for the *Adh* null allele and consequently in the absence of active ADH.

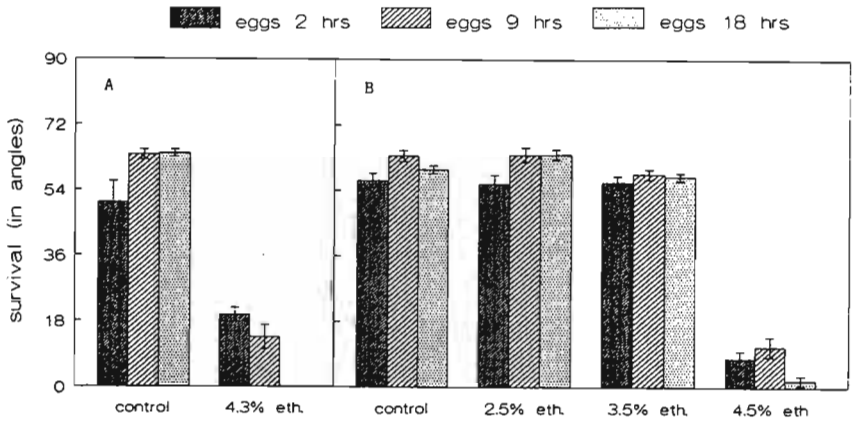

Figure 1. Mean survival percentages (expressed as angles) and their standard errors of eggs transferred at different ages to vials supplemented with different concentrations of ethanol. Means are based on 10 replicates.

The flies used for this purpose were the same as used by Kerver and Rotman (1987) in which the Adh^{n1} allele was introduced into the wildtype Adh^{S} background of the Groningen population. Egg collecting was done to conform to the method of Bijlsma-Meeles (1979). Adult flies were allowed to lay eggs on normal medium during a period of 4 hours (after which the flies were discarded) and were kept on this medium for various periods before they were transferred to the test vials containing 9 ml food. Eggs were transferred either immediately after the 4 hours egg laying period (mean age 2 hours) or at a mean age of 9 hours and 18 hours, respectively. Each vial was started with 50 eggs and from each vial the number of emerging adults was counted.

Normal medium contained 19 g agar, 54 g sucrose, 32 g dead yeast and 13 ml nipagin solution (10 g nipagin in 100 ml ethanol 96%) in 1 l water. (On account of this addition of 13 ml nipagin solution control food contains 1.3% ethanol). Other concentrations of ethanol food were made by adding the appropriate amount of ethanol to normal food while stirring vigorously.

A first test was performed on control food and food supplemented with three different amounts of alchol with final

concentrations of 4.3%, 5.8% and 7.3% ethanol. For each age class and each concentration 10 replicates with 50 eggs were established. It was found that the *Adh* null flies are very sensitive to ethanol and the two highest concentrations showed no survival at all. The results for the control and 4.3% ethanol are shown in figure 1A. It is clear that at 4.3% ethanol survival is low and at this concentration 18 hours old eggs produced no offspring. Because of this lack of survival at the high ethanol concentrations the experiment was repeated at lower concentrations. In this case the final concentrations (besides the control) were 2.5%, 3.5% and 4.5% respectively. The results of this test are shown in figure 1B.

The results from both tests show clearly that there is a sharp decline in survival between 3.5% and 4.3% ethanol. A two-way analysis of variance, with ethanol concentration and age as variables, was performed for all data from figure 1 lumped together and the result is shown in table 1A. There are significant effects due to both age and concentration, but there is also a highly significant interaction. This interaction seems to be due to the fact that differences in age affect survival differently in situations when there is a high survival compared to situations with a low survival. Therefore the data were divided in two sets that differ significantly from each other with respect to survival: A first set, the two controls and the lower ethanol concentrations (2.5% and 3.5%), showing a high survival and a second set, 4.3% and 4.5% ethanol, showing a low survival. These two sets were analyzed separately and the results are shown in table 1B and 1C respectively.

Table 1B shows that there are no significant differences in survival between both controls, 2.5% and 3.5% ethanol with respect to concentration. Mean total survival was around 75% which is normal even for wildtype flies at control food. This indicates that ethanol concentration of 3.5% and lower do not cause any additional mortality of Adh null eggs compared to normal food. This strongly contrasts with the situation observed for the second set: ethanol concentrations of 4.3% and 4.5% cause a high mortality and survival declines to less than 5%. These two concentrations, however, do not differ significantly with respect to survival (table 1C).

With regard to age the situation is different. Both analyses of variance show a significant effect of age on survival, though these are caused in a different way for the two sets. For the first, non-selective set, eggs that are transferred at an age of 2 hours show a mean survival of 66.9% which is significantly lower than eggs transferred after 9 and 18 hours which show a survival of 78.6% and 77.2% respectively. The reason why 2 hours old eggs show this lower survival has not been established yet. It is, however, known that when females are laying eggs very rapidly, the egg membranes may not yet be fully developed (Yoon and Fox, 1965). As a result the eggs may be more vulnerable and have a higher chance of becoming damaged during the process of transfer.

Table 1. Analysis of variance of the arc-sine transformed survival percentages. A: results for all data presented in figure 1 lumped together. B: results for the two control sets and the lower concentrations of ethanol, 2.5% and 3.5% together. C: results for the two higher concentrations of ethanol, 4.3% and 4.5%.

	source	SS	df	MS	F
A	age (a)	915.9	2	457.9	8.06***
	concentration (c)	104369.4	5	20873.9	376.38***
	interaction (axc)	3044.7	10	304.5	5.34***
	deviations	9204.7	162	56.8	
B	age (a)	1371.2	2	685.6	13.01***
	concentration (c)	241.1	3	71.4	1.35 NS
	interaction (axc)	522.6	6	87.1	1.65 NS
	deviations	5692.9	108	52.7	
C	age (a)	1748.0	2	874.0	13.40***
	concentration	152.3	1	152.3	2.34 NS
	interaction (axc)	308.7	2	154.3	2.37 NS
	deviations	3521.9	54	65.2	

*** P < 0.001; NS not significant at the 5% level.

For the second subset, 4.3% and 4.5% ethanol, also a significant effect of age is observed (table 1B). However, in this case the 18 hours old eggs show hardly any survival and mean survival (both concentrations lumped together) is only 0.03% which is not significantly different from zero ($t=1.43$, $df=19$). Although 2 and 9 hours old eggs show a low survival (mean survival 4.7% and 4.6% respectively) this is significantly higher than observed for 18 hours old eggs and also significantly different from zero ($t=3.72$, $df=19$ and $t=4.18$, $df=19$ respectively). This clearly indicates that eggs brought into contact with detrimental ethanol concentrations at younger age (2 and 9 hours) show a higher viability than eggs transferred at later age due to a phenotypic increase in ethanol tolerance. This seems especially true in case of the 2 hours old eggs, which in the control situation even showed a significantly lower survival than the other age groups. These findings are similar to the earlier observations by Bijlsma-Meeles (1979) and Kerver and Rotman (1987). As the eggs used in our experiment did not have active *Adh* we may conclude that in this case the phenomenon of increased ethanol tolerance is independent of the presence of active *Adh*. More elaborate experiments have to be done to see whether in ADH negative eggs this adaptation is of the same magnitude as in *Adh* positive eggs. Nevertheless the data presented in this paper indicate once more that adaptation to ethanol is not strictly a function of the level of *Adh* activity.

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Biyasheva, Z.M.[2], M.O. Protopopov[1], and E.S. Belyaeva[1]. [1]Institute of Cytology and Genetics, Novosibirsk, 630090, USSR, [2]Kazakh State University, Alma-Ata, 480121, USSR. Some characteristics of mutations in *sta* and *HM40* loci in the 2AB region of *D. melanogaster* X chromosome.

Cytogenetic mapping has arranged loci in the 2AB region from the left to the right as follows: *l(1)BA11, l(1)BA12, sta, ecs, dor, swi, l(1)HM38, l(1)HM40, l(1)HM32* (Aizenzon and Belyaeva, 1982). Three of the loci *ecs, dor* and *swi* affect ecdysteroid induction and were called a cluster (Belyaeva *et al.*, 1981; Belyaeva and Zhimulev, 1982). In the present study we investigate the development, effective lethal phases and pattern of ecdysteroid inducible puff formation of individuals carrying the mutations of two neighbors of this cluster - *sta* and *HM40* loci.

Ecdysterone ("Serva") in the Ephrussi-Beadle solution was injected into the third instar larvae homozygous for *sta* and *l(1)HM40* by glass micropipette. As a control, these mutants or *yellow* stock larvae were injected with Ephrussi-Beadle (E.B.) solution or were grown without any injection. The larvae with puparium formed were counted every other hour.

Complementation group *sta* contains two lethal mutations, *lt3* and *lt36*, which were induced by EMS in the *yellow* strain (Belyaeva *et al.*, 1982). The lethal period of development starts at the first instar larvae and lasts the whole period of development with the main peaks at the 1st and 2nd larval instar for *lt36* and prepupae for *lt3*. Pupariation in these mutants delays for 60-80h (Fig. 1) and has a lot of abnormalities. Some of them do not have pupal moulting and die at the stage of gas-bubble formation, others die at the stage of eye pigmentation and bristle formation. Those surviving to

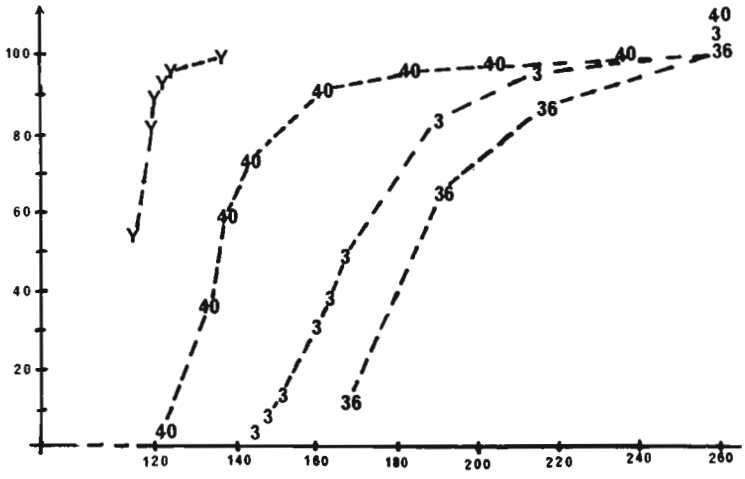

Figure 1. Pupariation of mutants: *yellow* (y), *y sta*^{lt3} (3), *y sta*^{lt36} (36), *y 1(1)HM40 w*^{a} (40). Abscissa - time in hours after eclosion from embryo; ordinate - number of individuals with puparium (%).

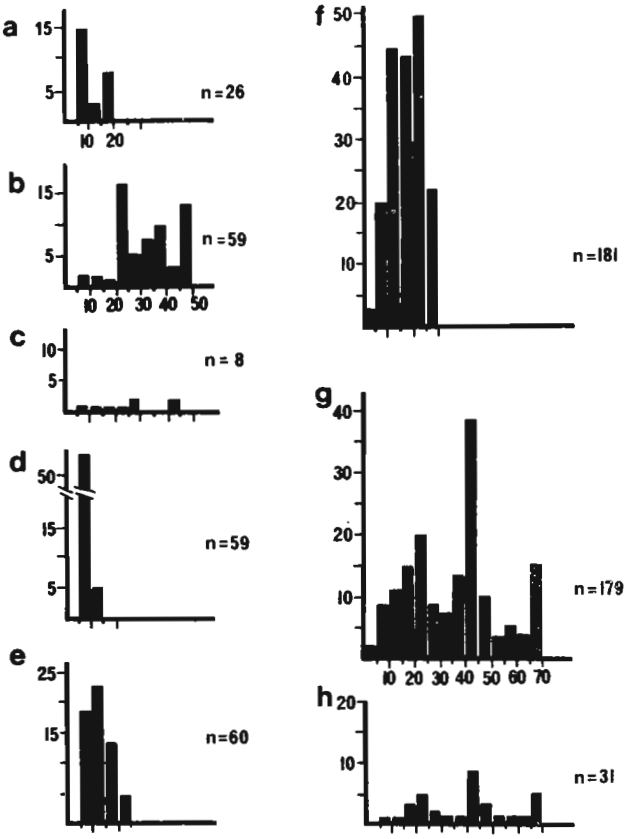

Figure 2. The influence of ecdysterone injection on puparium formation: a,d,f - after ecdysterone injection; c,h - E.B. injection; b,e,g - without injection. Abscissa - time in hours; ordinate - number of larvae with puparium formed.

a. HM40, ecd
b. HM40
c. HM40, saline
d. y, ecd
e. y
f. sta^{t3}, ecd
g. sta^{t3}
h. sta^{t3}, saline

imago formation die before or at the moment of eclosion. Those (2-5%) that managed to eclose live only a few days. When we use slightly overcrowded mixed cultures of *l/l* and *l*/FM6 (100-120 larvae per vial), larvae homozygous for *lt3* and *lt36* do not survive even till the third larval instar.

Late mortality (at the pupae stage) was observed for mutants of the *HM40* locus, containing one mutation, *l(1)HM40*. This mutation was obtained by Kramers by HMS treatment in *y* w^a strain (Aizenzon and Belyaeva, 1982). Morphological defects of this mutation are like those for the *sta* locus, but more individuals develop till formed imago, but they do not eclose. Mutation *l(1)HM40* causes a 20-30h delay of pupariation (Fig. 1). Salivary glands of mutants which were able to survive till prepupae were checked for the pattern of puff formation in 0h, 10h and prepupae, but the 63E1-3 and 74EF puffs, which correspond to these stages, significantly decrease in sizes ($P < 0.01$ and $P < 0.05$).

After ecdysterone injection into 140h sta^{lt3}/sta^{lt3} and 120h *l(1)HM40/l(1)HM40* larvae we observed the partial normalization of development (Fig. 2 a,f): puparium formation is not delayed but subsequent development stops and the imago does not eclose. The pattern of puff formation does not differ from control at the same stages, thus implying the process of injection does not cause extra puff formation (heat-shock, etc.). Therefore, lethal mutants of the *sta* and *HM40* loci are positively reactive to the exogeneous hormone and can be classified as ecdysterone deficient as previously described *dor* (Belyaeva *et al.*, 1985), *gt* (Schwartz *et al.*, 1984), *ecd-1* (Hansson and Lambertsson, 1984) and some other mutations.

We can't say if lack of ecdysterone in the case studied is the primary effect of mutations or the secondary event. In any case, the fact is interesting, because these two loci, *sta* and *HM40*, are closely adjacent to the cluster (distance between *sta* and *ecs* is 0.021 m.u. and between *swi* and *HM40* is 0.181 m.u. (Aizenzon and Belyaeva, 1982).

Acknowledgments: We are thankful to Dr. I.F. Zhimulev for his consultation in injection experiments and for critical reading of the manuscript.

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Brncic, Danko. Departamento de Biologia Celular y Genetica, Facultad de Medicina, Universidad de Chile. Inversion polymorphism in *Drosophila immigrans* from the Strait of Magellan.

In 1957, Brncic and Dobzhansky reported a list of Drosophilidae living in the extreme southern part of South America, near the Strait of Magellan: Punta Arenas (Lat. 53° 10'S) and Caleta Josefina, Bahia Inutil, in Tierra del Fuego Island (Lat. 53° 40'S). They found that all the native species of the family belong to the genus *Scaptomyza* that was represented by two very abundant species in the region: *S. denticauda* Malloch and *S. melancholica* Duda. The only species of the genus *Drosophila* was the cosmopolitan *D. funebris* Fabr. found in synanthropic environments. Further collections performed a few years later have shown no changes in the drosophilid fauna of the region. Nevertheless, more recent collections (Brncic, 1980) have revealed that, in addition to the scaptomyzids and *D. funebris*, two other cosmopolitan and domestic species of *Drosophila* have appeared, namely, *D. immigrans* Sturtevant and *D. melanogaster* Meigen, together with the invasive widely distributed European species *D. subobscura* Collin that has colonized Chile since 1978 (Brncic and Budnik, 1980).

Chilean populations of *D. immigrans* are polymorphic for three different chromosomal arrangements, besides the "standard" arrangements, due to the presence of two inversions in the right arm (Inv. B and C) and one in the left arm (Inv. A) of the second chromosome. These three inversions are widely distributed throughout the world (Toyofuku, 1951; Brncic, 1955; Richmond and Dobzhansky, 1968). Quantitative studies made in Chile (Brncic, 1955) and in Hawaii (Richmond and Dobzhansky, 1968) have shown interpopulational differences with regard to the frequency of the different gene arrangements, thus suggesting a process of microdifferentiation. In Chile, only the "standard" and the "B" gene arrangement had been observed all over the country, from the arid regions of the North to the cold rainforests in the South. By contrast, the "A" inversion has been found only in the Central and Southern regions and the "C" inversion was present in a local population from Valdivia (Lat. 39 degrees 48'S). Recently, a cytogenetic analysis of small samples of *D. immigrans* collected in a fruit store in the city of Punta Arenas in the Strait of Magellan (Jan. 1980, Dec. 1988) and in a greenhouse of the same city (Dec. 1989) revealed that those populations are polymorphic for the inversions "A" and "B" (Figs. 1-1 and 1-2). This finding seems to be important since samples of another domestic species, *D. funebris*, collected in Punta Arenas and Tierra del Fuego many years ago (Brncic and Sanchez, 1958), were shown to be

polymorphic for two widely distributed inversions, one of them being the inversion C-II-M which had been discovered in Russian populations by Dubinin et al. (1937) and in Sweden by Perje (1954). The observation that in another cosmopolitan and domestic species, *D. immigrans*, there are also inversions so well distributed as to reach the ultimate southern limit of its geographic distribution strongly supports the hypothesis that in many species of *Drosophila*, certain chromosomal morphs are incorporated as an almost permanent component of the genetic architecture of the species. This condition is not infrequently found in synanthropic species transported by man.

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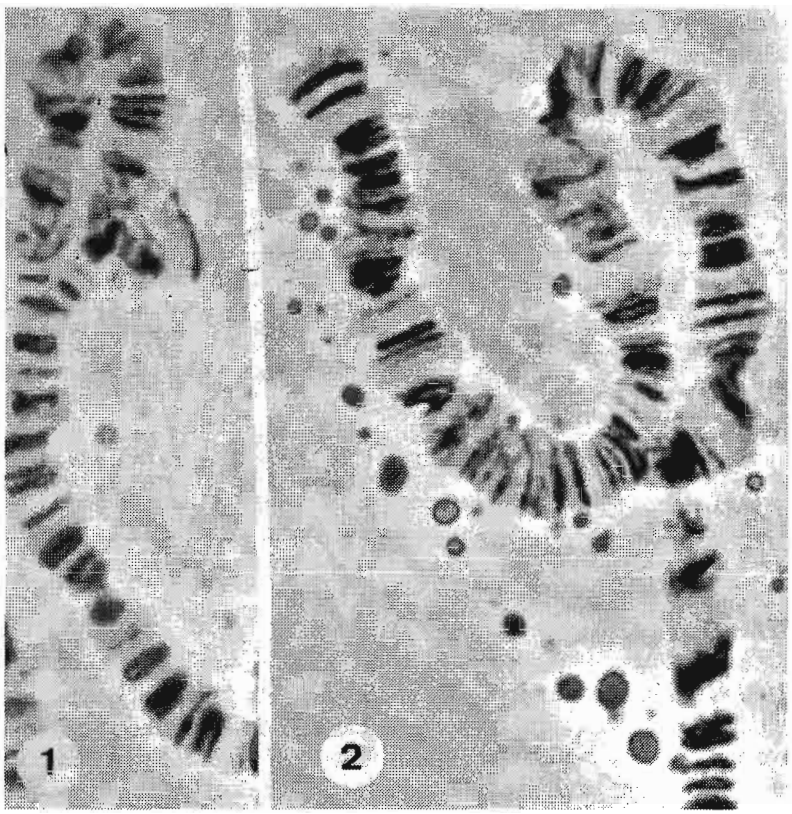


Figure 1. (1) Heterozygous inversion B in the right arm of the second chromosome. (2) Heterozygous inversion A in the left arm of the same chromosome.

References: Brncic, D. 1955, J. Heredity 56:59-63; Brncic, D. 1980, Ans. Inst. Patagonia (Chile) 11:293-299; Brncic, D. and M. Budnik 1980, DIS 55:20; Brncic, D. and Th. Dobzhansky 1957, Amer. Nat. 91:127-128; Brncic, D. and P. Sanchez 1958, Amer. Nat. 92:61-63; Dubinin, N.P., M.N. Sokolov and G.G. Tiniakov 1937, Biol. Zhurnal (Moscow) 6:1007-1054; Perje, A.M. 1954, Acta Zoologica (Sweden) 35:259-288; Richmond, R. and T. Dobzhansky 1968, Studies in Genet., The University of Texas Publ. 6818:381-386; Toyofuku, Y. 1951, Jap. Jour. Genetics 36:32-37.

Budnik, M. and L. Cifuentes. Departamento de Biologia Celular y Genetica, Facultad de Medicina, Universidad de Chile. Differences in sexual dimorphism between European and Chilean stocks of *Drosophila subobscura*.

Since 1980, when *D. subobscura* was first detected in Chile, various articles have been published dealing with the colonization process (for a full revision, see Budnik and Brncic, 1982; Brncic and Budnik, 1987).

In this note, the authors wish to inform on the results on sexual dimorphism. Quantitative characters such as wing size and egg-to-adult development time were studied in four European and four Chilean stocks of *D. subobscura* maintained for various generations in mass culture in the laboratory.

Experiments and results

The following stocks were used: a) European stocks originated from recollections in Sunne (Sweden, Lat. 59°50'N), Tübingen (W. Germany, Lat. 48°31'N), Cinisse (Italy, Lat. 38°08'N) and Almería (Spain, Lat. 36°49'N). b) Chilean stocks originated from Coyhaique (Lat. 45°34'5), Puerto Montt (Lat. 41°28'5), Laja (Lat. 37°10'5), and La Florida (Lat. 39°30'5). The experiments consisted in sowing 50 fertilized eggs of the same age from each stock of *D. subobscura* into vials containing 10cc of basic cornmeal yeast agar medium for *Drosophila*. For each stock 10 vials were used and a total of 500 eggs were sown. The vials were kept in a constant temperature chamber at 19°C $\pm$ 1. The emerging males and

females were recorded every day in order to estimate the egg-to-adult developmental time. For the wing analysis, the method of Pfriem (1983) was used. The left wing of 100 males and 100 females of each population were dissected and mounted on slides in Canada balsam and then observed under the microscope with an ocular micrometer of 100 divisions, each one corresponding to 0.03 mm. Two different measures were used (Fig. 1).

Table 1. Sex differences in the egg-to-adult development period (in days) of European and Chilean strains of D. subobscura.

Locality	Females N	Females $\bar{\chi}$ period of develop. and s.e.(in days)	Males N	Males $\bar{\chi}$ period of develop. and s.e.(in days)	t-test
Europe					
Sunne (Sweden)	149	26.21 ± 0.10	159	26.14 + 0.09	0.53
Tübingen (Germany)	167	27.07 ± 0.11	159	27.14 + 0.13	0.42
Cinisse (Italy)	177	30.09 ± 0.17	168	29.40 + 0.15	3.09**
Almería (Spain)	171	28.85 ± 0.23	156	28.41 + 0.25	1.27
Chile					
Coyhaique	148	27.64 ± 0.16	147	27.26 + 0.14	1.82*
Puerto Montt	174	28.93 ± 0.16	152	28.36 + 0.18	2.37**
Laja	151	26.88 ± 0.13	130	26.83 + 0.15	0.26
La Florida	183	29.26 ± 0.10	168	28.69 + 0.10	3.91**

*P <0.05 **P <0.01 ∞ D.F.

Table 1 shows that egg-to-adult developmental time is different in males and females but these differences are statistically significant only in one European population and as much as in three of the four Chilean populations. An analysis of variance (Table 2) shows that the geographic location is the important variable, not so the continent.

As to wing size, results in Table 3 also show that sexual dimorphism differs in each geographic location. An analysis of variance (Table 4) shows this to be the important variable, not so the continent.

Discussion

Some components of fitness, such as egg-to-adult developmental time and pre-adult viability in *Drosophila*, are highly variable traits and can be modified under laboratory conditions by factors such as temperature, food supply, density and intra-specific competition, among others (Barker, 1983). There also exist inter-population differences that have been interpreted as genetic responses to geographic conditions. In Chile, inter-population and inter-strain differences in pre-adult viability and egg-to-adult developmental time have also been detected in the endemic species *D. pavani* (Budnik and Brncic, 1972). Budnik and Cifuentes (1989), while studying the effect of metabolic waste products of three established species of *Drosophila* in Chile on larvae of *D. subobscura*, observed that females of this species presented longer developmental times than males. The different responses of females and males when confronted with situations of stress, are difficult to explain, but they might be of great importance for the population dynamics (Parsons, 1973; Price, 1975). It is important to underline the fact that in the Chilean populations of this species, differences are more significant than in European populations, which inclines us to think of a process of microdifferentiation.

Studies performed in the Chilean endemic species *D. flavoilosa* (Budnik et al., 1988) showed consistent differences in sexual dimorphism in wing size between populations. These differences might be attributed to different selection pressures on the sexes, as postulated by Atchley (1971) in *Chironomus* species. Bird and Shaffer (1972) found in *D. melanogaster* that selection to decrease sex dimorphism decreased female wing size, while selection to increase sex dimorphism decreased male wing length. X-chromosome linked genes might possibly be involved in these results. It should be pointed out that wings in the genus *Drosophila*, in addition to the obvious function in flight and dispersal, represent a fundamental element in courtship and mating. Therefore, male and female wing responses to different selective factors are not neccesarily expected to be equal.

Table 2. Nested analysis of variance to estimate the influence of locality, continent, and sex on egg-to-adult development period (in days) in *D. subobscura*.

Source of variation	D.F.	SS	F
Continent	1	2.09	0.003
Locality (Continent)	6	3655.03	173.58**
Sex	1	51.03	14.54**
Interaction between sex and locality	7	55.35	2.35**
Error	2550	8949.15	
Total	2565	12748.52	

*p <0.05; **p <0.0001

Acknowledgments: Supported by grants from University of Chile (B2308/8945) and Fondo Nacional de Ciencias - Chile. (FONDECYT 004 and 0967).

References: Atchley, W.R., 1971, Am. Nat. 105:455-466; Barker, J.S.F., 1983, The Genetics and Biology of *Drosophila*, Ashburner, M., H.L. Carson and J.N. Thompson, jr. eds. Academic Press, London and New York. Vol. 3c:286-341; Bird, M.A. and H.E. Shaffer, 1972, Genetics 72:475-487; Brncic, D. and M. Budnik, 1987, Genetica Iberica

Table 3. Means and standard errors for sexual dimorphism ($\chi = \Sigma$ females wing measure -Σmales wing measure / N) in wing length and width wings of European and Chilean populations of *D. subobscura*).

Locality	Latitude	N	Wing Length χ	Wing Length s.e.	Wing Width χ	Wing Width s.e.
Europe						
Sunne (Sweden)	50°50'N	100	4.60	0.17	3.65	0.14
Tübingen (Germany)	48°31'N	100	5.49	0.22	4.32	0.15
Cinisse (Sicily, Italy)	38°08'N	100	7.33	0.29	4.22	0.17
Almería (Spain)	36°49'N	100	5.08	0.31	3.84	0.16
Chile						
Coyhaique	45°34'S	100	5.53	0.31	3.68	0.20
Puerto Montt	41°28'S	70	5.18	0.25	3.81	0.17
Laja	37°10'S	99	4.92	0.25	3.52	0.18
La Florida	33°30'S	100	7.33	0.29	4.22	0.17

Table 4. Analysis of variance to estimate the influence of continent and locality on sexual dimorphism in wing length and wing width (SS = sum of squares).

Variable	D.F.	Wing Length SS	Wing Length F	Wing Width SS	Wing Width F
Continent	1	102.75	1.60	0.05	0.004
Population	6	385.42	9.59**	64.89	3.97*
Error	760	5088.48		2070.89	
Total	767	5591.68		2135.84	

*p <0.005 ; **p <0.0001

39:249-267; Budnik, M. and D. Brncic, 1972, Genetika (Yugoslavia) 4:281-285; Budnik, M. and D. Brncic, 1982, Actas V. Congr. Lat. Genetica, 177-188; Budnik, M. and L. Cifuentes, 1989, Brazil. I. Genetics 12:499-504; Budnik, M., D. Brncic and M. Acuna, 1988, Z. Zool. Syst. Evolut. forsch. 26:211-216; Parsons, P.A., 1973, Behavioural and Ecological Genetics: A study in *Drosophila*. Oxford University Press. Oxford, pp. 223; Pfriem, P., 1983, Genetica 61:221-232; Price, P.W., 1975, Insect Ecology, Wiley Interscience, New York, London, Sydney, Toronto, pp. 270.

Figure 1. Wing measures: Large (L). Wing width (W).

Chadov, B.F. Institute of Cytology and Genetics, USSR Academy of Sciences, Siberian Division, Novosibirsk 630090, USSR. Recovery of rearrangements in *Drosophila* based on a nondisjunction test.

In individuals containing metacentric autosome 2 and free arms F(2L) and F(2R) (Grell, 1970), the centromere of autosome 2 with its precentromeric heterochromatin is in triple dosage (Fig. 1). This duplication results in nondisjunction of the free arm with the metacentric and in the formation of aneuploid gametes 2/F(2L) and F(2R) as well as 2/F(2R) and F(2L) (Chadov and Chadova, 1981). The frequencies of aneuploid gametes resulting from 2-F(2L) or 2-F(2R) nondisjunctions are almost equal and they make up not more than 15% of the total gamete number. The remaining 70% is composed of euploid gametes 2 and F(2L); F(2R) (Chadov, et al., 1986). It was found also that an inversion in the metacentric drastically increases aneuploid gamete production. Paracentric 2L inversion increases the frequency of 2/F(2L) and F(2R) and paracentric 2R inversion that of 2/F(2R) and F(2L) oocytes. Both paracentric inversions or pericentric 2LR inversions increase the production of aneuploid oocytes of all types (Chadov et al., 1985). Subsequently, it was found that certain translocations also increase the nondisjunction of the metacentric with the free arm. The method suggests it is based on the ability of chromosome rearrangements to drastically increase the production of aneuploid oocytes in 2/F(2L); F(2R) females.

Fig. 2 shows schematically how rearrangements with a 2R break can be recovered with this method. The first step is to produce females containing appropriate metacentric and free arms, the second is to involve females in individual crosses to C(2R); F(2L) males. To obtain the same number of eggs from each tested female, three day old females were crossed and allowed to lay eggs for 3 days. These eggs were discarded. The same females were allowed to lay eggs for another 2 days. These eggs were collected to give rise to progenies. As the scheme shows, progeny arose only from aneuploid oocytes 2/F(2R) and F(2L). The third step is selection of vials with a great number of progeny. The 2/F(2L); F(2R) female without rearrangement yield a small number of offspring. Offspring number increases by 3-4 times, when the metacentric contains a rearrangement. To be more exact in selection, mean number of progeny produced by female was determined in the control cross (about 100 vials), in which 2/F(2L); F(2R) daughters with untreated metacentrics were crossed to tester males. In the experimental series, vials with progeny number 3 times the square root of the mean or greater were selected. When progeny number is small, determination is very easy in a glass vial without opening it. The last step of the procedure is to cross 2/F(2L); F(2R) sons (cn^+ phenotype) from the selected vial to normal females. Polytene chromosomes of larval progeny were analysed for determination of chromosomal rearrangements. A part of male progeny from the cross is used to raise a genetic line.

In the first experiment for rearrangement recovery with the nondisjunction test, S Px^2 males irradiated with gamma-rays (3,000R) were crossed to F(2L), l(2)gl pr; F(2R), l(2)ax females (Chadov et al., 1985). 972 S Px^2/F(2L); F(2R) daughters were crossed individually to F(2L), pr; C(2R), cn tester males. According to the progeny number

determined visually in a vial, 52 cultures (5.3%) with large progeny number were selected. Average progeny number per a female with no rearrangement in S Px^2 metacentric was 4.0 ± 2.0, and that for a female containing it was 13.8 ± 1.0. According to the polytene chromosome analysis 26 cultures had rearrangements in 2R arm (for stock list see Artjomova and Buzikanova, this issue).

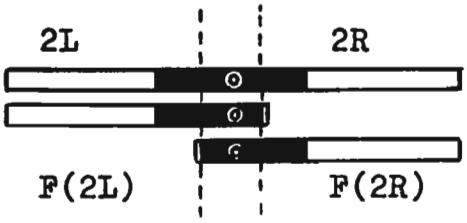


Figure 1. Free arms F(2L) and F(2R) in heterozygous condition. (I). Production of heterozygous females. (II). Individual crosses of heterozygous females with tester males. (III). Selection of vials with high number of progeny. (IV). Cytological analysis of salivary gland polytene chromosomes.

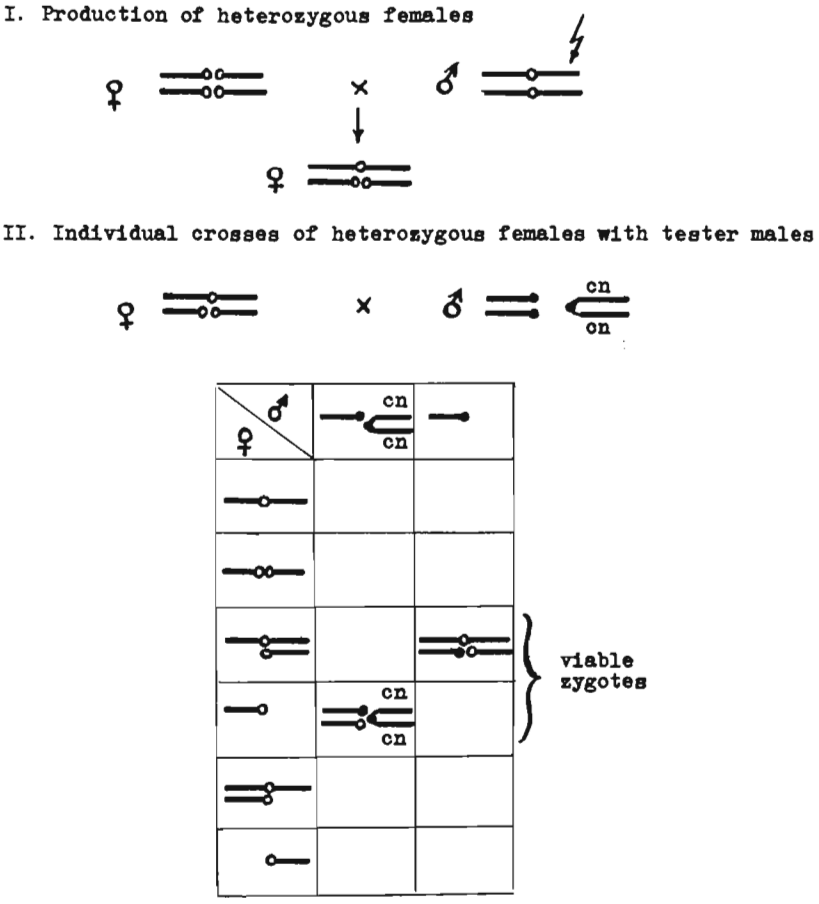


Fig. 2. Scheme of recovery of rearrangements with 2R break by means of the nondisjunction test.

The method suggested had a high resolution for inversion recovery. More than half of the rearrangements (54%) consist of 2R and 2LR inversions. It is probably more effective than the C-scan test (Roberts, 1970). Frequency of inversion formation produced by 3,000R irradiation was 1.4% in our experiment and it was 1.6% in Roberts' (4,000R irradiation). When taken into account that selection based on progeny number is simpler than the individual crossing-over analysis, the nondisjunction test appears to have an advantage over the C-scan test. Although translocation recovery was less than with the C-scan, the nondisjunction test can be suggested as a simpler procedure.

The method suggested has been described in detail (Chadov et al., 1985). It can be used for setting up a large collection of inversions in major autosomes 2 and 3. The procedure can possibly be developed for the recovery of X-chromosome inversions.

In the "New Mutants" section of this issue are presented new rearrangements in metacentric 2 induced by gamma-irradiation of male (Artojomova and Buzikanova) and of female (Artjomova) as well as rearrangements in the free arms F(2R) and F(2L) arising in irradiated males (Volkova and Buzikanova). A modification of the nondisjunction test to giving a higher resolution is described (Omelianchuk, et al.).

Acknowledgments: The author is grateful to Pr. R.G. Gethmann for supplying the free arm of autosome 2 and Pr. R.C. Woodruff for kindly providing *Drosophila* stocks.

References: Chadov, B.F. and E.V. Chadova, 1981, Dokl. Acad. Sci. USSR, 261:993-996 (In Russian); Chadov, B.F., Ju.N. Ivanov, and E.V. Artjomova, 1985, Genetica (USSR), 21:1143-1150; Chadov, B.F., E.V. Chadova, and E.A. Khotskina, 1986, Genetica (USSR), 22:62-79; Grell, E.H., 1970, Genetica 65:65-74; Roberts, P.A., 1970, Genetics 65:429-448.

Chadov, B.F. and L.V. Omelianchuk. Institute of Cytology and Genetics, USSR Academy of Sciences, USSR. Tetrad analysis of crossingover in disjunctional and nondisjunctional chromosomes of *Drosophila*.

There is ample evidence indicating that the chromosomes remaining nondisjunctional after the first meiotic division are crossovers (Merriam and Frost, 1964; Chadov and Chadova, 1981). These nondisjunctional chromosomes differ in crossingover level. The level depends, among others, on whether or not the cause of the nondisjunction is coorientation with the nonhomologue and, if so, with which of the nonhomologues (Chadov and Podoplelova, 1985; Chadov, et al. 1987, 1988). Crossingover estimates are needed for comparisons of crossingover in disjunctional and nondisjunctional chromosomes and of crossingover in different cases of meiotic nondisjunction.

Good estimates of crossingover are provided by the frequencies of exchange tetrads of different ranks. Weinstein (1936) had carried out tetrad analysis in the case of normal disjunction of homologues. According to Weinstein, the frequency of tetrads of rank i ($E_{1,2...i}$) and the frequency of exchange chromosomes of rank j ($a_{1,2...j}$), when the maximum rank of the tetrad and chromosome is 3, are expressed as:

$$\begin{aligned} a_0 &= E_0 + 1/2\,E_1 + 1/4\,E_2 + 1/8\,E_3 \\ a_1 &= \quad 1/2\,E_1 + 1/2\,E_2 + 3/8\,E_3 \\ a_2 &= \quad\quad 1/4\,E_2 + 3/8\,E_3 \\ a_3 &= \quad\quad\quad 1/8\,E_3 \end{aligned} \qquad (1)$$

Charles' transformation of Weinstein's equations (Steinberg, 1936) are conventionally used in tetrad analysis. In application to tetrad of not higher than rank 3, the equations become:

$$\begin{aligned} E_0 &= a_0 - a_1 + a_2 - a_3 \\ E_1 &= 2\,(a_1 - 2a_2 + 3a_3) \\ E_2 &= 4\,(a_2 - 3a_3) \\ E_3 &= 8a_3 \end{aligned} \qquad (2)$$

With these equations, estimates of frequencies of tetrads of different ranks can be based on the frequencies of chromosomes with non-, single-, double and triple crossingover recovered in the experiment.

Merriam and Frost (1964) have suggested the method of tetrad analysis of crossingover for the case of homologue nondisjunction. This is based on the determination of the marker composition of each of the nondisjunctional chromosomes in the dyad and the classification of dyads into 12 types. This classification is given below with the equations relating the frequencies of dyads and tetrads:

two noncrossover strands	$n_0 = E_0 + 1/4\,E_1 + 1/16\,E_2 + 1/64\,E_3$
a noncrossover strand and single crossover strand	$n_1 = 1/2\,E_1 + 1/8\,E_2 + 1/32\,E_3$
complementary single crossover strands	$n_2 = 1/4\,E_1 + 1/8\,E_2 + 3/64\,E_3$
a noncrossover strand and a double crossover strand	$n_3 = 1/4\,E_2 + 1/8\,E_3$
two noncomplementary single crossover strands	$n_4 = 1/4\,E_2 + 1/8\,E_3$
complementary double crossover strands	$n_5 = 1/16\,E_2 + 3/64\,E_3$
a double crossover and a complementary single crossover strand	$n_6 = 1/8\,E_2 + 1/16\,E_3$
a double crossover strand and a double crossover (one complementary strand)	$n_7 = 1/8\,E_3$
a triple crossover strand and complementary single crossover strand	$n_8 = 1/8\,E_3$
a triple crossover strand and a complementary double crossover strand	$n_9 = 1/32\,E_3$
a double crossover strand and noncomplementary single crossover strand	$n_{10} = 3/16\,E_3$
complementary triple crossover strand	$n_{11} = 1/64\,E_3$
a triple crossover strand and noncrossover strand	$n_{12} = 1/64\,E_3$

(3)

The equation formed after substitution of the values are computer solved to attain maximum likelihood.

Since 1981 (Chadov and Chadova, 1981), Chadov has applied another method for calculating tetrad frequencies in the case of chromosomal nondisjunction. Instead of dyads (Merriam and Frost, 1964), each nondisjunctional chromosome was taken as an observational unit. After determination of marker composition, all the nondisjunctional chromosomes were standardly classified as nonexchange and exchange chromosomes with one, two and more exchange(s). The frequencies (%) of the chromosomes of ranks 0, 1, 2, . . . were estimated, and those of tetrads

($E_{0,1,2...}$) were determined by Charles' formula (2).

To reemphasize, Charles' formula is a transformation of Weinstein's equation. We intend to show that Merriam and Frost's equations can also be reduced to those of Weinstein.

Let the number chromosomes with i exchanges be represented by A_i, and the number of dyads of class j - by N_j (see (3)). Then the following equations are true:

$$\begin{aligned} A_O &= 2N_O + N_1 + N_3 + N_{12} \\ A_1 &= N_1 + 2N_2 + 2N_4 + N_6 + N_7 + N_{10} \\ A_2 &= N_3 + 2N_5 + N_6 + 2N_7 + N_9 + N_{10} \\ A_3 &= N_8 + N_9 + 2N_{11} + N_{12} \end{aligned} \qquad (4)$$

Summing up, we obtain

$$\sum_{i=0}^{3} A_i = 2 \cdot \sum_{j=0}^{12} N_j$$

The frequencies of chromosomes (a_k) and dyads (n_e) calculated from the number of chromosomes and dyads are:

$$a_k = \frac{A_k}{\sum_{k=0}^{3} A_i} \quad \text{and} \quad n_e = \frac{N_e}{\sum_{j=1}^{12} N_j}$$

Hence,
$$\begin{aligned} a_0 &= n_0 + 1/2\, n_1 + 1/2\, n_3 + 1/2\, n_{12} \\ a_1 &= 1/2\, n_1 + n_2 + n_4 + 1/2\, n_6 + 1/2\, n_8 + 1/2\, n_{10} \\ a_2 &= 1/2\, n_3 + n_5 + 1/2\, n_6 + n_7 + 1/2\, n_9 + 1/2\, n_{10} \\ a_3 &= 1/2\, n_8 + 1/2\, n_9 + n_{11} + 1/2\, n_{12} \end{aligned} \qquad (5)$$

After introducing the values of n_i from Merriam and Frost's equations (3) into equations (5), we obtain Weinstein's formula (1). Thus both methods of calculation are equivalent.

The estimates of tetrad frequencies based on dyad analysis and on analysis by means of Charles' formula are very close. For instance, the tetrad frequencies in the case of primary X-nondisjunction obtained according to Merriam and Frost (1964) were as follows: E_0 = 26.0, E_1 = 24.5, E_2 = 47.6, E_3 = 2.0 % and those we obtained based on Merriam and Frost data and using Charles' formula are: E_0 = 28.6, E_1 = 24.5, E_2 = 46.8 and E_3 = 0 %. However, the procedure relying on Charles' formula is much simpler. It makes unnecessary classification of dyads, formation and computer solution of equations. Furthermore, it appears more appropriate in comparisons of crossingover associated with disjunction and nondisjunction. This is so because a single mathematical treatment is applied throughout.

Reference: Chadov, B.F. and E.V. Chadova, 1981, Dokl. Acad. Sci. USSR, 261:993-996, in Russian; Chadov, B.F., E.V. Chadova, E.A. Khotskina, S.A. Kopyl, 1988, Genetica (USSR), in press; Chadov, B.F., E.V. Chadova, E.A. Khotskina, M.L. Podoplelova, 1987, Genetica (USSR) 23:828-834; Chadov, B.F., M.L. Podoplelova, 1985, Genetica (USSR) 21:770-778; Merriam, J.R., J.N. Frost, 1964, Genetics 49:109-122; Steinberg, A.G., 1936, Genetics 21:615-624; Weinstein, A., 1936, Genetics 21:155-199.

Chambers, G.K., Victoria University, Wellington New Zealand, Seasonal variation in *Drosophila* species at a single site in Wellington, New Zealand.

In 1985/86 my colleagues and I initiated a series of *Drosophila* collections around the Wellington district (see Chambers et al., 1988). In our previous report it was noted that the range of species collected at each site varied considerably. Consequently, two more detailed studies were undertaken to attempt to discover the reason(s) for the marked heterogeneity in species composition between sites. In the first study (reported here) monthly collections were made at a single site between December 1985 and April 1988, and in the second (Moore and Chambers, 1991 DIS: this issue) collections were made in early and late summer at several different sites.

In the present study flies were collected by aspirator at the KAR site (permanent bait bucket in a suburban garden), brought back to the laboratory and scored (see Chambers et al., 1988 for procedures). Collections were

standardized to thirty minutes duration so that the total number of flies captured may be taken as a index of *Drosophila* species abundance. Collections made in this manner at a second KAR site separated by three metres from the regular site showed no difference in species composition nor abundance. Equally, collections made at the regular site within a few days of one another did not differ with respect to species composition nor abundance (data not shown). Therefore, I believe that collections made over the two and a half year experimental period accurately reflect the range of *Drosophila* species in the immediate vicinity of the KAR site.

The data from the collections are presented in Figure 1 and climatic data for the experimental period in Table 1. The pattern of seasonal fluctuation in *Drosophila* species diversity at the KAR site can be described as follows: the most common species, *D. immigrans*, reaches a population peak in mid summer and then declines as *D. melanogaster* becomes more abundant (population maximum in February). This pattern resembles that reported for seven sites between 1970-1973 around Melbourne, Australia, which has a climate quite similar to that of Wellington (McKenzie and Parsons, 1974). The recent colonist *D. pseudoobscura* was most common at the KAR site during spring months with a population peak just preceeding that of *D. immigrans*. Two species, *D. hydeii* and *D. busckii* were also captured quite frequently at the KAR site but were never present in sufficient numbers to discern any seasonal pattern in their abundance. Regrettably *D. simulans* was rarely, if ever, present in KAR collections even though it was often quite numerous at other local sites (Chambers et al., 1988). Hence it was not possible to test the prediction that *D. simulans* numbers peak in autumn (April/May) as they did in McKenzie and Parsons' (1974) study in Melbourne.

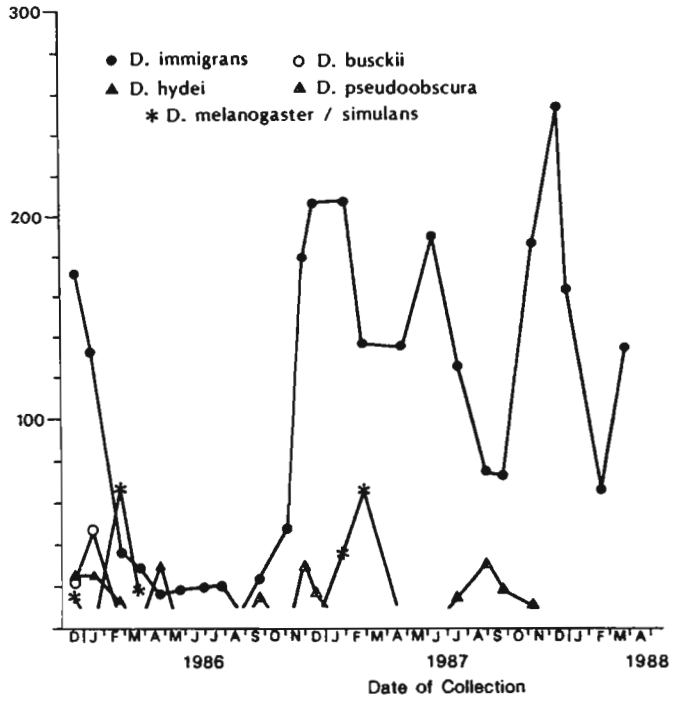
The pattern of seasonal fluctuation in *Drosophila* species abundance would seem to be quite predictable and largely driven by environmental fluctuations. These seasonal patterns go a long way to explain differences in species composition of earlier Wellington area collections (Chambers et al., 1988), although not entirely (see Moore and Chambers, this issue).

Two variations in the general seasonal pattern at the KAR site are worth noting. First, during the winter of 1987 *D. immigrans* remained relatively abundant (Figure 1). This may be due, in part, to the relatively high temperatures recorded for July and August 1988 (Table 1) but does not explain the persistence of *D. immigrans* through February to April 1987 since these months were colder than the corresponding period in 1986. Second, *D. melanogaster* numbers did not increase in late summer 1988. Examination of the climatic data

Table 1. Climate Data for Wellington, NZ 1985-1988.

Month	Temperature Mean °C	Temperature Range °C	Rainfall mm	Temperature Mean °C	Temperature Range °C	Rainfall mm
		1985			1986	
January	18.6	6.6	138	19.1	6.3	161
February	17.6	7.3	35	17.9	6.1	159
March	15.4	6.3	69	15.8	6.3	123
April	13.9	7.0	45	14.8	6.0	32
May	11.2	4.5	105	12.0	5.7	83
June	10.9	4.1	214	9.6	5.2	141
July	9.8	4.9	117	7.7	5.8	146
August	8.9	5.4	135	8.1	4.6	154
September	11.3	6.9	93	10.3	6.1	65
October	11.6	6.3	97	12.2	5.6	90
November	13.2	6.1	116	13.9	7.0	58
December	16.4	5.7	134	14.7	6.3	24
		1987			1988	
January	18.2	7.5	23	17.0	7.5	28
February	16.6	6.9	69	17.5	6.9	74
March	14.6	5.4	136	15.0	6.5	83
April	13.4	5.9	147	12.6	5.8	33
May	12.1	4.4	123			
June	9.9	5.1	65			
July	8.9	5.7	87			
August	10.0	5.7	57			
September	11.1	6.5	42			
October	12.3	6.6	116			
November	14.2	6.6	10			
December	15.4	6.4	8.5			

These data were provided by the New Zealand Meteorological Service for their Kelburn, Wellington, NZ Weather Station (approx. 8 km from KAR).



does not immediately suggest a reason why this should be so.

Longer term observations at more sites will be required before an adequate description of seasonal variation in *Drosophila* species abundance in Wellington can be provided. However, the small scale study carried out here has produced some intriguing preliminary data indicative that similar environmental factors probably regulate *Drosophila* population dynamics in Wellington and Melbourne.

Acknowledgments: The author is indebted to Ms. C. Dowdeswell and Mr. R.H. Moore for help with collections and to the fruiterers of Karori and Kelburn, Wellington, New Zealand, for providing fruit baits.

References: Chambers, G.K., Davies, S.L., Hodgetts, M., Moore, R.H. and Pomer, I.J., 1988, DIS 67:13-14; McKenzie, J.A. and Parsons, P.A., 1974, Aust. J. Zool. 22:175-187.

Chatterjee, R.N. Department of Zoology, University of Calcutta, India. Puffwise analysis of the gene activity in the 4th chromosome of male and female of *Drosophila hydei*.

never been critically tested. In the present report, the results of a locus-wise analysis of puffing activity and transcriptive activity patterns of different sites of the 4th chromosome of the male and female larval salivary gland chromosomes of *D. hydei* have been presented.

For the present experiments, salivary glands from third instar larvae of *D. hydei* were dissected in Drosophila Ringer (pH 7.2), fixed in aceto-alcohol (1:3) and stained in aceto-orcein. Temporary squash preparations were made and the puffing activity were measured (Chatterjee and Mukherjee, 1971). For present studies, 10 puff sites of the 4th chromosome have been considered (Figure 1). ^{3}H-uridine autoradioagraphy was performed to see the transcriptional activity of the puffs. For autoradiographic processing, the procedure as used in the laboratory has been followed (Chatterjee and Mukherjee, 1981).

Berendes (1965) noted that, in *Drosophila hydei*, some puff sites of the X chromosome behave differently in the two sexes. Works of Mutsuddi *et al.* (1987) have also indicated that the sexual physiology can influence the replication and transcriptive activity pattern of a number of X chromosomal sites. However, the transcriptive activity pattern of the autosomal puffs of males and females of *Drosophila* has

Table 1. Puffing activity indices and ^{3}H-uridine incorporation patterns in the 10 puff sites of the 4th chromosome of male and female salivary glands of *Drosophila hydei*.

puff site	Average puffing activity value in		Absolute grain number in	
	Female	Male	Female Mean ± S.D.	Male Mean ± S.D.
73BC	1.2	1.2	37.65 ± 9.30	40.94 ± 14.57
76B	2.1	1.95	31.90 ± 7.84	26.53 ± 5.01
81B	1.4	1.2	29.35 ± 7.17	20.24 ± 6.35
87C	1.5	1.5	31.60 ± 8.23	22.92 ± 7.99
88A	1.2	1.2	21.16 ± 4.79	14.65 ± 3.75
90B	1.2	1.2	30.75 ± 9.27	18.24 ± 5.30*
91A	1.8	1.6	28.75 ± 5.92	18.24 ± 10.58*
91C	1.6	1.4	22.90 ± 4.89	12.41 ± 5.21*
92B	1.2	1.1	19.26 ± 4.21	18.12 ± 6.47
93C	2.2	2.0	40.95 ± 9.11	37.17 ± 6.86

*Significantly different from respective male and female ($P < 0.05$).

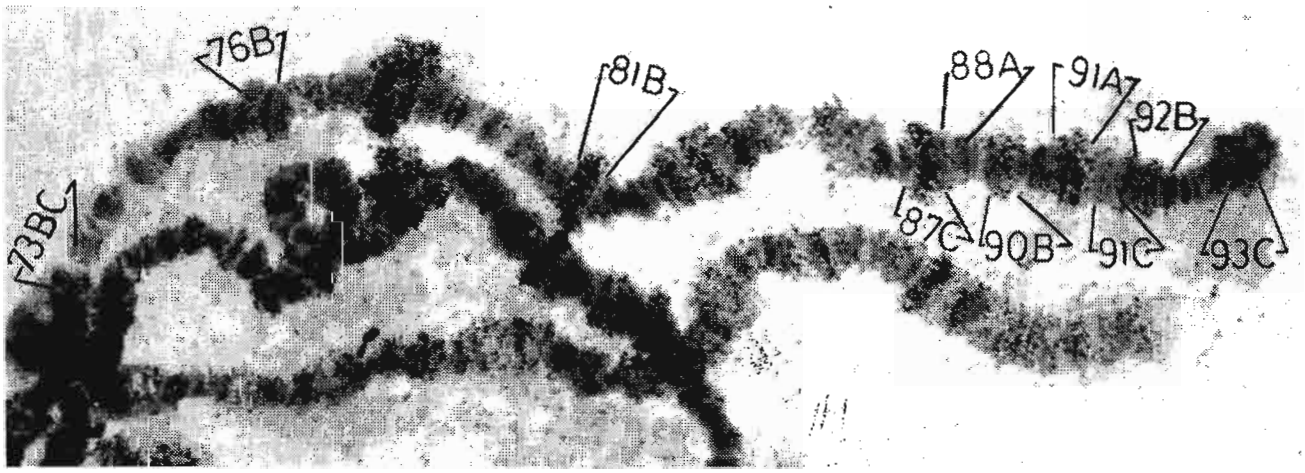


Figure 1. ^{3}H-uridine autoradiogram of a larval salivary gland of *Drosophila hydei* showing the transcriptive activity pattern of the 4th chromosomal puffs, considered in the present investigation.

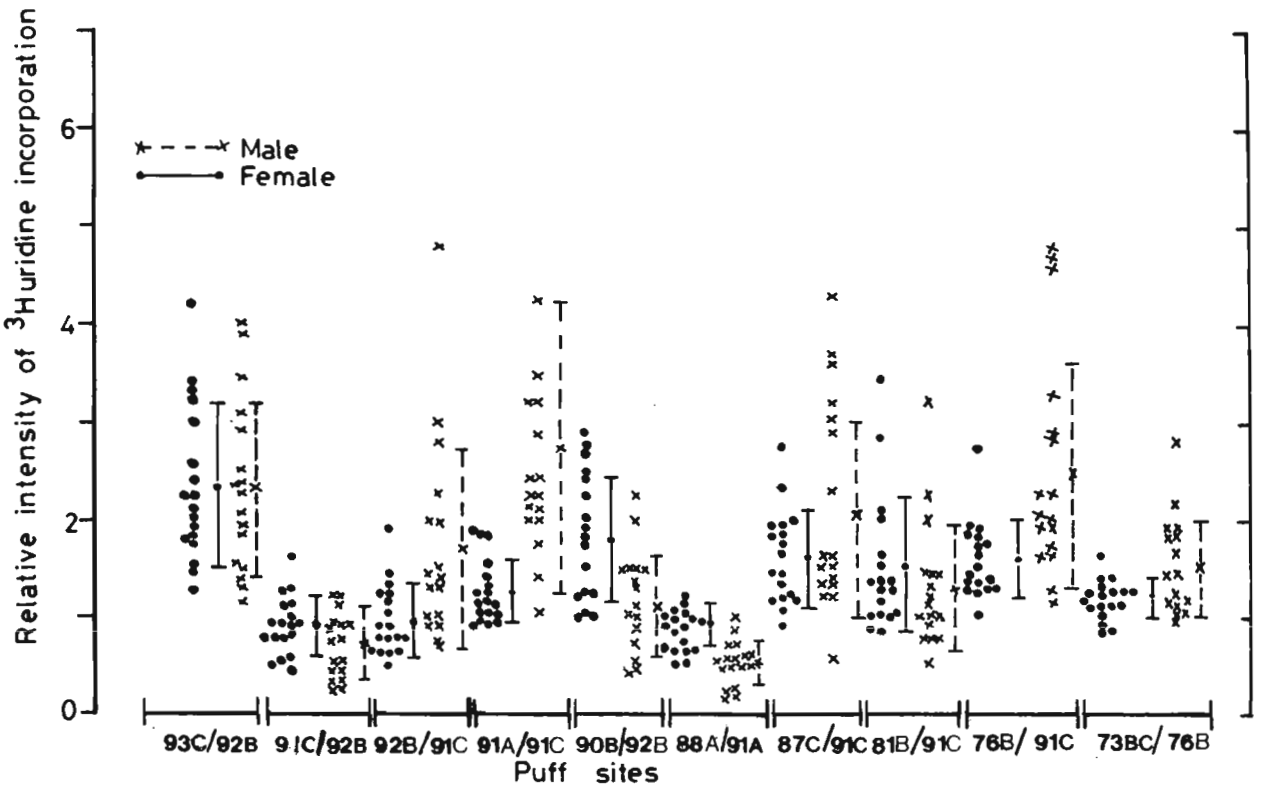


Figure 2. Relative rate of incorporation of ^{3}H-uridine into the 4th chromosomal puffs of male and female of *Drosophila hydei*.

Results presented in Table 1 show that in both sexes, most of puffs incorporate ^{3}H-uridine in accordance with the mean puffing activity indices. Furthermore, it is evident from the Table 1 that except the sites 90B, 91A and 91C, all other sites show a similar puffing activity and transcriptive activity pattern in male and female. A comparison of the relative transcriptive activity pattern of the puff sites of the 4th chromosome further reveal that the site 90B, 91A and 91C of the 4th chromosome show a considerable discordance of the activity pattern between male and female (Figure 2). These results have been interpreted to have suggested that a certain degree of disparity of autosomal puffing exists between the grain number in the two sexes. This discordance of the puffing activity pattern may be partially explained on the basis of inherent variability in the puffing pattern. However, this interpretation does not rule out the possibility of difference of sex physiology as the cause of discordance of grain intensity over some autosomal puffs.

Acknowledgment: This work is supported by a Council of Scientific and Industrial Research grant to RNC.

References: Berendes, H.D., 1965, Chromosoma (Berl.) 17:35; Chatterjee, R.N. and A.S. Mukherjee, 1981, J. Cell Sci. 47:295; Chatterjee, S.N. and A.S. Mukherjee, 1971, Chromosoma (Berl.) 36:46; Mutsuddi, D., M. Mutsuddi (Das) and A.K. Dutta Gupta, 1987, DIS 66:107.

Chatterjee, S. and B.N. Singh. Banaras Hindu University, Varanasi, India. Mating behaviour of *Drosophila biarmipes*.

Courtship behaviour in *Drosophila* is species specific and involves a number of elements performed sequentially. These signals enable the females to distinguish the conspecific from nonconspecific males. Various stimuli such as auditory, olfactory, tactile and visual may, together or in different combinations, function to inform the female of the species identity of the male (Ewing 1983). Males of a number of species exhibit epigamic pigmentation on their bodies which serve as visual signals during courtship.

Drosophila biarmipes belongs to the *suzukii* subgroup of the *melanogaster* species group, the males of which possess an apical dark black patch on their wings. We established a laboratory stock of *D. biarmipes* from a single female collected from Bhagalpur, Bihar, in October 1985. In our stock males show variation in the apical black wing patch.

Males without black wing patch have also been observed. It was found that males with black wing patch are more successful in mating than those without (Singh and Chatterjee 1987). During the present investigation we studied the mating behaviour of *D. biarmipes*, using two types of males, with and without the apical black wing patch.

Virgin females and males were collected and aged for five days. The black patch on the wings of males does not develop until 24 hours after eclosion. Only a few males without the black wing patch were found. Figure 1 shows the wings of males with and without the apical black patch. Small square chambers (25 x 25 mm) kept at 24±1°C served as observation cells which were covered by microscopic slides. A small hole was made on one side wall of the observation cell to enable the introduction of flies. A male and a female were introduced without etherization into the observation cell and courtship behaviour was observed. Observations were made for 60 minutes and if mating did not occur the pair was replaced by a fresh pair. In total 32 pairs were observed, 25 with males possessing the black wing patch and 7 with males without the wing patch.

Table 1. Mating time and duration of copulation in *D. biarmipes*.

Males	No. of pairs observed	No. of successful matings	Mating time (avg.)	Duration of copulation (avg.)
With wing patch	25	25	7 min 32 sec	12 min 12 sec
Without wing patch	7	2	40 min	12 min 1 sec

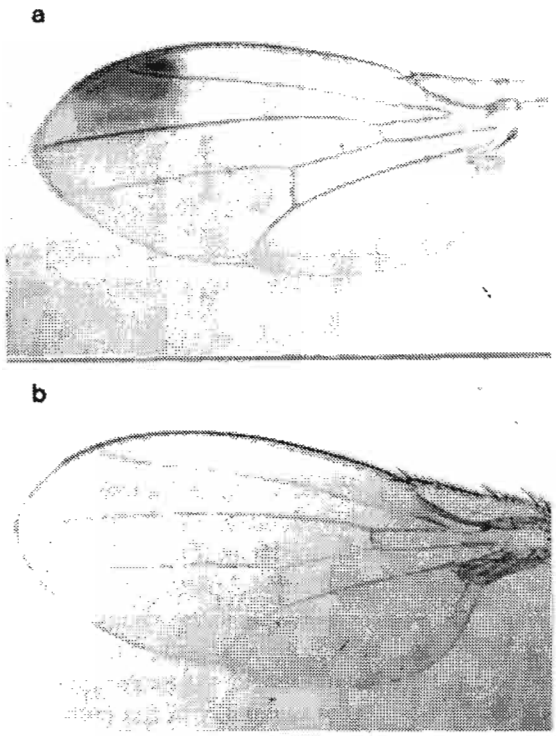


Figure 1. Wings of *D. biarmipes* males with and without apical black patch.

The male, after locating the female, orients itself at the rear side of the female. It then opens one wing vane closest to the head of the female and engages in wing vibration. The wing is opened 90 degrees and then vibrated. After each burst of vibrations the wing is typically returned to the resting position. The male then moves to the front of the female and engages in scissoring movements. The male simultaneously performs a to and fro dancing movement in front of the female, presumably displaying the black patch on its wings. Wing semaphoring is also sometimes observed between the scissoring movements. The male again moves to the rear of the female and vibrates its wings. This half circle movement is repeated a number of times after which the male moves to the back of the female, curls the tip of abdomen under and forward and attempts copulation. The female usually moves away or decamps. The male follows the female and resumes its courtship activities till copulation is achieved. When the female becomes receptive, it spreads its wings to accommodate the male. While copulating, the male keeps the front pair of legs below the wings of the female. Copulation lasts for 12 minutes and 12 seconds on an average for males with black wing patch.

The courtship activities of males without the black wing patch are similar to that of the males possessing the wing patch, but invariably the females show rejection responses. The males without wing patch are persistent in their courtship activities. While observation of courtship behaviour of males without wing patch it was found that copulation occurred in only two pairs out of seven within 60 minutes. However, in both the pairs mated the mating time (time elapsed between the introduction of the pair to the initiation of copulation) was longer than with the males possessing the apical patch. The average copulation duration with males without the apical black wing patch is 12 min. 1 sec. Table 1 shows the average mating time and duration of copulation between the two types of males. However, the mating time of males without patch is longer than those with wing patch.

It is known that if a male lacks one element of courtship, it may achieve copulation but must court the female for a longer time than does a normal male. Thus, as the males without the wing patch lacked the visual element of courtship display, they had to court the female for a longer time to stimulate the female beyond the acceptance threshold and

achieve copulation. We, therefore, propose that though other stimuli may also play a role, visual signals are important in the courtship behaviour of *D. biarmipes*. Similarly, the males of *D. suzukii* also possess an apical wing patch which is used as a visual signal during courtship (Fuyana 1979; Ewing 1983).

Acknowledgments: The financial support from the CSIR, New Delhi in the form of a Research Associateship to S. Chatterjee is thankfully acknowledged.

References: Ewing, A.W. 1983, Biol. Rev. 58:275-292; Fuyama, Y. 1979, Experientia 35:1327-1328; Singh, B.N. and S. Chatterjee 1987, Ethology 75:81-83.

Cicchetti, R., G. Argentin, C. Idili and B. Nicoletti. II University of Rome, Italy. A new allele at the *PGM* locus in *D. melanogaster*, detected by isoelectric-focusing (IEF).

Recently, the Pgm (phosphoglucomutase) locus has been investigated in *D. melanogaster* by the isoelectric-focusing method (Cicchetti et al., Atti A.G.I. 32:39-40, 1986), which has proved to be an useful tool to detect a non-electrophoretic genetic variability.

The authors, in fact, have been able to demonstrate, utilizing ampholene with a pH range between 5 and 8, a heterogeneity within the PGM-1.00,tr class; this one, in fact has shown two different isoelectric points (the one at pH 6.1 and the other at pH 5.9). By contrast, the allelic $Pgm^{1.00,ts}$, $Pgm^{0.70,ts}$ and $Pgm^{1.20,tr}$ classes were homogenous (isoelectric points respectively at pH 6.1, 6.4 and 6.1).

By a successive investigation, the same authors have demonstrated that the isoelectric-focusing variants segregate in Mendelian way and that they are genetically determined. Moreover, they are always transmitted in combination with their "original" electrophoretic and heat-sensitivity characters (Cicchetti et al., Biochem. Genet. 28:247-255, 1990).

In this paper, we report the analysis of 241 individuals from three Italian populations of laboratory (Dolianova, CA; Marzi, CS; Velletri, RM), performed to increase a *Pgm* allele sample. Single males have been homogenized and two small filter papers have been imbibed in the fly homogenate: the one has been undergone to electrophoresis and heat-denaturation, following the method of Trippa et al. (Nature 260:42-44, 1976); the other has been undergone to isoelectric-focusing, with the method reported by Cicchetti et al. (Atti A.G.I. 32:39-40, 1986; Biochem. Genet., loc. cit.).

The results are reported in Table 1. A new *Pgm* allele, $Pgm^{1.20,tr,5.9}$, has been found with a frequency equivalent to 0.012 (Table 1 and Cicchetti et al., New Mutants, this issue), whereas none $Pgm^{1.00,tr,5.9}$ allele has been recovered.

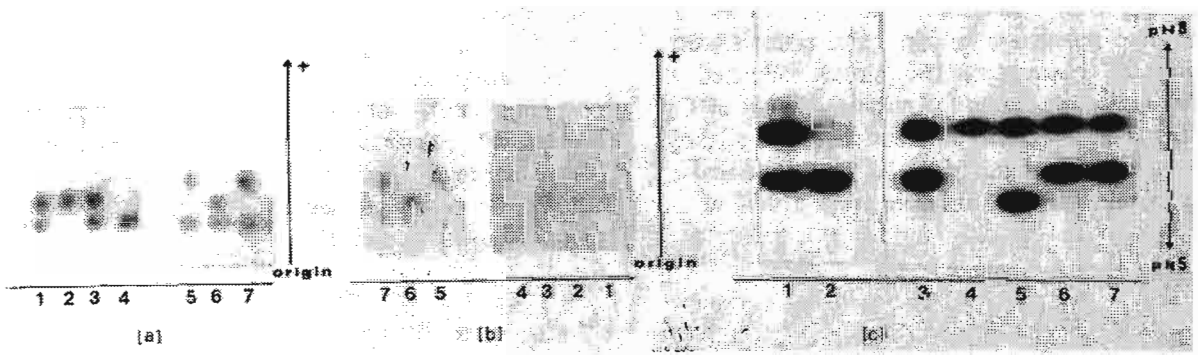
Table 1. Distribution of PGM electrophoretic, heat-sensitivity and isoelectri-focusing phenotypes, in the three populations examined.

PGM phenotypes	Populations DL	MR	VE
0.70,ts.6.4	1	1	1
0.70,ts,6.4/1.00,tr,6.1	16	12	13
0.70,ts,6.4/1.00,ts,6.1	2	-	1
0.70,ts,6.4/1.20,tr,6.1	-	-	1
0.70,ts,6.4/1.20,tr,5.9	-	-	1
1.00,tr,6.1	70	58	55
1.00,tr,6.1/1.20,tr,6.1	-	-	6
1.00,tr,6.1/1.20,tr,5.9	-	-	1
1.00,ts,6.1	1	-	-
1.00,ts,6.1/1.20,tr,6.1	-	-	1
1.00,ts,6.1/1.20,tr,5.9	-	-	-
1.20,tr,6.1	-	-	-
1.20,tr,6.1/1.20,tr,5.9	-	-	-
1.20,tr,5.9	-	-	-
Total	90	71	80

DL=Dolianova; MR-Marzi; VE-Velletri

Thus, 8 allelic variants at the PGM locus are determined, when this locus is analyzed combining the electrophoresis, the heat-denaturation and the isoelectric-focusing: $Pgm^{0.70,ts,6.4}$, $Pgm^{1.00,tr,6.1}$, $Pgm^{1.00,ts,6.1}$, $Pgm^{1.20,tr,6.1}$ and $Pgm^{1.20,tr,5.9}$ (Figure 1); $Pgm^{1.00,tr,5.9}$ (Cicchetti et al., Atti A.G.I. 32:39-40, 1986); $Pgm^{0.55,ts,6.8}$ and $Pgm^{0.70,ts,6.6}$ (Cicchetti et al., Biochem. Genet., loc. cit.).

Figure 1. The phenotypes detected at the PGM locus, by combining the electrophoresis (a), the heat-denaturation (b) and the isoelectric-focusing (c) methods. 1: 0.70,ts,6.4/1.00,ts,6.1 2: 1.00,tr,6.1
3 & 6: 0.70,ts,6.4/1.00,tr,6.1 4: 0.70,ts,6.4 5: 0.70,ts,6.4/1.20,tr,5.9
7: 0.70,ts,6.4/1.20,tr,6.1



Clark, M.A., C. McIntosh and D. Wittenberg.
Texas Wesleyan University, Fort Worth, Texas.

Modulation of CO_2-induced paralysis in *Drosophila melanogaster*.

In *Drosophila melanogaster*, animals homozygous for the gene *D1y* (McCrady and Sulerud, 1964) exhibit a prolonged paralysis following exposure to carbon dioxide. This phenomenon is superficially similar to the carbon dioxide sensitivity induced by infection with the sigma virus (Brun and Plus, 1980), but appears otherwise to be unrelated to it. The experiments reported here were done with the homozygous *D1y* stock TDR-BC^3, derived from TDR-orange by backcrossing through the male line to Cy/Pm;Sb/D females for three generations to remove maternally inherited sigma-like elements (Clark, McCrady and Fielding, 1979) that might influence the carbon dioxide sensitivity of the animals.

The conventional carbon dioxide exposure for assay of sensitivity is 15 minutes. However, even short periods of exposure to carbon dioxide may have significant effects on TDR-BC^3. Figure 1 represents the effect of increasing the exposure periods on the recovery of animals raised at 23°C. Exposure periods exceeding 5 minutes usually result in the death of the animals, with only a few flies eventually recovering several hours after their initial exposure. The wild-type reference stock Oregon-R usually recovers from narcosis within a few minutes under these conditions.

McCrady and Sulerud (1964) previously reported that in TDR-orange, the carbon dioxide-induced paralysis may be essentially eliminated by two conditions: elevation of the flies at incubation temperatures exceeding 26°C, and a previous sublethal exposure to carbon dioxide. These same conditions also alleviate CO_2 sensitivity in TDR-BC^3, indicating that both effects are related to the activity of the gene *D1y* rather than to that of the cytoplasmic elements of TDR-orange.

Table 1. Sensitivity to CO_2-induced paralysis of animals down-shifted from 28°C to 20°C at various developmental stages.

Stage at downshift	Sensitive/Total
L3	35/35
P1	23/23
P2	14/26
P3	1/30
P4	2/22

Table 2. Sensitivity to CO_2-induced paralysis of flies exposed for 24 hours to 20°C at various developmental stages.

Stage at 20°	Sensitive/Total
L3	1/37
P1	25/30
P2	27/32
P3	0/6
P4	2/22

Table 3. Sensitivity to CO_2-induced paralysis of flies exposed for 24 hours to 20°C at various developmental stages.

Stage at preexposure	Sensitive/Total
None	72/72
P7	0/38
P6-5	0/8
P4	20/20
P3	53/53
P2	60/60
P1	56/56
L3	59/59

All flies were collected as late 3rd instar larvae (L3) and pupated the day after collection. The designations P1, P2, etc. refer to pupal age in days. All animals were maintained at 28°C except for the periods indicated at 20°C. All animals were tested (15 minutes CO_2 at 14°C) within 24 hours after eclosion. Because of the slightly lower hardiness of flies raised at 28°C, only animals failing to recover within 30 minutes were classified as sensitive.

All animals were reared at 20°C. Late 3rd instar larvae (L3) and pupae (P1-7) were collected and exposed to carbon dioxide at 14°C for 15 minutes, then transferred to sucrose medium for completion of the life cycle. Pupae designated P7 eclosed the day after the first CO_2 exposure, with those designated P-6 to P-1 eclosing on successively later days. The small number of pupae in the P6-5 hatch may indicate that although exposure to CO_2 during the late pupal stage has no apparent effect on the viability of the flies, it may retard eclosion somewhat. Animals were challenged with a second exposure to CO_2 under the same conditions two days after eclosion. Flies failing to recover within 15 minutes were classified as sensitive.

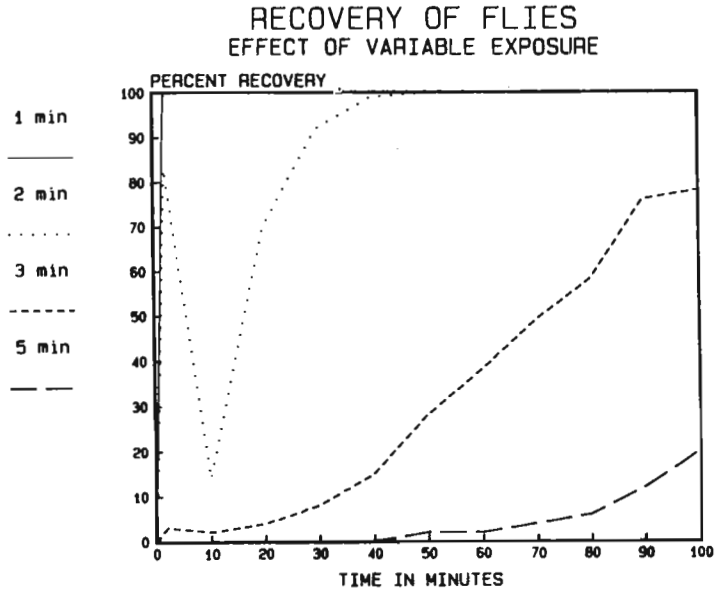


Figure 1. Recovery of flies exposed to carbon dioxide for various periods of time. Animals were incubated at 23°C, then exposed to pure CO_2 at 16° for the period of time indicated.

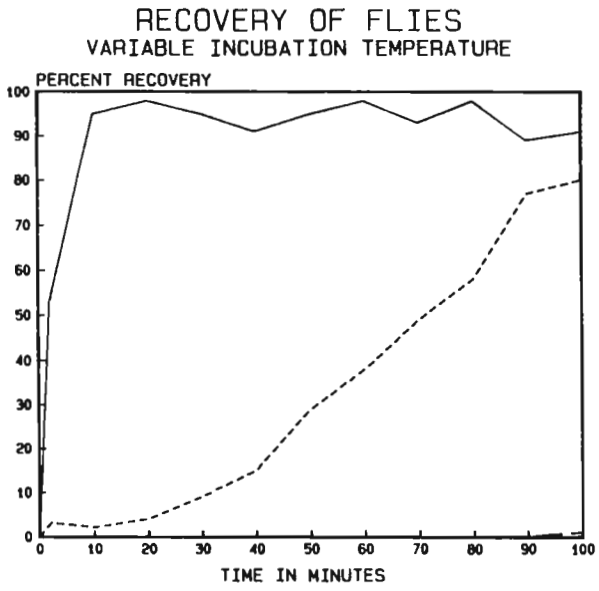


Figure 2. Recovery behavior of flies elevated at various incubation temperatures. The temperatures indicated on the figure are actual shelf temperatures for incubators set at 16, 22, and 28°C. All animals were exposed to CO_2 for 3 minutes.

Figures 3 to 5 show recovery behavior of flies preexposed to carbon dioxide. Animals raised at 22°C were preexposed to CO_2 as indicated and then tested for recovery from a second exposure (5 minutes at 16°C). All animals were maintained at 16°C for the period between exposures.

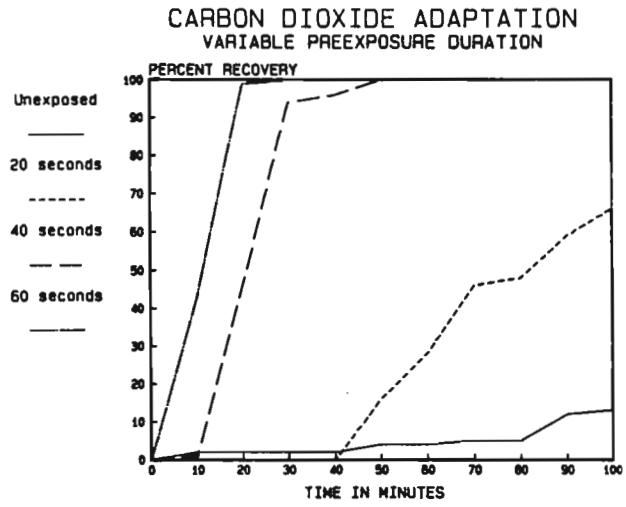


Figure 3. Effect of increasing duration of the preexposure period. Animals were exposed to CO_2 for the period of time indicated, then reexposed 2 1/2 hours after the end of the initial exposure.

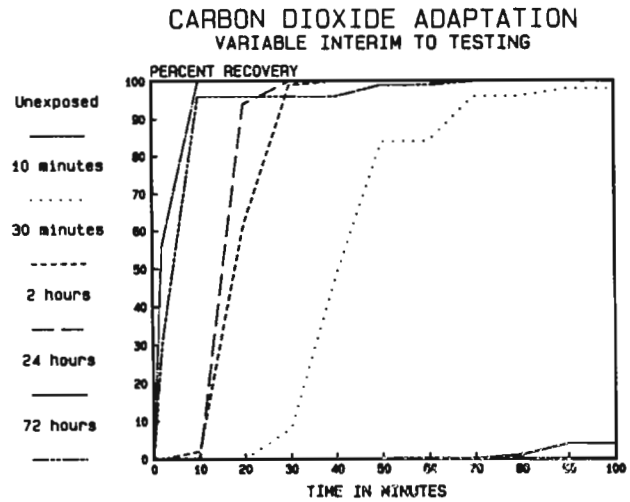


Figure 4. Effect of increasing duration of the period between preexposure and the testing exposure. All animals were given a 1 minute preexposure to CO_2 at 16°C, then retested after the time period indicated.

The "windows" for these two rescuing events are separated by several days; the two modes of rescue therefore appear to be directed against different targets. Tables 1 and 2 demonstrate that the period of sensitivity to the restrictive (20°C.) elevation temperature is during the first two days of the pupation period. A 24-hour exposure during this period appears to be sufficient to induce carbon dioxide sensitivity in most of the animals. Table 3 indicates that the period within which adaptation to carbon dioxide can be induced by a sublethal exposure begins only with the last few days of pupation, during which period no lethality appears to be associated with carbon-dioxide exposure.

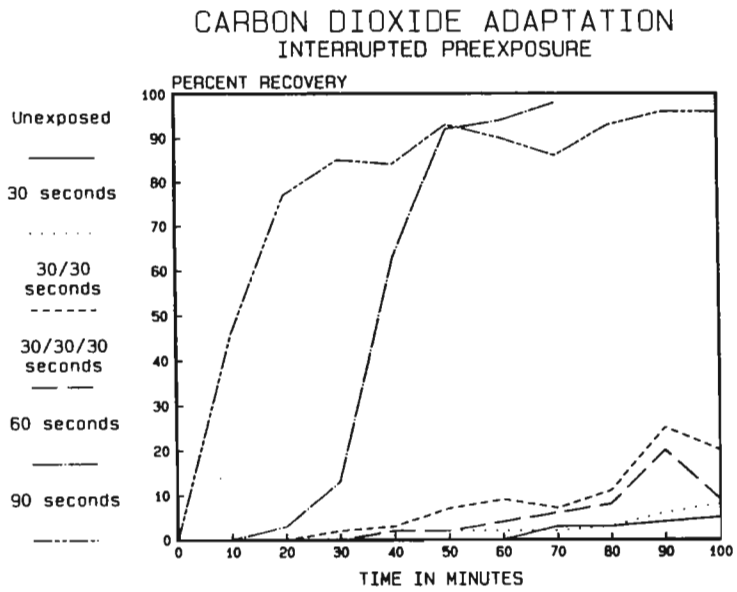


Figure 5. Effect of interrupted vs. continuous preexposure to carbon dioxide on recovery from subsequent exposure. Animals were exposed to CO_2 at 16°C for the periods indicated. The interval between repeated exposures was 10 minutes. All animals were retested 1 hour after their last exposure to CO_2.

Neither rescuing mechanism is an all-or-none phenomenon. Sensitivity to CO_2-induced paralysis increases with decreasing incubation temperatures and decreases with increasing length of prior exposure to carbon dioxide. Figure 2 represents typical effects of exposure to carbon dioxide for 3 minutes on animals raised at various incubation temperatures. Recovery behavior of animals exposed for shorter or longer periods of time exhibits similar relationships. Figures 3-5 represent the adaptation to carbon dioxide demonstrated by flies that have recovered from an earlier exposure to carbon dioxide. Figure 3 shows that carbon dioxide resistance increases with length of the previous exposure. Figure 4 shows that the adaptation response develops with time. Figure 5 shows that the adapting exposure must be continuous to be maximally effective; repeated short exposures have about the same effect as a single short exposure.

Since the primary defect leading to CO_2-induced paralysis is not known, it is difficult to speculate on the mechanisms for either high-temperature correction or CO_2-induced adaptation. Studies on the mechanism of CO_2-induced narcosis in crickets (Clark and Eaton, 1983) suggest that paralysis may be a consequence of neuron depolarization associated with increased intracellular acid load. Recovery would then require active pH regulation, presumably via proton or other ion pumps. This activity may be deficient in animals susceptible to CO2-induced paralyses. However, the brief low-temperature sensitivity period implicates a developmental rather than a simple physiological defect.

Acknowledgments: This work was supported by a Texas Wesleyan Faculty Development Grant to M.A. Clark from the Sid Richardson Foundation.

References: Brun, G. and N. Plus, In: The Genetics and Biology of Drosophila (M. Asburner and T.R.F. Wright, Eds.) Academic Press, 1980. v.2d:625-702; Clark, M.A. and D.C. Eaton, 1983, J. Neurobiol. 14:237-250; Clark, M.A., W.B. McCrady and C.L. Fielding, 1979, Genetics 92:503-510; McCrady, W.B. and R.L. Sulerud, 1964, Genetics 50:509-526.

Condie, J.M., J.A. Mustard and D.L. Brower.
Fort Lewis College, Durango, Colorado;
University of Arizona, Tucson, Arizona, USA.
Generation of anti-*Antennapedia* monoclonal antibodies and *Antennapedia* protein expression in imaginal discs.

We describe the generation and characterization of two monocolonal antibodies against a beta-galactosidase-*Antennapedia* (lacZ-*Antp*) fusion protein. These antibodies are specific for the lacZ-*Antp* fusion protein on Western blots, do not bind to beta-galactosidase or any other impurities in the bacterial lysates. Indirect immunofluorescence with either antibody shows a staining pattern in larval imag-

inal discs that is similar but not identical to previous *in situ* hybridization and polyclonal antibody results.

Protein was purified from IPTG-induced *E. coli* lysates using an anti-beta-galactosidase affinity column (Promega Biotec) as described by Carrol and Laughon (1987). A Balb/c mouse was immunized three times intraperitoneally at one week intervals (approximately 50 ug protein), followed by a final intravenous boost (approximately 10 ug protein). After one week, mouse spleen cells were fused with NSO myeloma cells and supernatants from the resulting hybridomas were screened by immunofluoresence on larval imaginal discs and nervous system (Glicksman and Brower, 1988). Two lines, 4C3 and 8C11, had indistinguishable staining patterns, characterized by a bright band of fluorescence in the thoracic region of the ventral ganglion and staining of the thoracic imaginal discs. Both lines have been recloned and appear to be stable.

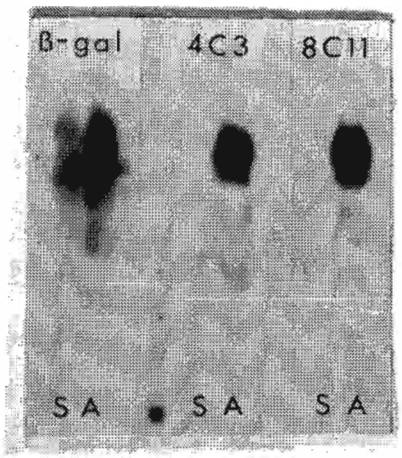
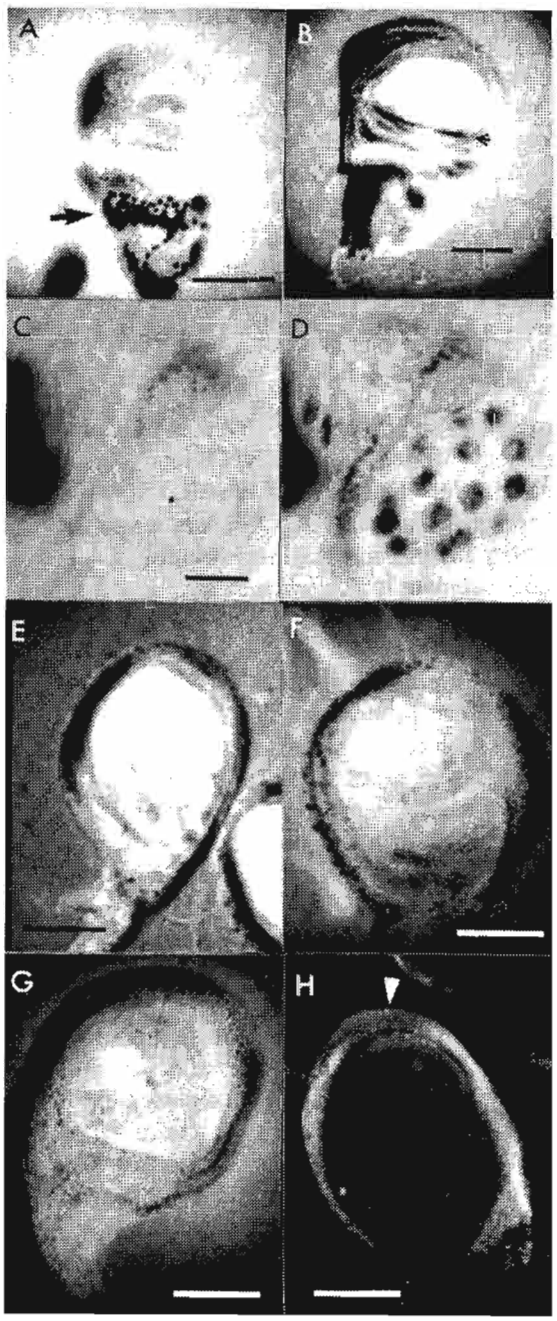


Figure 1. Above; Figure 2. Right.



To verify the specificity of the antibodies, ascites fluids were used to probe Western blots of IPTG-induced *E. coli* cell lysates containing the beta-galactosidase-*Antp* fusion protein (Fig. 1). The electrophoretic transfer and antibody staining were performed as described previously (Glicksman and Brower, 1988). Both antibodies recognize only proteins derived from bacteria containing the lacZ-*Antp* fusion (Fig. 1, lanes labeled "A"). Identical lysates from the same strain of bacteria containing a lacZ-*Scr* fusion (Fig. 1, lanes labeled "S") exhibit no antibody binding, even after long autoradiographic exposures. Blots probed with anti-beta-galactosidase (Promega Biotec) indicate that synthesis of the appropriate fusion protein was induced in both lines (Fig. 1, lanes headed beta-gal).

Antennapedia expression in larvae has been examined previously (Wirz et al., 1987), using a polyclonal anti-serum. The sensitivity of the monoclonal antibody, coupled with our video image processing system (Brower, 1987), allowed us to extend these earlier observations. Unless otherwise noted in the legend, the immunofluorescence images are processed, causing areas of staining to be dark. All of the thoracic imaginal discs express *Antennapedia* protein in distinct patterns. First, second and third leg discs all express the protein at relatively high levels around the periphery of the discs, corresponding to the proximal regions of the adult legs (Fig. 2E, G and F). In contrast to Wirz et al.,

we find that *Antennapedia* expression is not confined to either the anterior or posterior compartments. A significantly lower level of staining is seen on the medial edge in all leg discs (arrow in 2H). Confocal microscopy shows this most clearly (Fig. 2H). In addition to the epithelial staining, peripodial membrane nuclei stain in all the leg discs.

The dorsal discs show a somewhat different pattern. The wing disc stains most intensely in the anteriormost region, both dorsally and ventrally (Fig. 2B). Expression also occurs in the folds dorsal to the wing pouch, extending across the antero-posterior boundary (arrow, 2B). The anterior peripodial membrane also expresses *Antp* at significant levels. The pattern of expression in the haltere disc is analogous to that in the wing (Fig. 2A), though the intensity is much lower. The dorsal prothoracic imaginal nest (humeral disc) stains in an unusual pattern. A large group of the cells on one side of the trachea stain together with a narrow band of cells that extends around to the other side (Fig. 2C). That only a subset of the imaginal cells in the nest express the protein is demonstrated by comparing the immunofluorescence image (2C) to the DAPI-stained disc (2D). No significant staining is seen in the eye-antenna disc.

Note Added in Proof: We have recently been advised that immunostaining studies of the nervous system and of mutants that express *Antennapedia* ectopically suggest that the 4C3 antibody may not recognize all forms of the *Antennapedia* protein (Nipam Patel and Thom Kaufman, pers. commun.).

Acknowledgments: We thank John Birmingham, Peter Riley and Matt Scott for the bacteria containing the lacZ fusion plasmids, and Peter Bryant at U.C. Irvine for use of the confocal microscope. Supported by grants from the NSF (DCB-8608164) and the NIH (K04 HD00659 and S07 RR07002).

References: Carroll, S.B. and A. Laughon 1987, In "Expresion of Cloned Genes: A Practical Approach" (D. Glover, ed.) IRL Press, Oxford; Glicksman, M.A. and D.L. Brower 1988, Dev. Biol. 127:113-118; Wirz, J., L.I. Fessler, and W.J. Gehring 1986, EMBO J. 5:3327-3334.

Crossley, S.[1] **and J. Hirsch**[2]. [1]Department of Psychology, Monash University, Clayton, Vic. 3168 Australia. [2]University of Illinois, Urbana-Champaign, Illinois USNA. Observations on the generality and possible ADH-association of geotaxic behavior in *D. melanogaster*.

Selection for positive and negative geotaxis in *D. melanogaster* produced two genetically isolated populations in spite of relaxed selection (Ricker and Hirsch, 1988). This stability of a behavioral difference produced initially by artificial selection is remarkable and unexpected as the response, in one direction at least (positive geotaxis), is against the naturally occurring response favored by natural selection. No other example is known of a stable behavioral phenotype produced by artificial selection.

These geotaxic stocks are of considerable interest from a behavior-genetic and evolutionary standpoint. A first question to settle, before investigators in different laboratories can join these analyses, is whether the geotaxic phenotype is apparatus specific: do the stocks score high and low for geotaxis only in the maze used for selection or does the response generalize to a set of different situations; to a different kind of maze for example? Either way the answer is important. Any behavioral phenotype is the outcome of a set of interacting components of behavior. An accurate description of all components is essential to understanding the organisation of behavior (Manning, 1961, 1963; Crossley, 1975; McDonald and Crossley, 1982).

A second question concerns the segregation of the low and high geotaxis phenotypes following crossing, in particular whether the slow and fast alleles of the alcohol dehydrogenase (ADH) locus are characteristic of F_2 individuals scoring negatively and positively in the maze, as they are of the respective selected lines. Information of this kind is relevant to conclusions concerning the ADH-geotaxis association relationship derived from other lines of evidence including geotaxic behavior of ADH stocks.

Here we describe some preliminary experiments which address these questions.

The geotaxic flies were from strains selected divergently and intermittently for geotaxis for 600+ generations and then allowed free mating within each line for another 45 generations. These were the low line (positively geotaxic); and the high line (negatively geotaxic). Additional samples tested were: a wild-type line (Glen Waverley GW); hybrid A generation (F_1 progeny of a cross between 10 high line females and 10 low line males); hybrid B generation (F_1 progeny of a cross between 10 low line females and 10 high line males); F_{2A} generation (the progeny of the F_{1A} generation); and F_{2B} generation (the progeny of the F_{1B} generation). The Glen Waverley strain was derived from wild-caught females and had been maintained in the laboratory for three years. Stock maintenance was by bottle to bottle transfer and each generation was the progeny of at least 15 fertilized females per bottle. Room temperature was 22 + or - one degree C. and the incubator that housed the strains had high humidity, temperature 23°C, and 12:12 hour light dark cycle. Sexes were separated within eight hours of eclosion and aged in groups of ten per food vial. Flies were three days old when run through the maze, except where a few younger individuals were included to make up sample sizes.

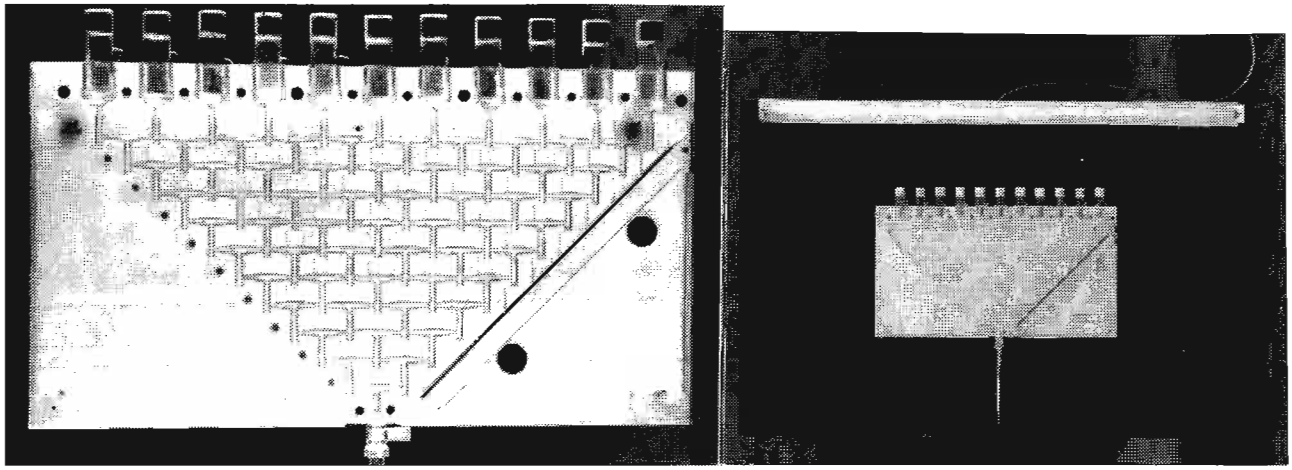


Figure 1. Maze for measuring the response of *D. melanogaster* to gravity. (a) The dimensions of the plexiglass sheets holding the maze together are 325 mm by 605 mm. (b) The maze *in situ* in front of a flourescent light.

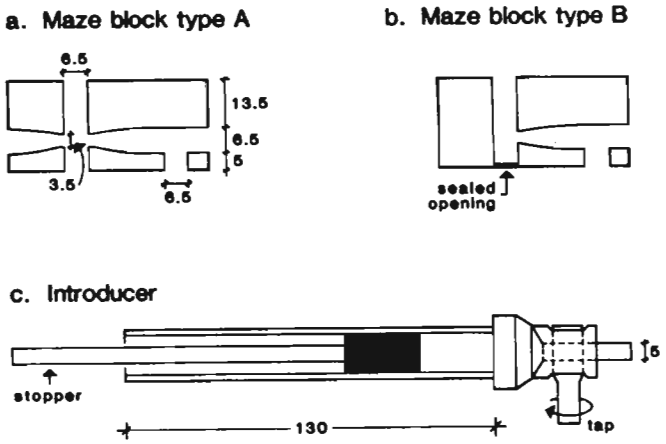


Figure 2. Components of the maze used for measuring responses of *D. melanogaster* to gravity. All measurements in the diagram are in millimeters. Overall dimensions of the blocks (a,b) are 50 by 44 by 12 mm thick. All passages taper from 6 mm to 3.5 mm at the narrowest point. (c) Introducer. The narrow end fits the entrance to the maze (Fig. 1).

The maze (Figure 1) was the prototype for those described by Hay and Crossley (1976). It consists of a base, cover, blocks from which the maze is built, exit tubes and a start tube.

The blocks are made of transparent plexiglass. Each block has a tubular passage drilled through it of variable width in the shape of T-junction. There are two kinds of T-junctions. In one both ends of the T connects laterally with openings in neighboring blocks (type A, fig. 2a), in the other only one end of the T connects in this way (type B, fig. 2b). The maze is built of blocks arranged in the same orientation with type A blocks forming the body of the maze and type B blocks the first row, comprising two blocks into one of which the starting blocks opens, and all blocks forming the upper and lower edges of the maze (Fig. 1a). Exit tubes are funnels designed to hold a 25mm diameter shell vial and the start tube is a plexiglass block with a single passage leading from an introducer that houses the flies, to the first T-junction in the maze. The introducer is a plexiglass tube with a funnel and tap at one end and a stopper in the form of a plunger at the other (Fig. 2c).

The base is an opaque white plexiglass sheet and the maze, once assembled, is held in position by a clear plexiglas cover attached to the base by screws and wing nuts. The advantage of this arrangement is that the maze is easily dismantled for cleaning between experiments. The whole is held vertically on a black wooden frame in front of a fluorescent tube, 270mm from the entrance to the exit funnels (Fig. 1b). The light draws the flies through the maze at the same time as illuminating all blocks uniformly. To achieve the latter, we covered the maze with a black cloth to prevent reflected light entering it from the side. The cloth hung over the frame and did not touch the maze. A second identical maze with its own light is held on the other side of the wooden frame, the two mazes being separated by a wooden partition. Immediately before running flies through the maze, food vials were placed into the exit tubes and held firmly in place with foam wedges (Fig. 1).

To study geotaxis, flies were tipped from their vials into the introducer through a funnel. This was removed and replaced by the stopper and the introducer was inserted into the start tube. A second replicate was set-up in the

introducer of the second maze in the same manner. The replicates acted as controls for each other, thus assuring that observations on the different genotypes studied were made under comparable conditions. Once setting up was completed the taps on the introducers were opened simultaneously and the flies ran into the maze. Rarely, stragglers lingered in the introducer and the stopper was advanced to bring them closer to the maze entrance.

Males and females were run separately through the maze. Experiments were started in late afternoon and dismantled the next morning, 16 hours later, by which time the flies had entered the food vials at the maze exits.

Once released, flies spread quickly across the maze. There was no evidence that they followed the outer edge of the maze (Walton, 1968) or hesitated to pass through narrow openings (Ewing 1963). Unlike in the Hirsch maze (1959), where there are cones to minimize moving backwards or vertically (up or down) through several blocks, in the Crossley maze, where there are no cones, they can, and sometimes do, move vertically through several blocks before advancing towards the exit or moving vertically again, also they can retrace their steps, but rarely do.

Table 1. Mean geotaxic scores and standard errors for females from different strains.

	Replicates				
Lines	1	2	3	4	5
Geotaxic					
Low	2.89 ± 0.3(83)	3.85 ± 0.3(135)	2.08 ± 0.3(73)	3.01 ± 0.4(75)	
High	9.69 ± 0.1(78)	8.80 ± 0.2(150)	6.91 ± 0.6(44)	9.14 ± 0.2(74)	8.96 ± 0.2(72)
Hybrid					
F_{1A}	7.18 ± 0.3(82)				
F_{1B}	5.00 ± 0.4(75)				
F_{2A}	7.21 ± 0.4(76)				
F_{2B}	5.86 ± 0.4(79)				
Wild-type					
GW	5.08 ± 0.3(116)	3.16 ± 0.4(81)	4.89 ± 0.4(74)		
	Overall Mean				
Geotaxic					
Low	2.96 ± 0.4				
High	8.70 ± 0.5				
Wild-type					
GW	4.36 ± 0.6				

Note: Sample sizes are given in parentheses. Values grouped in pairs within a column were the results of two samples tested at the same time in the 'back to back' mazes.

The mazes have 11 exits numbered zero, the lowest, through 10, the highest. The exit number indicates the number of choice points out of ten at which the individuals in the tube went up. A fly that moves up and then down through a vertical pathway in the Crossley maze, traversing narrowed sections, travels further to get through the maze than would usually occur in the Hirsch maze because of its cones. All flies score according to the number on their exit tube and the final score for each replicate is the composite score for all individuals tested. Occasionally, a fly moved back into the maze at dismantling. In such cases it was given the score of the tube it exited. Interestingly high line males and low line females responded to such dislodgement most noticeably. This suggests that selection may have influenced males and females differently, reactivity in one being balanced by inactivity in the other.

The geotaxic scores of the high and low lines and the scores of their hybrid generations and wild-type controls are given in Tables 1 and 2 for females and males, respectively. It is clear that the geotaxic lines do generalize their behavior to a situation different from that to which they adapated in response to selection. In agreement with earlier results, (Erlenmeyer-Kimling, Hirsch and Weiss, 1962), the high line does score higher than the low line and high females are more extreme than high males but, contrary to earlier results, the low males are more extreme than the low females. In the GW control lines, however, in every replicate males scored higher than females.

F_1 and F_2 hybrid generations gave average scores intermediate between those of the high and low lines. Reciprocal hybrids were different in the direction expected if there are some maternally inherited correlates of a different geotaxic phenotypes. Female F_2 hybrids showed scores comparable to those of their mothers, further evidence for some maternal correlates of the response to gravity.

For measuring ADH-activity, 10 males and 10 females of each line were used and both lines were measured twice. The F_2 generations were measured for ADH after they had run through the maze, samples being taken from both F_{2A} and F_{2B} at exits 0,2,5,8, and 10. Where fewer than 10 flies entered an exit tube, a smaller number was used.

The ADH study confirmed previous findings that the high line (negative geotaxis) is slow-ADH and the low line (positive geotaxis) is fast-ADH. All hybrids (F_{1A}, F_{1B}) were, as could be expected, heterozygous at the ADH-locus with genotype fast/slow. Segregation in the F_2 produced genotypes fast, slow and heterozygous (fast/slow) (Table 3). In the F_{2B} sample slow-ADH flies were found more often in the upper part of the maze as expected if this allele is associated

with the negative geotaxic phenotype and fast-ADH flies were more common in the lower part of the maze, also as expected. In the F_{2A} sample, however, the correlation is reversed: fast-ADH flies occurred more often in the upper exit tubes and slow-ADH individuals only occurred in the lower tubes. The GW strain used in this study was homozygous fast-ADH like the low line. One replicate of GW females was not dissimilar in maze behavior to the low line (Table 1), however, other GW samples showed maze behavior intermediate to that of the high and low lines. More work on ADH-activity and maze behavior is therefore required to unravel the relationship between the various phenotypes and to test hypotheses concerning linkage of the ADH locus and alleles influencing geotaxic behavior.

Table 2. Mean geotaxic scores and standard errors for females from different strains.

	Replicates			
Lines	1	2	3	4
Geotaxic				
Low	2.51 ± 0.3(102)	1.40 ± 0.2(150)	1.46 ± 0.3(61)	1.89 ± 0.3(74)
High	8.06 ± 0.2(55)	6.73 ± 0.6(55)	8.52 ± 0.3(71)	
Hybrid				
F_{1A}	7.32 ± 0.3(79)			
F_{1B}	6.81 ± 0.4(73)			
F_{2A}	5.93 ± 0.5(81)			
F_{2B}	7.07 ± 0.4(68)			
Wild-type				
GW	6.05 ± 0.3(137)	5.57 ± 0.4(72)	5.65 ± 0.4(78)	
	Overall Mean			
Geotaxic				
Low	1.82 ± 0.3			
High	7.77 ± 0.3			
Wild-type				
GW	5.76 ± 0.2			

Note: As for Table 1.

Table 3. The number of flies from F_{2A} and F_{2B} generations sharing geotaxic score and ADH-activity.

	Geotaxic Score					
ADH-activity	10	8	5	2	0	%
F_{2A}						
Fast	4	5	4	1	1	37
Fast/Slow	6	4	6	3	3	54
Slow	0	0	0	2	2	9
F_{2B}						
Fast	2	2	4	3	6	37
Fast/Slow	4	5	4	3	3	41
Slow	4	2	2	1	1	22

Note: Flies scoring 10, exit at the highest exit in the maze and those scoring 0 exit at the lowest.

Acknowledgments: We thank Dr. S. McKechnie for laboratory facilities used for ADH-activity testing, and Tania Ben-Meir, Cathy Cook and Cheryl Roberts for technical and research assistance.

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Announcements

"Evolucion Biologica" -- Scientific Organ of the Iberoamerican Association of Evolutionary Biology

"Evolucion Biologica" will publish original scientific articles in areas related to organic evolution, including population genetics, molecular genetics, evolutionary genetics, evolutionary cytogenetics, ecological genetics, population ecology, community ecology, systematics, taxonomy, biogeography, population biology (theoretical and/or experimental), paleontology, evolutionary geology, and all other areas which, in some way, include the field of organic evolution. In addition to articles which clarify evolution theory in general, "Evolucion Biologica" will also accept reviews, commentaries, critiques, Letters to the Editor, and bibliographical reviews. Articles can be written in the international languages of the Iberoamerican cultural area: Spanish, English, and Portuguese. Detailed instructions for authors can be obtained from the editor, Professor J.F. Hoenigsberg, Institute of Genetics, Universidad de los Andes, Bogota, D. E. Colombia.

Dalby, Brian and Kevin O'Hare. Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, U.K. w^{e59} - an allele of the *white* locus which has an insertion of an F element in the proximal portion of the gene and does not suppress the effect of *zeste* on expression of *white*.

In the course of studies on derivatives of the *white-one* (w^1) mutation of *D. melanogaster*, especially *white-eosin* (w^e), we obtained a stock with an allele of *white* known as w^{e59} from the Bowling Green stock center. This was entry 3783 of the stocklist in DIS 57 - *y ac sc pn* w^{e59}. On writing to the stock center for any further available information, Prof. R. Woodruff suggested that this was probably isolated in Muller's lab in Bloomington by I. Oster. However, based upon the existence of stocks carrying the *y ac sc pn* markers, w^{e59} could have been generated either by forward mutation from w^+, or by back mutation from w^1.

Preliminary DNA blotting experiments showed that in w^{e59}, there was an insertion in the same region of *white* where there is a Doc insertion in w^1 (A. Driver, S.F. Lacey, T.E. Cullingford, A. Mitchelson and K. O'Hare, in perparation). However, although there were some similarities, there were also significant differences. In order to clarify the relationship, if any, of w^{e59} to w^1, we undertook a molecular and genetic analysis of this allele.

Molecular analysis of w^{e59}

A more detailed map of the insertion in w^{e59} was constructed (see Fig. 1). A 3.7kb insertion was mapped to the region between the *Hind*III site at +3.2 and the *Bcl*I site at +3.9. The map of the insertion matched that of the F transposable element. A genomic library was constructed using lambda Dash as vector and *Bam*HI digested w^{e59} DNA as insert. This was screened with probes from the *white* locus and a recombinant phage whose insert included one of the junctions of the F element with the flanking *white* sequences was recovered and analysed by restriction enzyme mapping and DNA sequencing. This confirmed that the insertion was an F element, and located the position of insertion in the *white* locus (see Fig. 2). As only one end of the element was cloned, we cannot indicate the size, if any, of the duplication made upon insertion, but F elements are usually associated with duplications of 8 to 13 basepair (Din Nocera et al., 1983).

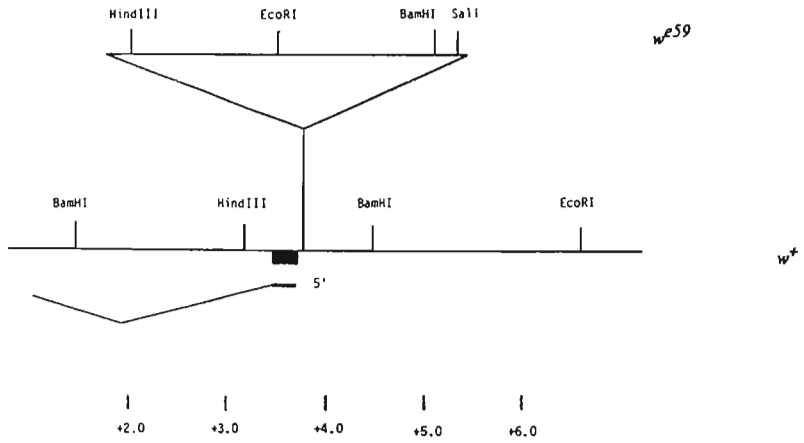


Figure 1. Map of the insertion in w^{e59}. The usual co-ordinate system for *w* is used, and the position of the 5' exon of the *w* transcription unit is shown. Transcription proceeds from right to left.

```
                                    +3760
                                      |                    *
w+      CCTCCTTCTCTGTCCACAGAAATATCGCCGTCTCTTTCGCCGCTGCGTCCGCTATCTCTT
we59    ATTCAATAAATAAAAGCAAAGTAAAATA17TCTCTTTCGCCGCTGCGTCCGCTGTCTCTT
F       ATTCAATAAATAAAAGCAAAGTAAAATAAV
```

Figure 2. The sequence of the *white* locus is that for Canton S. (O'Hare et al., 1984). The sequence of the F is that of 19F (Di Nocera et al., 1983), where A_V indicates a variable number of A residues are found in different elements. For the w^{e59} sequence, the first nucleotide beyond the oligo-A tract at the end of the F element corresponds to position +3760 and a polymorphism in the flanking *white* sequences is indicated.

Genetic analysis of w^{e59}

1) Phenotype: The eye phenotype is very similar to that of *white-honey* (w^h), with both males and females having yellow eyes. The adult ocelli and male testes sheath are colorless, although adult malpighian tubes are pigmented.

2) Dosage compensation: The eyes of females heterozygous with either w^1 or w^{1118} (a partial deletion of the *w* locus) are lighter than those of

hemizygous w^{e59} males. This indicates that there is dosage compensation in males.

3) Complementation with *white-spotted* (w^{sp}): Heterozygous w^{sp}/w^{e59} females have uniformly colored eyes which are darker than either homozygous w^{e59} or homozygous w^{sp} females. This indicates that w^{e59} partially complements w^{sp}.

4) Suppression by *zeste*: The phenotype of this allele is dependent upon *zeste*. The eyes of z^1w^{e59} males are lighter than those of z^+w^{e59} males and homozygous $^{z1}w^{e59}$ female flies have white eyes. Heterozygous z^1w^+/z^1w^{e59} females have yellow eyes similar to those of homozygous z^1w^+ females. We have not examined the effect of other *zeste* alleles.

A number of *w* alleles are known with insertions in the proximal portion of the gene. These include w^{sp}, and w^1. The F insertion in w^{e59} at +3760 falls between the site of the insertion of the Doc element in w^1 at +3700 and the site of insertion of the B104 element in w^{sp} at +4922. It is close to the position (+3687) where *w* transcription initiates as defined by S1 nuclease mapping (Stellar and Pirrotta, 1985). Sites within the *white* locus promoter region where the protein encoded by *zeste* binds have been mapped (Benson and Pirrotta, 1988). The insertion in w^{e59} lies between these upstream sites and the site where the *w* transcription starts.

When mutants which map to the proximal region of *w* are heterozygous with w^+ in a z^1 background, the flies have wildtype colored eyes. When mutants which map to the distal region are heterozygous with w^+ in a z^1 background, the flies have yellow eyes similar to those of homozygous z^1w^+ females (Green, 1959). This was one of the criteria used in the development of the genetic model of the *w* locus where the proximal region was regulatory and the distal region was structural. In this test, w^{e59} does not suppress the effect of z^1 upon the expression of *w*, and behaves like mutations of the distal region of *w*. We have not genetically mapped where within *w* the mutation in w^{e59} lies, nor have we observed any revertants of w^{e59}, so it remains possible that the phenotype is due to a point mutation in the distal portion of the gene, and that the F insertion is phenotypically silent. Phenotypically silent insertions of F elements have been observed in a number of genes, including *w* (Karess and Rubin, 1982). However, it seems reasonable to assume that an insertion of this size in the promoter region would affect the phenotype.

In w^{sp2}, some of the sites where the *zeste* protein binds are deleted while in w^{sp} there is an insertion into the region where the strongest binding sites are. In w^{e59}, the *zeste* binding sites are not directly affected, simply moved 3.7kb from the *w* transcription start site. In P[*w*] transposons where there was an insertion of 670bp between the major binding sites and the *w* transcription initiation site (Pirrotta, Steller and Bozzetti, 1985), the effect of z^1 was to reduce the pigmentation of the eye in homozygous flies. As these transposons are inserted at many different sites in the genome, and suppression by z^1 depends upon the two copies of *w* being paired, it was not possible to test them as we have done for w^{e59}, that is when heterozygous but paired with a w^+ gene. We conclude that in w^{e59}, the insertion in the promoter region results in a diminution of expression of *w*, but that the regulation of expression is essentially wildtype, with cis-acting sequences (especially *z* binding sites) being able to influence transcription from the promoter.

Acknowledgments: Most of these results were from a practical project carried out by B.D. as part of his requirement for the B.Sc. degree. K.O'H. is an MRC Senior Fellow. We thank Ron Woodruff for detective work on the derivation of this allele.

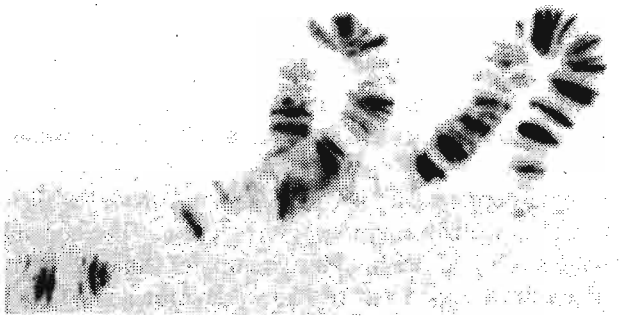
References: Benson, M. and V. Pirotta, 1988, EMBO J. 7:3907-3915; DiNocera, P.P., M.E. Digan and I. Dawid, 1983, J. Mol. Biol. 168:715-727; Green, M.M., 1959, Heredity 13:303-315; Karess, R.E. and G.M. Rubin, 1982 Cell 30:63-69; O'Hare, K., C. Murphy, R. Levis and G.M. Rubin, 1984 J. Mol. Biol. 180:437-455; Pirrotta, V., H. Steller and M.P. Bozzetti, 1985 EMBO J. 4:3501-3508; Steller, H. and V. Pirrotta, 1985 EMBO J. 4:3765-3772.

Das, A. and B.N. Singh. Banaras Hindu University, Varanasi, India. An inversion in an Indian population of *Drosophila melanogaster*.

Chromosomal polymorphism due to inversions is common in the genus *Drosophila*. Natural populations of *D. melanogaster* are often polymorphic for chromosome inversions (for references see the review by Lemeunier et al. 1986). In total nearly 326 inversions have been described in this species. The quantitative data on the frequencies of common cosmopolitan inversions have been reported for the natural populations of *D. melanogaster* from different regions (Stalker 1976; Mettler et al. 1977; Voelker et al. 1977; Inoue & Watanabe 1979; Knibb et al. 1981).

D. melanogaster is of frequent occurrence in India but chromosomal polymorphism in its Indian populations has not been studied. The present note describes an inversion in *D. melanogaster* from India. An isofemale line was initiated from a naturally impregnated female collected from Uchawa Lodge near U.P. College, Varanasi in February, 1987. After maintaining this line for 20 months in food bottles by transferring nearly 50 flies in each generation, its chromosomal analysis was made. The cytological analysis revealed the presence of an inversion in this stock. The inversion extends from 25E to 30C in the left arm of the second chromosome. This is the same inversion described for the first time by Stalker (1976) from Geranda, Mississippi, USA and designated as In(2L) F. Fig. 1 depicts the

microphotograph of this inversion in heterozygous condition. It is interesting to note that the inversion detected only from a North American population occurs in an Indian population.



	Standard Homozygote	Heterozygote	Inversion Homozygote
Observed	67	31	2
Expected	68.06	28.88	3.06
	$x^2 = 0.732$	d.f. = 1	P > 0.30

Figure 1. Microphotograph of heterozygous inversion in 2L of *D. melanogaster*.

From the laboratory stock 100 larvae were analysed. All the three karyotypes due to this inversion in 2L were distinguished. Their frequencies are shown in the table. The data show that all the three karyotypes are present in the laboratory stock although the inversion homozygotes occur with low frequency. The frequency of ST chromosomes is 82.5 percent and of chromosomes with inverted gene arrangement 17.5 percent. The observed number of inversion heterozygotes is more than their expected number but deviation from Hardy-Weinberg expectation is not significant (P > 0.30). Inoue (1979) reported that polymorphic inversions of *D. melanogaster* found at higher frequency in nature were almost eliminated from the cage populations after about 20 months. The present observation demonstrates that the inversion which is not cosmopolitan in distribution persisted for about 20 months in laboratory cultures and was maintained at a considerable frequency (17.5 percent). This suggests that inversion heterozygotes exhibit heterosis.

Acknowledgments: The financial support in the form of a JRF of the Centre of Advanced Study in Zoology to A.D. is thankfully acknowledged.

References: Inoue, Y. 1979, Jap. J. Genetics 54:83-96; Inoue, Y. & T.K. Watanabe 1979, Jap. J. Genetics 54:69-82; Knibb, W.R. et al. 1981, Genetics 98:833-847; Lemeunier, F. et al. 1986, In: The Genetics and Biology of Drosophila, N.Y. Acad. Press, V 3e:147-256; Mettler et al. 1977, Genetics 87:169-175; Stalker, H.D. 1976, Genetics 82:323-347.

Davies, A.G. and P. Batterham, University of Melbourne, Parkville, Vic., Australia. Analysis of an unstable P element insertion mutation at the lozenge locus in *Drosophila melanogaster*.

The mutant $1z^{pm2}$ (abbrev. PM2) was isolated in a P-M mutagenesis cross by M. Kidwell (Kidwell, 1987). The PM2 mutation has proved to be unstable in stock cultures and in P-M crosses where PM2 has been used as the P strain. The instability of this mutation has been investigated.

PM2 has a mild lozenge eye phenotype with apparent disorganization of facets only at the posterior rim of the eye. The mutation maps to the spectacle sublocus of 1z (Lindsley and Grell, 1967). The presence of a P element at the cytological location of 1z (8D4-8E2) in PM2 was confirmed by *in situ* hybridization of a P element probe to salivary gland polytene chromosomes.

The P element at the 1z locus was destabilized by crossing PM2 males with M strain females according to the cross described in Figure 1. Males in the second generation were scored for a lozenge phenotype that differed from that of PM2. In a total of 3201 males scored, 9 1z mutants phenotypically more extreme than PM2 were recovered. These 9 mutants could be separated into two classes based on the severity of their eye phenotype. If the spectrum of 1z eye phenotypes observed is divided into 5 classes (Figure 2) and the parental PM2 phenotype is class 2, then the two extreme classes recovered are representative of classes 3 and 4.

It was found that 7 of the 9 extreme secondary mutations were also unstable when crossed to M strain females (either C(1)DX,yf or FM7/EA113). In some cases, tertiary and quaternary mutations which are also unstable have been

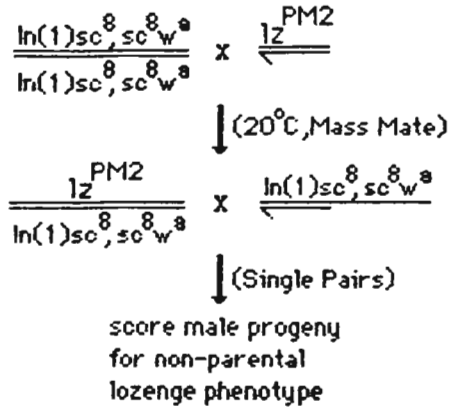


Figure 1. Crossing protocol for destabilizing the P element at the 1z locus in PM2 and subsequent recovery of non-parental derivatives.

produced (Figure 3). In crosses involving the 7 unstable secondary mutations, progeny representing all five phenotypic classes have been recovered. Also a number of mutants have produced phenotypically wild-type offspring. However, it should be noted that although these wild-type derivatives have a wild-type appearance in hemizygous and homozygous states, they fail to complement the deletion mutation $1z^{L}$. There is at least one example of a member of each phenotypic class showing instability except for the "wild-type" derivatives (Figure 4). This overall pattern of instability suggests that a P element capable of transposition is still present at the 1z locus in 7 of the 9 secondary derivatives and many of the tertiary and quaternary mutant derivatives. This is not consistent with the observations of other studies where secondary mutagenesis involving P element mobilization results in either precise or imprecise excision of the element to produce stable derivatives (Tsubota and Schedl, 1986; Salez et al., 1987). Recent reports have shown more complex events can be associated with P element mobilization (Geyer et al., 1988; Hawley et al., 1988). Such mechanisms could explain the pattern of instability observed with PM2 and its derivatives. Analysis at the molecular level should provide an interesting explanation for the observed continuum of lozenge phenotypes, particularly as each phenotype can apparently be produced by the insertion of a P element capable of transposition.

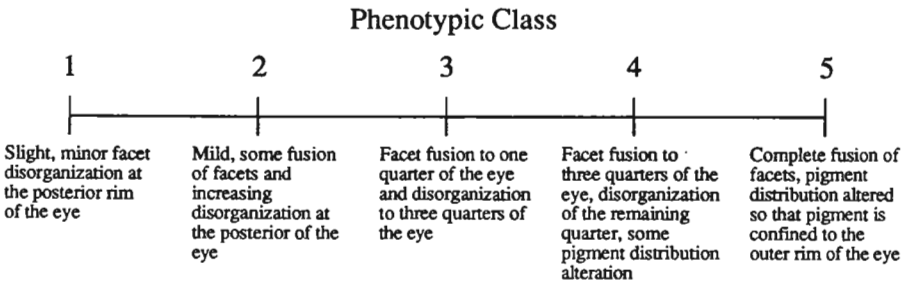


Figure 2. Phenotypic descriptions for the five classes of lozenge eye phenotype.

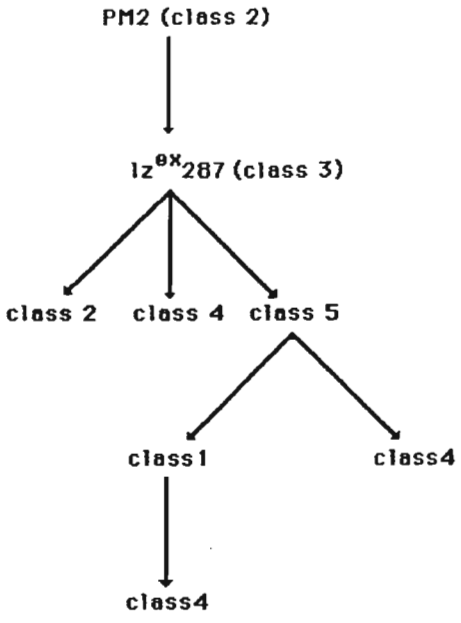


Figure 3. An example of the continued instability following a mutation event. Each arrow represents a mutation event which produced individuals with a non-parental eye phenotype, some of which showed further instability.

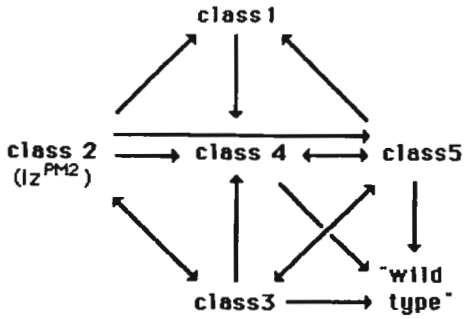


Figure 4. Representation of the combined mutation events that have occurred between mutants of the various phenotypic classes. Arrows indicate the observed class changes as a result of the mutation events.

References: Geyer, P.K., K.L. Richardson, V.G. Corces, and M.M. Green 1988, Proc. Natl. Acad. Sci. 85:6455-6459; Hawley, R.S., R.A. Steuber, C.H. Marcus, R. Sohn, D.M. Baronas, M.L. Cameron, A.E. Zitron and J.W. Chase 1988, Genetics 119:85-94; Kidwell, M.G. 1987, DIS 66:81-86; Lindsley, D.L. and E.H. Grell 1967, Carnegie Inst. Publ. 627; Salz, H.K., T.W. Cline and P. Schedl 1987, Genetics 117:221-231; Tsubota, S. and P. Schedl 1986, Genetics 114:165-182.

Dunkov, B.C., T.G. Georgieva and K.H. Ralchev. University of Sofia, Bulgaria. Diaphorase activity associated with *Drosophila* xanthine dehydrogenase.

Diaphorases (EC 1.6.) are enzymes which oxidise in vitro the reduced forms of NAD and/or NADP using different artificial electron acceptors as 2,6-dichlorophenolindophenol (DCIP), menadione and tetrazolium salts. The electrophoretic studies in *Drosophila* revealed the existence of three groups of diaphorases (Ralchev et al., 1987). The diaphorase-2 group of enzymes possessing intermediate electrophoretic mobility comprises two main fractions designated as DIA-2' and DIA-2". As the diaphorases in *Drosophila* have independent genetic control and on the other hand they probably are united mainly by their in vitro reaction with a common artificial substrate (DCIP, menadione), a reasonable question arises whether the diaphorase activity thus detected is not a side activity only associated with other dehydrogenases. In different organisms as *Chlamydomonas* (Perez-Vicente et al., 1987), *Aspergillus* (Lyon and Garret, 1978) and *Neurospora* (Lewis and Scazzoccio, 1977) an association of diaphorase activity with xanthine oxidising enzymes was ascertained. In order to test this possibility in *Drosophila* we used electrophoretic analysis of crude extracts followed by staining for diaphorase and xanthine dehydrogenase activities. The results obtained from the investigation of two *Drosophila* species (Fig. 1) indicate the coincidence of the band corresponding to DIA-2" with the single xanthine dehydrogenase one. In addition, our previous studies on diaphorase-2" have shown that this enzyme is only NADH specific and it has relatively high thermostability which is characteristic for xanthine dehydrogenases. The organ distribution of diaphorase-2" in *D. virilis* (Dunkov et al., 1987) is also similar to that one of *Drosophila* XDH which is synthesized in the fat body and Malpighian tubules and then transported to the eyes (Barret and Davidson, 1975; Reaume et al., 1989).

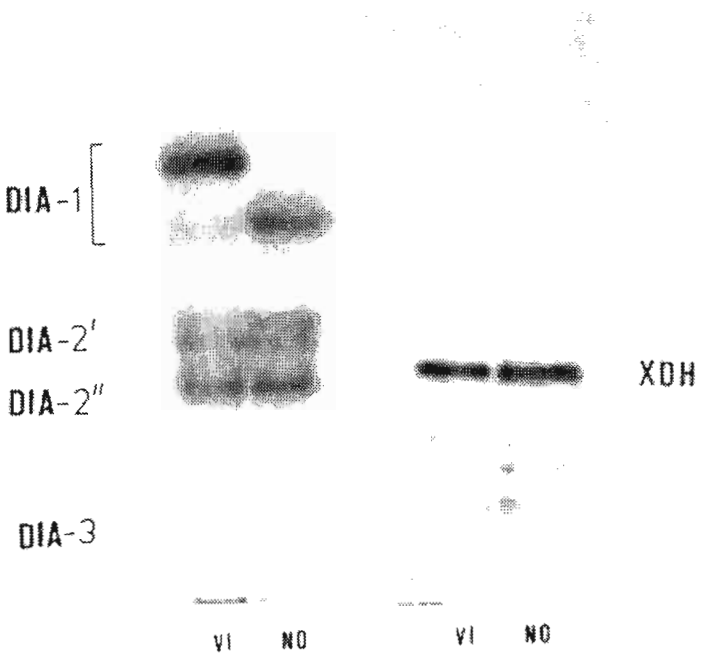


Figure 1. Nondenaturing polyacrylamide gel electrophoregrams stained for diaphorase (left) and xanthine dehydrogenase (right) activities. Gels were stained for DIA and XDH as described in Ralchev et al. (1987) and McCarron et al. (1974) respectively. VI = *D. virilis*, NO = *D. novamexicana*.

The fact that xanthine dehydrogenase gene-enzyme system in *D. melanogaster* is one of best studied systems enabled us to confirm our observations on the identity of DIA-2" and XDH by means of XDH null mutants. For this purpose extracts of flies from the lines ry^{506} and ry^{60} which are deletion mutants of the rosy locus were separated electrophoretically and stained for diaphorase

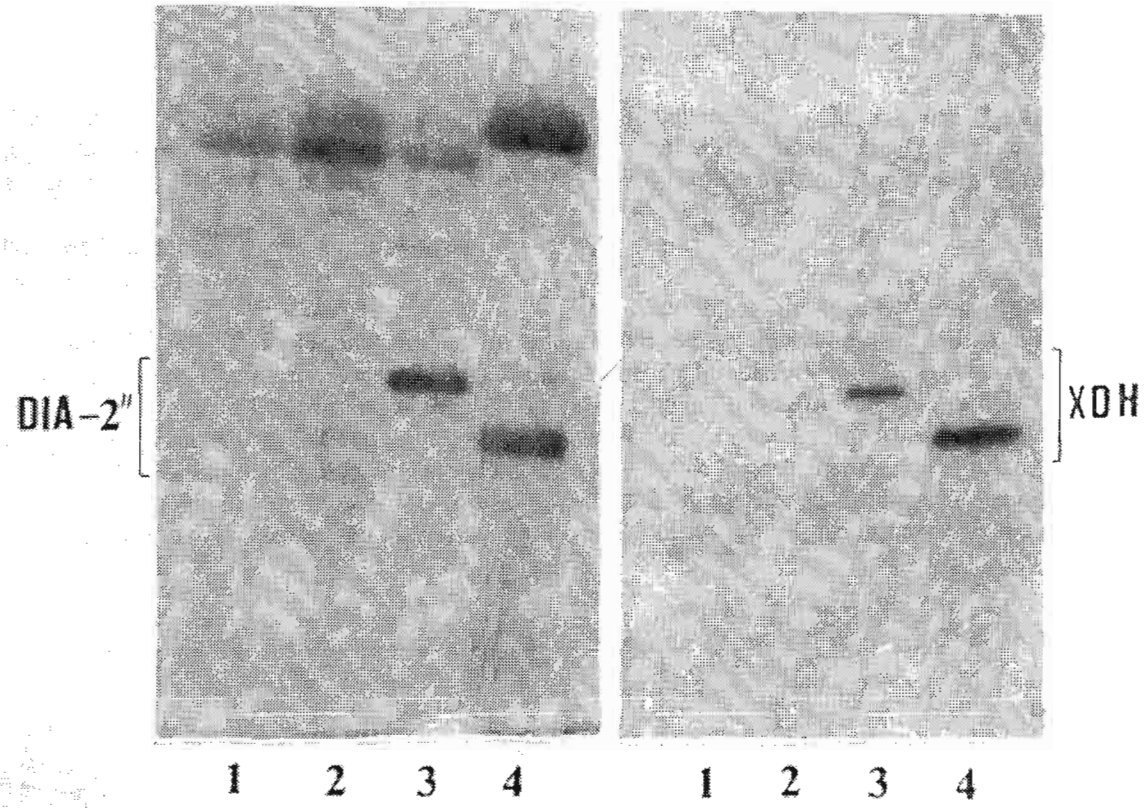


Figure 2. Nondenaturing polyacrylamide gel electrophoregrams stained for diaphorase (left) and xanthine dehydrogenase (right) activities. Lanes: 1, *D. melanogaster* ry^{60}; 2, *D. melanogaster* ry^{506}; 3, *D. m.* Canton S; 4, *D. virilis*.

and xanthine dehydrogenase activities. The electrophoregrams thus obtained (Fig. 2) confirm the identity of these two enzymes. Thus it is evident that the lack of XDH activity in these mutants leads to absence of the band corresponding to DIA-2", too. The question whether both activities of this enzyme are associated with the same active center remains open.

Acknowledgment: The authors are grateful to Dr. A. Chovnick, University of Connecticut, Storrs (USA) for kindly providing rosy mutants of *D. melanogaster*.

References: Barret, D. and N.A. Davidson 1975, J. Insect Physiol. 21:1447-1452; Dunkov, B.C., N.M. Ralcheva, K.H. Ralchev and E.P. Dimitrova 1987, Compt. Rend. Acad. Bulg. Sci. 40:69-72; Lewis, N.J. and C. Scazzocchio 1977, Eur. J. Biochem. 76:441-446; Lyon, E.S. and R.H. Garret 1978, J. Biol. Chem. 253:2604-2614; McCarron, M., W. Gelbart and A. Chovnick 1974, Genetics 76:289-299; Perez-Vicente, R., M. Pineda and J. Cardenas 1987, FEMS Microbiol. Lett. 43:321-325; Ralchev, K.H., B.C. Dunkov, A.S. Doichinov and M.J. Simeonovska 1987, Genetics and Breeding (Bulg. Acad. Sci.) 20:196-203; Reaume, A.G., S.H. Clark and A. Chovnick 1989, Genetics 123:503-509.

Eisses, K. Th. Department of Plant Ecology and Evolutionary Biology, Rijksuniversiteit Utrecht, The Netherlands. *Notch* and *rudimentary* mutants induced by female larval treatment with 2-methoxyethanol.

2-Methoxyethanol (2-ME) is a teratogenic agent for many organisms, including *Drosophila melanogaster* (Eisses, 1989). 2-ME was not known as a mutagenic agent, which was based on statistically conflicting results from treated adult *Drosophila* males (McGregor *et al.*, 1983). I used third instar larvae to test the teratogenicity of 2-ME in different *alcohol dehydrogenase* genotypes, and wanted to know if there were any epigenetic effects. Surprisingly *Notch* mutants were derived from two of the most affected females, crossed with untreated males. The mutation could be localized at the *Notch* locus by a noncomplementary cross with the recessive visible *facet-notchoid*. The mutants were called N^{86e1} and N^{86e2}, of which the former one is still alive. In one replication experiment *rudimentary* males turned up in the progeny of one out of 60 surviving treated females. In a complementation experiment with r^{9} and r^{39k} the phenotype and fertility of the homozygotes was much alike r^{39k}. The new mutant has been called r^{89k}, and is available as *C(1)DX, y w f / r^{89k}*. Both mutants are available at the Umea Drosophila Stock Center. In a second replicate experiment, one *Notch* mutant was found in the progeny of 43 treated females.

Acknowledgments: The *Notch* mutants were derived during a post-doctoral stage at the Biochemical Institute of the University of Oslo, Norway with a grant from the Royal Norwegian Council for Scientific and Technological Research. The hospitability of the Biological Institute, section General Genetics is greatly acknowledged.

References: Eisses, K.Th. 1989, Teratogen. Carcinogen. Mutagen 9:315-325; McGregor, D.B., M.J. Willins, P. McDonald, M. Holmstrøm, D. McDonald and R.W. Niemeier 1983, Toxicol. Appl. Pharmacol. 70:303-316.

Escriche, B. and F.J. Silva. Universidad de Valencia, Spain. Pteridine deposition in "in vitro" cultured heads of *Drosophila melanogaster.*

The organ culture in *Drosophila melanogaster* is a powerful tool to study the metabolic pathways, because it lets us investigate in a live-like system but under more controlled conditions.

We are planning to use these techniques like an intermediate system between "in vivo" and "in vitro" biochemical tests in the study of the pteridine pathway.

We used a fast and simple system to measure oxidized pteridines by HPLC with fluorescent detection. The mobile phase was 4% methanol with 50mM phosphate buffer pH 3.2, the column was a reverse-phase Altex Ultrasphere-ODS (5um, 250x4.6mm) and we used a 360 nm excitation filter and 425 nm cut-off filter.

The "in vitro" cultures were made with *Or-R* fly-heads, placing 2 synchronous adult or pupa heads (one male and one female) in a sterile microplate with 100 ul of Schneider's Drosophila Medium (SERVA).

Figure 1 shows the results obtained for the deposition of biopterin and its dependence on the developmental stage of the insect when the culture was started. The normal pattern of deposition "in vivo" was used as a control.

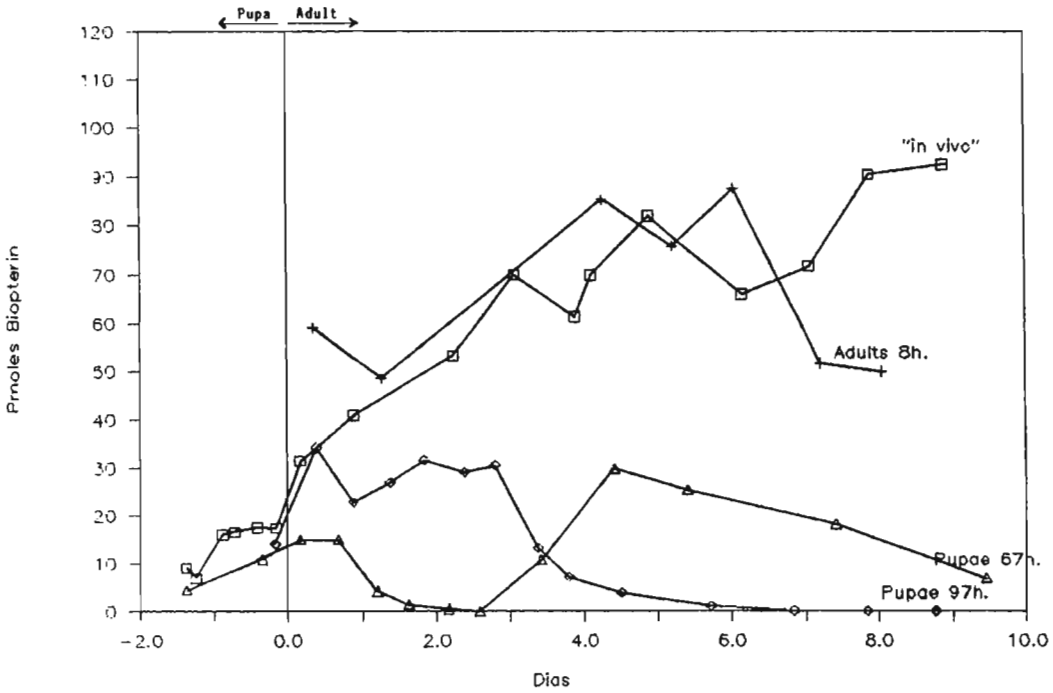


Figure 1. Deposition of the biopterin in "in vitro" cultured heads in different stages of development.

We only show the pattern of biopterin for each type of culture because the other pteridines measured follow the same pattern. An exception is isoxanthopterin deposited "in vivo", which remains constant whereas the other pteridines steadily increase.

We may see that according to the age of the insects different deposition patterns are formed. Thus 8 hours adults show a similar pattern to the one formed "in vivo"; however the deposition in pupae seems to be similar to "in vivo" pattern at the beginning, but diverging at later stages.

These results seem to indicate that the adult heads already have their metabolic system to give a "normal" deposition pattern. Whereas the pupa heads do not have developed the complete metabolic system and they are not able to develop it in these conditions.

Because the studies on insect organ culture have been performed with imaginal discs it is difficult to compare our results with other authors' data. However their conclusions are similar to ours.

M. Horikawa (1959) had already observed for the brown pigment that a metamorphic hormone seems to be an essential substance for pigmentation in cultures and P. Mandaron (1973) studied the need of ecdysone to develop embryonic structures.

Authors who described the deposition of pteridines in imaginal discs are I. Scheneider (1964) and E.W. Hanley et al. (1967), who concluded that a similar pigment deposition was found between 65 hours pupae explants and "in vivo". These results do not agree with the ones shown in Figure 1, probably due to the use for these authors of a not very efficient system for the analysis of pteridines.

References: Hanley, E.W., C.W. Fuller and M.S. Stanley 1967, J. Embryol. Exp. Morph. vol. 17, 3:491-499; Horikawa, M. 1959, Cytologia 23:468-477; Mandaron, P. 1973, Develop. Biol. 31:101-113; Scheneider, I. 1964, J. Exp. Zool. 156:91-104.

Etges, W.J. Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA.

Seasonal variation among gene arrangements in *Drosophila robusta.*

When encountered, seasonal changes in observed frequencies of gene arrangements in natural populations of *Drosophila* are often considered results of genotypic changes driven by natural selection (Dobzhansky, 1948; Carson and Stalker, 1949; Levitan, 1973). Populations of *D. robusta* that have been monitored for temporal variation in gene arrangement frequencies vary in the extent of seasonal or long term genetic changes. Missouri populations show few chromosomal responses to seasonal variation and virtually no changes in gene arrangement frequency over time intervals as long as ten years (Carson, 1958). Pennsylvania, Virginia and Tennessee populations show seasonal changes (Levitan, 1973) with the latter population also showing long term frequency changes (Etges, 1984). Differences in the capacity for such microevolution is clearly of interest, yet much more information is needed on the frequency and overall pattern of temporal changes among populations, as well as the mechanisms involved.

Table 1. Inversion frequency differences between a Fayetteville, BY, and Ozark Mountains, SL, population of Drosophila robusta. Both population were sampled during October and November, 1987.

Gene arrangement	BY	SL	X^2
XL	0.615	0.154	
XL-1	0.221	0.480	51.62^{****}
XL-2	0.164	0.366	
n	104	123	
XR	0.192	0.041	
XR-1	0.337	0.049	52.95^{****}
XR-2	0.471	0.910	
n	104	123	
2L	0.320	0.313	
2L-1	0.523	0.453	3.99^{ns}
2L-2	0.133	0.167	
2L-3	0.024	0.067	
n	128	150	
2R	0.867	0.827	0.87^{ns}
2R-1	0.133	0.173	
n	128	150	
3R	0.344	0.253	2.72^{ns}
3R-1	0.656	0.747	
n	128	150	

**** $P < 0.0001$, ^{ns}not significant

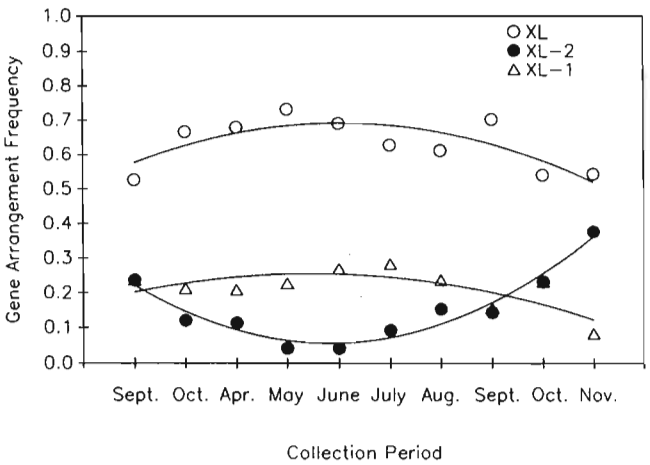


Figure 1. Seasonal shifts in gene arrangement frequencies of the left arm of the X chromosome in a population of *D. robusta* from Fall 1987 to 1988. Quadratic regressions of frequency on collection period are plotted for each gene arrangement: for XL, r^2 = 0.59, for XL-1, r^2 = 0.56, and for XL-2, r^2 = 0.94.

Two populations of *D. robusta* were surveyed for inversion polymorphisms in northwestern Arkansas. No published data of *D. robusta* inversion frequencies in Arkansas is currently available, and the closest populations within the same geographical area, the Ozark Plateau, studied by Stalker, Carson, and their students have not been recently resurveyed, In Fayetteville, a population inhabiting a woodlot near a residential area was sampled weekly for 13 months. A population from the Ozark National Forest, about 60 miles southeast of Fayetteville, was sampled 5 times over a two month interval. Flies from both populations were collected by sweep-netting over buckets of fermenting bananas. Females were separated into individual vials and supplied with a wild caught male. Immature adults were pairmated in the lab and 1 larva from each cross was karyotyped.

Table 2. Seasonal variation among gene arrangements in a population of Drosophila robusta. Collections started in the Fall of 1987 and continued until the Fall of 1988. The number of sex chromosomes, n^X, and autosomes, n^a, sampled are given for each collecting period. x^2 statistics from tests of homogeneity across collecting periods are shown.

Collecting Period	XL	XL-1	XL-2	XR	XR-1	XR-2	n^X	2L	2L-1	2L-2	2L-3	2R	2R-1	3R	3R-1	n^a
1987																
9/24-10/8	0.526	0.237	0.237	0.158	0.342	0.500	38	0.375	0.542	0.083	0.000	0.875	0.125	0.354	0.646	48
10/9-10/22	0.667	0.212	0.121	0.212	0.333	0.455	66	0.288	0.512	0.163	0.037	0.863	0.137	0.338	0.662	80
1988																
4/9-5/16	0.679	0.208	0.113	0.132	0.340	0.528	53	0.397	0.471	0.103	0.029	0.897	0.103	0.323	0.677	68
5/17-5/31	0.732	0.226	0.042	0.216	0.342	0.442	190	0.309	0.555	0.089	0.047	0.898	0.102	0.411	0.589	236
6/1-6/30	0.690	0.268	0.042	0.262	0.399	0.339	168	0.284	0.574	0.113	0.029	0.843	0.157	0.407	0.593	204
7/1-7/31	0.627	0.281	0.092	0.184	0.424	0.392	217	0.364	0.477	0.121	0.038	0.848	0.152	0.390	0.610	264
8/1-8/30	0.611	0.236	0.153	0.111	0.500	0.389	72	0.383	0.436	0.128	0.053	0.819	0.181	0.298	0.702	94
9/1-9/30	0.702	0.155	0.143	0.131	0.511	0.357	84	0.377	0.481	0.104	0.038	0.877	0.123	0.434	0.566	106
10/1-10/23	0.538	0.231	0.231	0.000	0.539	0.461	13	0.438	0.500	0.062	0.000	0.688	0.312	0.438	0.562	16
10/24-11/17	0.542	0.083	0.375	0.125	0.458	0.417	24	0.200	0.500	0.267	0.033	0.867	0.133	0.400	0.600	30
		$x^2 = 55.53^{****}$			$x^2 = 28.51^{+}$				$x^2 = 26.40^{ns}$				$x^2 = 10.12^{ns}$		$x^2 = 7.45^{ns}$	
						Total n^X =	925								Total n^a = 1146	

$^{+}$ - $0.1 < P < 0.05$, **** - $P < 0.0001$, ^{ns} - not significant

X chromosome gene arrangement frequencies differed among the two samples, but the autosomal frequencies were remarkably homogeneous (Table 1). Throughout one year, Fall 1987 through Fall 1988, only X chromosome gene arrangement frequencies changed, particularly those on the left arm (Table 2; Fig. 1). Gene arrangement XL-2 showed the strongest seasonal shift, decreasing in frequency during the summer. The frequency of XL-2 dropped in October, 1987, but increased throughout Fall 1988. The Fayetteville population and the closest Ozark population, Steelville, Missouri, studied by Carson (1958) shared all gene arrangements but the frequency differences were quite large.

Repeated observations of such seasonal patterns will be necessary before any firm conclusions concerning selectively caused variation can be made. Certainly, the summer of 1988 was an extreme year characterized by drought and above average temperatures. Consideration of linkage with gene arrangements on opposite chromosome arms will also be necessary because linkage disequilibrium can be quite strong in natural populations of *D. robusta* (Levitan, 1973).

References: Carson, H.L., 1958, Adv. Genet. 9:1-40; Carson, H.L. and H.D. Stalker, 1949, Evolution 3:322-329; Dobzhansky, Th., 1948, Genetics 33:158-176; Etges, W.J., 1984, Evolution 38:675-688; Levitan, M. 1973, Evolution 27:215-225.

Falk, Raphael and Shula Baker. Department of Genetics, The Hebrew University of Jerusalem.

Segregation of centric Y-autosome translocations in *Drosophila melanogaster*.

In an attempt to identify autosomal disjunction determinants in *Drosophila* males and females we screened for translocations between a doubly marked Y-chromosome (B^sYy^+) and the centric sections of chromosome 2 (Falk, R., S. Baker and A. Rahat, 1985a. *Genet. Res. 45*, 51-79. Falk, R., A. Rahat and S. Baker, 1985b. *Genet. Res. 45*, 81-93). Fifteen Experimental Stocks (ES's), each with a different half-translocation element (T-element) and three "identical" additional elements (A - a CyO, *pr* cn^2 autosome; F - a F(2L), *dp* or F(2R)VH2, *bw* free chromosome; X - a $Y^SX.Y^L$, In(1)EN,*y B* or a C(1)RM, y^2 *su*(w^a) w^a sex chromosome), were tested with four tester stocks for all eight possible segregation patterns. Segregation patterns in males were different from those in females. In males T-elements either carried or were devoid of sex-chromosome disjunction determinants. Disjunction of the T-A elements in males varied from nearly complete dependence to complete independence, indicating differences in autosomal disjunction determinants on the T-elements. In females no meaningful differences in segregation patterns were detected. All showed a preference for the three 2:2 segregation patterns.

In order to further elucidate the presence of disjunction determinants and their possible organization two additional series of 3KR X-ray induced T-elements were recovered. Disjunction patterns of the ES's established from these T-elements in males and females were determined (series II & III. Series I was that described by Falk et al. 1985a & b).

The screening method of Falk et al. 1985a was improved, so that more putative T-elements could be recovered as ES's. B^sYy^+; *dp b cn bw* irradiated males were mated to 0/C(1), y^2 *su*(w^a) w^a; CyO, *pr* cn^2 females. Cy F1 daughters

were mated to $0/Y^{S}X.Y^{L}$, In(1)EN,*y B*; C(2L)SH1/F(2R)VH2,*bw* males. The few progeny obtained were almost exclusively the putative carriers of the desired T-elements. These were crossed to recover ES's of the constitution $0/Y^{S}X.Y^{L}$,In(1)EN,*y B*/C(1),y^{2} *su*(w^{a}) w^{a}; CyO, *pr* cn^{2}; F(2R)VH2,*bw* or F(2L)*dp*; T-element, or in short: X A F T.

Series	f_1 females screened	Putative T(Y;2) events L	L&R	R	Total	%	ES's L	R	Sterile & complex
I*	3340	10	4	3	17**	.54	9	5	2
II	1426	1	10	12	23	1.61	7	9	10
III	1479	2	9	7	18	1.22	7	9	5
Total	6245	13	23	22	58	.91	23	23	17

*Reported by Falk et al. 1985a.
**One T-element from another experimental series, treated with 4KR.

ES's in series II & III were mated only to three tester stocks: *A*: *y*. *B*: *y*;C(2L)SH1/F(2R)VH2,*bw*. *C*: F(2L)*dp*/C(2R)RM,*cn*. Thus only six of the eight segregation patterns were studies (see Fig. 2 of Falk et al. 1985a). The frequencies of the remaining two patterns (VII & VIII) proved negligible in series I. The number of progeny was calibrated for missing types and lethality to corrected numbers of progeny per culture bottle. The disjunction coefficient was calculated for each pair of elements (X, A, F and T) as the *proportion* of the total corrected number of flies of the given ES in which the elements disjoined.

Segregation coefficients for males and females from all ES's are shown in the table on the next page.

In addition to the 3 ES's that changed arms as discussed in Falk et al. 1985a another ES (298-3L)* was detected. Five ES's were male-sterile. For 22 T-elements the breakpoints were determined on the mitotic map by the method of Gatti and Pimpinelli, 1983 (*Chromosoma 88*, 349-373 and personal communication).

A total of 27 stocks were examined in which a left T-element from one ES was combined with a right T-element of another ES (with no F-elements).

Distribution of cytological break-points in translocation events.

Experimental stock	Break-point in: Y-chrom.	2nd chrom.	Experimental stock	Break-point in: Y-chrom.	2nd chrom.
a. 2LY.y^{+}/2R.YB^{S}			c. 2L.YB^{S}/2RY.y^{+}		
4R	1	39 p.e.	750	10	44
256-23L&1R	9	37	3L	0	40 p.e.
277-11L&5R	14	39	11R	8-9	47
			17R	17	43
b. 2L.Yy^{+}/2RY.B^{S}			18L	6-9	47
442	26	37	298-1L&3R	1	46 p.e.
5L	24	47-48	319-1R	11	46
12L	22	48			
105L	24	40	d. 2LY.B^{S}/2R.Yy^{+}		
107L	24	46	101	24	38
240-4L&13R	26	46-47	101R	22	38
287-3L&1R	1,2,19-20	46 p.e.	261-1&8R	24	39
289-9L&6R	20	43			
346-5L	21	39			

p.e. = strong B position effect

Disjunction coefficients in males with combination of different left- and right-arm translocation elements

Stocks combined	X-TL	A-TL	X-TR	A-TR	TLTR	X-A	Total
277-11L/240-13R		sterile					
287-3L/8R		sterile					
256-23L/5R		sterile					
256-23L/240-13R		sterile					
287-3L/256-16R	-.62	.93	.60	-.49	.56	.69	39
240-4L/289-6R	-.24	.93	.83	-.34	.41	.31	120
4750/2R	-.13	.90	.89	-.12	.22	.17	1167
4750/261-8R	-.06	.81	.79	-.07	.26	.21	1889
240-4L/256-1R	.02	.93	.91	.02	.05	-.02	1884
4442/4R	.06	.99	.97	.05	-.04	-.06	2372
3L/319-1R	.09	.99	.96	.07	-.06	-.10	2122
4442/256-16R	.13	.95	.93	.12	-.07	-.10	1334
4442/240-13R	.19	.99	.96	.18	-.17	-.20	1696
4750/261-8R	.25	.90	.88	.24	-.14	-.16	2106
5L/256-16R	.35	.68	.61	.30	.02	-.10	1206
346-3L/4R	.39	.64	.62	.39	-.03	-.09	1366
240-4L/4R	.41	.99	.96	.39	-.38	-.41	1707
298-1L/261-1R	.44	.69	.65	.45	-.14	-.25	1560
289-9L/289-6R	.46	.87	.86	.48	-.35	-.33	138
256-23L/256-1R	.50	.05	.13	.59	.36	.28	32
4442/9R	.60	.98	.96	.60	-.58	-.60	2060
256-23L/4R	.66	.53	.40	.54	-.07	-.23	1786
277-11L/4R	.68	.38	.20	.52	.10	-.10	1815
277-11L/277-5R	.74	.36	.24	.63	.01	-.12	2245
277-11L/256-1R	.78	.81	.56	.66	-.47	-.59	78
256-23L/277-5R	.80	.03	-.07	.70	.26	.14	1036
287-3L/287-1R	.85	.32	.21	.81	-.13	-.19	2248

Segregation coefficients for males (left) and females (right) from all ES's.

Stock #	X-T	A-T	F-T	A-F	X-F	X-A	Total	X-T	A-T	F-T	A-F	X- F	X-A	Total
Control (no T-element, two F-elements)														
1017	.03	.78	- .53	.74	.00	.03	2588	.28	.15	.45	.39	.04	.53	800
Left Arm T-elements														
442	.01	.67	-.46	.79	-.03	.03	984	-.15	.54	.66	-.19	.44	.60	677
240-4L	.01	.80	-.10	.31	.07	.02	2272	.21	.16	.68	.16	.04	.60	881
3L	.05	.62	-.10	.47	-.02	.04	668	.11	.36	.49	.15	.29	.51	1278
289-9L	.81	.67	-.39	.72	.51	-.47	1622	.12	.32	.61	.06	.17	.54	872
312	.83	.56	-.33	.77	.35	-.41	1722	.21	.31	.54	.16	.15	.41	367
298-1L	.83	.39	-.22	.83	.25	-.23	1907	.12	.42	.50	.08	.30	.43	1184
18L	.85	.43	-.27	.84	.26	-.29	2676	.36	.26	.40	.34	.17	.38	688
287-3L	.87	.18	.03	.79	.05	-.07	1910	.46	.14	.42	.44	.05	.39	858
148	.87	.10	.09	.80	-.01	.01	2136	.37	.11	.58	.32	-.04	.49	422
13L	.88	.45	-.36	.91	.36	-.34	1865	.54	.60	-.21	.61	.44	-.14	140
105L	.88	.27	-.10	.84	.16	-.15	2520	.31	.19	.43	.38	.16	.31	784
5L	.90	.37	-.24	.88	.26	-.27	2514	.06	.48	.48	.05	.36	.45	630
890	.90	.52	-.42	.89	.42	-.45	3351	.38	.11	.50	.38	.01	.48	620
12L	.90	.27	-.17	.91	.17	-.17	1781	.48	.24	.30	.46	.11	.25	744
112	.91	.47	-.35	.88	.36	-.37	2431	.66	.03	.33	.64	-.03	.29	392
170	.91	.48	-.42	.94	.43	-.41	1945	.31	.33	.37	.30	.28	.33	652
750	.91	.62	-.54	.91	.52	-.54	1738	.38	.45	.19	.36	.34	.16	374
110	.91	.51	-.42	.90	.41	-.44	1530	.36	.40	.28	.33	.23	.24	579
107L	.92	.41	-.33	.92	.33	-.33	1931	.03	.37	.69	-.06	.19	.57	974
346-5L	.92	.33	-.22	.88	.24	-.26	2908	.22	.37	.46	.18	.25	.38	985
980	.95	.21	-.15	.94	.16	-.17	1975	.43	.15	.44	.42	.02	.42	624
277-11L	.96	.19	-.11	.92	.12	-.16	1436	.44	.16	.68	.17	-.14	.38	857
256-23L	.98	.23	-.19	.95	.19	-.22	2246	.44	.29	.46	.25	.07	.26	936
Right-Arm T-elements														
726(L*)	.00	.76	- .34	.58	.03	.03	1925	.30	.08	.55	.36	.02	.52	417
126(L*)	.01	.86	-.53	.67	.04	.01	1982	.37	-.02	.65	.37	-.12	.59	618
311(L*)	.02	.67	- .25	.58	.01	.04	1627	.15	.20	.67	.13	.12	.63	682
298-3L*	.06	.42	- .29	.87	.02	.01	1690	.20	.58	.12	.29	.50	.14	756
5R			sterile					.08	.44	.25	.30	.57	.10	926
8R			sterile					.39	.24	.48	.28	.06	.34	688
12R			sterile					.47	.48	.12	.39	.32	.04	1353
16R			sterile					.57	-.15	.73	.42	-.30	.60	542
289-6R			sterile					.87	-.22	.38	.84	-.30	.34	878
287-1R	.35	.54	-.34	.81	.20	-.07	2284	-.22	.45	.82	-.27	.36	.74	708
277-5R	.87	.75	-.62	.86	.69	-.64	677	.41	-.02	.65	.37	-.12	.58	1087
101	.92	.51	-.37	.85	.42	- .44	1644	.12	.59	.30	.11	.47	.27	1294
17R	.93	.31	-.26	.95	.28	- .25	1700	.19	.63	.33	.03	.39	.17	682
256-1R+	.92	.39	-.31	.92	.37	- .34	1133	.15	.22	.69	.08	.10	.56	856
256-16R+	.96	.41	-.38	.97	.40	- .40	1294	.42	.15	.42	.43.	.08	.38	690
319-1R	.95	.13	-.12	.99	.17	- .17	1752	.47	.41	.21	.38	.25	.10	694
9R	.96	.81	-.79	.98	.77	- .77	1850	.22	.56	.21	.23	.43	.18	451
4R	.96	-.06	.08	.98	-.05	.05	1847	.18	.27	.47	.27	.20	.43	597
11R	.96	.20	-.16	.95	.18	-.18	1102	.60	.18	.27	.56	.09	.22	853
101R	.97	.37	-.34	.97	.34	-.34	2590	.12	.59	.30	.11	.47	.27	1294
261-1R+	.99	.54	-.52	.99	.52	-.53	2250	.29	.24	.52	.25	.12	.45	640
261-8R+	.96	.54	-.52	.98	.55	-.56	1652	.31	.28	.40	.32	.19	.36	779
240-13R	.98	.47	-.46	.99	.47	-.47	226	.57	.19	.44	.37	-.07	.23	1060
880	.99	.85	-.84	1.00	.84	-.84	2145	.01	.39	.65	-.04	.28	.57	754
2R	1.00	.26	-.26	1.00	.26	-.26	2163	.57	.00	.52	.48	-.15	.41	824

* ESs in which the right arm T-element turned left arm T-element (see Falk et al. 1985a).
\+ Two independent extractions from same putative T-elements.

In some ES's the CyO chromosome was exchanged for a non-inverted chromosome 2 with a dominant marker (*Bl, L,* or Pin^{Bx}) and the disjunction pattern in males was examined. This did not change the disjunction pattern significantly.

The results confirm that there is a different disjunction pattern for given T-elements in males and in females. In males there was a clear dichotomy with respect to X-T disjunction. 32 ES's carried sex-chromosome determinants. The combined cytological and genetic analysis indicated that the $B^{s}Yy+$ carried three regions of collochore activity, at both marked ends, distinct from the markers (of X-chromosome origin) and at section 20, to the left of the centromere. Breaks in the immediate vicinity of B^{s} at the tip of the long arm of the Y-chromosome induced strong *B*-position effects. The independent disjunction of the T- and F-elements in males indicated that they have different disjunction determinants. Although there were clear differences in the strength of A-T disjunction coefficients in males, these could

not be meaningfully correlated to the sites of the break-points in the proximal sections of chromosome 2. Disjunction patterns in females revealed again predominance of the three 2:2 segregation patterns, with no clear specificity for the various T-elements involved. The segregation patterns changed, however, significantly when two T-elements of different origin were combined (instead of one T- and one F-element). It appears that there is a strong competitive interaction between the T-elements, mainly for X-T disjunction, the "looser" seems to gain advantage in A-T disjunction.

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Salivary gland cells of *Drosophila* may serve as facultative mycetocytes.

Mycetocytes, or cells containing non-pathogenic microorganisms, are found in specific parts of the body in a number of insects. These intracellular microorganisms, generally bacteria or yeasts, in most cases act as symbionts that supply the insect with useful molecules (Wigglesworth, 1972). Usually, mycetocytes are situated in the midgut, Malphighian tubules, or in the body cavity, within the gonads and fat body (Milburn 1966; Chapman 1985; Dean et al. 1985). However, mycetocytes have never been described from the salivary glands of *Drosophila* larvae.

Materials and methods. Wild type (Oregon R) *Drosophila melanogaster* larvae of 3rd instar were used for all observations. Exact age of investigated larvae corresponded to individual puff stages (PS-1 to PS-15) according to puffing patterns on polytene chromosomes (Ashburner 1972; Ashburner and Berendes 1978). Dissected salivary glands were fixed in glutaraldehyde, postfixed in OsO_4 dehydrated in ethanol and embedded in Durcupan epoxy resin (Fluka AG, Buchs, Switzerland). Ultrathin section stained with uranyl acetate and lead citrate were investigated under Telsa BS 500 and Jeol 1200 EX electron microscopes.

Results and discussion. During the investigation of transversal as well as longtudinal sections of salivary glands we have found that in a single gland a few cells can contain living yeasts or yeast-like microorganisms. They are randomly scattered through the cytoplasm (Figure 1). Frequently they are seen in intimate association with spindle granules (Figure 1) or in their vicinity (Figure 2). Some yeasts are clustered into membrane-lined vacuoles, and thus separated from the surrounding cytoplasm (Figure 3). These clusters regularly contained round-shaped small yeast cells, while individual yeasts, in most cases, were seen as large kidney-shaped microorganisms. Some of the small yeasts placed in the clusters had an undifferentiated nucleus. This indicated the ability of the yeasts to undergo maturation in the salivary glands. The occurrence of the yeasts is independent on the developmental stage of the salivary glands. Yeasts were found in interecdysial salivary glands producing secretory granules [PS-1] (Figure 1) as well as in salivary glands after secretion of glycoprotein glue is completed and glands start undergo the histolysis [PS-15] (see Figure 3).

Besides agar, cornmeal and sucrose, the yeasts are a major source of food for developing *Drosophila* larvae (Ashburner and Thompson 1978; Ransom 1982). During the larval feeding the yeasts are taken in and digested by intestinal tract. However, a few yeasts may also move into the lumen of salivary gland which is part of the digestive system. If yeasts are not released back into the culture medium or intestinal tract, they can be carried passively by the fluid of the lumen to a vicinity of any salivary gland cell. It was shown by Schomann (1937) that in some insects the future mycetocytes (cells which are still without microorganisms) of the gut epithelium extrude protoplasmic processes into the lumen and engulf symbiotic yeasts which the insect have just consumed. Salivary gland cells of larval *Drosophila* extrude many cytoplasmic microvilli into the secretory lumen (von Gaudecker 1972; Lane et al. 1972; Berendes and Ashburner 1978), and thus they can engulf yeasts passively carried in the fluid.

In the textbooks of insect pathology much importance is placed on the inability of symbiotic microorganisms to invade any of the host cells except for the mycetocytes or special regions of midgut epithelium (Brooks 1963). This is interpreted as being a case of the regulation of symbiotes by host cells. On the other hand, under experimental conditions, this regulation ability is lost and the microorganisms may invade other tissues, behaving like parasites during infection. In the case of the yeasts in salivary glands of *Drosophila* the parasitic infection is clearly ruled out. Results indicate the ability of salivary glands to recognize yeasts as symbiotes which are not pathogenic for them. Moreover, intracellular survival and differentiation of symbiotic microorganism are strongly dependent on the mycetocytes (Koch 1960). These facts suggest that salivary gland cells have behaved like mycetocytes. Furthermore, salivary gland cells bear a striking resemblance to truely mycetocytes not only in their histological appearance but also in their metameric arrangment, cyclic growth, high degree of polyploidy (polyteny), and evident involvement in intermediary metabolism (Koch 1960).

The cytoplasm of typical mycetocyte is reduced in extent because symbiotic microorganisms are too abundant (Whitcomb et al. 1974). In addition, many large vacuoles with symbiotes are often present within the mycetocyte,

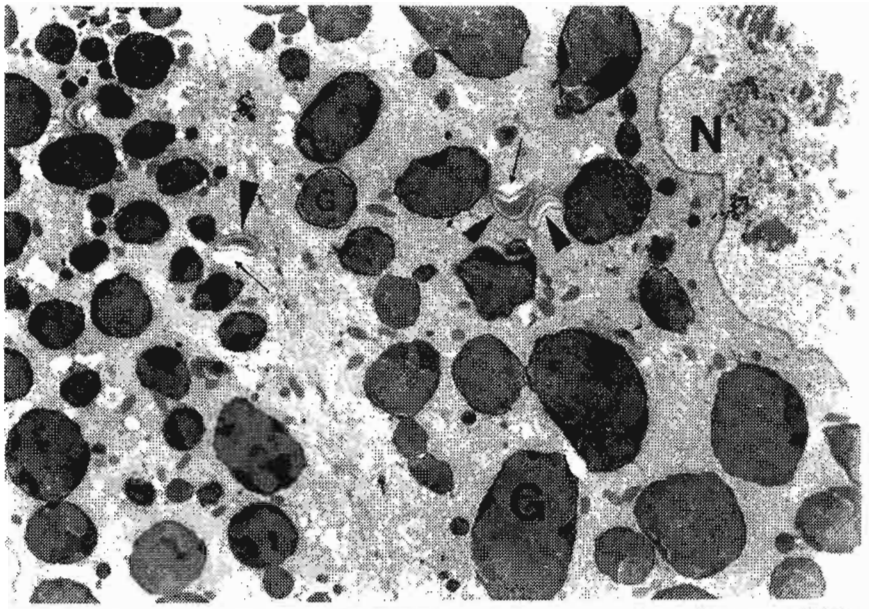

Figure 1.

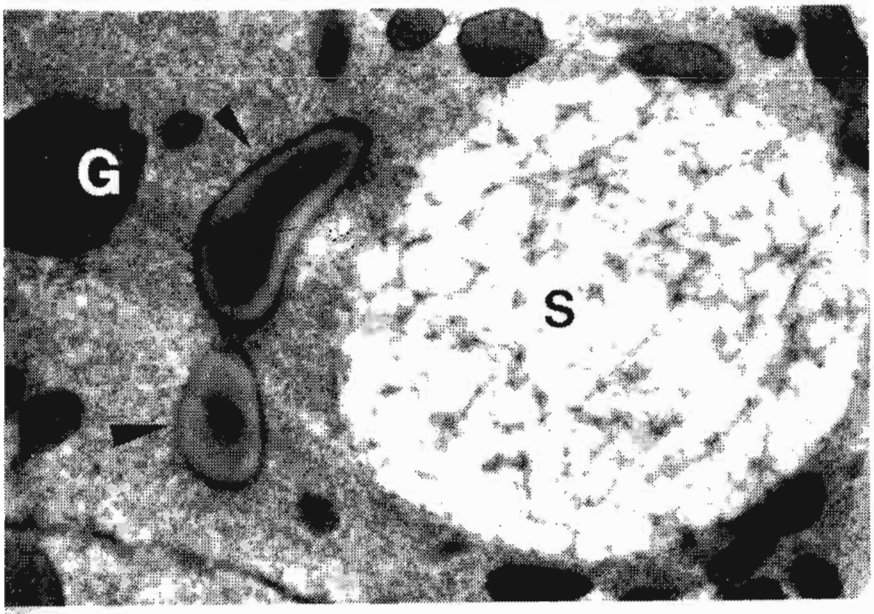

Figure 2.

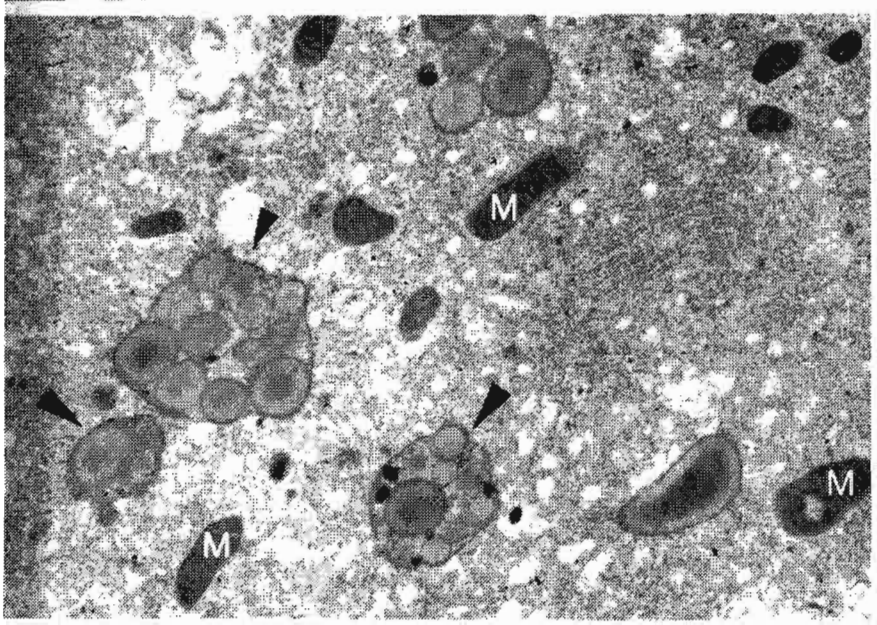

Figure 3.

containing a variety of less readily identified objects, which may include other types of microorganisms, expecially bacteria, and/or degenerating symbiotes (Grinyer and Musgrave 1966). Since we did not observe any large bacteria-containing vacuoles in this study, we propose that salivary gland cells of *Drosophila* may serve only as facultative mycetocytes.

It is known, that mycetocyte or the ability of the cell to be a mycetocyte is formed before it receives its complment of symbiotes (Brooks 1963). Any treatment which prevents symbiotes from getting into the such a cell leaves the "sterile mycetocytes" or anlage of "sterile mycetocytes". Because of the importance of symbiotes for intermediary metabolism, this situation can result in an arrested stage of insect development. Among the complicated varieties of symbioses found in insects, Buchner (1957) reported one in which the symbiotes appear to have been lost in phylogeny but the empty (=sterile) mycetocytes still remain. Although, from our research we cannot conclude that salivary gland cells represent sterile mycetocytes, the results reported here may facilitate new ways in the research of phylogenic and ontogenic development, as well as understanding the physiology of *Drosophila* salivary glands.

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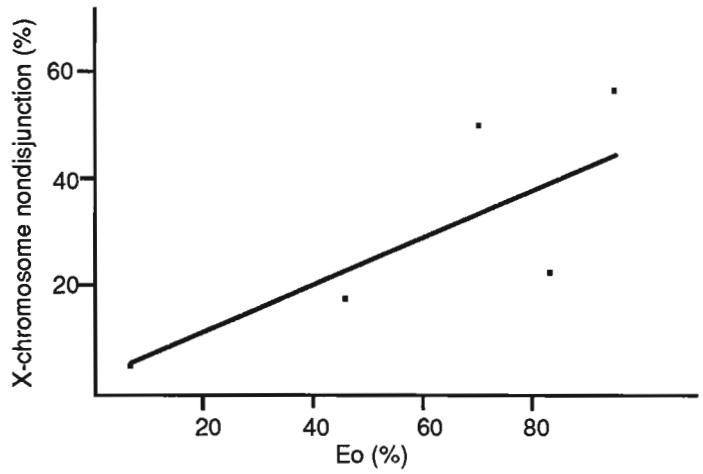
Nonexchange chromosomes produced by inversion heterozygosity and by meiotic mutants are both recognized by the distributive mutant, *nod*.



The distributive system, an alternate segregation pathway in *Drosophila melanogaster*, provides for the regular disjunction of nonexchange chromosomes. One would predict that the phenotypes of mutants in the distributive system would be exacerbated by increasing the number of nonexchange chromosomes entering the distributive pool. This prediction has recently been confirmed for two such mutants, *nod* (Zhang and Hawley, in preparation) and *Axs* (Zitron and Hawley, 1989). For both mutants X-chromosome nondisjunction rises dramatically with increasing Eo's (chromosomes that have not undergone exchange). Data available for *Axs* indicate that the two parameters are related in a linear fashion. In experiments characterizing both mutations, various numbers of Eo

Figure 1. Graph of data presented in Table 1 showing that nondisjunction rises as Eo tetrads increase, for both inversion heterozygosity and meiotic mutants.

tetrads were generated by constructing X chromosomes heterozygous for a variety of X-chromosome inversions. To test whether the alterations in nondisjunction seen resulted from increasing the Eo frequencies, or were due to an unrecognized effect of inversion heterozygosity, perhaps analogous to the interchromosomal effect, we used a different method of varying Eo frequency. Double mutant stocks were constructed using the well characterized meiotic mutants *mei-282* and *mei-219*, and *nod*^{a}. X-chromosome and fourth chromosome nondisjunction were then assayed in double mutant females. In addition, data are available for *mei-9, nod*^{a} double mutants (Baker and Carpenter, 1972). The data in Table 1 and Figure 1 show that *nod*-induced X-chromosome nondisjunctions rise in the presence of meiotic mutants, and that the increase seen is proportional to the frequency of Eo's that the particular meiotic mutant generates. We therefore conclude that *nod* females recognize non-exchange chromosomes derived from meiotic mutants, although our data do not allow us to determine if the two types of nonexchange chromosomes are treated in precisely the same qualitative or quantitative manner.

Table 1. Correlation of Eo frequency and non-disjunction in *nod* and *nod*, meiotic mutant females.

Genotype	X non-disjunction	4 non-disjunction	Eo frequency	Total Progeny	Source
nod^{a} / *nod*^{a}	.03	.86	.05	6778	Carpenter, 1973
nod^{a}; *mei-282* / *nod*^{a} *mei-282*	.19	.78	.46	425	this study
mei-9^{b},*nod*^{a} / *mei-9*^{b},*nod*^{a}	.25	.69	.83	4562	Bakerand Carpenter, 1972
FM7a,nod^{b27} / *nod*^{a}	.53	.83	.71	1828	Zhang and Hawley,in preparation
nod^{a},*mei-218* / *nod*^{a},*mei-218*	.57	.69	.95	125	this study

References: Baker, B. and A.T.C. Carpenter, 1972, Genetics 71:255-286; Carpenter, A.T.C., 1973, Genetics 73:393-428; Zitron, A. and R.S. Hawley, 1989, Genetics 122:801-821.

Fukui, H.H., University of Minnesota, Minneapolis, MN, USA. Selection for return of female receptivity produced a correlated response in pupation height.

Lines selected for fast and slow return of female receptivity from a recently-collected wild type *Drosophila* fast and slow *melanogaster* population (COMP stock, described in Gromko and Newport, 1988) were subjected to a chromosome substitution analysis. By following the appropriate mating scheme (Pyle, 1978; Fig. 1), every possible homozygous chromosomal combination of chromosomes I, II, and III from the fast and slow lines was produced (Fukui and Gromko, 1991). In the course of the experiment, variation in pupation height was observed among different combinations of the chromosomes. Thus, the following experiment was carried out: the flies (15 males and 15 females) from the each chromosome substitution stock were allowed to mate and lay eggs for four days in a cage (10.5 cm X 12.5 cm X 5.5 cm) with four food cups. On the fourth day, all the food cups were separated from the cage, and the flies in the cup discarded. Then, the level of food surface was marked on the cups, and a plexiglas tube (2.8cm in diameter;

Table 1. Mean (±S.E.) of pupation height. Chromosome combination of each substitution stock (represented by a letter) is described in Fig. 1. Four stocks with fast chromosome II's are the first 4 letters (A, B, E, and F), the stocks with slow chromosome II's are the last 4 letters (C, D, G, and H).

Chromosome Substitution Stock	A	B	E	F	C	D	G	H
Pupation Height (cm)	2.2 (± 0.1)	1.5 (± 0.1)	2.8 (± 0.2)	2.6 (± 0.1)	1.6 (± 0.1)	1.6 (± 0.1)	1.4 (± 0.1)	1.2 (± 0.1)

Table 2. Results of three-way ANOVA on chromosome effects on correlated responses to selection for fast and slow remated day in pupation height. Only levels of significance are shown. Non-significant interactions are omitted.

N	Chrom. I	Chrom. II	Chrom. III	Interaction	
960	0.0003	0.0001	0.002	C1 x C2	0.0001
				C1 x C2 x C3	0.02

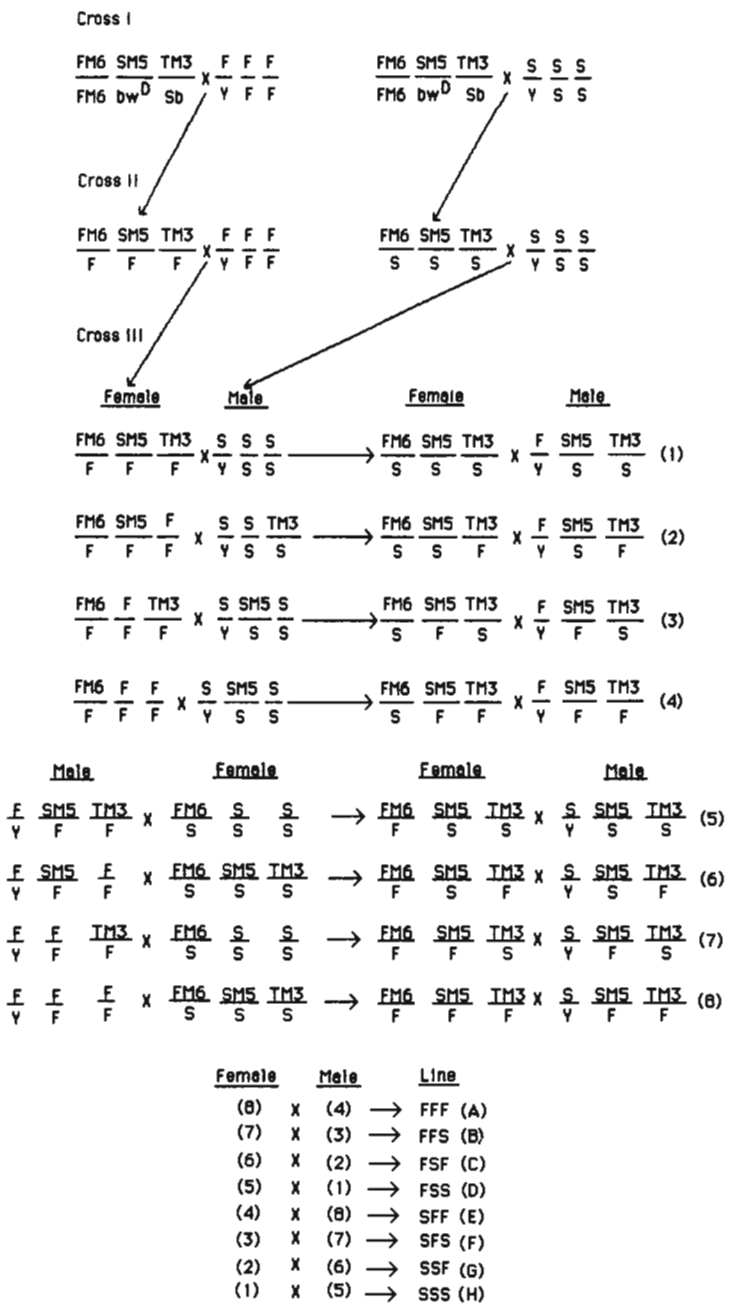


Figure 1. Mating scheme utilized to produce the eight chromosome substitution lines, consisting of every possible homozygous chromosomal combination of chromosomes I, II, and III from the fast (F) and slow (S) selection lines.

9.5cm in length) was taped on the mouth of the cup. Pupation height of the first 30 pupae in each cup were measured to the nearest 0.1 cm. Chromosomal effects were determined by a three-way ANOVA (Sokal and Rohlf, 1981, p. 374). The main effects of each of the three chromosomes had 2 levels (fast and slow). The Statistical Analysis System packages (SAS Institute, Inc., 1985) were employed for this analysis. The flies were reared and tested simultaneously at 23-25°C and on 12:12 light:dark cycle.

Mean pupation height with S.E. is summarized in Table 1. All three chromosomes were found to have highly significant effects on this attribute with significant two-way and three-way interchromosomal interaction terms (Table 2). Variation among the food cups nested within line was also significant ($F_{[24,928]}=3.55$, $P=0.001$) suggesting the presence of environmental variation. However, chromosome II contributed the largest effects on this attribute; it accounted for 35.0 percent of the variation observed. Chromosomes I and III accounted for 5 and 4 percent, respectively.

The choice of a suitable pupation site by third-instar larvae has been shown to be an important fitness trait in *D. melanogaster*. Pupae remain immobile for several days and are subjected to variation in environmental conditions associated with height of the pupation site, which may affect survival (Casares and Carracedo, 1987). Bauer and Sokolowski (1985) showed that differences in pupation height were affected by a maternal effect and by chromosomes II and III (chromosome I was not tested) in a stock newly derived from nature. The effect of chromosome II was about three times stronger than that of chromosome III. The influence of chromosome II on this behavior was demonstrated by Wong et al. (1985) in a laboratory strain. Wong et al. (1985) also showed correlations between larval foraging strategies and pupation height. Selection for pupation height has been found to produce correlated changes in adult and larval behavior traits. Sokolowski and Hansel (1983) showed that a larval foraging behavior ("rover" and "sitter") was positively correlated with pupation height. The rover/sitter variation is also correlated with adult search tactics (Nagel and Bell, 1987). Markow (1979) demonstrated that adult geotaxis behavior might utilize genetic variation affecting behavioral processes common to pupation height selection. The current data support the previous studies and additionally show a possible involvement of chromosome I. Furthermore, it was demonstrated that selection for adult mating behavior induced correlated responses in an important larval fitness trait.

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Gallagher, P.D. and T.A. Markow. Arizona State University, USA. Phototactic behavior and light-dependent mating in *Drosophila* species.

A good deal of variability exists among *Drosophila* species in phototactic behavior as measured in Hirsch-Hadler classification mazes (Dobzhansky and Spassky 1976; Markow and Smith 1977; 1979). This variability is of interest in light of reports by Grossfield (1966) that some *Drosophila* species exhibit light dependence in their mating behavior while others do not. Grossfield (1971) divided *Drosophila* species into three groups on the basis of their light dependence: Group I - Light-independent; Group II - Facultative Dark Mating; and Group III - Dark-repressed. We asked if there is any relationship between the degree of photopositive or photonegeative behavior in a classification maze and the degree of light dependence in mating behavior in *Drosophila* species.

Table 1. Pooled phototaxis scores or Drosophila species

		Females			Males		
Group	Species	$\bar{x}$ ± SE		(n)	$\bar{x}$ ± SE		(n)
I	D. immigrans (F-1)	8.63 ± 0.16		(600)			
I	D. immigrans (F-3)	9.76 ± 0.17		(600)			
I	D. immigrans (F-7)	8.59 ± 0.22		(600)			
I	D. immigrans (F-10)	9.88 ± 0.21		(600)			
I	D. immigrans (F-18)	9.91 ± 0.15		(600)			
I	D. pseudoobscura (Kofa)	8.18 ± 0.09		(1020)	8.60 ± 0.11		(897)
I	D. pseudoobscura (Tempe)	7.91 ± 0.10		(832)	8.76 ± 0.13		(634)
I	D. hydei (Tempe)	8.98 ± 0.13		(684)	6.09 ± 0.13		(647)
I	D. virilis (1051)	10.99 ± 0.08		(855)	11.52 ± 0.08		(947)
I	D. melanogaster (Tempe)	7.21 ± 0.09		(1214)	6.54 ± 0.10		(1105)
I	D. melanogaster (CS)	6.88 ± 0.08		(947)	5.99 ± 0.09		(1064)
II	D. robusta (111.2)	4.23 ± 0.16		(436)	3.88 ± 0.25		(230)
II	D. simulans (Tempe)	9.59 ± 0.12		(688)	9.86 ± 0.12		(579)
II	D. persimilis (0111.1)	6.69 ± 0.10		(518)	7.91 ± 0.01		(606)
II	D. prosaltans (0901)	10.10 ± 0.10		(794)	12.03 ± 0.09		(633)
II	D. mulleri (1371)	8.60 ± 0.13		(487)	7.20 ± 0.15		(293)
III	D. auraria (0471.1)	11.41 ± 0.10		(799)	11.52 ± 0.14		(488)
III	D. gibberosa (1181)	14.22 ± 0.07		(674)	14.43 ± 0.07		(556)
III	D. pavani (1241)	10.96 ± 0.09		(939)	11.06 ± 0.11		(761)
III	D. melanica (1141)	5.52 ± 0.14		(361)	6.73 ± 0.38		(101)

Flies used in this study were either collected recently by the authors (*D. pseudoobscura* - Kofa Mountains, Arizona; *D. pseudoobscura* - Tempe, Arizona; *D. hydei* - Tempe, Arizona; *D. melanogaster* - Tempe, Arizona; and *D. simulans* - Tempe, Arizona) or obtained from the *Drosophila* species stock center at Bowling Green State University (all others, stock numbers appear after species names in Table 1). Five species were tested for Group I, five for Group II and four for Group III. For several species more than one strain was tested. For *D. melanogaster*, the recently captured Tempe strain as well as the Canton-S (CS) laboratory strains were used. For *D. pseudoobscura* two strains were used and for *D. immigrans* five strains (females only) were tested. Testing several strains of one species was carried out to get an idea of the degree of intraspecific variability for photomaze behavior.

Flies were reared on standard cornmeal medium at 24°C, the sexes were separated under light ether anesthesia and stored until 4 days of age. At that time, males and females were tested separately in phototaxis mazes (Markow 1975). At least three replicates of approximately 200 flies were tested on the maze for each strain.

The design of the maze is reported upon extensively elsewhere (Markow 1975). Flies entering the maze are confronted with 15 light dark choices and emerge in one of 16 numbered collecting tubes at the end. Those flies having made only dark choices emerge in tube number one while individuals making only light choices appear in tube sixteen. The number of flies in the collecting tubes are counted and a mean phototaxis score is computed. Photoneutrality is represented by a score of 8.5 while a score of 1.0 is photonegative and 16.0 is photopositive.

Phototactic scores for replicates of a given strain were not significantly different from each other and are therefore presented in Table 1 as pooled values. Males of *D. immigrans* were not tested. Some variability was detected among the five strains of *D. immigrans* and the two strains of *D. melanogaster* and *D. pseudoobscura*. The photoscores of Group I and Group II flies are similar to each other in that they tend to be rather photoneutral with the exception of *D. robusta*. However, with the exception of *D. melanica*, the species in Group III are consistently more photopositive than the flies from the other groups. Several species, such as *D. melanica*, *D. robusta*, and *D. mulleri*, exhibited a tremendous mortality during the 24 hour maze trials.

It would make sense for flies which require light for courtship and mating to exhibit a preference for light habitats while no preference might be expected among species which are light independent. The photoscores of several species, in particular *D. robusta* and *D. melanica*, appear to pose exceptions to this expectation. However, Markow (1979) showed that under conditions of elevated temperatures and dryness, flies of a number of species become photonegative. *D. robusta* and *D. melanica* both displayed increased mortality during the maze testing, suggesting that these two species may be near their temperature or humidity tolerance thresholds and that perhaps the survivors were adjusting their phototaxis behavior to avoid light and potentially desiccating places. Our data suggest that, given these other environmental factors, the classification proposed by Grossfield (1972) for mating behavior may be related to the more broad ecological picture of species diversity as detected by basic habitat preference behaviors.

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Georgieva, S.G. and T.I. Gerasimova. N.K. Koltsov Institute of Developmental Biology and N.I. Vavilov Institute of General Genetics, Moscow, USSR. Deletion mutations and their reversions at the *white* locus in transposition bursts.

The *white* locus appeared to be a hot spot for unstable mutations which occurred among closely related strains originating from the ct^{MR2} strain. The characteristic feature of these strains is the existence of transposition bursts, i.e. simultaneous multiple transpositions of mobile elements belonging to different classes (Gerasimova, et al., 1984, 1985). We performed a molecular analysis of several *white* mutations and reversions. The pedigree of the strains analyzed is presented in Fig. 1a. The genomic DNA libraries were prepared from Oregon R stock (wild type), the w^{MR19} strain and its revertant $w^{+}sn^{L2}$. The clones containing the *white* locus were selected by hybridization with a 1.5kb SalGI fragment from the *white* locus which had been kindly provided to us by Dr. Rubin. The restriction map of the *white* locus from Oregon R was identical to that described by Levis, et al. (1982). The w^{MR19} strain contained an insertion of mobile element B104(roo) from 1.0 to 2.5kb downstream from the 3' end of the *white* locus. However it remained unclear whether it influences the *white* gene expression (Fig. 2). Besides that, the PvuII-SacI segment of the *white* locus 700 bp long was found to be decreased in size up to 250 bp. This segment consists mostly of coding sequences. The restriction map of some region in the revertant $w^{+}sn^{L2}$ was indistinguishable from that of the wild type. Thus, the reversion was associated with the reconstitution of the B104 element. Thereafter we analyzed several strains of the ct^{MR2} family using different fragments of the *white* locus as probes. In five of the ten mutants analyzed, the

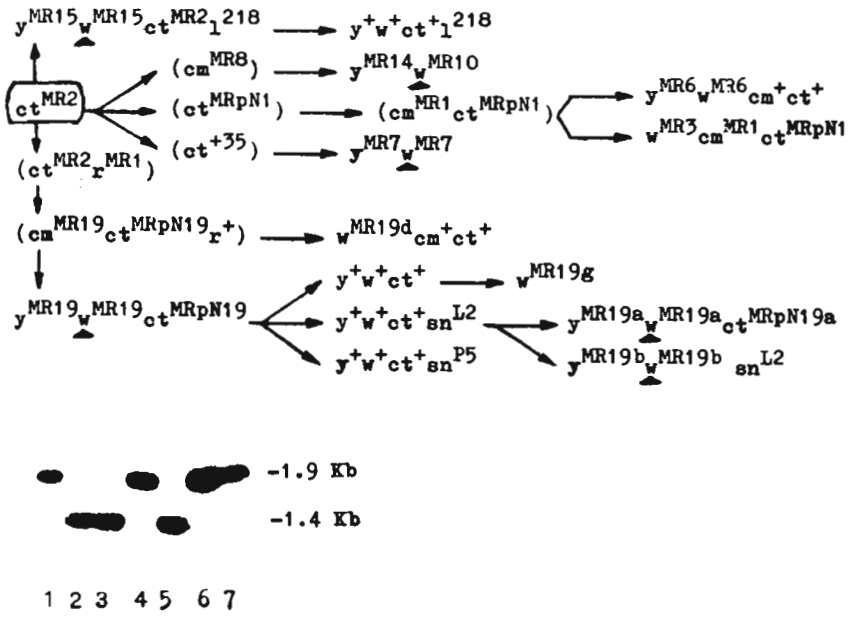

Fig. 1a. The pedigree of analysed strains. The *white* mutations carrying the deletion in PvuII-SacI fragment are underlined by black triangles.
1b. Hybridisation of 1.9kb SalGI-BamHI fragment, containing the PvuII-SalI fragment with genomic DNA of analyzed stocks digested with BamHI and SalGI.
1,4,6,7 - DNA from stocks with normal BamHI-SalGI fragment; 2,3,5 - DNA from stocks with BamHI-SalGI fragment of decreased size.
1 - ct^{+35}; 2 - $y^{MR19a}w^{MR19a}ct^{MRpN19a}$; 3 - $y^{MR14}w^{MR10}$; 4 - $cm^{MR19}ct^{MRpN19}$; 5 - $y^{MR19b}w^{MR19b}sn^{L2}$; 6 - $y^{+}w^{+}ct^{+}sn^{L2}$; 7 - $w^{MR19d}cm^{+}ct^{+}$.

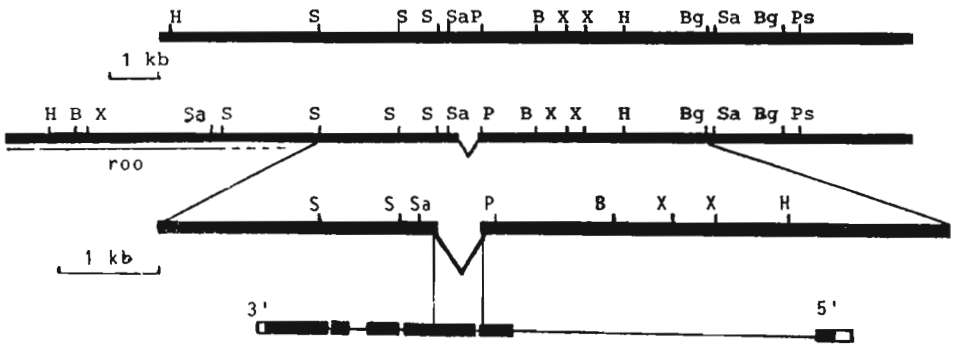

Fig. 2. The restriction map of the *white* locus:
a. from the strains of Oregon R and $y^+w^+ct^+sn^L$;
b. from the strain $y^{MR19}w^{MR19}$ ct^{MRpN19}. Below is a transcriptional map of the *white* locus.

PvuII-SacI fragment was shorter than the normal one and similar in size to that in the w^{MR19} allele (Fig. 1b). These mutants also contained B104 downstream to the *white* locus. Three reversions obtained from these mutations contained unchanged *white* locus. To detect more precisely the deletion borders we sequenced the PvuII-SacI fragment from w^{MR19}. The deletion was found to be located between +86 and -378 bp of the O'Hare, et al. (1984) map, its length being equal to 462 bp. The borders are located in the second and third exons of the locus *white* and represented by the following sequences . . . AGGCGGACTATTCTTCTGCAACG/AGCGACACATACCGGCGCCC - (422 bp) . . AGCAAATGTCAGCACACGAT/CATCGGTGTGCCCGGCAGGG . . . No prominent direct or inverted repeats can be detected by making the involvement of the homologous recombination in deletion. Obviously, the reversion of deletion could happen if somewhere the mutated genome contains the deleted sequence. Possibly it was transferred either to autosomes or to extrachromosomal DNA. It should be diluted in the course of obtaining the homozygous strains and this fact might prevent its detection on Southern blots. However, it should be present in some individuals of a population and serve as a factor inducing w^+-revertants by means of the gene conversion. The contamination in our strains was excluded by several tests. For example, in several cases of mutation changes at the *white* locus, some other recessive markers have been conserved (Fig. 1). The results of the present work indicate that the transposition bursts compose a wide set of different events, including not only the transpositions of mobile genetic elements, but also the processes of genetic recombination and gene conversion.

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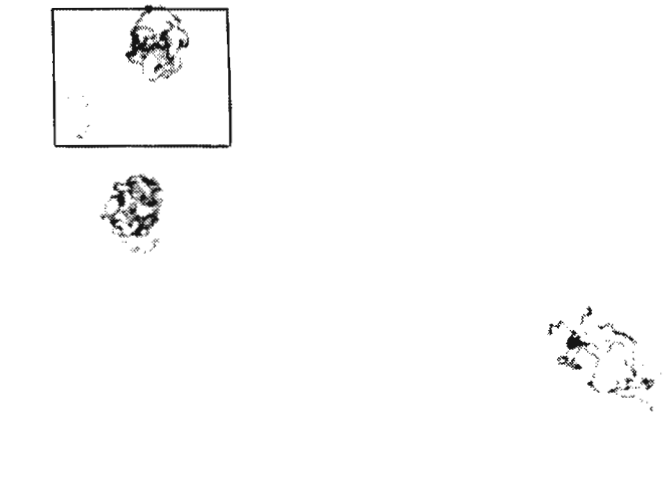
Ghosh, M. and A.S. Mukherjee. University of Calcutta, India. Induction of hyper-hyperactivity in Oregon R^+ male X chromosome by haemolymph proteins from In(1)BM^2 [rv] male.

Earlier it has been reported that In(1)BM^2[rv] male, a modulator mutant of *Drosophila melanogaster*, exhibits a mosaic pattern of X chromosomal morphotypes viz., Flabby (F), Intermediate (I), and Normal (N) (Ghosh and Mukherjee, 1986; Mukherjee and Ghosh, 1986). When larval salivary glands from In(1)BM^2 [rv] male are cultured (in vitro), they maintained the mosaic pattern of the X chromosome (Ghosh and Mukherjee, 1987). Interestingly when Oregon R^+ male salivary glands are cultured (in vitro) in the presence of whole gland extract from In(1)BM^2[rv] male, induction of In(1)BM^2[rv] male like mosaic X's takes place in high frequency (Ghosh and Mukherjee, 1987).

In the present investigation, ten In(1)BM^2 [rv] male third instar larvae were sacrificed and 20 ul samples of haemolymph were collected. Phenylthiourea was added to prevent darkening of the haemolymph. Haemolymph was collected with the help of a microsyringe to avoid contamination of fat body and other unwanted abdominal material, 0.001 M PMSF was then added, and centrifuged at 2000 rpm for 10 minutes. The pellet was discarded and the clean supernatant containing haemolymph proteins was collected. The 20 ul drop of haemolymph proteins was then placed in a grooved slide and one contralateral gland from a pair was incubated in the haemolymph protein and the other gland was incubated in *Drosophila* Ringers to serve as the control for the same period. Incubation of the glands was performed at 4°C for 30 min, 45 min, or one hour. Altogether three treated and three control sets of experiments were performed and each set of experiments was repeated 3 times. After proper incubation at 4°C, the glands were washed thoroughly with Ringers (pH 7.0), fixed in aceto-methanol, and conventional squash preparation following aceto-carmine, aceto orcein staining was performed. Preparations were examined under a Zeiss microscope and photographs were taken.



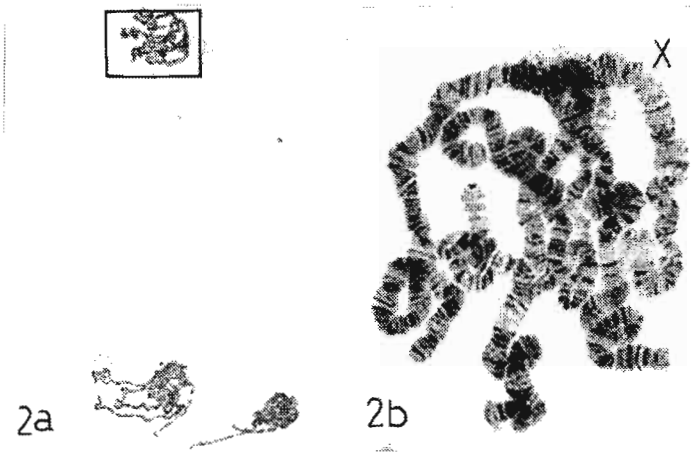


Table 1. Frequency [%] of X chromosomal morphotypes of Oregon R male salivary gland nuclei after incubation in Ringer or haemolymph of In(1)BM^2 (rv) male. A total of 12 pairs of Oregon R salivary glands were used in each experiment

Incubating medium	Time of incubation	No. of nuclei examined: Different X chromosomal morphotypes [% freq.] F	I	N	Total no. of nuclei
Ringer	30 min.	-	-	1287 [100%]	1287
Ringer	45 min.	-	-	1492 [100%]	1492
Ringer	1 hour	-	-	1295 [100%]	1295
Haemolymph of In(1)BM^2 (rv) male	30 min.	26 2.0%	130 20.03%	1140 77.9%	1296
"	45 min.	63 4.99%	253 20.03%	947 74.99%	1263
"	1 hour	276 20.02%	413 29.97%	689 50.0%	1378

F = Flabby, I = Intermediate, N = Normal.

Figure 1: [a] Photomicrograph of salivary gland chromosomes of Oregon R male shows normal X chromosomes in all the nuclei (under low magnification); [b] one of the nuclei in Fig. 1a (inset) is magnified.

Figure 2: [a] Photomicrograph showing the induced mosaic X morphotypes of Oregon R male salivary gland nuclei after incubation in haemolymph of In(1)BM^2[rv] males (under low magnification); [b] one of the nuclei in Fig. 2a (inset) is magnified showing flabby X morphotype.

Data reveal that there is no induction/change in the X chromosomal morphotype in Oregon R$^+$ male salivary glands when incubated in Ringers either for 30 min, 45 min, or 1 hr, i.e., 100 percent of the nuclei show the normal X chromosomal conformation (Fig. 1a and b). On the other hand, when protein from In(1)BM^2[rv] male was used, they exhibited the three X chromosome morphotypes in a mosaic pattern. These morphotypes can be clearly distinguished as Flabby (F), Intermediate (I) or Normal (N) (Fig. 2a & b) as are normally found in In(1)BM^2[rv] males cultured at 18°C. The frequencies of flabby and intermediate X's are increased with the increased incubation time. The induction

of flabby and intermediate X's is maximum for 1 hr incubation series (Table 1).

The results reveal that [1] haemolymph proteins of In(1)BM^2[rv] male induces Oregon R male salivary gland X chromosomes to show mosaic X morphotypes i.e., flabby, intermediate and normal as found in salivary gland nuclei of In(1)BM^2 [rv] males and [2] frequency of induction of flabby and intermediate Xs increased with the increase of incubation time under the same experimental condition. The results suggest that the In(1)BM^2 mutant produces certain active enhancer protein factors in excess of that in the wild type and this induces the activity state of the X sex specifically.

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Gibson, G. and Gehring, W.J.
Biozentrum, University of Basel, CH-4056 Basel, Switzerland. P-element-meditated enhancer detection in imaginal discs and third instar larval brain of *Drosophila melanogaster*.

A new technique for randomly generating fly strains in which different cell types specifically express beta-galactosidase has recently been described by O'Kane and Gehring (1987). It was used to build a considerable array of markers for analyzing patterns of gene expression during embryogenesis (Bellen et al., 1989) and oogenesis (Grossniklaus et al., 1989). Here, we complete the analysis of the screen by describing the variety of beta-galactosidase staining patterns observed in the imaginal discs and brains of third instar larvae in the more than 500 different strains. Similar screens have also been described for embryogenesis (Bier et al., 1989) and oogenesis (Fasano and Kerridge, 1988).

The technique we have used has been termed "enhancer detection", and relies upon the tissue-specific expression of a lacZ reporter gene which is carried on a P-transposable element (O'Kane and Gehring, 1987). This gene is driven by a weak promoter, which is influenced by genomic transcriptional regulatory elements adjacent to the site of insertion of the P-element. A single copy of the enhancer detector P[lArB] generated by P-element mediated transformation (Spradling and Rubin, 1985) was mobilized to 523 different locations using the "jump-start" technique (Robertson et al., 1988; Cooley et al., 1988). The vast majority of the resulting transposant lines contain a single insertion of P[lArB], and display unique temporal and spatial control of beta-galactosidase expression in embryos (Bellen et al., 1989). Considerable evidence exists to suggest that many of the observed beta-galactosidase expression patterns reflect the transcription pattern of genes lying nearby to the P[lArB] insertions (Wilson et al., 1989).

It is worth emphasizing a number of salient features of the enhancer-detection technique (see Wilson et al., 1990, for a review). Firstly, it is very quick and simple, and relatively inexpensive. Secondly, an astonishingly high yield of an incredible variety of patterns can be obtained: in our experience, up to 65% of all P[lArB] insertions yield temporally and spatially restricted patterns of beta-galactosidase expression. Thirdly, since the screen is for patterns of gene expression rather than gene activity, it is possible to detect genes which would escape traditional genetic screens because of redundancy, or because they only affect internal structures. Fourthly, using constructs such as P[lArB], it is possible to rapidly isolate DNA adjacent to the site of insertion by "plasmid rescue", and hence commence molecular analyses of interesting loci. Fifthly, it is equally possible to carry out genetic analysis of genes lying near insertions, by using the jump-start technique to mobilize the P-element and scoring for mutants which arise as a result of imprecise excision. Sixthly, around 10% of the P-element insertions are homozygous lethal (see also Cooley et al., 1988), implying that the technique can be used for simultaneous screens for patterns and (possibly) associated lethal mutations (Bellen et al., 1989). Finally, a couple of limitations of the technique should be mentioned: clearly, not all of the patterns will necessarily correspond to the pattern of expression of a single nearby gene; and nor will they always reflect the complete developmental expression of a gene product, for example in cases where a gene may use two separately controlled promoters, or where extensive post-transcriptional regulation occurs.

In order to screen the 523 strains for potential imaginal-disc cell markers, we performed a quick assay for beta-galactosidase activity in the anterior halves of late third instar larvae. Two or three wandering third instar larvae from each transposant line constructed by Bellen et al. (1989) were cut at the base of the crop, and the heads were inverted, fixed for 15' in 1% glutaraldehyde in PBS and then stained overnight at 37°C (see Ashburner, 1989, for detailed protocol). The imaginal discs and brain were then dissected with fine forceps and mounted in PBS. Such preparations last only one day at 4°C before their quality is affected by diffusion of the converted substrate. As a control, we examined the staining of larvae of the ry^{506} strain, which does not contain a P-element insertion. This revealed that there is very strong endogenous beta-galactosidase activity in the anterior spiracles of third instar larvae, in the dorsal prothoracic (humeral) discs, and somewhat lighter staining in the larval cuticle and in the adhering haemocytes over the centres of older imaginal discs. Consequently, we did not attempt to score staining in these tissues.

After the initial screen, the staining was repeated for 130 lines which showed potentially "interesting" patterns, and 119 of these have been retained specifically for their larval staining pattern. The classification of the remaining 395 lines, which were not rechecked, is consequently only approximate.

Table: 1 Classification of larval staining patterns

Class		Frequency	
		N	%
(i)	No staining observed	110	21
(ii)	Staining only in the larval brain	79	15
(iii)	Ubiquitous staining	72	14
(iv)	Miscellaneous patterns	22	4
(v)	Recurring pattern	134	26
(vi)	Spatially restricted patterns in imaginal discs	106	20
	Total	523	100

Table 2: Frequencies of different pattern elements

Sub-Class	Pattern Element	Specific	General
Leg / Antenna	all or most	1	8
	concentric circles or ring	9	25
	incomplete circles	2	6
(16 lines)	line of cells	4	6
	base of disc	1	9
	antennae only	1	2
Wing / Haltere	cross-like patterns	2	2
	margin (D-V boundary?)	2	3
	pouch / blade	1	2
(16 lines)	periphery of pouch	3	13
	thoracic portion	6	17
	other patches	5	6
Eye	morphogenetic furrow	7	11
	posterior to morph. furrow	17	30
(20 lines)	presumptive head capsule	1	14
	Bolwig's organ (?)	-	3
PNS	all (?)	6	-
(14 lines)	part (?)	8	-
Brain	all over ventral ganglion	8	75
	part of brain hemishere	8	61
(13 lines)	stripes in ventral ganglion	1	6
	ventral midline	3	5

The data for the remaining 40 lines which stain pattern elements in all discs is included in the "general" column.

The types of staining observed in third instar larvae were arbitarily divided into six classes of staining : (i) no specific staining observed (although some of these lines may stain just a few cells, or stain at later or earlier stages in disc development which were not examined); (ii) staining only in the brain; (iii) ubiquitous but only moderate staining in all discs and the brain; (iv) miscellaneous patterns (for example, cells scattered over all parts the brain, or diffuse stain in the discs); (v) a recurring pattern consisting of staining in the posterior region of the eye, the centers of the leg and antennal discs, faint staining in the wing disc, and throughout the brain (the explanation for this type of staining is unclear, although it may be due to a regulatory element located within the P[lArB] vector which is only active in some genomic environments - a recurring pattern has also been reported in embryos (Ghysen and O'Kane, 1989) for a different, but largely overlapping, set of lines); (vi) spatially restricted staining in the imaginal discs (106 lines; see below). The frequencies of each class are shown in Table 1. Although we have observed staining in the genital discs of a number of lines, we have made no systematic attempt to score the staining in these discs.

The beta-galactosidase expression patterns for a total of 119 lines which may prove to be valuable as imaginal cell-specific markers are summarized in Table 2. For 66 of these lines, four "sub-classes" of staining were (again arbitrarily) defined: "Leg/Antenna", "Wing/Haltere", "Eye", and "Peripheral Nervous System" ("PNS"). A fifth sub-class ("Brain") consists of 13 class (ii) strains which are noteworthy for highly region specific staining in the larval brain. The remaining 40 of the class (vi) lines were not sub-classified, since they show staining in significant portions of all of the imaginal discs. Since there is still some overlap (in that in most of the lines, staining is not restricted to just one type of imaginal disc), the assignment of a strain to a sub-class merely implies that in that strain, that sub-class of disc stains considerably more than any other sub-class does.

It is striking that the patterns of staining observed display a high degree of regional specificity, such that we were further able to identify regions of staining which we have termed "pattern elements". The staining in the vast majority of lines can be considered to a rough approximation as a summation of one or more pattern element(s). It is however important to realize that a pattern element refers merely to a region of staining, and not to a precisely defined set of cells - for example, the leg pattern element "concentric circles" indicates that one or more concentric circles of beta-galactosidase activity is observed in the leg and antennal discs. Table 2 distinguishes between the number of lines in which a specific pattern element forms a major part of the staining in that line, and those in which it forms just a small part of it. Thus, the "specific" column of Table 2 lists the number of lines which stain both a particular pattern element, and have been assigned to the sub-class of staining to which the pattern element belongs; while the "general" column shows the number of remaining lines which stain the pattern element. For example, of the 34 lines which stain

concentric circles in the leg discs, 9 belong to the sub-class "leg-antenna", while the remaining 25 come from other class (vi) lines. Since many lines show more than one pattern element, the numbers in each column add up to more than the number of lines examined.

To assist with the interpretation of Table 2, Figure 1 shows schematic representations of the "pattern elements" identified in each sub-class of imaginal disc, while Figure 2 presents 15 examples of interesting staining patterns. We have also selected four lines which might be used as general markers for imaginal disc development as they stain all of the discs heavily, but most larval tissues only lightly. These lines have stable insertions on the wild-type 1st or 3rd chromosomes, the 2nd CyO balancer, or the 3rd TM2 balancer chromosomes. One line has also been retained because the peripodial membrane of all of the discs expresses beta-galactosidase.

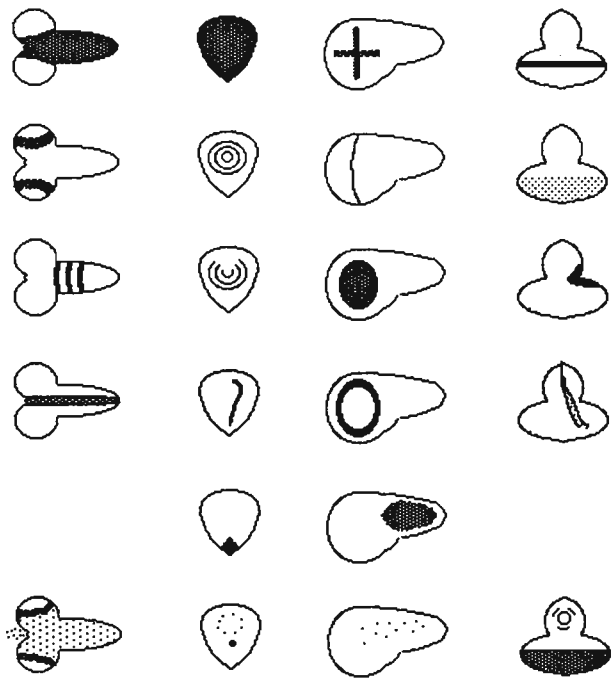

Figure 1. Schematic representation of pattern elements. Left column, top-to-bottom (brain): all over ventral ganglion; part of brain hemisphere; stripes in ventral ganglion; ventral midline. Middle left (leg discs): all/most; concentric circles; incomplete circles; line of cells; base of discs. Middle right (wing disc): cross-like; wing margin; wing pouch; periphery of pouch; thoracic portion of disc. Far right (eye discs): morphogenetic furrow; posterior to the morphogenetic furrow; head structures; Bolwig's organ (?). Bottom row: PNS pattern.

Assuming that the P-element insertions generated in our large-scale cross are truly random, we estimate that about 40% of the genes encoded by the *Drosophila* genome may be expressed in developing imaginal discs. This estimate is based on the identification of 200 lines (classes (iii),(iv) and (vi)) which stain the imaginal discs in mid-to-late third instar larvae, out of 502 lines which show specific staining during embryonic and/or oocyte development. If we only include type (vi) patterns (those which stain specific regions of the imaginal discs including the PNS) the proportion would be 25%. Such figures are in good agreement with earlier estimates based on genetic studies, which suggest that 20-25% of genes of *D. melanogaster* may be expressed in the imaginal discs (Shearn, 1978). However, we have only isolated one line in which the beta-galactosidase is exclusively expressed in the imaginal discs, whereas Shearn estimated that the expression of 10-12% of the genes may be restricted to the discs. We note, though, that even if the relationship between lacZ and *Drosophila* gene expression holds, there may not be a strict correspondence between expression and function in the particular tissues. Our study supports the prediction that the vast majority of genes expressed in the imaginal discs may also be active during embryonic development and/or oogenesis. We also note that there seem to be no preferences for chromosomal location of any of the classes of patterns reported here: all classes are distributed evenly across the three major chromosomes.

Considering just the class (vi) patterns, 45% (40 out of the 92 lines showing expression in portions of the discs, excluding those which stain the PNS in the discs) expressed beta-galactosidase strongly in portions of all discs. Typically, in any given line, the regions of each disc that are stained are related: for example, the periphery, or the base, or the center of each disc. However, in a few cases the staining defies simple explanation. A number of more specific observations concerning all class (vi) lines can also be made:

(a) The leg and antennal discs are clearly related, since clear differences between the beta-galactosidase expression patterns in these two types of disc were seen in only twelve cases. Interestingly, these twelve lines include five of the sixteen lines which stain predominantly in the leg/antennal discs. The differences generally relate to the number of circles which stain in each disc, but include other pattern elements as well. The three different types of leg disc always stain equivalently.

(b) No obvious differences between haltere disc and wing disc staining were observed, although it should be noted that minor differences may have escaped our attention.

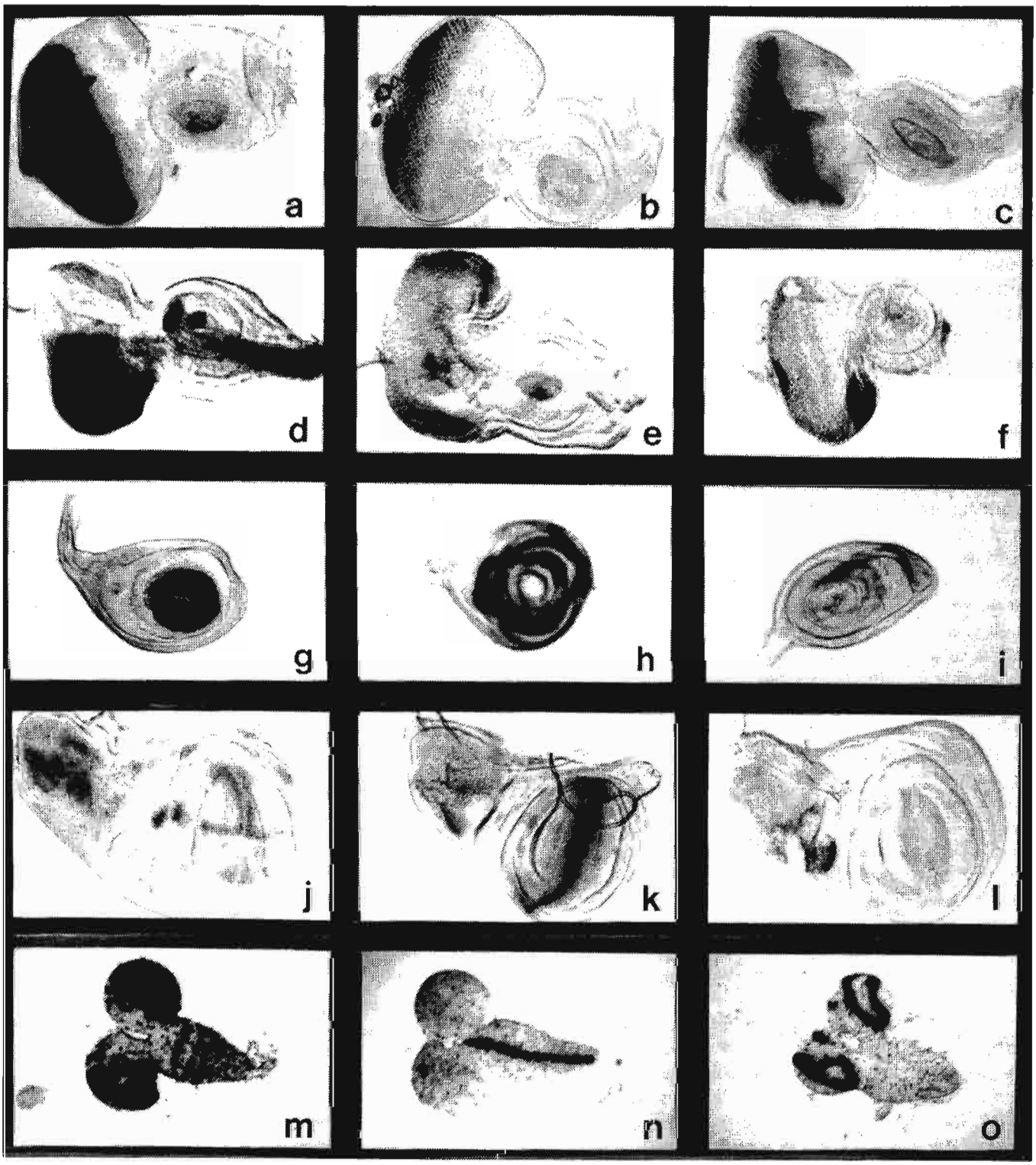

Figure 2. Range of pattern elements. Eye-Antenna: (a) strong posterior eye, (b) weak posterior eye, (c) morphogenetic furrow, (d) dorsal eye plus Bolwig's organ (?), (e) presumptive bristles (?), (f) occiput/ocellus. Legs: (g) single ring, (h) concentric circles, (i) circles plus line. Wing: (j) cross-like, (k) wing margin, (l) patches in thoracic portion. Brain: (m) stripes in ventral ganglion, (n) mid-line, (o) optic lobes (?) in brain hemispheres.

(c) Most of the wing/haltere subclass also lightly stain pattern elements in the leg/antennal and/or eye discs (in only two lines is expression in third instar larvae restricted to the wing/haltere discs, whereas 14 lines stain only in the leg/antennal discs). This observation possibly supports the notion that the wing discs have derived during evolution from primitive leg discs (Wigglesworth, 1973). It was not possible to draw any firm correlations relating pattern elements derived from different sub-classes.
(d) Excluding the PNS-specific lines, 20 lines stain predominantly in the posterior eye, of which six do not stain leg or wing discs at all - these lines all seem to stain different cells, and may identify genes involved in photoreceptor formation and other aspects of ommatidial development.
(e) All of the lines staining the imaginal discs also stain specifically in the brain, including a number identifying structures such as the mid-line and the presumptive optic lobes, but no correlations between larval brain and imaginal disc staining can be drawn. The variety of staining patterns observed in the brain of class (vi) lines was as great as the variety seen in the class (ii) lines, that is, those which specifically stain the brain.
Two observations concerning the staining in the PNS can be made:
(f) 10 out of 14 presumptive PNS lines (classified by comparison with the imaginal disc staining of the P[lac, ry^+]A37 strain described by Ghysen and O'Kane, 1989) also stain subsets of cells posterior to the morphogenetic furrow of the eye disc; and
(g) 11 of these 14 PNS lines (80%) also stain the ring gland, which we estimate is true for a maximum of only 30% of the remaining class (vi) lines, although ring-gland expression was not systematically noted.

We have looked for relationships between the classes of embryonic patterns and patterns in the presumptive adult tissues. No strict relationships could be identified, and it is evident that any type of maternal or embryonic expression pattern can evolve into any class of imaginal disc expression pattern. However, a few relatively weak tendencies can be recognized. Thus, a significantly higher proportion of PNS patterns in the imaginal discs derive from strains showing embryonic PNS expression than from any other class of embryonic expression pattern. Similarly, class (ii) patterns tend also to occur in lines which also show embryonic CNS expression; those uniformly staining the discs also often stain all over the pre- and/or post-blastoderm embryo; and lines not staining in the embryo typically do not stain in larvae.

It is also important to realise that, although we have only described specific patterns of expression in this report, it is probable that a number of interesting genes will be expressed ubiquitously in the imaginal discs. For example, we have already identified the Dras-1 gene, a *Drosophila* homologue of the Ha-ras oncogene (Segal and Shilo, 1986), from amongst the class (iii) lines (Bellen et al., submitted).

We have reported the application of a technique designed to detect tissue-specific activity of developmentally regulated genes, to the study of late third instar larval brain and imaginal disc development. The range of expression patterns observed is quite remarkable, both for the fact that "pattern elements", in which staining is often seen, can be defined; and because such pattern elements usually make "developmental sense", staining important boundaries (for example, the wing margin which separates the dorsal and ventral compartments of the wing disc) and regions (for example circles in the leg discs, which are presumably related to the formation of leg segments). However, it is equally important to note that the majority of the patterns of expression do not correspond to known compartmental restrictions, implying that there are elaborate genetic mechanisms controlling pattern formation over and above those operating to ensure compartment formation. Furthermore, we would like to emphasize that many of the patterns are just as useful for marking temporal as for spatial relationships. This is most obvious in the case of lines which stain the morphogenetic furrow. We have also observed differences in beta-galactosidase expression patterns of some of the markers in discs of different ages.

It may be expected that some of the markers will define new cell-types and/or developmental domains, as well as opening up new avenues for the study of the role of enhancer elements in directing spatially restricted gene expression. These staining patterns are of immediate use as cell-markers for various processes which occur during determination and morphogenesis of the imaginal discs, as well as the larval brain (see for example Ghysen and O'Kane, 1989). In addition, the strains generated here and in future experiments should allow the cloning (Wilson et al., 1989) and genetic analysis (Bellen et al., 1989) of a large number of developmentally important genes, many of which may have escaped detection in classical genetic screens.

Most of the lines described here are now available from the Howard Hughes P-element Stock Center at Bloomington, Indiana.

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Announcements

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Hannah-Alava, A. Genetics Institute, University of Turku, Finland. Another aristapedia-dominant?

A neutron-induced antennal leg mutant of *D. melanogaster* was named Aristapedia-dominant (Ar,ss^{Ar}) by Le Calvez (1948) on the basis of 3rd-chromosomal localization, morphological changes of the antennae and heterozygous mutant expression, who considered it to be an allele of aristapedia (ss^{a}). The ambiguity in localization, i.e., no recombination in 3R, was explained by Le Calvez as the consequence of a simultaneously-induced inversion (with salivary breakpoints in 3R at 84A5-6 and 92A5-6) which suppressed crossing-over. In the same year, Yu and Lewis (E.B. Lewis, 1956, c.f., Lindsley and Grell, 1968; Lindsley and Zimm, 1985, 1987) on the basis of their cytological studies of two Antennapedia mutants (Antp^{Yu} and Antp^{B}) concluded that the critical fracture for expression of Antp was in the 83-84 region. As one of the break-points of the Le Calvez mutant is 84A5-6, it was renamed Antp^{LC}. The validity of considering Antp^{LC} an Antp allele has been supported by other data: Antp^{50} maps genetically between the centromere (+47) and pink (p:48.0) in 3R (Hannah-Alava, c.f., Lindsley and Grell, l.c.; Lindsley and Zimm, 1985) and the Antp gene complex (ANT-C) maps cytologically in 3R to 84B1-2 (R.A. Lewis, et al., 1980). In as much as spineless (ss) and aristapedia (ss^{a}) map in 3R to 58.5 genetically and 89C in the salivaries (Fig. 1) Antp^{LC} cannot be an allele of ss^{a}.

As yet it is not known whether the ss-ss^{a} locus is single (Struhl, 1982) or complex (Hexter, et al., 1967). Lindsley and Grell (l.c.) list only 12 ss and ss^{a} among the mutations, all spontaneous and presumably all point mutations. Of these two were only ss and of the ten designated as ss^{a}, six were also ss. An X-ray induced rearrangement, T(1;3)ss^{v}, (found by E.B. Lewis) which was variegated for ss and mutant for ss^{a}, as well as an ss iso-allele (found by Piternick) were also included among the mutations. Other rearrangements -- notably In(3LR)P88 of the TM6 balancer chromosome -- with breakpoints in the 89B-D region and having an ss or ss^{a} phenotype in appropriate heterozygotes were also recorded by Lindsley and Grell (l.c.). Lindsley and Zimm (1987) reported that two more of the rearrangements with 89B-D breakpoints expressed the ss-ss^{a} phenotype. They also recorded three new deficiencies and a translocation having an "ss-ss^{a}" phenotype when heterozygous for ss and/or ss^{a} alleles.

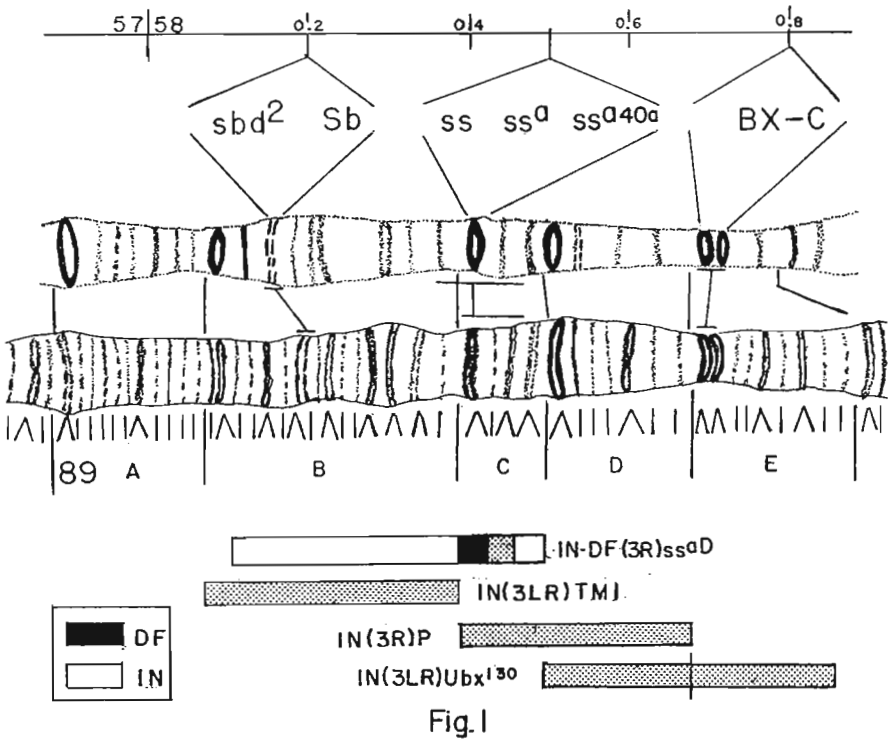

Fig. 1

Figure 1. Upper: Genetic and cytological location of the sbd-Sb, ss-ss^{a} and BX-C loci (after E.B. Lewis, 1951, 1963, 1978). Lower: Salivary map after Bridges (1941) and four chromosomal rearrangements with breakpoints in section 89. Stippling indicates that the breakpoint is only known to be in that region. The dominant, ss^{aD}, original; the data for the rest from Lindsley and Zimm (1985, 1987).

In a large-scale experiment on the induction of 3rd-chromosome specific-locus mutations and recessive lethals (Hannah-Alava, 1964; Puro, 1964a, b) only ten ss-ss^{a} mutations were recovered, all from the postmeiotic broods of offspring from irradiated (3000r) wild-type (Ore-RS, isogenic 3rd) males mated to "ss- or ss^{a}-tester"/TM1 Me females: of these one was ss, one was ss^{a} and seven were ss-ss^{a} (including one X; 3 and one Y; 3 translocation). The tenth, found by Puro, was an unusual variant: a male (Ore-RS 3rd/"ss-tester") having extreme "spineless-Minute" bristles and antennal legs similar to ss^{aB} (Fig. 2b). In a backcross (by the author) of this male to females from the "ss-tester"/ TM1 Me ri sbd^{1} stock, both the mutant/"ss-tester" and the mutant/Me offspring had ss^{aB}-like antennae and other pleiotropic expressions of ss-ss^{a}; but about half of them were "ss-M" and the rest had extreme stubble-forked (Sb-f) bristles. Further crosses established that the mutant was dominant - hence ss^{aD}

-- and homozygous lethal. The mutant/Me flies, however, were poorly viable and fertile, the "ss-M" ones so much so that few matings produced any offspring and the "ss-M" strain was soon lost. An ss^{aD} "Sb-f"/Ubx strain was established from crosses of ss^{aD} "Sb-f"/"ss-tester" males with In(3LR)Ubx^{130}/TM1 Me females. The mutant/Ubx flies were viable and fertile and had strong "Sb-f" bristles; the expression of ss^{a} was more like ss^{a-H} (Fig. 2c; c.f., Goldschmidt, 1951) than ss^{a} (Fig. 2e) and often were "antennal legs". The most extreme types (Figs. 2i-l) were found in the mutant/ss^{a} compounds, and in these the antennal leg was tibial rather than tarsal, but terminating in a brush or claws. All of these results point to the probability that the mutant was a dominant at the ss-ss^{a} locus. This inference was substantiated by localization tests. The "Sb-f" and antennal leg phenotypes -- which segregated as a unit -- mapped genetically to 3R between p (48.0) and sr (62.0) [and later bx (58.8)] thus approximately at the ss locus (58.5). There was little indication of excessively reduced recombination in the p-bx region. The mutant was therefore considered to be a dominant ss^{a} i.e., ss^{aD}.

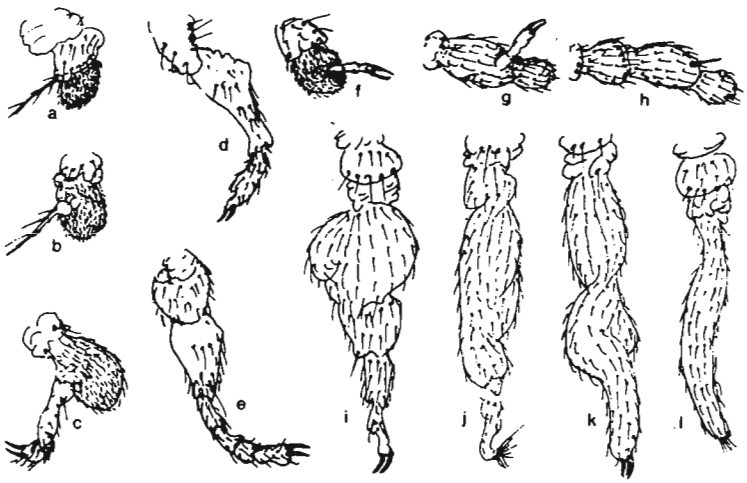

Fig. 2

Figure 2. The normal antenna (a); Different degrees of expression of an antennal leg characteristic of ss-ss^{a} mutant alleles: homozygous ss^{aB} (b), ss^{a-H} (c), ss^{a40a} (d), ss^{a} (e), and heterozygous ss^{aD} (f-l). The drawings for ss^{a-H} and ss^{aD} original; data and literature for the rest from Lindsley and Grell (1968) and Waddington and Clayton (1952).

Cytologically, the mutant appears to be related to a deficiency of the 89C1-2 and possibly also the 89C 3-5 bands within an inversion with breakpoints after 89B2-3 and before 89D1-2 of the 3R salivary chromosome (Fig. 1). Since 89C is the probable position of the ss complex of loci (Lewis, 1963, 1978) it seems reasonable to conclude that ss^{aD} was a "mutation" of the ss-ss^{a} locus.

Because in the earlier crosses there had been a rather regular and independent segregation of the "Sb-f" and "ss-M" phenotypes, related in a complex way to the sterility of the ss^{aD} flies, it is possible that the bands in 89C that had been deleted were inserted into a new position either in the same or another chromosome. Before this hypothesis could be tested the ss^{aD} Sb-f" mutation was lost.

In spite of the homozygous lethality of ss^{aD}, it was not lethal with any of the mutant-marked "tester" and balancer chromosomes, only semilethal with In(3LR)TM1 Me ri sbd^{1}, In(3R) Payne and two (one, ss^{aH2}, variegated for ss and mutant for ss^{a}) of the eight recessive "ss-ss^{a}" mutations (Hannah-Alava, unpublished). However, the criterion of lethality had been very stringent, so the recovered "aristapedia" flies, at least some of them, could have been escapers similar to those reported for sbd by Spillmann and Nothiger (1978).

The expression of an antennal leg in the ss^{aD} heterozygotes (Fig. 2) depended to a large extent upon the mutant(s) in the homologous chromosome. The expression of an antennal leg in $ss^{aD}/+$, ss^{aD}/ss, ss^{aD}/ss^{P} (one of the "ss-ss^{a}" mutations), ss^{aD}/In(3R)Payne and ss^{aD}/In(3LR)TM1 was similar to or a little more than ss^{aB} (Fig. 2b). Although variable in degree, ss^{aD} and Ubx or bx trans-heterozygotes usually had somewhat larger and more differentiated antennal legs, but with more tarsal- than tibial-like expression (Figs. 2b-d). In ss^{aD}/Sb heterozygotes, the antennal legs could even be more tibial-like especially in bristle pattern (Figs. 2g-h). Such tibial-like antennal legs have also been depicted by Le Calvez (1948) for $Antp^{LC}$ heterozygotes and by Balkaschina (1929) for ss^{a} homozygotes. The most extreme, and frequently atypical, expression of the antennal leg (Figs. 2i-l) was in the compounds of ss^{aD} with the recessive ss^{a} alleles, either spontaneous or induced, and (depending upon the combination) ranging in expression from those similar to ss^{aSp} (Lindsley and Grell, l.c.) with five tarsal joints, to a single long segment (Fig. 2l) usually terminating in a tarsal brush or claws.

The Minute-like and Stubble-forked phenotypes are more difficult to interpret. The "Minute" could have been an extreme expression of ss, perhaps, even due to the deficiency in the ss-ss^{a} region, or even to an independent mutation. The "Stubble-forked" could have been a pleiotropic expression of ss^{aD}, or an independent mutation at or near the sbd-Sb

complex (Fig. 1) or even to a position-effect mutation, of either Sb or ss^a because of the accompanying inversion. Initially, the poor viability and fertility of ss^{aD}/TM1 Me ri sbd^1 was assumed to involve the sbd-Sb complex locus, but other evidence does not support this supposition: even though Sb is homozygous lethal, a deficiency for the Sb locus does not produce a dominant phenotype; sbd^1/Sb is lethal, but sbd^2/Sb is more extreme in expression than Sb/+ (data from Lindsley and Grell, 1968). Whatever the cause of the extreme "Stubble-forked" bristles the conclusion that ss^{aD} is due to a deficiency or a dominant mutation of one or more loci of the ss-ss^a complex appears to be valid.

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Harshman, L.G., M.M. Green, W. MacKay, G. Bewley, and G. Edlin. University of California, USA.
Relative survival of catalase deficient genotypes on irradiated *Drosophila* food.

Irradiation of medium produces hydrogen peroxide and various oxygen compounds or radicals which are responsible for bacterial toxicity and mutagenicity (Imlay and Linn 1986; Linn and Imlay, 1987). Catalase is capable of ameliorating the mutagenic and toxicological effect of irradiated microbial medium (Wyss *et al.*, 1948). In this study we examined the effets of irradiating *Drosophila* medium on the relative survival of catalase deficient *D. melanogaster.*

The *Drosophila* medium used was made of cornmeal (6.2%), semolina (3.1%), sucrose (3.6%), dextrose (7.1%), agar (1.4%) and dead yeast (1.5%). Medium was subjected to 1.5 Mega Rad of gamma radiation from a Cobalt 60 source. The treated vials (n=29) and unirradiated control vials (n=28) were painted black on the outside to retard the decomposition of hydrogen peroxide by light. Immediately after irradiation young flies (5 days or less) were placed on medium (10 females and 5 males per vial) for several days at room temperature (22-25°C). The genotypes of emerging progeny were scored for approximately a week after first eclosion.
Parental Cross:

Df(3L) Cat^{DH104} ri sbd e^2/cp in Cat^{n1}ri p^P male x cp in Cat^{n1} ri p^P/TM3 female

A 2x2 contingency table analysis comparing the number of catalase deficient progeny (catalase hemizygotes [Cat^{n1}/Df(3L)Cat^{DH104}] and homozygotes [Cat^{n1}/Cat^{n1}]) with heterozygotes (the deficiency or null over TM3) emerging from control and irradiated medium indicates that it is unlikely there is independence between genotype and medium type ($p<0.001$). There are relatively less catalase deficient progeny emerging from irradiated medium. In other experiments investigators have shown that *D. melanogaster* sensitivity to the deleterious effects of hydrogen peroxide increases when catalase activity is relatively low (Bewley and Lubinsky, 1980; Mackay and Bewley, in press). The present experiment was not conducted under axenic condition and microbes carried onto the medium by adult flies could catabolize the agents produced by irradiation, which would reduce the magnitude of the observed effect.

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Hillesheim, E. and S.C. Stearns. Zoological Institute, Rheinsprung 9, CH-4051 BASEL, Switzerland.
Repeatability of life history traits in *Drosophila melanogaster* estimated on standard medium.

When one compares successive results under the same standardized laboratory conditions, one needs to know whether environmental variation in the standardized conditions influences the results, especially for repeated measurements and for selection experiments. *Drosophila melanogaster* is often reared under constant conditions (temperature, humidity, medium), and it is widely suspected

that substantial fluctuations in phenotype values widely suspected that substantial fluctuations in phenotype values are caused by differences in microenvironment. Variation among batches of food as well as among vials may be an important source of uncontrolled environmental fluctuations. To reduce effects of uncontrolled environmental fluctuations, it is standard practice to use a control line, especially in selection experiments. However the use of the control line values as a correction factor implicitly assumes that all genotypes react to these uncontrolled environmental fluctuations in parallel.

To test this assumption and to estimate the magnitude of genotype by environment interactions for life history traits arising from uncontrolled environmental variation, we meausred the repeatability of body size (dry weight) and developmental time on different yeast concentrations by preparing each medium three times. We used a slightly modified recipe from Backhaus et al. (1984) shown in table 1.

Table 1: Recipes for 1 kg medium. Because the yeast concentration of the medium was defined as the content of Heliosan brewer yeast, we called recipe 1 a 1% yeast concentration, recipe 2 a 4% yeast concentration.

Recipe 1 (in distilled water for a total of 1 kg)

10.0 g	Agar (poudre)
30.0 g	Sucrose (extra pure)
60.0 g	Glucose (monohydrate)
0.5 g	$MgSO_4 \times 6H_2O$
0.5 g	$CaCl_2 \times 2H_2O$ (for analysis)
10.0 ml	Of a 10% Nipagin alcohol solution
6.0ml	Propionic acid
10.0g	Heliosan brewer yeast (pharmacy quality)
5.0 g	Difco yeast extract

Recipe 2 = recipe 1 with following alteration

20.0 g	Agar (poudre) instead of 10.0 g
40.0 g	Heliosan brewer yeast instead of 10.0 g
+ 50.0 g	cornmeal

Table 2. Larval viability values in per cent (%). Mean value for 12 vials each containing 12 larvae, i.e. 100% viability means a total of 144 emerged flies. 2a. 1% yeast concentration; 2b: 4% yeast concentration.

2a: Larval viability in per cent at 1% yeast concentration

batch	line A	line B	line C
1	80.6	83.3	26.4
2	84.7	88.0	25.0
3	80.6	84.0	24.3

2b: Larval viability in per cent at 4% yeast concentration

batch	line A	line B	line C
1	87.5	81.9	54.2
2	82.6	90.3	81.9
3	84.0	92.4	78.5

Table 3. Model II nested ANOVA. Analyses separated by yeast concentration and sex. Differences among vials were tested with MSvial (line*batch)/MSerror. All other F-tests were performed with MSvials (line*batch) as error term (cf. Sokal and Rohlf, chapter 11, 1980). All reported MS values are type III for unbalanced data (GLM procedure from SAS Institute 1985). * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

3a: Results of an analysis of variance at 1% yeast concentration

dry body weight

	male			female		
Source	DF	MS	F	DF	MS	F
line	2	0.04647	22.9***	2	0.24154	84.99***
batch	2	0.00293	1.44	2	0.00747	2.63
line*batch	4	0.00751	3.70***	4	0.00573	2.02
vial (line*batch)	96	0.00203	1.61***	77	0.00284	1.31
error	323	0.00126		316	0.00216	

developmental time

	males			females		
Source	DF	MS	F	DF	MS	F
line	2	3.12375	6.23**	2	0.93221	1.51
batch	2	2.28277	4.56*	2	4.03720	6.53**
line*batch	4	0.51503	1.03	4	1.29439	2.09
vial (line*batch)	96	0.50106	1.14	77	0.61799	1.30
error	23	0.43807		316	0.47481	

3b: Results of an analysis of variance at 4% yeast concentration

dry body weight

	males			females		
Source	DF	MS	F	DF	MS	F
line	2	0.09338	72.51***	2	0.14229	83.37***
batch	2	0.00307	2.39	2	0.01545	9.06***
line*batch	4	0.00495	3.85**	4	0.00441	2.58*
vial (line*batch)	99	0.00129	1.10	97	0.00170	0.98
error	399	0.00117		440	0.00174	

developmental time

	males			females		
Source	DF	MS	F	DF	MS	F
line	2	2.60551	24.73***	2	15.2974	78.20***
batch	2	4.74901	45.08***	2	2.12520	10.86***
line*batch	4	0.60738	5.77***	4	0.47275	2.42
vial (line*batch)	99	0.10535	1.16	98	0.19560	0.87
error	400	0.09105		41	0.22446	

Methods: Three different homozygous lines (A, B, C) established by Gebhardt (Gebhardt and Stearns 1990) were used. The flies were kept at 25°C, 70% r.h. and reared under controlled low density. Twelve fertilized females, 5-7 days old, laid eggs for 4 hours on agar medium with 2 drops of fresh yeast. After laying, flies and fresh yeast were removed. To control for the shift in developmetnal time due to egg retention, larvae which hatched within 16 hours after the laying period were discarded. 24 hours after the laying period, the remaining larvae were transfered into the test vials (12 larvae per vial) and kept until hatching at 25°C and 70% r.h. Emerging flies were gathered at 6 hour intervals using a collecting machine (Stearns et al. 1987). Developmental time was calculated as the difference between the midpoint of the egg laying and the collection intervals. Dry weight of each emerged fly was measured to the nearest of 0.01 mg after drying for 3 hours at 50°C.

Test vials contained 2 ml medium without fresh yeast. We tested two types of medium (Table 1), one with a 1% and a second with a 4% yeast concentration, to make sure that the yeast concentration was not limiting larval viability, body weight or developmental time. The 4% medium was supplemented with 10 g agar/kg and 50 g cornmeal (see Table 1). Larvae were placed on 3 different batches of each medium (the same recipe prepared 3 different times). For each line on each medium and for each batch we had 12 replicate vials (2 types of medium * 3 lines * 3 batches * 12 replicate vials * 12 larvae = 2592 larvae).

Larval viability: Table 2 shows the larval viability of the three different lines (A, B, C) for each batch of both media. At 1% yeast concentration there were no significant differences within the lines among the 3 batches. Line A and C (X^2 = 129.6, df = 1, $p < 0.001$) and line B and C (X^2 = 139.84, df = 1, $p < 0.001$) differed significantly, but A and B did not differ in viability. Also at 4% yeast concentration, A and B did not differ, nor were there differences among the 3 different batches within either line. Line A (X^2 = 4.8, df = 1, $p < 0.05$) and line B (X^2 = 7.51, df = 1, $p < 0.01$) both differed significantly from line C. In comparisons of viability between 1% and 4% yeast concentration, lines A and B did not differ, but line C did (X^2 = 95.7, df = 1, $p < 0.001$).

These results show that lines differ in how their larval viability reacts to changes in yeast concentration and/or cornmeal presence. Line C, a very sensitive line, responded positively to the medium with more yeast and cornmeal. Added cornmeal can affect nutrient gain among lines if they have different amylase isozymes with different enzymatic activities (Klarenberg et al. 1988). This could explain the higher larval viability of line C.

The other 2 lines showed no increase in larval viability at higher yeast concentrations. Because larval viability reaches a level of 80 to 90%, which is the maximum we observe for this experimental design, we concluded that these

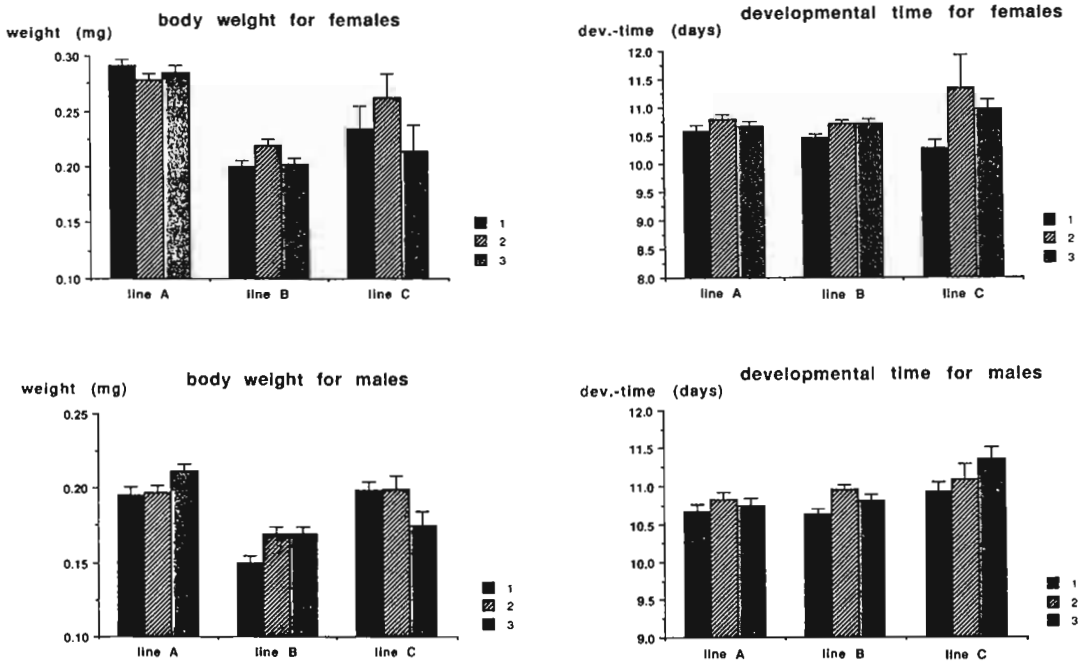

Figure 1. Means and SEs of dry weight and developmental time for both sexes of thre three lines on 1% yeast medium. Weight = dry weight; dev. time + = developmental time.

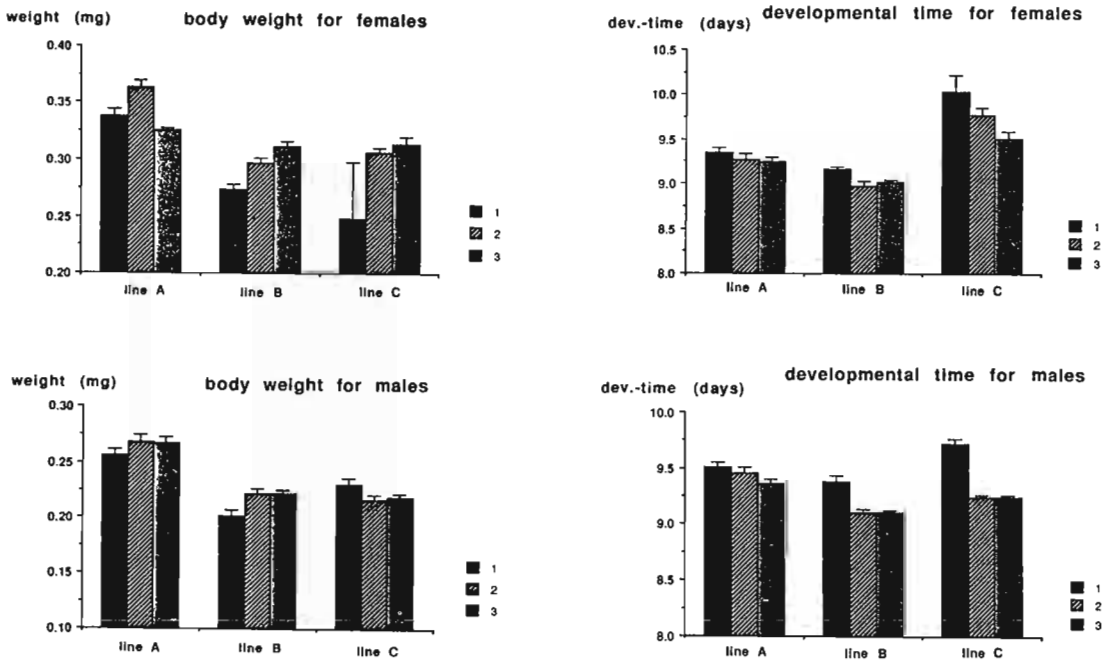

Figure 2. Means and SEs of for dry weight and developmental time for both sexes of the three lines on 4% yeast medium.

yeast concentrations do not limit larval viability in lines A and B. Our viability values are within the range found in other experiments (Gebhardt and Stearns 1988).

Effects of repeated cooking procedure: Figures 1 and 2 show the effects of repeated cooking on developmental time and dry weight for each sex and both yeast concentrations. We found significant effects of line and batch and significant interactions (line*batch) for some traits, but not in all cases (Table 3). The pattern of significance was not consistent across all traits and sexes. This means that the lines perform differently on different food batches - mainly for developmental time (Table 3). Because the differences caused by different batches were not parallel, the line*batch interaction is not predictable (see Figures 1 and 2). Males seem to be more sensitive to this kind of environmental variation.

Interpretation: We did this experiment to help us understand the importance of variation among batches of food for a planned selection experiment, where genotype by environment interactions would influence the repeatabilities of response to selection. We also wanted to know how well larvae from different strains survived on food of different quality. If the viability among lines is not similar, the emerged flies may represent only certain genotypes, not a probe from all potential genotypes of this line.

Hageman et al. (1990) have shown the potential selective influence of ethanol in the medium if you select for alcohol dehydrogenase. Their and our results demonstrate that *Drosophila melanogaster* is sensitive even to slight environmental differences. These flies responded to batch effects as well as to line*batch effects. Microenvironmental differences due to different vials are well known and were detected, but in our results these effects were smaller than line*batch effects (compare the corresponding F-values - except for developmental time of males at 1%) and only significant for one case (dry weight in males at 1% yeast concentration).

Conclusions: We drew two conclusions. First, selection for body size and/or developmental time will be influenced by uncontrolled variation among batches of medium. Second, correcting selected lines by an unselected control line to minimize fluctuation from one generation to the next caused by uncontrolled environmental variation introduces a mixture of effects. The validity of correcting with a control line depends on the difference between its sensitivity to environmental variation and the sensitivity of the selected lines for the same environmental variation. For example, consider a control line that shows a sensitive response caused by line*batch interaction similar to line B in Figure 1 (body weight for females) and a selection line that reacts as line C (highest body weight for batch 2). The response of the control line and of line C on different batches is similar. However, another selection line, eg. line A, could well have nearly the opposite response (lowest body weight for batch 2). A correction with the control line for line C will be a real correction wheras for line A the measurement error will increase, not decrease.

In selection experiments, different genotypes express different phenotype values in a given environment, but there are always uncontrolled microenvironmental variations. To deal with microenvironmental effects among vials, one normally uses a number of replicate vials for each genotype. It is also standard practice to compare genetic differences among lines and across generations by correcting with a control line. But genotypes differ in their sensitivity to uncontrolled microenvironmental variation and do not necessarily react in parallel. Only if genotype by environment interactions are parallel in selected lines and control line will a correction improve the results. Therefore selection experiments with control lines should always be analyzed with and without correction. Differences between the analyses will indicate genotype by environment interactions among the lines.

Caution is especially appropriate when results from different laboratories or different years are compared.

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Hilliker, A.J., D.F. Eberl, S.N. Trusis-Coulter, C.B. Sharp, and B.J. Duyf. Department of Molecular Biology and Genetics, University of Guelph, Ontario, Canada. Translocations between chromosomes 2 and 3 of *D. melanogaster*.

Available translocations are indexed according to polytene breakpoints in Table 1 for easy reference. The detailed cytology of each translocation is provided in Table 2.

Table 1. Index of translocations according to individual polytene breakpoint

Polytene Section	Translocations with a break in this section
21	6, 12, 20, 30, 102, 8r17
22	34, 61, 62, 102, 148, 6r3, 8r4, 8r5, 8r20, 8r32
23	87, 117, 144, 8r17
24	7, 9, 66, 6r32, 8r4
25	4, 23, 106, 8r18, 8r35, 8r33
26	
27	8r1, 8r11
28	42, 44, 6r29
29	112
30	29, 60, 89, 98, 111, 152, 6r25
31	6r34
32	31, 119, 6r10, 6r25, 6r26, 8r22, 8r40
33	77, 86, 100, 140,
34	21, 105, 118, 147, 152, 6r15, 8r3
35	47, 50, 67, 68, , 89, 105, 6r14, 6r28, 8r12, 8r32, 8r38
36	53, 84, 90, 95, 122
37	8r32
38	2, 45, 51, 59, 94
39	79
40	
2het	8, 11, 13, 22, 28, 33, 35, 37, 39, 40, 41, 46, 48, 52, 58, 64, 70, 71, 72, 73, 76, 80, 88, 92, 99, 108, 109, 120, 127, 132, 134, 141, 6r2, 6r5, 6r9, 6r17, 6r21, 6r24, 6r31, 6r36, 8r7, 8r8, 8r16, 8r19, 8r21, 8r26, 8r27, 8r34, 8r37, 8r41
41	114, 6r4, 8r36
42	3, 5, 26, 43, 116, 130, 8r3
43	1, 19, 36, 103, 6r1, 8r14, 8r24
44	10, 32, 133, 8r15
45	56, 6r12, 8r15
46	21, 6r27, 8r31
47	15, 49, 129, 131, 6r19, 8r9, 8r15
48	78, 85, 124
49	24, 6r6, 6r8, 6r13, 6r30, 8r18, 8r32, 8r33
50	6r35, 6r35
51	18, 6r22, 6r23, 8r18, 8r33
52	104, 6r20
53	14, 21, 112, 128
54	71, 6r16
55	38, 62, 6r3, 6r22, 8r30, 8r36, 156
56	27, 54, 8r6, 8r23, 8r33
57	25, 93, 114, 136, 8r3
58	63, 6r3, 6r27, 6r33, 8r13, 8r31, 8r36
59	16, 55, 71, 6r8, 6r33
60	3, 155, 6r18, 8r4, 8r4
61	78, 6r29, 8r5
62	53, 86, 102, 6r30, 8r11, 59
63	44, 102, 6r21, 8r20, 8r24, 8r38
64	38, 61, 76, 133, 6r20, 6r21, 8r32
65	47, 72, 73, 104, 111, 8r23, 8r30

66	26, 62, 112, 141, 6r19, 8r5, 8r17, 8r32, 8r34
67	46, 84, 114, 6r1, 6r17
68	27, 6r33, 8r19, 8r24
69	6r30
70	54, 58, 105, 8r15, 8r41
71	23, 94, 105, 129
72	32, 63, 6r36, 8r26
73	8r36
74	20, 124
75	6r18, 8r22, 8r27
76	3, 8r13, 8r19
77	6r16, 8r5
78	2, 7, 51
79	14, 31, 6r30, 8r3
80	6r6
3 het	9, 11, 16, 22, 24, 29, 34, 35, 39, 41, 42, 45, 48, 50, 55, 60, 64, 67, 89, 90, 92, 93, 98, 106, 108, 109, 118, 120, 122, 130, 134, 136, 147, 6r9, 6r14, 6r23, 6r26, 6r27, 6r28, 8r1, 8r4, 8r8, 8r14, 8r16, 8r18, 8r37
81	8r32
82	5, 6, 43, 77, 119, 120, 140, 6r13
83	3, 12, 13, 30, 36, 112, 6r9, 6r15, 8r22
84	3, 10, 68, 95, 131, 6r31, 6r35, 8r6
85	32, 52, 66, 85, 87, 6r1, 6r1, 156
86	100
87	1, 4, 19, 21, 25, 56, 71, 79, 80, 87, 148, 6r12, 6r15, 6r24, 8r32
88	49, 66, 128, 6r3, 6r4, 6r32
89	6r5, 6r8, 8r12, 8r21
90	120
91	33, 6r9, 6r10
92	15, 70, 6r2, 8r31, 8r40
93	117, 155
94	103, 127, 6r32, 6r8, 6r19
95	40, 6r34, 8r22
96	18, 112, 116, 6r12, 6r22, 6r25
97	132, 6r25, 8r9
98	3, 8, 23, 28, 37, 88, 155, 8r5, 8r7, 8r17
99	99, 144, 152, 6r32
100	155, 8r33, 8r35
het(unassigned)	32, 112, 114, 6r22, 6r22, 6r25, 8r5, 8r11, 8r15, 8r24, 8r32, 8r38
Y	6r8, 6r19, 8r17, 8r19, 8r31, 8r33
Chr 4	6r21, 6r22?, 8r32?

Table 2. Translocations and their breakpoints listed according to translocation number

Translocation[a]	Cytology[b]
T(2;3)1	43F1-2; 87D4-13
T(2;3)2	38DE; 78B
T(2;3)3	60C2-7; 76A2-3 + T (2;3)42A6-19; 83D5-E1 + In(3)84F12-16; 98C3-D1 New order: 21 to 42A/83D to 84F/98C to 100 60A to 60C/76A to 83D/42A to 60C/76A to 61
T(2;3)4	25BC; 87B4-5
T(2;3)5	42B1-4; 82C2-D1
T(2;3)6	21B2-8; 82F8-83A1
T(2;3)7	24D2-E1; 78C
T(2;3)8	2het; 98D1-2
T(2;3)9	24D1-2; 3het
T(2;3)10	44C5-D1; 84D3-8
T(2;3)11	2het; 3het
T(2;3)12	21E2-F1; 83C2-D1
T(2;3)13	2het; 83E
T(2;3)14	53D3-E1; 79E2-5
T(2;3)15	47B; 92D3-9
T(2;3)16	59C5-D1; 3het
T(2;3)18	51D2-7; 96E5-9
T(2;3)19	43B1-C1; 87D3-E1
T(2;3)20	21B; 74C
T(2;3)21	46F5-47A1; 87B + In(2)34A; 53D New order: 21 to 34A/53D to 46F/87B to 61 60 t0 53D/34A to 46F/87B-100
T(2;3)22	2het; 3het
T(2;3)23	25A2-4; 71F2-72A2; 98D1-2 New order: 21 to 25A/98D to 71F/98D TO 100 60 TO 25A/71F to 61
T(2;3)24	49CD; 3het
T(2;3)25	57A3-B1; 87D1-2
T(2;3)26	42B1-4; 66A
T(2;3)27	56F2-5; 68C
T(2;3)28	2het; 98F1-2
T(2;3)29	30C; 3het
T(2;3)30	21A; 83D2-4
T(2;3)31	32D3-5; 79F3-6
T(2;3)32	43E; 72F3-5; 85F-86A; het New order: 21 to 44E/72F to 61 60 TO 44E/85F to 72F/het het/85F to 100
T(2;3)33	2het; 91F
T(2;3)34	22F2-23A1; 3het
T(2;3)35	2het; 3het
T(2;3)36	43B; 83A
T(2;3)37	2het; 98B
T(2;3)38	55F3-56A1; 64F
T(2;3)39	2het; 3het
T(2;3)40	2het; 95F
T(2;3)41	2het; 3het
T(2;3)42	28A; 3het

T(2;3)43	42A; 82F
T(2;3)44	28A; 63E
T(2;3)45	38B; 3het
T(2;3)46	2het; 67E
T(2;3)47	35AB; 65F
T(2;3)48	2het; 3het
T(2;3)49	47D4-8; 88B
T(2;3)50	35D; 3het
T(2;3)51	38A1-2; 78A1-2
T(2;3)52	2het; 85E5-F1
T(2;3)53	36B2-C1; 62B8-10
T(2;3)54	56C; 70B
T(2;3)55	59C3-D3; 3het
T(2;3)56	45F; 87E
T(2;3)58	2het; 70A
T(2;3)59	38A2-B2; 62F2-63A1
T(2;3)60	30C2-7; 3het
T(2;3)61	22D; 64C
T(2;3)62	22F3-23A2; 55E; 66A8-20 New order: 21 to 22F/66A to 100 60 to 55E/22F to 55E/66A to 61
T(2;3)63	58D; 72D
T(2;3)64	2het; 3het
T(2;3)66	24D3-5; 85C; 88B New order: 21 to 24D/85C to 88B/85C to 61 60 to 24D/88B to 100
T(2;3)67	35B9-C2; 3het
T(2;3)68	35B; 84B
T(2;3)70	2het; 92EF
T(2;3)71	2Rhet; 87A + In(2)59D; 54D New order: 21 to 2Rhet/87A to 100 61 to 87A/2Rhet to 59D/54D to 59D/54D to 60
T(2;3)72	2he; 65D
T(2;3)73	2het; 65F
T(2;3)76	2het; 64E
T(2;3)77	33F3-5; 82D
T(2;3)78	48C1-2; 61C
T(2;3)79[c]	39DE; 87E2-F2
T(2;3)80	2het; 87E
T(2;3)84	36C; 67D1-9
T(2;3)85	48F; 85C
T(2;3)86	33B; 62A
T(2;3)87	23D; 85C; 87B New order: 21 to 23D/85C to 61 60 to 23D/87B to 85C/87B to 100
T(2;3)88	2het; 98C2-D2
T(2;3)89	30A; 35A; 3Rhet New order 21 to 30A/35A to 30A/3Rhet to 61 60 to 35A/3Rhet to 100
T(2;3)90	36C5-E2; 3het
T(2;3)92	2het; 3het
T(2;3)93	57B14-C1; 3het
T(2;3)94	38B; 71E
T(2;3)95	36C; 84B
T(2;3)98	30EF; 3het
T(2;3)99	2het; 99A
T(2;3)100	33B; 86B
T(2;3)102	21F; 22F; 62F; 63E New order: 21A to 21F/63E to 62F/21F to 22F/63E to 100 60 to 22F/62F to 61
T(2;3)103	43F2-44A2; 94D4-E7
T(2;3)104	52D; 65B
T(2;3)105	34C; 71F + t(2;3)35B; 70C New order: 21 to 34C/71F to 100 60 to 35B/70C to 71F/34C to 35B/70C to 61
T(2;3)106	25E2-F2; 3het
T(2;3)108	2het; 3het
T(2;3)109	2het; 3het
T(2;3)111	30A2-7; 65C1-2
T(2;3)112	83E; 29F + 53C; 66A + 96A; het New order: 21 to 29F/83E to 66A/53C to 60 61 to 66A/53C to 29F/83E to 96A/het het/96A to 100
T(2;3)114	41F; 67B + 57F; het New order: 21 to 41F/67B to 61 60 to 57F/het het/57F to 41F/67B to 100
T(2;3)116	42A2-B1; 96C2-D1
T(2;3)117	23D; 93E4-F2
T(2;3)118	34C; 3het
T(2;3)119	32F2-33A1; 82D
T(2;3)120	2het; 3het + In(3)82CD; 90A2-B1
T(2;3)122	36F; 3het
T(2;3)124	48C; 74BC
T(2;3)127	2het; 94D1-2
T(2;3)128	53D; 88D
T(2;3)129	47A1-2; 71C1-2
T(2;3)130	42B2-C1; 3het
T(2;3)131	47A2-B1; 84D4-9
T(2;3)132	2het; 97B6-c2
T(2;3)133	44D5-e1; 64E
T(2;3)134	2het; 3het
T(2;3)136	57D; 3het
T(2;3)140	33B; 82C
T(2;3)141	2het; 66B
T(2;3)144	23A1-2; 99A7-B1
T(2;3)147	34C3-6; 3het
T(2;3)148	22D1-2; 87D
T(2;3)152	30A2-7; 34D; 99B5-C1 New order: 21 to 30A/99B to 61 60 to 34D/30A to 34D/99B to 100
T(2;3)155	60D1-2; 93A1-2; 98C1-2; 100D2-E1 New order 21 to 60D/100D to 100F 60F to 60D/93A to 98C/100D to 98C/83A to 61
T(2;3)156	55E; 85A2-6
T(2;3)6r1	43A3-19; 85E + In(3)67E; 85A4-11
T(2;3)6r2[d]	2het; 92E5-F9
T(2;3)6r3	22B2-5; 88B2-C1 + In(2)55CD; 58D
T(2;3)6r4	41BC; 88D

T(2;3)6r5 2het; 89A
T(2;3)6r6 49A; 80A
T(2;3)6r9[d] 2het; 3het + In(3)83C; 91F
T(2;3)6r10[d] 32A2-B1; 91F
T(2;3)6r12 45F-46A; 87F-88A; 96E
New order: 21 to 45F-46A/96E to 100
61 to 87F-88A/96E to 87F-88A/45F-46A to 60
T(2;3)6r13 49B; 82F
T(2;3)6r14 35B; 3het
T(2;3)6r15[d] 34A; 83BC; 87D
New order: 21 to 34A/83BC to 61
60 to 34A/87D to 83BC/87D to 89C/96A to 89C/96A to 100
T(2;3)6r16 54C; 77D
T(2;3)6r17 2het; 67AB
T(2;3)6r18[d] 60B; 75C
T(2;3)6r20 52C; 64C1-2
T(2;3)6r22[d] het; 96AB + Tp(2;3?)51E6-11; 55F8-12; het
New order: 21 to 51E6-11/55F8-12 to 60
het/51E6-11 to 55F8-12/het
81 to 89C3-7/96A to 89C3-7/96A to 96AB/het
het/96AB to 100
Possible translocation between 3L het and chr. 4
T(2;3)6r23 51D2-E1; 3het
T(2;3)6r24 2het; 87C7-D2
T(2;3)6r25[d] het; 96F4-8 + Tp(2,3)30D; 32A; 97F3-11
New order: 21 to 30D/32A to 30D/97F3-11 to 96F4-8/het
60 to 32A/97F3-11 to 100
61 to 89C/96A to 96F/het
T(2;3)6r26 32CD; 3het
T(2;3)6r27 46BC; 58E; 3het
New order: 21 to 46BC/3het
3het/58E to 46BC/58E to 60
T(2;3)6r28 35A; 3het
T(2;3)6r29 28D4-E1; 61B
T(2;3)6r30 49E3-5; 69A3-5 + In(3)62B-D; 79E
T(2;3)6r31[d] 2het; 84B1-4
T(2;3)6r32[d] 24F; 94D +In(3)88A; ; 99A8-B4
T(2;3)6r33 58B3-5; 59B3-8; 68F6-8
New order: 21 to 58B3-5/59B3-8 to 60
61 to 68F6-8/58B3-5 to 59B3-8/68F6-8 to 100
T(2;3)6r34 31A2-B1; 95CD
T(2;3)6r35 50A; 50EF;84D
New order: 21 to 50A/50EF to 60
61 to 84D/50EF to 50A/84D to 100
T(2;3)6r36 2het; 72E1-F4
T(2;3)8r1 27F; 3het
T(2;3)8r3 34B2-6; 79A2-3 + In(2)42B; 57B3-5
T(2;3)8r4 22A2-B1; 24C2-D1; 60A2-B1; 60E; 3het
New order: 21 to 22A2-B1/24C2-D1 to 60A2-B1/60E to 60 F
61 to 3het/60A2-B1 to 60E/22A2-B1 to 24C2-D1/3het to 100
T(2;3)8r5[d] 22AB; 61C; 66A; 77E; 98B; het
New order: 21 to 22AB/98B to 96A/89C to 96A/89C to 77E/98B to 100
61A to 61C/66A to 77E/22AB to 60
61C to 66A is inserted into het
T(2;3)8r6 56E; 84BC
T(2;3)8r7 2het; 98C2-D1
T(2;3)8r8 2 het; 3 het
T(2;3)8r9 47E; 97C3-D1
New order: 21 to 27F/62D2-6 to 100
60 to 27F/het
61 to 62D2-6/het
T(2;3)8r11 27F; 62D2-6; het
New order: 21 to 27F/62D2-6 to 100
60 to 27F/het
61 to 62D2-6/het
Deficiet for 44D to 45D3-8
T(2;3)8r12[d] 35D4-F1; 89D2-E2
T(2;3)8r13[d] 58A; 76B
T(2;3)8r14 43C2-E1; 3het
T(2;3)8r15 44D; 45D3-8; 47D; 70B4-7; het
New order: 21 to 44D/het
61 to 70B4-7/het
60 to 47D/45D3-8 to 47D/70B4-7 to 100
deficient for 44D to 45D3-8
T(2;3)8r16 2het; 3het
T(2;3)8r18 25D; 3het + In(2)49F; 51D-F
T(2;3)8r20 22B3-7; 63E3-4
T(2;3)8r21 2het; 89B2-C1
T(2;3)8r22[d] 32A2-B1; 75C1-2 + In(3)83E-84B; 95A1-2
T(2;3)8r23 56A2-B1; 65E2-F1
T(2;3)8r24 43C; 68AB +63 CD; het
T(2;3)8r26 2het; 72B
T(2;3)8r27[d] 2het; 75B7-D1
T(2;3)8r30 55F1-56A2; 65CD
T(2;3)8r32[cde] 37D3-7; 87D + Tp(2;3)22A; 35EF; 49AB; 64D-F; 66AB; 81het
New order: 21 to 22A/het
4^{s}?het/22A to 35EF/49AB to 37D3-7/87D to 89C/96A to 89C/96A to 100
het/49AB to 60
61 to 64D-F/81 to 87D/37D3-7 to 35EF/66AB to 64D-F/66AB to 81^{P}
T(2;3)8r34 2het; 66C1-2
T(2;3)8r35 25A4-B2; 100B
T(2;3)8r36 41; 55E; 58E; 73
New order: 21 to 41/58E to 55E/58E to 60
61 to 73/41 to 55E/73 to 100
T(2;3)8r37[d] 2het; 3het

T(2;3)8r38	35; 63F; het	
	New order:	21 to 35/63F to 100
		het/35 to 60
		61 to 63F/het
T(2;3)8r40	32A2-B2; 92A4-10	
T(2;3)8r41	2het; 70C1-2	
T(Y;2;3)6r8	49D-F; 59B-D; 89AB + T(Y;3)94B; Y	
	New order:	21 to 49D-F/59B-D to 49D-F/89AB to 94B/Y
		61 to 89AB/59B-D to 60
		Y/94B to 100
T(Y;2;3)6r19	47B9-14; 66B + T(Y;3)94B;Y	
T(Y;2;3)8r17[d]	21EF; 98A + Tp(Y;2;3)23D; 66AB;Y	
	New order	21 to 21EF/98A to 96A/89C to 96A/89C to 66AB/23D to 21EF/98A to 100
		60 to 23D/Y
		61 to 66 AB/Y
T(Y;2;3)8r19	2het; 68AB; 76A;Y	
	New order:	All euchr. breaks are associated with het
T(Y;2;3)8r31	46BC; 92A11-14 + T(Y;2)58E;Y	
T(Y;2;3)8r33	25E; Y+ T(2;3)51DE; 100AB + In(2)49AB;56A	
T(2;3;4)6r21	2het; 63C + T(3;4)64D; 102D1-4	

[a] Those translocations labelled by numbers with the prefices "6r" or "8r" were induced in *cn bw* with 6000 and 8000 rads, respectively, of γ radiation, while those labelled only with a number were induced in Ore-R with 2000 rads.

[b] New orders involving heterochromatic (het) breakpoints are shown with the centromeric regions intact. This is because it is not known in which chromosome arm the heterochromatic break resides.

[c] The cytology reported here is tentative.

[d] These rearrangements also contain In(3R)89C3-7; 96A [possibly In(3R)Payne] which must have been segregating in the *cn bw* stock which was mutagenized.

[e] The breakpoint in section 81 is very proximal. The proximal broken end is not distinguishable from heterochromatin and is therefore labelled only "het" in the new order.

Hirsch, J. and M. Holliday. University of Illinois, Urbana-Champaign, Illinois USNA. Response to divergent selection for excitatory conditionability in *D. melanogaster*.

Figure 1.

We reported two failures followed by success in our attempts to breed selectively for conditionability.

The attempts to use classical conditioning as a phenotype in the genetic analysis of Diptera have been fraught with problems and confusion (Hirsch and Holliday 1988, Ricker et al. 1986). So, the results we report now have both methodological and substantive importance. Holliday and Hirsch (1984, 1986a&b) reported the first successful procedure for achieving classical excitatory conditioning in identified individual *D. melanogaster*. With that accomplished, we attempted to breed selectively on the basis of individual differences in performance.

Our first attempt to breed selectively for conditioning with acquisition as the criterion produced selected lines the "conditioning" performance of whose animals was indistinguishable from excitation when compared with proper control groups, despite the fact that the conditioning performance of the foundation population *is* distinguishable from that of proper excitation controls.

As reported in Holliday and Hirsch (1986a,b), the class of flies that exhibits successful acquisition contains two subclasses, one that extinguishes and another that does not. Therefore, in the later attempts to breed for conditionability, we included successful extinction in the

selection criterion, i.e., acquisition plus extinction (the second attempt, which bred only for a "good" line, was lost to infertility). As shown in Figures 1, 2 and 3, with the new criterion divergent selection now succeeds in producing a conditioning line with a high level of performance. The result represents a truly behavior-genetic accomplishment in that neither behavior (conditioning), nor genetic, theory alone suggests what has now proved to be the correct selection criterion.

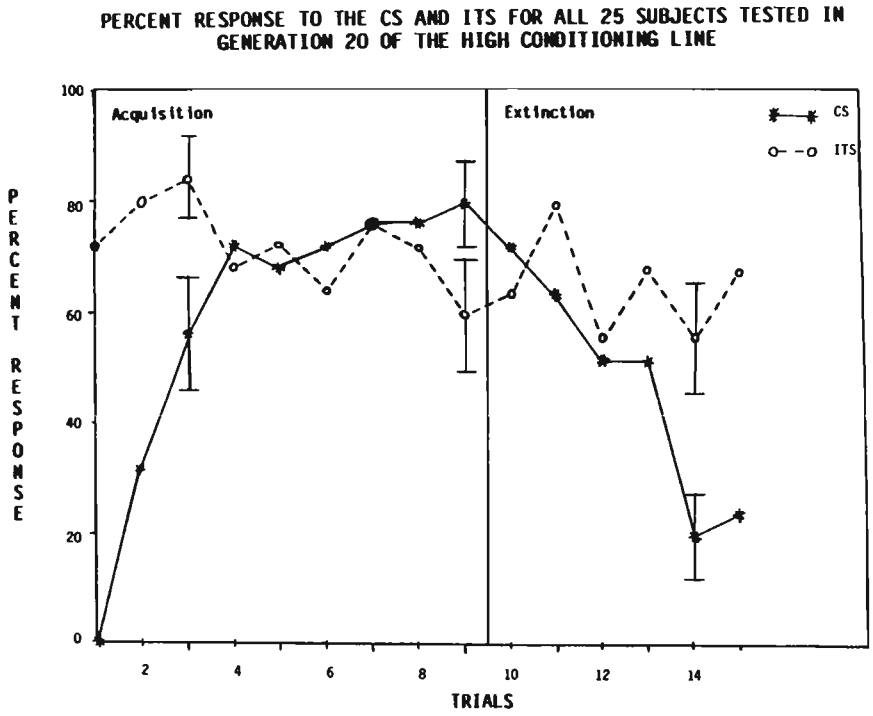

Figure 2.

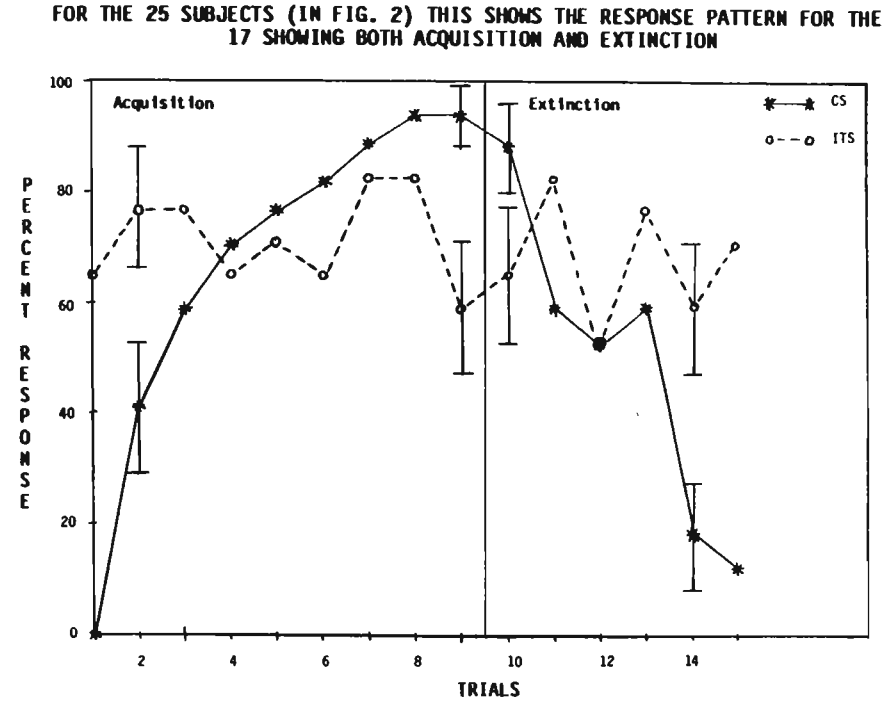

Figure 3.

We have also produced a line selected for poor conditioning in which in most but not all generations there is a small proportion of animals that do condition, 0.00 to 0.08. Interesting properties of these results to which we draw attention are: (1) In the High Conditioning Line the curve in Fig. 2 representing the response to the conditioned stimulus (CS) now climbs above that for the response to the intertrial stimulus (ITS), which is measuring, discharging and controlling the sucrose-induced central excitatory state (CES) on each trial (for a discussion of the relation between CES and conditioning see Holliday and Hirsch 1984, 1986a; Ricker et al. 1986). In fact, the acquisition curve for the subset of "good conditioners" now approaches 100% responding (see Fig. 3), a level comparable to what has been reported for the honeybee (Bitterman et al. 1983). (2) Unselected wild-type Control Line conditioning curves are shown in Holliday and Hirsch (1984, 1986a&b), comparing the CS response curves for the High (Generation 20) and Control Lines Yields $X^2 = 45.24$, $p < 0.001$ and $X^2 = 14.88$, $0.01 < p < 0.05$ for acquisition and extinction, respectively. So, they are markedly different. Comparing the ITS response curve for the subset of 17 "good conditioners" from the High Line (shown in Fig. 3) to the ITS curve from the poor conditioning line, i.e., the excitatory levels for the two cases which might be expected to show the greatest difference, reveals no difference between these two extreme possibilities ($X^2 = 5.92$, where for a $p < 0.05$ X^2 would have to = 23.7).

We now have control of CES and are manipulating the conditioning effects independently of it, both behaviorally and genetically.

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Homyk, T. Jr., . University of Virginia, Charlottesville, VA. Interactions between two ether shaking mutant strains produces a novel, age dependent behavioral phenotype.

During a survey for intergenic interactions between mutations in separate genes affecting muscle development (Homyk and Emerson, 1988), a novel interaction was discovered among the F_1 female progeny of crosses involving *Flu/FM6* female and Sh^{101}/Y male parents. When raised at 29°, the *Flu* +/+ Sh^{101} doubly heterozygous mutant progeny hopped and flew poorly compared to either single heterozygote. When maintained at 22°, these doubly mutant progeny walked and climbed normally up through seven days after eclosion. After this time, however, there was a gradual loss of function of the mesothoracic legs, first noticeable in their lack of use for climbing.

In one experiment, twenty individual *Flu* +/+ Sh^{101} mutant heterozygotes were followed which survived four weeks post eclosion. By two weeks of age, both mesothoracic legs had become paralyzed in five individuals, one mesothoracic leg had become paralyzed in two and one prothoracic leg had become paralyzed in yet another. All legs in the remaining twelve flies were normal. At the end of three weeks, paralysis of both mesothoracic legs was evident in twelve, one mesothoracic leg was paralyzed in two, and one mesothoracic and one prothoracic leg were paralyzed in one of the double mutants. All legs were normal in the remaining five doubly mutant heterozygotes. By four weeks post eclosion, nineteen of the doubly mutant heterozygotes had developed paralysis of both mesothoracic legs and one was paralyzed in one mesothoracic and one prothoracic leg.

In general, paralysis was accompanied by formation of a black spot, resembling a wound reaction, at the region of the trochanter and the femural coxal joint. In two repetitions of the cross, similar dark spots were occasionally observed in the probosci and prothoracic legs. It is notable that none of the Sh^{101}/*FM6* sibs from these crosses showed the paralytic phenotype, suggesting that one factor involved in the interaction is on the X-chromosome bearing the *Flu* mutation.

Sh^{101}/Sh^{101} and *Flu/FM6* mutants from the parental strains as well as Sh^{101}/+ and *Flu*/+ single heterozygous mutant progeny, from crosses of the respective parental strains to Oregon-R, did not express the phenotype when tested identically. Two of thirty-one *Flu/Y* hemizygous males from the latter crosses, however, did develop paralysis in one mesothoracic leg during a four week observation period.

Limited genetic mapping of the interacting factor in the Sh^{101} parent strains was performed using one *y cv v f* Sh^{101} and one *y cv v f* Sh^{+} recombinant strain. In this study, only relevant heterozygous progeny from the cross of the *y cv v f* Sh^{101} recombinant strain to *Flu/FM6* females developed paralysis of their mesothoracic leg. This data suggest that the Sh^{101} mutation in this strain is responsible for the novel interaction.

No genetic analysis has yet been performed to further identify the factor involved in the interaction in the *Flu/FM6* strain, although the results above show that it is X-linked. As noted by Deak et al. (1982), the *Flu* mutation causes a dominant ether induced leg shaking phenotype similar to that described for *Sh* mutations. That the genetic interaction observed here results from the interaction of the mutant *Flu* and Sh^{101} mutant gene products presents an interesting possibility. Since *Sh* encodes a component of K^+ channels (Tempel et al., 1987; Pongs et al., 1988; Kamb et al., 1988), this would imply that *Flu* might encode some product involved in K^+ channel function. In this regard, it is notable that the location of *Flu* on the X-chromosome of *D. melanogaster* (Deak et al., 1982) is distinct from the location of *Shaker* and from the locations of three additional *Shaker*-like genes recently reported by Butler et al., (1989).

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Imasheva, A.G., Lazebny, O.E. and **Zhivotovsky, N.I.** Vavilov Inst. of General Genetics, Moscow, USSR. Quantitative wing variation in two wild populations of *D. melanogaster*.

As part of an extensive survey of variation in natural populations of *D. melanogaster* of the Soviet Union, two populations of the European part of the USSR were examined for variation in morphometric wing characters.

Flies were collected at two localities situated near Kishinev (Moldavia) and Vilino (Bakhchisaray region) in late September 1988. For each locality 30 iso-female lines were established from wild-caught females fertilized in nature. From the progeny of each of the founder females, 5 males and 5 females were used as parents for the second generation. 50 first-instar F_2 larvae of each line were placed in a separate vial to avoid density effects. After emergence 5 females were sampled from each vial thus giving a total sample of 150 flies per locality. Left wing of each fly was removed, mounted on a slide and measureed using an ocular micrometer. 10 measurements were recorded on each wing (Fig. 1). Wings of the founder flies were also measured.

Table 1 shows means and standard deviations for the ten characters in Kishinev and Vilino populations. Generalized population parameters calculated according to Zhivotovsky (1980) are presented in Table 2.

Table 1. Means ($\bar{\chi}$) and standard deviations (s.d.) of 10 wing characters in two populations of *D. melanogaster*.

Population	Item	Characters 1	2	3	4	5	6	7	8	9	10
Kishinev Parents	$\bar{\chi}$	102	111	118	80	69	86	88	37	79	49
	s.d.	7.68	8.27	7.54	5.45	4.79	5.76	5.67	2.67	5.74	2.92
F_2	$\bar{\chi}$	98	106	113	76	66	84	85	36	76	47
	s.d.	3.29	3.55	3.27	2.56	2.44	2.86	2.60	1.47	2.36	1.67
Vilino Parents	$\bar{\chi}$	98	108	115	77	67	83	85	36	77	47
	s.d.	7.35	8.40	8.11	5.56	5.14	5.74	5.90	2.55	5.41	3.48
F_2	$\bar{\chi}$	96	104	110	75	64	83	86	36	75	45
	s.d.	4.30	4.58	4.04	3.01	2.57	2.81	2.55	1.33	2.19	1.75

Table 2. Generalized means ($\bar{\chi}$), variances (s.d.^{2}), coefficients of variation (CV) and correlation coefficients (r) of 10 wing characters in two populations of *D. melanogaster*.

Population		$\bar{\chi}$	s.d.^{2}	CV	r
Kishinev	P	77.7	3.53	2.36	0.936
	F_2	74.7	2.40	2.07	0.740
Vilino	P	75.3	3.35	2.49	0.937
	F_2	73.4	2.90	2.32	0.733

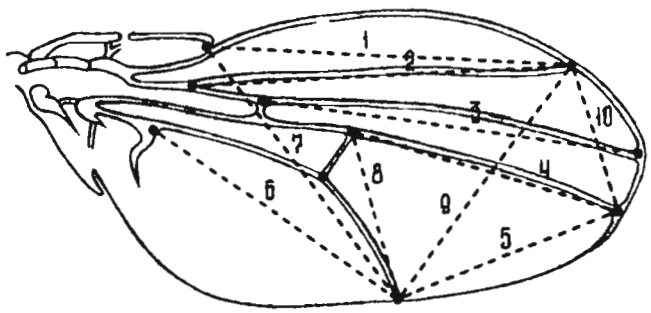

Figure 1. Wing measurements.

Means of each of the ten characters as well as their generalized means are greater in the Kishinev as compared to the Vilino population both for wild-caught (parents) and laboratory-reared (F_2) flies. This is not surprising as Kishinev is situated some 300 km to the north of Vilino. In *Drosophila*, wing length is strongly correlated with body size (Reeve and Robertson, 1953). Fruit flies seem to obey the Bergmann rule which states that body size in animal species increases with latitude. There are at least two well-documented clines showing latitudinal increase in wing length in *D. melanogaster*: between Europe and equatorial Africa (David, et al., 1977) and along the East coast of the United States (Coyne and Beecham, 1987). We believe that Kishinev and Vilino populations constitute a part of a similar cline. Independent occurrence of such parallel clines on different continents suggests natural selection as the cause of geographic variation in wing/body size in *D. melanogaster*.

Phenotypic variation in the wing characters, both taken separately (Table 1) and as a character complex (Table 2), is higher in the wild-caught than in laboratory-reared flies which evidently reflects more variable environmental

conditions encountered by flies in nature. However, this difference is much smaller than reported earlier (David, 1979).

Correlation between the characters is substantially weaker in F_2 as compared to the parental generation. Analysis of principal components of the covariance matrices shows that lower values of correlation coefficients in laboratory-reared flies result from the decrease of the fraction of extreme phenotypes with large (M_+) and small (M_-) values of all characters (Zhivotovsky, 1988).

Table 3. Coefficients of correlation between 10 wing characters (Kishinev population). Above diagonal - parents; below diagonal - F_2.

	1	2	3	4	5	6	7	8	9	10
1	—	0.978	0.931	0.847	0.865	0.931	0.938	0.841	0.920	0.654
2	0.903	—	0.951	0.871	0.888	0.932	0.945	0.878	0.941	0.711
3	0.780	0.756	—	0.933	0.921	0.896	0.907	0.892	0.931	0.776
4	0.625	0.617	0.737	—	0.936	0.800	0.807	0.921	0.885	0.713
5	0.615	0.588	0.684	0.792	—	0.809	0.826	0.855	0.918	0.734
6	0.696	0.723	0.706	0.523	0.407	—	0.982	0.859	0.879	0.700
7	0.715	0.715	0.722	0.540	0.430	0.902	—	0.872	0.903	0.744
8	0.357	0.377	0.415	0.588	0.280	0.560	0.622	—	0.895	0.732
9	0.714	0.651	0.627	0.579	0.664	0.529	0.623	0.442	—	0.804
10	0.219	0.224	0.485	0.401	0.477	0.369	0.449	0.243	0.450	—

Table 4. Coefficients of correlation between 10 wing characters (Vilino population). Above diagonal - parents; below diagonal - F_2.

	1	2	3	4	5	6	7	8	9	10
1	—	0.977	0.930	0.926	0.902	0.922	0.924	0.802	0.942	0.776
2	0.937	—	0.893	0.911	0.892	0.915	0.916	0.779	0.929	0.768
3	0.788	0.793	—	0.947	0.919	0.919	0.923	0.821	0.948	0.867
4	0.549	0.544	0.669	—	0.952	0.906	9.916	0.867	0.952	0.883
5	0.392	0.431	0.527	0.725	—	0.848	0.873	0.760	0.943	0.844
6	0.735	0.719	0.704	0.407	0.192	—	0.980	0.864	0.899	0.799
7	0.700	0.663	0.590	0.359	0.181	0.864	—	0.862	0.919	0.837
8	0.401	0.387	0.346	0.516	0.211	0.511	0.620	—	0.832	0.754
9	0.590	0.620	0.488	0.638	0.660	0.395	0.499	0.532	—	0.859
10	0.089	0.105	0.363	0.449	0.486	0.258	0.267	0.242	0.438	—

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Islam, M. Saiful. Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh. The directional and relaxed selection for hatchability in *Drosophila melanogaster*.

Among the three principal patterns of selection, viz., stabilizing, disruptive and directional, that are important in quantitative genetics, the directional one can be extremely useful in improving the yield of metric traits of an organism (Clayton *et al.*, 1957; Falconer, 1953, 1973; Hossain *et al.*, 1974; Nagai *et al.*, 1978). The directional selection, which can be split up into the forward and reverse regimes, involves breeding from those individuals closest to the extremes, and the elimination of those that manifest the phenotypes less desirable in terms of performance. The purpose of this selection, therefore, is to increase the frequency of the most favourable genes in the population. Several experiments with plants and animals show that the effect of selection may be different when selection is carried in high and low lines. This has been the cases of selection for oil content of maize kernels (Woodworth *et al.*, 1952); abdominal bristles (Mather and Harrison, 1949), sternopleural bristles (Thoday and Boam, 1961; Spickett, 1963; Haque *et al.*, 1984; Rahman, 1985), thorax length (Robertson, 1955), fertility (Hossain *et al.*, 1974) and body-size (Baptist and Robertson, 1976) in *Drosophila*; body size and body-weight inheritance in mice (Falconer, 1977; Eklund and Bradford, 1977) and so on.

Table 1. Effects of the forward, reverse and relax selection on hatchability of the *CyL/+* females of *D. melanogaster*.

Generations		Forward selection		Reverse selection*		Relax selection**	
		High line (mean ± S.E.)	Low Line (mean ± S.E.)	High line (mean ± S.E.)	Low Line (mean ± S.E.)	High Line (mean ± S.E.)	Low Line (mean ± S.E.)
P	55.60 ± 4.57	—	—	65.98 ± 2.67	42.40 ± 2.58	66.50 ± 1.91	42.81 ± 2.38
1		56.11 ± 3.95	51.69 ± 3.47	65.09 ± 2.60	44.01 ± 2.43	63.66 ± 2.68	44.85 ± 2.67
2		57.60 ± 3.70	48.99 ± 2.81	62.68 ± 3.64	45.28 ± 2.37	61.18 ± 3.89	46.19 ± 2.76
3		58.46 ± 3.41	46.65 ± 2.87	60.48 ± 3.98	48.05 ± 2.85	59.54 ± 3.77	49.99 ± 3.25
4		58.89 ± 2.39	45.38 ± 2.56	59.38 ± 3.66	52.13 ± 3.62	59.39 ± 2.69	52.20 ± 3.40
5		59.72 ± 2.47	43.65 ± 2.27	58.70 ± 3.01	55.45 ± 3.60	58.82 ± 3.08	52.78 ± 2.71
6		61.70 ± 3.19	43.56 ± 1.80	58.32 ± 2.30	57.56 ± 3.57	57.23 ± 3.30	54.40 ± 2.58
7		62.58 ± 3.38	42.90 ± 2.42	57.10 ± 2.98	58.21 ± 3.31	56.19 ± 3.93	54.73 ± 3.98
8		65.23 ± 3.12	43.47 ± 2.80	55.18 ± 2.88	59.52 ± 2.01		
9		66.17 ± 3.00	42.64 ± 1.98	55.38 ± 2.81	59.69 ± 2.26		
10		66.48 ± 3.16	42.47 ± 2.56	54.93 ± 3.12	59.09 ± 2.28		
11		65.98 ± 2.67	42.40 ± 2.58	52.60 ± 3.20	59.30 ± 1.97		
12		66.10 ± 2.82	42.07 ± 2.81	51.87 ± 1.94	59.97 ± 1.95		
13		66.22 ± 3.15	42.94 ± 2.77	49.72 ± 2.47	60.14 ± 1.99		
14		66.55 ± 1.83	42.76 ± 2.79	47.19 ± 2.35	60.28 ± 2.23		
15		66.77 ± 2.02	42.69 ± 2.45	46.54 ± 2.31	61.00 ± 2.19		
16		66.51 ± 2.43	42.79 ± 2.49				
17		66.63 ± 2.20	42.94 ± 2.51				
18		66.50 ± 1.91	42.81 ± 2.38				

P = Parental generation;
* Initiated after 11th generation of forward selection;
** Initiated after 18th generation of forward selection.

Table 2. Significance tests for mean hatchabilities between different generations of the forward, reverse and relax selection regimes in *D. melanogaster*.

Selection regimes	Generations Tested	High line t-values	Low line t-values
Forward	F_1 - F_{10}	3.1579 (<0.01)	3.4573 (<0.01)
	F_{10} - F_{18}	0.2952 (ns)	1.8372 (ns)
Reverse	P - F_{10}	2.9819 (<0.01)	10.1118 (<0.001)
	F_{10} - F_{15}	2.2234 (<0.05)	0.9874 (ns)
Relax	P - F_4	2.1352 (<0.05)	2.9834 (<0.01)
	F_4 - F_7	1.0492 (ns)	2.0193 (ns)

Figures in parentheses indicate probabilities at 22 df;
ns = not significant; P = Parental generation.

Of course, perplexing cases occasionally arise where no response to artificial selection is observed in population, even though genetic variability is present (Falconer and King, 1953; Rasmuson, 1955; Hill, 1977). The present experiments were designed to investigate the effects of the forward and reverse selection (directional) for the high and low productivity in *D. melanogaster* measured by the mean hatchabilities recorded in each generation, and of the relax selection that was helpful in evaluating the strength and nature of the directional selection under study. The primary objective of the forward selection program was to obtain lines which were homozygous for polygenic modifiers causing an increase and decrease in hatchabilities in the high and low lines respectively. While, the reverse selection was aimed at to give a possible explanation of the plateau noted in both high and low lines of the forward selection regime.

Single pair matings between the virgin Cy L/+ females (derived by crossing Cy L/Pm flies with wild-type, +/+ flies) and unmated +/+ males were used throughout the experiment in the forward and reverse selection lines. For each line in every generation, consecutive 24-hrs egg collections were taken in 12 replicates (vials of 6 cm x 3 cm size) provided with the classical Agar-molasses-cornmeal-yeast food medium, to which powdered charcoal was added to increase the contrast between the eggs and the medium that facilitated egg counting. For the measurement of hatchabilities, the number of eggs laid and the corresponding number of hatched eggs were recorded by observing them under 30x magnification of a binocular microscope in good-illumination. In the forward selection regime, the high line was maintained by taking the flies from the vial showing the highest mean hatchability in each generation; like wise, the low line was continued by taking the flies of the lowest mean hatchability. Meanwhile, after 11 generations, flies from the high and low lines were subjected to reverse selection regime, in which the high and low lines were maintained by choosing the flies just opposite to those for the forward lines. A suspension of selection for 7 successive generations was made after 18th generation of the forward selection. During such relax selection, however, no selection, neither in the high nor in the low line, was in operation when the flies in respective lines were allowed to mate at random. All the experimental flies were reared in the incubator at $25 \pm 0.5^{\circ}C$.

Responses to the forward, reverse and relax selection have been presented in Table 1. The mean hatchability in the forward selection regime increased gradually from 55.60 ± 4.57 in the parental generation to 66.50 ± 1.91 and decreased

to 42.81 ± 2.38 in the high and low lines respectively in course of 18 successive generations. Apparent plateau was noted after 10 generations in the high and after 9 generations in the low line. In the reverse selection regime, the high line decreased from 65.98 ± 2.67 to 46.54 ± 2.31 and the low line increased from 42.40 ± 2.58 to 61.00 ± 2.19 during a period of 15 generations. Response to the relax selection regime was interesting in that both high (decreased from 66.50 ± 1.91 to 56.19 ± 3.93) and low (increased from 42.81 ± 2.38 to 54.73 ± 3.98) lines recovered almost to the unselected parental generation level (55.60 ± 4.57) within 7 generations. Significance test values (Table 2) show that response to forward selection in both high and low lines was significant ($P < 0.01$) during the first 10 generations, thereafter, insignificant responses were the indices of plateau; responses to reverse selection were significant except for F_{10}-F_{15} in the low line, and responses to relax selection were significant up to F_4 generation in both lines.

That the population having the largest genetic variability offers the greatest chance for improvement by selection has been justified by the forward selection regime in the present experiment. Similar results have been reported for sternopleural bristles (Spickett, 1963; Rahman, 1985) and fertility (Hossain *et al.*, 1974) in *Drosophila* and for body-weight (Falconer, 1973) and nursing ability (Nagai *et al.*, 1978) in mice. Moreover, it has been shown that selection accompanied by temperature-shock at pupal stage may increase the hatchability (Islam and Hossain, 1987) and the sex-ratio in favour of males (Islam and Siddique, 1988) in *Drosophila*. In this experiment, apparent limits were reached after about 11 generations beyond which further selection gave no response. It appears that, despite the appearance of a plateau, there was still heterozygosity in both lines at some loci controlling the hatchability which could be utilized in the reverse selection. The fact that the original selection limits were not reached was tested by reversing the direction of selection after 11 generations. The result was an exchange of levels in the mean hatchabilities by both lines. The apparent selection limits were, therefore, due to the exhaustion of relevant genetic variability. The most probable alternate explanation is that the observed selection limits were reached because of a conflict between the natural and artificial selection. To test this, selection was relaxed. As a result, the mean hatchabilities in both lines were found to have converged to virtually the same as the original unselected level. It is very likely, therefore, that the directional selection for the trait under study was powerfully opposed by the natural selection, and when the former was removed, the latter rapidly restored the original level. These results conform to those reported by Mather and Harrison (1949), Robertson (1955), Yamada *et al.* (1958) and Hossain *et al.* (1974).

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Ivannikov, A.V. Inst. of Cytology and Genetics, Novosibirsk, USSR. Synantropic *Drosophila* species in the Central Tajikistan.

The catching of synantropic flies in different antropogenic landscapes in the Central Tajikistan (USSR) was performed in 1988-1989. To collect flies, 30 points were evenly set throughout the Hissar Valley and Dushanbe-city (fruit and vegetable gardens, human habitats, enterprises for vegetable and fruit processing, vine factories). Single fly collections were made in the valleys of the Javan-Su and the Vahksh rivers (in similar landscapes). The material obtained represented five species relating to three subgenera of the *Drosophila* genus:

Drosophila (Dorsilopha) busckii Coquillett, 1901
Dros. (Drosophila) funebris Fabricius, 1787
Dros. (Dros.) hydei Sturtevant, 1921
Dros. (Dros.) immigrans Sturtevant,1921

Dros. (Sophophora) melanogaster Meigen, 1830

D. melanogaster is being distributed widely and on a mass scale. *D. funebris* and *D. busckii* are rarely found and only isolated instances are registered. Presence of *D. immigrans* and *D. hydeii* is typical of store houses and enterprises for vegetable and fruit processing.

Jaenike, J., University of Rochester, Rochester New York, USA, Across-environment heritability of wing length.

Gillespie and Turelli (1989) have suggested that much additive genetic variance for polygenic characters under stabilizing selection may be maintained in a population if the additive contributions of alleles depend upon the environment in which they are expressed. Such genotype-environment interactions may be revealed by correlating the phenotype of parents reared in one environment with that of their offspring reared under different conditions. For instance, Prout (1958) found a negative, but nonsignificant, correlation between the wing length of wild-caught males of *D. melanogaster* and their lab related offspring. In contrast, Coyne and Beechem (1987) found a significant positive correlation between the wing lengths of wild caught parents and their lab-reared offspring in this species. Here I present some old data that bears on this question.

Inseminated females of the *D. affinis* subgroup were collected on and around Deer Isle, Maine in June and July of 1972. They were allowed to oviposit in vials containing Carolina Instant Drosophila medium for a period of three days at 21°C., after which they were removed. Their wing lengths were measured from the anterior crossvein to the tip of the third longitudinal vein. Offspring developed at 21°C. When they had emerged, the wing lengths of 10 (or as many as were available if less than 10) female F1 were noted.

For none of the three species studied was there a significant relation between the number of offspring that emerged and average wing length, thus suggesting that neither larval facilitation nor crowding had a major influence on offspring wing length (Table 1). The correlations between the wing lengths of wild-caught mothers and their lab-reared daughters were nonsignificant in all three species (Figure 1).

The lack of a positive heritibility of wing length across environments may be explained in a number of ways.

Table 1. Correlations between number of offspring emerging in a family and mean wing length of F1 females.

Species	Number of families	Mean number of offspring (n)	Correlation between n and mean wing length
D. affinis	73	14.9	0.05
D. algonquin	91	17.9	0.09
D. athabasca	186	27.1	0.06

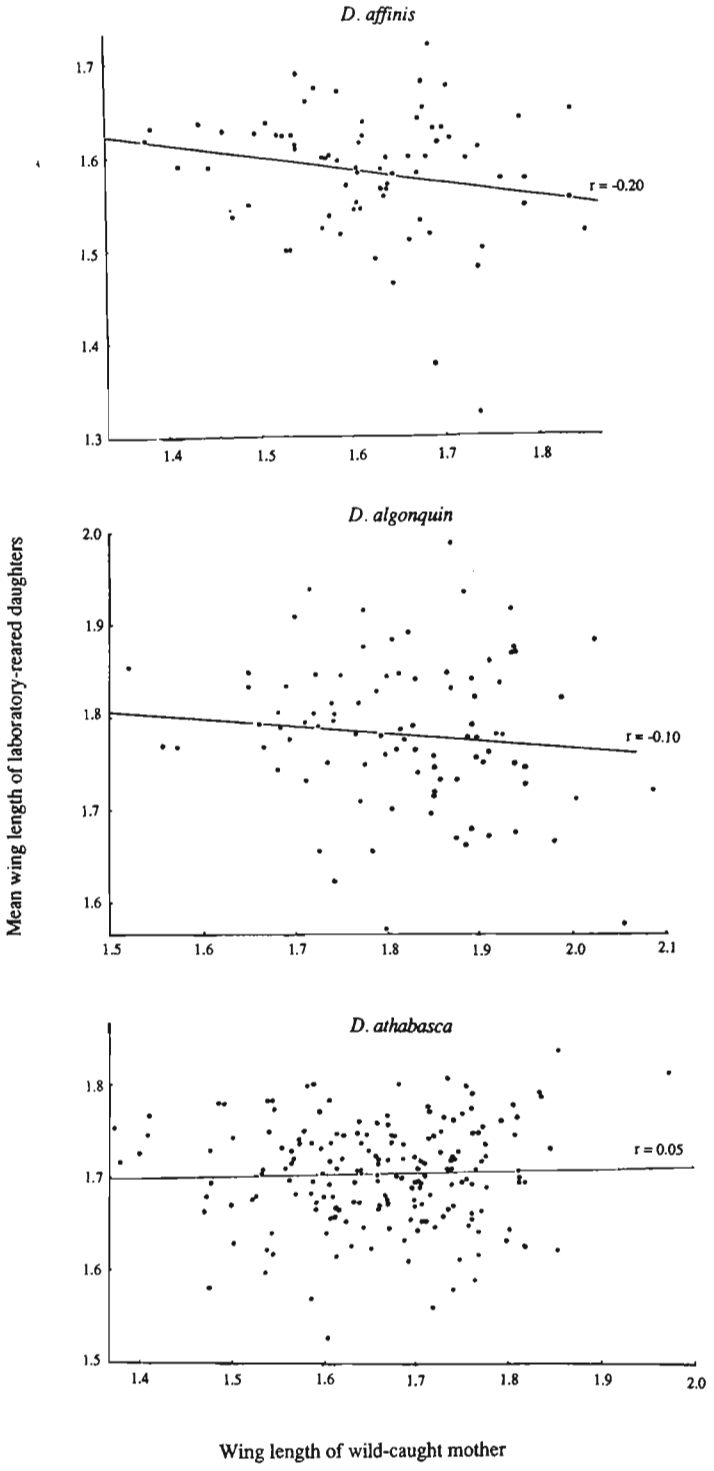

Figure 1. Correlations between wing lengths of wild-caught females and lab-reared daughters in three members of the *D. affinis* subgroup. All measurements are in mm.

1. It is possible that all threee species lack variance for wing length. This is most unlikely, as it has been found in all other *Drosophila* species that have been studied under controlled conditions. 2. Another rather improbable explanation is that there is negative assortative mating with respect to wing length in the wild. 3. As Gillespie and Turelli (1989) suggest, genotype-environment interactions will reduce parent-offspring correlations when they are reared in different environments. 4. Finally, overwhelming environmental effects, either in the wild (females having developed on a variety of breeding sites at different temperatures and degrees of larval crowding) or the lab (e.g., cultures being inoculated with different microbial species by different mothers) may mask any underlying positive genetic correlation between wild-caught mothers and their lab-reared offspring.

References: Coyne, J.A. and E. Beecham, 1987, Genetics 117:727-737; Gillespie, J.H. and M. Turelli, 1989, Genetics 121:129-138; Prout, T., 1958, DIS 32:148-149.

Jain, Jaagrati[1] and R.P. Sharma.[2] [1]Division of Vegetable Crops and [2]Biotechnology Center, Professor, Nucl. Res. Lab, Ind. Agr. Res. Inst., New Delhi-110012, India. Effect of neurotoxins on 'stambha' temperature sensitive paralytic mutants of *Drosophila melanogaster.*

The effect of two neurotoxins, veratrine -- a gating modifier of sodium channels and tetradotoxin (TTX) -- a sodium channel antagonist, has been studied by feeding adult flies of *stm* mutants and Oregon-k. The results have revealed that of the three mutants, only *stm-A* exhibits reduced sensitivity to veratrine and no significant difference in sensitivity to TTX, as compared to Or-k flies. The reduced sensitivity to veratrine could be due to a qualitative alteration in the veratrine binding site of sodium channels which impairs its functioning.

Introduction: In recent years, the use of neurotoxins has gained much importance in outlining the various components of nervous system and their function and also to provide an insight into the various molecular mechanisms underlying the behavior. The value of neurotoxins lies in their unique mechanism of interactions with neuronal systems. A number of neurotoxins have been employed to characterize neurological defects of behavio.ural mutants (Armstrong and Binstock, 1965; Yeh *et al.* 1976; Satow and Kung, 1976 a,b, Jan *et al.*, 1977; Tanouye *et al.*, 1981; Hall, 1982). In the present study, the effect of two neurotoxins such as veratrine and tetradotoxin has been studied on stambh (*stm*) the second chromosome temperature-sensitive paralytic mutants (Shyngle and Sharma, 1985) and wild type (oregon-k) flies of *Drosophila melanogaster* to elucidate the possible molecular lesions underlying the paralytic behaviour.

Materials and Methods:

Stambh mutants: The three 'stambh' mutants used in the present study are recessive, monogenic, non-allelelic temperature sensitive paralytic mutants located on the second chromosome (Shyngle and Sharma, 1985). Mutants *stm-A* and *stm-B* show a summation effect of temperature on paralysis, whereas *stm-C* shows a sharp transition temperature for paralysis. Of the three mutants, *stm-A* also shows a temperature dependent, maternally determined embryonic lethality.

Oregon-k (Or-K); It was used as control.

Neurotoxin treatment:

Veratrine: Veratrine (Sigma) at concentrations ranging from 10 ug/ml to 1000 ug/ml, made in *Drosophila* Ringer solution was fed to 4-5 days old males of *Drosophila*. The treatment was effected by feeding the neurotoxin for 10-12 hrs. to pre-starved males through saturated filter paper discs for 20 hrs. Each treatment had two replicates of 50 flies and was repeated five times. The treatment was terminated by transferring the males to fresh food. A count on the number of dead males was made after 15 hrs. Kill curves for Or-k and *stm* flies were plotted against various concentrations of veratrine. The medium lethal dose (LD_{50}) of veratrine was calculated by using probit analysis (Finney, 1971).

Tetradotoxin: Of the three *stm* mutants, only *stm-A* showed less sensitivity to veratrine than Or-k flies, effect of TTX was studied only on this mutant.

TTX (Sigma) was dissolved in 0.05 M citrate buffer at pH 4.8 (Kelly, 1974) and employed at concentrations ranging from 1 ug/ml to 9 ug/ml. The feeding procedure, period of feeding and the calculation procedure for LD_{50} value was the same as outlined for veratrine treatment.

Results:

Effect of veratrine: The kill curves plotted in Fig. 1 indicated an increase in the percent kill of flies with increase in veratrine concentration. The dose-lethality relationship was found to be linear when expressed as log concentration and probit of kill (Fig. 2). Accurate values of LD_{50} with fiducial limits (Table 1) were obtained for the three *stm* mutants and Or-k flies using probit analyses. These results showed that LD_{50} for *stm-B* and *stm-C* flies were within the fiducial limits of LD_{50} value of OR-k flies. *stm-A* on the other hand, had a higher LD_{50} value with fiducial limits beyond the LD_{50} range of Or-k. These results suggested that *stm-A* flies are relatively less sensitive to veratrine as compared to other two

mutants and Or-k.

Effect of tetradotoxin: The effect of TTX, expressed as percent kill of flies (Fig. 3) showed an increase in the percent lethality with increase in TTX concentration. A dose of 9 ug/ml proved to be completely lethal to both Or-k and *stm-A* flies. The LD_{50} value for *stm-A* obtained by plotting TTX concentrations against percent lethality, was not significantly different for Or-k control.

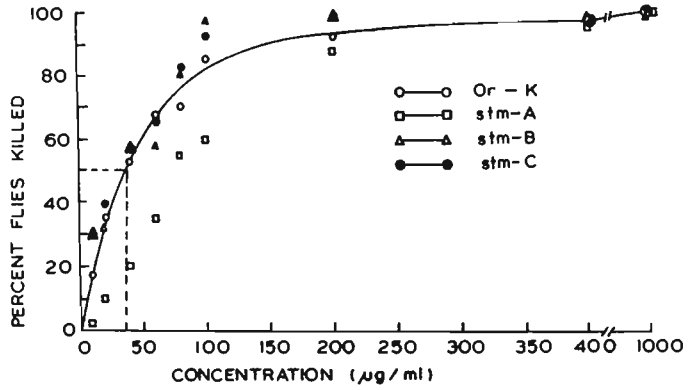

Figure 1. The effect of veratrine feeding on survival of Or-K and *stm* flies.

Table 1. The LD_{50} values of veratrine for Or-k and stm mutants obtained by using probit analysis (Finney, 1971).

Strain	Estimated LD_{50} (ug/ml)	Fiducial limits for LD_{50} values	
		Lower limit	Upper limit
Or-k	33.57	41.78	26.98
stm-A	77.15	89.95	66.07
stm-B	24.52	26.45	22.73
stm-C	26.00	35.08	19.23

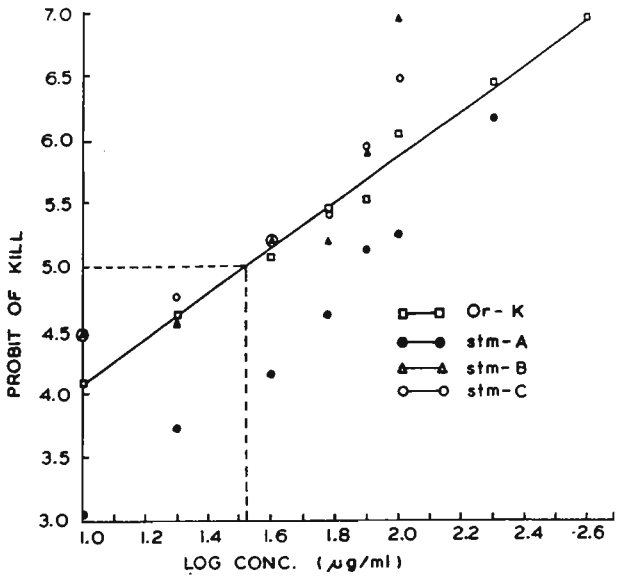

Figure 2. Relation between probit of kill of male flies and dose of veratrine (log conc. ug/ml).

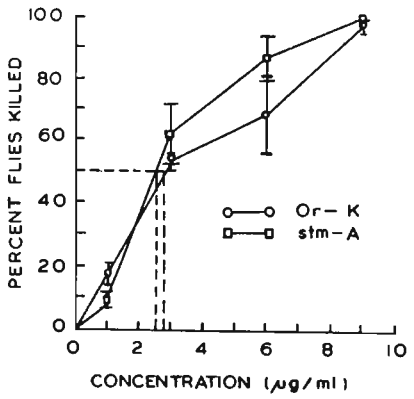

Figure 3. The effect of tetradotoxin feeding on Or-K and *stm-A* male flies.

Discussion: The property of neurotoxin specificity in binding to various molecular components of the neural system has been utilized to unravel the nature of lesion in *stm* mutants. The results obtained show that of the three *stm* mutants studied, only *stm-A* depicts differential sensitivity to neurotoxins used. This suggests that the molecular lesions in these mutants are most probably different. Neurotoxins such as tetradotoxin (TTX) and saxitoxin (STX) have been employed successfully (Wu and Ganetzky, 1980; Gitschier *et al.*, 1980; Hall *et al.*, 1981; Kauver, 1982) to characterize the molecular nature of lesions in $para^{ts}$ and nap^{ts} paralytic mutants. Effect of veratridine (employed in the form of veratrine in this study) was studied by Albuquerque and Warnick as early as 1972. They suggested that it probably binds to the regulatory or activation site and possibly acts as a gating modifier of sodium channels. Subsequently, Catterall (1975[16], 1977[17], 1980[18]) showed that veratridine increases the sodium channels. TTX, on the other hand, binds to an ion transport site of

sodium channels and blocks the generation of action-potential (Kao, 1966). In the light of this available information, it is tempting to propose that the reduced veratrine sensitivity of *stm-A* flies observed in the present study might be due to an altered veratrine binding regulatory site of sodium channels. Since *stm-A* flies did not differ in their TTX sensitivity from Or-k flies, the density of distribution of sodium channels or TTX binding ion transport site seems to be unaltered in *stm-A*. Further, the reduced veratrine sensitivity of *stm-A* flies at permissive temperature of 25°C suggests that the altered product of *stm-A* gene is present even at 25°C but may not be so severely altered as to interfere with the normal behaviour of *stm-A* flies. The paralytic behaviour at higher temperature can be ascribed to a more severe temperature mediated alteration in veratrine binding sites.

Based on our earlier studies on kinetics of paralysis and recovery (Shyngle and Sharma, 1985) and the present results on neurotoxin sensitivity, it is proposed that a single gene change probably brings about a temperature-dependent qualitative alteration in the regulatory site of sodium channels, which becomes irreversible beyond a certain limit of exposure time to restrictive temperature. Further studies are in progress to test the validity of this preposition.

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Sperm length diversity in *Drosophilidae*.

In a 30-year old issue of DIS, Yanders and Perras (1960 and 1963) first reported the existence of 'impressive' differences in sperm length between *Drosophila* species. Considering the most recent measurements of *Drosophila littoralis* sperm length up to 19 mm (Joly *et al.*, 1989), that would be equivalent in proportions to a 10-meter sperm in men, their qualifying remains highly pertinent. In a 20-year old issue of DIS, Policansky (1970) first mentioned the striking co-occurrence of sperm bundles of different sizes in a testis in *D. pseudoobscura* and *D. persimilis*. This striking phenomenon has been mostly documented and coined as 'polymegaly' by Beatty and coworkers (Beatty and Sidhu, 1970; Beatty and Burgoyne, 1971; Beatty, 1975). Further evidence given by Sanger and Miller (1973), Kurokawa *et al.*, (1974), Joly (1989) and Joly *et al.* (1989) definitely shows that within-ejaculate sperm length dimorphism is a trait common to all the *obscura* group species (so far 18 species have been investigated) and that it seems absent in the outgroups. However, there is one noticeable exception with *D. teissieri*, one of the eight closely related species of the *melanogaster* subgroup, which uniquely shows a striking within-ejaculate sperm dimorphism together with a geographic variability of the overall sperm length (Joly *et al.*, 1990).

Table 1. Variation of sperm length in different organisms.

Species	length (um)	reference
Hippopotamus amphibius	33.49	1
Sperm whale	40	2
Hump-backed whale	52	2
D. pseudoobscura	56 (short sperm)	3
Chimpanzee	57	4
Human	58	4
Gorilla	61	4
Orangutan	67	4
D. teissieri Tai	1000 (short sperm)	5
D. mauritiana	1036	3
D. simulans	1124	3
D. sechellia	1649	3
D. teissieri Tai	1700 (long sperm)	5
D. melanogaster	1898	3
D. teissieri Selinda	2000 (very long sperm)	5
Discoglossus pictus	2300	4
Heterocyris incongruens	10000	4
Scutigera forceps	14000	6
D. hydei	15000	5
Notonecta glauca	16500	7
D. littoralis	19297	5

1. Cummins 1983; 2. Cousteau & Paccalet 1986; 3. Joly et al. 1989; 4. Smith 1984; 5. Joly 1989; 6. Ansley 1954 ; 7. Afzelius 1970

We thought it is of interest to present here a synoptic and illustrated view of sperm length diversity in Drosophilidae in comparison with the range of variation of sperm length in the animal kingdom (Table 1 and Figures 1 and 2). We aim to stress that the between-species variation in Drosophilidae ranging from about 50 um to 20,000 um is at least as wide as the range of variation (from 33.5 um to 16,500 um) of the rest of the animal kingdom including both invertebrates and vertebrates (exclusive of organisms

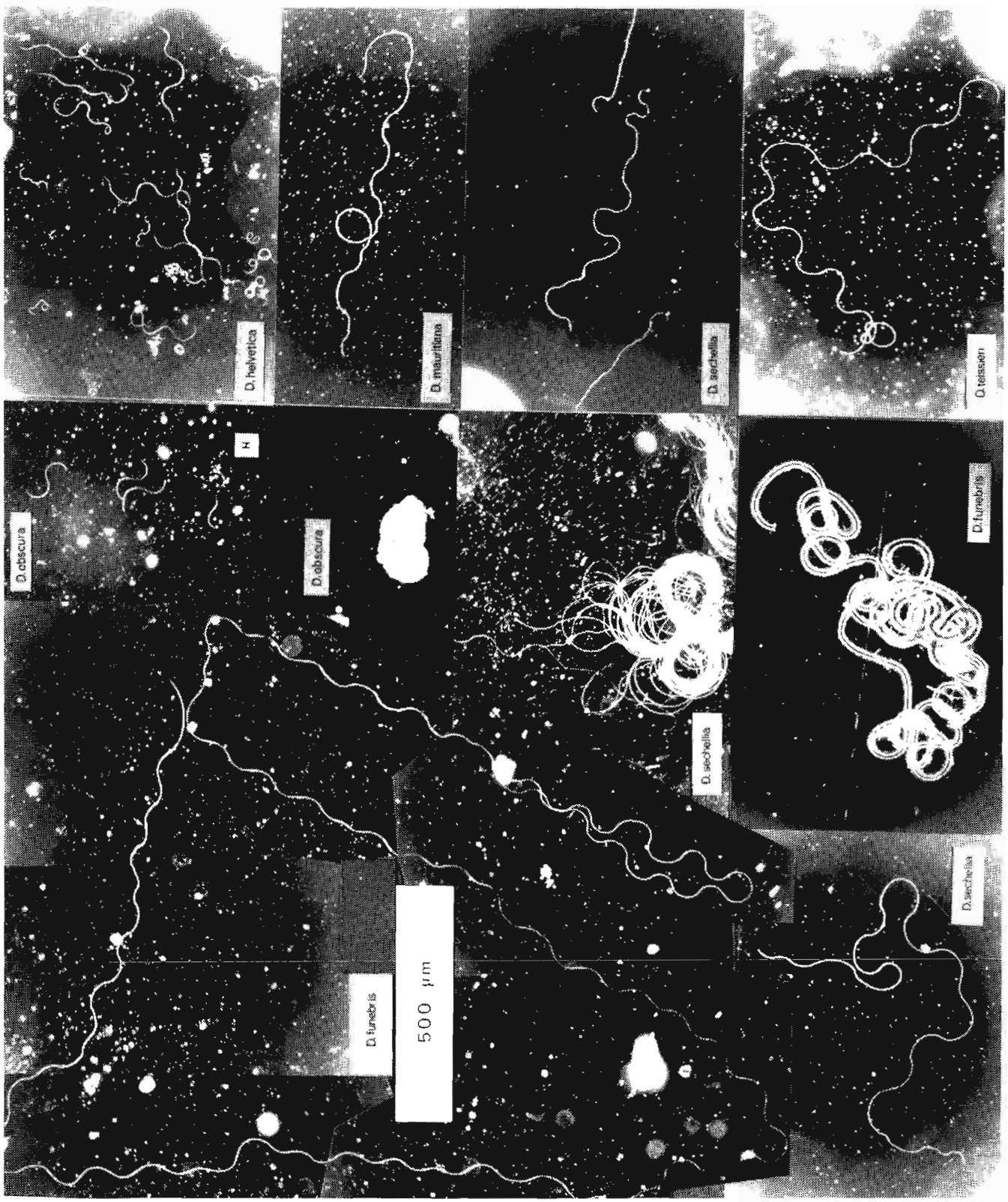

Figure 1. Diversity of sperm length in several species of Drosophilidae. Note the existence of sperm length dimorphism in *D. helvetica. D. funebris* and *D. obscura* are also represented by their seminal receptacles which should be compared with their respective sperm length. All photographs are at the same magnification. Scale bar is 500 um.

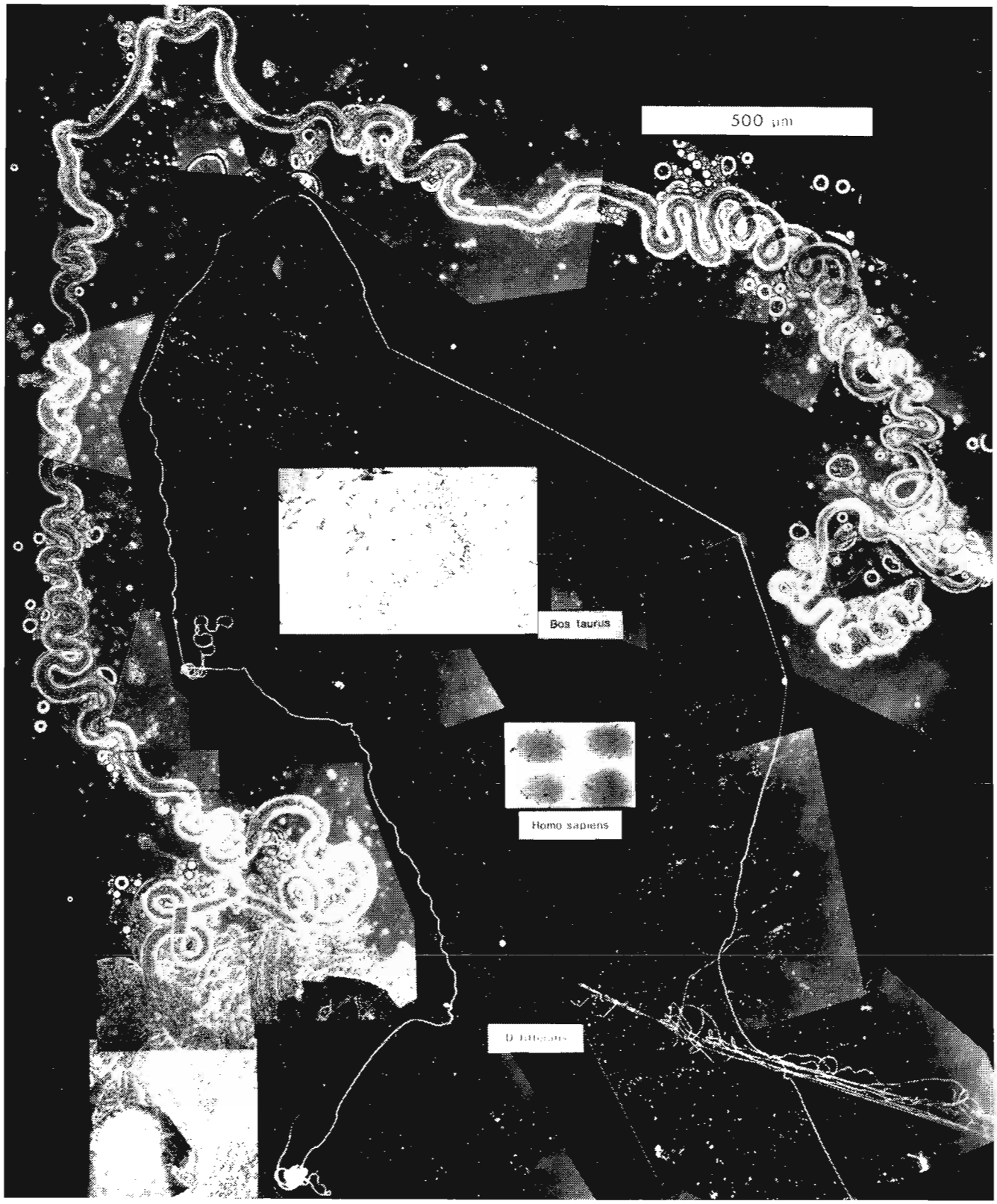

Figure 2. Sperm fragment and seminal receptacle in *D. littoralis* in comparison with bull and human sperm at the same magnification. Scale bar represents 500 um. A direct comparison can be made between figures 1 and 2.

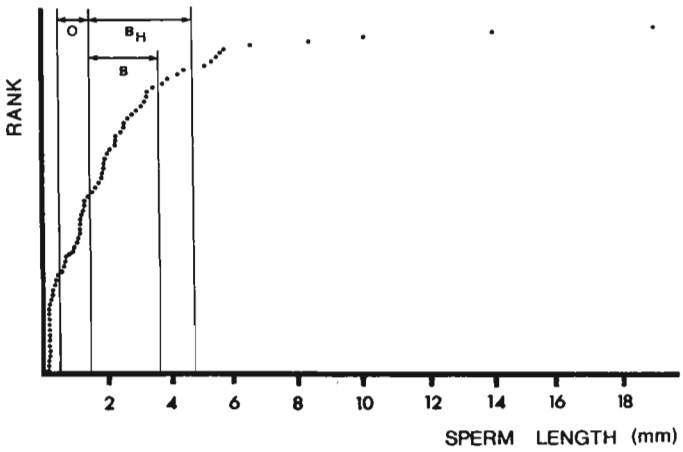

Figure 3. Sperm length diversity among *Drosophila*. Each point represents one species. Species are classified according to the mean values of sperm length in mm irrespective of bimodality or unimodality of the distribution patterns. From the smallest to the longest, the species represented here are: *D. obscura* .113 mm, *D. microlabis* .115, *D. kitumensis* .135, *D. ambigua* .138, *D. subobscura* .142, *D. latifasciaeformis* .150, *D. tristis* .152, *D. persimilis* .158, *D. madeirensis* .166, *D. pseudoobscura* .168, *D. helvetica* .170, *D. affinis* .177, *D. bifasciata* .185, *D. guanche* .202, *D. coracina* .210, *D. azteca* .240, *D. throckmortoni* .320, *D. quadrivittata* .360, *D. narragansett* .565, *Scaptomyza graminum* .580, *Amiota variegata* .630, *D. algonquin* .635, *Amiota dispina* .640, *D. bryani* .900, *D. athabasca* .910, *D. mauritiana* 1.036, *Microdrosophila purpulata* 1.060, *D. tolteca* 1.065, *Leucophenga maculata* 1.080, *D. busckii* 1.100, *D. simulans* 1.124, *Leucophenga megnipalpis* 1.160, *D. erecta* 1.210, *Scaptomyza pallida* 1.257, *D. pallidifrons* 1.270, *D. sternopleuralis* 1.290, *Leucophenga argentosa* 1.380, *D. orena* 1.436, *D. lutescens* 1.520, *D. teissieri* 1.606, *D. sechellia* 1.649, *D. grimshawi* 1.664, *D. yakuba* 1.681, *D. ficusphila* 1.840, *D. pulaua* 1.850, *D. melanogaster* 1.898, *D. sulfurigaster* 1.980, *D. subtilis* 2.020, *D. auraria* 2.220, *D. suzukii* 2.220, *D. ananassae* 2.300, *D. kohkoa* 2.410, *Liodrosophila aerea* 2.470, *D. kepulauana* 2.490, *D. curviceps* 2.600, *D. alboralis* 2.780, *D. nokogiri* 3.040, *D. bahunde* 3.1444, *D. bakundjo* 3.173, *D. histrio* 3.230, *D. nasuta* 3.420, *D. pengi* 3.710, *Zaprionus tuberculatus* 3.729, *D. nigromaculata* 3.850, *D. albomicans* 4.330, *D. niveifrons* 5.060, *D. sordidula* 5.290, *Mycodrosophila shikokuana* 5.320, *D. rufa* 5.370, *D. lacertosa* 5.440, *D. virilis* 6.470, *D. funebris* 8.290, *D. hydei* 10.000, *D. repleta* 14.000, *D. littoralis* 19.297.

Are noted the amplitude of variations of oocyte length (O), body length of *Drosophila* adults with (B_H) or without Hawaiian species (B).

producing male gametes without tail). Considering that Drosophilidae is nothing but a small family within the huge Diptera order, which is itself a small fraction of the class of insects, this makes fruitflies a highly and valuable material for studying the evolution of sperm length.

Figure 3 summarizes the mean value of sperm length for 75 species of Drosophilidae ranked in ascending order and irrespective of their phylogenetic relationships. Each point represents one species. The species belonging to 8 genera including both Drosophilinae and Steganinae are listed in the figure caption according to sperm size: 48 species have been taken in the literature, namely Sanger and Miller (1973), Hatsumi and Wakahama (1986), Hihara and Kurokawa (1987), and 27 have been measured or remeasured by ourselves (Joly, 1987; Joly, 1989; Joly et al., 1989). From the lowest mean value (i.e. 56 um in the short sperm class of *D. pseudoobscura* to the highest value or sperm fragment ever measured 19,000 um in *D. littoralis*) the growth factor is 340. If we consider the lowest measure of individual sperm (i.e. 20um in *D. pseudoobscura*) the growth factor is approximately 1000. In other term, sperm length in some species is 1/100th of the fly's body length whereas it may be as much as siz times the fly's body length in some others. Interestingly, more than half the species have male gametes the length of which greatly exceeds that of the fly's body. Hence, sperm gigantism is common in Drosophilidae and this may appear paradoxical regarding the expectations of theory of anisogamy (Parker, 1984). Although the longest sperm are found in the largest species of the *Drosophila* subgenus, there is not actual positive correlation with the body size of the fly, a conclusion that is consistent with that of Kurokawa, et al. (1974). As an example, the sperm of the Hawaiian picture-winged species *D. grimshawi*, the body size of which is of inordinate length for a Drosophilidae (4.5 - 5.5mm), is only 1670 um that is ten times less than *D. hydei* or *D. littoralis* sperm. Otherwise, the growth factor of the size of the oocyte in Drosophilidae is four times from 400 um to 1700 um with most species between 400 and 800 um (Kambysellys and Heed, 1971; Lachaise, 1980). Although such a between-species variation is noticeable it cannot be comparable with the relevant sperm increase. In that respect, it should be noticed that 50 per cent of the species (i.e. 38 over 75) have a sperm which is far and away beyond the oocyte size, in many species it is 10 to 25 times longer. As a comparison the sperm-whale, hump-backed whale or human ooctye is only 100-200 um for a sperm length being 40, 52 and 58 um respectively. There is no clear relationship between sperm length and taxonomic position therefore sperm length increase should more plausibly be seen as an evolutionary innovation that has convergently occurred in many taxa.

Tiny, giant, or dimorphic sperm appear to be three alternative species-specific patterns in Drosophilidae and the question is now to understand what evolutionary force can generate and maintain them.

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Announcements

Guide to Contributors

Drosophila Information Service prints short research and technique articles, descriptions of new mutations, stock lists, directory information, and other material of general interest to *Drosophila* researchers. The current publication schedule for regular issues is annually in July. Special issues will also be prepared on an irregular schedule.

Manuscripts, orders, and inquiries concerning the regular annual DIS issue should be sent to James Thompson at the address at the front of this issue.

Submission: Submissions are accepted at any time, but the deadline for the annual July issue will be about 1 April or until the issue is full. To help minimize editorial costs, proofs will not be sent to authors, unless there is some question that needs to be clarified or they are specifically requested by the author at the time of submission.

Manuscripts should be submitted in duplicate. If possible, a 5.25" or 3.5" diskette with the manuscript in ASCII, in a major IBM-compatible word processing format such as WordStar 2000 or WordPerfect, or in Macintosh Word would be very much appreciated.

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Citation of References: Citation should be by name and date in the text of an article (Smith, 1989; Smith et al., 1990). At the end of the article, references should be listed alphabetically by author; titles will not be included except for books, unpublished theses, and articles in press.

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W.-E. Kalisch, Institut für Genetik, Medizinische Fakultät, Ruhr-Universität Bochum, F.R.G.

Bibliography on the *D. nasuta* subgroup.

In this bibliography I have tried to cover the literature of the *D. nasuta* subgroup up to January, 1990. Since most of the data, so far, are comparative studies which include different members of the *D. nasuta* subgroup, a bibliography of the total subgroup is given together with some additional, strongly related papers. This includes basically the members: *D. n. nasuta*, *D. n. albomicans*, *D. kohkoa*, *D. n. kepulauana*, *D. pulaua*, *D. pallidifrons*, *D. s. sulfurigaster*, *D. s. bilimbata*, *D.s.albostrigata*, *D. nixifrons*, *D. niveifrons* and *D. neonasuta* (The terms *D. albomicana* and *D. albomicans* are used synonymously in literature). Papers of graduate students, the main results of which are not published so far, are included together with the advisor's name. If a paper is not written in English, the original title and an (more or less acceptable) English translation of the title are listed. Part of the material presented here was kindly contributed and/or critically read by several Drosophilist's working with *nasuta*.

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Kamping, A. and van Delden, W. Biology Centre, Department of Genetics, University of Groningen, Haren, The Netherlands. Biochemical differences between *alpha-GPDH* genotypes in *Drosophila melanogaster.*

The alpha-Glycerophosphate dehydrogenase (*alpha-Gpdh*) locus is generally considered to be one of the least variable enzyme loci in *Drosophila*, as only a few species were found to be polymorphic (Lakavaara *et al.*, 1977). The relatively conservative nature of the system is possibly due to its multiple roles in metabolism. alpha-GPDH plays a central role in deriving energy from carbohydrates and is of importance for: the energy metabolism of flight muscle (Sacktor and Cockran 1957); the biosynthesis of phospholipids and triglycerides (Gilbert, 1967); the regulation of the NAD^+/NADH ratio in the cytoplasm (O'Brien and MacIntyre 1972). *D. melanogaster* is one of the few species which show variation for *alpha-Gpdh*. Almost every wild population segregates for two common alleles, Slow (S) and Fast (F). In addition to these two alleles, we have regularly observed two additional mobility variants in a natural population in Groningen, The Netherlands. The Super-Fast (F^S) (see also Bewley *et al.* 1984) and Super-Slow (S^S) alleles migrate on polyacrylamide faster than the F and slower than the S allele respectively. The frequencies of the F^S and S^S alleles in the Groningen population are < 1%.

Four strains, each homozygous for one of the *alpha-Gpdh* alleles were derived from the Groningen population. alpha-GPDH activity, Km and Vmax were measured to study biochemical differences between the allozymes.

alpha-GPDH activity was measured in a 0.05 M Glycine NaOH buffer (pH 9.5) with a final concentration of 5 mM NAD^+ and 10 mM alpha-GP. The increase in absorbance was measured in a spectrophotometer at 340 nM and 30°C.

Kinetic properties were studied by varying either co-enzyme concentration or the substrate concentration. The number of replicate samples was 3 or 4 for each measuring point. The amount of protein was measured by the method of Bradford (1976) using bovine serum albumin as a standard. Km and Vmax were measured by the Lineweaver-Burk method.

In Figure 1, alpha-GPDH activities of the homozygotes at different substrate concentrations are presented. The SS and S^sS^s homozygotes had the highest enzyme activity. SS is more active than the FF genotype. This finding is in agreement with Barnes and Laurie-Ahlberg (1986) and McKechnie and Geer (1986). All genotypes, including the heterozygotes (not presented), were inhibited by high concentrations of alpha-GP.

From Table 1 it is clear that differences in Vmax values existed between the genotypes. SS showed the highest Vmax, the heterozygotes were intermediate to the corresponding homozygotes.

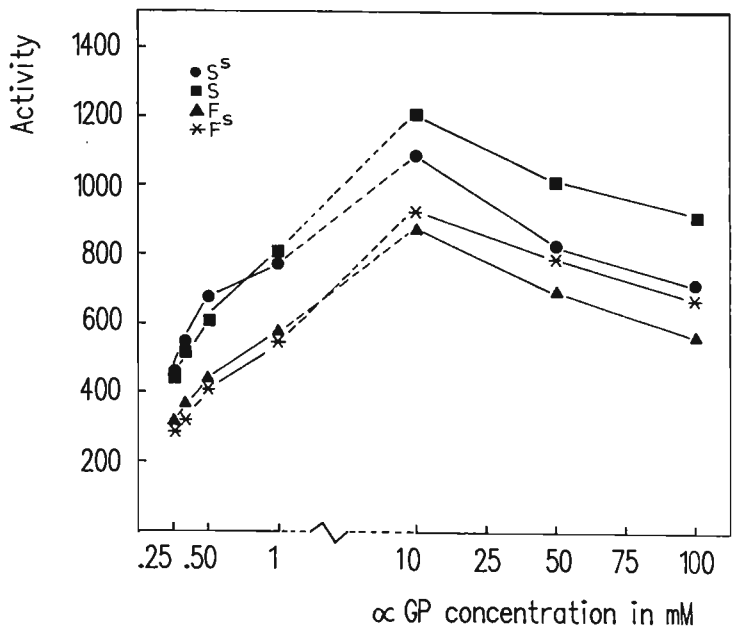

Figure 1. alpha-GDPH activity of adult *alpha-Gpdh* homozygotes at different substrate concentrations. Activity is given in nanomoles of cofactor metabolized per minute per mg protein at 30°C.

Table 1. Km and Vmax values of α–Gdph genotypes. Km is given as co-enzyme- and α–GP millimolarity. Vmax is given in nanomoles of cofactor metabolized per minute per mg protein at 30°C.

genotype	Vmax NAD+	Km NAD+	Vmax αGP	Km αGP
S^sS^s	1074	.36	1026	.29
SS	1239	.36	1160	.42
FF	854	.38	870	.44
F^sF^s	949	.37	887	.53
S^sS	1174	.41	1120	.37
S^sF	977	.37	883	.35
S^sF^s	1015	.42	898	.37
SF	1044	.41	962	.45
SF^s	1035	.41	1001	.48
FF^s	880	.41	887	.53

The Km's for NAD^+ showed only very small differences between the genotypes, thus no differences in affinity to alpha-GPDH were present. The Km's for alpha-GP pointed at higher affinities for S^sS^s than for SS and FF while F^sF^s exhibited the lowest affinity of the homozygotes.

These *in vitro* genotypic differences in biochemical properties imply structural distinctions in the allozymes, which may lead to physiological and fitness differences.

References: Barnes, P.T. and C.C. Laurie-Ahlberg 1986, Genetics 112:267; Bewly, G.C. *et al.* 1984, Comp. Biochem. Physiol., Vol 79B:23-32; Bewly, G.C. *et al.* 1974, J. Insect Physiol. Vol. 20:153-165; Bradford, M.M. 1976, Anal. Biochem. 72:248-254; Gilbert, L. 1967, Adv. Insect Physiol. 43:69-211; McKechnie, S.W. and B.W. Geer 1986, Biochem. Genet. 24:859-872; Lakovaara, S. *et al.* 1977, Evolution 31:319-330; O'Brien, S.J. and R.J. MacIntyre 1972, Biochem. Genet. 7:141-161; Sacktor, B. and D. Cockran 1957, Biochem. Biophys. Acta 25:699-753.

Kamping, A. and Van Delden, W. Biology Centre, Department of Genetics, University of Groningen, Haren, The Netherlands. Differences in hybridization rates between *D. melanogaster* and *D. simulans* from different geographical origins.

In a previous paper (Kamping and Van Delden 1988) it was shown that hybridization between *D. melanogaster* and *D. simulans* occurred repeatedly in a sympatric population in nature. It seemed that the frequencies of hybrid crosses were negatively correlated with *D. simulans* frequencies. The outcome of the hybrid crosses (*mel* female x *sim* male or *sim* female x *mel* male) was in agreement with the

hypothesis that females of the ancestral species discriminate against males of the derived species while females of the derived species accept males of the ancestral species (Kaneshiro 1976). In the present study we used two sympatric populations of different geographical origin, to estimate hybridization under experimental conditions. One population was derived from a fruitmarket in Groningen, The Netherlands, in September 1987 (indicated as Gron), the other population was started with flies captured in August 1987 in Vernet-les-Bains, eastern Pyrenees in France (indicated as Vernet). The *D. simulans* frequencies were 13% and 43% for the Gron and the Vernet natural population respectively. Experimental populations were started with isofemale lines of the two species from each geographical origin. Hybridization was tested in a no-choice situation. Single pair crosses for each possible combination within and between the two species and origins were set up (50 replicates each).

Table 1. Percentages homogamic and heterogamic matings of *D. melanogaster* and *D. simulans* producing viable offspring. Figures in brackets indicate percentages mating without offspring.

		males			
		D. melanogaster		*D. simulans*	
Females	Origin	Gron	Vernet	Gron	Vernet
D. melanogaster	Gron	94	100	8	14
	Vernet	100	98	6	-
D. simulans	Gron	2	-	80 (20)	70 (14)
	Vernet	-	-	74 (10)	92 (4)

The results are presented in Table 1. Between and within the *D. melanogaster* populations almost all combinations of males and females produced offspring. For *D. simulans* the percentages successful matings were relatively low, however the percentages of matings without offspring (between brackets in Table 1) suggest that possibly incompatibility genes are involved.

From Table 1 it is clear that successful matings between *D. melanogaster* and *D. simulans* are asymmetrically distributed. Under these testing conditions the cross *D. melanogaster* female x *D. simulans* male is more successful than the reciprocal cross which follows the expectation based on the hypothesis of Watanabe and Kawanishi (1979). This experimental finding is in contrast with the outcome of the hybrid crosses in the natural Gron population (Kamping and Van Delden 1988).

D. melanogaster Vernet female x *D. simulans* Vernet male crosses were unsuccessful, possibly due to selection against hybridization in both species in the wild Vernet population. The frequencies of both species in this population were almost equal. *D. melanogaster* Vernet females do mate with *D. melanogaster* Gron males, so the fact that the cross *D. melanogaster* Vernet female x *D. simulans* Vernet male is unsuccessful is not only due to a lower receptivity of the *D. melanogaster* Vernet females (Carracedo *et al.* 1987). In contrast to the Gron population (Kamping and Van Delden 1988) no hybrids nor evidence for hybrid crosses were observed in the natural sympatric Vernet population.

Although interspecific hybridization between *D. melanogaster* and *D. simulans* was relatively low, it is interesting to know why some flies mate with flies of a sibling species and others do not. The *D. melanogaster* flies which had mated with the *D. simulans* were compared with a random sample of the same *D. melanogaster* population (control) using Adh and alpha-Gpdh as marker genes. For the Gron populations the mated *D. melanogaster* flies (combined data) were significantly different from the control group. Both the Adh^S and the alpha-$Gpdh^F$ allele frequencies were significantly higher in the flies which mated with *D. simulans* than in the control group. From the Vernet population only a single Adh/alpha-Gpdh genotype (Adh^{FS}, alpha-$Gpdh^{FF}$) mated with *D. simulans*. Possibly factors which influence mating behavior are linked to these allozyme loci.

References: Carracedo, M.C., P. Cesares and E. San Miguel, 1987, Genome 29:334-339; Kamping, A. and W. Van Delden, 1988, Dros. Inf. Serv. 67:53; Kaneshiro, K.Y. 1976, Evolution 30:740-745; Watanabe, T.K. and M. Kawanishi, 1979, Science 205:906-907.

Khechumian R.K.[1], **E.O. Mndjoyan**[1], **A.A. Galoyan**[1], **T.G. Bakayeva**[2], **L.I. Korochkin**[2], **O.A. Malevanchuk**[3], **and G.N. Yenikolopov**[3]. [1]Institute of Biochemistry, Yerevan, USSR. [2]Institute of Developmental Biology, Moscow, USSR. [3]Institute of Molecular Biology, Moscow, USSR. Evolutionary conservation of the esterase S gene of *D. virilis*.

The *virilis* group of the genus *Drosophila* represents a unique model for the investigation of the basis of genetic polymorphism and its possible adaptive significance in evolutionary process. This is possible because fertile hybrids between different members of the group may be obtained. The esterase gene-enzyme system has been studied extensively by a variety of genetic, biochemical and molecular means. We are especially interested in the tissue-specific esterase S of *D. virilis* which is synthesized only in ejaculatory bulbs during a strictly defined and genetically determined period of individual development (Korochkin 1980), particularly because the Est^6 locus of *D. melanogaster* which is in many aspects (including DNA and protein

sequence) homologous to the Est^S locus of *D. virilis*, is one of a few for which a certain adaptive significance of the enzyme polymorphism has been shown. This adaptive fitness mainly concerns the reproductive process and, in particular, modulation of the fly's mating behaviour. We have used the cloned gene for esterase S of *D. virilis* (Yenikolopov *et al.* 1983) for restriction analysis of the genomes of different species of the *virilis* group and other species of the genus *Drosophila* and found a high degree of conservation of the gene within the *virilis* group.

Labeled cloned DNA was blot hybridized with the chromosomal DNA of 15 *Drosophila* species, treated with HindIII, PstI, EcoRI, BamHI, and other restriction enzymes. Specimens included some highly related species within the *virilis* group and some species of other groups and subgenera of the genus *Drosophila*. Comparison of signal intensities demonstrated a correlation of the probe hybridization efficiencies with the relative phylogenetic distance between the species. Moreover, comparison of the length of restriction fragments of the DNA of various species identified by the probe suggests a high degree of conservation of the sequences corresponding to the esterase gene within the *virilis* group. Some bands are present throughout most of the panel, some are specific for groups of 2-3 species, some are unique for a particular species, but any given species has a number of bands which overlap with the band pattern of other (>1) species. This distribution was used to construct a phenogram of nucleotide divergence among restriction-map variants using the approach of Nei and Li (1979).

When a short (ca. 500 bp) subclone corresponding to the active-site peptide of esterase S is used as a probe, 2-3 bands of various intensity are revealed, to suggest that related genes can be detected within the species genomes. These signals probably correspond to the Est^X gene of *D. virilis* located in the close proximity to Est^S (ca. 800 bp in the 3'-direction). Interestingly, the species of the *repleta* phylad give almost identical bands when probed with the active-site probe, whereas *virilis* phylad produce more varying signals.

Northern blot analysis of the RNA from species belonging to the *virilis* group demonstrates a very similar pattern of transcription, with a high prevalence of the gene activity in males (5-50 times higher than in females), to produce mRNA of a constant length of about 1900 bp. One can also see extra bands of lower intensity, which suggests that alternative promoters and alternative splicing are just as in the case of *D. virilis*.

Taken together, the analysis of the esterase genes and their transcripts demonstrates a high degree of the esterase gene conservation within the related species and rapid divergence within distant species of *Drosophila*. This conservatism of the Est^S locus within the *virilis* group is especially interesting since recent results have shown that the tissue-specific esterase is important for the reproductive behaviour and reproductive fitness of flies; the conservation of the gene and the protein can support the maintenance of the unique possibility to obtain fertile hybrids, characteristic for the *virilis* group.

References: Korochkin, L.I. 1980, In: Isozymes, Vol. 4, Alan R. Liss, Inc., N.Y., pp. 159-202; Nei, M. and W.H. Li 1979, Proc. Natl. Acad. Sci. USA 76:5269-5273; Yenikolopov, G.N. *et al.* 1983, EMBO J. 2:1-7.

Khechumian R.K.[1], E.O. Mndjoyan[1], A.A. Galoyan[1], L.I. Korochkin[2], O.A. Malevanchuk[3] and G.N. Yenikolopov[3]. [1]Institute of Biochemistry, Yerevan, USSR. [2]Institute of Developmental Biology, Moscow, USSR. [3]Institute of Molecular Biology, Moscow, USSR.

The very high stability of the Est^S locus of *D. virilis*.

The genome of *D. virilis* is exceptionally stable and this is expressed in the absence of naturally occurring inversions and translocations (Patterson and Stone 1951). Such a stability of the genetical material, scored by the genetic and cytological means, can be detected at the molecular level in terms of DNA structural conservation. Analysis of restriction site polymorphisms can be used to measure genetic variability in natural populations, species etc. We have used a cloned fragment of *D. virilis* genome (Yenikolopov *et al.*, 1983) containing the esterase S gene, a related Est^X gene, an unidentified gene and flanking sequences to analyse restriction site polymorphisms in a worldwide sample of 20 lines of *D. virilis*, and have revealed an unusual stability in this region. Southern analysis of genomic DNA was performed using EcoRI, HindIII, PstI, BamHI and Sa1GI restriction endonucleases and a 15-kb long DNA fragment as a probe. None of these lines contained any alterations when checked for point mutations as could be revealed by the disappearance of restriction sites or the appearance of new ones. We used two estimators of population genetic variation at the nucleotide level - $\hat{\theta}$ of Hudson (1982) and $\hat{\pi}$ of Nei and Tajima (1981). Taking one mutation as an upper limit, $\hat{\Theta}$ is less than 0.0009 and $\hat{\pi}$ is less than 0.0008. The estimated value is by an order of magnitude lower than that for the *white* locus of *D. melanogaster* - 0.015 (Langley and Aquadro 1987), the ADH locus of *D. melanogaster* - 0.006 (Aquadro *et al.* 1986), and the ADH locus of *D. pseudoobscura* - 0.066 (Schaeffer *et al.* 1987).

When checked for large insertions, only one line was shown to contain a 3.5 kb long extra fragment of DNA. This corresponds to the frequency of insertions equal to 0.002, again much lower than that for *D. melanogaster* genes. One

must also take into account that only one mobile element of *D. virilis* has been found so far (Evgen'ev *et al.* 1982) in spite of an extensive search.

We believe that demonstrated extremely low genetic variability at the nucleotide level is manifested at the chromosomal level in the exceptional stability and lack of gross rearrangements (inversions and translocations) in natural populations of *D. virilis*.

References: Aquadro, C.F. *et al.* 1986, Genetics 114:1165-1190; Evgen'ev, M.B. *et al.* 1982, Chromosoma 85:375-386; Hudson, R.R. 1982, Genetics 110:711-719; Langley, C.H. and C.F. Aquardo 1987, Mol. Biol. Evol. 4:651-663; Nei, M. and Tajima 1981, Genetics 97:145-163; Patterson, J.T. and W.S. Stone 1952, In: Evolution in the genus *Drosophila*, MacMillan, N.Y.; Schaeffer, S.W., C.F. Aquadro and W.W. Anderson 1987, Mol. Biol. Evol. 4:254-265; Yenikolopov, G.N. *et al.* 1983, EMBO J. 2:1-7.

Klarenberg, A.J.[1], B.L.A. Buiter de,[2] and W. Scharloo.[2]
[1]Zoologisches Institut der Universitat, Munchen, F.R.G., and [2]Rijksuniversiteit Utrecht, The Netherlands. Regulatory variants of beta-galactosidase *Drosophila melanogaster.*

Variation in regulatory genes for tissue-specific expression in midguts of *D. melanogaster* is not a peculiarity of the alpha-amylase gene-enzyme system (Abraham and Doane 1978; Klarenberg et al. 1986; Klarenberg et al. 1988). Beta-Galactosidase shows similar production patterns. Genetic variation of these midgut patterns in strains isogenic for the second chromosome shows that beta-galactosidase is controlled by its own tissue-specific regulatory genes located at or in the neighborhood of beta-galactosidase structural gene(s). The function of midgut beta-galactosidase is digestion as indicated by successful chemical selection for beta-galactosidase activity on lactose food medium (Fargnoli et al. 1987; Klarenberg, unpubl.). Beta-galactosidase is expressed in anterior and posterior parts of larval and adult midguts. No electrophoretic variation for beta-galactosidase was found. High levels of regulatory variation for beta-galactosidase in wild type cage populations of *D. melanogaster* was revealed by observing tissue-specific gene-expression in the midguts of adults and larvae under different dietary conditions. Genetic variation in dietary regulation of beta-galactosidase in midguts of third-instar larvae was detected by comparing midgut expression on yeast-lactose and cornmeal-sucrose food. Our mapping data indicate that the regulatory gene(s) for beta-galactosidase midgut expression are closely linked to the structural gene for beta-galactosidase (beta-Gal-1) at 2-20.3 (Knipple and MacIntyre 1984).

References: Abraham and Doane 1978, Proc. Natl. Acad. Sci. USA 75:4446-4450; Fargnoli et al. 1987, Biochem. Genet. 25:327-333; Klarenberg et al. 1986, Genetics 114:1131-1145; Klarenberg et al. 1988, Comp. Biochem. Physiol. 89B:143-146; Knipple and MacIntyre 1984, Mol. Gen. Genet. 198:75-83.

Kokoza, V.A., Baricheva, E.M., and Katokhin, A.V.
Inst. of Cytology and Genetics, Novosibirsk, USSR. Cloning and chromosomal localization of conservative brain specific sequences in *D. melanogaster.*

The high grade of evolutionary conservativeness of neurospecific proteins and therefore of neurospecific gene sequences is one of the characteristic features of neural tissue. In order to clone evolutionarily conservative *Drosophila* neurogenes the genomic library of *D. melanogaster* (Maniatis et al., 1978) was screened using polysomal poly(A)$^+$- RNA from mouse brain. As a result 15 recombinant clones were selected containing conservative brain specific sequences (CBS) of *Drosophila*. All cloned CBS had individual restriction patterns (excluding J4 and J8) and were shown to be transcribed in the heads of adult flies using Southern-blot hybridization (Figure 1).

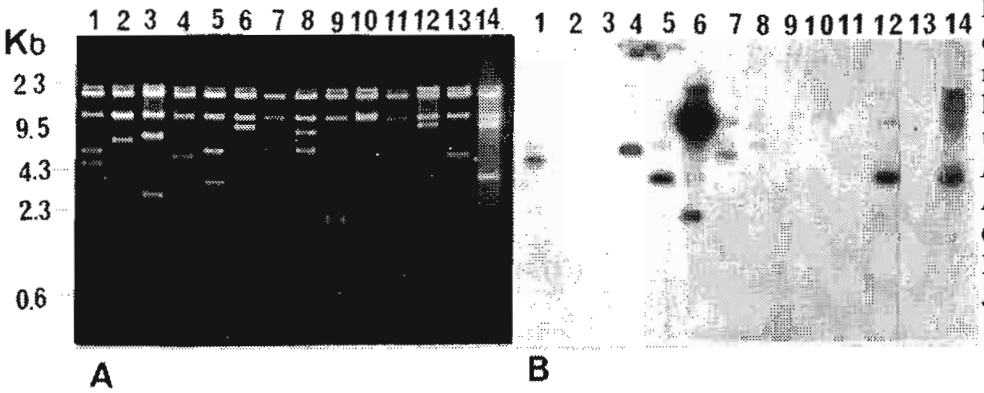

Figure 1. Restriction analysis of cloned CBS by EcoRI endonuclease (A) and Southern-blot hybridization of cloned CBS using(^{32}P)-poly(A)$^+$-RNA from *Drosophila* heads (B). Lanes: 1 - A1; 2 - B3; 3 - B4; 4 - D2; 5 - E1; 6 - E8; 7 - E9; 8 - F4; 9 - H2; 10 - H4; 11 - J1; 12 - J4; 13 - J7; 14 - J8.

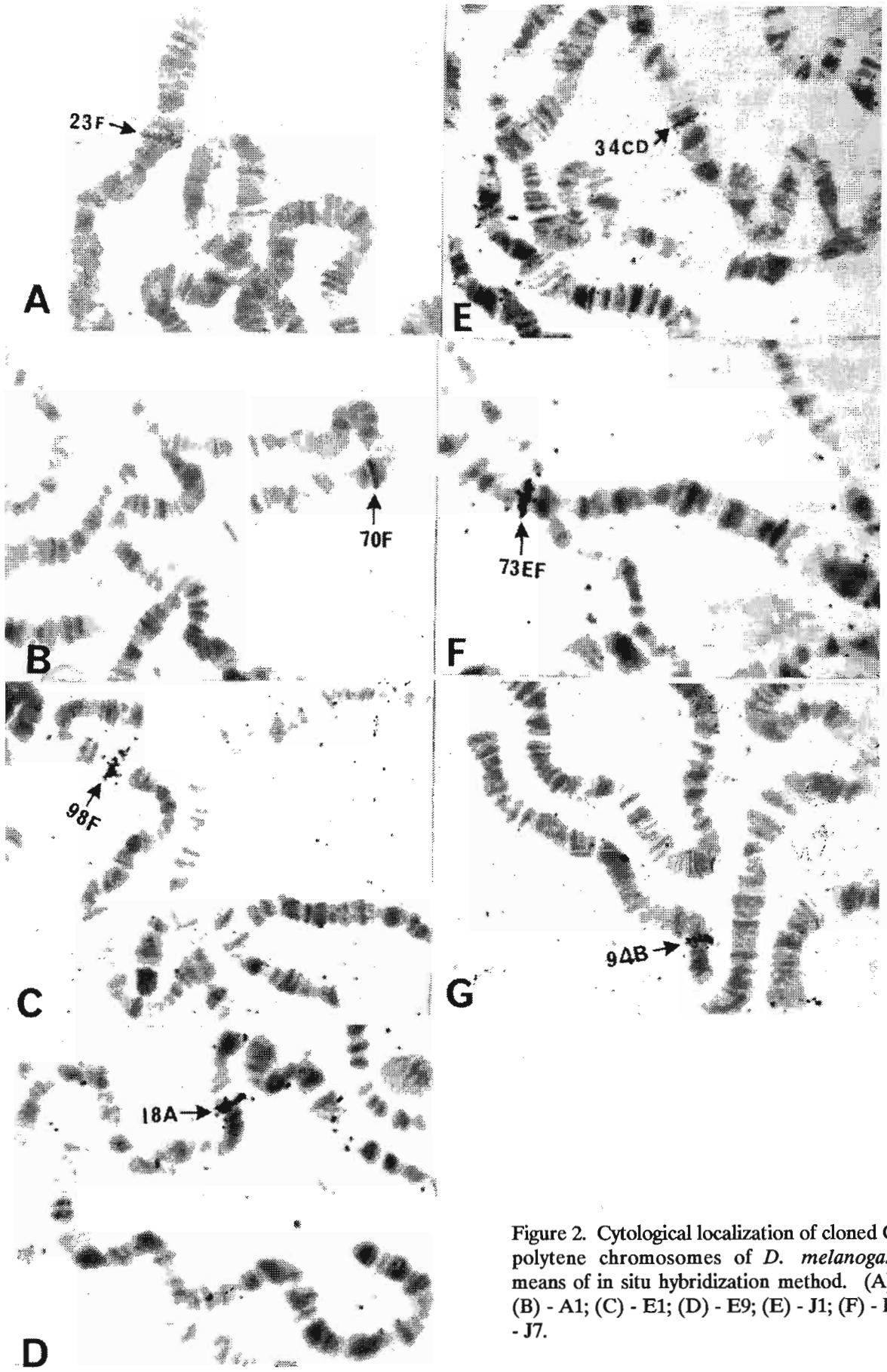

Figure 2. Cytological localization of cloned CBS on polytene chromosomes of *D. melanogaster* by means of in situ hybridization method. (A) - H2; (B) - A1; (C) - E1; (D) - E9; (E) - J1; (F) - B3; (G) - J7.

Table 1. Cytological localizations of 7 CBS of the first group.

CBS name	A1	B3	H2	E1	E9	J1	J7
Cytological region	70F	73EF	23F	98F	18A	34CD	94B

Table 2. Cytological localizations of CBS of 2nd group in 3 strains.

	Cytological regions and structures		
CBS name	Canton-S	Oregon-R	giant
E8	chromocentre nucleolus 102C, 22F-23A, 31A	chromocentre nucleolus 102C, 6A, 89B	chromocentre nucleolus 102C
D2	chromocentre 63B, 22B	chromocentre 63B, 22B	63B

CBS were localized on *Drosophila* polytene chromosomes in 3 stocks: Oregon-R, Canton-S and giant ($gt\ w^a/gt^{13z}$) with the help of in situ hybridization method. According to in situ hybridization data, the examined sequences could be divided into three groups:

1. The first group included 7 CBS which hybridized with the unique chromosomal regions in all the three stocks. Data on the localization of these CBS are presented in Table 1 and Figure 2.

CBS of the group were not organized in clusters but distributed thoughout the chromosomes. It should be noted that CBS of this group did not hybridize with chromocentre.

2. The second group involved two CBS: D2 and E8. They showed hybridization with two chromosomal regions as well as with the chromocentre (and E8 sequence - with nucleolus also). Localization of these CBS was not the same in different strains (Table 2).

3. The third group consisted of 6 CBS. Each sequence had, in addition to the chromocentre, from 10 to 100 hybridization sites on the polytene chromosomes, the sites being different in three stocks. This allowed us to consider the sequences to contain the same mobile elements.

References: Maniatis, T., R.C. Hardison, E. Lacy, J. Lauer, C. O'Connell, D. Quon, G.K. Sim and A. Efstradiatis, 1978, Cell 15:687-701.

Kosuda, K. Biological Laboratory, Faculty of Science, Josai University, Sakado, Saitama, Japan. The tumor formation in *Drosophila melanogaster* females.

A large number of melanotic tumor strains have been reported in *Drosophila* and other insects since Bridges (1916) described one in *Drosophila melanogaster* for the first time. In the course of study for the mating activity of the aged individuals, a new type of melanotic tumor formation was found in one strain of *D. melanogaster* (Kosuda, 1988). The tumor forming character is heritable from generation to generation. This mutant strain, named C-104 for convenience, is a highly inbred one and derived from a single female in a natural population at Szentendre near Budapest, Hungary, in the summer of 1986. The tumors can be detected under the microscope in the abdominal cavities of female flies, mainly in the vicinity of spermathecae. They cannot be observed in male flies so far. Consequently, the phenotypic expression is considered to be sex-limited. The tumors are usually attached to either or both spermathecae, and subsequently they appear to encapsulate either or both spermathecae (Plate 1). They can be externally visible as

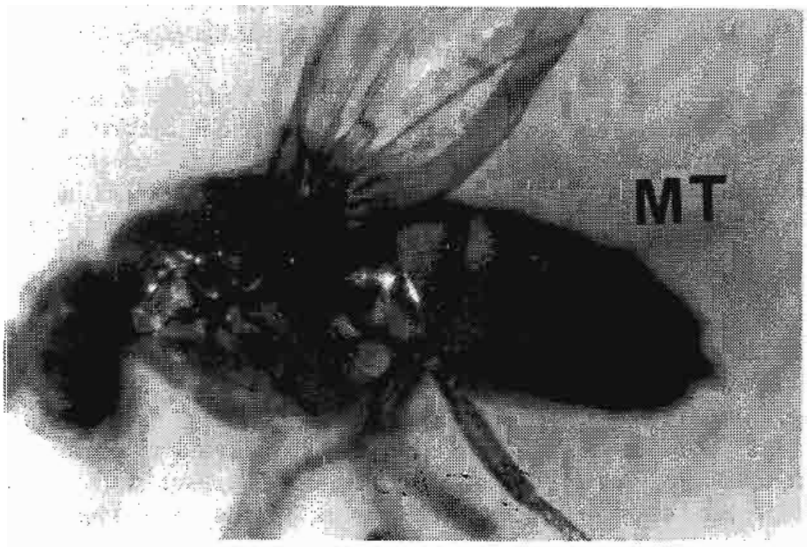

Figure 1. Melanotic tumor development in female abdomens. MT: melanotic tumor.

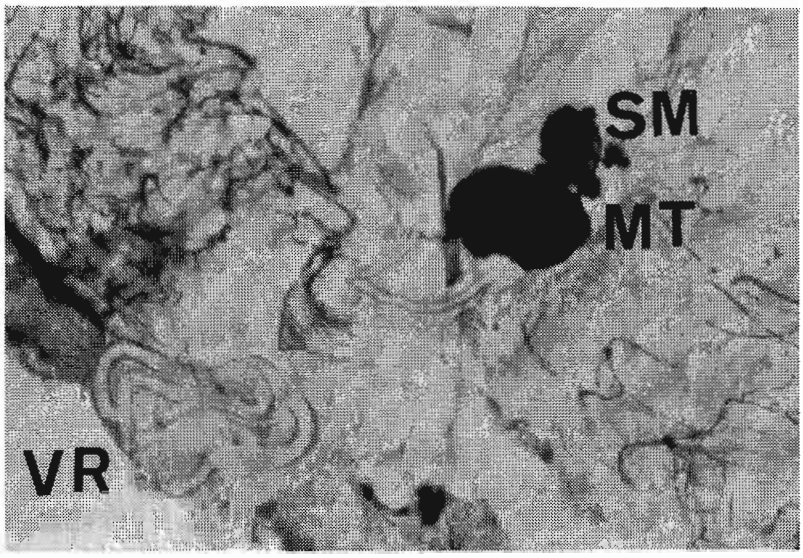

Figure 2. A pair of female spermathecae encapsulated and melanized by melanotic tumors. MT: melanotic tumor, SM: spermatheca, VR: ventral receptacle.

dense black bodies with unaided eyes, when they grow up well (Plate 2). It is of particular interest that these tumors develop as female flies get old. It should be noted that the tumor formation cannot be observed during the larval-pupal stages. As a matter of fact, female flies of the C-104 strain are not lethal either at the time of embryo or during the larval-pupal stages, and their fertility is fairly good. It is not yet determined at present whether the tumors in this strain are no more than melanotic aggregates of haemocytes or they are truly benign neoplastic transformatin of adult blood cells. Actually, more than twenty mutations of neoplastic transformation were reported (Gateff, 1978, 1982). As the present melanotic tumor is very similar to the vertebrate neoplasm in many respects, the C-104 strain may provide a model system for study of the basis of tumorigenesis, if the genetic factors are involved as causative agents, for a wealth of genetic information is available in *D. melanogaster*. Further details will be published elsewhere.

References: Bridges, C.B., 1916, Genetics 1:1-52; Gateff, E., 1978, In: Genetics and Biology of Drosophila. Vol. 2B (Ashburner and Wright, Eds.) Academic Press, London pp. 181-276; Gateff, E., 1982, Adv. Cancer Res. 37: 33-74; Kosuda, K., 1988, Jap. J. Genet. 63:572-573.

Krebs, R.A. Department of Zoology, Arizona State University, Tempe, Arizona. Body size of laboratory and field populations of *Drosophila mojavensis*.

Size has been an important theme in many investigations of *Drosophila* mating systems (Markow 1985, 1988; Partridge and Farquhar 1983; Santos et al. 1988) or host plant adaptation (Etges 1989). Species with geographically separated populations are useful for studying the environmental and genetic components of size variation. Here I report size variation in one such species, *D. mojavensis*. Although size differences among races of *D. mojavensis* have been noted (Brazner 1983; Etges and Heed 1987), no data on a wide range of populations under similiar rearing conditions have been reported.

Laboratory strains were obtained from a wide range of geographic regions encompassing all races of *D. mojavensis*: Race A from Southern California; BI, from mainland Sonora, Mexico; BII, from Baja California, Mexico; and race unclassified, from Santa Catalina Island, California (Heed and Mangan 1986). Flies used for measurements were reared on standard cornmeal-molasses-yeast media in half-pint milk bottles seeded with live yeast. Most strains had been maintained in the laboratory for about a year in large population numbers for use in other experiments (e.g. Krebs and Markow 1989). However, measurements of flies from San Carlos, Mexico, and Santa Catalina$_2$ were made for first generation offspring of field collected flies.

Field collected flies were obtained by aspirating flies at random from their host plant in the cases of the San Carlos (organ pipe cactus) and Desemboque (agria cactus) populations. Santa Catalina Island flies were collected from two necrotic prickly pear fruits within the first four days after being brought into the laboratory. The larval development of the flies had therefore been completed in nature.

Thorax length is a standard measure of overall body size in *Drosophila* and is highly correlated with other linear traits such as wing length in *D. melanogaster* (Robertson and Reeve 1952) and body weight in *D. mojavensis* (Markow et al. in press). Thorax length was measured with an ocular micrometer (Reeve 1950) set at 30 units per millimeter. Flies were measured to the nearest half unit.

Table 1. Mean thorax size (mm) of laboratory reared *Drosophila mojavensis* from different races and widely separated localities within the races. Standard errors are approximately 0.01 mm for all means.

Origin	Race	Size males (N)	Size females (N)	Host Plant
Vallecito Stage Station California, USA	A	0.97 (46)	1.00 (32)	Barrel cactus
Las Bocas Sonora, Mexico	BI	0.96 (46)	1.06 (29)	Organ pipe
San Carlos Sonora, Mexico	BI	0.98 (34)	1.06 (36)	Organ pipe
Santa Rosa Mountains Arizona, USA	BI	0.95 (329)	1.01 (307)	Organ pipe
St. John the Baptist Mountains, Arizona, USA	BI	0.97 (67)	1.05 (34)	Organ pipe
Desemboque Region Sonora, Mexico	BI	0.96 (296)	1.03 (302)	Agria
San Lucas, Baja California, Mexico	BII	0.91 (343)	0.97 (312)	Agria
Punta Prieta, Baja California, Mexico$_1$	BII	0.94 (336)	0.99 (326)	Agria
Punta Prieta, Baja California, Mexico$_2$	BII	0.94 (80)	0.97 (97)	Agria
Santa Catalina Isl. California, USA$_1$	Uncl.	0.91 (56)	0.98 (33)	Prickly pear
Santa Catalina Isl. California, USA$_2$	Uncl.	0.96 (29)	1.01 (33)	Prickly pear

Table 2. Thorax lengths (mm) of flies collected in the field. Standard errors are approximately 0.01 mm for all means.

Origin	Race	Size Males (N)	Size Females (N)
Desemboque Region Sonora, Mexico	BI	0.97 (22)	1.03 (16)
San Carlos, Sonora, Mexico	BI	0.90 (54)	0.97 (26)
Santa Catalina Isl California, USA$_2$	Uncl.	0.67 (24)	0.72 (22)

Flies from Baja and Santa Catalina Island strains are smaller than those of the mainland Sonoran desert or Southern California (Table 1). Size variation was also present among field collected flies (Table 2; 2-way ANOVA for size and sex; $F=405$, $P< 0.0001$). While females were larger than males ($F=92$, $P<0.0001$), no strain x sex interation was observed ($F=0.48$, ns). Field collected flies of one strain, Desemboque, were equivalent in size to those reared in the laboratory. Field collected flies from San Carlos, and to a greater degree those from Santa Catalina$_2$, however, were significantly smaller than F_1 individuals reared on laboratory media ($F=830$, $P<0.0001$ for rearing environment from a 3-way GLM for the variables: strain, sex, and environment). A significant strain x environment interaction also was present because of the relatively greater size increase of the F_1 generation of Santa Catalina flies relative to San Carlos F_1.

In nature *D. mojavensis* uses only necrotic fruits and pads of the prickly pear on Santa Catalina Island, and flies I measured were all reared from two small fruits. No columnar cactus is present on that island. The San Carlos flies were collected from necrotic organ pipe cactus, *Stenocereus thurberi*, and the Desemboque flies from necrotic agria, *S. gummosus*. Size differences between flies collected from these two columnar cacti species suggests these host plants may differ nutritionally, and this difference affects the size of *D. mojavensis* flies. The size differences among laboratory reared Santa Catalina, Baja peninsular and mainland Sonoran desert flies suggests that a genetic component to size differences among natural populations also exists, but environmental factors, such as type of host plant, have greater influence on size.

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Fluctuating asymmetry in a selection experiment: effects of directional and stabilizing selection.

Fluctuating asymmetry (non-directional deviations from bilateral symmetry) has been widely used as a measure of developmental pathways (see Palmer and Strobeck, 1986 for a review). In *D. melanogaster*, artificial directional selection was shown to decrease developmental stability as indicated by the increase in fluctuating asymmetry in the course of

selection (Thoday, 1958). On the other hand, increased fluctuating asymmetry was reported for phenotypically extreme individuals in different organisms (Soule and Couzin-Roudy, 1982; Leary et al., 1984). Since stabilizing selection by definition acts on extreme phenotypes, a question arises what would be its effects in a population if applied simultaneously with directional selection. The objective of the present study was to assay developmental homeostasis of *Drosophila* lines subjected both to directional and stabilizing selection.

The base population used in our experiment was derived from a cross involving 11 inbred laboratory lines homozygous for ri (3 - 47) - a recessive mutation which causes an interruption of the second longitudinal wing vein (L2). Experimental lines were set up by randomly taking from the base population 15 pairs of founder flies for each line. The character under directional selection was the length of the proximal part of L2 (Fig. 1) measured on left wings. Selection coefficient was 80% (30 flies of each sex out of 150 scored). Stabilizing selection was conducted on a complex of morphometric wing characters (Fig. 1), with a coefficient of 50%, only on females (15 flies out of 30 scored, see Imasheva et al., 1989 for details of selection procedure).

Three experimental designs each replicated 3 times were used:

(1) Directional and stabilizing selection (DS lines)

(2) Directional selection (D lines)

(3) Unselected controls (C lines).

Each replicate line was kept in a 1/2 pint milk bottle with standard medium. Selection was continued for 16 generations.

In the base population and in generations 6, 12 and 16 the length of the proximal part of the L2 was measured on both wings in a sample of 50 females from each line. FA was calculated as the mean squared difference between right and left wings. The results averaged over replicate lines are presented in Fig. 2. In the unselected controls, FA was essentially unchanged throughout the experiment. As to the selection lines, in early generations FA increased under both selection regimes, regardless of stabilizing selection. However, in later generations there was divergence between stabilized and unstabilized lines. In the D lines (under directional selection) the upward trend in FA continued, suggesting further deterioration of developmental homeostasis. These results are in agreement with those of Thoday (1958) who selected for sternopleural chaeta number. In the DS lines (under both directional and stabilizing selection) after the initial increase FA declined and remained slightly above the control level. The possible interpretation is that stabilizing selection counteracted the effects of directional selection in such a way that developmental stability of the DS lines did not decrease.

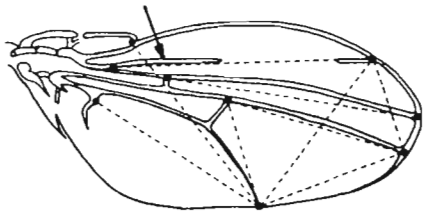

Figure 1. Selected wing characters.
Arrow indicates proximal segment of L2 vein.

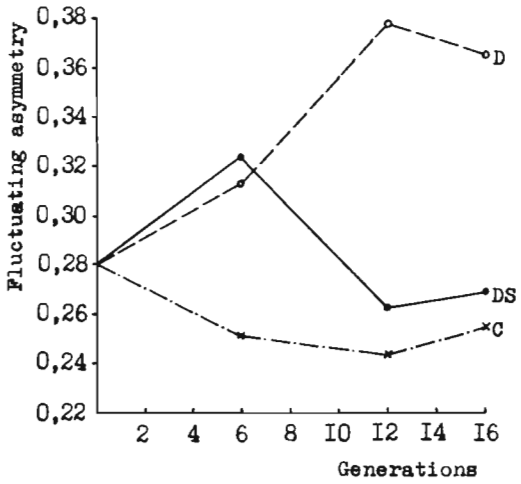

Figure 2. Fluctuating asymmetry in lines under directional (D), directional and stabilizing (DS) selection, and in unselected controls (C).

Two alternative explanations may account for this effect of stabilizing selection.

(1) Directional selection might bring about disruption of coadaptive gene complexes leading to segregation of extreme phenotypes with high FA (Mather, 1983). Elimination of these by stabilizing selection will result in decrease of FA in the population.

(2) Stabilizing selection could increase canalization of individual development by favoring individuals with better buffering capacity.

Our results do not allow to choose between these possibilities. The first one, however, seems to be more plausible in view of the evidence that canalizing effects are extremely difficult to produce by means of stabilizing selection (Prout, 1962).

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Lee, T.J. and N.W. Kim, Chung-Ang University, Seoul, Korea. *Drosophilidae* collection from South Korea.

The collections of *Drosophilid* flies were made at twelve localities such as Mt. Halra, Is. Wan, Mt. Chiri, Mt. Deoku, Mt. Palkong, Mt. Juwang, Mt. Kyelyong, Mt. Sokli, Mt. Taebaik, Mt. Sori, Mt. Ohdai and Mt. Sulak during a period ranging from April 1985 to November 1988. As the Korean members of the Drosophilidae family are recorded here in total of 83 species belong 10 genera. They are as follows: genus *Amiota* (5 species), genus *Stegana* (4 species), genus *Leucophenga* (7 species), genus *Paraleucophenga* (1 species), genus *Microdrosophila* (5 species), genus *Mycodrosophila* (9 species), genus *Liodrosophila* (1 species), genus *Nesiodrosophila* (3 species), genus *Scaptomyca* (4 species) and genus *Drosophila* (44 species). The genus *Drosophila* are included 6 subgenus such as subgenus *Hirtodrosophila* (10 species), subgenus *Dorsilopha* (1 species), subgenus *Lordiphosa* (6 species), subgenus *Scaptodrosophila* (3 species), subgenus *Sophophora* (7 species) and subgenus *Drosophila* (17 species).

Among the 83 species obtained in present study, 10 are new to Korea. They are as follows: *A. elongata, St. nigripennis, St. taba, St. ctenaria, St. longifibula, My. erecta, N. okadai, D. pseudotenuiciuda, D. throckmortoni* and *D. curvispina*.

In the collection for this survey, the following species were captured in northern localities more than in southern localities: *A. chungi, A. dispina, A. elongata, D. biauraria, D. bifaciata, D. kuntzei* and *D. testacea*. This fact suggests that these habitats are in the high altitudes or northern localities of Korea. The following species were captured in southern localities more than in northern localities: *Mi. fuscata, Mi. matsudairai, Mi. urashimae, Mi. purpurata, D. puncticeps, D. throckmortoni, D. nipponica* and *D. cheda*. This fact suggests that these habitats are in southern localities of Korea. The widely distributed species in Korea are *A. okadai, L. orientalis, Sc. pallida, D. busckii, D. coracina, D. melanogaster, D. auraria, D. triauraria, D. unispina, D. suzukii, D. bizonata, D. angularis, D. brachynephros, D. nigromaculata, D. histrio, D. virilis* and *D. lacertosa*. Among these species mentioned above, *D. busckii, D. melanogaster, D. virilis, D. suzukii, D. auraria, D. immigrans* and *D. nigromaculata* which are domestic or semidomestic species were captured near the human-habitations. In the collection for this survey, the following species were captured in small number: *A. elongata, St. ctenaria, St. longifibula, My. biceps, N. raridentata, N. magnidentata, N. okadai, D. nigripennis, D. pseudotenuicauda* and *D. curvispina*.

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Non-uniform distribution of mobile genetic elements along *Drosophila melanogaster* chromosomes.

The distribution of 10,525 *in situ* hybridization sites of copia-like, P, I and some other mobile elements (ME) was studied in X-chromosomes and autosomes of different *Drosophila* strains (Table 1). For some ME, the number of copies in X-chromosome was previously shown to be fewer than in autosomes (3, 11-13). Table 1 demonstrates that this phenomenon is also typical for X-chromosome in the gross analysis of ME distribution in the genome. On the contrary, the right arm of chromosome 3 is enriched with ME sites. The analysis of small chromosome regions containing two adjacent cytologic map sections shows that ME against DNA content are rare to be found in segments 14 + 15 and 16 + 17 of the X-chromosome. Interestingly, the same segments of X-chromosome recombine in meiosis 2 to 4 times less efficiently (1). Besides, the number of poly(dC-dA) * poly(dG-dT) sequences is much less here as compared with the other regions of X-chromosome (14). These sequences are potentially able to generate the Z-form of DNA (15), they are recombination "hot spots" (16-18), and they are absent from weakly recombining centromeric heterochromatin and chromosome 4 of *D. melanogaster* (14). In autosomes as well, there are regions relatively poor in ME. This phenomenon is specially typical for segments 26 + 27, 28 + 29, 54 + 55, 88 + 89. At the same time, regions considerably enriched with ME were revealed in each chromosome. In X-chromosome, this is segment 18 + 19; 34 + 35 in the left arm and 42 + 43 in the right arm of chromosome 2, and 84 + 85 and 86 + 87 in the right arm of chromosome 3. The amount of ME tends to increase in the chromosomal regions proximal to the centromere.

Thus, the above data on the gross distribution in chromosomes of approximately two dozens of ME families demonstrates well their non-uniform distribution both among different chromosome arms and in different regions within chromosomes. It was earlier proposed (7) that ME are responsible for at least a substantial part of ectopic contacts. A comparison of ectopic contact frequencies of different chromosome regions (19) with ME chromosomal distribution (Table 1) shows that their correlation along the genome on the whole is small but significant (the correlation coefficient is $r = +0.44$). One can therefore suppose that the non-uniform distribution of ME along the

Table 1. ME distribution along segments of X-chromosome and autosomes.

Parameter	Chromosome	Chromosome segments (section number of the cytologic map)									
	X	2+3	4+5	6+7	8+9	10+11	12+13	14+15	16+17	18+19	Total
1		188	199	148	156	194	134	49	70	281	1419
2		13.2	14.0	10.4	11.0	13.7	9.4	3.5	4.9	19.8	
3[a]		12.5	11.5	11.8	11.2	13.1	12.7	8.0	7.9	11.2	
4		1.06	1.22	0.88	0.93	1.05	0.74	0.44	0.62	1.77	
5[a]		8.5	19.1	8.0	14.9	15.1	17.5	4.2	4.3	8.5	
	2L	22+23	24+25	26+27	28+29	30+31	32+33	34+35	36+37	38+39	
1		223	198	110	94	175	206	389	303	393	2091
2		10.7	9.5	5.3	4.5	8.4	9.9	18.6	14.5	18.8	
3[a]		11.4	10.9	7.7	10.2	8.9	9.3	15.2	13.3	13.1	
4		0.94	0.87	0.69	0.44	0.94	1.06	1.22	1.09	1.44	
	2R	42+43	44+45	46+47	48+49	50+51	52+53	54+55	56+57	58+59	
1		388	157	260	236	260	244	103	310	227	2185
2		17.8	7.2	11.9	10.8	11.9	11.2	4.7	14.2	10.4	
3[a]		13.4	9.2	11.1	10.9	12.8	10.6	10.0	13.2	8.8	
4		1.33	0.78	1.07	0.99	0.93	1.06	0.47	1.08	1.18	
	3L	62+63	64+65	66+67	68+69	70+71	72+73	74+75	76+77	78+79	
1		230	315	282	207	186	119	297	119	182	1937
2		11.9	16.3	14.6	10.7	9.6	6.1	15.3	6.1	9.4	
3[a]		13.3	14.4	17.1	9.1	11.3	6.9	9.9	8.3	9.7	
4		0.89	1.13	0.85	1.18	0.85	0.88	1.55	0.73	0.97	
	3R	82+83	84+85	86+87	88+89	90+91	92+93	94+95	96+97	98+99	
1		360	461	488	214	224	264	220	250	412	2893
2		12.4	15.9	16.9	7.4	7.7	9.1	7.6	8.6	14.2	
3[a]		10.3	14.9	14.4	12.9	7.3	11.1	8.2	11.0	9.9	
4		1.20	1.07	1.17	0.57	1.05	0.82	0.93	0.78	1.43	

Notes: 1 - absolute number of ME sites revealed in the corresponding chromosome segment; 2 - % of the number of ME sites in the given segment with regard to the number of sites in the whole chromosome; 3 - DNA contents in the segment according to (2); 4 - the relation of ME % to the % of DNA in the segment; 5 - segment length in % of genetic map, determined by the position difference on the map of markers closest to cytologic segment boundaries (according to (1)); a - data on DNA content and on % meiotic recombination were calculated by multiplying by the corresponding coefficient so as the sum in the line would make up 100%. The DNA content and recombination frequency ratios between different regions of chromosome remains unchanged.

Data sources: MDG 1 (3305 sites) - (3-7); MDG2 (65 sites) - (7); MDG3 - (714 sites) - (3,4,7); MDG4 (4 sites) - (7); *copia* (2979 sites) - (4,7,8); Dm132 (577 sites) - (8); Dm67 (826 sites) - (8); *hopel* (839 sites) - (8); Dm25 (69 sites) - (9); Dm101F (43 sites) - (10); P (293 sites) - (4,8); I (454 sites) - (5,8); Dm2027 (33 sites), Dm2028 (10 sites), Dm2029 (153 sites), Dm2066 (34 sites), Dm2068 (35 sites), Dm2074 (16 sites), Dm2078 (76 sites) - (7).

genome will affect the spatial organization and the interaction of the chromosomes and their regions in interphase nuclei. Besides, the data show that the level of DNA sequences polymorphism dependent on integrating ME and crossing over efficiency might be strongly different for different regions of chromosomes. This is especially true for region 14-17 of the X-chromosome, in which the recombination, poly (dC-dA) * poly (dG-dT) and ME contents are reduced. DNA polymorphism drop might be either the cause or the consequence of the accumulation in this region of adaptively important loci, any damage of them substantially lowers the fitness of the organism.

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High variability of *in situ* hybridization intensity of the mobile element *hopel* and of telomere-specific probes.

The morphology of telomeric regions in polytene chromosomes often varies in different *Drosophila* strains, and sometimes even in the larvae of one strain or in different nuclei of one larva (1,2). According to Roberts, this phenomenon is due to a different degree of polyteny of telomeric regions. In total, this can lead to marked under-replication of telomeric sequences (3,4). In studies of *D. melanogaster* telomere-specific sequences (5,6) our attention was occupied by a more than usually pronounced variation of *in situ* hybridization efficiency over different chromosome sites within one nucleus and over identical sites within different nuclei. Figure 1 shows two adjacent nuclei of the salivary glands of a larva from the natural population after the *in situ* hybridization of DNA fragment Dm4568 consisting of two non-homologous telomere-specific sequences Dm2103 and Dm665. There is evidence of the equal intensity of hybridization with all but one telomere (the hybridization with telomeric region 100F of the 3R chromosome differs much in the two nuclei). Figure 2 demonstrates the hybridization with site 62A of two different nuclei from one preparation of the mobile element *hopel* that we cloned from DNA fragment Dm4579 also containing telomere-specific sequences. The element *hopel* is a variant of element Dm1360 (7). The photograph shows that the intensity of hybridization with site 62A in the two nuclei is different at a similarly high efficiency of hybridization with the chromocenter. Similar results were also obtained for other cloned DNA fragments containing telomeric sequences and having adjacent mobile elements. We believe that these data can be evidence in support of Roberts' opinion concerning variable polytenization of chromosome telomeric regions, and the data also indicate that the variably polytenized regions could also exist in internal chromosome regions.

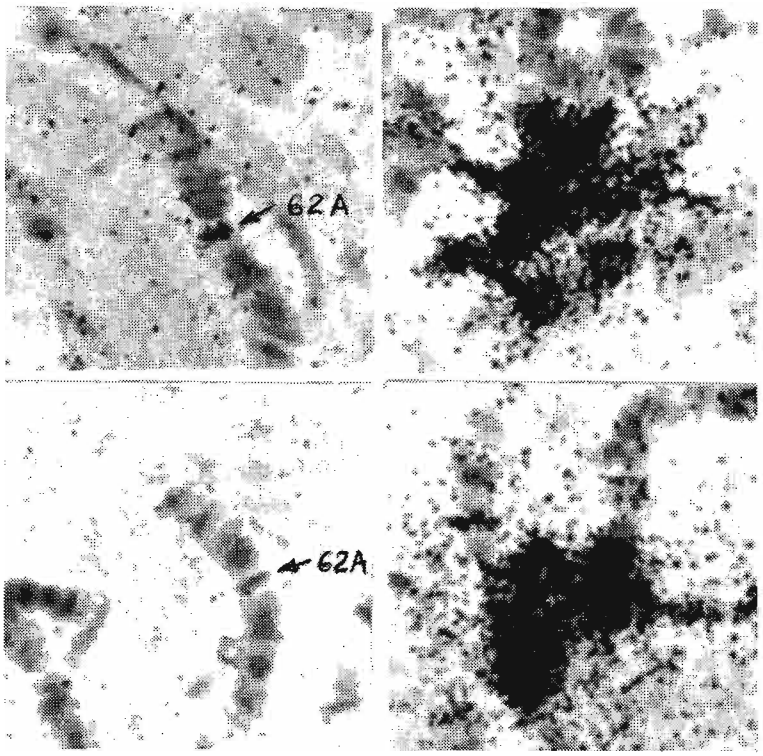

Figure 2. Varying hybridization with site 62A of the mobile element *hopel* in different nuclei of one larva. The hybridization with the chromocenter of the same nuclei is shown to the right.

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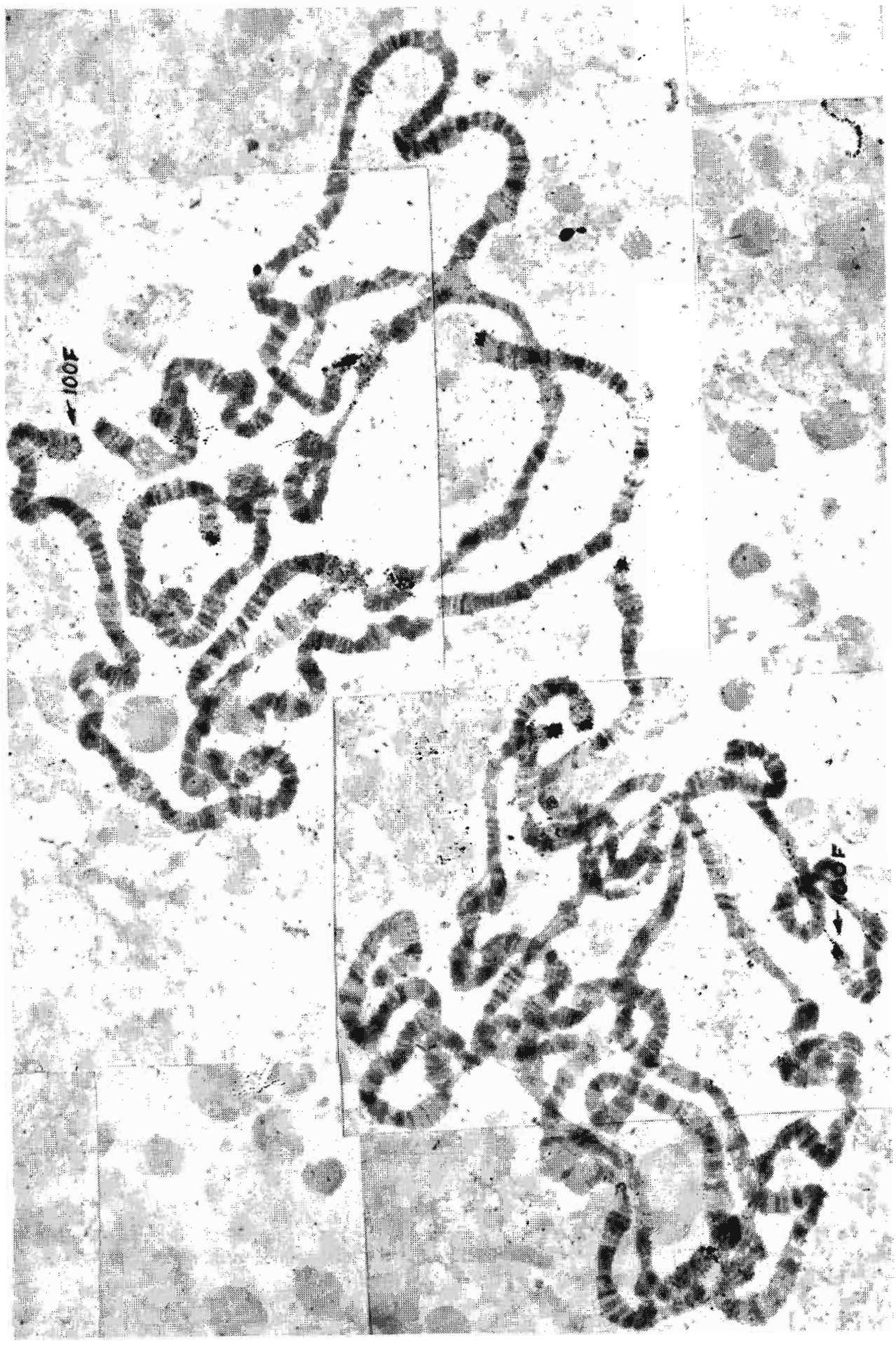
Figure 1 (facing page). The hybridization of DNA fragment Dm4568 with telomeric regions of adjacent nuclei of a larva from the Lerik natural population (arrows indicate greatly different hybridization with region 100F of the 3R chromosome).


100F
80F

Leisner, J. Department of Ecology and Genetics, University of Aarhus, Aarhus, Denmark. Linkage analysis of the *alpha-Amy* and *Pt-10* loci on *D. pseudoobscura.*

Prakash and Lewontin (1968) located the *Pt-10* locus on the third chromosome. By using two strains, homozygous for different *alpha-Amy* (alpha-amylase) and *Pt-10* (larval protein-10) alleles and fixed for the Chiricahua inversion (CH), it has been possible to map the *Pt-10* locus relative to the *alpha-Amy* locus.

The electrophoretic survey was carried out by using 5% acrylamide gels with pH adjusted to 8.9. Staining for alpha-amylase was done by incubating the gel in a 1% starch solution for 30 minutes and then in a I/KI solution for another five minutes. For protein-10 the staining condition was a 0.1% coomassie blue solution in a mixture of acetic acid, methanol and water in the proportions 10:25:65. The 3339.20 CH strain is fixed for both the 0.84 *alpha-Amy* allele and the 1.04 *Pt-10* allele, whereas the 3339.18 CH strain is fixed for the 1.00 *alpha-Amy* allele and the 1.06 *Pt-10* allele. Two replicate crosses between 3339.18 CH males and 3339.20 CH females and two replicates of the reciprocal cross were made. F-1 females were then testcrossed to either 3339.18 CH or 3339.20 CH males. The F-1 females were only checked for the amylase genotypes, but the offspring were scored for both amylase and protein-10 genotypes. Examination of 224 flies showed 24 recombinants between the two loci, which gives a genetic distance of 10.7 map units in the CH inversion. All other genetic positions for the third chromosome have been determined for the Standard-inversion (ST) (Tan 1937; Yardley 1974). The data presented cannot easily be transformed to the ST-inversion. It is not possible to estimate such a transformation from the observation of linkage-disequilibrium in nature between the *alpha-Amy* and the *Pt-10* loci and the different inversions. The reason for this is that these data can be explained as a result of natural selection and not only as a result of genetic drift and linkage (Prakash and Lewontin 1968).

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Lofdahl, K.,[1] **D. Hu,**[1,2] **and J. Hirsch**[1]. [1]University of Illinois, Urbana-Champaign, Illinois, USNA; [2]Peking University, Beijing, China. Partial reproductive isolation between lines of *Drosophila melanogaster* long selected for high or low geotaxis.

Several studies have convincingly demonstrated that significant partial reproductive isolation can be produced by disruptive selection when hybrids have zero fitness (e.g., Koopman, 1982/1950; Knight, Robertson and Waddington, 1956), though some others have been received with skepticism (Hirsch, 1976). Here artificial selection has been acting directly against hybridization.

In models of the origin of reproductive isolation during allopatric speciation, however, natural selection is believed to act directly on other characters related to environmental adaptation. Reproductive isolation thus arises as a by-product of adaptation of two subpopulations to different environments (Muller, 1939). Better to understand the process of allopatric speciation, experiments must explore how genetic changes involved in environmental adaptation relate to genetic change that produces homogamy. This rationale led to the report of Thoday and Gibson (1962) (reviewed in Thoday, 1972) whose primary finding of complete reproductive isolation is still controversial (Scharloo, 1971; Hirsch, 1976).

In 1958 bidirectional selection for high and low geotaxis behavior was begun from a single base population (Erlenmeyer-Kimling, Hirsch and Weiss, 1962). Since this involved divergent selection for behavioral adaptation to two different environments, we tested whether any reproductive isolation might have arisen between these two behavioral phenotypes. No gene flow occurred between high and low geotaxis lines after the first generation. A 15-unit geotaxis maze (Hirsch, 1959) was used to classify phenotypes on a scale of 0-15. After more than 650 generations of intermittent selection, geotaxis scores were highly divergent (High Line: 14.88 females, 14.07 males; Low Line: 1.97 females, 2.02 males) and, when selection is relaxed, these behavioral phenotypes have remained stable since about 1978, suggesting that new phenotypic optima (i.e., new behavioral adaptations) have evolved in the High and Low geotaxis populations (Ricker and Hirsch, 1985, 1988b).

A preliminary test for significant reproductive isolation between High and Low geotaxis lines at Generation 585 was performed as a student project by Linda Skoog in this laboratory. Using the mating chambers of Elens and Wattiaux, 1964) with a multiple choice test (10 females and 10 males each per chamber from both lines), she found an index of reproductive isolation of $I = 0.18 \pm 0.07$ for 3-day old flies (where $I = 0$ for random mating and $I = 1$ for complete reproductive isolation). Approximately 80 generations later, we replicated her test, but for comparability used the same sample sizes as Henderson and Lambert (1982, 11 females and 11 males each per chamber from both lines),

and restricted the observation period to the morning (8:00 am to noon), the time in their circadian cycle of maximum courtship activity. The High and Low geotaxis lines still show significant (partial) reproductive isolation similar in amount to that found by L. Skoog. I = 0.13 ± 0.04 (3-day old flies) and I = 0.18 ± 0.05 (5-7 day old flies). This result contrasts with the random mating found between two *D. melanogaster* wild-type control lines (ADH^{F1} and ADH^{US} in the same experiment (I = 0.03 ± 0.08, see Table 1). Random mating was also found among many *D. melanogaster* populations despite their divergence in other adaptive traits as reported by the wild-wide survey of Henderson and Lambert (1982). Therefore, the partial reproductive isolation found between geotaxis lines in the present study, where the behavioral adaptation under selection is known, as are some of its genetic correlates (Ricker and Hirsch 1988a & b), offers further possibilities for understanding the early stages of allopatric speciation.

Table 1. Measurement of partial reproductive isolation between high and low geotaxis lines.

	N	I	ZI	χ^2:	♀♀ vs ♂♂
1	188	0.18 ± 0.07	1.44 ± 0.18	6.17,	$p < 0.025$
2 a	489	0.13 ± 0.04	1.43 ± 0.13	10.99,	$p < 0.001$
2 b	363	0.18 ± 0.05	1.43 ± 0.13	11.54,	$p < 0.001$
3	146	0.03 ± 0.08	1.12 ± 0.19	0.41,	$p > 0.50$

I: Reproductive isolation index;
ZI: Joint isolation index;
χ^2: Test of independence in mate preference between ♀♀ and ♂♂ and within geotaxis lines
1: Previously collected student (Linda Skoog) data
2a: 3-day old - current data
2b: 5-7 day old - current data
3: Two-wild type control (random mating) populations

We plan additional experiments to determine whether any genetic correlation exists between mate preference and geotaxis behavior. The lines are available from this laboratory to interested investigators who contact Jerry Hirsch.

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Lofdahl, K.,[1] D. Hu,[1,2] and J. Hirsch[1]. [1]University of Champaign, Illinois USNA; [2]Peking University, Beijing, China. Rapid response to divergent selection for geotaxis from the F_2 of a cross between long selected high and low geotaxis lines in *D. melanogaster*.

Evidence exists that long-term bidirectional selection for extreme expression of geotaxis behavior in two genetically isolated populations of *D. melanogaster* has involved evolutionary changes in the genetic correlates of this behavior (Ricker and Hirsch, 1985a & b, 1988a & b). One change is the appearance of recently detectable interactions among different chromosomes (Ricker and Hirsch 1988a). A second change is a reversal of the usual relationship between geotaxis score and fitness [the extreme phenotype now has higher fitness than intermediate phenotypes in the low line (Ricker and Hirsch, 1988b)]. Perhaps the number of genes differentiating the High and Low geotaxis lines is now very low, possibly as few as 2-4 major loci (Ricker and Hirsch, 1988b). This result contrasts with the original gradual response to selection which suggested a typical polygenic system underlying the individual differences in this behavior (Erlenmeyer-Kimling, Hirsch and Weiss, 1962). Thus, to assess this possibility we have tested whether changes in the gene correlates of this behavior would be reflected in the rate of response to selection. Additionally, we designed our experiment to address the possibility (suggested by a reviewer of Ricker and Hirsch, 1988b) that presently the gene pools of the High and Low geotaxis lines are the result of an accidental cross between them near generation 415 that had gone unnoticed (see Fig. 1 in Ricker and Hirsch, 1985a or Figs. 2 & 3 in Ricker and Hirsch, 1985b).

Therefore, High and Low geotaxis lines that have been intermittently selected more than 650 generations were crossed to compare the response to selection from the F_2 generation with that in the original base population. Replicated divergent selection for high or low geotaxis score in a 15-unit maze began in the F_2 generation of the High females x Low males cross. The highest (or lowest) scoring 25 females and 25 males were selected from the approximately 150 females and 200 males tested in each line every generation. Results of this second cycle of divergent selection are presented in Figure 1. The response to selection is now much faster from the F_2 than it was from the original base population (Erlenmeyer-Kimling, Hirsch and Weiss, 1962). The rate of behavioral evolution is also much

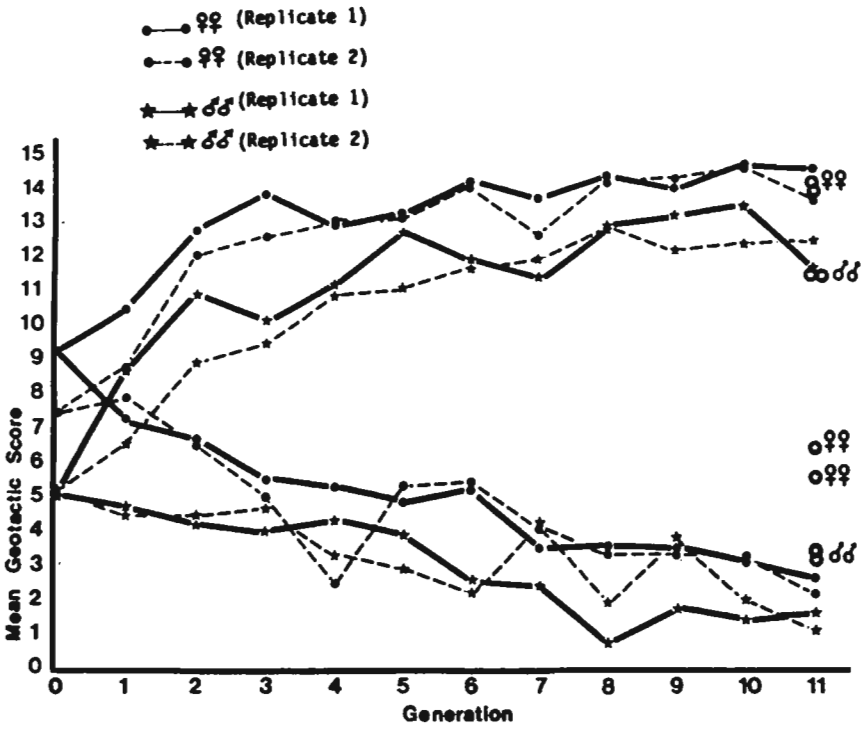


Figure 1. Results of bidirectional selection in replicate lines from an F_2 cross of High x Low geotaxis lines of *D. melanogaster*.

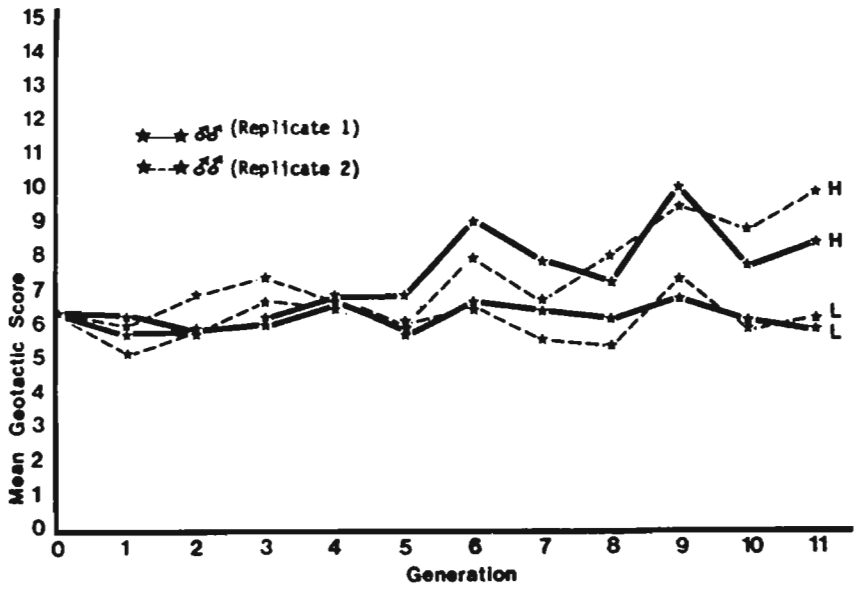


Figure 2. Results of bidirectional selection in replicate lines from the Control Line (Champaign wild-type) at an intensity of selection greater than or equal to that used with the F_2 cross shown in Figure 1. (Only males are shown; results for females are similar).

faster than that presently observed in a wild-type stock (Champaign line) under a similar intensity of divergent selection (Fig. 2). The more rapid response to selection from an F_2 could be due entirely or in part to linkage disequilibrium of geotaxis related genes in the F_2 generation (Mather, 1983). A second possibility that could account for this rapid response in the F_2 reselected lines was suggested by Ricker and Hirsch (1988b), namely that, by the start of the present experiment (Generation 652), only a few segregated genes are correlated with differences between the High and Low geotaxis lines. They suggested two causes of such a reduction in the number of effective factors: (1) changes at "modifying" loci and (2) evolution of major loci that are each a complex of closely-linked genes. Because our results are consistent with either the involvement of fewer genes or linkage disequilibrium between geotaxis gene correlates in the F_2 generation, further experiments will try to find loci that cause differences between high and low geotaxis lines by mapping with electrophoretic methods.

The High and Low reselected lines were also studied further to understand a remarkable change in the relation between fitness and geotaxis phenotype in the original High and Low lines. The High and Low geotaxis lines now show stable phenotypes with no tendency to regress toward the wild-type mean when artificial selection is relaxed. Prior to 1978, regression of the phenotype toward the wild-type mean usually occurred (Dobzhansky and Spassky, 1969; Ricker and Hirsch, 1988b). We relaxed artificial selection for 7 generations beginning in Generation 4 of our reselection from the F_2 cross. Here we again found the new high and low phenotypes to be evolutionarily stable (see Fig. 1). This stability persists in the High and Low lines despite the opportunity for genetic recombination that occurred

in breeding the F_2. At present, reverse selection is underway to assess the amount of genetic variance still remaining in the High and Low geotaxis lines reselected from the F_2. If these lines are genetically variable for this behavior, natural selection may have established a new phenotypic optimum for geotaxis in these High and Low lines (Ricker and Hirsch, 1988b). If there is a response to reverse selection, relaxed selection in the reversed lines will test whether natural selection now favors a return to a high (or low) geotaxis phenotype in the lines reselected from the F_2. The lines are available from this laboratory to interested investigators who contact Jerry Hirsch.

References: Dobzhansky, T. and B. Spassky 1969, Proc. Nat. Acad. Sci. USA 62:75-80; Erlenmeyer-Kimling, L., J. Hirsch and J.M. Weiss 1962, J. Comp. & Physiol. Psych. 55:722-731; Mather, K. 1983, in M. Ashburner, H.L. Carson, and J.N. Thompson, Jr. (Eds.). THE GENETICS & BIOLOGY OF *DROSOPHILA*, 3c:155-221, London:Academic Press; Ricker, J.P. & J. Hirsch 1985a, DIS 61:141-142; Ricker, J.P. & J. Hirsch 1985b, J. Comp. Psych 99:380-390; Ricker, J. & J. Hirsch, 1988a, Behav. Gen. 18:13-25; Ricker, J. & J. Hirsch 1988b, J. Comp. Psych. 102:203-214.

Lozovskaya, E.R., V.Sh. Sheinker, and M.B. Evgen'ev. Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow, Vavilov str., 32. New type of hybrid dysgenesis discovered in *Drosophila virilis*.

In recent years independent systems of hybrid dysgenesis have been discovered in *Drosophila melanogaster*. The activity of P-element, I-element and hobo is responsible for different dysgenic traits described in these systems (Bingham, P.M., M.G. Kidwell and G.M. Rubin, 1982; A. Bucheton, R. Paro and H.M. Sang, 1984; Yannopoulos, G., N. Stamatis, M. Monastirioti, P. Hatzopoulos and Ch. Louis, 1987).

Interestingly, that "typical" dysgenesis has not been observed in other *Drosophila* species studied, although some of the dysgenic features, e.g. male recombination or sterility of F_1 progeny, were demonstrated (Kidwell, M.G., J.F. Kidwell, and J.A. Sved, 1977; Kidwell, M.G., 1987).

Here, we report a new system of hybrid dysgenesis found in *Drosophila virilis* when strains of different origin were crossed. Preliminary results were published (Lozovskaya, E.R. and M.B. Evgen'ev, 1987).

The following wild type strains were used: 1 (Kutaisi, USSR), 9 (Batumi, USSR), S9 (Seishel Islands) and 101 (Japan). The following marked strains were used: 104-gl; 110-tb gp-L_2,st (USA); 149-b,tb gp-L_2, cd, pe (Japan); 160- b, tb gp-L_2, cd, pe, gl (obtained by crossing strains 104 and 149); 109- w Bx y (USA). All strains were obtained from *Drosophila* collections of the Institute of Developmental Biology of the USSR Academy of Sciences. The genetic markers mentioned were described earlier (Gubenko, I.S. and M.B. Evgen'ev, 1984).

We analyzed the level of sterility of F_1 progeny from different reciprocal and intrastrain crosses (Table 1). Parental strains were crossed en masse. Then F_1 flies were individually mated with fully fertile strain 9. Gonads of sterile flies were examined. Table 1 indicates that all strains used may be divided into three groups: (1). 149 and 160; (2). 2, 9, S9, 104 and 109; (3). 101 and 110. When females of group 2 are mated to males from group 1 the percentage of sterility is very high, while in reciprocal crosses the correspondent value is rather low. When flies from group 3 are mated to the stocks from group 1 or 2 the level of sterility does not differ significantly from that observed in intrastrain crosses.

The investigation of testes and ovaries in sterile F_1 flies revealed bilateral atrophy of gonads (Fig. 1). In the flies from reciprocal and intrastrain crosses such atrophy was never observed (or was observed in a very low level).

Analysis of progeny from 9 female x 160 male crosses exhibiting high level of F_1 sterility enable us to observe a great deal of different changes in phenotype. Some of them have been proven to be due to mutations. Some of them resemble by phenotype well-known mutations of *D. virilis*, the others were obtained for the first time. It's noteworthy that some of the mutations, e.g. sn, w, arc, etc., were repeatedly isolated from the progeny of independent dysgenic crosses. Most of the mutations observed were found in clusters thus being the result of premeiotic event. The insertional nature of the mutations have been proven firstly by finding the revertants for sn-locus and secondly, by Southern blotting comparing genomic DNA from strains 9, 160 and a strain carrying the mutation of w-locus, obtained in dysgenic cross between these strains.

Thus, we may conclude that *D. virilis* has its own system of hybrid dysgenesis partially resembling that of P-M dysgenesis described in *D. melanogaster*. Probably strains 149 and 160 carry some mobile genetic element responsible for dysgenic traits observed. We call this element U-element (Ulysses) and strains 149 and 160 U-strains, while strains without active U-element should be called U^o-strains.

Figure 1 (facing page). Atrophy of gonads due to hybrid dysgenesis in *D. virilis*. a & b - strain 9, normal testis and ovaries. c & d - testis and ovaries of F_1 progeny from crosses of females 9 to males 160. (x180).

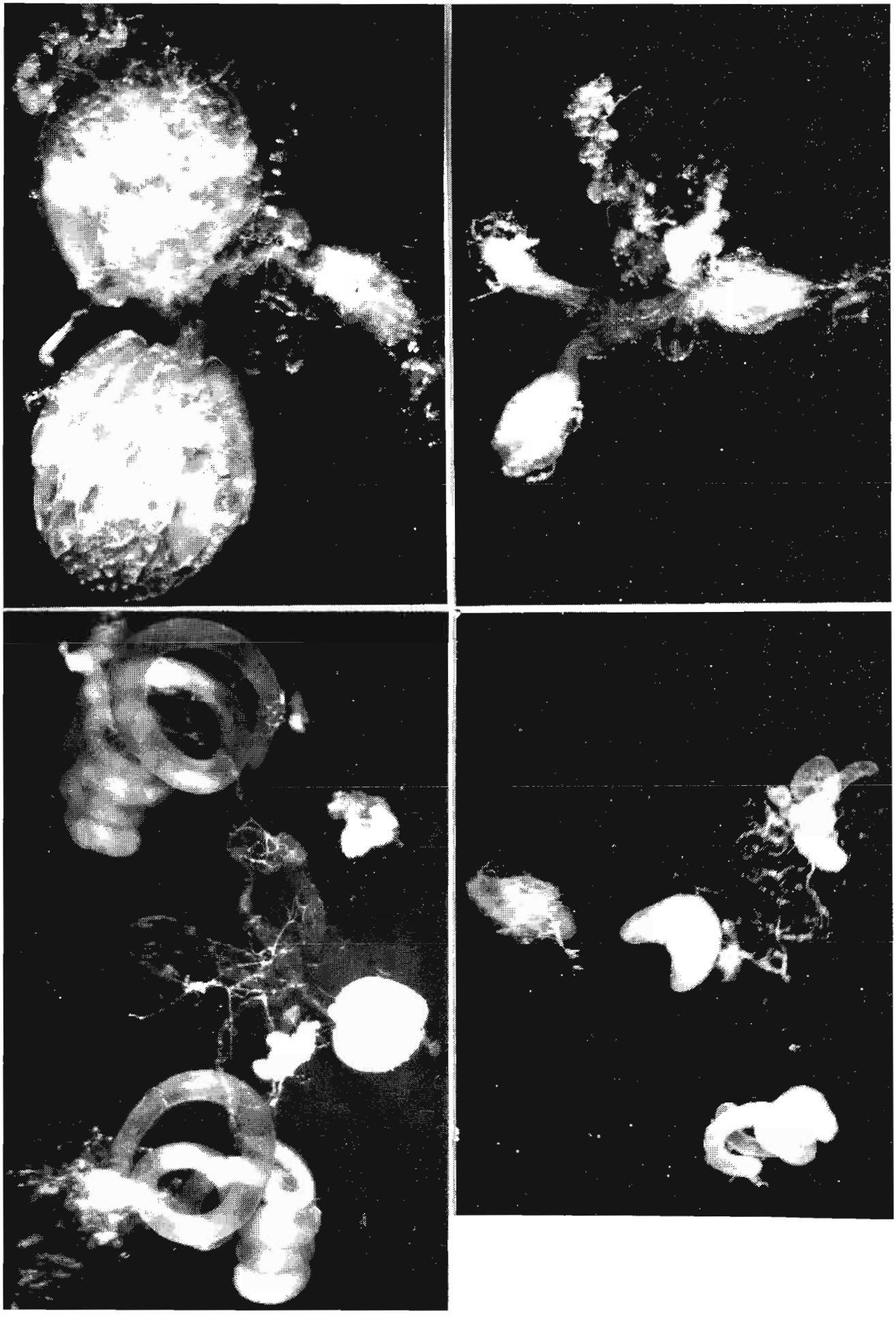


Table 1. Percent F_1 progeny sterility in some crosses of different strains of *Drosophila virilis*.

	2	9	104	109	S9	101	110	149	160
2	0 0	0 0	0 13				0 0	15(40) 93(42)	96(82) 100(55)
9	0 0	0 0	0 0	0 0	0 0	0 0	0 0	3(40) 16(32)	96(480) 94(120)
104		0 0							91 100
109	0 0	0 21					0 7	0 20	52 83
S9		0 0			0 0				83 38
101 110									0 7
110	0 0	0 0		0 5				0 0	0 0
149	0 0	0 25		0 0					0 8
160	0 10	7 5		20 10	9 5	8	8 17	0 0	20 10
Group			2			3		1	

Females above the line, males below the line. In each case not less than 20 flies were examined. In the brackets the numbers of flies are indicated in the cases where fertility of the progeny was significantly reduced.

It should be noted that intensive studies failed to demonstrate the presence of *D. melanogaster*'s P- or I-element in *D. virilis*.

The study of U-element is now in progress.

References: Binham, P., M.G. Kidwell and G.M. Rubin 1982, Cell 29:995-1004; Bucheton, A., R. Paro and H.M. Sang 1984, Cell 38:153-163; Yannopoulos, G., N. Stamatis, M. Monastorioti, P. Hatzopoulos and Ch. Louis 1987, Cell 49:487-495; Kidwell, M.G., J.F. Kidwell and J.A. Sved 1977, Genetics 86:813-833; Kidwell, M.G., The Genetics and Biology of *Drosophila*, N.Y., Acad. Press, 1983, pp 127-139; Lozovskaya, E.R. and M.B. Evgen'ev 1987, Proc. Acad. Sci. of the USSR 296:727-731; Gubenko, I.S. and M.B. Evgen'ev 1984, Genetics 65:127-139.

Mal, Arpita and A.S. Mukherjee. Calcutta University, India. The pattern of protein synthesis in the presence of puromycin in *Drosophila melanogaster*.

The nucleosomal structure undergoes a considerable change during the cell cycle (Gurley et al., 1978). Proteins play a significant role in such change in the chromatin organization. Rao and Sperling (1979) showed that a pool of protein factor is built up during the late G-I to early S-phase. When the pool reaches a certain level or threshold, the pool somehow triggers the initiation and DNA replication starts. Puromycin is an inhibitor of protein synthesis, it activates potential initiation sites and evokes new initiation of replication of *Drosophila* (Murkherjee and Chatterjee, 1984). In order to understand the pattern of protein pool differentiated under the condition of puromycin treatment that renders the replicative system to be potentially able to activate new initiation, the present investigation was undertaken to isolate and fractionate the proteins from *Drosophila* following treatment with puromycin.

Third instar larvae of *Drosophila melanogaster* were used as the experimental material. In the first set (Set A) of experiments, salivary glands were incubated with puromycin (100 ug/ml) followed by ^{3}H-lysine (1mCi/ml; sp.activity 5600.0 mCi/mol) labelling for 1 hour. In the second set (Set B) of experiment, glands were treated with puromycin and pulsed with ^{3}H-lysine simultaneously for 1 hour. Salivary gland incubated in the buffer Ringer (pH 7.0) without puromycin and labelled with ^{3}H-lysine were used as control set. After termination of the incubation (by adding a drop of 10% cold TCA), the glands were washed three times with 10% TCA. Tissues were transferred from the slide to a 1.5 ml eppendorf tube filled with 10% TCA and sedimented in the eppendorf centrifuge. The pellet was washed in cold TCA (20 min), then in 95% ethanol, followed by treatment with methanol:chloroform mixture (1:1), and subsequently dried in 37°C.

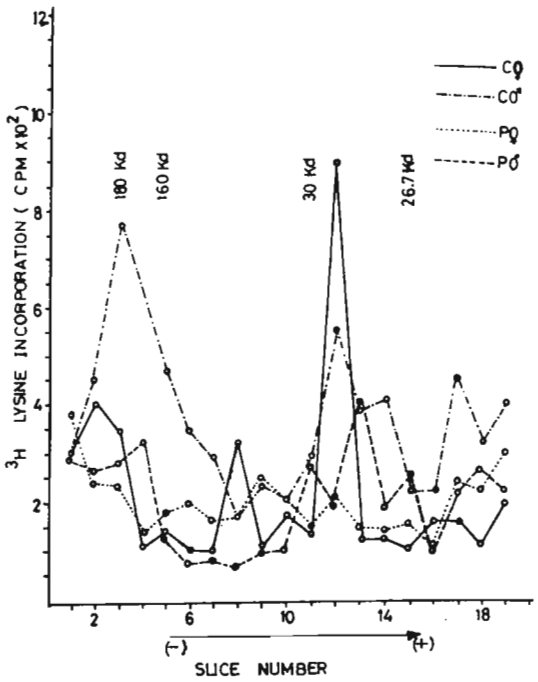


Figure 1. Distribution of radioactivity in SDS gel after separation of proteins extracted from 3rd instar salivary glands of *Drosophila melanogaster* (male and female) after 20 min incubation with Ringer (pH 7.0)/puromycin (100 ug/ml) followed by 1 hour incubation with 20 uCi tritiated lysine. C = Control, P = Puromycin treated.

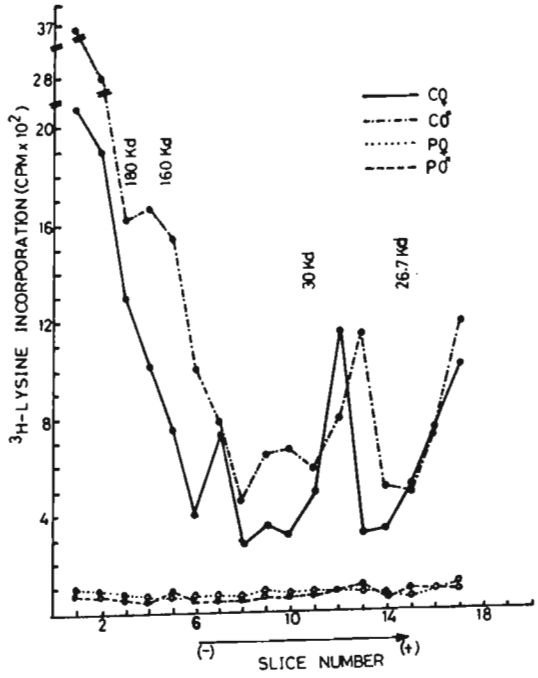


Figure 2. Distribution of radioactivity in SDS gel after separation of proteins extracted from 3rd instar salivary glands of *D. melanogaster* (male and female) after 1 hour simultaneous incubation with Ringer (pH 7.0)/puromycin (100 ug/ml) and 20 uCi of a mixture of tritiated lysine. C = Control, P = Puromycin treated.

SDS polyacrylamide gel electrophoresis was carried out with 20-30 ul (conc. 1 mg/ml) of sample per lane according to Laemmli's method (Laemmli, 1970). Samples were heated for 2 minutes in boiling water bath and loaded onto vertical SDS polyacrylamide (12.5%) slab gel. The run was performed for 4 to 5 hours with 2 mA/lane. After completion of run the gel was fixed and stained with coomassie blue. The banding pattern of proteins in the gel were more or less similar in control and puromycin treated samples. Each lane of the gel was sliced into 5 mm pieces from origin to dye-front and each piece was placed in scintillation vials with 0.5 ml hydrogen peroxide and kept over night at 65°C. After the gel was dissolved the vials were brought to room temperature. 4 ml of scintillation cocktail was added and mixed gently. Radioactivity was measured in the Packered scintillation Counter (Prias).

In set A, the radioactivity measurement revealed two distinct peaks (Fig. 1) in control set in samples from both males and females (corresponding to the bands with the molecular weight of 160-180 kd and 26.7-30 kd). In contrast, the proteins extracted from puromycin-treated glands show drastically reduced radioactivity and no such peaks are found as in control.

In set B, the radioactivity measurement revealed two distinct peaks (Fig. 2) in controls, especially in the two regions of banding pattern, one at 160-180 kd, another at 26.7-30 kd. The radioactivity in the high molecular weight peak is greater in male than in female, and that of the low molecular weight peak is higher in the female than in the male. A third peak appears in the control female at an intermediate position. In contrast, the proteins extracted from puromycin-treated glands show moderate to low radioactivity and low peak heights in the same corresponding regions as in control. It is suggested that these proteins/polypeptides might play a role in augmenting the initiation or inhibiting the chain elongation during DNA replication.

The following conclusion is tentatively made on the basis of the results obtained so far. Puromycin treatment establishes a different pool of proteins within the replicating cell, such that the proteins required for the chain growth are repressed and those required for initiation are allowed to predominate.

References: Gurly, L.R. et al. 1978, Acad. Press pp. 37-60; Laemmli, U.K. 1970, Nature 227:686; Mukherhjee, A.S. and C. Chatterjee. 1984, J. Cell Sci. 65:95-108; Rao, P.N. and K. Sperling. 1979, Chromosoma [Berl.] 45:121-131.

Mal'ceva, N.I., Kozlova, T.Yu., Zhimulev, I.F. Institute of Cytology and Genetics, the USSR Academy of Sciences, Novosibirsk-90, USSR. Cryptic polyteny in the giant nuclei of the *gnu Drosophila melanogaster* embryos.

Embryos whose mothers are homozygous for *gnu* (a recessive, maternal-effect lethal mutation) undergo DNA replication but no nuclear division. This causes the formation of a small number of giant nuclei in the syncytial blastoderm (Freeman et al., 1986; Freeman and Glover, 1987). The interior spatial organization of such type of nuclei has been of particular interest for the present study. Some authors reported the data suggesting more or less regular arrangement of chromatids in such type of Dipteran nuclei (Bier, 1960; Hammond and Laird, 1986). To elucidate this problem the 10A1-2 band genomic clones of the X chromosome -- M2364, M2034 obtained by chromosome walking -- were hybridized to the *gnu* embryo nuclear preparations. In case of a bundle-like array of chromatic fibers the labelling was expected to be compact, otherwise it should be diffuse.

Table 1. Distribution of different label patterns of nuclei as subject to clone and temperature rate.

Clone	Back-ground labelling	Over back-ground	One region of intensive labelling	Two regions of intensive labelling	Three regions of intensive labelling	Individual clusters of label throughout the whole nucleus
236	2	1	7	4		1
2034		1	6	1		1
all	2	2	13	5		2
236		2	3	4	1	
2034	1	3	2			
all	1	5	5	4	1	
total	3-7.5%	7-17.5%	18-45%	9-22.5%	1-2.5%	2-5%

The fly cultures were maintained at 25°C and 18°C. The eggs deposited by the females homozygous for the *gnu* mutation at 25°C and 18°C were allowed to develop during the night at 18°C. After dechorionation the embryos were transferred to Carnoy fixative. Twenty minutes after being transfixed the vitelline membranes were removed, and embryos, 2-3 at a time, were transferred onto glass slides in 45% acetic acid for squashing. DNA:DNA in situ hybridization was performed by the technique of Gall and Pardue (1971) with the addition of 10% dextran sulfate at 65°C. The preparations were rinsed at 55-60°C.

The quantitative results on the 40 nuclei studied are represented in Table 1. The label pattern is diffuse in a quarter of all the nuclei, in the remainder it is local. The most typical nuclei are shown in Figure 1. The intensity of labelling appears not to be dependent on the chromatin density in the nuclei (Figure 1). In a series of works on different organisms the increase of chromatid synapsis was shown under the action of low temperature as well as the better chromosome morphology was observed (Bier, 1960; Nagl, 1969; Ribbert, 1979). In the present experiment the lowering the temperature did not enhance chromosome conjugation enough to see classic banding chromosomes (Table 1). However our results are evidence of partial conjugation of chromatids in the majority of cases, and arrangement of chromatin in a longitudinal order.

Acknowledgments: The authors are very indebted to Dr. C. Nusslein-Volhard for providing us with the *gnu* stock and O.A. Kharlamova for translating the manuscript into English.

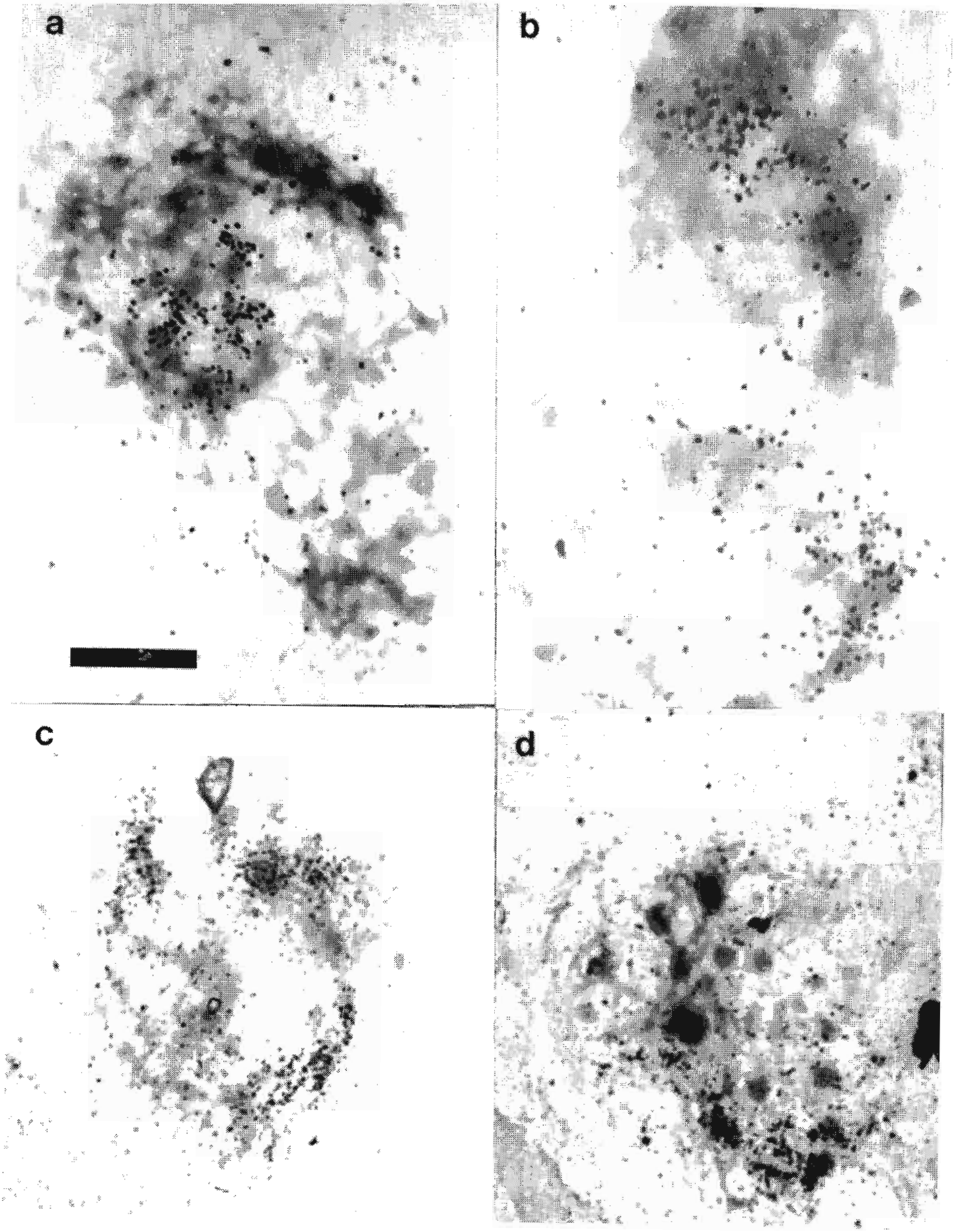
References: Bier, K., 1960, Chromosoma 11:335-364; Freeman, M., C. Nusslein-Volhard and D. Glover, 1986, Cell 46:457-468; Freeman, M. and D.M. Glover, 1987, Genes and Development 1:924-930; Gall, J.G. and M.L. Pardue, 1971, Methods Enzymol. 21:470-480; Hammond, M.P. and C.D. Laird, 1985, Chromosoma 91:267-278; Nagl, W., 1969, Nature 221:70-71; Ribbert, D., 1979, Chromosoma 74:269-299.

Figure 1 (facing page). Main types of nuclear labelling: a, one region of intensive labelling; b, two main regions; c, three main regions; d, numerous regions. Scale represents 10 micra.

Malevanchuk, O.A., N.I. Peunova, P.V. Sergeev, G.N. Yenikolopov. V.A. Engelhardt Institute of Molecular Biology, USSR Academy of Sciences, Moscow, USSR. The Est^S locus of *D. virilis* contains two related esterase-like genes.

Several esterase isozymes are known for *D. virilis* (Korochkin, 1980) but interrelations within this class of enzymes are not clear. Some of them, which are tightly linked on the genetic map, can either be encoded by distinct genes, or present splice forms of the same gene. We have previously cloned the Est^S gene coding for *D. virilis* tissue-specific esterase S (Yenikolopov et al., 1983) and now describe the localization of the second esterase-like gene in the close proximity to the Est^S gene.

Southern analysis of the total *D. virilis* genomic DNA reveals several (up to 10) additional signals when a long (ca.


a
b
c
d

15kb) cloned region of *D. virilis* DNA is used as a probe. These signals are indicative of hybrids with a different degree of homology as they respond differently to the elevation of washing temperature. The pattern differs when a short (ca. 500bp) subclone, which contains the region of the Est^S gene coding for the active site polypeptide, is used. This presumably more evolutionary conservative fragment, when used as a probe, reveals only one fragment forming a highly stable duplex. Adjacent fragments when used as probes, do not reveal additional new fragments except the expected ones, which suggests that the homology region for the two genes is rather short and does not exceed 0.5kb. We have cloned the genomic fragment containing a region of homology with Est^S and the physical and transcriptional maps (obtained by S_1-analysis and sequencing) for the corresponding region in *D. virilis* genome are shown in Fig. 1. Two genes are separated with only 800bp-long stretches of DNA with a high AT-content. They have the same direction of transcription and are similar in their size (appr. 1900bp-long mRNA) and exon-intron structure. These data suggest a duplication origin for this tandem. The discovered gene (designated as Est^X) starts to be transcribed in imago immediately after hatching and is used with an equal efficiency in males and females. Sequence comparison of several functionally important regions of two genes shows different rates of divergence of various parts of genes. While the similarity of the 5'-flanking regions is mainly limited to AT-rich tracts, the coding region reveals higher degree of relatedness. For example, 28 out of 50 N-terminal amino acids (56%) are the same in the mature proteins (both contain signal peptide-like sequences; Est^S is known to be secreted into the ejaculatory bulb cavity) and 86 out of 150 coding nucleotides (57%) coincide. The coding regions of the active sites carry even higher degree of similarity while the C-terminal halves, in accordance with hybridization data, have much less in common, probably as a result of exon shuffling in the evolution of the genes. Comparison of the presumptive active-site peptides of the two genes with a consensus sequence for the active site peptides from ten esterases of various origin (*Drosophila*, man, ray, eel, chicken, horse) shows a substantial homology, especially to *D. melanogaster* Est^6, whose role is similar to that of Est^S (Oakeshott et al., 1987).

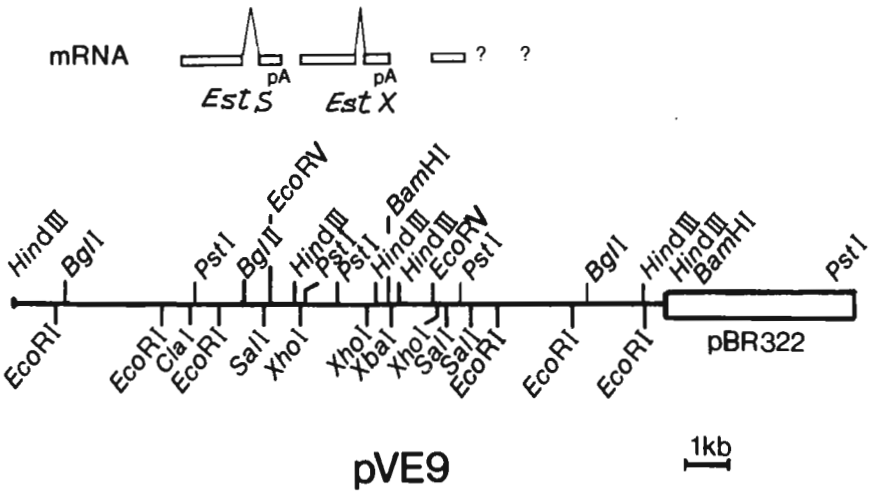


Figure 1. Physical and transcriptional maps for the Est locus of *D. virilis*. pA - mRNA polyadenylation site.

At present, we cannot determine which of the *D. virilis* esterases is encoded by the newly discovered gene. Genetic mapping data and data on the transcriptional pattern in ontogenesis point to the esterase-B as the most probable candidate, but one has to consider the possibility that multiple polypeptide isoforms are generated via alternative splicing and the use of alternative promoters.

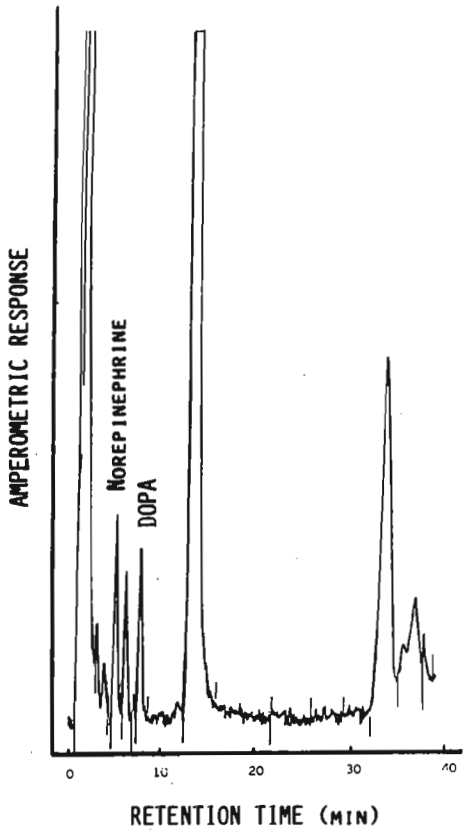
References: Korochkin, L.I. 1980, In: Isozymes, vol. 4, Alan R. Liss, Inc. N.Y., pp. 159-202.; Oakeshott, J.G., et al., 1987, Proc. Natl. Acad. Sci. USA 84:3359-3363; Yenikolopov, G.N., et al., 1983, EMBO. J. 2:1-7.

Martinez-Ramirez, A.C., F.J. Silva and J. Ferre. University of Valencia, Spain. Analysis of DOPA and norepinephrine in *Drosophila melanogaster* by high-performance liquid chromatography.

Biogenic monoamine neurotransmitters have been detected in nervous tissue from many insects. A simple procedure of analysis by reversed phase high-performance liquid chromatography has been applied to the estimation of catecholamines from *Drosophila melanogaster* extracts.

The chromatographic system consisted of a Nova-Pak C_{18} column (150 x 3.9mm, 5um) and a Waters Model 510 pump. The mobile phase consisted of a methanol-buffer mixture (2:98 v/v) with the following buffer composition: 50mM sodium phosphate, 0.7mM octyl-sulfate and 0.5mM EDTA (pH 3.0).

Samples were collected, homogenized in 200ul of 0.1M HCl (4ul per larva) and centrifuged for three minutes at



15,000g. To the supernatant, 40% $HClO_4$ (1/20 by vol.) was added to precipitate the proteins. After another centrifugation at 15,000g, the supernatant is collected and injected directly (20ul). The flow rate was 1.4 ml/min and the catecholamines were detected with a Methrom 656/641 electrochemical detector, working at an oxidation potential of +700 mV.

Figure 1 shows a typical chromatogram of an extract of wild type third instar larvae. The amount of DOPA and norepinephrine per larva was estimated to be around 18.9 and 14.4 pmoles, respectively.

Figure 1. Chromatogram of an extract from wild type third instar larvae.

McRobert, S. Swarthmore College, Swarthmore, Pennsylvania. *Drosophila* species collected in Bozeman, Montana.

In what has become an ongoing project to collect *Drosophila* in various locations throughout the United States, I trapped flies near the town of Bozeman, Montana, during the summer of 1988.

Table 1. Species trapped in Bozeman, Montana.

Species	No. of Males	No. of Females	Total
D. athabasca	29	10	39
D. occidentalis	7	5	12
D. testacea	1	3	4

The flies were captured in clear plastic cups suspended from trees (McRobert and Tompkins, 1983) or metal coffee cans set on the ground. The bait used was a mixture of banana, yeast and red wine. The traps were set near a stream at the base of the Bridger Mountains. The elevation at the trapping site was about 5,500 feet.

The traps were checked in the afternoon or at dusk during the months of June and July. The ground traps were the most effective and the majority of flies were collected at dusk. Flies were identified immediately after capture and were then either kept in culture or released.

The most notable aspect of this collection was the small number of species and individual flies found in this area. This could be due to the fact that Montana's climate is cool with extremely severe winters. These factors, along with a scarcity of natural foods, may limit the species that can inhabit this region.

A project has been initiated to compare the sexual behaviors of the three species of *Drosophila* found in this region.

References: McRobert & Tompkins 1983, DIS 59:143.

Mecheva, I. and E. Semionov. Institute of Genetics and Institute of Molecular Biology, Bulgaria. Localization of ribosomal DNA in diploid and polytene nuclei of *Drosophila simulans* and *Drosophila mauritiana*.

In this work we have studied in detail the localization of the genes coding for (18S + 28S) ribosomal RNA (rRNA) in the chromosomes of two *Drosophila* species from *melanogaster* subgroup. It is known, that in each of the two sex chromosomes X and Y

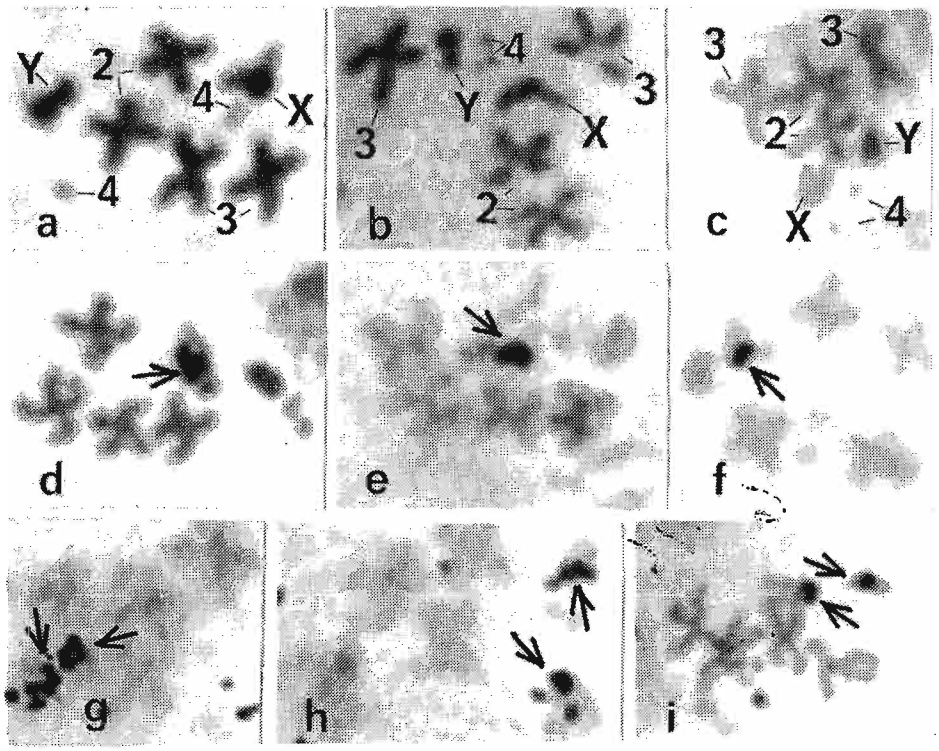
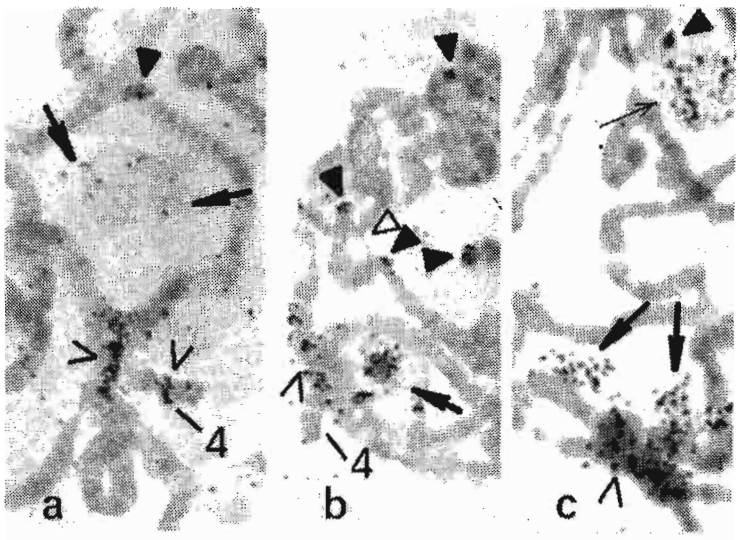


Figure 1a-i. Metaphase chromosomes from colchicined ganglion cells. (a-c) = karyotypes of *D. melanogaster*, *D. simulands* and *D. mauritiana*, respectively. Figures and letters indicate different chromosomes. Giemsa staining. (d-f) = hybridization of the probes: (d) the 28S gene fragment (plasmid HO9), (e) *ivs-I* (pC2) and (f) *ivs-II* (R23) to the chromosomes of *D. simulans*. (g-i) = the hybridization of the same plasmids as in (d-f), respectively, to the chromosomes of *D. mauritiana*. Arrows mark labelled chromosome sites.

of *D. melanogaster* there are 200-250 tandemly arranged ribosomal DNA (rDNA) repeating units (Ritossa and Spiegelman, 1965; Tartof, 1971). Both of these clusters are heterogeneous - part of X chromosome rDNA repeats are interrupted in the 28S gene sequence by intervening sequence type I and type II (*ivs-I* and *ivs-II*), while in the Y chromosome nucleolar organizer (NO), only *ivs-II* in part of the genes was found (for details see: Glover, 1981). *Ivs-I* are flanked by short direct repeats and are capable to transposition in restricted degree. In the polytene chromosomes of *D. melanogaster* these sequences were found not only in the nucleoli, but in the chromocenter and in region 102C of chromosome 4, as well (Kidd and Glover, 1980; Roiha, et al., 1981). Besides, they were detected in the form of sites with incomplete type of labelling (ITL), where the *ivs-I* probe hybridized only to a part of the chromosomal diameter (Semionov and Kirov, 1986). Chromosome localization of these sites varies. For two other members of the *melanogaster* subgroup -- *Drosophila simulans* and *Drosophila mauritiana* the number of rRNA genes was determined (Tartof, 1979), and the presence of both types of the insertions in their genomes was demonstrated for blotting-hybridization (Cohen et al., 1982; Roiha et al., 1983). At the same time, cytological data allow to propose that the number of NOs in these closely related species may be different from that of *D. melanogaster* (Durika and Krider, 1977). The question about the representativity of the intervening sequences in their NOs is answered yet. The approach of these questions by the direct method of in situ hybridization was the aim of the work presented here.

It is known, that the karyotypes of *D. melanogaster*, *D. simulans* and *D. mauritiana* are very similar, and include two pairs of big submetacentric autosomes (chromosomes 2 and 3), a pair of dot-like chromosomes 4 and a pair of heteromorphic (in males) sex chromosomes X and Y (Fig. 1a-c). In spite of this similarity, in *D. simulans* the hybridization of all probes were found only with the X chromosomes (Fig. 1d-f), whereas in *D. mauritiana*, a species evolutionary much closer to *D. simulans* than to *D. melanogaster* (Tartof, 1979), the NOs were detected in both sex chromosomes (Fig. 1g-i). However, it should be pointed out that in this species, unlike *D. melanogaster*, the *ivs-I* repeats are presented in both NOs (Fig. 1h). Our results show also that in the polytene nuclei of *D. simulans* and *D. mauritiana*, the *ivs-I*+ and *ivs-II*+ rDNA repeats are present in the nucleoli associated with the NOs, as well as in the "additional" nucleoli attached to the ITL sites, in accordance with the results found earlier for *D. melanogaster* (Fig. 2a-c). For these species *ivs-I* were found also in the chromocenters, but not in chromosome 4 (Fgi. 2b, c).

Acknowledgments: We are grateful to Dr. V. Mitrofanoff for providing the *Drosophila* stocks, and to Drs. D.



Glover and A. Kolchinsky for the cloned DNA sequences.

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Figure 2a-c. The *ivs-I* hybridization of polytene chromosomes of (a) *D. melanogaster*, (b) *D. simulans* and (c) *D. mauritiana*. The NO-associated nucleoli are marked with thick arrows. The thin arrow indicates an additional nucleolus. Dark and open arrowheads indicate sites of varying (ITL) and uniform hybridization, respectively. The closed arrowhead indicates an ectopic contact fiber, which connects two ITL sites.

Medina, M. and C.G. Vallejo. Instituto de Investigaciones Biomedicas, CSIC. Facultad de Medicina, UAM, Madrid, Spain. The activity and subcellular localization of glucose-6-phosphate dehydrogenase and glucose phosphate isomerase during embryogenesis of *Drosophila*.

The regulation of the pentose phosphate cycle and that of the glycolysis have been considered important the regulation of development. The binding of the enzymes to cell structural elements has been proposed as a mechanism to developmentally regulate the enzymatic activity in sea urchin and *Spisula* eggs. We here present data in *Drosophila* on the activity and subcellular localization during development of glucose-6-phosphate and glucose phosphate isomerase, enzymes belonging respectively to the pentose phosphate pathway and the glycolytic pathway.

Two metabolic changes, reduction of pyridine nucleotides (Epel, 1964; Whitaker and Steinhardt, 1981) and increased carbohydrate metabolism (Isono and Yasumasu, 1968; Krahl, 1956) have been observed after fertilization in the sea urchin.

Glucose-6-phosphate dehydrogenase is considered to exert the control of the pentose phosphate pathway. Both in the sea urchin (Isono, 1963; Swezey and Epel, 1986) and the surf clam *Spisula solidissima* (Ii and Rebhun, 1982), the association of the enzyme to structural elements has been suggested to regulate its activity. The enzyme activation and solubilization have been observed in both cases a few minutes after fertilization.

It is not clear whether the mechanism of dissociation accompanies activation in other eggs. We present here data in *Drosophila* on the activity and association to particulate material during development of glucose-6-phosphate dehydrogenase, a key enzyme of the pentose phosphate pathway and glucose phosphate isomerase, a glycolytic enzyme.

Materials and Methods. *Drosophila melanogaster* (Oregon-R strain) was cultured at 25°C as usual (Elgin and Miller, 1977). Mature oocytes were obtained (Petri et al., 1977) taking advantage of the fact that mature oocytes adhere to glass while the immature, do not. Embryos were collected routinely for 2 h, considering these embryos to be on average 1 h-old. The collected embryos were allowed to develop the additional time required (for example, 5 h, to obtain 6 h-old embryos).

Homogenization and fractionation. Embryos and oocytes were dechorionated, unless otherwise indicated, prior to hand homogenization in 2-4 vols of a medium (Vallejo et al., 1981) composed of 0.3 M sucrose, 15% Ficoll 400, 25 mM Hepes buffer, 60 mM KCl, 15mM NaCl, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1 mM sodium borate, adjusted to pH 7.5. This

medium has been proven to preserve the embryonic subcellular structures (Vallejo et al., 1981; Roggen and Slegers, 1985). Subcellular fractionation was accomplished by centrifuging the homogenates at 2500 rpm 20 min (500xg fraction). The supernatant was further centrifuged at 15000 rpm 30 min (27000xg fraction) and the resulting supernatant was spun at 38000 rpm for 60 min giving a pellet (100000xg fraction) and a supernatant (soluble fraction).

Enzymes assays. Glucose-6-phosphate dehydrogenase and glucose phosphate isomerase were determined as described (Vallejo et al., 1970). One unit is the amount of enzyme able to transform 1 umol of substrate per min at 37°C. 1 g of embryos contains 10^5 animals.

Table 1. Subcellular distribution and activity of glucose-6-phosphate dehydrogenase during embryogenesis of *Drosophila*.

	1hr	3.5hr	7hr	19hr
500 g fraction	-	-	-	-
27,000 g fraction	4%	15%	5%	5%
100,000 g fraction	3%		24%	1%
		85%*		
Soluble fraction	93%		71%	94%
Activity, Units /10^6 animals	7.3	6.5	3.7	3.5

The subcellular fractions were obtained and assayed as described in Materials and Methods. * The activity was assayed in the 100,000 x g fraction and soluble fraction together.

Table 2. Subcellular distribution and activity of glucose phosphate isomerase during embryogenesis of *Drosophila*.

	1hr	3.5hr	7hr	19hr
500 g fraction	24%	35%	32%	28%
27,000 g fraction	14%	16%	10%	13%
100,000 g fraction	10%	7%	10%	9%
Soluble fraction	52%	42%	48%	50%
Activity, Units /10^6 animals	179	202	272	159

The subcellular fractions were obtained and assayed as described in Materials and Methods.

The activity of glucose-6-phosphate dehydrogenase (Table 1) decreased from the first determination, 1 h after fertilization. The activity was mainly soluble, although a small percentage (5-30%) was found particulate. The activation in sea urchin and *Spisula* eggs has been reported to occur a few minutes after fertilization. The same probably holds true for *Drosophila* since at 1 h post-fertilization the enzyme is active and soluble. Since it is found soluble, the posterior decrease in activity is probably due to degradation and not subjected to regulation by association to particulate structures. The uncertainty in evaluating shorter times of development in *Drosophila* has precluded the study at a few minutes after fertilization.

Glucose phosphate isomerase has been studied as a glycolytic marker (Table 2). As shown, the activity increased slightly from the first determination and decreased again by the end of embryogenesis. About 50% of the activity remained associated to particulate material throughout embryogenesis. Glucose phosphate isomerase is considered a soluble enzyme except for the case of *Trypanosoma* where part of the activity is located in the glycosome (Opperdoes, 1988). By analogy with observations in sea urchin (Isono, 11963; Swezey and Epel, 1986) and surf clam (Ii and Rebhun, 1982), the partial particulate location in *Drosophila* may suggest that the enzyme can be further activated during development. This could be in agreement with the observation that glycolysis is activated later than the pentose phosphate cycle (Isono and Yasumasu, 1968).

The results presented in *Drosophila* suggest that the association to structural elements may be a mechanism to regulate during development the enzymatic activity not only of the pentose phosphate cycle but also of glycolysis.

Acknowledgments: This work has been supported by a grant from Comisión Asesora de Investigación Científica y Técnica.

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Metaphase karyotype of *Zaprionus argentostriata*.

D. brindavani reported from Mysore by Rajeshwari and Krishnamurthy (1971) has been identified by Prof. T. Okada as *Zaprionus argentostriata* (Bock) = *Drosophila argentostriata* Bock (1966) = *Zaprionus multistriata* Sturtevant (1927). It was also recorded as *Zaprionus multistriata* Dwivedi, Singh and Gupta (1979). It is now included under subgenus Aprionus, Okada (1983) (Personal Communication, Prof. T. Okada). We herein report the metaphase karyotype of *Zaprionus argentostriata* collected from Mysore.

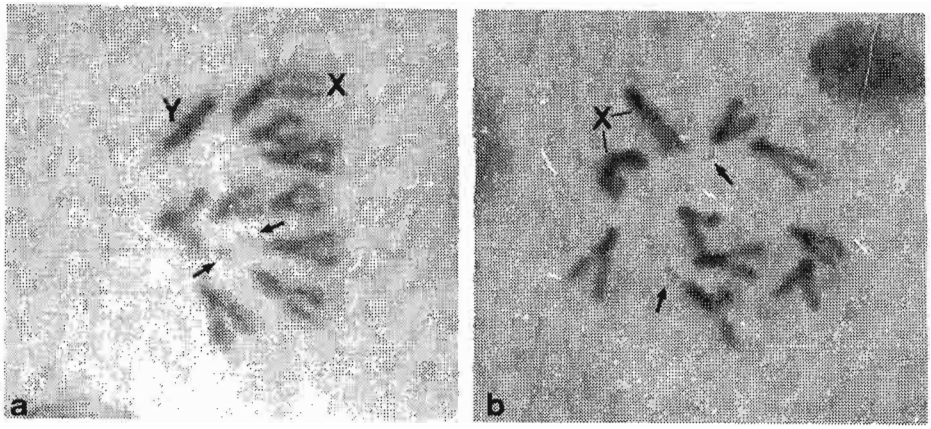

Figure 1. Mitotic chromosomes of *Zaprionus argentostriata* (a) female and (b) male. Arrows indicate dot chromosomes.

Permanent air dry preparations of the neural ganglia of third instar larvae were made as described by Ramachandra and Ranganath (1985). The male and female karyotypes are represented in Fig. 1a and 1b. the metaphase karyotype consists of 2n=12 with five pairs of rods and a pair of lightly stained dots. The X's represent one of the pair of rods. The Y is darkly stained rod-shaped chromosome, unlike the submetacentric Y seen in *Zaprionus indianus* (Kumar and Gupta, 1987).

Acknowledgments: The authors are grateful to The Chairman, Department of Studies in Zoology for his help and encouragement; to Prof. T. Okada, for his advice in identifying the species; to Mr. M.G. Vasudeva Rao for preparing the photographs and to the University Grants Commission for financial assistance.

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Peculiarities of the Va/Ba balanced strain of *Drosophila subobscura*.

One important method which may be used to obtain chromosomes in homozygous condition from trapped wild flies of *D. subobscura* is the balanced lethal strain Va/Ba. In this work we would like to comment on some peculiarities of this strain.

This strain is characterized by carrying one O chromosome with two overlapped induced inversions (VIII+210) and with Va ch cu markers. Its homologue has the standard arrangement and the Ba mutant (Sperlich et al., 1977). The mutant genes Va and Ba were described in detail by Koske and Maynard Smith (1954). Both genes present very variable expressivity, especially the Ba gene: sometimes only one macrochaeta is absent but the bristle socket is always present.

On the other hand, the tarsal joint missing in many Va flies was reported by the Department of Biometry of the University College (London) in 1946. In our experiments, the fly population was trapped in Bordils (about 100 Km N E from Barcelona). Crosses were carried out in order to obtain homozygous chromosomes in the F3 generation. Out of 142 lines analyzed, 8 (5.633%) presented many heterozygous F3 flies Va/+ with tarsal fusion in the two articles carrying the sex combs. This tarsal fusion is different from that observed in the af mutant of *D. subobscura* (Mestres, 1985).

Another interesting characteristic of the Va/+ heterozygous flies from this F3 is the appearance of a few individuals with only one wing. In fact, from the 142 lines from Bordils, 6 (4.225%) presented many flies with only one wing. On the side where the wing was missing an undifferentiated protuberance with bristles can be observed. Many flies with this trait also appeared in the population of Gilroy (California, about 100 Km S E from San Francisco). To sum up, from 195 analyzed lines, 8 (4.102%) presented many individuals with only one wing. The recurrence of the phenomenon in similar proportion in the two populations analyzed seems to imply that the trait is a development

characteristic of the flies descended from the Va/Ba strain.

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Methyl methanesulphonate fails to induce recombination in *Drosophila melanogaster* males.

Methyl methanesulphonate (MMS), a potent chromosome breaking agent, was tested for its ability to induce recombination in males of *Drosophila melanogaster*, where this phenomenon is normally absent. Treatments were given to the developing larvae with 0.5% MMS during their entire life. 0.5% MMS solution was mixed with standard *Drosophila* food in ratio 1:9. The *dumpy black cinnabar* (*dp b cn*) and Oregon -K wild-type strains of *D. melanogaster* were used.

From 18 F_1 (Oregon-K + */dp b cn*) untreated males, each mated with 3-4 *dp b cn* females, a test cross progeny of 2035 flies was studied. The progeny included 2 male (1 + *b cn* and 1 *dp b* +) and 3 female (2 + *b cn* and 1 + + *cn*) recombinants (Table 1). In this experiment, 0.245% spontaneous male recombination was recorded. The 21 MMS-treated F_1 males yeilded a test cross progeny of 2467. The progeny included 4 male (1 + *b cn*; 1 *dp* + + and 2 + *b* +) and 2 female (1 + + *cn* and 1 *dp* + +) recombinants (Table 1). The 0.05% MMS thus induced 0.284% recombination in males of *D. melanogaster*.

The difference observed in frequencies of male recombination in untreated and 0.5% MMS-treated F_1 flies was not significant ($p < 0.001$). The 0.5% MMS thus failed to enhance frequency of recombination over the control in males of *D. melanogaster* when treatments were given to the developing larvae during their entire life period.

Table 1. Induction of recombination in males of *D. melanogaster* with 0.5% methyl methanesulphonate (MMS).

	Control		MMS	
F_1 males tested	18		21	
Pooled test cross popu. size	2035		2467	
F_1 males yielding recomb.	3		5	
Percent recombination	0.245		0.284	
Phenotypes of recomb.	males	females	males	females
+ *b cn*	1	2	1	0
dp + +	0	0	1	1
+ + *cn*	0	1	0	1
dp b +	1	0	0	0
+ *b* +	0	0	2	0
dp + *cn*	0	0	0	0
Total recombinants	2	3	4	2

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Phenotypic spectrum of recombinants induced in heterozygous males of *Drosophila melanogaster* and their heterozygous sons.

Performance of ethyl methanesulphonate (EMS)-treated F_1 *Drosophila melanogaster* males of genotype Oregon K+/ *dumpy black cinnabar (dp b cn)* and that of their untreated wild-looking TC_1 sons (having probably the same genotype as the F_1 males) has been compared with respect to the phenotypic spectrum of recombinants in their respective test cross progenies. A standard wild-type laboratory strain, Oregon-K, and a mutant *D. melanogaster* stock homozygous for 3 second-chromosome recessive markers, *dumpy* (*dp*: 2-13.0), *black* (*b*: 2-48.5) and *cinnabar* (*cn*: 2-57.7) were used. It was decided to treat the developing F_1 individuals with 0.75% EMS, mixed with food in ratio 1:9, in the second one third part of larval life (i.e., 54-86h after deposition). After 54h of egg deposition at 25°C, the developing F_1 larvae were flushed out with distilled water and physically transferred with a camel-hair brush on to the medium with or without EMS. After allowing the larvae to feed there for 32h, they were again flushed out and transferred on to the standard food medium. Each untreated or EMS-treated larva, thus, underwent two transfers, here referred to as "physical stress". A control experiment was run simultaneously where neither EMS was added to the food nor usual two transfers of larvae were done. In the present investigation, three sets of experiments - control, physical stress and 0.75% EMS - were thus performed. A two-day-old F_1 male was crossed with 3 *dp b cn* females, on standard food medium, to get the first test cross (TC_1) progeny. Every TC_1 progeny was individually studied for recombinant phenotypes. A few of the wild-looking TC_1 males (having probable genotype +/ *dp b cn*) were again test crossed to obtain the second test cross (TC_2) progeny, on standard food medium. All the TC_2 progenies were individually screened for recombinant phenotypes.

The TC_1 progenies of 14, 15 and 39 F_1 males of *D. melanogaster*, randomly selected from control, physical stress and EMS experiments, comprised of 3375, 5952 and 13887 individuals, respectively. As many as 6, 10 and 52

wild-looking TC_1 males (of probable genotype $+/dp\ b\ cn$) were randomly selected from TC_1 progenies of only those control physical stress- and EMS-treated F_1 males that had produced recombinants; these TC_1 males respectively produced 868, 1443 and 9028 flies in TC_2 progeny. The frequencies of recombination revealed in all the three experiments by the F_1 males (in the TC_1 progeny) and TC_1 males (in the TC_2 progeny) were: control; F_1 0.267%, TC_1 0.0%, physical stress; F_1 1.125%, TC_1 0.97%, EMS; F_1 1.706%, TC_1 1.395%. Frequencies of male recombination produced by F_1 and TC_1 males in the three experiments were not significantly different from each other which showed that enhanced male recombination caused by physical stress (with or without EMS) was transmitted in quantitative terms to the next generation.

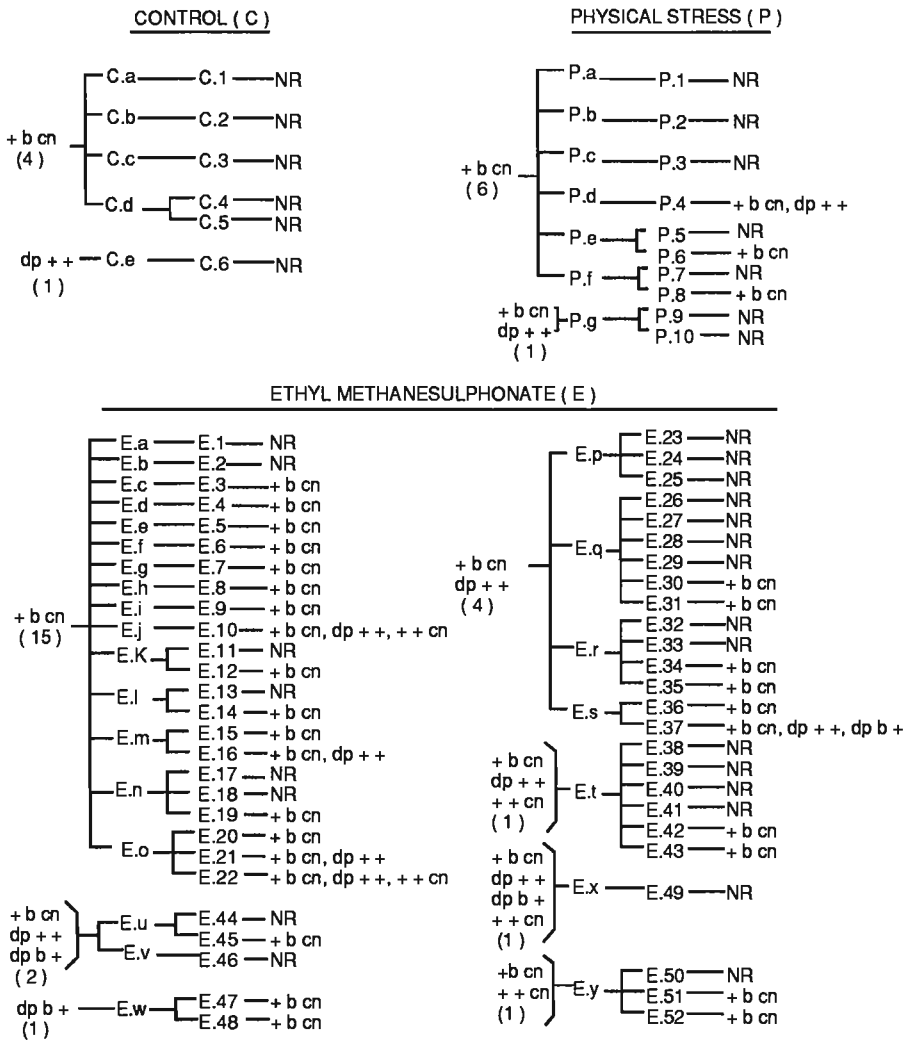


Figure 1. Recombinant phenotypes produced by individual *D. melanogaster* F_1 ($+/dp\ b\ cn$) and TC_1 (probably $+/dp\ b\ cn$) males in their TC_1 and TC_2 progenies, respectively. Figures within parenthesis indicate number of F_1 males yielding given recombinant(s) in their TC_1 progenies. See text for description, NR, no recombinant produced.

Since TC_1 and TC_2 progenies of each F_1 and TC_1 males were recorded separately, it was possible to examine the presence or absence of a particular recombinant phenotype in a particular TC_1 or TC_2 progeny. Recombinant phenotypes produced by heterozygous F_1 and TC_1 males of *D. melanogaster*, in their respective TC_1 and TC_2 progenies are given in figure 1 which also traces ancestory of different TC_1 males to their fathers (F_1 males). The F_1 males have been designated C.a, C.b . . . in control, P.a, P.b . . . in physical stress and E.a, E.b . . . in EMS experiments. The TC_1 sons test crossed have been designated C.1, C.2. . . in control, P.1, P.2. . . in physical stress and E.1, E.2 . . . in EMS experiments. In the control experiment, no TC_1 male produced any recombinant in its progeny while 4 and 1 F_1 males yielded $+\ b\ cn$ and $dp\ +\ +$ recombinant types in their progenies, respectively (Fig. 1). In the physical stress experiment, no recombinant type was observed in the progeny of 7 TC_1 males whereas their fathers (F_1 males) had produced $+\ b\ cn$ and/or $dp\ +\ +$ type(s) in their progenies. Recombinant type $+\ b\ cn$ was produced by 2 different TC_1 and their respective fathers (F_1 males). One TC_1 male produced recombinant type $dp\ +\ +$ in addition to $+\ b\ cn$ produced by its father. In the EMS experiment, various phenotypic spectra of recombinants by 52 TC_1 males were observed as compared to F_1 males. Twenty-three of these TC_1 males did not yield any recombinant type in their progeny while their F_1 males had given some recombinant type(s). The same recombinant type, $+\ b\ cn$, was produced by 12 different TC_1 males and their fathers. Two TC_1 males produced recombinant type $+\ b\ cn$ which was different than the one ($dp\ b\ +$) their fathers had produced. Five TC_1 males produced one or two additional whereas 10 TC_1 males produced one or two less recombinant types as compared to F_1 males. Out of 52 TC_1 males analysed in the EMS experiment, in 13 cases

where two or more TC_1 males descending from the same F_1 male were testcrossed, two behaved in similar fashion in producing the same recombinant type or in not yielding any recombinants whereas 11 behaved differently from one another in producing different recombinant types in their TC_2 progenies. In a nutshell, one-fifth of the TC_1 males studied in the physical stress and about one-fourth in the EMS experiment produced qualitatively same spectrum of recombinants as done by their F_1 fathers suggesting qualitative inheritance of induced male recombination only in certain cases. The cases where two or more TC_1 sons of an EMS-treated F_1 male were test crossed and the TC_1 brothers descending from the same F_1 male produced a different phenotypic spectrum of recombinants of those that were responsible for appearance of recombinants in the TC_1 progeny. The physical stress or EMS treatment applied may randomly induce recombination in certain gametic cells of certain individuals but that does not imply that a given male, in whose progeny some recombinants were found, was genetically predisposed to recombination. We did not find any such correlation in the present study because not all the events responsible for exchange of markers in a particular generation seemed to repeat themselves in the next one, in *D. melanogaster*.

Miglani, G.S. and V.P. Singh. Punjab Agricultural University, Ludhiana, India. Production of novel recombinant types in the progenies of male recombinants of *Drosophila melanogaster*.

This communication reports appearance of novel phenotypes in the progenies of hydroxylammonium sulphate (HAS)-induced male recombinants of *D. melanogaster* when test crossed for genotypic/phenotypic confirmation. A standard wildtype, Oregon-K, stock and a mutant stock homozygous for three second-chromosome recessive markers, *aristaless* (*al*:1-0.0), *dumpy* (*dp*:2-13.0) and *black* (*b*:2-48.5), were used in the present experiment. HAS (0.5%) was mixed in the medium (1:9) on which the developing F_1 (Oregon-K + /*al dp b*) individuals were reared during their entire larval life. A two-day old F_1 male was crossed with three *al dp b* females to get the first testcross progeny (TC_1). Each TC_1 male recombinant was again crossed with three *al dp b* females to obtain second testcross generation (TC_2) for confirming their genotypes/phenotypes. A TC_1 male recombinant was considered as verified when it produced the recombinant and *al dp b* phenotypes in its TC_2 progeny.

Table 1. TC_2 progenies of the verified TC_1 male recombinants of *D. melanogaster* which show a novel phenotype.

Phenotype of TC_1 male recombinant		TC_2 Phenotype	TC_2 Males	TC_2 Females	Percent
Control					
al dp +		*al dp b*	33	35	49.27
		al dp +	20	24	31.88
		+ dp +	11	15	18.85
+ dp +		*al dp b*	30	28	39.19
		+ dp +	27	20	31.76
		+ dp b	21	22	29.05
HAS					
al dp +	1.	*al dp b*	40	43	51.23
		al dp +	32	33	40.12
		+ dp +	4	10	8.65
	2.	*al dp b*	30	39	47.92
		al dp +	20	20	34.03
		+ dp +	12	14	18.05
	3.	*al dp b*	30	35	50.39
		al dp +	22	29	32.56
		+ dp +	12	10	17.05
	4.	*al dp b*	33	36	44.23
		al dp +	31	32	40.38
		+ dp +	12	12	15.38
	5.	*al dp b*	25	26	42.50
		al dp +	20	25	37.50
		+ dp +	14	10	20.00
	6.	*al dp b*	23	29	42.62
		al dp +	20	21	33.61
		+ dp +	14	15	23.77

The 27 recombinants recovered among 2802 TC_1 flies included 7 (5 + *dp b*, 1 *al dp* + and 1 + *dp* +) and 72 recombinants recovered among 4323 TC_1 in HAS experiment included 20 (12 + *dp b* and 8 *al dp* +) males. Out of 7 male recombinants verified in control experiment, 5, all + *dp b* type, produced only expected two phenotypes whereas the remaining two (1 *al dp* + and 1 + *dp* +) produced an additional novel phenotype each: an additional phenotype, + *dp* + was produced by TC_1 male recombinant *al dp* + whereas + *dp b* type was produced by TC_1 male recombinant + *dp* + (Table 1). Out of 20 TC_1 male recombinants verified in HAS experiment, 14 (12 + *dp b* and 2 *al dp* +) TC_1 recombinants produced only the two expected phenotypic classes. The remaining six, all of phenotype *al dp* +, produced an additional phenotype + *dp* + (Table 1).

Production of additional phenotype *dp b* in TC_2 progeny of a spontaneously induced TC_1 male recombinant + *dp* + (genotype + *dp* + / *al dp b*) of *D. melanogaster* could be the result of a crossing-over like event in the male. It may be noted here that in this TC_2 progeny only one novel phenotype + *dp b* had appeared whereas another possible complementary product, *al* + +, was conspicuous by its absence. The cluster of novel phenotype *dp b* might have appeared due to chromosome

breakage and its reunion at a stage when gonadal multiplication was occurring before onset of meiosis. Production of a novel phenotype + *dp* + by seven (1 spontaneous and 6 HAS-induced) male recombinants of phenotype *al dp* + could not be attributed to crossing-over like events. If the three phenotypes (*al dp b, al dp* + and + *dp* +) appearing in TC_2 progenies of one control and 6 HAS-induced TC_1 male recombinants (Table 1) are pooled, the number of individuals of *al dp b* type equals the sum of number of individuals of *al dp* + and + *dp* + types ($p<0.05$). This suggests that the novel phenotype, + *dp* +, in some way originated from *al dp* + type. If it is believed to be so, *al* locus seems to be the target where through some, yet unidentified influence of HAS at *al* or some other locus, *al* allele mimicks wild type allele. Since mutation *al* shows incomplete penetrance, possibility of involvement of incomplete penetrance of *al* mutation in production of novel phenotype + *dp* + by *al dp* + TC_1 males is not ruled out. Alternatively, HAS, through its effect on some determinant(s) of hybrid dysgenesis system, may have led to the production of novel phenotype + *dp* +. This suggestion is based on the fact that certain phenomenon such as transmission-ratio distortion, chromosomal aberrations and sex-ratio deviation known to be associated with hybrid dysgenesis, have been observed in addition to non-reciprocal male recombination in TC_1 progenies of HAS-treated F_1 (+ /*al dp b*) males of *D. melanogaster*.

Miglani, G.S., V.P. Singh and K. Preet. Punjab Agricultural University, Ludhiana, India. Induction of recombination with hydroxylammonium sulphate in *Drosophila* males.

Hydroxylammonium sulphate (HAS), an allied compound of hydroxylamine and a potent chromosome breaking agent, has been tested for its ability to induce recombination in males of *D. melanogaster*. The 0.5% HAS was mixed with the medium in ratio 1:9 and fed to the developing F_1 larvae of genotype Oregon-K+/ *dumpy black cinnabar* (*dp b cn*) during their entire life. A two-day old F_1 male was crossed with 3 *dp b cn* females to get the first test cross (TC_1) progeny. Out of 27 F_1 males test crossed, 17 gave 31 recombinants of 4 types (+ *b cn*, *dp* + +, + + *cn* and *dp b* +) and out of 32 HAS-treated F_1 males, 26 yielded 69 recombinants of the same 4 types in TC_1 progenies of 4147 and 6544 individuals, respectively (Table 1). The most prevalent recombinant recovered in TC_1 progenies of untreated and HAS-treated F_1 males was + *b cn*. The control and HAS-treated F_1 males yielded higher ($p < 0.05$) frequencies of recombination in *dp - b - cn* region in control (0.74) and HAS-treated (1.05) F_1 males were not significantly different from each other. The following noteworthy observations were made regarding number of F_1 males producing a particular number of recombinant classes, ratio of complementary phenotypes, segregation pattern and male-female ratio in TC_1 progenies:

1. In control experiment, 10, 14 and 3 F_1 males gave 0, 1 and 2 recombinant classes, respectively. And, 6, 13, 10 and 3 F_1 males in HAS experiment produced 0, 1, 2 and 3 recombinant classes, respectively.
2. The untreated F_1 males yielded higher ($p < 0.05$) number of + + + flies as compared to *dp b cn* type whereas HAS-treated F_1 males yielded these parental phenotypes in 1:1 ratio. Both the untreated and HAS-treated F_1 males produced a higher frequency ($p < 0.01$) of + *b cn* individuals as compared to *dp* + + type. The TC_1 progeny of untreated and HAS-treated F_1 males had + + *cn* and *dp b* + complementary classes in 1:1 ratio.
3. At the *dp* locus, the control F_1 males exhibited non-Mendelian segregation ($p < 0.001$) whereas HAS-treated F_1 males showed Mendelian segregation. Both at *b* and *cn* loci, the control and HAS-treated F_1 males revealed Mendelian segregation.
4. The control F_1 males produced males and females of + + +, + + *cn* and *dp b* + types in 1:1 ratio, whereas *dp b cn*, + *b cn* and

Table 1. Induction of recombination with 0.5% HAS in males of *D. melanogaster*.

		Control		HAS	
F_1 males crossed		27		2	
Pooled test cross popu. size		4147		6544	
F_1 males yielding recombinants		17		26	
Per cent recombination in region					
	dp - b	0.62		0.78	
	b - cn	0.12		0.27	
TC_1 progeny		Males	Females	Males	Females
	+ + +	1048	1087	1617	1655
	dp b cn	935	1073	1560	1643
	+ *b cn*	4	18	13	23
	dp + +	0	4	4	11
	+ + *cn*	0	3	3	9
	dp b +	0	2	0	6
	+ *b* +	0	0	0	0
	dp + *cn*	0	0	0	0
	Total	1987	2187	3197	3347

dp + + types produced females in majority ($p < 0.01$) over the males. The HAS-treated F_1 males yielded the two sexes of types + + +, an *dp b cn*, *dp* + + and + + *cn* in 1:1 ratio. However, the *dp b* + type produced by the HAS-treated F_1 males revealed females in majority ($p < 0.01$) over the males. Considering all the phenotypes together in the TC_1 progenies, in control experiment, females were in majority ($p < 0.01$) over the males whereas in HAS experiment, males and females appeared in 1:1 ratio.

Miglani, G.S., V.P. Singh and Kanwal Preet. Punjab Agricultural University, Ludhiana, India. Hydroxylammonium sulphate induces nonreciprocal recombination in males of *D. melanogaster*.

The 0.5 per cent hydroxylammonium sulphate (HAS) was fed to *D. melanogaster* F_1 Oregon K +/*aristaless dumpy black* (*al dp b*) larvae in food in ratio 1:9. Twenty untreated and twenty-five HAS-treated F_1 males yielded in their TC_1 progenies 27 and 72 recombinants, respectively. The most prevalent recombinants produced by control and HAS-treated F_1 males were + *dp b* and *al dp* +, respectively. All the 7 and 20 TC_1 male recombinants recovered, respectively, from untreated and HAS-treated F_1 males were confirmed genotypically/phenotypically by further test crossing. HAS-treated F_1 males produced (1.66%) a significantly higher ($p < 0.05$) frequency of verified male recombination over the control (0.96%). HAS induced non-reciprocal recombination in both *al-dp* and *dp-b* regions studied.

The untreated F_1 males produced higher ($p < 0.05$) whereas HAS-treated F_1 males produced lesser ($p < 0.05$) number of wildtype flies as compared to *al dp b*, in their TC_1 progenies. The control F_1 males exhibited non-Mendelian segregation only at *al* locus whereas HAS-treated F_1 males exhibited non-Mendelian segregation at the *al, dp* and *b* loci.

The pooled TC_1 progenies of untreated and HAS-treated F_1 males revealed greater number of females over males. Most of the HAS-induced *al dp* + TC_1 male recombinants produced a novel phenotype, + *dp* +, in their TC_2 progenies. Aberrations involving second and third chromosomes were detected in the larvae sampled from TC_2 progenies of TC_1 male recombinants. These aberrations may have intrachromosomal and/or interchromosomal effects on induction of recombination with HAS in males of *D. melanogaster*.

Moltó, M.D., M.J. Martínez-Sebastián and R. de Frutos. Departmento de Genética, Universidad de Valencia, Spain. Localization of the hsp70 gene in *Drosophila guanche*.

The chromosomal location of the hsp70 gene in *Drosophila guanche* has been determined by "in situ" hybridization. Late third instar larvae of TF2, a *D. guanche* strain, cultured at 19°C, were subjected to 34°C during a period of 30 minutes. Squash preparations of polytene chromosomes from heat shocked larvae were performed as described by Pardue (1985). The oligolabeling method (Feinberg and Vogelstein, 1983) was applied for the radiolabeling of the 122.1 probe. Fragment 122 contains two hsp70 genes in diverging orientation and is derived from the 87A locus of *D. melanogaster* (Goldschmidt-Clermont, 1980). Hybridization was performed in aqueous salt solution at high temperature (Pardue, 1985) and the autoradiography and Giemsa staining of the chromosomes was carried out as described by Steinemann *et. al.* (1984).

As can been seen in Figure 1 the hsp70 coding sequences are located at two loci on the 0 chromosome of *D. guanche*. The comparison of the characteristics of the hsp70 gene of this species with those of *D. subobscura*, a closely related species, and *D. melanogaster*, shows that the hsp70 locus is duplicated in all three species and is located in the same chromosome element (0 in species of the *obscura* group and 3R in *D. melanogaster*).

In *D. subobscura* the most active puffs after heat shock (85AB, 89A and 94A) are located in the 0 chromosome (Pascual and de Frutos, 1988). The 85AB puff is also active under non-induced conditions but increases in frequency after heat shock. The same response has been found in *D. guanche* with the exception that locus 94A never shows puff activity (Moltó *et. al.*, 1988). By "in situ" hybridization, using the hsp70 gene of *D. melanogaster* as a probe, positive hybridization on 89A and 94A loci has been found in *D. subobscura* (de Frutos *et. al.*, in preparation). This suggests that the 89A and 94A loci also code for hsp70 in *D. guanche*, but after heat shock only 85AB and 89A are detected at the cytological level.

In respect to the standard gene arrangement of *D. subobscura*, the 0 chromosome of *D. guanche* carries two overlapping inversions: the 0 arrangement and another which involves the region between 84D/85A and 93D/94A (Moltó *et. al.*, 1987). Figure 2a shows the arrangement established in this chromosome. The "in situ" hybridization results suggest a new arrangement (Figure 2b); 94A and 95AB loci are side by side and appear as a single active puff after heat shock. As can be seen in Figure 1 only the distal side of this puff shows hybridization (94A locus). The

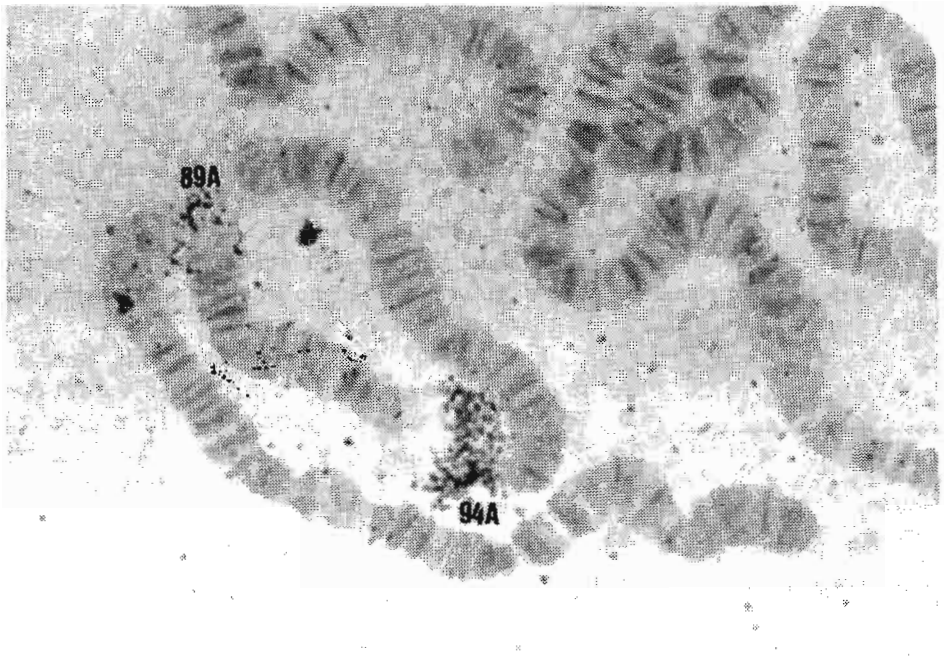
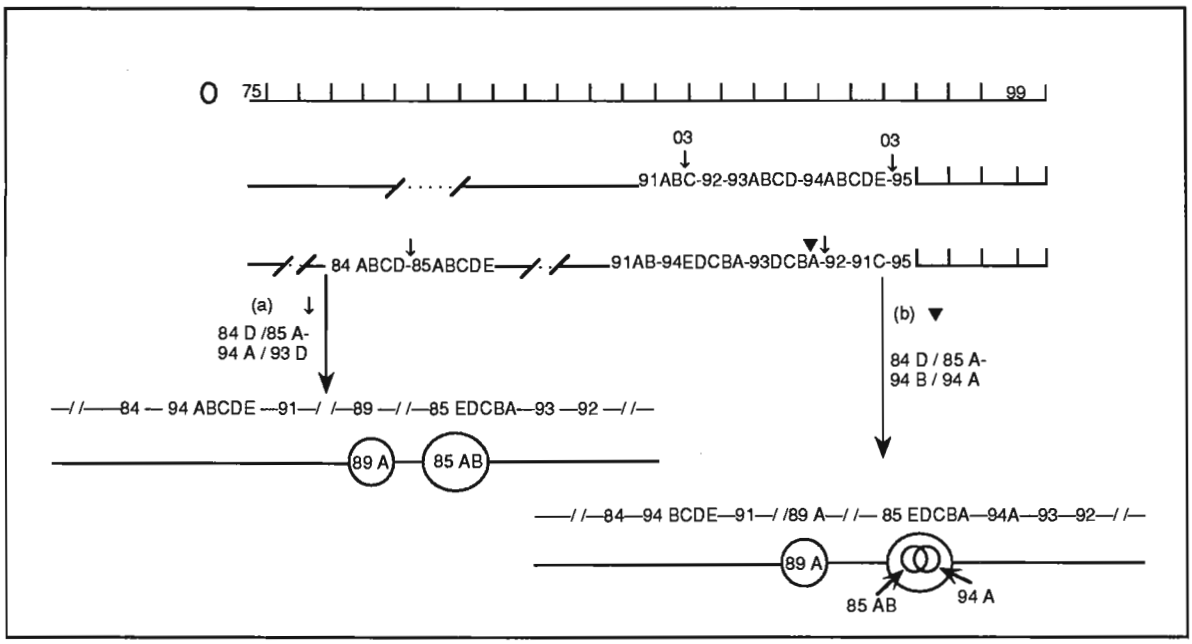


Figure 1 (above). Autoradiography of *D. guanche* polytene chromosome O hybridized with ^{3}H-DNA of 122.1 probe.

Figure 2 (below). Gene arrangement on the O chromosome of *D. guanche*: (a) arrangement proposed by Molto et al. (1987). (b) a minor change in the downstream breakpoint of this arrangement is proposed in this work.

proximal side of the puff is the 85AB locus active after heat shock but not coding for hsp70, as occurs in *D. subobscura*.

This specific inversion on the 0 chromosome of *D. guanche* has been detected by other authors but given different boundaries for the downstream breakpoint (see Moltó *et. al.*, 1987). None of the breakpoints proposed are in agreement with the results of "in situ" hybridization. Therefore, we propose a minor change in the 0 chromosome gene arrangement of *D. guanche* proposed by Molto *et. al.* (1987): the downstream breakpoint 93D/94A is changed to 94B/94A.



Acknowledgments: This work was supported by a grant from Conselleria de Cultura, Educacio i Ciencia de la Generalitat Valenciana.

References: Feinberg, A.P. and B. Vogelstein 1983, Analytical Biochemistry 132:6-13; Goldschmidt-Clermont, M. 1980, Nucleic Acids Research 8(2):235-253; Molto, M.D., R. de Frutos and M.J. Martinez-Sebastian 1987, Genetica 75:55-70; Moltó, M.D., R. de Frutos and M.J. Martinez-Sebastian 1988 Chromosoma 96:382-390; Pardue, M.L. 1985 In Nucleic Acid Hybridization, Ed. by Hames, B.D. and Higgens, S.J. Oxford:IRL Press pp:179-202; Pascual, L. and R. de Futos 1988 Chromosoma 97:164-170; Steineman, M., W. Pinsker and D. Sperlich 1984 Chromosoma 91:46-53.

Moore, R.H. and G.K. Chambers. Victoria University, Wellington, New Zealand.

Drosophila species diversity in the Wellington, Wairarapa and Horowhenua districts of New Zealand.

We have previously reported (Chambers, et al., 1988) that *Drosophila* species diversity varies considerably between collection sites in our region of New Zealand. Preliminary data from a longitudinal study at a single site (KAR: Karori) in 1985-1986 (Chambers, 1989; DIS this issue) suggested that predictable seasonal patterns of fluctuations in the population size of each species might be largely responsible for the observed variation. Based on these results a series of collections were made at several sites in the Wellington, Wairarapa and Horowhenua Districts (Table 1).

Table 1. *Drosophila* Collection Sites

Wellington District	
BRO	- Brooklyn: compact heap in suburban garden
KAR	- Karor: permanent fruit bait in suburban garden
UHU	- Upper Hutt: fruit bait in suburban garden
Wairarapa District	
APB	- Apple Barrel: vegetable dump in market garden, Greytown
BAM	- Brown Acres: Market garden, Manakau
TAT	- Tates Orchard: fruit bins in orchard, Greytown
Horowhenua District	
STP	- Strawberry Plant: fruit dump at packing house, Te Horo

Our collection and analysis methods were as previously described (Chambers et al., 1988) except that *D. melanogaster* and *D. simulans* were not scored electrophoretically and consequently totals for these two species were pooled. Data from our collections are given in Tables 2 and 3.

Our first collections (12/86 and 87) at four sites BRO and KAR in Wellington (Table 2) and APB and TAT in Wairarapa (Table 3) confirmed our earlier findings (Chambers et al., 1988). *D. melanogaster/D. simulans* are rather rare in the Wellington district compared with the Wairarapa district and numbers of these two species increased in late summer (February) compared to early summer (December). Encouraged by these preliminary results more extensive collections were made during the 1987-1988 season.

Examination of the data in Tables 2 and 3 shows that although *D. melanogaster/D. simulans* were virtually absent from the Wellington collections in 1987-1988 these were often abundant in Wairarapa collections and were, as expected, more common in February and March than in December. Taken together with the data in Chambers et al. (1988) and Chambers (1989) this observation suggests that each *Drosophila* species reaches peak numbers at characteristic time: *D. busckii* and *D. pseudoobscura* around October, *D. hydei* and *D. immigrans* around December and *D. melanogaster/D. simulans* around February/March. It is quite likely that numbers of *D. melanogaster* peak before those of *D. simulans* as reported by

Table 2. Numbers and proportion of *Drosophila* species collected at Wellington sites.

Species	Site and Date of Collection									
	BRO[1]			UPO			KAR[1]			
	12/86	12/87	2/87	2/88	12/87	12/88	12/86	12/87[3]	2/87[2]	2/88
D. immigrans	0.98	0.97	0.98	0.95	0.60	0.47	0.92	0.94	0.75	0.79
D. melanogaster } *D. simulans* }	-	-	-	-	-	-	-	0.01	0.22	-
D. hydei	-	-	-	0.02	-	-	-	0.03	0.02	0.11
D. busckii	-	-	-	-	0.33	0.32	-	-	-	-
D. pseudoobscura	0.02	0.03	0.02	0.03	0.07	0.21	0.07	0.03	0.01	0.11
Total	208	109	96	107	43	19	224	448	460	84

[1] Data are taken from Chamber (1988)
[2] Pooled data for two collections 1/2/87 and 25/2/87
[3] Pooled data for two collections 11/12/87 and 28/12/87

McKenzie and Parsons (1974) for *Drosophila* populations around Melbourne, Australia. Detailed electrophoretic analyses of species compositions at Wairarapa sites will be required before we can confirm this hypothesis.

The seasonal fluctuation in species numbers is well illustrated by the 1987-1988 data for the APB Wairarapa site (Table 3). In October 1987 the most common species was *D. pseudoobscura* (97% of all individuals), in December 1987 *D. hydei* was the most common species (71%), in February 1988, *D. immigrans* (95%) and in March 1988 *D. melanogaster/D. simulans* (88%). Striking as these seasonal patterns seem to be they cannot always predict which species will be collected from a particular site at any particular time. Imposed on the regular seasonal pattern of species increase and decline are climatic and environmental changes from year to year (see Chambers, 1989 for data on the KAR site). Environmental differences between sites may also be extremely important (see McKenzie and Parsons, 1974). However, much more extensive data would be required before we could undertake a detailed analysis of such factors. Nonetheless, we believe that the small scale study reported here points to some interesting seasonal patterns of *Drosophila* species diversity in New Zealand which are helpful in predicting when and where to obtain specimens of particular species.

Acknowledgments: We are indebted to the many horticulturalists and private citizens who allowed us to make collections on their properties.

References: Chambers, G.K. 1991, DIS this issue; Chambers, G.K., S.L. Davies, M. Hodgetts, R.H. Moore and I.J. Pomer 1988, DIS 67:13-14; McKenzie, J.A. and P.A. Parsons 1974, Aust. J. Zool. 22:175-187.

Table 3. Numbers and proportions of *Drosophila* species collected at Wairarapa and Horowhenua sites

Species	Site and Date of Collection 10/87	12/86	12/87	1/88	2/87	2/88	3/88
APB							
D. immigrans	0.02	0.11	0.23	n.c.	0.32	0.95	0.05
D. melanogaster / *D. simulans*	-	0.09	-		0.05	-	0.88
D. hydei	0.01	0.75	0.71		0.63	0.05	0.07
D. busckii	-	0.04	-		-	-	-
D. pseudoobscura	0.97	0.01	0.06		-	-	-
Total	178	206	171		202	44	291
BAM							
D. immigrans	0.28	n.c.	0.02	0.08	n.c	n.c	0.46
D. melanogaster / *D. simulans*	0.12		0.38	0.62			0.25
D. hydei	0.34		0.57	0.20			0.22
D. busckii	0.16		-	0.09			0.08
D. pseudoobscura	0.11		-	0.01			-
Total	161		183	175			212
STP							
D. immigrans	0.28	n.c.	0.12	-	n.c	n.c	-
D. melanogaster / *D. simulans*	-		0.02	0.37			-
D. hydei	0.00		0.85	0.63			-
D. busckii	0.71		-	-			-
D. pseudoobscura	0.01		0.01	-			-
Total	364		299	65			0
TAT							
D. immigrans	0.10	0.11	-	0.13	0.11	n.c.	0.06
D. melanogaster / *D. simulans*	-	0.50	-	0.83	0.87		0.91
D. hydei	-	0.39	-	0.02	0.01		0.03
D. busckii	-	0.05	-	-	-		-
D. pseudoobscura	0.90	0.02	-	0.02	-		-
Total	10	219	0	48	273		324

n.c.: no collection

Mutsuddi, M. and A.S. Mukherjee. University of Calcutta, India. On the female-determining factors on the X chromosome of *Drosophila melanogaster*.

In *D. melanogaster*, the X-chromosomes contain a preponderance of female determining factors, and the notion was long conceived that such factors are scattered along the X chromosomes (Dobzhansky and Schultz 1934; Pipkin 1940). In recent years, substantial evidence was provided which supports the idea that major female determining factors are localized distally on the X chromosome, and simultaneous presence of two distal I elements, viz., 3F3-4B1 and 6F (cytological map position for Sxl, Lucchesi and Manning 1987), are required in two doses to allow normal female differentiation (Steinmann-Zwicky and Nöthiger 1985). Furthermore,

the segment 3F3-4B1 could not be duplicated in males, because such a situation would activate the Sxl locus, and in turn, would implement female-mode of development and basal-level of X-transcription (Steinmann-Zwicky and Nöthiger 1985).

In the present investigation, complements with X chromosomal segmental aneuploids were generated to identify other female determining factors over the X chromosome. 1X aneuploids with proximal and interstitial duplications were constructed by using different X;Y translocation stocks, as cited in Maroni and Lucchesi (1980) and Stewart and Merriam (1975).

Earlier observations from this group (Mutsuddi *et al.* 1983) and others (Steinmann-Zwicky and Nöthiger 1985) documented that the genotype with 1X/dp.16A-20F (25% duplication) promotes male differentiation (Fig. 1a). However, in the present investigation, when the distal X-fragment 1A-3F (15% duplication) was annexed to the aforesaid genotype, this aneuploid class (1X/dp.1A-3F;16A-20F; 40% duplication), though survived poorly for a few hours after eclosion, directed towards a female-mode of development (Fig. 1b). Noticeably, the duplicated segments of this genotype excepted both the important loci 3F3-4B1 and 6F. On the other hand, 1X aneuploids carrying 62% X-fragment as a duplication from the proximal end (dp.80-20F), responded to a male-mode of development (Fig. 1c; Steinmann-Zwicky and Nöthiger 1985). These observations may be sufficient to indicate the feminizing effect of the segment 1A-3F in these aneuploids. Similar results on some other aneuploid classes also corroborated this view. As for instance, while the genotype with 1X/dp.1A-7D (33% duplication) was reported to develop female structures (Steinmann-Zwicky and Nöthiger 1985, Fig. 1d), the elimination of the segment 1A-3E from this aneuploid class (present genotype 1X/dp.3F-7D; 18% duplication), as we have observed, resulted in male development (Fig. 1e). Furthermore, aneuploid progeny with 1X/dp.3F-8C (23% duplication) also developed as males (Fig. 1f). Note, in these aneuploid males (1X/dp.3F-7D and 1X/dp.3F-8C), the duplicated fragment included both 3F-4B and 6F (Sxl) subdivisions.

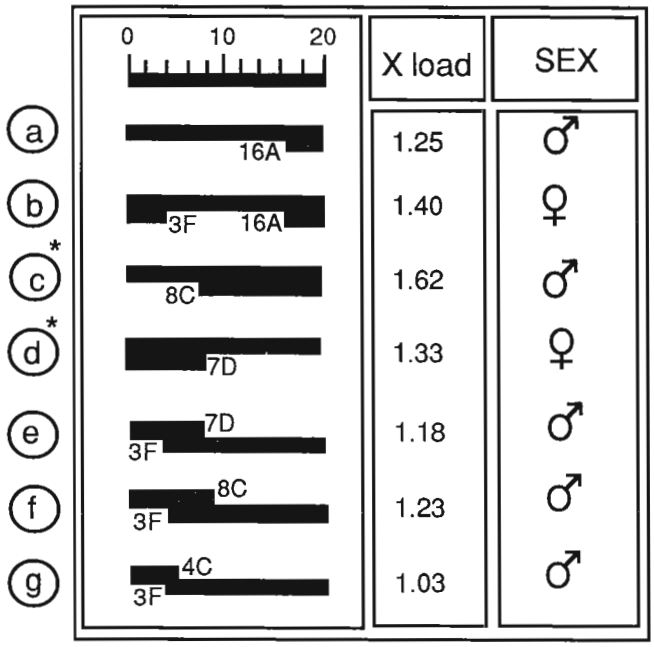


Figure 1. Diagram representing X chromosomal composition, X-load and sex of different 1X aneuploids.
*observation of Steinmann-Zwicky and Nothiger (1985).

Lastly, we succeeded in constructing the genotype with one X and a small duplication, encompassing the segment 3F3-4B1 (generated by crossing translocation X;Y A2 x B29). This aneuploid (1X/dp.3F-4C), in contrast to the expected developmental pattern (Steinmann-Zwicky and Nöthiger 1985), was found to be a healthy adult male (with respect to all sexual characters) (Fig. 1g), and was even fertile. The contribution of the segment 3F3-4B1 on the activation of Sxl, therefore, remains controversial.

Acknowledgments: We are thankful to Prof. J.R. Merriam and Dr. R.C. Woodruff for kindly providing T(X;Y) stocks. This work is supported by a C.S.I.R. fellowship to Mausumi Mutsuddi.

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Naseerulla, M.K., M. Jayashankar, and S.N. Hegde. Department of Zoology, University of Mysore, Manasagangotri, Mysore, India. *Drosophilid* fauna from Mahadeswara hills, Salem and Yercaud Hills.

An inquiry into the literature on the *Drosophila* fauna of Indian subcontinent shows that although extensive studies have been made on the *Drosophila* fauna in different parts of the country, the south eastern parts remain unexplored. This part of the country has the eastern ghats with charact-

Table 1. Distribution of Different Species of Drosophila in Mahadeswara Hills, Salem and Yercaud Hills (* New species described by the authors).

LOCALITIES:	MAHADESWARA HILLS 1040 m				SALEM						YERCAUD HILLS								TOTAL	
	wild		Domestic		Bamboo Forest		Sarvara-yana Hills		Domestic		400m		675m		900m		1,100m		1,225m		1,500m			
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
Subgenus : *Sophophora*:																									
D. malerkotliana	122	134	—	—	219	213	178	196	91	88	34	24	43	24	178	124	73	125	344	378	39	78	1321	1384	2705
D. bipectinata	4	2	—	—	1	1	—	—	6	10	—	—	—	—	—	—	—	—	—	—	—	—	11	13	24
D. takahashii	1	3	—	—	—	—	—	—	—	—	—	—	1	—	9	5	—	—	41	29	—	—	52	37	89
D. rajasekari	11	8	—	—	—	—	—	—	—	—	—	—	—	—	4	—	—	—	6	9	—	—	21	17	38
D. melanogaster	—	—	17	13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17	13	30
D. ananassae	1	2	39	25	—	—	—	—	24	26	2	—	—	—	2	—	6	10	—	—	32	98	106	161	267
D. kikkawai	8	8	—	—	3	5	—	—	14	13	3	—	5	4	—	—	3	—	16	10	15	76	67	116	183
D. neoelegans	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	5	7	5	12
Drosophila:																									
D. n. nasuta	6	5	—	—	1	1	7	4	—	—	—	—	—	—	5	7	—	—	—	—	3	1	22	18	40
D. bridavani	3	2	—	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4	8
D. immigrans	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	11	—	—	5	11	16
D. repleta	19	17	—	—	52	71	101	124	—	—	—	3	—	—	6	—	—	—	—	—	—	—	178	225	393
Scaptodrosophila																									
D. nigra	3	6	—	—	—	—	—	—	—	—	—	—	—	—	1	7	1	—	—	—	—	—	5	13	18
Hirtodrosophila																									
*D. longivittata**	—	—	—	—	—	—	—	—	4	6	—	—	—	—	—	—	—	—	—	—	—	—	4	6	10
Genus : *Phorticella*																									
P. lineosa	—	—	—	—	—	—	4	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	2	6
Zaprionus	—	—	—	—	—	—	—	—	6	10	—	—	—	—	—	—	—	—	—	—	—	—	6	10	16
Total No. of flies	178	187	56	38	277	293	290	326	145	153	39	27	49	28	205	143	83	135	412	437	96	258	1830	2035	
Grand Total	365		94		570		616		298		66		77		348		218		849		354				3855
No. of species per site	10		2		6		4		6		4		3		7		4		5		5				

eristic flora and fauna. A survey of *Drosophila* fauna was made in three parts of eastern ghats region, viz, Mahadeswara hills (12°.2'-12°4' E latitude and 77°-77°.5' N longitude, Salem (11°6'-11°.8' E latitude and 78°2'-78°.4' N longitude), and Yercaud Hills (11°6'-11°.8' E latitude and 78°2'-78°.4' N longitude). Flies were collected by trapping them with banana baits and net sweeping over rotting fruits from either wild or domestic or both localities in the month of June 1988. The details of collection localities, the species collected and their numbers are presented in table 1.

A total of 16 species were collected in these localities. Of these, 8 species belong to the subgenus *Sophophora*, 4 to *Drosophila*, 1 to *Scaptodrosophila*, 1 to *Hirtodrosophila* and 2 to *Phorticella*. *Hirtodrosophila* is new and named as *Drosophila longivittata* because of the presence of longitudinal stripes on the body. It is closely related to *D. quadrivittata*. Among the species trapped, *D. malerkotliana* is the most common and abundant species (2705) while *D. repleta* scores second with 393 flies. There was an ascendency in the number of flies with the increase in altitude except at the top of Yercaud Hills. The density of *Drosophila* depends on the vegetation. At lower altitudes and at 1,500 m the vegetation is sparse and hence low densities.

Most of the species collected belong to either *melanogaster* species group of subgenus *Sophophora* or *immigrans* species group of the subgenus *Drosophila*. This is in agreement with the view of Bock and Wheeler 1972) and others that both these species groups have originated and diversified extensively in South East Asia.

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References: Bock, I.R. and M.R. Wheeler 1972, Univ. Tex. Publ. 7213:1-102.

Mutsuddi, M. and A.S. Mukherjee. University of Calcutta, India. Specificity of the interruption of X chromosome in determining male or female-level activity vis-a-vis level of compaction in *Drosophila*.

In *D. melanogaster*, the polytene X chromosomes display different patterns of chromatin compaction in the two sexes. While in females, a reasonably similar pattern of chromatin packing is visualized among all chromosomes, the X chromosome in males possesses a comparatively less compacted state of chromatin, relative to autosomes (Lakhotia and Mukherjee, 1970). These two different states of X condensation are considered to be related to its different levels of gene function (Mukherjee, 1982).

We have reported earlier (Mutsuddi et al., 1983) that in X chromosomal segmental hyperploids, the decondensed nature of the X chromosome is maintained as long as the duplicated fragment reaches to the limit of 62% from the proximal end (dp.8C-20F, Fig. 1a). The aneuploid progeny having 82% proximal duplication (dp.4C-20F), on the

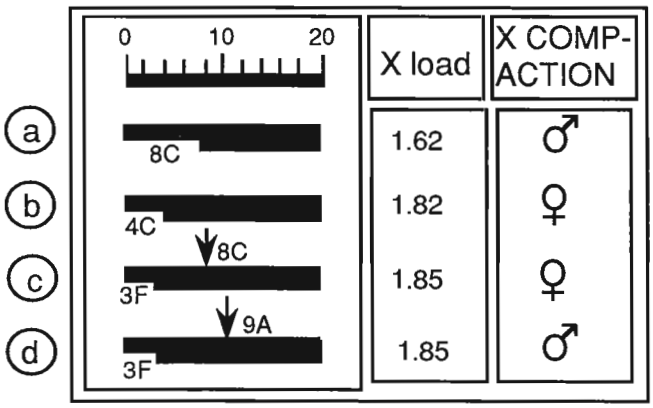

Figure 1 (above). Diagram representing X chromosomal composition, X-load and level of X condensation in different lX aneuploids. Arrow denotes the break.

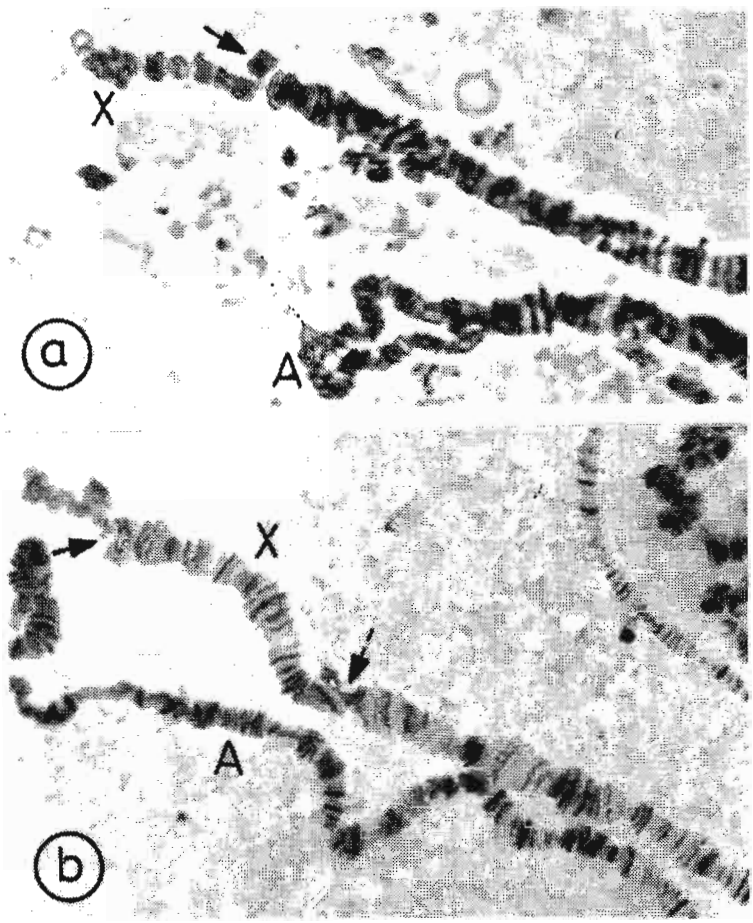

Figure 2 (right). Salivary gland squash preparations depicting the relative diameter and stainability of the X chromosomes (X) relative to those of autosomes (A), in two aneuploids, (a) 1X/dp.4C-20F and (b) 1X(1A-9A; 9A-20F)/ dp.3F-20F. Note, "female-like" (a) and "male-like" (b) appearance of the X chromosomes. Solid arrow denotes the end of duplicated X-fragment, and dotted arrow represents the break.

contrary, displays "female-like" condensation in its X chromosome (Figs. 1b, 2a). These results imply that with respect to proximal duplications, the state of X condensation is determined by the quantum of the additional X-fragments involved in duplication. An interesting situation with both the "male-" and "female-like" X condensation has been observed here in an aneuploid, presumably due to being a break at two different loci.

The genotype of this aneuploid differs in two respects from those examined earlier (Mutsuddi et al., 1983). First, the duplication size (dp.3F-20F; 85% duplication) is longer than the previously tested largest one (dp.4C-20F; 82% duplication), and secondly, the continuity of the "entire-X", unlike that of the earlier genotypes, is interrupted by breaks (Figs. 1c-d, 2b) at different locations. While in one class, the X chromosome is interrupted in 8C locus (Fig. 1c), it is at 9A (Figs. 1d, 2b) in the other. The genotypes (1X/dp.3F-20F) are thus 1A-8C; 8C-20F/dp.3F-20F and 1A-9A; 9A-20F/dp.3F-20F (generated by crossing translocation X; Y A2 x J8 and A2 x B149), respectively.

An examination of larval salivary gland chromosomes (Das et al., 1982) revealed completely opposite patterns of the X chromosome condensation in these two classes. As for instance, the former class of genotype (1A-8C; 8C-20F/ dp.3F-20F) exhibited its natural inclination towards the "female-like" chromatin condensation of X, while the other class (1A-9A; 9A-20F/dp.3F-20F), contrary to expectation, was of "male-like" disposition in its chromatin packing (Fig. 2b). Therefore, the quantum, that is known to act as determinant of the X chromatin condensation, while found to be applicable in the former class, the type of chromatin conformation of the X observed in the other class shows that an interruption at locus 9A could be sufficient to subdue this effect of X-quantum and to divert the programme of X chromatin packing towards the female level, indicating that the load or the quantum of the X in the complement might not be the determining factor for the male or female-level chromatin compaction.

Acknowledgments: We are thankful to Prof. J.R. Merriam and Dr. R.C. Woodruff for kindly providing us T(X;Y) stocks. This work is supported by a C.S.I.R. fellowship to Mausumi Mutsuddi.

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Amylase gene family homologies in *D. melanogaster* and *D. miranda*.

The alpha-Amylase gene family in *D. melanogaster* is located in chromosome 2R and consists of two copies of the structural gene at the *Amy* locus in section 54A and a putative "pseudogene" in 53CD (1-3). The duplicated, divergently transcribed gene copies at 54A lie about 4 kb apart and are both functional in homozygous strains that produce two discrete amylase isozymes (2). In *D. miranda*, the functional Amy locus is in chromosome X^2, at a site homologous to section 73A in chromosome 3 of its sibling *Drosophila* species - *pseudoobscura* and *persimilis* (4-8). A duplication of the *Amy* gene (5) which fails to produce a functional product in transient somatic expression assays (7) is located between the transcribed copy in *miranda* and a truncated sequence with homology to the *Amy* coding region of *melanogaster* (5,6).

In situ hybridization studies (1,4,6,8), summarized in Table 1, suggested that two other sites in the X^2 chromosome of *D. miranda*, and homologous sites in *D. pseudoobscura* and *D. persimilis*, might contain inactive sequences belonging to the Amylase gene family, namely sites at 78C and 81BC of chromosome 3 in the latter two species and homologous sites in the X^2 of *miranda*. (The 73A, 78C and 81BC sites are numbered differently in a new cytological map of the X^2 chromosome of *miranda* (10). However, they lie within regions that display homologous chromosomal pairing in *miranda-pseudoobscura* hybrids (8,11) so we use the banding numbers for *pseudoobscura* to keep the interspecific relationships clear.) The lambda-Dm65 probe in Table 1 contains duplicated *Amy* gene copies as is characteristic of *D. melanogaster* (2,3); its 3.8 kb EcoRI fragment, subcloned into pDm3.8, includes *Amy-p*^{1} (proximal gene copy) plus flanking sequences extending ca. 200 bp upstream and ca. 2 kb downstream of the coding region (2,3).

The lambda-Dm32 probe in Table 1 contains the *Amy* "pseudogene" of *melanogaster* which produces no transcript, at least not in third instar larvae or mature adults (1,2,12). In Southern analysis, the internal 3.8 kb EcoRI fragment of lambda-Dm32 hybridizes with pDm38.8 and pDm5.6, which carry the respective Amy-p^1 and Amy-d^3 (distal) genes of lambda-Dm65 (2). Within this 3.8kb fragment of lambda-Dm32, is a 1 kb EcoRI-BamHI sequence with homology to mouse amylase cDNA. Several kb of DNA extend beyond these regions of homology within the insert of lambda-Dm32.

The probes in Table 1 contain more than *Amy* gene sequences, so there has been uncertainty about whether the 78C and 81BC chromosomal sites in the *Drosophila* sibling species - *miranda, pseudoobscura* and *persimilis* - contain DNA belonging to the Amylase gene family or not (6). It is reasonable to assume that there are no *Amy* family

sequences at the 81BC site because pDm3.8 (containing *Amy-p*[1]) does not hybridize to it in any of these three sibling species (8). Thus, the strong homology of the 81BC site to lambda-Dm32 probably reflects conserved sequences within its insert that are unrelated to *Amy*.

Table 1. In situ hybridization of probes containing Amy family sequences to specific sites in polytene chromosomes of four species of *Drosophila*.

Tritiated Probe	Source of Insert DNA	*Amy* Family Member	Site of Hybridization*		
			D. species	Chromosome	Site
λDm65 [^{3}H-cRNA]	*melanogaster*	Amy -p[1], Amy-d[3]	*melanogaster*	2R	54A
			pseudoobscura	3	73A
				3	78C
			persimilis	3	73A
				3	78C
			miranda	X[2]	73A
				X[2]	78C
pDM3.8 [^{3}H-DNA]	λDm65	Amy-p[1]	Same as λDm65		
λDm32 [^{3}H-cRNA]	*melanogaster*	pseudogene(?)	*melanogaster*	2R	53CD
[^{3}H-DNA]			*pseudoobscura*	3	81BC
			persimilis	3	81BC
			miranda	X[2]	81BC
p Dmp3.8 [^{3}H-DNA]	λDm32	pseudogene(?)	*melanogaster*	2R	53CD
			other	species:	inconclusive**

* Standard conditions (1) of high stringency were used intraspecifically and, for ^{3}H-DNA probes, interspecifically as well. For ^{3}H-cRNA of λDm65, reduced stringencies were used interspecifically, i.e., hybridization of 40-41° C (in 50% formamide) with washes at 60° C, omitting the final wash in 0.05 X SSC.
** The pDmp 3.8 probe may not contain all of the Amy "pseudogene" sequences from λ Dm32 (see text).

The situation for the 78C site is not so clear. However, new evidence suggests that the 78C site in *D. pseudoobscura* is unlikely to contain *Amy* family sequences (9). In a first attempt to clarify the situation for *D. miranda*, we report here results of a Southern analysis that utilized better defined probes from the *Amy* region than had been employed previously for *in situ* hybridization and clone isolation. We used these same probes to investigate *Amy* homologies within the 3.8 EcoRI fragment of lambda-Dm32, which comprises the insert of pDmp3.8, the last probe listed in Table 1.

Southern analysis of the *Amy* "Pseudogene" in *D. melanogaster*. DNA from pDmp3.8 was mapped for a variety of restriction enzyme sites by means of single and double digestions. Restriction fragments of pDmp3.8 were electrophoretically separated in 0.8% agarose gels and subjected to Southern blot analysis using ^{32}P-labeled probes from the *Amy* region of *melanogaster*. These probes, which are shown in Figure 1 and contain sequences from the 3.8 kb and 5.6 kb EcoRI fragments of lambda-Dm65, had been subcloned into Bluescript plasmid vectors (Stratagene) (7). One contains the highly conserved internal 0.7 kb BamHI fragment from the coding region of *Amy-d*[3] in lambda-Dm65. It is essentially the same, for practical purposes, as the homologous sequence in *Amy-p*[1] and is called the 0.7 kb probe below. The second carries the 0.9 kb BamHI-EcoRI fragment of lambda-Dm65 which encompasses ca. 700 bp of the coding region of *Amy-p*[1] at its 5' end, plus ca. 200 bp of upstream, noncoding flanking sequence. The third probe contains the 0.8 BamHI fragment of lambda-Dm65 which covers ca. 100 bp from the 3' end of the coding region in *Amy-p*[1] and ca. 800 bp of its adjacent downstream sequence.

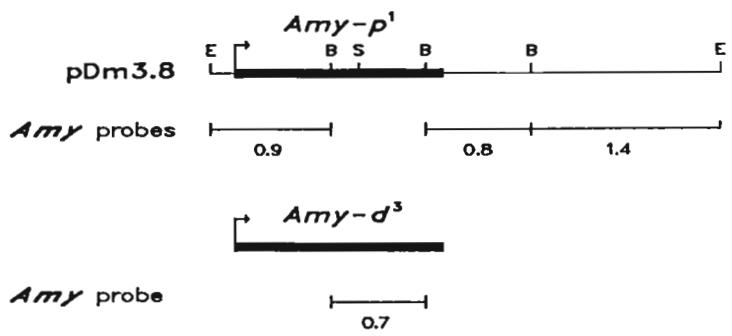

Figure 1. Probes from lambda-Dm65 used in Southern blot analyses. Top: Partial restriction map of the 3.8 kb EcoRI fragment from lambda-Dm65 which contains Amy-p[1] and is inserted in pDm 3.8. Bottom: Coding region of *Amy*-d[3] from the 5.6 kb EcoRI fragment of lambda-Dm65 inserted in pDm5.6. Restriction sites shown are: BamHI (B), EcoRI (E) and SalI(S). Thickened bars denote coding sequences and bent arrows, the direction of transcription. Subcloned fragments used as *Amy* probes are shown below each map with their sizes given in kilobases.

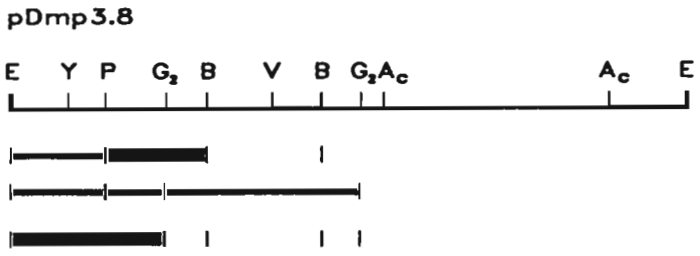

Figure 2. Restriction map of pDmp3.8. This subclone of lambda-Dm32 contains putative *Amy* pseudogene sequences from *D. melanogaster*. Restriction sites include: AccI (Ac), BamHI (B), BgIII (G_2), EcoRI (E), PstI (P), PvuII (V), and XbaI (Y). There are no BgII, HindIII, SalI, or XhoI sites within the mapped sequence. Black bars below the map indicate regions of hybridization in Southern transfers to the 0.7 probe in Figure I, which contains only amylase coding sequences. Bar thickness denotes the intensity of hybridization of probe to restriction fragment.

Transfer membranes carrying restriction fragments of pDmp3.8 were sequentially hybridized to the above probes (Fig. 1) under the reduced stringency conditions originally employed in the isolation of lambda-Dm65 and lambda-Dm32, i.e., hybridization at 37°C in buffer with 50% formamide, and the final wash in 1 X SSC at 50°C (1). Results are illustrated by the restriction map of pDmp3.8 in Figure 2. No consistent hybridization of the 0.9 kb or 0.8 kb probes to any given fragments in pDmp3.8 could be demonstrated. The 0.7 kb probe, however, showed strong intensity of hybridization to certain sequences in pBmp3.8, even under conditions of higher stringency (final wash in 0.2 X SSC, 55°C). Under the latter conditions, *Amy* probes from *D. melanogaster* hybridize specifically and consistently to *Amy* sequences from species of the *pseudoobscura* group.

The 0.7 kb *Amy* probe (Fig. 1) contains only *Amy* sequences from the 3' half of the coding region. Thus, the above results suggest that an *Amy* pseudogene, or part of one, very likely does exist at the 53CD site in chromosome 2R of *D. melanogaster*, at some distance from the functional *Amy* structural gene locus in 54A. If so, additional sequences of this pseudogene may extend beyond the insert in pDmp3.8, based on the skewed position of the restriction fragments which hybridized to the 0.7 kb probe (see left side of the map in Fig. 2). In support of this view, Levy et al. (2) showed weak hybridization of the 0.65 kb EcoRI fragment in lambda-Dm32 (pseudogene clone) with pDm3.8 and pDm5.6 which, as noted, carry the respective proximal and distal *Amy* genes. This 0.65 kb EcoRI fragment flanks the appropriate side of the putative pseudogene sequence in pDmp3.8 for the latter to extend into it. It should be noted, however, that the same 0.65 kb fragment failed to show homology to mouse amylase cDNA (2). Unfortunately, we are unable at present to examine this situation further because the lambda-Dm32 clone containing the fragment in question is no longer available.

Southern Analysis of the Putative *Amy* "Pseudogene" Sequences at 78C in *D. miranda*. A series of 7 unique, nonoverlapping clones carrying genomic fragments from *D. miranda* were isolated by virtue of their homology to pDm3.8 (5,6). To show this cross-species homology, somewhat reduced stringencies were used in their isolation, i.e., final wash in 0.2 X SSC at 55°C. Under high stringency conditions, each of these clones specifically hybridizes in situ to the 78C site in the X^2 chromosome of *miranda* (6,8). We analyzed one of them, lambda-Dmi34, in Southern transfers for homologies to *Amy* sequences in the previously described probes of *melanogaster* origin (Fig. 1) which contain the 0.7 kb and 0.9 kb fragments from pDm5.6 and pDm3.8, respectively. Also used was a probe containing the 1.4 kb EcoRI-BamHI fragment from pDm3.8 (Fig. 1). Filters were washed under the same conditions used to isolate lambda-Dmi34 (see above).

Results of this analysis were fairly clear, but did not conclusively resolve the question of whether or not an *Amy* pseudogene, or relics of one or more of them, is (are) present at the 78C site in the X^2 of *D. miranda*. They may be summarized as follows: (1) strong homology was displayed between one or more fragments of lambda-Dmi34 and the probe containing the 1.4 kb BamHI-EcoRI fragment located downstream of the coding region in the *Amy-p*[1] gene of pDm3.8; and (2) hybridization of only very weak intensity occurred between fragments from the same digests of lambda-Dmi34 and probes containing amylase coding sequences.

Thus, it appears that the strongest homology between lambda-Dmi34 and the insert in pDm3.8 resides in the sequence 3' to the proximal *Amy* gene from *D. melanogaster* and that this homology is what permitted the isolation of lambda-Dmi34. If true, there may be no sequence related to the Amylase gene family at the 78C site in the X^2 after all. The other 6 unique clones isolated, using pDm3.8 as probe (5,6), which contain genomic fragments from *miranda* and hybridize specifically to its 78C site in X^2, remain to be tested. We suspect they will give results similar to those for lambda-Dmi34 because the 0.7 kb probe containing only amylase coding sequences from *melanogaster* has failed to hybridize *in situ* to the 78C site in the polytene X^2 chromosome of *miranda* (8).

Conclusion. Southern blot analysis utilizing probes which contain amylase coding sequences permitted us to obtain

more convincing evidence than previously available for the presence of an *Amy* pseudogene within section 53CD of chromosome 2R in *D. melanogaster*. However, the precise size of this apparent member of the Amylase gene family remains to be determined, and final proof of its pseudogene status awaits its sequencing.

Data obtained from *D. miranda* cast serious doubt on the existence of Amylase gene family sequences outside of the 73A site in chromosome X^2. A conserved sequence of unknown function, located adjacent to the centromere-proximal, 3'-end of the *Amy-p* gene in *D. melanogaster*, shares strong homology to sequences located at the 78C site in the X^2 of *miranda*.

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O'Hare, Kevin. Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ, U.K. On the identity of *white-eosin* and *white-cherry*.

In their studies on the physical map of alleles of the *white (w)* locus of *D. melanogaster*, Zachar and Bingham (1982) could not detect any difference between *white-eosin* (w^e and *white-cherry* w^{ch}). This was surprising, as w^e arose in a culture of *white-one* (w^1) in August 1911 (Morgan and Bridges, 1916) while w^{ch} was found as a cluster of males in October 1912 in a cross between *vermilion* females and *miniature* males (Safir 1913). Even at the time of isolation, it was not possible to phenotypically distinguish w^{ch} from w^e. Experiments since then have not revealed any significant differences in either the phenotype of the alleles, or their behavior in various genetic tests (for example, see Green 1959).

We have been working on the structure of w^1 and its derivatives, including w^e, and have found that in w^1 there is a Doc insertion in the proximal region of *w*, and in w^e there is a second small insertion within the Doc insertion. These results are in accord with those of Zachar and Bingham (1982). We have also examined a mutant stock called *white-eosin-two* (w^{e2}) which is reported to be an independent derivative of w^1 which is phenotypically similar to w^e [Hefner (1925) cited in Lindsley and Grell (1968)], and a number of other stocks of w^{ch}. By DNA blotting we compared the structure of the proximal region of *w* in all these mutants with that of w^e and could not detect any differences. Furthermore, we have made use of a second chromosome modifier mutation present in one of the w^{ch} stocks, *Suppressor of white-cherry* [*Su*(w^{ch}] to test these alleles and again find no difference between w^e, w^{e2} and the different stocks of w^{ch}. In all cases, males carrying the *Su*(w^{ch}) mutation had darker eyes than their brothers without the suppressor chromosome. This phenotype was similar to that of w^e females (w^e males are not dosage compensated, and so have lighter eyes than w^e females). Females heterozygous for w^{ch} and carrying the *Su*(w^{ch}) chromosome had dark brown eyes while their sisters lacking the suppressor chromosome had eyes like w^e females. Green (1959) has already reported the interaction between *enhancer of white-eosin* [*e*(w^e)] and these alleles (see table 1). Given that the interaction of enhancers and suppressors is so allele specific, this is further genetic evidence for the identity of these alleles.

Table 1. Properties of *w* alleles. UM - European Drosophila Stock Center, Umea, Sweden. BG- Bowling Green Stock Center, Bowling Green, Ohio, USA . DTM - Danielle Thierry-Mieg.

stock	source	dosage compen-sation	*e*(w^e)	*Su*(w^{ch})
$w^{e}m$	BG	No	e	s
w^{e2}	BG	No	e	s
$w^{ch}{}_{wy}$	UM	No	e	s
y $w^{ch}{}_{wy}$	DTM	No	e	s
w^{ch};*In(2L)Cy In(2R)Cy, Cy* cn^2/*Su*(w^{ch})	UM	No	e	s

e - enhanced s - suppressed

We cannot conclude from this that there never were novel alleles for w^{e2}, or for w^{ch}, although contamination remains a possible explanation. An equally plausible explanation is that there has been mislabelling of stocks over the years, as happened for w^e in Edinburgh, where it was called w^a by mistake and is now called w^{aE} (MacKendrick, 1953 cited in Lindley and Grell 1968).

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Orr, H. A. The University of Chicago, Chicago, Illinois. Genetic basis of postzygotic isolation between *D. melanogaster* and *D. simulans*.

All hybrids between *D. melanogaster* and *D. simulans* are either inviable or sterile: the cross *D. melanogaster* female x *D. simulans* male produces females (who are sterile), but no males. The reciprocal hybridization produces males who are sterile), but no females. This complete postzygotic isolation has hindered attempts to analyze the genetic basis of reproductive isolation between these species.

Nonetheless, some facts are known: First, because F_1 females are viable in only one direction of the hybridization, Sturtevant (1920, 1929) pointed out that female inviability must involve the cytoplasm, and suggested that a maternal effect is involved. Sturtevant (1929) also found that the cross of *D. melanogaster* attached-X female X *D. simulans* male produced males (X_sY_m, where s = of *D. simulans* origin, and m = of *D. melanogaster* origin), but no females ($X_mX_mY_s$)--the opposite of the results obtained in a normal *D. melanogaster* female X *D. simulans* male cross. The sex chromosomes are thus obviously involved in both male and female inviability. This result was confirmed by Pontecorvo (1943), who further showed that the major autosomes are also involved in male and female inviability.

Here I report the results of crossing *D. simulans* attached-X (yellow white) females to *D. melanogaster* wild-type males. This cross allows the production of $X_sX_sY_m$ females and X_mY_s males on a background of *D. simulans* cytoplasm. As shown in Table 1, many adult $X_sX_sY_m$ females, but no X_mY_s males, were recovered. I tested the fertility of these hybrid females by mass mating them with many wild-type *D. melanogaster* and *D. simulans* males. No larvae were observed after 10 days at 22°C. Microscopic examination of the reproductive tracts of 93 of these females (see Orr [1987] for details) revealed that 89 had been inseminated, while 4 had not. Thus all 89 mated females were completely sterile.

These results confirm Sturtevant's (1920, 1929) claim that the cross of *D. simulans* XXY female X *D. melanogaster* male yields some exceptional $X_sX_sY_m$ females, many regular males (X_sY_m), but no exception males (X_mY_s). Unfortunately, Sturtevant's *D. simulans* females did not carry an attached-X: thus it was not clear whether his failure to recover exceptional males reflected the inviability of these males, or the rare production of O/Y eggs by XXY females. The latter possibility was plausible as only 8 exceptional females were ever recovered (these females result from the desired segregation of XX vs. Y in the mothers) (Sturtevant, 1929). Recovery of so few exceptional females also prevented any reliable assessment of their percent fertility.

The present results show that F_1 female (cyt. = s) inviability involves an X-linked allele from *D. melanogaster*: female hybrids having a *D. simulans* mother are inviable if they carry a *D. melanogaster* X, but are viable if they carry two *D. simulans* X's. As Sturtevant (1920) pointed out, however, the inviability of X_mX_m (cyt = m) females (see above) proves that at least one zygotically-acting *autosomal* locus is also involved. The location of the gene(s) responsible for the maternal effect on female viability remains unknown (the possibility that an endosymbiont in the *D. simulans* cytoplasm -- not maternal nuclear genotype -- is responsible for the non-reciprocal female inviability seems unlikely given that X_mX_m females carrying *D. melanogaster* cytoplasm are also inviable). The present results further show that hybrid female sterility involves at least one dominant autosomal allele from *D. melanogaster*: females with cytoplasm, both X chromosomes, and a haploid complement of autosomes from *D. simulans* remain completely sterile.

Furthermore, male inviability is clearly independent of the source of cytoplasm: X_mY_s males are inviable whether their cytoplasm derives from *D. melanogaster* or from *D. simulans*. Conversely, as Sturtevant (1920, 1929) showed X_sY_m males are viable regardless of the source of cytoplasm. Thus, unlike female viability, there is no evidence of any maternal effect on male viability.

Table 1. Number of adult female and male *D. melanogaster* - *D. simulans* hybrids recovered in various crosses.

Cross	Females	Males
sim y w $\hat{X}\hat{X}$Y ♀ X mel ♂	147	0
mel y^2 Hmr ♀ X sim ♂	217	26
sim y w $\hat{X}\hat{X}$Y ♀ X mel y^2 Hmr ♂	9	5

I also used the *D. simulans* attached-X stock to address an ancillary question. Hutter and Ashburner (1987) isolated an X-linked mutation in *D. melanogaster, Hmr*, that partially rescues hybrid male viability in the cross of *D. melanogaster Hmr* female X *D. simulans* male. The rescued males remain completely sterile. By crossing *D. simulans* attached-X females to *D. melanogaster Hmr* males, one can determine whether *Hmr*'s rescue effect depends upon a maternal or cytoplasmic effect, i.e. are X_mY_s males who carry *D. simulans* cytoplasm also rescued?

Preliminary crosses confirmed that *Hmr* partially rescues male viability on a background of *D. melanogaster* cytoplasm, as expected (Table 1; cross performed and progeny reared at 18°C). Unfortunately, the *D. simulans* attached-X (*y w*) female X *D. melanogaster* y^2 *Hmr* male cross proved extremely difficult. Dissection of several of the *D. simulans* females revealed strong mating isolation. After three weeks, all the tested females remained uninseminated. After repeated crossing, only 14 F_1 hybrids were obtained. Nonetheless, the results clearly show that *Hmr* does rescue hybrid male viability on a background of *D. simulans* cytoplasm: five yellow-body wild-eye males were recovered (Table 1; cross performed and progeny reared at 18°C). Thus, *Hmr*'s rescue effect does not involve any maternal or cytoplasmic effect. X_mY_s males are rescued whether or not their mothers carry a copy of the *Hmr* allele or are from *D. melanogaster*.

Unfortunately, the five males recovered were completely sterile. Their testes were extremely atrophied and produced no sperm. This lack of fertile *D. melanogaster* - *D. simulans* hybrids obviously represents the greatest remaining obstacle to the analysis of reproductive isolation in this hybridization.

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Oudman, L. and A. Kamping, University of Groningen, Haren, The Netherlands. ADH and alpha-GDPH in relation to anoxia in in *Drosophila melanogaster*.

Resistance to anoxia might give larvae of *Drosophila melanogaster* an advantage when exploring the deeper layers of the medium during feeding. Rechsteiner (1970) supposed that alpha-glycerol-3-phosphate dehydrogenase (alpha-GPDH) plays a role in regenerating NAD^+ during anaerobiosis in the fat body (which has a low level of lactate dehydrogenase). Alcohol dehydrogenase (ADH) too plays a role in the NAD^+/NADH metabolism, and might have a metabolic relation to alpha-GPDH (Cavener and Clegg, 1981).

The influence of these enzymes on the resistance to anoxia was investigated using *Adh* and *alpha-Gpdh* null mutants: Adh^{n1} (Grell et al., 1968) and *alpha-*$Gpdh^{nNC3}$ (Dickson Burkhart et al., 1984), respectively. Control wild type flies were used from the laboratory strain Groningen 83 which was founded in 1983 with females from a fruit market in Groningen, The Netherlands.

Eggs were incubated in plastic vials of 22 x 75 mm with 8 ml medium of agar, sucrose and dead yeast (Van Delden et al., 1978) at 25°C. To create an anaerobic situation the medium with larvae was covered with water on the fourth day, when the larvae were in the middle of the third larval instar. (Before the experiment the water was boiled during 15 minutes to extract gases, and cooled down to 25°C). Water was removed after 30, 60 or 90 minutes. Control vials were not inundated. For each genotype (3) and treatment (4) three replicate vials, with 25 eggs each, were incubated. Table 1 gives adult survival per genotype and treatment, expressed as the percentage of the number of flies of the same genotype that eclosed in the control vials. Table 2 gives an analysis of variance of the data.

From Table 2 it is clear that inundation time is the only significant effect. No significant differences between the sexes or genotypes were found. So neither ADH nor alpha-GPDH activity seems to be a limiting factor during anoxia.

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Table 1. Survival expressed as percentage from control.

Time (min.)	wild type	*Adh* null	α-*GPDH* null
0	100	100	100
30	80	83	77
60	46	68	33
90	13	7	12

Table 2. Analysis of variance of the numbers of eclosed adults per vial.

	SS	DF	MS	F	sign.
time (t)	988	3	329	92	***
genotype (g)	8	2	4	1	ns
sex (s)	0	1	0	0	ns
t x g	35	6	6	2	ns
t x s	14	3	5	1	ns
g x s	3	2	1	0	ns
t x g x s	11	6	2	1	ns
residual		171	48	4	

ns: not significant. ***: $p < 0.001$

Paik, Y.K., M.S. Lyu and C.G. Lee. Dept. of Genetics, Hanyang University School of Medicine, Seoul, Korea. Distribution of P factor activity and cytotype in two Korean wild populations of *D. melanogaster*.

Recently, the distribution of the chromosomal P factors and the extrachromosomal cytotypes in natural populations of *D. melanogaster* has been analyzed worldwide by many workers. Presented here are the results of our survey of two Korean wild populations of this species for the gonadal sterility. These populations were mainly M and Q types, the frequency being almost equal; P type was practically null and polymorphism of the M cytotype was apparent.

Materials and Methods: Two natural populations of *Drosophila melanogaster* were studied in Taegoo (35.52°N, 128.35°E) and the island of Cheju (33.20°N, 126.32°E). The samples were collected in large orchards between mid-September and mid-October 1987.

Table 1. A strain classification system in this study

Cross A (CS ♀♀ X U ♂)	Cross B (U ♀ X CS ♂♂)	Cross A* (U ♀ X Har ♂♂)	Cross B* (Har ♀♀ x U ♂)	Strain classification
GD	N	N	N	P
N	N	GD	N	M
N	N	N	N	Q

GD, % GD sterility is >10; N, % GD sterility is <10.

Two tester strains (a strong P strain Harwich and the standard M strain Canton S) were used to assay the GD sterility of the wild strains. Wild-caught males were used immediately for crosses with the M strain females. Wild-caught females were used to establish isofemale lines. The lines were kept under standard laboratory conditions by single pair mating; males and virgin females were collected at generations 4 (G_4) and 13 (G_{13}) following capture. They were then tested for GD sterility at two different times to see if type change had occurred during the laboratory culture.

The following standard, reciprocal crosses were made for the detection of GD sterility at 29°C: Cross A*: U female x P males, Cross B*: P females x U male, Cross A: M females x U male, Cross B: U female x M males. Having analyzed all the results of these four reference crosses (Table 1), each female line was then classified as P, Q or M strain according to the criteria.

A cutoff point at 10% (Kidwell 1983) was selected to define the potential sterility of the parents in question, U, in crosses A and A*, since no background sterilities were observed in the two control crosses (B and B*).

For cross A and B* tests, a single male from each line was mated with two Canton-S virgins: for crosses A* and B, a single female from each line was crossed with two Harwich males. Each cross was made at 29°C in a vial with medium. Parents were discarded on the seventh day after mating, and progenies were allowed to eclose at 29°C. On the eleventh day, emerged F_1 flies of both sexes were transferred to fresh vials with medium at 25°C and allowed to mature for three or four more days. After aging, 48 F_1 females of each mating were placed individually into wells of a 96-cell tissue culture plate (Engels and Preston 1980). Each well in the plates was dispensed with 0.2ml of special medium (1.2% agar, 5% sucrose, 0.2% propionic acid, stained with red or yellow food coloring) containing a small amount of live yeast as an egg laying stimulant. After three days the plates were chilled to anesthetize the flies and then scored for egg production. A female which had laid one or more eggs was scored as fertile, whereas, all the females producing no eggs were individually dissected and only those females with two dysgenic ovaries were classified as sterile.

Results and Discussion: Genotypes of wild males: Figure 1 shows the frequency distribution of gonadal sterility among the F_1 daughters of individual wild males sampled at Taegoo (TG) and Cheju (CJ). In a sample of 48 daughters of each male crossed to Canton-S virgins (Cross A), GD sterility was estimated by the percent of infertile females with bilaterally dysgenic ovaries.

It is clearly shown in the figure that all wild males, excepting one from each population, had 100% fertile daughters in this cross. The overall mean sterility frequency was 0.46% among 10,224 daughters from the TG males and 0.47% among 10,080 from the CJ males, suggesting that true P elements with GD sterility function were practically null or nearly absent in these populations. Even the two exceptional males from the TG (1/213) and CJ (1/210) samples revealed very weak P activity - less than 50% F_1 female sterility on crossing with Canton-S females; their sterility level ranged from 20% to 30% among 48 F_1 daughters tested per line. Of the other geographical areas thus far reported, the Mediterranean wild populations analyzed by Anxolabehere *et al.* (1982) presented a similar picture (0.2% of mean sterility frequency in Tantaval village and Nasr' Allah populations).

Males sampled from the isofemale lines of the TG (270 strains) and the CJ (139 strains) at G_4 and G_{13} were tested for the presence of P factors using cross A in which 48 F_1 daughters were tested per line, as in the case of wild-caught

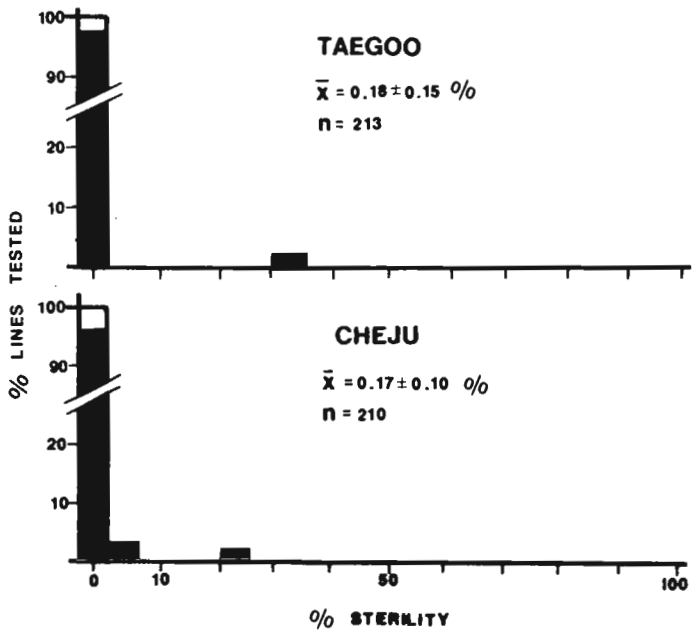

Figure 1. Distribution of GD sterility frequencies in 48 F_1 daughters of individual wild males crossed with Canton-S females. Closed square and open square indicate the frequency distributions found in the sterility tests for the wild-caught males and the isofemale-derived males sampled at G_4 and G_{13}. n, number of the wild-caught males tested. x, overall mean sterility.

males. As illustrated also in Figure 1, all males of these isofemale lines tested at G_4 and G_{13} completely lacked the ability to induce GD sterility. This result is comparable to that reported by Kidwell *et al.* (1983) who found no wild males with GD sterility in Australian populations of *D. melanogaster*. Cross A GD sterility level reported by Yamamoto *et al.* (1984) for Japanese populations was also low, but showed a much wider range, from zero to 50% in mating with M females. In the tests of North American California populations of this species, Simmons (1986) found an average of 14.3% of cross A GD sterility.

Figure 2 shows the results of the tests for the determination of cytotype and genotype of isofemale lines at G_4. The daughters from cross A were all fertile. In cross A* in which 270 TG and 139 CJ isofemale lines were used, average sterility percent in the TG and CJ strains was 31.98 ± 2.59 and 32.84 ± 3.39, respectively. The present results of crosses A* indicated that 46-48% of the tested isofemale lines at G_4 in both populations were M strains, showing 10-100% of F_1 daughter GD sterility on crossing with Harwich P males. This suggests the existence of cytotype polymorphism in the Korean populations of *D. melanogaster*. On the other hand, the remaining strains (52-54%) were typical Q, and showed no F_1 daughter sterility on crossing with Harwich males or Canton-S M females. The variations in the GD sterility level observed between the two populations are not significant by Mann-Whitney U test.

Determination of the type of the foregoing isofemale lines was also carried out at G_{13}. As shown in Figure 3, some changes were observed in the mean values of GD sterility (cross A*) in both the TG and CJ lines as compared with the corresponding values found at G_4. The mean of GD sterility (39.04%) in the TG lines at G_{13} was significantly higher than that (31.98%) found at G_4 ($P<0.001$), and the mean value (38.14%) of the CJ lines at G_{13} was also higher than that (32.84%) at G_4, but the deviation being statistically insignificant ($P > 0.05$). 53.7% (145/270) of the TG and 67.6% (94/139) of the CJ lines tested showed little change during the laboratory culture up to G_{13}.

As is seen in Figure 4, of the 124 TG lines determined as M type at G_4, 44 lines (36%) changed to Q type, and 81 lines (55.5%) out of 146 lines determined as Q type at G_4, to M type. Among 45 (32%) CJ lines where type changes were observed, M to Q and Q to M changes have occurred with frequency of 23.9% (16/67) and 40.3% (29/72), respectively. The type conversion reported by Hihara *et al.* (1985) in isofemale lines established from Japanese wild populations showed a similar rate (37.5%) for the M or M' to Q and much lower one (25.0%) for the Q to M or M' after two years of small mass culture.

In the present study, on crossing with Harwich males, the Q-derived M strains of G_{13} showed various degrees of F_1-female sterility ranging from 10% to 100% (Fig. 4). This could be considered a result of random loss of Q elements (defective or silent P elements), during the laboratory culture for 9 generations and supports the stochastic loss hypothesis of Engels (1981). Contrary to this, M to Q changes might have resulted from an increase in copy number of a few Q elements in M state chromosome when they frequently transpose into new chromosomes under M cytotype. The work of Engels and Preston (1980) on the artificial selection of M cytotype suggests that a small number of P or Q factors in M state chromosome transpose frequently under intensified M cytotype condition, eventually bringing about an appreciable increase in the frequency of P or Q type.

The results of *in situ* hybridization test in the recent study (Paik, 1988) revealed that all or an absolute majority of modern Korean wild-strains, genetically characterized as M type hitherto, were of pseudo-M (M') containing P element-

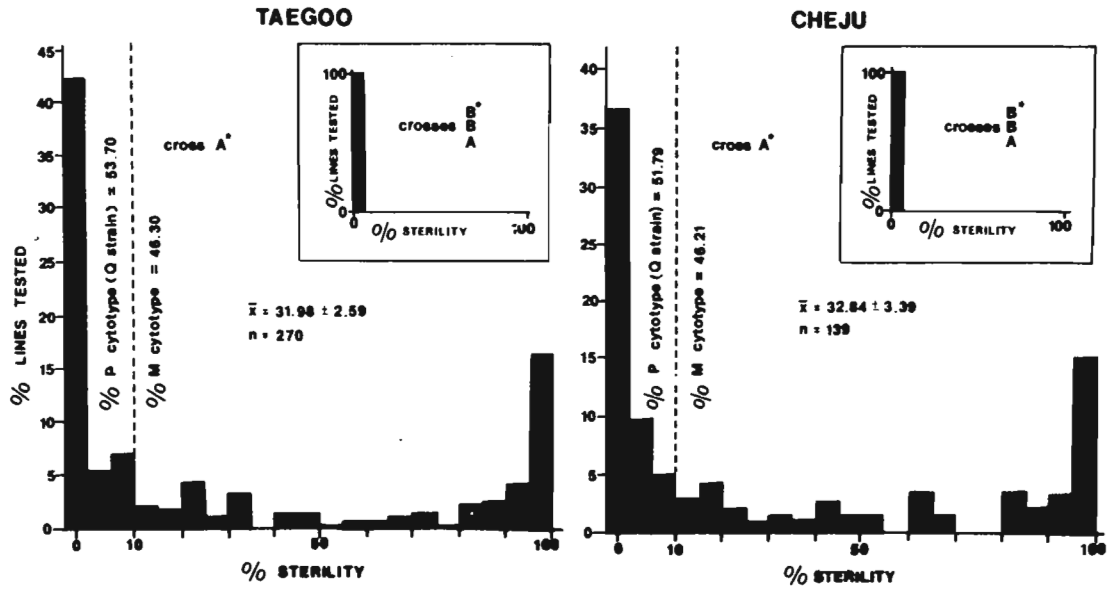

Figure 2. Distribution of GD sterility frequency estimated in female progenies of individual G_4 granddaughters (A^* and B) and grandsons (A and B^*) of isofemale lines. Sterility was measured by mean percent bilateral ovarian dysgenesis in 48 daughters from individual crosses with Harwich males (A^*) and Canton-S females (A). Crosses B and B^* served as control. n, number of G_4 granddaughters and grandsons tested in each of the four reference crosses. x, average percent sterility estimated in cross A^*.

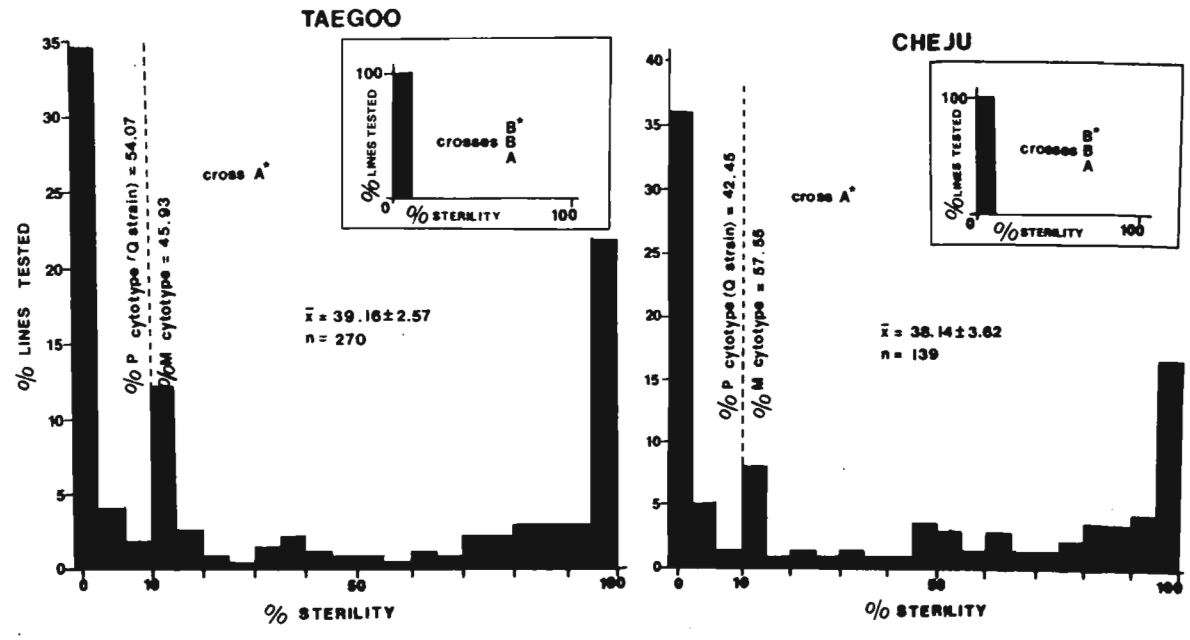

Figure 3. Distribution of GD sterility frequency estimated in female progenies of individual G_{13} granddaughters (A^* and B) grandsons (A and B^*) of isofemale lines. See the captions of Fig. 2. for GD sterility measurement and other symbols.

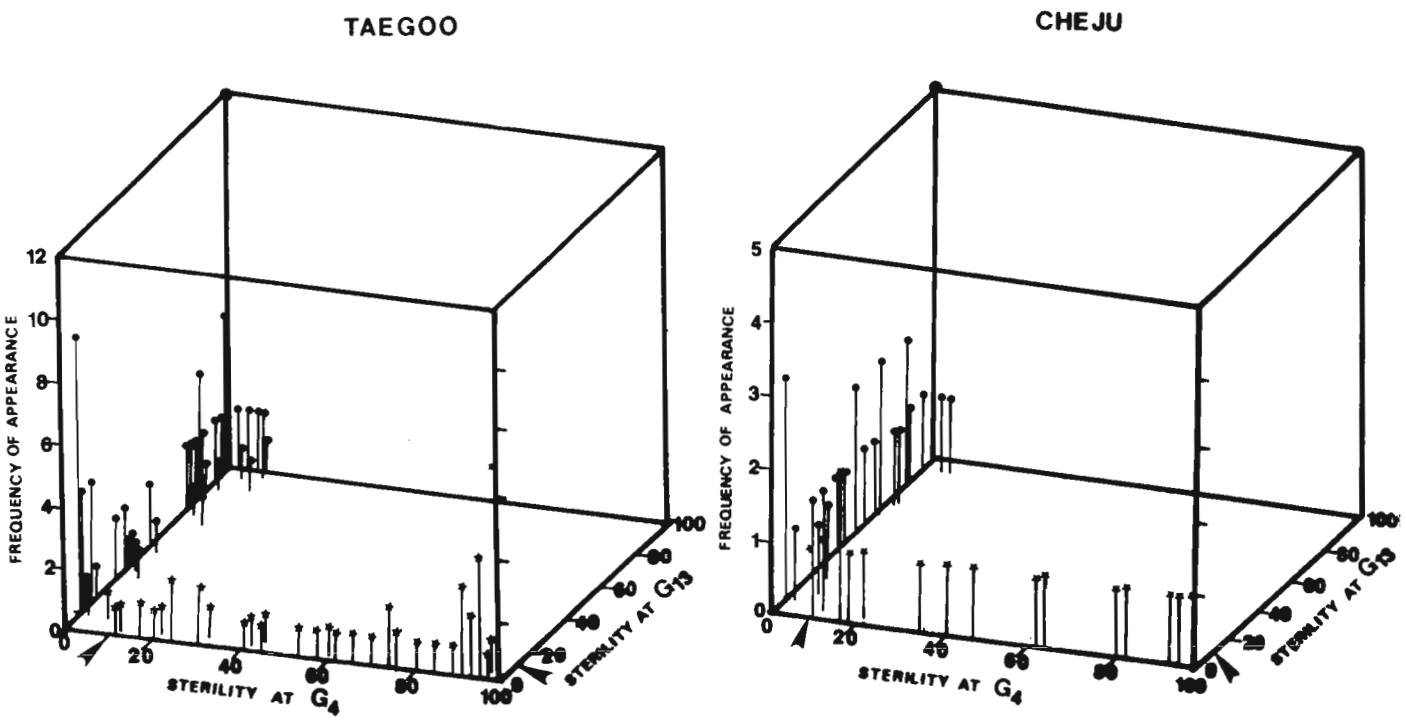

Figure 4. Type changes of P-M system in TG and CJ isofemale lines over a period of 9 generations (G_4 - G_{13}). Frequencies of the changes from Q to M (81 Taegoo and 29 Cheju lines) and from M to Q (44 Taegoo and 16 Cheju lines) are represented by —● and —★, respectively. ✓ indicates the 10% cutoff point for sterility and cytotype determination.

hybridizing sequences in their major chromosomes. This coincides with the result reported by Anxolabehere *et al.* (1985) in a similar study for the Mid-east populations and proves the Kidwell's (1983) prediction.

The relative frequencies of P-M phenotypes in the Korean populations differ from those so far reported in other continents. Only the Middle East populations (Anxolabehere *et al.*, 1985) present a most comparable picture, although M type predominates Q in them (In the Korean populations M and Q are almost equal in frequency). Nevertheless, the present results clearly confirmed the clinal pattern of a gradually decreasing frequency of P strain from west to east.

Although we do not know the precise reasons for this difference in Korean populations at present, the absence of P strains in the present-day Korean natural populations, coexistence and coprosperity of M' and Q types in the wild, our finding of a wide range of polymorphism for M cytotype, and frequent occurrence of cytotype switch (P $\rightleftharpoons$ M) in the isofemale lines, all suggest that Korean populations of *D. melanogaster* are now in a transient stage from Q to M' or *vice versa* since the introduction to Korea of the P-element family. It requires more data from both population and molecular levels, however, to explain the introduction process of the transposable elements into Korea and their genetic divergence thereafter.

Acknowledgment: Authors are grateful to professor T. Mukai, Department of Biology, Kyushu University, Japan, for kindly provided tester stocks of *D. melanogaster*. We are also indebted to the Korea Research Foundation and Korea Science and Engineering Foundation for their financial support.

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Paik, Y.K., H.S. Yim, and C.G. Lee. Dept. of Genetics, Hanyang University School of Medicine, Seoul, Korea. *In situ* hybridization of biotin-labeled P-elements on polytene chromosomes from a M/Q population of *D. melanogaster* in Korea.

A total of 201 isofemale lines from a Korean M/Q population of *D. melanogaster* were studied for chromosomal distribution of P transposable elements. All lines were maintained at 19°C by single pair mating, and two larvae from each line were studied at generation 4 following the capture of wild females, using the technique of *in situ* hybridization on polytene chromosomes. Chromosome preparations were hybridized with a probe -- biotin-labeled 4.7kb BamHI fragment of pπ25.1 containing the 2.9kb P element, together with fragment of flanking genomic DNA from the cytogenetic locus 17C. Nick translation of the probes from clone pπ25.1, hybridization and detection of hybridized DNAs were carried out according to Engels' protocol (obtained personally from Prof. T. Mukai) and the manual of the BRL system (BRL, 1988, Gaithersburg).

Figure 1 presents three examples showing the hybridization sites for the labeled P element on major chromosome arms in different strains. Table 1 shows the pooled average number of the copies per chromosome arm of P elements observed in 118 Q and 83 M strains.

Each hybridization site for a given isofemale line was counted on two nuclei, and the total number was determinated by counting the number of copies observed at the same sties of the same chromosomes in the two as well as that detected in one nucleus but considered missing in the other.

Table 1. Pooled average number of the copies per chromosome arm of P elements scored in Korean isofemale lines.

Strains		n	Chromosome arms					Total
			X	2L	2R	3L	3R	
M		83	11.58	6.65	9.06	7.71	9.79	44.87
	SD		2.16	1.33	2.05	1.76	1.74	4.95
Q		118	11.79	6.71	9.47	7.71	9.79	45.42
	SD		2.22	1.48	1.77	1.81	1.82	4.89
M+Q		201	11.69	6.68	9.30	7.71	9.82	45.23
	SD		2.19	1.42	1.90	1.79	1.79	4.93

n, number of isofemale lines examined.

As seen in Table 1, the X chromosome has the higher frequency of P elements than any of the other arms. The order of copy number in both M and Q strains is as follows: X> 3R> 2R> 3L> 2L.

These results are generally consistent with those found in the Tunisian population of *D. melanogaster* by Ronsseray and Anxolabehere (1986), although the number of copies of P in this study is greater than that found by them (1986, n=31.33).

This may indicate that the different chromosome arms are not equally susceptible to the presence of P elements, even though statistical significance test of inter-arm variation is yet to be made in the future. More detailed results await future publication.

Acknowledgment: The senior author is grateful to Prof. T. Mukai for providing the clones, pπ25.1. We are also indebted to the Korean Science and Engineering Foundation for financial support.

References: Ronsseray, S. and D. Anxolabehere 1986, Chromosoma 94:433-440.

Figure 1 (see next page). Examples of *in situ* hybridization of biotin-labeled P elements in Korean Q (a) and M (b) strains and US P strain Harwich (c). 17C, hybridization site observed in the cytogenetic locus 17C region. n, total numbers of copies of P elements counted.

Paik, Y.K., C.G. Lee, and H.S. Yim. Dept. of Genetics, Hanyang University, School of Medicine, Seoul, Korea. Southern blot hybridization of Korean strains of *D. melanogaster* to P element sequences.

Genomic DNAs from 35 M and 15 Q strains (4th generation isofemale lines) derived from a Korean wild population of *D. melanogaster* were subjected to Southern blot analysis according essentially to Woodruff *et al.* (1987).

Figures a and b are some examples showing the results of probing Southern filters with a biotin labeled 4.7kb BamHI fragment of Pπ25.1 containing the 2.9kb P element. The genomic DNAs were digested with HindIII (Figure a) and EcoRI (Figure b). As shown in these examples, all the Korean strains examined contained multiple copies of DNA sequencies homologous to the US P-element used as probe. This observation was consistent with that found in *in situ* hybridization (data shown separately in this volume of DIS).

The most important conclusion from this study is that all these natural strains including strong M (lanes 4 and 8 in Figure a) carried as many positive bands as the US P strain Harwich (lane 2 in Figure a; lane 3 in Figure b), but the hybridization patterns differed from each other.

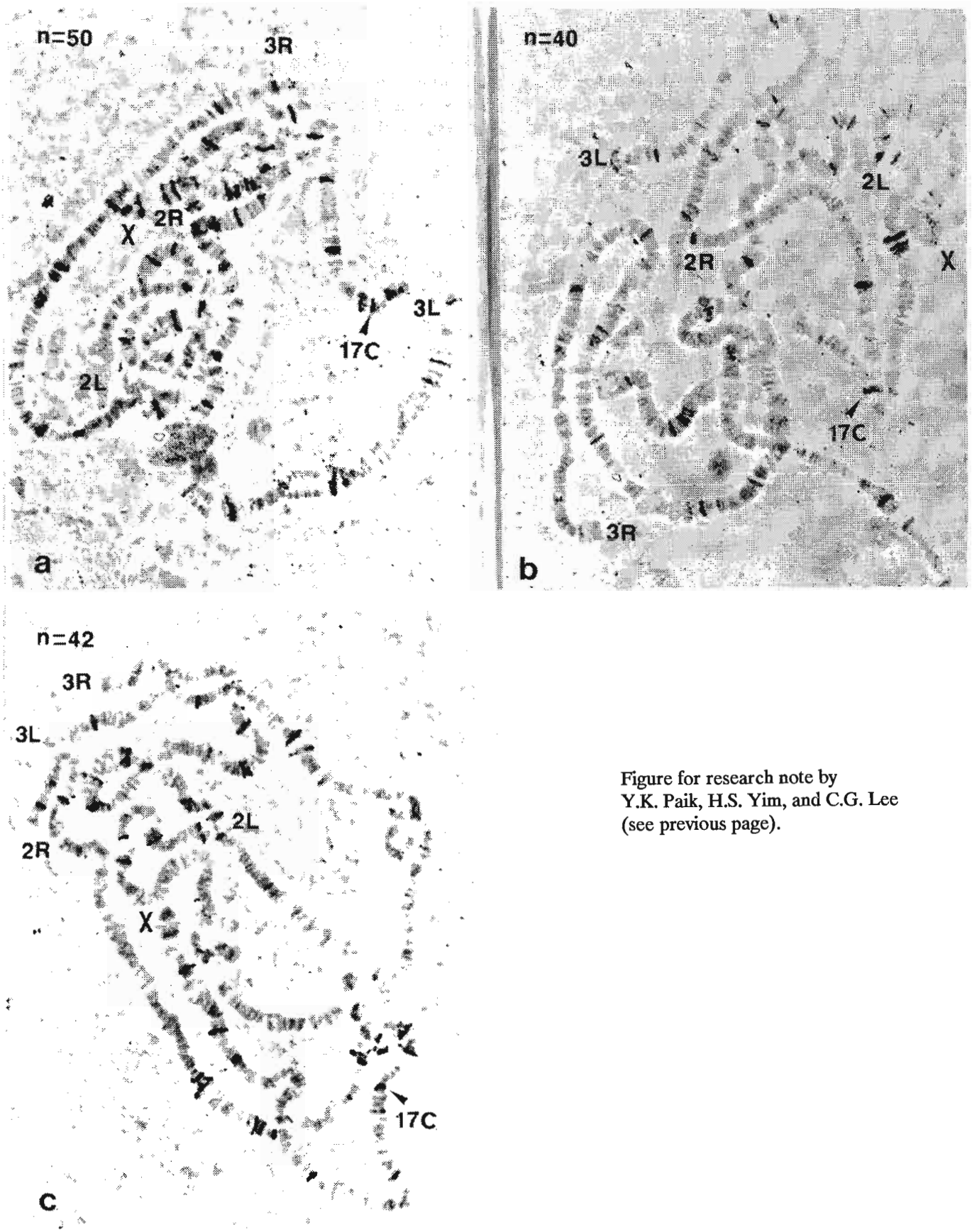

Figure for research note by
Y.K. Paik, H.S. Yim, and C.G. Lee
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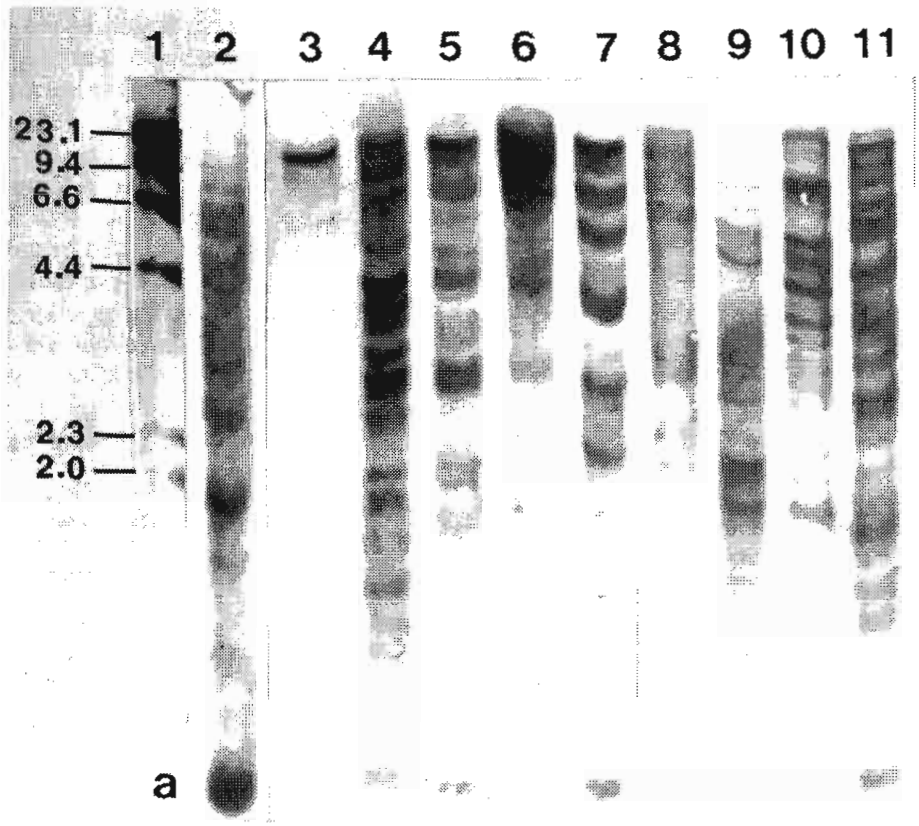

The summarized results and analysis of diversity of the members of the P element family observed in the present study will be published at a later date elsewhere.

Acknowledgment: The senior author is grateful to Prof. T. Mukai and Dr. Shirami for providing the clones, Pπ25.1 and Canton-S.

Reference: Woodruff, R.C., J.N. Thompson, jr., A.A. Szekely and J.S. Gunn 1987, DIS 66:173-177.

Figure a. HindIII digest of genomic DNAs from ten *D. melanogaster* lines (lanes 2-11). These digests were hybridized with a biotin-labeled 4.7kb BamHI fragment of Pπ25.1 containing the 2.9kb P element. Lane 1, size marker of HindIII digested lamda DNA; lane 2, standard P strain Harwich; lane 3, standard M strain Canton-S; lanes 4 and 8, strong M strains,; lanes 5 and 9, intermediate M strains; lanes 6 and 10, weak M strains; lanes 7 and 11, Q strains.

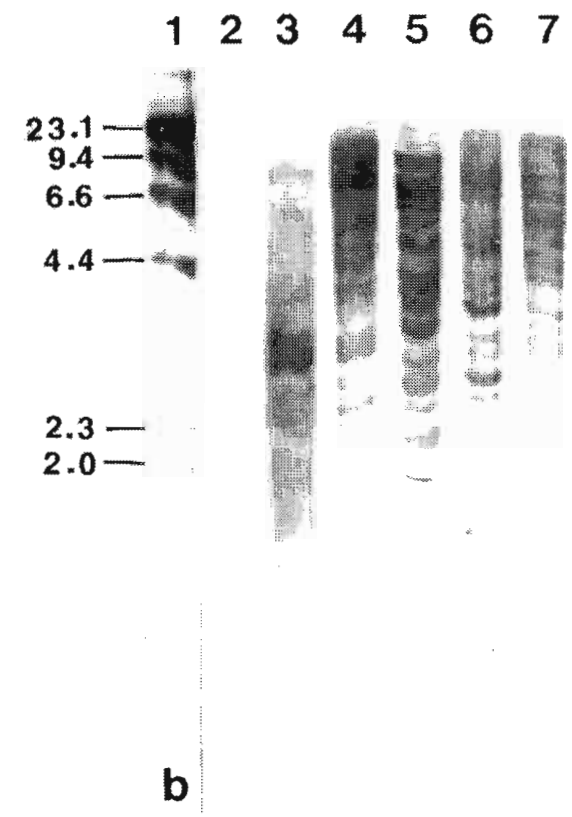

Figure b. EcoRI digest of genomic DNAs from six *D. melanogaster* lines (lanes 2-7). Lane 1, HindIII digested lamda DNA; lane 2, standard M strain Canton-S; lane 3, standard P strain Harwich; lanes 4,5,6,7, Q strains.

Pandey, M. and B.N. Singh, Banaras Hindu University, Varanasi, India. A preliminary study on pupation site preference in *Drosophila ananassae*.

The study of behavior is relatively a new field of investigation and research in behavior genetics largely began as a by-product of other investigations in a number of organisms including *Drosophila*. Two of the four life stages of *Drosophila* exhibit behavior -- larva and adult. Although sexual and nonsexual behavior of adults have been extensively studied (Grossfield, 1978; Ehrman, 1978; Spieth and Ringo, 1983), the behavior of larvae has been largely ignored until recently. In view of the fact that the total fitness is heavily influenced at the larval stage, behavior genetic study of *Drosophila* larvae has been initiated recently. The pupation site preference is an important step in *Drosophila* preadult development because the place selected by larvae can have a decisive influence on their subsequent survival (Sameoto and Miller, 1968). Thus pupation site preference is interesting and important primarily because it affects the survival of pupae. A number of investigations on pupation site preference in various *Drosophila* species such as *D. melanogaster, D. simulans, D. willistoni, D. pseudobscura, D. nigrospiracula* and *D. mettleri* have been carried out by different investigators (Sokal, 1966; Sameoto and Miller, 1968; De Souza et al., 1968; Markow, 1979; Fogleman and Markow, 1982; Sokolowski, 1985). In most of the studies, the pupation site preference (PSP) has been investigated by measuring the pupation height (the distance a larva pupates above the surface of the food medium).

Pupation site preference is affected by numerous factors such as moisture, light, temperature, density, sex, development time and species measured (Sokal et al., 1960; Mensua, 1967; Sameototo and Miller, 1968; Markow, 1979; Ringo and Wood, 1983; Sokolowski and Hansell, 1983; Bauer, 1984). The methods used to measure pupation height may also influence the results (Sokolowski and Hansell, 1983). A number of studies have been reported which demonstrate the influence of genetic factors on pupation site preference in different *Drosophila* species (Sokal, 1966; Markow, 1979; De Souza et al., 1968; Ringo and Wood, 1983; Bauer and Sokolowski, 1985; Casares and Carracedo, 1986; Garcia- Florez et al., 1989).

Drosophila ananassae, a cosmopolitan and domestic species is very common in India. The genetics and cytology of this species have been studied (for reference see the reviews by Moriwaki and Tobari, 1975; Singh, 1985; 1988). Recently, the behavior genetic study of *D. ananassae* has been initiated by Singh and his coworkers (Singh et al., 1985; Singh and Chatterjee, 1985a,b, 1986, 1987, 1988a,b, 1989; Chatterjee and Singh, 1987; 1988) who have demonstrated the existence of sexual isolation and sexual selection within *D. ananassae*. During the present study, we investigated the pupation site preference in *D. ananassae* by measuring pupation height in two strains derived from natural populations.

Table 1. Mean pupation height (in mm.) in two wild strains of *Drosophila ananassae*.

PAT-90		GOA	
Replicate number	Pupation height ± SE (mm)	Replicate number	Pupation height ± SE (mm)
1	3.8 ± 0.4	1	1.7 ± 0.5
2	2.7 ± 0.5	2	5.1 ± 0.6
3	4.7 ± 0.8	3	2.5 ± 0.5
4	5.8 ± 0.7	4	3.1 ± 0.5
5	3.3 ± 0.5	5	2.0 ± 0.6
6	5.4 ± 0.8	6	2.6 ± 0.6
7	3.5 ± 0.1	7	3.0 ± 1.1
8	5.7 ± 1.0	8	2.1 ± 0.5
9	3.9 ± 0.7	9	2.5 ± 0.8
10	8.8 ± 1.7	10	1.1 ± 0.4
	4.8 ± .06		2.6 ± 0.3

Table 2. Mean pupation height (in mm) for females and males in two wild strains of *Drosophila ananassae*.

Strain	Pupation height ± SE for females (mm)	N	Pupation height ± SE for males (mm)	N
PAT-90	4.6 ± 0.3	53	4.7 ± 0.6	47
GOA	2.6 ± 0.4	40	2.6 ± 0.3	59

N, total number

To measure pupation heights, two mass culture wild strains of *D. ananassae* used are: PAT-90 - established from females collected from Patna, Bihar in March 1990; GOA - established from females collected from Goa in March 1985. The simple food medium (agar-yeast-brown sugar-maize powder-propionic acid-nepagin) was used for maintaining these strains and also during the course of this study. Experiments were conducted in a culture room maintained at approximately 24°C temperature with 70% average relative humidity and approximately 12 hrs light and dark cycle. Virgin females and males were collected from each strain and aged for 4-5 days separately. Ten females and ten males were placed for mating in a food vial for 24 hrs and then they were transferred to a petridish containing food medium for egg-laying. After 48 hrs, flies were removed from the petridish. After larval eclosion, ten first instar larvae were removed and carefully seeded in a fresh food vial (size - 100 mm length and 25 mm diameter) stoppered with cotton plug. At the end of pupation time, the heights of pupae were measured. Ten replicates were carried out for each strain and thus 100 larvae were used. In order to detect sex difference in pupation heights, each pupa was transferred to a food vial and after adult emergence, its sex was noted.

The mean pupation heights for both strains are shown in Table 1. The mean pupation height for PAT-90 strain is

4.8 mm and for GOA strain 2.6 mm. To test the difference in pupation heights between two strains, t-test was performed. The t value of 3.27 shows significant difference ($p < 0.01$). Thus the two strains of *D. ananassae* measured show significant difference in pupation height. The mean pupation heights for females and males in both strains are given in Table 2. In both strains there is no difference in pupation heights between females and males.

From these results it is evident that there is interstrain variation with respect to pupation height in *D. ananassae* which seems to be attributable to genetic heterogeneity between the strains tested as they were derived from different localities. However, there is no sex difference in pupation height. Pupation sit preference in *D. ananassae* will be investigated in detail by using a large number of strains. The influence of various environmental and genetic factors on pupation site preference will also be studied.

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Paricio, N., L. Pascual, M.J. Martínez-Sebastián and R. de Frutos. Departamento de Genetica, Universidad de Valencia, Spain. Sequences homologous to P elements of *Drosophila melanogaster* are widely distributed in *Drosophila subobscura*.

P elements are a family of transposable genetic elements that have been found in certain strains of *Drosophila melanogaster*, and have been linked to the P-M system of hybrid dysgenesis (Bregliano and Kidwell, 1983). P elements are absent from other species of *melanogaster* group, but have been found in species from *willistoni, saltans,* and *obscura* groups (Daniels and Strausbaugh, 1986; Anxolabéhère *et al.*, 1985; Lansman *et al.*, 1985).

Drosophila subobscura is one of the species belonging to the *obscura* group. To determine whether sequences homologous to P elements of *Drosophila melanogaster* are widespread in *Drosophila subobscura*, 38 strains of this species from the whole area of distribution of this species were analyzed. The presence of P element sequences was detected by the squash blot technique (Tchen *et al.*, 1985) using as probe the P element of *Drosophila melanogaster* isolated from pπ25.7 BWC plasmid. In this work, the strong P strain of *Drosophila melanogaster*, Harwich, was used as a positive control, and the true M strain, Gruta, was used as a negative control.

As can be seen in Fig. 1, sequences homologous to P elements occur in each of the *Drosophila subobscura* strains examined. The presence of these sequences in the whole area of distribution of *Drosophila subobscura* suggests that they are probably ancient in this species. On the other hand, the low intensity of hybridization signals obtained in all the strains, compared with the signal detected in the strain Harwich of *Drosophila melanogaster*, indicates a small number of these sequences in *Drosophila subobscura* genome and/or a low level of homology between *Drosophila subobscura* and *Drosophila melanogaster* P elements.

References: Anxolabéhère, D., D. Nouaud, and G. Périquet 1985, Genet. Sel. Evol. 17(4):579-584; Bregliano, J.C. and M.G. Kidwell 1983, In: Mobile genetic elements, 363-410, Ed Shapiro; Daniels, S.B. and L.D. Strausbaugh 1986, J. Mol. Evol. 23:138-148; Lansman, R.A., S.N. Stacey, T.A. Grigliatti and H.W. Brock 1985, Nature 318:561-563; Tchen, P., D. Anxolabéhère, D. Nouaud, and G. Périquet 1985, Anal. Biochem. 150:415-420.

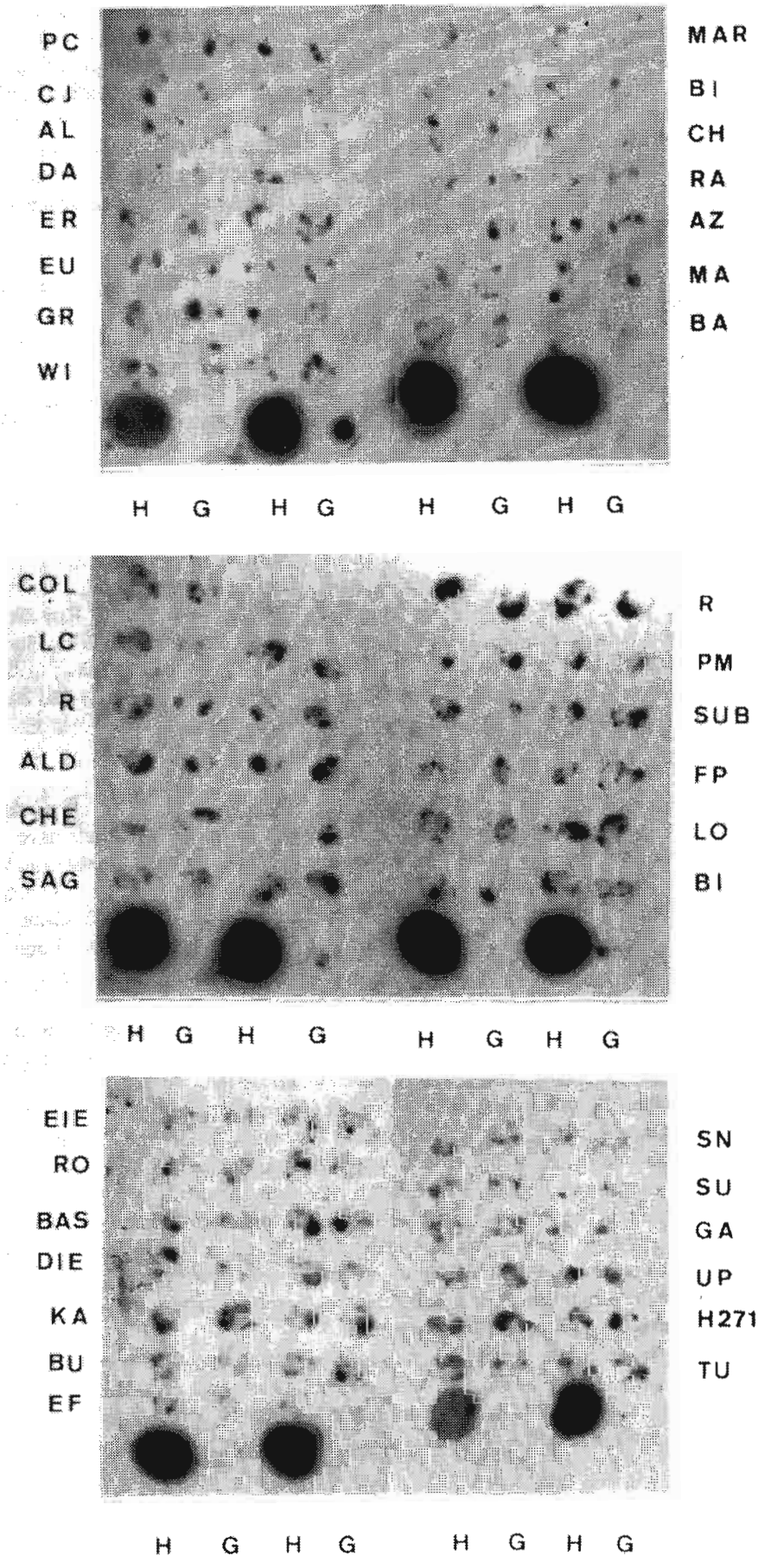


Figure 1. Squash blot of 38 strains of *D. subobscura* from different geographical origins. (COL: Godella, LC: La Canada, R: Ribarroja, ALD: Aldaya, CHE: Cheste, SAG: Sagunto, PM: Palma de Mallorca, SUB: Barcelona, RA: Canary Islands (Spain); FP: Formia-Ponza (Italy); LO: Lokrum (Yugoslavia); BI: Bizerte (Tunis); PC: Port Coquitlan (Canada); CJ: Cave Junction, AL: Alta Loma, DA: Davis, ER: El Rio, EU; Eureka, GR: Gil Roy, WI: Winters (USA); CH: Cheouan, MAR (Morocco); AZ: Azores, MA: Madeira (Portugal); BA: Bariloche (Argentina); EIE: Eierbrecht, RO: Rochefort, BAS: Basilea, DIE: Dietikon, KA: Kaferberg, BU: Bungui, EF: Effretikon, (Switzerland); SU: Sunna, SN: Sundsvall, GA: Gävle, UP: Uppsala, (Sweden); H271: Helsinki (Finland); TU: Tübingen (Germany); Control strains of *D. melanogaster*: H. Harwich and G. Gruta.

Parkash, R. and Shamina. M.D. University, Rohtak, India. **ACPH allozymic variation in *Drosophila* species.**

A single polymorphic zone of ACPH (E.C. 3.1.3.2) activity appears on the gels in all the drosophilids analysed. The electrophoretic phenotypes of single individuals are represented by segregating single-band variants (electromorphs) and triple-band patterns in six drosophilids (*D. melanogaster, D. takahashii, D. nepalensis, D. malerkotliana, D. bipectinata, D. ananassae*).

The number of observed segregating single-band variants and triple-band patterns were found to differ in various species. The species specific ACPH electromorphs display characteristic electrophoretic mobilities. The genetic basis of electrophoretic phenotypes of the parents and progenies of several species specific single pair matings can be summed up as follows: (a) the crosses between individuals having different electrophoretic single band variants always resulted in triple-banded pattern; (b) crosses between individuals both having triple-band patterns produced three kinds of offspring with segregating single-bands and triple-band patterns in accordance with Mendelian proportions of 1:2:1; (c) genetic crosses involving segregating single-band variants and a triple-band pattern resulted in 1:1 ratio of parental electrophoretic phenotypes in the progeny. Thus data on genetic crosses indicate that the observed ACPH isozyme polymorphism in each of the six drosophilids is controlled by a single ACPH locus. The single-band variants and triple-band patterns represent homozygous and heterozygous genotypes respectively. The occurrence of hybrid band in the heterozygotes indicates that acid phosphatases in these species are dimeric. The ACPH patterns do not vary in the two sexes and thus are coded by an autosomal locus.

Table 1. Data on allelic frequencies, observed and expected heterozygosity, effective number of alleles (n_e), Wright's inbreeding coefficient (f) and G values for log-likelihood x^2 test for fit to Hardy-Weinberg expectations at the ACPH locus in seven *Drosophila* species.

Species	Frequencies of ACPH alleles					Het	n_e	f	G-value
	F'	F	S	S'	rare	Obs./Exp.			
D. melanogaster	—	.05	.95	—	—	.07/.09	1.10	0.23	4.5 *
D. busckii	—	.06	.94	—	—	.12/.11	1.13	-0.09	1.20 n.s.
D. nepalensis	—	.91	.06	.03	—	.17/.16	1.20	-0.03	2.6 n.s.
D. takahashii	.07	.80	.10	.01	.02	.40/.34	1.53	-0.16	18.2 n.s.
D. bipectinata	.13	.36	.41	.10	—	.65/.69	3.23	0.06	54.7 *
D. malerkotliana	.16	.47	.23	.13	.01	.64/.69	3.23	0.07	9.18 n.s.
D. ananassae	.14	.44	.24	.02	.07 & .09	.73/.72	3.57	-0.01	1.03 n.s.

* Significant at 5% level; n.s. = non-significant

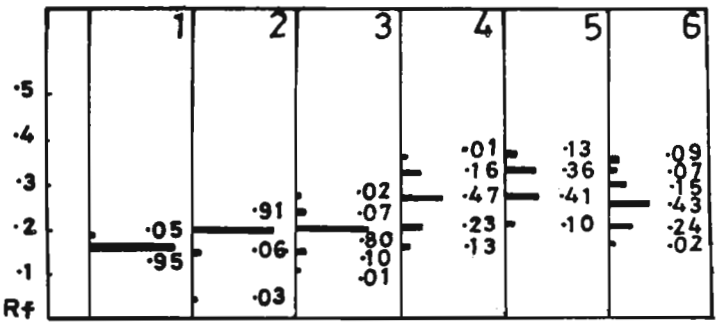


Figure 1. Schematic representation of patterns of number, electrophoretic mobility (RF) and frequencies of species specific allelic isozymes (allozymes) at the acid phosphatase locus (ACPH) in various drosophilids. Each lane diagramatically represents all the allelic variants of a species. RF values are based on comparative gel analysis. The length of bars, denoting allozymes, refer to frequencies of ACPH allelic variants. *Drosophila* species include: 1-*D. melanogaster*; 2-*D. nepalensis*; 3-*D. takahashii*; 4-*D. malerkotliana*; 5-*D. bipectinata*; 6-*D. ananassae*.

The data on observed and expected heterozygosity, effective number of alleles (n_e), Wright's inbreeding coefficient (f) and log-likelihood X^2 values for fit to Hardy-Weinberg expectations at the ACPH locus are given in Table 1. The data on ACPH allozymes (electromorphs) indicate two types of distribution patterns. Three *Drosophila* species (*D. melanogaster, D. busckii* and *D. nepalensis*) display the occurrence of one most common ACPH allele (frequency > 90%) and one rare allele (frequency < 10%). However in three *Drosophila* species (*D. malerkotliana, D. bipectinata* and *D. ananassae*), the occurrence of two or three common alleles is followed by few or more less frequent and/or rare alleles. The present studies indicate that patterns of genetic variability differ significantly at a polymorphic ACPH locus in closely and distantly related drosophilids. The occurrence of such ACPH genic heterogeneity patterns in drosophilids implies that ACPH allelic diversity may not be functionally alike or neutral. Although physiological functions of acid

phosphatases are not yet well established but their ubiquitous occurrence merits their diverse metabolic roles. Thus, the frequency distribution patterns (Bell shaped/J shaped) as well as species specific population genetic structure at the ACPH locus may be interpreted in terms of the action of natural selection mechanisms. The occurrence of significantly low genetic variation (almost S=0, F = 0.05) at ACPH locus in *D. melanogaster* and *D. busckii* (which constitute the cosmopolitan species as compared to other drosophilids) might be interpreted on the basis of colonization history of these species.

Parkash, R. and M.Sharma. M.D. University, Rohtak, India. Heat stability polymorphism at the ACPH locus in two *Drosophila* species.

Application of gel electrophoresis and heat denaturation technique has revealed allelic heterogeneity within electropmorph classes at acid phosphatase locus (ACPH) in three sympatric natural population samples of *D. takahashii* and *D. nepalensis*. The heat denaturation technique of Trippa *et al.* (1978) involved heating the ACPH enzyme in situ in the starch gel at 57°C for 15±1 minutes after electrophoretic separation.

The electrophoretic ACPH phenotypes in *D. takahashii* and *D. nepalensis* are governed by four autosomal co-dominant alleles, $ACPH^{90}$, $ACPH^{95}$, $ACPH^{100}$ and $ACPH^{105}$ in order of increasing electrophoretic mobility from the origin. $ACPH^{100}$ is the most common allele in both the species, followed by less frequent as well as rare alleles (Table 1). On the basis of heat denaturation technique, the most common allele ($ACPH^{100}$) has been found to occur as isoelectrophoretic thermoresistant (tr) and thermosusceptible (ts) variants which occur with polymorphic frequencies in both the species populations analysed. The distribution of thermostability patterns for $ACPH^{105}$, $ACPH^{95}$ and $ACPH^{90}$ are asymmetrical, i.e., the variants are thermoresistant (tr) only in case of *D. takahashii* while these are thermosusceptible in *D. nepalensis* (Table 1).

Table 1. Data on ACPH allelic frequencies, heterozygosity and effective number of alleles (n_e) on the basis of electrophoretic analysis alone and post-electrophoretic heat denaturation test in three natural populations of *D. takahashii* and *D. nepalensis* from India.

		Frequencies of thermostability (tr & ts) ACPH variants														
		105		100		95		90		Total Freq.		Heterozygosity				
Species	Populations	tr	ts	tr	ts	tr	ts	tr	ts	tr	ts	H	H'	n_e	$n_{e'}$	$n_{e'}/n_e$
A. *D. takahashii*																
	Roorkee (U.P.)	—	—	0.72	0.14	0.12	—	0.02	—	0.86	0.14	0.25	0.45	1.3	1.8	1.36
	Bagdogra (W.B.)	.055	—	0.712	0.124	0.094	—	0.01	—	0.876	0.124	0.29	0.46	1.4	1.87	1.31
	Hasimara (W.B.)	.07	—	0.68	0.14	0.14	—	0.01	—	0.86	0.14	0.31	0.50	1.45	1.97	1.36
B. *D. nepalensis*																
	Roorkee (U.P.)	—	—	0.82	0.09	—	0.06	—	0.03	0.82	0.18	0.16	0.32	1.2	1.46	1.22
	Bagdogra (W.B.)	—	—	0.88	0.09	—	0.02	—	0.01	0.88	0.12	0.06	0.21	1.06	1.28	1.20
	Hasimara (W.B.)	—	—	0.836	0.093	—	0.057	—	0.14	0.836	0.164	0.13	0.289	1.15	1.408	1.22

tr and ts are thermoresistant and thermosusceptible respectively. H and n_e heterozygosity and effective number of alleles on the basis of electrophoretic analysis alone; H' and n_e' are the same based on heat denaturation test.

The different population samples have depicted a nearly homogeneous pattern of distribution of thermoresistant (tr) and thermosensitive (ts) isoelectrophoretic variants of the most common allele in *D. takahashii* and *D. nepalensis*. The cryptic isoelectrophoretic variation has not changed the overall pattern of allelic variation at the ACPH locus in both the sympatric *Drosophila* species populations. The occurrence of heat stability polymorphism with high frequency in all the populations suggest that natural selection might be responsible for the maintenance of such cryptic genic variation.

References: Trippa, G., A. Catamo, A. Lombordozzi and R. Cicchetti 1978, Biochem. Genet. 16:229.

Parkash, R. and S. Sharma. M.D. University, Rohtak, India. Atypical ACPH isozyme patterns in three *Drosophila* species.

The ACPH (E.C.3.1.3.2) electrophoretic phenotypes in each of the three *Drosophila* species (*D. jambulina, D. punjabiensis* and *D. busckii*) have revealed either three-

banded or five-banded ACPH patterns and the mobility patterns are species specific. The progeny, even up to 20th generation, of isofemale lines depicting particular triple-banded patterns did not reveal segregation of the involved ACPH bands in any of these two species. Genetic crosses involving two distinct types of triple-banded ACPH phenotypes resulted in F_1 individuals having five-banded ACPH patterns. However, crosses among five-banded individuals resulted in offsprings having two distinct types of triple-banded patterns and five-banded patterns in accordance with Mendelian segregation proportion of 1:2:1.

In most *Drosophila* species, a single polymorphic autosomal ACPH locus codes for segregating single-band variants or allozymes or electromorphs in homozygotes and triple-banded patterns in heterozygotes due to dimeric subunit structure of acid phosphatases. However, the genetic basis of three-banded and five-banded ACPH profiles of *D. busckii, D. jambulina* and *D. punjabiensis* has revealed monogenic control but the isozyme patterns are not in agreement with genetically coded allelic isozyme patterns. Therefore, it has been suggested that the observed ACPH patterns of *D. busckii, D. jambulina* and *D. punjabiensis* might be coded by duplicate alleles. Such an argument finds support from studies of Ohno (1970) in animals and that of Gottlieb (1979) in plants. Costa et al. (1979) have reported the occurrence of multiple esterase isozymes due to duplicate alleles in *D. melanogaster*. Another possible explanation for such atypical ACPH patterns could be that the three-banded patterns represent epigenetically modified or conformational isozymes in a putative homozygote. Such non-genetic origin of multiple isozyme patterns have been reported for EST-6, XDH and LDH (Cochrane and Richmond, 1979; Finnerty and Johnson, 1979; Onoufriou and Alahiotis, 1982). Thus, it seems difficult to argue the occurrence of 3-band and 5-band ACPH patterns either on the basis of duplicate alleles or conformational isozymes. Further empirical studies involving dissociation-reassociation experiments and gel densitometric analysis of various ACPH band patterns need to be employed to resolve the question whether ACPH profiles in *D. busckii, D. jambulina* and *D. punjabiensis* are under monogenic or digenic control?

References: Cochrane, B.J. and R.C. Richmond 1979, Biochem. Genet. 17:167-183; Costa, R., G.A. Danieli, and E. Morbini 1979, Experientia 35:26-27; Finnerty, V. and G. Johnson 1979, Genetics 91:695-722; Gottlieb, L.D. 1982, Science 216:373-380; Ohno, S. 1970, Evolution by Gene Duplication, Springer-Verlag; Onoufriou, A. and S.N. Alahiotis 1982, Biochem. Genet. 20:1195-1209.

Parkash, R. and M. Vashisht. M.D. University, Rohtak, India. ADH allozymic variation in nine *Drosophila* species.

Alcohol dehydrogenase (ADH.E.C.1.1.1.1.) constitutes an important gene-enzyme system in *Drosophila* because of its role in detoxification and/or utilization of alcohol in the natural habitat of the organism. Starch gel electrophoretic analysis of homogenates of single individuals from isofemale lines of six *Drosophila* species have revealed that ADH locus is polymorphic for two allelic variants (allozymes) and homozygous strains depict two-banded conformational ADH isozyme patterns (Table 1). Three species (*D. jambulina, D. punjabiensis*, and *D. immigrans*) have revealed monomorphism at ADH locus.

Table 1. Data on observed ADH genotypes, allelic frequencies, heterozygosities, effective number of alleles (n_e) and G values for log-likelihood x^2 test for Hardy-Weinberg expectations at thie ADH locus in *Drosophila* species.

	ADH genotypes							Sample	Allelic frequencies					Heterozygosities	
Species	1,1	2,2	3,3	4,4	1,2	2,3	3,4	Size	1	2	3	4	obs/exp	n_e	G-value
D. melanogaster	8	60	—	—	40	—	—	108	.26	.74	—	—	.37/.38	1.62	0.14
D. takahashii	—	84	6	—	—	24	—	114	—	.84	.16	—	.28/.27	1.36	2.85
D. nepalensis	—	116	12	—	—	48	—	176	—	.80	.20	—	.27/.32	1.47	4.30*
D. malerkotliana	—	—	8	70	—	—	36	114	—	—	.23	.77	.31/.36	1.55	1.10
D. bipectinata	—	—	12	54	—	—	40	106	—	—	.30	.70	.38/.42	1.73	1.19
D. ananassae	—	—	16	60	—	—	48	124	—	—	.32	.68	.38/.44	1.77	1.61

Three *Drosophila* species (*D. jambulina, D. punjabiensis* and *D. immigrans*) are monomorphic at ADH locus.
* Significant at 5% level.

The gel slices stained for ADH revealed three zones of activity due to non-specificity and overlapping band patterns of alcohol-oxidizing enzymes (ADH, AO and ODH). On the basis of comparison of gel slices stained for ADH, AO and ODH, it was found that ODH and AO constitute the two anodal zones while the single cathodal zone is true ADH. The ADH zymograms of three species (*D. jambulina, D. punjabiensis* and *D. immigrans*) show two-banded phenotypes which constitute a monomorphic zone. However, the ADH zymograms of other six species reveal two-banded and four-banded phenotypes which are identical in both the sexes.

The distribution of ADH genotypes, allelic frequencies, observed and expected heterozygosity, effective number of alleles and log-likelihood X^2 test for fit to Hardy-Weinberg expectations at the ADH locus in various drosophilids are given in Table 1. The ADH locus is effectively polymorphic in six drosophilids on the basis of the criterion that the most common allele does not exceed 0.95. Except *D. nepalensis*, all the species polymorphic at the ADH locus have shown fit to Hardy-Weinberg equilibrium and did not reveal rare alleles. Most of the species depict occurrence of two common ADH alleles and high heterozygosity value. The maintenance of two common alleles at the ADH locus may be explained on the basis of balancing natural selection. However, both field and laboratory studies on several eco-geographical populations of these drosophilids need to be analysed to assess the role played by evolutionary forces in the maintenance of genic diversity at the ADH locus.

Parkash, R. and J.P. Yadav. M.D. University, Rohtak, India. Low Variation at alpha-GPDH locus in oriental drosophilids.

Horizontal starch gel electrophoretic analysis of single individuals from naturally-caught as well as laboratory strains of some oriental drosophilids revealed very low amount of allelic variability and heterozygosity at a single locus coding alpha-glycerophosphate dehydrogenase (alpha-GPDH). Alpha-GPDH revealed two-banded conformational isozymes in homozygous strains. The most common alpha-GPDH allozymes are unique in *D. immigrans, D. busckii* and *Zaprionus* species. However, except *D. melanogaster*, none of the fifteen drosophilids revealed alpha-GPDH polymorphism. The species belonging to *takahashii* species group and *Zaprionus* revealed occurrence of a rare allozyme (*Zaprionus* species include: *Z. indianus, Z. sepsoides* and *Z. tuberculatus*).

All the species analysed possess a single zone of alpha-GPDH activity which is represented mostly by two-banded and rarely four-banded patterns. The alpha-GPDH patterns are species specific on the basis of the occurrence of the most frequent variant. Five *Drosophila* species (*D. ananassae, D. malerkotliana, D. bipectinata, D. immigrans* and *D. busckii*) are monomorphic for alpha-GPDH while three *Zaprionus* species and five other *Drosophila* species (*D. melanogaster, D. takahashii, D. nepalensis, D. lutescens, D. prostipennis*) have shown mostly two-banded but rarely four-banded alpha-GPDH patterns.

The alpha-GPDH variant 108 seems to be fixed in five *Drosophila* species (*D. ananassae, D. malerkotliana, D. bipectinate, D. jambulina* and *D. punjabiensis*) while alpha-GPDH^{105} and alpha-GPDH^{106} are restricted to *D. immigrans* and *D. busckii* respectively. Four *Drosophila* species belonging to *takahashii* species group (*D. takahashii, D. nepalensis, D. lutescens* and *D. prostipennis*) have depicted a frequent allele (alpha-GPDH^{108}, 0.91 to 0.98) and a rare allele (alpha-GPDH^{104}, 0.02 to 0.09) while both these alleles are frequent in *D. melanogaster*. In the three species of *Zaprionus*, alpha-GPDH^{104} is the most frequent while alpha-GPDH^{108} is a rare allele. Thus, alpha-GPDH electrophoretic patterns have revealed species specificity. Except *D. melanogaster*, most of the species have shown low amount of heterozygosity at the alpha-GPDH locus. Thus out of all the species analysed, only *D. melanogaster* fulfills the criterion of being effectively polymorphic at the alpha-GPDH locus.

Peel, David J. and Martin J. Milner. Dept. of Biology and Pre-clinical Medicine, Bute Medical Buildings, University of St. Andrews, St. Andrews, Fife, KY16 9TS, Scotland, United Kingdom. Karyotype analysis of imaginal disc cell lines.

Permanent cell lines have been established from *Drosophila melanogaster* imaginal discs (Currie *et al.* 1988). Lines exist from both leg and wing discs and have been growing in culture for some time. An analysis of the chromosome complement of the cells was undertaken to see whether or not the cells showed any signs of karyotype instability in culture.

Cells were plated out at a concentration of 3×10^6 cells in a 5cm petri dish (Nunc) and left for a couple of days to ensure that the cells were in active exponential growth. The cells were incubated, harvested and fixed as in Schneider (1973) but chromosome spreads were carried out differently as below.

Cells were incubated with 0.06 ug/ml colcemid (Demecolcine-SIGMA D-7385) for 18 hours. After this incubation the cells were pipetted off the culture surface. A volume of distilled water (about 4mls) was then added to the culture volume and left for 20 minutes with another volume of distilled water being added after 10 mins and again after 15 mins. The fixative used was a mixture of glacial acetic acid:methanol (1:3). A few drops were added to the cell suspension now heavily diluted with distilled water, this dilution caused the cells to swell making the chromosomes more visible later on. The cells were centrifuged down for 10 minutes at 1000 r.p.m. Then a small amount of fixative was added to the pellet

(0.1 ml), which was then allowed to stand for 20 minutes at room temperature before a larger volume of fixative (2.9 ml) is added. The pellet was mixed gently by blowing air through the solution using a pipette, thus avoiding bursting the swollen cells and consequently losing the chromosome complement.

The cells were centrifuged, washed in fresh fixative, pelleted again and resuspended in a small amount of the supernatant fixative. Slides were pre-cooled on a block of dry ice for 5-10 mins. so that a thin sheet of frost covered them. The slides were taken off and allowed to warm slightly until the frosting started to disappear. Then two drops of the cell suspension were dropped onto the slide, any excess blotted off and the slide gently warmed over a spirit burner. After drying the slides were stained for 10 mins in a 3% Giemsa solution in buffer at pH 7.2. Karyotypes were then scored.

The karyotypes of the cells in culture showed that the vast majority have a diploid chromosome complement. The percentage of diploid cells in the wing cell line CME W2 was 92%, the remainder being made up of aneuploid cells, this culture was at passage number 20 and had been in culture for approximately 10 months. Most of the cells that showed a change in the chromosome complement showed a doubling of the complement (4n). The leg line CME L2 which had been in culture for about 5 months and had a passage number of 15 showed a similar percentage of diploid cells (Figure 1). This shows that even after a substantial time in culture (10 months), that the chromosome complement of the cells remains fairly constant and that the culture conditions do not cause any karyotype instability.

Figure 1. Diploid metaphase plate of a cell from CME L2 after 15 passages.

References: Currie, D.A., M.J. Milner, and C.W. Evans 1988, Development 102:805-814; Schneider, I. 1973, Chapter 15, p. 788-790, In: Tissue culture:Methods and application, eds. Kruse, P.F. and M.K. Patterson, Academic Press.

Piñeiro, R., J.I. Izquierdo, Maria C. Carracedo and P. Casares. Departamento de Genetica, Universidad de Oviedo, c/ Julian Claveria 33071 Oviedo, Spain. Preliminary results on selecting for female receptivity in *Drosophila melanogaster.*

A freshly caught population of *Drosophila melanogaster* was examined for female receptivity, and a great variability with a genetic component for receptivity was detected (to be published). From this population we started a selection procedure to increase and decrease female receptivity. Selection was practiced only in females and their receptivity against a tester standard male from the same population (the M7 isofemale line) was obtained as follows: One female and two M7 males, virgins and aged for 3 days, were kept in a small vial and continuously observed over a 30 min. period. Receptivity was estimate as the time elapsed until copulation (in sec). The number of non-copulating females within this time (failures) was also registered.

From the base population, 200 females were tested and four independent selection lines were started simultaneously with the following criteria. The 40 females showing the smallest time-to-copulation (TC) values were selected in order to start 2 lines of high receptivity, H1 and H2 (20 females per line). The 20 females copulating with the largest TC values were selected to start a line of low receptivity, the L line. Another low-receptivity line was started from the non-copulating females, twenty of which were taken at random to establish the F line (failure line). In this way, the L and F lines were established both with females showing low receptivity, but L-line females copulated and F-line females did not within the period of the test.

The selected copulating pairs were in all cases gently shaken to interrupt copula before sperm transfer, the M7 males were discarded and the females kept in bottles with males of the same line and age. Therefore, only females were selected each generation, and the same M7 males were used along the generations. Once the four lines were started, 100 females per line were tested each generation and 20 females per line selected with the appropriate criterion.

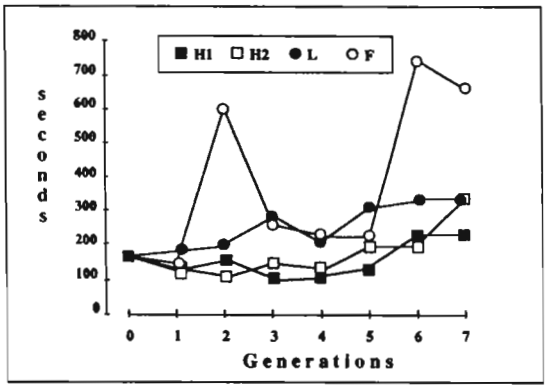


Figure 1. Response to selection for increased (H1 and H2) and decreased (L and F) female receptivity in *Drosophila melanogaster*.

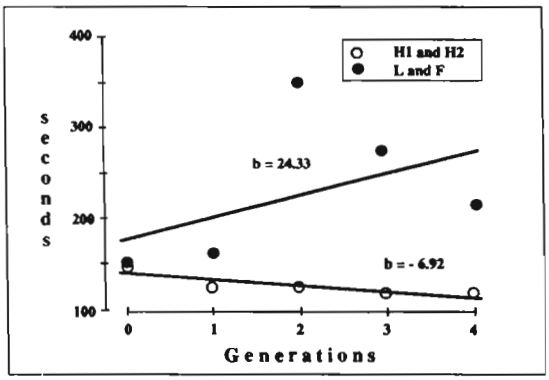


Figure 2. Regression lines for the average of the H1 and H2 (high receptivity) and L and F (low receptivity) selected lines, for the first generations of selection.

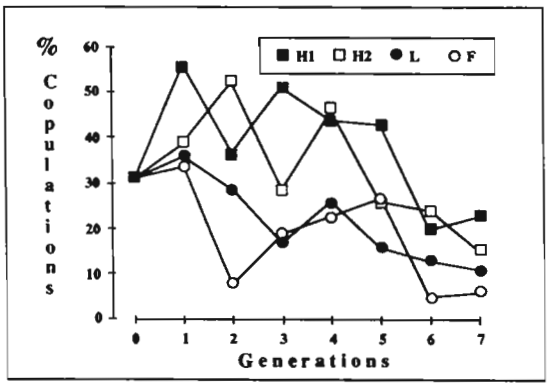


Figure 3. Number of copulations observed in the first five minutes of courtship along the generations.

The distribution of the raw data (in sec) is strongly skewed and a number of transformations have been applied by different authors. Our data were subjected to the log transformation (Dow, 1976) which brought about an approximately normal distribution with a small tail corresponding to slowest times. The average time to copulation of the 30% fastest individuals was taken each generation as the measure of receptivity. The values, after being transformed to the original scale, are graphically shown in Figure 1. The progress of the selection was substantial up to generation 4, (Figure 2), with lines H1 and H2 showing greater receptivity than lines L and F. This response suggests that genes for receptivity with additive action were present in the base population.

From generation 4 to 7, no progress of selection was apparent (Figure 1), although the differentiation between lines achieved in the first generations of selection was still evident, the H1 and H2 lines being consistently more receptive than L and F lines. The difference between lines was statistically significant ($X^2_3 = 20.35$) from a Friedman test (Siegel, 1956) applied to the 4 lines for the 7 generations, and is graphically manifest in Figure 1.

Together with the lack of response to selection from generation 5, it was observed that, along generations, the average of pairs copulating in 30 min progressively decreased in the H1, H2 and L lines, from 78% at generation 1 to 53% at generation 7. In addition, the time to copulation increased as we can see in Figure 3 where the number of pairs copulating in the first five minutes is shown. From direct observations of courtship it becomes evident that M7 males were sexually less active and more quiescent along generations, with scarce courtship directed towards the females. Neither endogamic depression nor bottleneck effect could be invoked to explain such a behavior, since the M7 line was maintained with more than 250 pairs each generation exhibiting good fertility and fecundity. This unexplained change in behavior appears to be a quite general finding when studying *Drosophila* sexual behavior (Spieth and Ringo, 1983).

Owing to this change in M7 males, the number of copulations observed was so small that the criterion for selecting the L line could not be maintained for a long time. At this point, selection was temporarily stopped, and a new standard male was taken at random to substitute the M7 line. The change was successful, for the number of successful matings at generation 8 was as high as 92.5% for the H1 and H2 lines and 75% for the L line. Selection is currently in progress.

Acknowledgments: This work was supported by the Ministry of Education and Science of Spain (DGICYT Grant No. PB87-0596), and by the University of Oviedo, Spain (COFA Grant No. 89/1606).

References: Dow, M.A. 1976, Behav. Genet., 6:385-389; Siegel, S. 1956, Non Parametric Statistics for the Behavioural Sciences, MacGraw Hill, New York; Spieth, H.T. and Ringo, J.M. 1983, In: The Genetics and Biology of Drosophila, Vol. 2C, (Ashburner, H., H.L. Carson, and J.N. Thompson, jr., Eds.), Academic Press, New York, pp. 222-284.

Pokholkova, G.V., I.V. Solovjeva, E.S. Belyaeva. Institute of Cytology and Genetics, Siberian Branch of the USSR Academy of Sciences, Novosibirsk, USSR. Lethal mutations of the X chromosome 9F12-10A7 region induced by P-M hybrid dysgenesis.

Mutations of 12 genes were previously found to locate at the cytological interval 9F12-10A7 of the X chromosome (Zhimulev *et al.*, 1987). Our objective in the present study was to induce insertion mutations at the essential loci and *vermilion* gene followed by comparing their genetic properties with those of EMS-induced ones.

The insertion mutations were induced in the P-M hybrid dysgenesis system (Engels, 1982). Dysgenically induced mutations were shown to arise due to P element insertions (see Bingham *et al.*, 1982). The mating scheme used for the induction of insertion mutations is outlined in Figure 1. It permits the isolation of mutations which fall within the limits of a deficiency Df(1)v^{L3} that uncovers the X chromosome region 9F12-10A7 (Zhimulev *et al.*, 1981). The ratio of the *B* females to B^+ ones scored in the progeny of female Df(1)v^{L3}/FM6 individually mated to a dysgenic male has served as a selection criterion. If the number of B^+ females was much less than that of *B* females the mutation was classified as hypomorph lethal, in the absence of the B^+ class females - as lethal. In case the B^+ females had bright scarlet eyes the mutation was thought to arise in *v* gene.

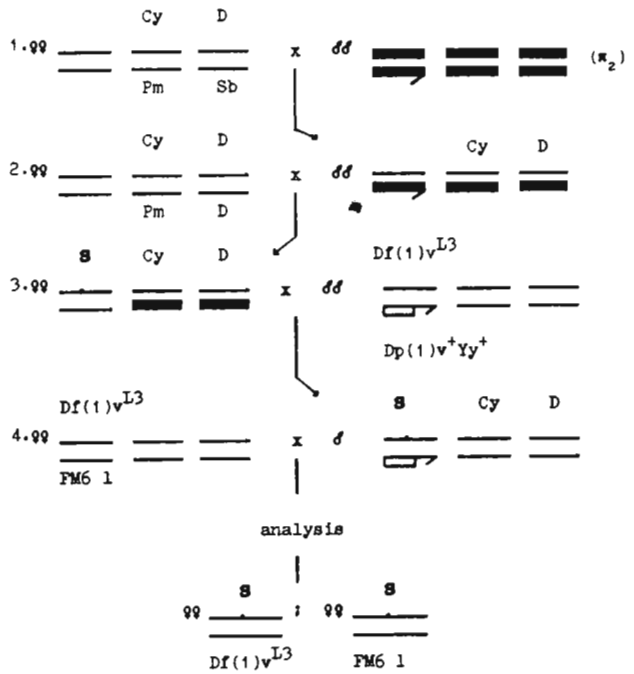


Figure 1. Mating scheme for the induction of insertion mutations, solid bars - chromosome of the P cytotype (π_2), crosses: 1-2 - hybrid dysgenesis; 3 - selection of insertion mutations within the limits of Dpv$^+$Yy$^+$; 4 - selection of insertion mutations within the limits *Df(1)*v^{L3}; S - insertion mutation.

From a total of 11702 chromosomes of dysgenic males we isolated 19 lethals (designated by S). Fifteen of 19 mutations were analysed (*S1, S3, S5, S10, S14, S15, S19, S21, S26, S27, S32, S35, S37, S38, S40*) by in situ hybridization with P DNA using the method previously described (Gall and Pardue, 1971). In all cases, except *S32*, the label was found in the 10A1-2 band, indicating that the mutations arise due to P element insertions. Complex chromosomal rearrangements (translocations and deletions) were not found. The mutations were characterized according to viability of mutant females carrying one or two doses of the mutant allele at 18°C, 25°C and 29°C. The ratios of B:B$^+$ for homo- and hemizygotes are listed in Table 1. According to an effect on viability the mutations could be divided into two classes - lethals and hypomorph lethals. The first class, comprised of *S6, S19, S27, S36, S40* caused lethality of both females and males irrespective of dose or temperature conditions. Mutations of the second class (*S1, S3, S4, S5, S10, S15, S17, S21, S26, S32, S37, S38*) reduced viability of hemizygous (carrying one dose) females, this effect being enhanced by high temperature. Males and homozygous females of these strains survived at all temperatures, had no morphological abnormalities and were fertile. The ratio of lethals to hypomorph lethals is 1:3. The high frequency of hypomorphs has to be taken into account while evaluating frequencies of lethal insertion mutations induced in the P-M hybrid dysgenesis system.

We localized the recovered mutations using a set of preexisting deficiencies and some mutations at the already known loci. Lethal insertion mutations were distributed only among four genes: *l(1)BP1, l(1)BP5, l(1)BP8, l(1)BP7,* (Figure 2), i.e. nonrandomly. No

correlation was found between target loci for P elements and highly mutable loci revealed by using common mutagens (loci highly responsive to EMS are *l(1)BP4, l(1)BP5, v*, see Zhimulev *et al.*, 1985). Nonrandom distribution of insertion mutations in some other chromosomal regions has been reported previously (Eeken *et al.*, 1985; Simmons and Lim, 1980; Campbell *et al.*, 1985; Kidwell, 1988).

The analysis of interaction of the insertion mutations with lethal ones previously induced in the loci *l(1)BP1, l(1)BP5, l(1)BP8, l(1)BP7* shows that complementation patterns remain the same in *l(1)BP1, l(1)BP5, l(1)BP7*, but become more complex in the *l(1)BP8* group.

Summing up, the comparison of genetical characteristics of mutations induced by different mutagens suggests that they can differ not only in the frequency of induction in particular genes, but in the type of their action.

Table 1. Effect of insertion mutations on viability of homo- and hemizygous females. Ratio B: B+.

		homozygotes (S/S)			hemizygotes (S/Df)		
		♀♀ S/FM6 x ♂♂ S/y or S/Dp			♀♀S/FM6 x ♂♂ Df(1)v^{L3}/Dp		
loci	mutations	18°C	25°C	29°C	18°C	25°C	29°C
1(1)BP1	S19	198:0	126:0	97:0	190:0	296:0	78:0
	S27	192:0	156:0	99:0	104:0	197:0	87:0
1(1)BP5	S5	194:188	179:186	164:154	154:121	97:34	102:6
	S10	110:136	186:200	172:196	320:349	518:14	103:2
	S14	184:0	120:0	130:0	125:0	140:0	122:0
	S15	185:210	198:212	125:127	102:56	186:127	121:0
	S21	175:164	148:154	110:110	184:172	147:60	94:1
	S26	104:88	174:154	72:49	286:302	593:278	122:2
	S32	113:102	186:190	178:180	187:0	215:0	165:0
	S35	102:91	111:115	84:70	169:186	113:70	156:0
	S37	120:118	115:116	138:140	232:79	334:158	184:15
	S38	95:108	146:148	92:82	431:425	427:191	79:10
1(1)BP8	S1	192:202	184:164	142:150	183:123	183:23	125:1
	S4	152:158	243:266	164:155	123:83	661:96	112:0
	S6	125:0	169:0	186:0	185:0	222:0	188:0
	S17	304:310	148:130	132:96	174:120	384:50	123:0
	S36	103:0	190:0	184:0	100:0	102:0	94:0
	S40	120:0	134:0	102:0	150:0	180:0	87:0
1(1)BP7	S3	105:84	196:184	172:164	158:20	91:9	114:0

References: Zhimulev, I.F., G.V. Pokholkova, A.V. Bgatov, G.H. Umbetova, I.V. Solovjeva, Yu. E. Khudyakov, and E.S. Belyaeva 1987, Biol. Zentralbl. 82:25-40; Engels, W.R. 1983, Annu. Rev. Genet. 17:315-344; Bingham, P.M., M.G. Kidwell and G.M. Rubin 1982, Cell 29:955-1004; Zhimulev, I.F., G.V. Pokholkova, A.V. Bgatov, V.F. Semeshin, and E.S. Belyaeva 1981, Chromosoma (Berl.), 82:25-40; Eeken, J.C.J., F.H. Sobels, Y. Huland, and A.P. Schalet 1985, Mutation Res. 150:261-275; Simmons, M.J. and J.K. Lim 1980, Proc. Natl. Acad. Sci. USA, 77:6042-6046; Campbell, S.D., A.J. Hilliker, and J.P. Phillips 1985, Genetics 112:205-215; Kidwell, M.G. 1987, DIS 66:81-86.

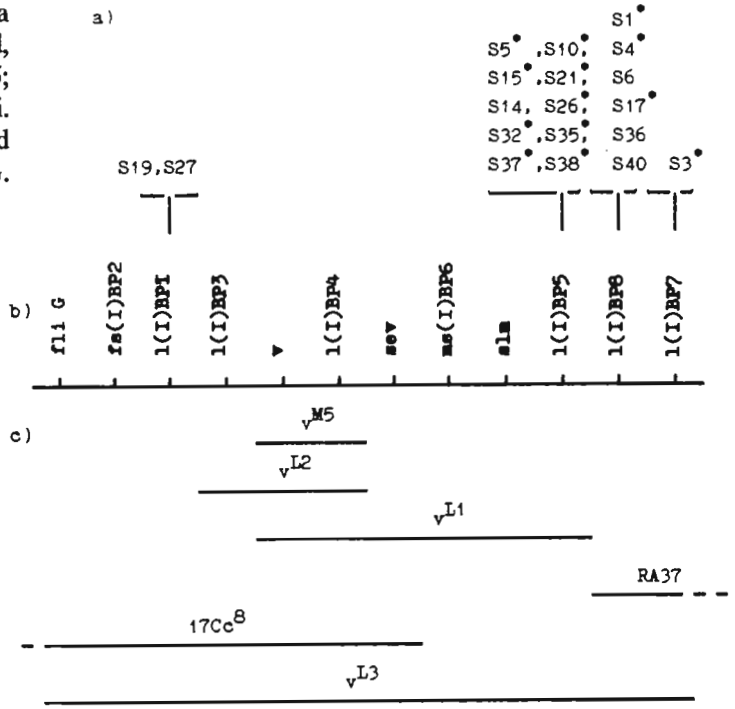
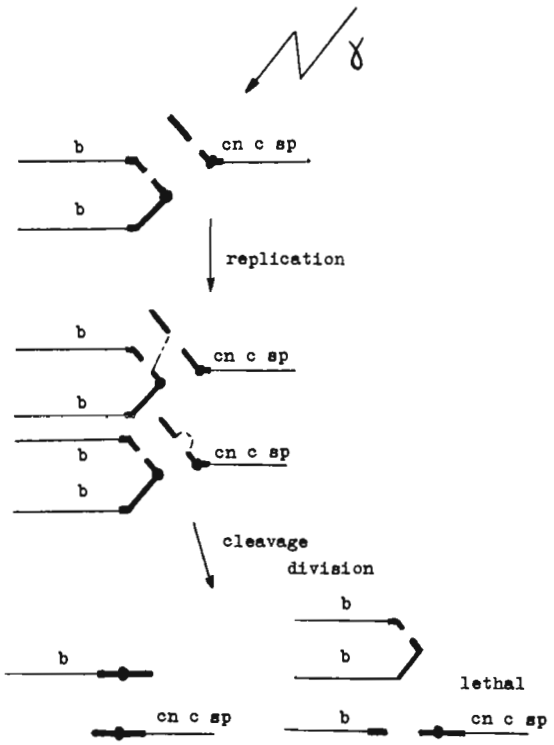


Figure 2. Mapping of insertion mutations.
a) lethal insertion mutations (* - hypomorphs);
b) genetical map of the region
c) deletions that uncover different genes

Ponimaskin, E.G. and L.V. Omelyanchuk. Institute mature of Cytology and Genetics Novosibirsk 630090, USSR. A new case of rearrangements formation by post-replicative repair of gamma-ray damaged chromosome.



During half-translocation identification in gamma-rayed sperm of *Drosophila melanogaster*, described in the accompanying paper, we identified 10 cytologically proven cases of half-translocation formation. Five other cytologically examined cultures unexpectedly had no half-translocations, they had left and right acrocentrics of 2 autosome instead. The question arose: what is the origin of the telomeric region healing the arm of C(2L) to produce a new left acrocentric? The suggested mechanism of this phenomenon is shown in Figure 1. It includes a chromosome break in heterochromatic portion of the compound and a break in the heterochromatic portion of the acrocentric. After fertilization with such sperm male chromosomes together with those breaks are replicated. Rejoining of heterochromatic fragment of acrocentric with one arm of the compound gives the left acrocentric and the restitution of parts of the second acrocentric gives the right acrocentric. In the first cleavage division, those acrocentrics can segregate together in a nucleus, the other nucleus is with nonviable chromosome constitution. Only the nucleus containing two acrocentrics produces an adult fly. A similar interpretation invoking chromatid exchange occurring after replication in the zygote was proposed by Leigh and Sobels (1969) and Belote and McKeown (1985).

References: Leigh, B. and F.H. Sobels 1969, Genen en Phaenen 13:9-10; Belote, J. and M. McKeown 1985, DIS 61:33-34.

Ponimaskin, E.G. and L.V. Omelyanchuk. Institute of Cytology and Genetics, Novosibirsk 630090, USSR. Comparison of frequencies of half-translocations and translocations induced in mature sperm of *Drosophila melanogaster*.

An attempt was made to isolate translocations with break positions corresponding to break positions of half-translocations described in the accompanying paper.

Females with genetic constitution C(2L)RM,dp F(2R)1, bw were crossed with gamma-irradiated (Co^{60} 3000R) males bearing C(2L)RM,b F(2R)1,cn c sp. The F_1 males with phenotype b were then individually crossed with females In(2L)Cy In(2R)Cy,S^2 Cy dp^{lv1} pr Bl cn^2 L^4 sp^2/In(2LR)px^{52g}, cn px^{52g} crs^{20}. Males bearing translocation between heterochromatic areas of C(2L) and F(2R) would have a metacentric consisting of 2L arm of compound and 2R arm of the paternal acrocentric. The right maternal acrocentric and 2L arm of compound linked with the heterochromatic fragment of the paternal acrocentric. Such males would give progeny in crosses with the above females, and males without this kind of translocation would not.

In our experiment we tested 574 males and found 4 males with translocations. This is about twice greater than half-translocation frequency induced by the same dose, at the same stage, within almost the same heterochromatic area of C(2L) and F(2R). This disagrees with Panshin (1941). According to Panshin the translocation frequency was 15 times less than half-translocation frequency mainly because he used the position-effect test to identify translocations. In contrast, our data agree with those of Muller and Herskowitz (1956) that show greater frequency of translocations than the frequency of half-translocations. But according to them the difference between translocations and our data (it is necessary to take only one of two possible classes of half-translocations) is four times greater than theirs. It seems that they lost a piece of half-translocation because of somatic mosaicism of a centric euchromatic nonrejoined fragment of half-translocation. In our experiment we have no such euchromatic fragment because exchanges identified were in heterochromatin.

We interpret frequencies of half- and full translocations in terms of classical theory. Let q be the probability of rejoining or restitution of two broken chromosome ends. If we have two spatially close chromosome breaks, or four broken chromosome ends, the probability of rejoining two ends and non-rejoining two other ends will be 2q(1-q). The probability of rejoining of all four ends is q^2. It is assumed that all variants of rejoinings and restitutions with the same number of ends are equally probable and N is the mean number of spatially close chromosome breaks in spermatozoa, then the frequency of translocation is $f_t = Nq^2/3$ and the frequency of half-translocation is $f_{ht} = Nq(1-q)/3$. Using our values f_t and f_{ht}, we obtain q=0.74 and N=0.039.

References: Panshin, I.B. 1941, Dokl. Acad. Nauk USSR, 33:319-322; Muller, H.J. and I.H. Herskowitz 1956, DIS, 30:141-142.

Ponimaskin, E.G. and L.V. Omelyanchuk. Institute of Cytology and Genetics, Novosibirsk 630090, USSR. Selection of half-translocations in mature sperm of *Drosophila melanogaster*.

Half-translocations are usually identified in female meiosis (Parker and Williamson, 1976). An approach to the selection of half-translocations induced in mature sperm was developed by Panshin (1941). We present another approach to it.

Females with structurally normal second chromosomes with markers bw sp in one experiment and In(2L)Cy In(2R)Cy S^2 Cy dp^{lv1} pr Bl cn^2 L^4 sp^2/In(2LR) px^{52g} cn px^{52g} crs^{20} in another experiment were individually crossed with gamma-irradiated (Co^{60}) males bearing C(2L)RM,b F(2R)1,cn c sp. After 24h, the females were put in bottles for egg laying and allowed to develop for progeny counting. Cytological examination of mitosis shows that 10 of the 15 analysed cultures are true half-translocations involving 2L and 2R arms.

Estimated frequency of half-translocations taking into account survival at given dose (Edington and Randolph, 1958) and appearance of the combination C(2L) F(2R) in 50% of spermatozoa is shown in Table 1.

Table 1.

Dose (R)	0	1000	2000	3000	4000
Estimated frequency of half-translocations	$>1.5 \times 10^{-4}$	6.8×10^{-4}	1.5×10^{-3}	2.9×10^{-3} 2.5×10^{-3}	5.5×10^{-3}

An attempt was made to induce half-translocations in males with genetic constitution C(2L)RM4,bw C(2R)RM4,px with a dose of 3000R. One cytologically proven half-translocation was obtained from 57676 eggs. When survival and appearance of combination C(2L) C(2R) in 25% of spermatozoa is taken into account, frequency of identified half-translocations in this system is 2.67×10^{-4} about an order of magnitude less than the previously estimated frequency.

There are two possibilities for half-translocation formation in the system C(2L) F(2R):

1. Loss of centric heterochromatic fragment of F(2R) and acentric arm of C(2L).
2. Loss of acentric heterochromatic fragment of F(2R) and centric arm of C(2L).

Low frequency of half-translocation identification in the system C(2L) C(2R), where centric arm of a compound must be lost, means that possibility 2 is not true.

References: Parker, D.R. and J.H. Williamson 1976, In: The Genetics and Biology of *Drosophila*, Vol. 1C; Panshin, I.B. 1941, Dokl. Acad. Nauk 30:437-443; Edington, C.W. and M.L. Randolph 1958, Genetics 43:715-727.

Protopopov, M.O., E.S. Belyaeva, I.V. Tretyakova, and I.F. Zhimulev. Insititute of Cytology and Genetics, Novosibirsk, 630090, USSR. Molecular map of the 2B region of *D. melanogaster* X chromosome.

The 2B region of *D. melanogaster* X chromosome is known to contain a cluster of genes affecting ecdysteroid induction. The *ecs* gene plays a key role in ecdysterone sensitivity of cells (Belyaeva *et al.*, 1981) and acts as a trans-regulator for other genes (Crowley *et al.*, 1984; Lepesant *et al.*, 1986; Dubrovsky and Zhimulev, 1988). The *ecs* locus was cloned and its physical map was published (Chao and Guild, 1986; Belyaeva *et al.*, 1987). More extensive cloning of the 2B puff was done and a resulting map of molecular and cytological analysis of the region is given here. The chromosomal walk, *in situ* hybridization and mapping chromosomal rearrangements' breakpoints were done as previously described (Belyaeva *et al.*, 1987).

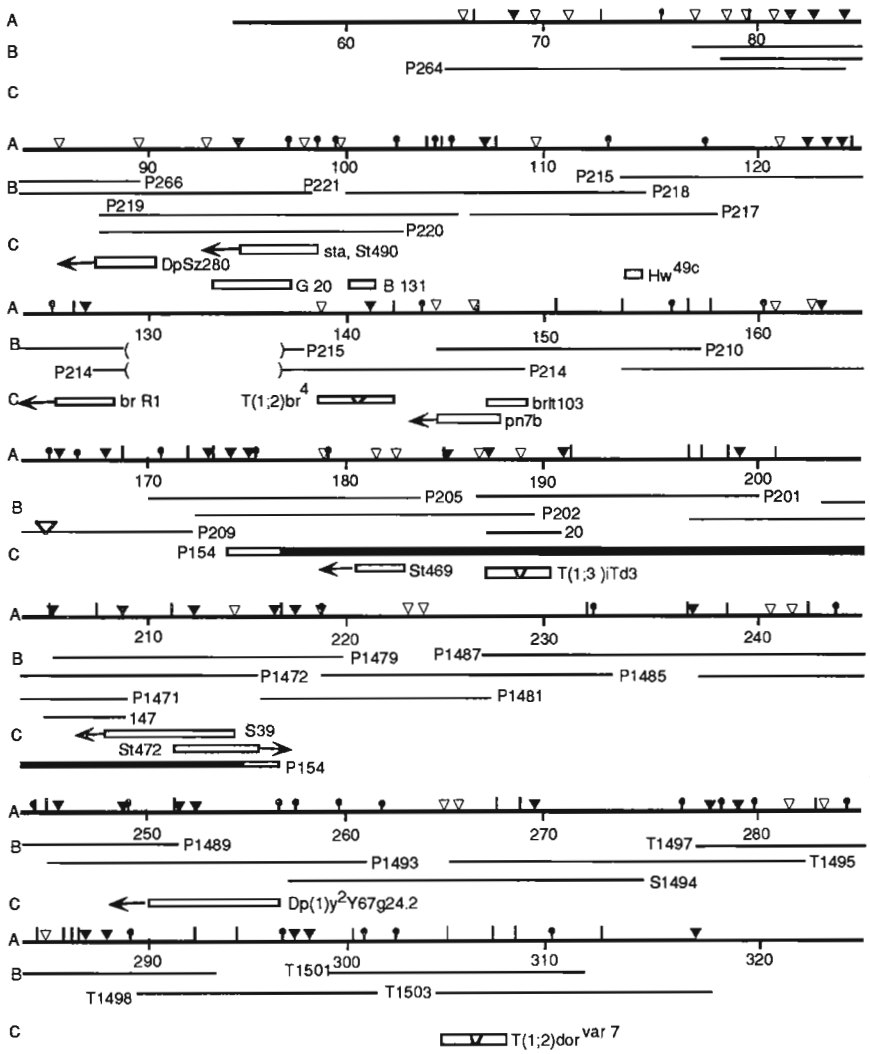


Figure 1. Restriction map of the DNA from the 2B region and the position of chromosome rearrangements. A - Restriction map, EcoRI, HindIII, SalI and BamHI sites are designated by dark triangles, bars, dark circles and open triangles, respectively. B - Extent of the clones. C - Location of chromosome rearrangements: *DpSz280* - Dp(1;Y)y^2*Sz280*, *G20* - T(1;Y)*G20*, *B131* - T(1;Y)*B131*, *sta* - Df(1)*sta*, *St490* - Df(1)*St490*, *Hw*^{49c} - In(1)*Hw*^{49c}, *br*^{R1} - Df(1)*br*^{R1}, *pn7b* - Df(1)*pn7b*, *brlt103* - In(1)*br*^{lt103}, *St469* - Df(1)*ST469*, *P154* - Df(1)*P154*, *S39* - Df(1)*S39*, *St472* - Df(1)*St472*. Solid line represents DNA removed by Df(1)*P154*; arrows show the directions of the deficiencies and duplications; limits of the breakpoint locations are shown by open lines.

Approximately 260 kb of contiguous genomic DNA was isolated from 2B3-7 region (Fig. 1). The walk starts in the proximal part of the band 2B3-5 and ends in the distal part of the band 2B7-8 (Balyaeva *et al.*, 1987; this paper, Fig. 3). Several new breakpoints of chromosomal rearrangements were mapped: T(1;2)*br*^{4}, Dp(1;Y)y^2*67g24.2.* (Protopopov *et al.*, 1988), T(1;3)*ITd3* (Fig. 2), Dp(1;Y)y^2*Sz280* and T(1;2)*dor*^{var7}. Dp(1;Y)y^2*Sz280* contains a region from 1A to 2C1-2 with inner deficiency between 2B3-5 and 2B7-8 bands. It covers genetically loci from *y* to *sta* and does not cover *ecs, dor* and *swi* (Belyaeva *et al.*, 1982). It was reported in cited paper that the duplication covers also loci *l(1)HM38, l(1)HM40* and *l(2)HM32*, but now it does not complement with lethals from these loci and cytologically the loss of fragment from 2B7-8 to 2C1-2 is visible.

Dp(1;Y)y^2*Sz280* has a break in 2B3-5 band which was mapped at coordinates 87-90 kb (Figs. 1 and 3). Since males heterozygous for Df(1)*sta* and Dp(1;Y)y^2*Sz280* are viable and have normal phenotype, one can conclude the fragment (4-10 kb) between breaks of the rearrangements is not necessary for viability.

T(1;2)*dor*^{var7} cause a position effect of the 2B region and the loci situated in it (Zhimulev *et al.*, 1986). We have cloned the euchromatic sequence which is broken by the translocation (Figs. 1 and 3). The break is about 100 kb far from the proximal end of the locus *ecs*.

Dp(1;Y)y^2*67g24.2* covers the loci *ecs, dor* and *swi* and Df(1)*S39* does not damage *dor* and *swi* (Belyaeva *et al.*, 1982). So, one can conclude the latter two loci occupy segment about 45 kb - the distance between the breaks of *Df(1)S39* and Dp(1;Y)y^2*67g24.2* (Figs. 1 and 3).

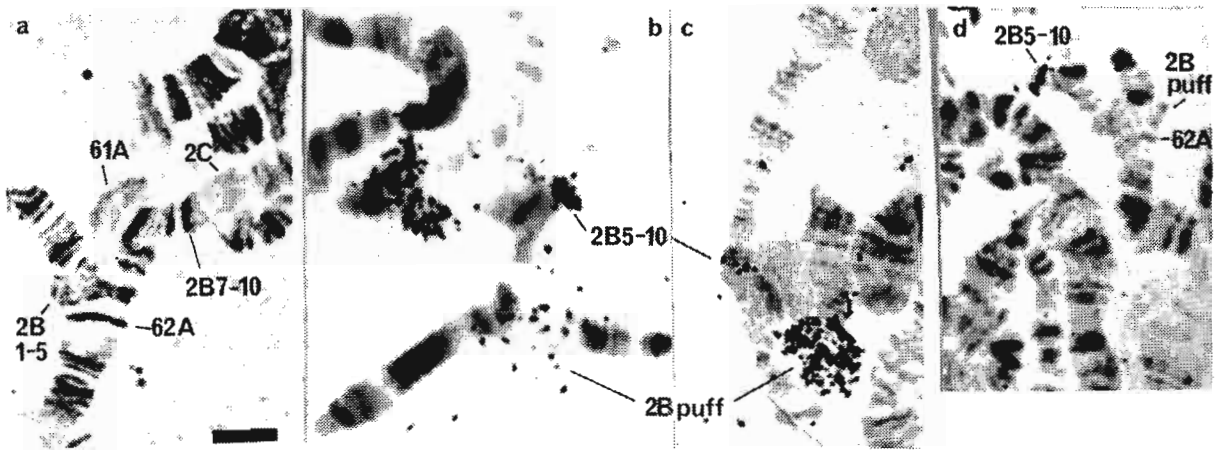


Figure 2. Cytological mapping (a) and *in situ* hybridization of DNA clones with T(1;3)*ITd3* (b-d). b-clone P201, c- clone P202, d- clone P1471.

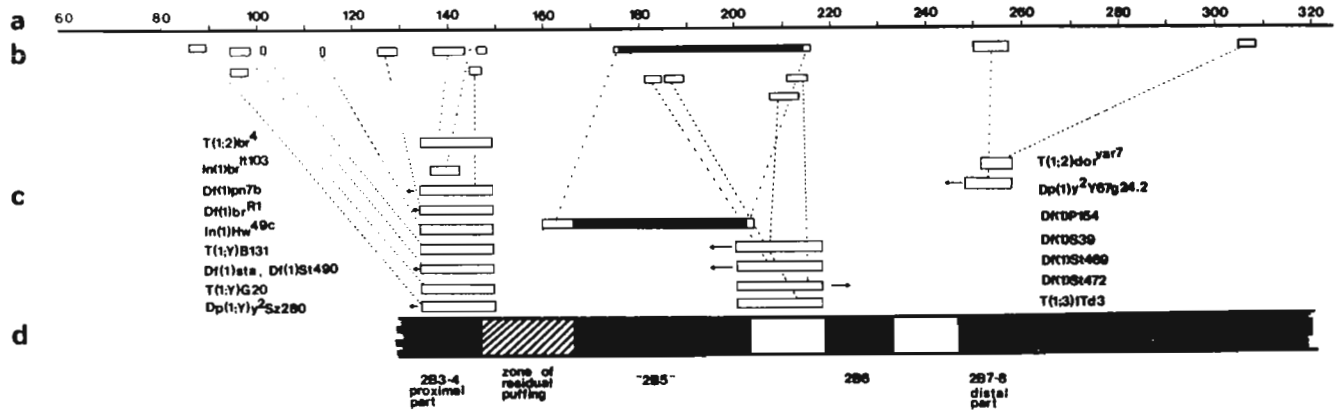


Figure 3. Molecular and cytogenetic map of the 2B puff. a - Physical map of DNA (kb). b,c- Mapping of chromosome rearrangements on the physical (b) and cytological (c) maps. Limits of localization of breakpoints are indicated by open rectangles, arrows indicate the direction of deficiencies and duplications. Limits of material removed by Df(1)*P154* are shown by black rectangle. d - Cytological map of the 2B puff region.

T(1;2)br^4 is an allele of the *br* complementation group of the *ecs* locus (Belyaeva *et al.*, 1981). It has a break at position 137-144 kb (Figs. 1 and 3).

Genetically T(1;3)*ITd3* fails to complement with all mutations of the *ecs* locus. The break is located in position 188-189 kb (Figs. 1 and 2). It means that minimal size of the *ecs* locus is even larger than it was suggested in previous paper (Belyaeva *et al.*, 1987). Now the minimal size of *ecs* should be increased from 65 kb to 75 kb - the distance between the breaks of In(1)Hw^{49c} and T(1;3)*ITd3* (Figs. 1 and 3).

References: Belyaeva, E.S., I.E. Vlassova, Z.M. Biyasheva, V.T. Kakpakov, G.Richards, and I.F. Zhimulev 1981, Chromosoma 84:207-219; Belyaeva, E.S., M.G. Aizenzon, I. Kiss, T.D. Gorelova, S.D. Pak, G.H. Umbetova, P.G.N. Kramers, I.F. Zhimulev 1982, DIS 58:184-190; Belyaeva, E.S., M.O. Protopopov, E.M. Baricheva, V.F. Semeshin, M.L. Izquierdo, I.F. Zhimulev 1987, Chromosoma 95:295-310; Chao, A.T. and G.M. Guild 1986, EMBO J. 5:143-150; Crowley, Th.E. P.H. Mathers, E.M. Meyerowitz 1984, Cell 39:149-156; Dubrovsky, E.B., I.F. Zhimulev 1988, Develop. Biol. 127:33-44; Lepesant, J.-A., F. Maschut, J. Kejzlarova-Lepezant, K. Benes, C. Yanicostas 1986, Arch. Insect. Biochem. Physiol. suppl. 1:133-141; Protopopov, M.O., E.S. Belyaeva, E.B. Kokoza, E.M. Sharapova, E.M. Baricheva, O.V. Demakova, V.E. Grafodatskaya, I.F. Zhimulev 1988, Genetica (USSR) 24:1614-1623; Zhimulev, I.F., E.S. Belyaeva, O.V. Fomina, M.O. Protopopov, V.N. Bolshakov 1986, Chromosoma 94:492-504.

Rancourt, D.E., B. Duncker, P.L. Davies and V.K. Walker. Queen's University, Kingston, Ontario, Canada. A flightless host for biological containment of P mediated transformants.

Germline transformation experiments in *Drosophila melanogaster* have predominantly involved the transfer of native DNA sequences or promoter regions that have been ligated to bacterial coding regions such as beta-galactosidase chloramphenicol acetyltransferase, or the neomycin resistance gene. Since these bacterial sequences are thought to be fairly benign, little attention has been focused on the biological control of transformants. We have been using *Drosophila* as a model organism to test the biological effects of expressing fish antifreeze protein genes in heterologous systems (Rancourt et al., 1987). Since the expression of such a foreign gene product has the potential to confer a selective advantage to transformant flies in the wild, we have restricted our gene transfer experiments to host strains that are genetically crippled, and therefore confined to the laboratory (Walker, 1989). To achieve this we have introduced into our original host strain (*b cn; ry*^{8}) dominant, homozygous-viable mutations which restrict flight. Because these mutations are dominant it also ensures that heterozygotes resulting from illicit matings with wild type flies also remain confined to the laboratory.

Initially the dominant, homozygous-viable flightless mutation, Wrinkled (*W*), was introduced into *b cn; ry*^{8} by selecting for a recombination event on the third chromosome between *W* (3-46.0) and *ry*^{8} (3-52.0). Although *W* proved to be an effective handicap for restricting flies to the laboratory, this stock was less fit than the original host strain (*b cn; ry*^{8}), as G_0's derived from an injection series experienced great difficulty in hatching and eclosion. Although up to 30% of the injected embryos survived injection and developed into first instar larvae, only 10-20% of these managed to escape from their chorions. This high mortality resulted in a significant reduction in transformation efficiency.

Another dominant flightless mutation, Intermediary flight muscle (3)3 (*Ifm(3)3*) (Tansey et al., 1987), was introduced into *b cn; ry*^{8} by selecting for a recombination event on the third chromosome between *ry*^{8} (3-52.0) and *Ifm(3)3* (3-55.0). The multiply marked *b cn; ry*^{8} *Ifm(3)3* stock is vigorous and easily transformed, and thus serves as an ideal host, not only for the transfer of foreign genes, but also for aberrantly expressed *Drosophila* genes. We intend to donate this stock to the Bowling Green Stock Center.

Acknowledgments: The stocks *b cn; ry*^{8}, *Ifm(3)3* and *W* were kindly contributed by John Bell (University of Alberta), Robert Storti (University of Chicago) and the Bowling Green Stock Center, respectively.

References: Rancourt, D.E., V.K. Walker and P.L. Davies 1987, Molec. and Cell. Biol. 7:2188-2195; Tansey, T., M.D. Mikus, M. Dumoulin, and R.V. Storti 1987, EMBO J. 6:1375-1385; Walker, V.K. 1989, Gene Transfer in Insects In: Adv. in cell Culture, Vol. 7 (Maramorosch and Saito, eds.) pp. 87-124.

Rasooly, R.S.* and L.G. Robbins. Genetics Program and Department of Zoology. Michigan State University, East Lansing, MI 48824. Mitotic instability of a P-element ribosomal cistron insertion.

Rex is a dominant, maternal-effect locus on the X chromosome which induces exchange between two separated blocks of ribosomal DNA in a single, paternally-derived chromosome (Robbins, 1981; Swanson, 1987). The *Rex*-induced event takes place in the first or second mitotic division of the zygote, leading to either inversion or deletion of the DNA between the two *bb* loci undergoing exchange.

We investigated whether a single ribosomal cistron could serve as one half of a *Rex* target by using an X chromosome containing both a normal *bb* locus and a single rDNA cistron inserted distal to y^+. The single cistron ([*rib7*]) had been inserted by P-element transposition using a defective P-element vector that lacks transposase activity (Karpen, Schaefer and Laird, 1988). In order to be able to score *Rex* exchanges using [*rib7*], we constructed a [*rib7*]*Dp(1;1)sc*^{V1}, [*rib7,ry*$^+$] y^+ *v f car su(f).y*$^+$ chromosome. Females who were homozygous *y* (with or without other markers) were then crossed to [*rib7*]*Dp(1;1)sc*^{V1}/Y males. Regular progeny are y^+ daughters and *y* sons. Non-disjunctional progeny are *y* females and y^+ *v car* males. Mitotic exchanges that delete the material between [*rib7*] and the normal *bb* locus would generate y^+ X-chromosome fragments. Such exchanges occurring before first division in the zygote would yield y^+car^+, X/fragment sterile males. If the exchanges occur before second division, then gynandromorphs would result.

Our initial cross with *Rex/Rex* mothers gave significant numbers of the predicted y^+car^+ sterile males (Table 1), suggesting that a single cistron is sufficient to serve as a *Rex* target. However, in a control experiment using *y/y* mothers, we found the same frequency of y^+car^+ sons (Table 1). Thus, the [*rib7*]*Dp(1;1)sc*^{V1} chromosome itself appears to be unstable.

There are three possible sources of this observed instability: the duplicated ribosomal cistron, the P-element transformation vector, or the original *Dp(1;1)sc*^{V1},y^+ *v f car su(f).y*$^+$ chromosome used to construct our target

chromosome. Duplicated ribosomal cistrons on the X are not generally unstable. We have used many such chromosomes in our study of *Rex*, and only find exchanges when maternal *Rex* is present. In order to test whether the parent *Dp(1;1))sc*^{V1} chromosome is itself unstable, we crossed *Rex/Rex* females with *Dp(1;1)sc*^{V1} males. No y^+car^+ sons were observed.

Table 1. Intra-chromosome exchange frequencies.

Cross			Regular Progeny		Non-Disjunctional Progeny		Fragment-Bearing Progeny	
			female	male	female	male	males	Percent [a]
y cv v f Rex / *y cv v f Rex*	x	*[rib7]Dp(1;1)sc*^{V1} / Y	1751	1544	5	11	8	0.45%
y / *y*	x	*[rib7]Dp(1;1)sc*^{V1} / Y	3460	3343	16	24	12[b]	0.35%
y cv v f Rex / *y cv v f Rex*	x	*Dp(1;1)sc*^{V1} / Y	1493	1395	4	9	0[c]	0.0%[c]

[a] Percent exchange is calculated as exchange males/(regular females + exchange males), since the exchange product can only be formed in X/X zygotes.

[b] Five exchange products were recovered from one of the 57 single-female matings scored.

[c] One *cv y f* male was recovered that was a patchy y^+ mosaic. This exception is not, however, analogous to *Rex*-induced events, since *Rex*-induced exchange events yield gynandromorphs but not mosaic males.

We suggest, therefore, that the exchange events we observe with *[rib7]Dp(1;1)sc*^{V1} are due to the P-element transformation vector. Although it has not been reported previously, a low frequency of mitotic instability may be a characteristic even of defective P-elements. We do not, however, know whether other P elements might have been inadvertently introduced in the course of the experiment.

We do not know what exchange event is taking place, since the y^+car^+ progeny are all sterile. Although the exchange could be a *Rex*-like intrachromosomal event, it is also possible that the exchange is interchromosomal, between the Y-chromosome *bb* locus and *[rib7]*. The interchromosomal exchange would, of course, have to be a male germline, possibly pre-meiotic, event. This possibility is lent some credence by our recovery of one cluster of y^+car^+ progeny in the control cross.

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Note on the chromosome arrangements of *Drosophila nebulosa*.

Drosophila nebulosa, a non-sibling species of the *willistoni* group, has a wide Neotropical distribution and is very common in xeric regions of Brazil (Pavan, 1946; Da Cunha et al., 1953). Few studies, however, have been done on its chromosomal polymorphism. Here, we provide a synthesis of all the inversions already encountered -- including our new findings in South Brazilian populations -- in this fly's chromosomal set, together with some pictures that should help their identification during cytogenetic analysis in further studies (Fig. 1).

The polytene chromosomes of *D. nebulosa* were first described by Pavan (1946), who reported the occurrence of nine inversions: 8 (A, B, C, D, E, F, G, H) on the third chromosome and one on chromosome XL. Da Cunha et al. (1953 and personal communication) found three other inversions (I, J, K) also on chromosome III. While studying wild and urban populations of this species in Southern Brazil (Porto Alegre city area, 30° 02' S - 51° 14' W), we encountered, in addition to some of the inversions mentioned above, a new one (L in Fig. 1) and a new arrangement (M), involving the overlapping inversions H and G (Fig. 2). Therefore, up to now, thirteen paracentric inversions (and fourteen heterozygous arrangements) have been found to account for the chromosomal variability of *D. nebulosa*.

The present survey came from a series of studies on *Drosophila nebulosa*, under behavioral, genetical and

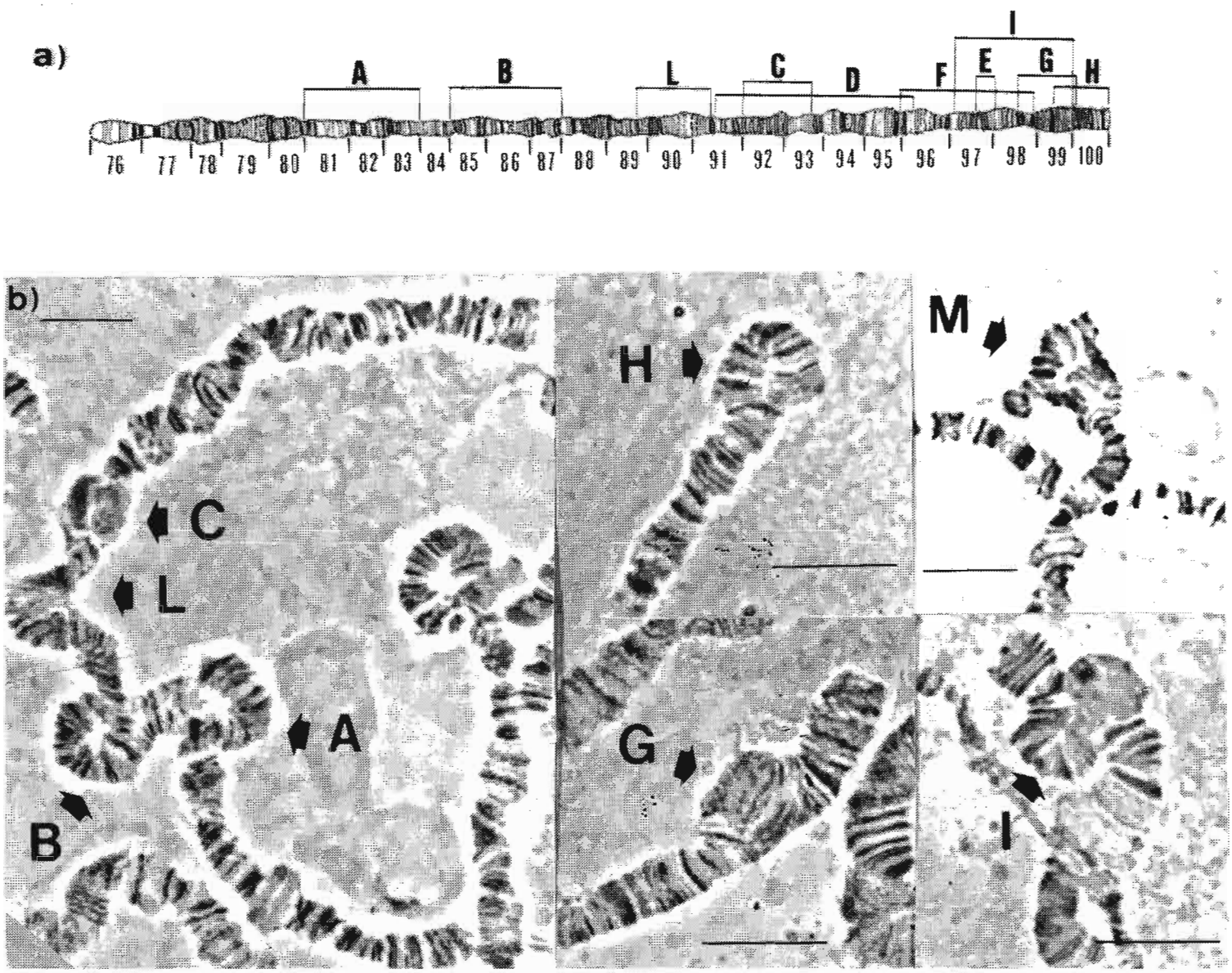

Figure 1. (a) Graphic presentation of the reference map of the third chromosome of *D. nebulosa* (Pavan, 1946) showing inversion breakpoints; (b) Photographs of the heterozygous arrangements found in populations of Southern Brazil. Bar represents 10 um.

Figure 2 (see next page). Explanatory drawing (a), photographic mounting (b) of third chromosome sections involved in arrangement M and some configurations (c - f) of this arrangement in Southern Brazilian populations. Bar represents 10 um.

ecological approaches (Regner, 1988; Regner and Valente, in prep.; Bonorino and Valente, 1989; Bonorino and Valente, in prep.; Valente et al., 1989) in which relationships among urbanization, mating behavior and chromosomal polymorphism as well as interactions among the latter, climactic factors and urbanization are being investigated.

Acknowledgments: Grants: CNPq, FINEP, FAPERGS and PROPESP-UFRGS.

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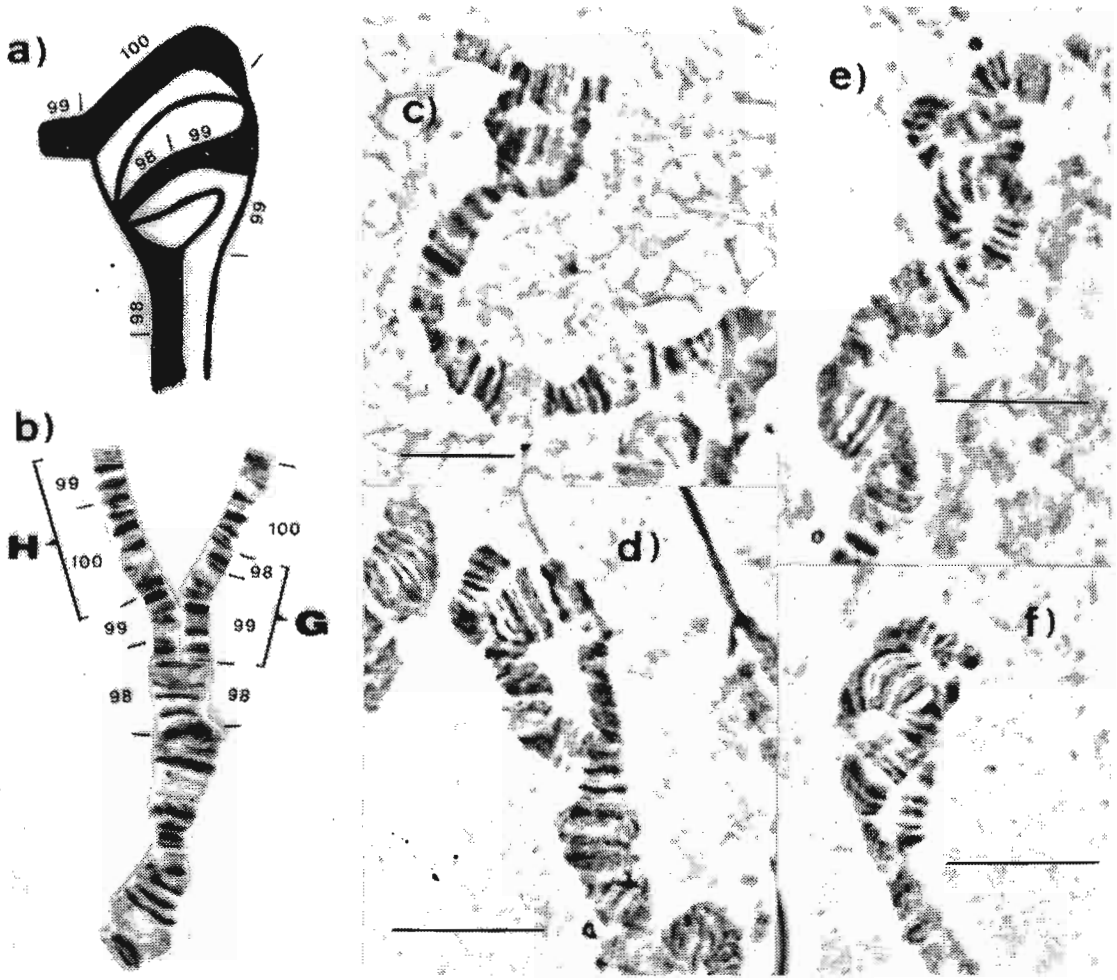

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Rearing temperature and epicuticular lipid composition in *Drosophila mojavensis*.

Insect epicuticular lipids, especially hydrocarbons, reduce cuticular permeability and are also important in a variety of other functions as well. These compounds act as aggregation pheromones, aphrodesiacs, and also function in sex recognition to mediate mating behaviors (Blomquist and Jackson, 1979; Jallon and David, 1987). The varied activities of hydrocarbons (HC) subject them to a complex interaction of different selection pressures. Toolson and Kuper-Simbron (1989) recently found evidence suggesting epicuticular HC composition was subject to both natural and sexual selection in a laboratory strain of *Drosophila pseudoobscura*.

Presently we are investigating possible interactions among the various adaptive functions of epicuticular HC of *D. mojavensis*. Previous investigations of *D. pseudoobscura* have shown pupal rearing temperatures affect adult epicuticular HC composition (Toolson, 1982). Further evidence suggests climatic factors such as temperature and humidity may underlie strain differences in the HC composition of *D. mojavensis*, a xeric-adapted, cactophilic *Drosophila* (Markow and Toolson, in press). As part of a study of epicuticular HC's in this species we ask the following questions: Can epicuticular HC composition be modified by temperature changes, and is there genetic variability for HC shifts in

response to temperature? We address these questions by examining the effect of high and low temperatures on the epicuticular HC's of adult *D. mojavensis*.

Table 1. Compositional changes of the two major alkadienes in 5 strains of *D. mojavensis* at three rearing temperatures. C35:2 refers to a 35 Carbon atom alkadiene and C37:2 refers to a 37 Carbon atom diene. TOTAL is the sum of all cuticular hydrocarbons and "R" refers to the ratio of C35:2/C37:2 dienes. For each sample, N=20 flies.

		DIENES (ug per sample)							
		Females				Males			
Temp. Regime	Line	C35:2	C37:2	TOTAL	"R"	C35:2	C37:2	TOTAL	"R"
17°C	2	.35	.07	.53	5.36	.41	.04	.58	9.35
	3	.46	.06	.64	7.27	.46	.04	.58	10.36
	5	.39	.09	.52	4.33	.39	.04	.52	9.39
24°C	2	.40	.20	.67	2.00	.56	.16	.79	3.55
	3	.55	.16	.78	3.39	.49	.09	.63	5.65
	4	.70	.28	1.04	2.51	.60	.16	.83	3.69
	5	.79	.17	1.05	4.64	.60	.10	.79	5.76
	7	.42	.16	.65	2.63	.40	.08	.54	5.30
31°C	2	.46	.23	.75	2.00	.42	.12	.59	3.46
	7	.28	.18	.49	1.54	.29	.11	.44	2.63
17->24 °C	2	.43	.29	.77	1.49	.62	.13	.89	4.70
	3	.51	.29	.85	1.77	.52	.12	.75	4.42
	4	.41	.21	.67	1.91	.37	.09	.50	3.97
	5	.52	.30	.89	1.73	.58	.14	.81	4.01
	7	.31	.25	.65	1.73	.36	.10	.54	3.60
31->24 °C	2	.45	.26	.77	1.74	.52	.10	.71	4.96
	3	.51	.29	.85	1.77	.55	.12	.78	4.47
	4	.49	.24	.79	2.03	.42	.12	.57	3.52
	5	—	—	—	—	—	—	—	—
	7	.67	.35	1.11	1.97	.83	.16	1.14	5.17

Females were collected from a Sonoran desert population in June 1987 (strain SC687, San Carlos, Mexico), and used to set up 5 isofemale lines (SC-2, SC-3, SC-4, SC-5, SC-7). The flies were maintained in half-pint bottles on a standard cornmeal-molasses-agar media seeded with live yeast. Subcultures of these five isofemale lines were reared under three different temperature regimes (17°C, 24°C, and 31°C) for approximately 16 generations. After eclosion, flies were sexed and maintained in 8-dram vials (15 flies/vial) at 24°C for 8-10 days until sexually mature. Lipids were extracted from groups of 20 males and 20 females with hexane (Toolson, 1982). Hydrocarbons were separated from other lipids by eluting the extract with hexane through a 7 x .05 cm silicic acid mini-column (Toolson, 1982), and analyzed by gas chromatography.

We found larval rearing temperature affected adult epicuticular HC composition. Changes

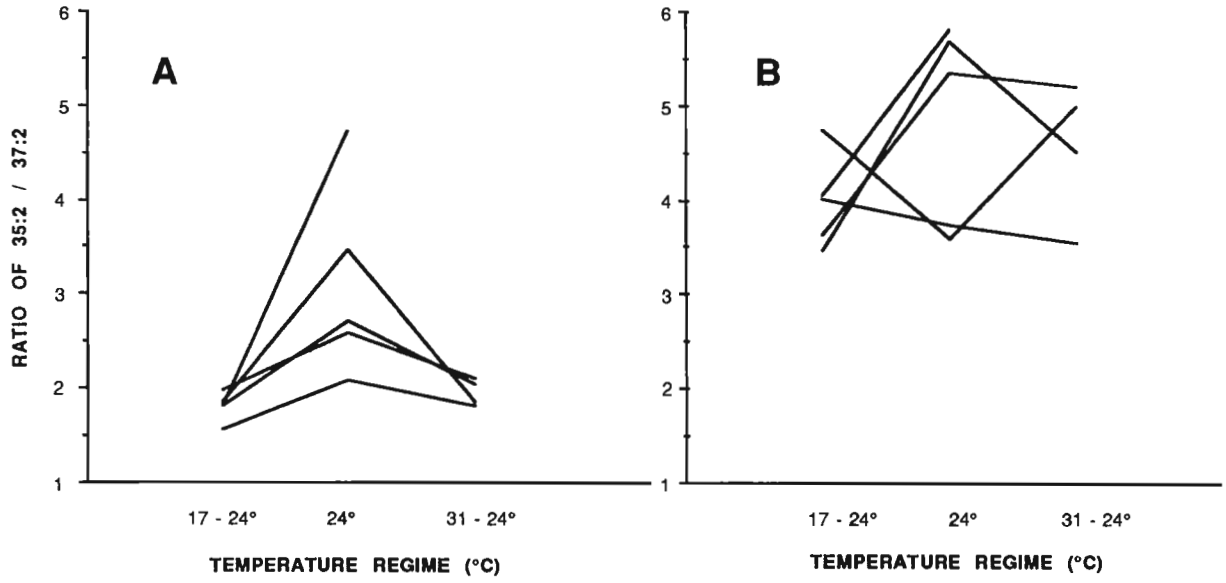

Figure 1. Effect of rearing temperature on female (A) and male (B) $C_{35:2}/C_{37:2}$ diene ratio. Norms of reaction for flies of five strains reared under three temperature regimes and maintained at 24°C until sexually mature. Strain 5 was not viable after six generations at 31°C.

were greatest in the alkadiene fraction. This fraction consisted mostly of dienes ranging from C33 to C37, although only two dienes, $C_{35:2}$ and $C_{37:2}$, accounted for over 70% of the total hydrocarbon fraction in both males and females (Table 1). Although total amounts of the two dienes were not significantly different among the various temperatures, the ratio "R" of $C_{35:2}/C_{37:2}$ was significantly different (Figures 1A, 1B). Sex differences were present for all strains in the HC's ($p<0.0001$), although strains differed in response to temperature ($p<0.01$). The ratio was greater in extracts from males than females at all temperatures, and males had less of the C37 diene even when they had more total dienes than females. In both sexes, as temperature increased, the ratio of $C_{35:2}/C_{37:2}$ decreased. Higher constant temperatures were generally associated with a shift to greater proportions of longer chain dienes, although not necessarily greater total diene amounts.

These experiments demonstrate the potential of temperature to act as a selective force in cuticle lipid composition. Long-term rearing at high and low temperatures produced predictable, repeatable changes in adult epicuticular HC of *D. mojavensis*. The norms of reaction for "R" plotted in Figure 1 indicate that there is considerable genetic variation both with respect to the biosynthetic pathways involved in the synthesis and deposition of epicuticular HC and in the acclimatory response of flies' epicuticular HC profiles to temperature changes. Thus, in *D. mojavensis*, as in *D. pseudoobscura*, there exists the potential for epicuticular HC profile to evolve in response to selection pressures, both natural and sexual.

Shifts in epicuticular HC profile that accompany changes in rearing temperature confirm the inference from earlier studies (Toolson, 1982, 1988; Hadley, 1977; Toolson and Hadley, 1979) that temperature can act as a selection pressure on epicuticular HC profile. The epicuticular HC's of *D. mojavensis* are unusual in that $C_{33:2}$ - $C_{37:2}$ alkadienes comprise most of the hydrocarbons (Blomquist and Dillwith, 1985). The shift toward greater proportions of longer hydrocarbons in flies reared at higher temperatures is qualitatively similar to what has been reported in many other insect species, including *D. pseudoobscura* (Toolson, 1982). Such changes in epicuticular HC profile are typically associated with predictable changes in cuticular permeability (Toolson, 1982; Hadley, 1984; Toolson and Kuper-Simbron, 1989). Whether or not that is the case in *D. mojavensis* remains to be determined.

Markow and Toolson (in press) have evidence that epicuticular HC profile is also subject to sexual selection in *D. mojavensis* males. They also found rearing temperature significantly affected a male's "attractiveness" to females. Males with higher "R" values had greater mating success (Markow and Toolson, in press). Coupled with these findings, our results point to temperature as a powerful selective force on epicuticular HC composition. These findings corroborate the inference of Toolson and Kuper-Simbron (1989) that epicuticular HC composition in *Drosophila* is subject, and evolutionarily responsive, to a variety of natural and sexual selection pressures. We are presently designing experiments that will use temperature effects on epicuticular HC profile and on mating behavior in *D. mojavensis* to study the interactions of natural and sexual selection in shaping physiological adaptations to the environment.

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Rim, N.R. Chonbuk National University, Chonju Korea. Time and the allelism of Lethal Genes in Korean *D. melanogaster* populations.

According to time interval, behavior of lethal genes in natural populations of *D. melanogaster* was studied in the present work. Samples used in this study were collected in every early November for five years from 1982 to 1986, at a vineyard near Chonju locating on southern part of Korea. To detect lethal genes in both chromosome 2 and 3 simultaneously, wild males only were selected and then balanced by means of A_1B_{18} technique used first by Wallace *et al.* (1966).

The results of frequency tests for lethal genes are presented in Table 1. As shown in this table, chi-square test for homogeneity shows significant difference between annual samples due to reverse the frequency order of complete-lethal and semi-lethal in 411 and 511 among the samples, although the frequencies of lethal lines taken together complete- and semi-lethals do not differ statistically from each other.

Table 1. Gene frequencies, in both second and third chromosomes as a single genome unit, for five annual samples (1982-1986) of *D. melanogaster* from Chonju area, a Korean locality.

Samples	N	CLe	SLe	Qua	N & S	CLe + SLe
211	203	.4975	.3448	.1478	.0098	.8424
311	247	.4615	.3648	.1377	.0323	.8300
411	167	.3054	.4970	.1437	.0539	.8024
511	232	.3836	.4397	.1509	.0259	.8233
611	202	.4059	.3911	.1584	.0446	.7970
Total	1,051	.4158	.4044	.1484	.0314	.8202
Homogeneity	χ^2 = 24.105		P = 0.020			

CLe: complete-lethal line ; SLe: semi-lethal line ; Qua: quasi-normal line
N & S: normal and super-normal line

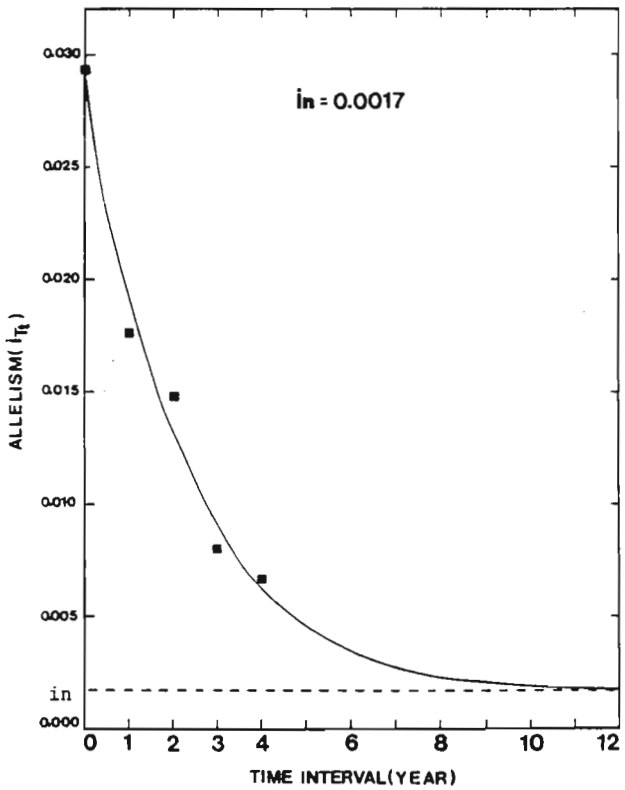

Figure 1. Relationship between time and the allelism of lethal genes in Korean *D. melanogaster* populations.

Table 2. Time series allelism of lethals within and between five *D. melanogaster* samples taken annually from Chonju, a Korean locality.

Time interval	Types of cross	No. of crosses	No. of allelism	Allelic rates (%)
	Within population			
0	211 - 211	1,346	69	5.13
	311 - 311	614	16	2.61
	411 - 411	340	8	2.35
	511 - 511	744	11	1.48
	611 - 611	924	12	1.29
	Total	3,968	116	2.92
	Between populations			
1	211 - 311	971	23	2.37
	311 - 411	815	24	2.58
	411 - 511	839	9	1.07
	511 - 611	923	7	0.87
	Total	3,548	63	1.77
2	211 - 411	720	17	2.36
	311 - 511	1,128	11	0.98
	411 - 611	855	12	1.40
	Total	2,703	40	1.48
3	211 - 511	1,022	9	0.88
	311 - 611	972	7	0.72
	Total	1,994	16	0.80
4	211 - 611	801	5	0.62
	Grand total	13,014	240	1.84

The data given in Table 2 is the results of the test for allelism of lethal genes according to the time interval between the five annual collections. Total 13,014 crosses out of 95,266 possible combinations with the complete-lethals analysed were practically carried out. The average frequency of total allelism was 1.84 percent. The allelism of lethals show to decrease significantly as the time interval between the samples increases. Previously, Wallace (1966) has conjectured a relationship between time interval and allelism rate in the sampling of first and second groups of lethals as proposed in equation, $iTt=in+iF(1-K)^t$ where iTt is the frequency of allelism of lethals at t generations apart, iF the allelism resulting from common descent and component of iTt, in is the allelism caused by independent lethal mutations, and K a constant related to the rate of turnover of lethals within the population. In this equation, the allelism rate caused by independent mutation (in) can be calculated through an equation, $in=n^{-1}(m/Q)^2$ where n is the number of loci, m the number of lethal genes in each chromosome ($m=-ln(1-Q)$), and Q the frequency of lethal chromosomes. In the present study, the number of loci (n) to cause lethal mutation was granted to 1,000 in chromosome 2 and 3 together. By substituting the values of $n(1000)$, $m(0.5375)$ and $Q(0.4158)$ into the equation, the value of in was estimated to be 0.0017. Therefore, by subtracting the value of in from the observed frequencies of allelism (iTt), the allelic frequencies of common descent (iF) were obtained. To take the corrected iT curve, the iF values were corrected by regression analysis, and then added the values of in (0.0017). So, the value of iT at zero interval of time was calculated to be 0.0291 and that at four-year interval to be 0.0061.

In Fig. 1, an exponential curve for the relationship between time and the allelism of lethals is plotted by the corrected values of iT. The curve passing through the observed frequencies decreases progressively toward the limiting

value of 0.0017, as time interval increases.

From the above equation proposed by Wallace, K a constant value for the curve shown in Fig. 1 was estimated to be 0.019 from the corrected values of iT, in which time interval held as one generation is for 15 days and a year for 24 generations.

Prout (1966) supported the conjecture by Wallace to be correct, and under the assumption he provided a prediction that Wallace's K is closely related to the selection impinging on heterozygotes (s: selection coefficient) and the rate of lethal mutation (u) as in the following equation: $K=\sqrt{s^2 + 4u}$ (the sign of the root is given by the sign of s). However, among the three circumstances simplified K by Prout, the lethals in this local population studied did not correspond to any of semi-dominant, complete recessive or heterotic, because of that in the present results s^2 induced from K(0.019) was negative (-0.0396). Therefore, it is assumed that most of lethals in *D. melanogaster* populations from Korea having clear four seasons are maintained by both balanced and neutral loads in spite of involuntary chaos of populations with drastic changes of seasonal environments.

Acknowledgment: This work was supported by a grant from the Korea Science and Engineering Foundation in 1989.

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Rodin, S.N., T.A. Kozhemyakina and D.P. Furman. Institute of Cytology and Genetics, Novosibirsk, USSR. Mobile elements as an influence upon the penetrance of the *scute* mutation in *D. melanogaster*.

The *achaete-scute* supergene complex is a very convenient model for studying the genetic determination of quantitative characters, the classic problem, as the *scute* alleles are so various phenotypically, as floating penetrance is a typical feature of the mutations, and as the character itself, the number of bristles falls easily under quantitative estimations.

Some time before, we induced in the P-M hybrid disgenesis system a unique series of 10 *scute* alleles which is characterized both by the reduction of one pair of humeral bristles (h^{1-2}) and at the same time varying penetrance (from 10-20% to 90-100%). The way of inducing the mutations makes it plausible to correlate their appearing and their varying manifestation with an activity of mobile elements. To verify the supposition we took five original lines and synthesized from 1 to 4 isogenic derivates (11 in sum total). Isogenization was carried out with the use of the *M5;Cy/Pm;D/Sb* line. The *mdg*1,2,3,4,*copia*,*B*104, P-element localization pattern was reproduced after the method of hybridization *in situ* with salivary gland chromosomes of larvae from the corresponding lines. At least 5 larvae of each genotype have been analysed.

Data gathered in Table 1 evidence that the penetrance of the *scute* mutation depends on its genetic background: from any basic line one can get isogenic stocks in which the level of humeral bristle reduction deviates from that in their ancestor. Moreover, the lines with originally different reduction levels may produce isogenic lines with close penetrance (see, for instance, sc^{59P}, sc^{33P}, $sc^{59\text{-}10}$ and $sc^{133\text{-}16}$) and *vice versa*, the lines with close h^{1-2} reduction frequencies may yield derivates in which the respective values of the character are strongly different (sc^{146P}, sc^{147}, $sc^{146\text{-}8}$, $sc^{147\text{-}7}$).

If the phenotypical features revealed are connected with the characteristics of *mdg* localization, then we may expect that:

1) isogenic lines with different reduction levels but coming from a common ancestor should have different numbers of mobile elements;

2) at the same time isogenic derivates with close frequencies of bristle reduction but coming from different parental lines should resemble one another in the patterns of *mdg* localization sites.

Take *mdg2* as an example of the "*mdg* pattern - penetrance" dependence (Tables 2 and 3). A quantitative analysis of the results obtained proves that there is a room indeed for both of the presumptions.

1) Really, there are 14 sites to the fore over which the $sc^{59\text{-}5}$ line with a low bristle reduction level is at variance with its isogenic relations; there are 16 sites of that kind defined for the $sc^{59\text{-}10}$ line with a high bristle reduction level.

2) Comparison of combined data (Table 3) reveals some complications in the scheme, yet here we can as well see at least several regions the availability or lack of *mdg* wherein may correlate with another level of penetrance (e.g. 21F, 22A, 22B sites fore higher reduction levels (Fig. 1) and 49CD, 51D, 51E, 57F, 70C for lower and average).

Thus, the fact of interline *differences* by the *mdg* pattern localization and correlating changes of reduction level is quite explicit. As for interline *similarities* the *mdg* distribution for cases of close penetrance, that is a tendency to be experimentally verified.

Table 1. Characteristics of the scute mutation, induced in the P-M hybrid disgenesis system, and its isogenic derivates.

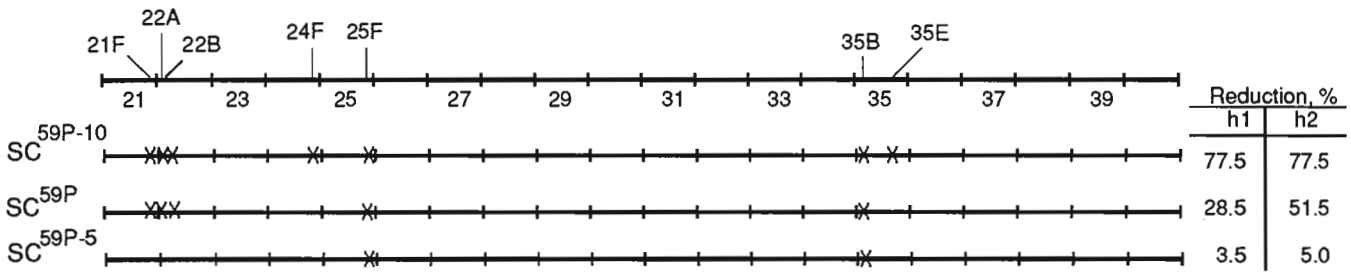
Line	Bristle reduction frequency in females, %	
	h^1	h^2
sc^{55P}	48.5	58.5
55-9	41.5	48.5
sc^{59P}	28.5	51.5
59-4	20.5	34.0
59-5	3.5	5.0
59-10	77.5	77.5
sc^{133P}	94.0	93.5
133-1	73.0	73.5
133-16	72.0	73.5
133-10	77.0	73.5
133-11	83.5	84.0
133-3	48.0	47.0
sc^{146P}	72.0	69.5
146-2	60.0	57.7
146-8	82.0	80.5
sc^{147P}	76.0	76.0
147-7	48.0	48.0

Table 2. Localization of mdg2 labeled sites in the chromosomes of the sc^{59P} basic line and its isogenic derivates.

chaetae	lines			
	59	59-5	59-4	59-10
	Bristle Reduction, %			
h^1	28.5	3.5	20.5	77.5
h^2	57.5	5.0	34.0	77.5
	Labelled sites			
18BC	+	+	+	+
25F	+	+	+	+
35B	+	+	+	+
51B	+	+	+	+
58EF	+	+	+	+
63E	+	+	+	+
65F	+	+	+	+
74A	+	+	+	+
78EF	+	+	+	+
84A	+	+	+	+
88B	+	+	+	+
88C	+	+	+	+
96D	+	+	+	+
49CD	+	+	+	
51D	+	+	+	
51E	+	+	+	
70C	+	+	+	
43F	+	+		
57F	+	+		
63A	+	+		
19DE	+		+	+
58BC	+		+	+
21F	+			+
22A	+			+
22B	+			+
73C	+			+
83C	+			+
96F	+			
47D		+		
47B				+
24E				+
35E				+

Table 3. Distribution of mdg2 over isogenic derivates from different basic lines.

chaetae	Lines										
	59-5	59-4	55-9	147-7	146-2	133-1	133-10	133-16	59-10	146-8	133-1
	Bristle Reduction, %										
h^1	3.5	20.5	41.5	48.0	60.0	73.0	77.0	72.0	77.5	82.0	83.5
h^2	5.0	34.0	48.5	48.0	57.5	73.5	77.5	73.5	77.5	80.5	84.0
	Labelled Sites										
18BC	+	+	+	+	+	+	+	+	+	+	+
58EF	+	+	+	+	+	+	+	+	+	+	+
74A	+	+	+	+	+	+	+	+	+	+	+
19DE		+	+	+	+	+	+	+	+	+	+
58C		+	+	+	+	+	+	+	+	+	+
25F	+	+	+	+	+	+	+		+	+	+
78EF	+	+	+	+		+	+	+	+	+	+
96D	+	+	+		+	+	+	+	+	+	+
88C	+	+	+	+	+	+		+	+	+	+
47D	+			+	+	+	+	+	+	+	+
88B	+	+	+	+		+		+	+	+	+
47B			+	+		+	+	+	+	+	+
35B	+	+	+	+	+	+			+		
84A	+	+		+		+	+		+		
54B	+	+		+	+	+			+		
73C				+	+	+	+	+	+		
65F	+	+			+	+	+		+		
63E	+	+							+	+	+
83C					+	+	+		+		
43F	+		+							+	+
63A	+				+					+	+
21F								+	+	+	+
22A								+	+	+	+
22B								+	+	+	+
70C	+	+	+								
49CD	+	+		+							
51D	+	+		+							
51E	+	+		+							
28D				+		+		+			
24E				+		+		+	+		
57F	+		+								
64A					+	+					
90B							+	+			
86C			+								+
68C			+								
35E									+		
87D									+		
60A										+	
60E										+	
48B										+	
88E						+					
99D						+					
69F								+			
91A								+			



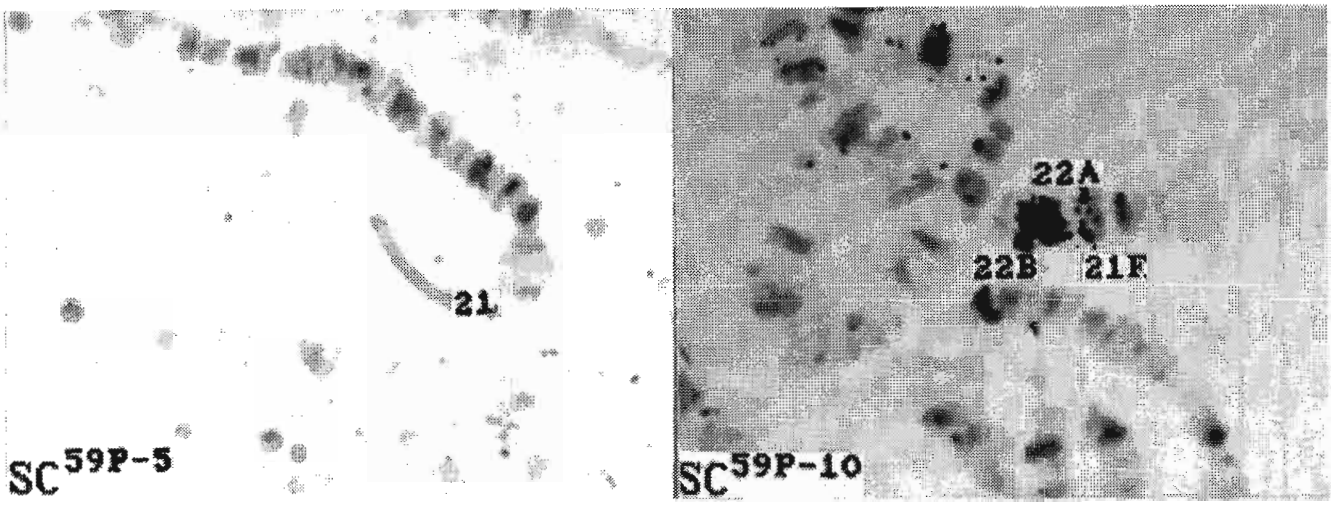


Figure 1. Hybridization of *mdg2* with 2*l* salivary gland chromosomes from sc^{59P-5} and sc^{59P-10} larvae *in situ*.

Sakul, B.U and H. Sakul. Department of Anatomy, Faculty of Medicine, University of Ankara, Ankara, Turkey, and Department of Animal Science University of California, Davis, California. Detection of the effects of carcinogenic methyl methane-sulphonate by a short-term test.

Summary: Methyl methanesulphonate (MMS) is an alkylating agent, having a direct effect without metabolic activation (1,2,3). MMS is currently used in synthesis of of chemicals in chemical industry and also in detection of of some illnesses (4,5). MMS may also cause mutation by its harmful effects on DNA (deoxyribonucleic acid), as some other carcinogenic chemicals may in our environment (1,3, 6,7). These effects may be transferred into the next generations if they occur in *germ* cells, and may also cause tumors in the somatic cells (3).

As in the most sophisticated tests, DNA is the main basis of this short-term Drosophila test, since the DNA is the genetic material of almost all the living objects (8). Once it is assumed that the damage caused by the outside effects, and the repairing systems are the same in the DNAs of different organisms, then it is obvious that a factor which may damage the DNA of a bacteria or Drosophila may damage the DNA of a mammal as well.

This study aims at detecting the mutagenic and carcinogenic effects of MMS in a short-term test in *Drosophila melanogaster*, using the "Brood Pattern Analysis".

Material And Methods: The material of this study is composed of *D. melanogaster* (Type Oregon K) which is a highly inbred type as a result of a close inbreeding for many years, and is genetically homogenous. In order to determine LD-100 of MMS (Sigma USA), a solution containing 2.5 x 10^{-3}M. in 2% of sucrose was prepared. Then it was exposed to the groups of 30 males (2-4 days old) for 24 h. (9). After this period, it was observed that all of the males were dead. For this reason, another solution containing 2.5 x 10^{-4}M. was prepared and exposed to the same number of males for the same time to determine LD-50 of MMS. This second solution caused the death of 50% of the flies. Subsequently the solutions containing 2.5 x 10^{-5}M., 2.5 x 10^{-6}M., 2.5 x 10^{-7}M., and 2.5 x 10^{-8}M. were prepared and exposed to different male groups of 30 flies for the same time using the same methods. The glass tubes (2.5 x 10cm) containing the treated males were put into the refrigerator ($25 \pm 1^{\circ}$C and 50-60% humidity). Since MMS is easily vaporized, the males may

Table 1. Number of live females obtained from different brood patterns.

Dosage	Sex	Brood-1	Brood-2	Brood-3	Total
Control	M	442	448	421	1311
	F	424	452	410	1286
2.5 x 10^{-5}M	M	268	265	191	724
	F	244	294	222	760
2.5 x 10^{-6}M	M	253	303	283	839
	F	317	328	301	946
2.5 x 10^{-7}M	M	324	351	308	983
	F	341	362	24	1027
2.5 x 10^{-8}M	M	378	398	358	1134
	F	382	403	361	1146
Total males alive		1665	1765	1561	4991
Total females alive		1708	1839	1618	5165
Total flies alive		3373	3604	3179	10156

Table 2. The "s" value of various alkylating agents at different temperatures.

Alkylating agent	20°C	37°C
Dimethylsulphate	0.86 (0-10°C)	—
Methyl methanesulphonate	0.86	0.83
Ethyl methanesulphonate	0.67	0.64
N-Methyl-N-nitrosourea	0.42 (25°C)	—
Isoprophyl methanesulphonate	0.31	0.29
N-Ethyl-N-nitrosourea	0.26 (25°C)	—

Reference: Vogel and Natarajan (1979)

take it in by both inhalation and feeding (10). Because the solutions contain sucrose, it makes it easier to take MMS orally into the body (11). At the end of the specified period, males were transferred to the glass bottles, having a volume of 250 ml and sterilized Lewis conditions (11). Three bottles were alloted to each dosage and control groups, and 10 males were put into each bottle. Then the first series of Brood Pattern bottles were obtained by placing an equal number of virgin females to the number of males into each bottle. After four days the males in the first series of Brood Pattern bottles were transferred to other bottles which also contained the sterilized Lewis conditions, in order to obtain the second series of Brood Pattern bottles. However, the females were discarded at that time. And similarly, new virgin females were added to the second series of Brood Pattern bottles. After four days, the same procedure was followed to get the third series of Brood Pattern bottles.

The progenies of each brood pattern bottle were counted by sex. Chi-square analysis and analysis of variance techniques were employed to analyse the data. Duncan's Multiple Range Test was applied following the analysis of variance, in order to find which groups differed the most from the others for the criteria studied (12,13,14).

Results: The number of females and males alive, obtained from each of the three brood pattern bottles are shown in Table 1 by dosage groups.

No statistically significant differences were found between the expected and the observed number of live females ($P>0.05$). Similarly, the differences between the number of live males for each dosage group of each pattern were not found to be significant ($P>0.05$).

The results of the analysis of variance using the data on alive females and males of each brood pattern were as follows: The differences between the number of live females obtained from MMS-treated males were found to be significant for all of the dosages and the control groups of each brood pattern. The differences with respect to the number of live males of different brood patterns were found to be significant within the brood patterns ($P<0.01$) although it was not significant between the different brood patterns. The results of the Duncan test suggested that there was an increasing decline in the number of live males and females from the dosage of 2.5 x 10^{-5}M (Fig. 1 and 2). The analyses on the progeny of MMS-treated males showed that the difference between the sexes with respect to the effects of MMS were not significant ($P>0.01$).

Discussion: In this study, the effects of MMS, an alkylating agent, on *D. melanogaster* were investigated, using the "Brood Pattern Analysis". For this purpose, the progenies of MMS-treated males were counted and these counts were used to get an estimate of the incidence of death caused by MMS (6,15).

The reactivity of a substance is known as the "Swain-Scott factor" which expresses the dependence of the primary reaction rate of an alkylating agent on the nucleophilicity of the receptor atom(s), and is represented by "s" (16) (Table 2).

Compounds with low "s" values, typified by the nitrosamide ENU, alkylate more efficiently at centers of low nucleophilicity, such as the 0-6 of guanine, when compared to agents of higher nucleophilic strength (1,6,17). It is also known that MMS causes some mutations such as transition, transversion, deletion, translocation, partial and entire chromosome losses (1,3,6,7,17,18,19,20,21,22,23).

In our opinion, the damage is in spermatogenesis, and for this reason, the number of live flies was decreased in this experiment. The decrease in the number of live flies by an increase in dosage confirmed this hypothesis (Fig. and 2).

The results of the chi-square analysis showed no significant differences between the number of expected and observed live flies ($P>0.05$) which may be interpreted as there was no significant experimental error in the study. The

results of the analysis of variance showed that the differences among dosages, and between the dosages and control groups were statistically significant ($P>0.01$). This suggests that the effects of MMS were significant whereas the differences in concentration between the dosages were not big.

The lowest number of live flies was recorded in the dosage of 2.5×10^{-5}M (Table 1). This dosage was followed by 2.5×10^{-6}M, 2.5×10^{-7}M and 2.5×10^{-8}M dosages. These results are in accordance with the results of Aaron and Lee (24), Vogel and Natarajan (1) and Vogeland Leigh (22).

Although the overall difference between the dosages in both sexes was found significant, it was observed that the differences between broods were significant in females only ($P<0.01$). This tells us that MMS does not effect only the gonosomal chromosomes. If it was the case then we would have expected to see more females than males. It has already been shown that the genome of *D. melanogaster* has 50-60 genes only, which were sensitive to MMS, and most of the genes were in autosomal chromosomes (25,26).

The results obtained from our test suggest that MMS may be the reason of various mutations in the DNA of *D. melanogaster*. Since the structure of DNA is similar in all living organisms, these types of alkylating agents may also damage the human being.

Consequently as the carcinogenic effects of mutagenic chemicals are already known (27,28), use of these chemicals must be restricted for the sake of mankind and a healthy world.

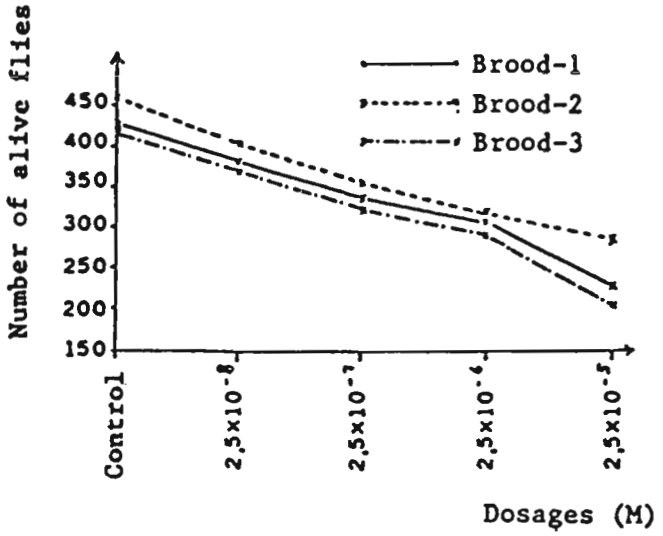


Figure 1. Comparison of the number of live females obtained from brood patterns.

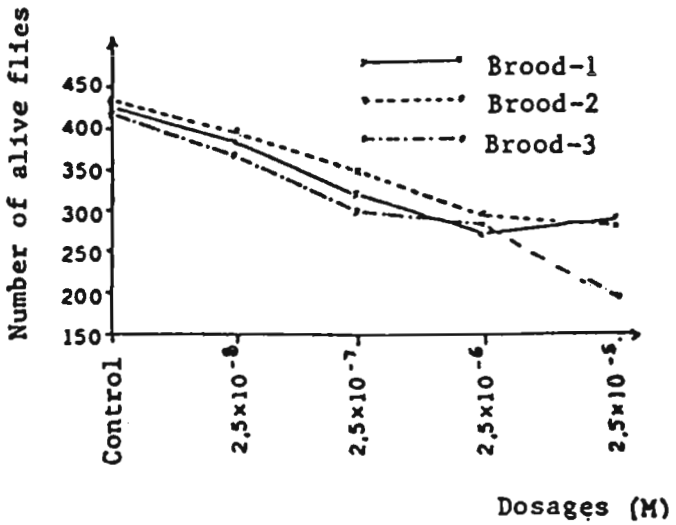


Figure 2. Comparison of the number of live males obtained from brood patterns.

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Sharma, A.K., Punjab Agricultural University, Ludhiana, India. Genotype environment interaction for acid phosphatase in *Drosophila*.

For studying genotype-environment interaction of the *acid phosphatase* (*Acph*-I) locus of *D. melerkotliana* (i) crude extracts of three genotypes, *S/S*, *F/F* and *F/S* and (ii) three allozymes, two homodimeric and one heterodimeric, isolated from heterozygote were characterized over a wide range of hydrogen ion concentration (pH) ranging from 4.70 to 7.10. In order to have information about genotype-environment interaction, at each value, the specific activity of acid phosphatase in slow homozygotes (*S/S*) was taken as 1.00 and the specific activity of the other two genotypes (*F/F* and *F/S*) were expressed as multiples of slow. Secondly, at each pH value, the specific activity of the *F/F* genotype was taken as 1.00 and that of *F/S* expressed at multiple of cast.

It was found that the specific activity of phosphatase in crude extracts in *F/F* ranged from 1.04 to 2.14, in *F/S* it ranged from 2.16 to 8.55. The specific activity of the *F/S* genotype in relation to specific activity of the *F/F* genotype ranged from 1.63 to 4.70. The specific activities of *F/F* and *F/S* genotypes in relation to the *S/S* genotype showed differential activity at almost all the pH values and the same was the case with *F/S* when the specific activity of *F/F* genotype was taken as one. Further, the individual values (at almost all the pH values) of these genotypes deviated significantly from their respective means. These observations indicated that each genotype (*F/F* and *F/S*) responds differently from the *S/S* genotype to each of the different changes in pH values, thus showing genotype-environment interaction. The same is true for the *F/S* genotype in relation to the *F/F* genotype.

The specific activities of fast homodimeric and heterodimeric allozymes isolated from heterozygotes were compared in relation to slow homodimeric allozyme, and those of heterodimeric allozyme in relation to fast homodimeric allozyme. Most of the values tested at different pH values deviated significantly from their respective mean values and thus showed interaction between allozymes and environment.

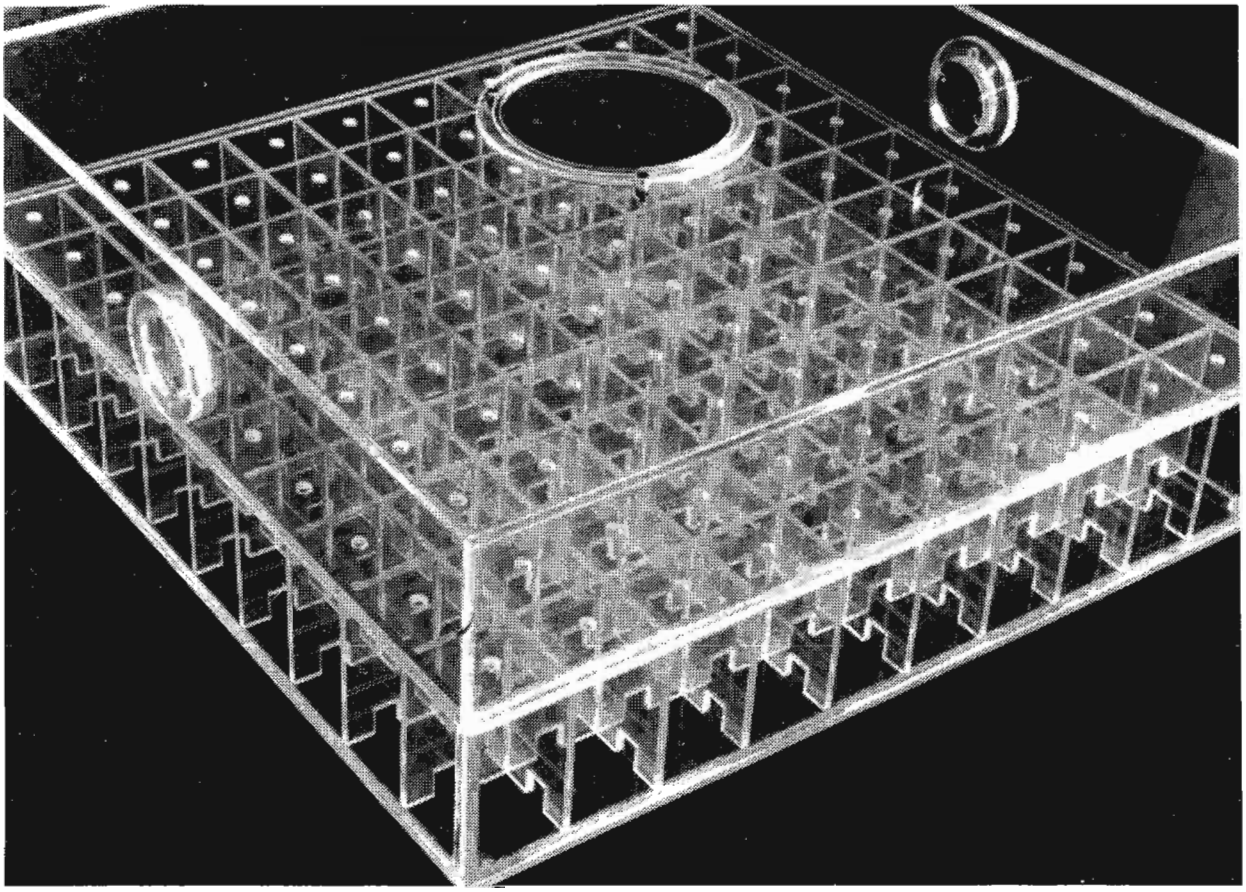
The differential response of genotypes as well as the allozymes isolated from heterozygotes to particular changes in the environmental conditions (different hydrogen ion concentrations) is simply a reflection of genotype and environmental interaction.

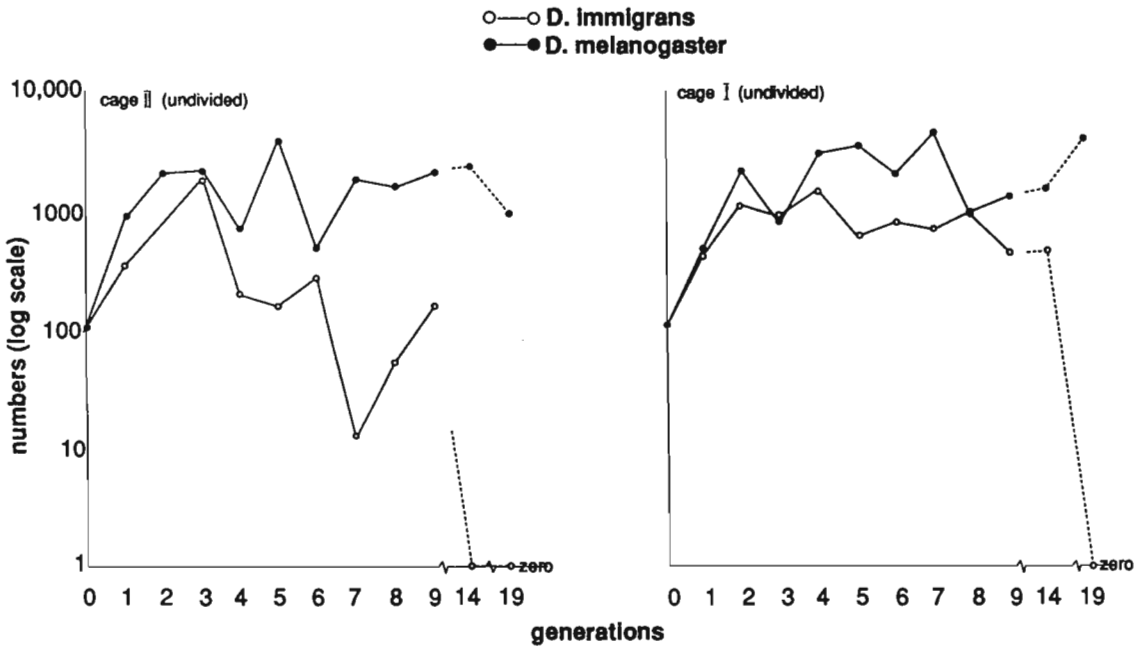
Shorrocks, B. University of Leeds, Leeds, England. Coexistence on a patchy environment: a cage experiment.

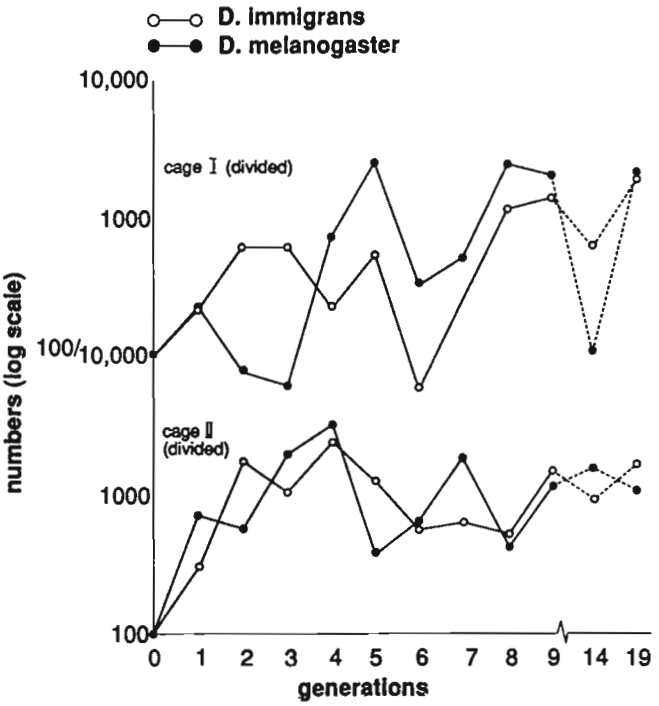
Drosophilids lay their eggs in breeding sites which are discrete, consisting of small, separate units, and are ephemeral, persisting for only one or two generations. In a series of papers (Shorrocks *et al*, 1979; Atkinson and Shorrocks 1981; Shorrocks *et al*. 1984; Atkinson and Shorrocks 1984; Shorrocks and Rosewell 1986, 1987), we have shown, using computer simulation and field measured parameters, that two species exploiting such breeding sites might never exclude one another globally despite strong local competition between the larvae if the two species have aggregated and independent distributions. The present investigation reports the results of cage experiments designed to test the above predictions in a laboratory system.

The populations were maintained in perspex cages (Figure 1) with internal partitions dividing the floor of the cage into 100 cells or patches. Two types of cage were available, "divided" and "undivided". The latter cages had small openings, below the level of the Drosophila food, as illustrated in Figure 1. These allowed larvae to move from one patch to another, effectively eliminating the divided nature of the environment. The "divided" cages did not have these openings. The species used were *D. melanogaster* and *D. immigrans*, maintained in both single and two species populations. Adults were transferred from old to new cages every 14 days and allowed to oviposite for 12 hr before being removed. This apparently artificial regime is a necessary devise to try and simulate the kind of distribution seen in the wild, inside a small confined cage. All experiments were carried out in cooled incubators at a temperature of 24 $\pm$ 1°C, with a light regime of 8 hr light and 16 hr dark.

Single species populations of both *D. melanogaster* and *D. immigrans*, in both "divided" and "undivided" cages, persisted under the experimental conditions. The extinctions reported below cannot therefore be attributed to unfavourable environmental conditions. Figures 2 and 3 show the results from the two-species cages. After week 9 the flies were transferred but not counted in order to save time. Counting and identification was carried out on weeks 14 and 19. For the "divided" cages the computer model of Atkinson and Shorrocks (1981) predicts that both species should persist, at about 1000 flies per species. The cage results fit this prediction. For the "undivided" cages the computer model




D. immigrans
D. melanogaster
cage II (undivided)
cage I (undivided)
10,000
1000
100
10
1
numbers (log scale)
zero
zero
0 1 2 3 4 5 6 7 8 9 14 19
0 1 2 3 4 5 6 7 8 9 14 19
generations



predicts that *melanogaster* should persist (at about 5000 flies) while *immigrans* should become extinct by about generation 20. Again the cage results fit the prediction.

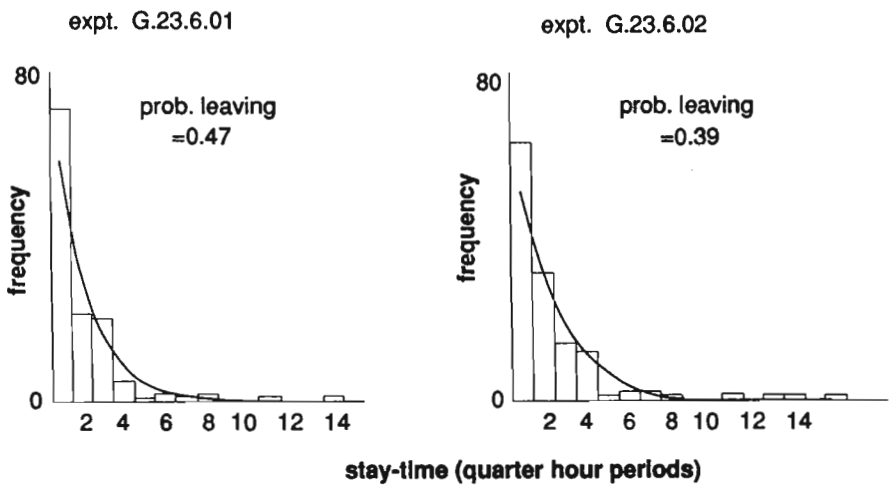
These cage experiments clearly demonstrate that in a "divided" environment with ephemeral patches, the inferior competitor (*immigrans*) persists along with the superior species (*melanogaster*). In contrast, in the "undivided" environment the inferior species is eliminated. What is more, using parameter values for the two species measured under cage conditions the simulation model of competition predicts final outcomes (coexistence or exclusion) and general population levels that agree with the cage results.

References: Atkinson, W.D. and B. Shorrocks 1981, J. Anim. Ecol. 50:461-471; _____ 1984, Am Nat. 124:336-351; Shorrocks, B. and J. Rosewell 1986, J. Anim. Ecol. 44:527-541; _____ 1987, BES symposium no. 27:29-51; Shorrocks, B., W.D. Atkinson and P. Charlesworth 1979, J. Anim. Ecol. 48:899-908; Shorrocks, B., J. Rosewell, K. Edwards and W.D. Atkinson 1984, Nature 189:732-735.

Shorrocks, B. University of Leeds, Leeds, England.

Distribution of clutch size and patch-leaving probability in *D. melanogaster*.

Drosophilids lay their eggs in breeding sites which are discrete and are ephemeral. We have shown (Shorrocks *et al.* 1979; Atkinson and Shorrocks 1981; Shorrocks *et al.* 1984; Atkinson and Shorrocks 1984; Shorrocks and Rosewell, 1986, 1987) that two species exploiting such patches might never exclude one another globally despite strong local competition between the larvae, if the two species have aggregated and independent distributions. Atkinson and Shorrocks (1984) suggest that a likely cause of the aggregated distributions seen in both laboratory and field, is compounding of two distributions representing two different behavioral events. Egg-laying visits to breeding sites could be Poisson distributed and the number of eggs laid at a visit (clutch size) could conform to a discrete form of the negative exponential curve. This latter distribution would arise if there were a constant probability of a female leaving the breeding site after each egg was laid. We report here some experiments which attempt to investigate the distribution of clutch size and the probability of leaving a patch.



Experiments were carried out in a dark-room (24°C ± 2°C). The cages used were those described in the previous note (Figure 1). Cells contained half a grape, to simulate a natural breeding site, held upright by a bed of fine sand. Only the middle 64 cells were used, in order to eliminate edge effects. The females used in each experiment (25) were removed from stock cultures two days before, anaesthetized with CO_2, identified, sexed and placed in fresh culture bottles until the morning of the experiment. At 9:00 a.m. they were released into the

experimental cage without using CO_2. The cage was placed under a frame supporting a stip-light along each side, ensuring even illumination for all the patches. Every 15 min the number of females in each of the 64 patches was recorded, until 5:00 p.m. when the experiment was ended. The next morning the number of eggs laid on each patch was recorded. Five experiments were carried out using *melanogaster* and five were carried out using *simulans*.

In all 10 experiments the relationship between stay-time (cumulative female 1/4 hr) and total eggs laid per patch was positive and significant. This suggests that females lay eggs at a constant rate from the moment they arrive at a patch to the moment they leave.

Although these experiments are course-grained (female position only recorded every 1/4 hr), short-term continuous observations indicate that, within a 15 min period, females move rather infrequently. We have therefore assumed that a female present on a patch on two consecutive occasions is the same female and has not left the patch between readings. Figure 1 shows the distribution of stay-time for two selected experiments. All 10 trials in fact showed this characteristic negative exponential distribution. By comparing each consecutive time period for each patch it is possible to calculate the probability of leaving a patch during a 15 min interval. For the experiments in Figure 1, this gave values of 0.47 and 0.39. Assuming this leaving probability is a constant, we can calculate an expected distribution of stay-time which is also shown on Figure 1. The agreement between observed and expected is good (exp. G23.6.01, X^2 = 8.54, $p > 0.05$ and exp. G.23.6.02, $X^2 = 9.63$, $p > 0.05$). Females appear to show a constant probability of leaving a patch per 15 min period. This result, in combination with the linear relationship between stay-time and egg number suggests that female drosophilids show a constant probability of leaving after each egg is laid. This supports part of the explanation put forward by Atkinson and Shorrocks (1984) for the aggregation of eggs over patches.

References: Atkinson, W.D. and B. Shorrocks 1981, J. Anim. Ecol. 50:461-471; _____ 1984, Am Nat. 124:336-351; Shorrocks, B. and J. Rosewell 1986, J. Anim. Ecol. 55:527-541; _____ 1987, BES symposium no. 27:29-51; Shorrocks, B., W.D. Atkinson and P. Charlesworth 1979, J. Anim. Ecol. 48:899-908; Shorrocks, B., J. Rosewell, K. Edwards and W.D. and W. Atkinson 1984, Nature 189:732-735.

Shyamala, B.V. and H.A. Ranganath. Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570006, India. Inverted gene sequences in three species of the *montium* subgroup of *Drosophila*.

Inversion polymorphism represents a facet of the dynamic nature of the genetic system of a species and/or of a population. *D. truncata* (Okada, 1964), *D. nagarholensis* (Prakash and Reddy, 1980), and *D. agumbensis* (Prakash and Reddy, 1978) of the *montium* subgroup of *Drosophila* have been analysed for chromosomal polymorphism. We herein present an abstract picture of the inversion polymorphism in these three species.

The polytene chromosomes in these three species show five long and a short arm corresponding to two pairs of metacentrics, one pair of acrocentrics and a pair of dot chromosomes of the mitotic metaphase. Temporary squash preparations of the salivary glands stained with 2% lacto aceto orcein were used for screening the inversions. The species under study were collected from different parts of south India.

For *D. truncata*, only one population (Charmadi ghats) was available. It was found to be highly polymorphic. It showed a total of ten inversions. Of these, three were complex gene arrangements involving more than two inversions, four were overlapping inversions and three were simple inversions. These inversions were found distributed in all the five long arms of the polytene chromosomes.

For *D. nagarholensis*, three populations were screened. A total of four simple inversions were recorded. These inversions were found to be located in two of the five long arms, two in each arm. The populations from Shiradi ghats and Nallur showed only one inversion while the population from Biligirirangana Hills showed all the four inversions.

Only one population of *D. agumbensis* from Mysore could be screened. It showed three inversions, of which one was a complex inversion and the remaining two were simple inversions. The complex and one simple inversion was located on one arm while the other simple inversion was on another arm.

The results of this preliminary survey are indicative of the fact that these species with restricted distribution can form fertile systems for further studies on inversion polymorphism.

Acknowledgment: We wish to thank the Chairman, Department of Zoology, Manasagangotri, Mysore for his help and encouragement. BVS is thankful to University Grants Commission, New Delhi, for the financial support.

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Singh, A.K. and R.N. Singh. Banaras Hindu University, Varanasi, India. A new inversion in Indian *Drosophila ananassae*.

Drosophila ananassae exhibits a high degree of chromosomal polymorphism. More than fifty paracentric inversions, ten pericentric inversions and fourteen translocations have been reported in this species from different regions of the world (for references see the review by Singh, 1988). However, only three paracentric inversions namely subterminal (alpha or In(2L)A) in 2L, terminal (delta or In(3L)A) in 3L and basal (eta or In(3R)A) in 3R are coextensive with the species. Chromosomal polymorphism has also been studied in Indian populations of *D. ananassae* and several paracentric and pericentric inversions and translocations have been detected. Evidence for geographic differentiation of inversion polymorphism in Indian populations has been obtained (Singh, 1974, 1984a,b, 1989).

In this note we report a new paracentric inversion in an isofemale line of *D. ananassae* serialized as MR-20 in our laboratory which was initiated from a single female collected in Madurai, Tamil Nadu in December 1984. According to the reference map of polytene chromosomes of *D. ananassae* constructed by Ray-Chaudhuri and Jha (1966), the inversion extends from 2A to the end of 9B in the right arm of the second chromosome. The microphotograph of the new inversion (heterozygous) which has been named as zeta (ZE) is shown in Figure 1. Figure 2 depicts the location of zeta inversion in 2R and alpha in 2L. The zeta inversion occupies nearly 63 percent region of 2R. The alpha inversion occupies approximately 70 percent of 2L. The chromosome distance between alpha and zeta inversions is nearly 32 percent of the total length of the second chromosome.

Figure 1. Microphotograph of a new inversion (heterozygous) in the right arm of the second chromosome of *D. ananassae*.

Figure 2. Location of AL and ZE inversions in 2L and 2R, respectively.

Due to the presence of zeta inversion in 2R, three karyotypes, ST/ST, ST/ZE and ZE/ZE could be distinguished. From MR-20 stock, 205 larvae taken randomly from the culture bottles were analysed. The observed and expected (via Hardy-Weinberg rule) numbers of homo- and heterokaryotypes and the frequencies of ST and ZE gene arrangements are given in Table 1. There is no significant deviation from Hardy-Weinberg expectation ($P>0.05$).

Table 1. Observed and expected numbers of different karyotypes and frequency of different gene arrangements due to Zeta (new) inversion in 2R.

Total no. of larvae analysed		Karyotypes ST/ST	ST/ZE	ZE/ZE	Gene Arrangements ST	ZE
205	Obs.	91	84	30	65	35
	Exp.	86.61	93.28	25.11		
		$\chi^2 = 2.10$	d.f. = 1	P>0.05		

Since the stock is also polymorphic in 2L due to the presence of alpha inversion, all the three karyotypes ST/ST, ST/AL and AL/AL were scored and the data on the combinations between 2L and 2R karyotypes were obtained in order to test the intrachromosomal associations. Table 2 incorporates the observed and expected numbers of different

Table 2. Observed and expected associations of 2L and 2R karyotypes in MR-20 stock.

2R		2L ST/ST	ST/AL	AL/AL	Total
ST/ST	Obs.	19	54	18	91
	Exp.	10.21	46.17	34.62	
ST/ZE	Obs.	4	50	30	84
	Exp.	9.42	42.61	31.96	
ZE/ZE	Obs.	0	0	30	30
	Exp.	3.37	15.22	11.41	
Total		23	104	78	205
		$\chi^2 = 70.28$	d.f. = 4		P<0.001

combinations between 2L and 2R karyotypes. The expected numbers have been calculated from the marginal totals of an RxC contingency table under the assumption of random combination of karyotypes. The X^2 value shows highly significant variation between observed and expected numbers which suggests that the two linked inversions AL and ZE in the opposite limbs of the second chromosome of *D. ananassae* are associated non-randomly (linkage disequilibrium). Thus a new inversion in 2R (ZE) which is maintained at considerable frequency in a laboratory stock, shows linkage disequilibrium with AL inversion (cosmopolitan) in 2L.

Acknowledgments: The financial support in the form of a Senior Research Fellowship of CSIR, New Delhi to AKS is thankfully acknowledged. We also thank Dr. (Miss) Sujata Chatterjee for maintaining the isofemale line in the laboratory.

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Feeding habits of some Kumaun *Drosophilidae*.

All the organisms have a definite form, that is they occupy space so they can be considered to exhibit certain morphologic characters. Similarly every individual possesses some specific characters regarding their habit and habitats, specificity of their selective feeding and breeding sites, fully adapted to survive in its particular environment and possess its own pattern to live in a particular niche. A fair number of *Drosophila* flies can be collected from overripe fruits viz., banana, guava, citrus fruits, decaying vegetables and other sources like fungi. In addition to this a good majority of *Drosophila* species feed and breed in the laboratory on artificial food medium.

Table 1.

Sl. No.	Name of Drosophilid species	Name of fungal species
	GENUS - *DROSOPHILA* SUBGENUS - *Drosophila*	
1.	*Drosophila immigrans*	*Tubercularia coccicola* and *Mucor racemosus*
2.	*Drosophila lacertosa*	*Tubercularia coccicola* and *Mucor ambigans*
	GENUS - *DROSOPHILA* SUBGENUS - *Sophophora*	
3.	*Drosophila jambulina*	*Mucor nigricans*
4.	*Drosophila malerkotliana*	*Rhizopus nigricans*
5.	*Drosophila nepalensis*	*Mucor racemosus*
6.	*Drosophila takahashii*	*Rhizopus nigricans* and *Mucor ambigans*
	GENUS - *ZAPRIONUS*	
7.	*Zaprionus indiana*	*Penicilium chryogenum*

Given an abundance of resources, a population may be expected to restrict its niche to those resources that it can most profitably and safely exploit, yet specialization can only occur if resources are sufficiently abundant, long lasting and predictable to be available throughout the consuming stages of an organisms life cycle (Dethier, 1954; Levins and MacArthur, 1949; Schoener, 1971). Associations between host ephemerality and trophic generalization have been noted among some herbivorous insects, with a greater prevalence of polyphagous species on annuals and more monophagous species on herbaceous and woody perennials (Otte and Joern, 1977; Cates, 1980, 1981; Lawton and Strong, 1981). The drosophild fauna of an area serves as a sensitive indicator of the degree of ecological disturbance which has occurred. The ecological tolerance differs in each species and every individual has a tendency to move to sites which afford the optimal conditions for its successful living.

The group of species collectible on fungi or by baiting with fungi is somewhat heterogeneous, the fungal habitat also apparently has been invaded by several evolutionary lines. The full contribution of *Drosophila* in genetics will be incomplete without knowing its relation with ecology. Lacy (1984) stated that the population biology of the mycophagous guild of Drosophilidae flies is an attempt to learn how some species in this taxon respond ecologically and genetically to the challenges posed by a fungal feeding niche. Fungi have diversity of chemical defenses, some fungi produce one or more toxins which may have serious effect. Mycophagous insects evolved the means to avoid or detoxify the poisonous compounds and for successful survival they adapt themselves too.

During the present studies an attempt has been made to analyse the gut contents of some common Drosophilid species occurring in Kumaun region, so as to know their feeding habits. The Drosophilid flies were collected by net sweeping from different geographical localities of Kumaun region and were brought to the laboratory, washed thoroughly with distilled water and 70% alcohol and dried. The gut contents were cultured on P.D.A. (Potato Dextrose Agar) medium. The result of the culture has been shown in Table 1. The present result shows that all the Drosophilid studied so far, instead of feeding on common food substances also feed on some toxic and pathogenic fungi. Particularly

Tubercularia coccicola has been found pathogenic in human beings and *Penicilium chryogenum* is pathogenic to seeds. Perhaps these Drosophilids have developed the means to detoxify the toxic effects of these fungi.

Acknowledgment: We gratefully acknowledge Dr. M.C. Pant, Head of the Department, for facilities and to the U.G.C. for financial support.

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Singh, B.N. Banaras Hindu University, Varanasi, India. Chromosome inversions in *Drosophila ananassae* population from Siliguri, West Bengal.

Drosophila ananassae, a cosmopolitan and domestic species, presents a high degree of chromosomal polymorphism. In total nearly fifty paracentric inversions, ten pericentric inversions and fourteen translocations have been detected in this species (for references see the reviews by Singh, 1985, 1988). Out of several paracentric inversions known in this speices, only three are cosmopolitan in distribution (Shirai and Moriwaki, 1952; Futch, 1966; Singh, 1970). The three cosmopolitan inversions are subterminal (alpha or In(2L)A) in 2L, terminal (delta or In(3L)A) in 3L and basal (eta or In(3R)A) in 3R. The chromosomal analysis of twenty-five Indian populations have shown that the frequency of various gene sequences and the level of inversion heterozygosity vary among different populations (Singh, 1974, 1984a,b, 1989a,b). Furthermore, Indian natural populations of *D. ananassae* show a considerable amount of genetic divergence at the level of chromosomal polymorphism (Singh, 1984c, 1986).

The present note describes the results of chromosomal analysis of *D. ananassae* population from Siliguri, West Bengal. Flies were collected from Siliguri in March 1989. All the females collected from natural population were cultured individually in food vials and chromosomal analysis of F_1 larvae was done using acetocarmine method. The present quantitative analysis is based on the identification of karyotype of only one F_1 larva from each wild female.

Table 1. Observed and expected numbers of different karyotypes in *D. ananassae* populations from Siligh.

Total no. of larvae examined		KARYOTYPES											
		2L				3L				3R			
		ST/ST	ST/AL	AL/AL	X^2	ST/ST	ST/DE	DE/DE	X^2	ST/ST	ST/ET	ET/ET	X^2
28	Obs.	0	6	22		12	12	4		17	11	0	
	Exp.	0.32	5.36	22.32	0.43	11.57	12.86	3.57	0.13	18.08	8.84	1.08	1.63

Table 2. Frequencies (in percent) of different gene arrangements and mean number of heterozygous inversions per individual in *D. ananassae* population from Siliguri.

Total no. of chromosomes examined	GENE ARRANGEMENTS						Mean no. of heterozygotes inversions individual
	2L		3L		3R		
	ST	AL	ST	DE	ST	ET	
56	10.71	89.29	64.29	35.71	80.36	19.64	1.04

Chromosomal analysis of *D. ananassae* population from Siliguri revealed the presence of all three cosmopoliatn inversions. The observed and expected (via Hardy-Weinberg proportions) numbers of 2L, 3L and 3R karyotypes are given in Table 1. The X^2 values show insignificant deviation from Hardy-Weinberg expectation. Table 2 presents the data on the frequencies of different gene arrangements in 2L, 3L and 3R due to the rpesence of three cosmopolitan inversions and the mean number of heterozygous inversions per individual. The frequency of AL inversion is nearly 90 percent while the chromosomes with delta and eta inversions are less frequent than those with standard sequence. The mean number of heterozygous inversions per individual is 1.04. Thus, the natural populations of *D. ananassae* from Siliguri situated in West Bengal exhibits chromosomal variability due to the presence of three cosmopolitan inversions which have been detected earlier in most Indian populations analysed.

Acknowledgments: I thank Mr. Aparup Das for collecting *D. ananassae* flies from Siliguri.

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Singh, B.N. Banaras Hindu University, Varanasi, India. A new translocation in Indian *Drosophila ananassae*.

Drosophila ananassae, a cosmopolitan and domestic species, presents a high degree of chromosomal polymorphism (for references see the reviews by Singh, 1985, 1988). A large number of pericentric inversions and translocations which are rare in other species of *Drosophila*, have been detected in natural populations of *D. ananassae*. This reflects unusual mutational properties of this species. Freire-Maia (1961) suggested that it has developed some special mechanisms through which it can retain, in its natural populations, chromosome rearrangements which are disadvantageous.

The present note describes a new translocation detected from an Indian natural population of *D. ananassae*. The flies were collected from Gauhati in November 1989. A new translocation was detected in a single F_1 larva of a naturally impregnated female collected from Gauhati. The larva was heterozygous for the reciprocal translocation which involves the left limbs of the second (2L) and third (3L) chromosomes and it has been designated as T (2L-3L) 15. Figure 1 shows the microphotograph of heterozygous translocation. The break points are near 1C in 2L and 4C in 3L in the reference map constructed by Ray-Chaudhuri and Jha (1966). The 2L chromosome is homozygous for alpha or In (2L)A inversion (AL/AL) whereas the 3L is homozygous for the standard sequence (ST/ST).

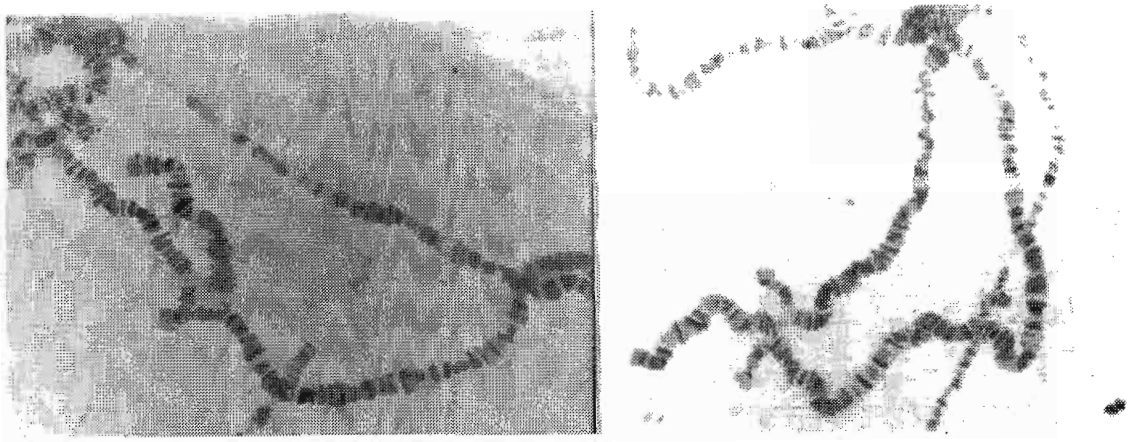

Figure 1. Microphotograph showing heterozygous translocation T(2L-3L)15 in *D. ananassae*.

Thus including the new translocation reported here, a total of fifteen translocations are known in *D. ananassae*. All the translocation detected in *D. ananassae* are listed in Table 1.

Acknowledgments: I thank Mr. Aparup Das for collecting *D. ananassae* flies from Gauhati.

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Table 1. Translocations detected in *Drosophila ananassae*.

	Translocation	Investigators
1.	IIIL - IV	Kikkawa 1938
2.	IIL - IIIL	Dobzhansky & Dreyfus 1943
3.	IIR - IIIR	Freire-Maia 1961
4.	IIIL - IV	Ray-Chaudhuri & Jha 1966
5.	(XL - 2R)A	Futch 1966
6.	(XR - 2R)	Sajjan & Krishnamurthy 1970
7.	(2R - 3R)	Sajjan & Krishnamurthy 1970
8.	(XR - 2L)8	Reddy & Krishnamurthy 1972b
9.	(2L - 3L)9	Reddy & Krishnamurthy 1972a
10.	(2L - 3L)10	Sajjan & Krishnamurthy 1972
11.	T(Y - 2)A	Hinton & Downs 1975
12.	T(Y - 2)B	Hinton & Downs 1975
13.	T(Y - 2)A	Hinton & Downs 1975
14.	T(2 - 3)B	Hinton & Downs 1975
15.	T(2L - 3L)15	Present Study

Singh, B.N. Banaras Hindu University, Varanasi, India. Sex ratio in *Drosophila ananassae*.

The heterogametic sex in *Drosophila* forms equal proportions of female- and male-producing gametes which results in approximately equal ratios of females and males. However, a significant deviation from 50:50 ratio has been found in certain species. In *Drosophila willistoni* and some other species, an abnormal sex ratio when predominantly or exclusively female progeny are produced, has been found which is transmitted through maternal cytoplasm. On the other hand, the abnormal sex ratio found in *D. pseudoobscura* is carried through the male line and is chromosomally determined (for details see Levine, 1969).

In laboratory populations of *D. melanogaster*, Reed and Reed (1950) found more females than males among 25,906 flies counted and the deviation from 50:50 ratio was highly significant. The females develop faster and therefore tend to exploit the available food supply better. Levitan (1954) also found more females than males in cage populations of *D. robusta* as a result of differential mortality favoring the females during the adult period.

Drosophila ananassae is a cosmopolitan and domestic species. It is a genetically unique species as it possesses many unusual features (Singh, 1985, 1988). In order to know sex ratio in *D. ananassae*, 22 wild laboratory stocks initiated from mass cultures were used for counting the flies. The stocks used are: MR-Madurai; JM-Jammu: JP-Jamalpur; BO-Bombay; EK-Ernakulam; GO-Goa; QL-Quilon; BR-Baripada; GH-Ghazipur; B-Bhubaneswar; PU-Puri; BH-Bhagalpur; MD-Madras; CA-Calcutta; VN-Varanasi; SH-Shillong; KK-Kanyakumari; BP-Biralapur; R-Rameswaram; AG-Agra; PT-Patna and SG-Siliguri. All these stocks were cultured in food bottles and adult females and males were counted. The number of bottles used varied for different stocks and the number of flies also varied in different bottles.

Table 1. Numbers of females and males in wild laboratory stocks of *Drosophila ananassae*.

Stocks	Females	Males	Total	χ^2
MR	447	535	982	7.89**
JM	614	597	1211	0.24
JP	687	715	1402	0.56
BO	642	734	1376	6.16*
EK	731	789	1520	2.22
GO	658	676	1334	0.24
QL	894	917	1811	0.30
BR	1240	1183	2423	1.34
GH	830	930	1760	5.68*
B	783	842	1625	2.14
PU	599	676	1275	4.65*
BH	551	564	1115	0.15
MD	626	688	1314	2.93
CA	638	570	1208	3.83
VN	612	774	1386	18.94***
SH	611	636	1247	0.50
KK	440	436	876	0.02
BP	632	572	1204	2.99
R	1181	1184	2365	0.002
AG	716	823	1539	7.44**
PT	839	891	1730	1.56
SG	771	815	1586	1.22
Total	15742	16547	32289	20.06***

*P < 0.05 **P < 0.01 ***P < 0.001

Table 1 shows the number of females and males in various wild laboratory stocks of *D. ananassae*. In most of the stocks, the number of males is more than that of females. The deviation from 50:50 ratio is statistically significant in six stocks. When the data of all the stocks have been pooled, males are more than females (total number of 32,289 flies counted) and the difference is highly significant ($P < 0.001$). This suggests that there is a significant excess of males in *D. ananassae*. Thus *D. ananassae* differs from *D. melanogaster* and *D. robusta* in which a high excess of females has been found.

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This bibliography includes the papers published on *Drosophila ananassae*. However, the abstracts of symposia/ conferences have not been included. I apologize to the readers for inadvertent omission of any paper. I thank Sujata Chatterjee and Arvind Kumar Singh for their help in preparation of this bibliography.

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[1]Molecular Biology Unit, Tata Institute of Fundamental Research, Homi Bhabha Road, Bombay 400 005, India; and [2]Department of Biology, Yale University, P.O. Box 6666, New Haven, CT 06511, USA. How do the fine structures of neuropils of larva and adult differ in *Drosophila*?

The gross shape and appearance of the larval and adult central nervous systems (CNS) of *Drosophila* are quite different (Bodenstein, 1965; Miller, 1965). However, at the cellular and sub-cellular levels, differences in the morphology of larval and adult systems are not so apparent. As for many other insects, *Drosophila* larvae and adult both have neurons whose soma form a rind/cortex around a core of intricately tangled mass of arborizations, usually known as neuropil. Neuropils in the larva and adult which are devoid of cell bodies, at first sight look very much alike at the fine structural levels. The aim of this study was to know how the fine structure of larval and adult neuropils differ in *Drosophila*.

Newly emerged, second or third instar larvae and 3-5 day old adult *Drosophila melanogaster* Canton Special strain grown at 22°C were used. The CNS was dissected out from larva submerged in Karnovsky's fixative, which was used as the first fixative (Karnovsky, 1965). Dalton's fixative was used as a postfixative (Dalton, 1955). Rest of the electron microscopy methods used were essentially similar to those employed in our earlier studies (Singh and Singh, 1984).

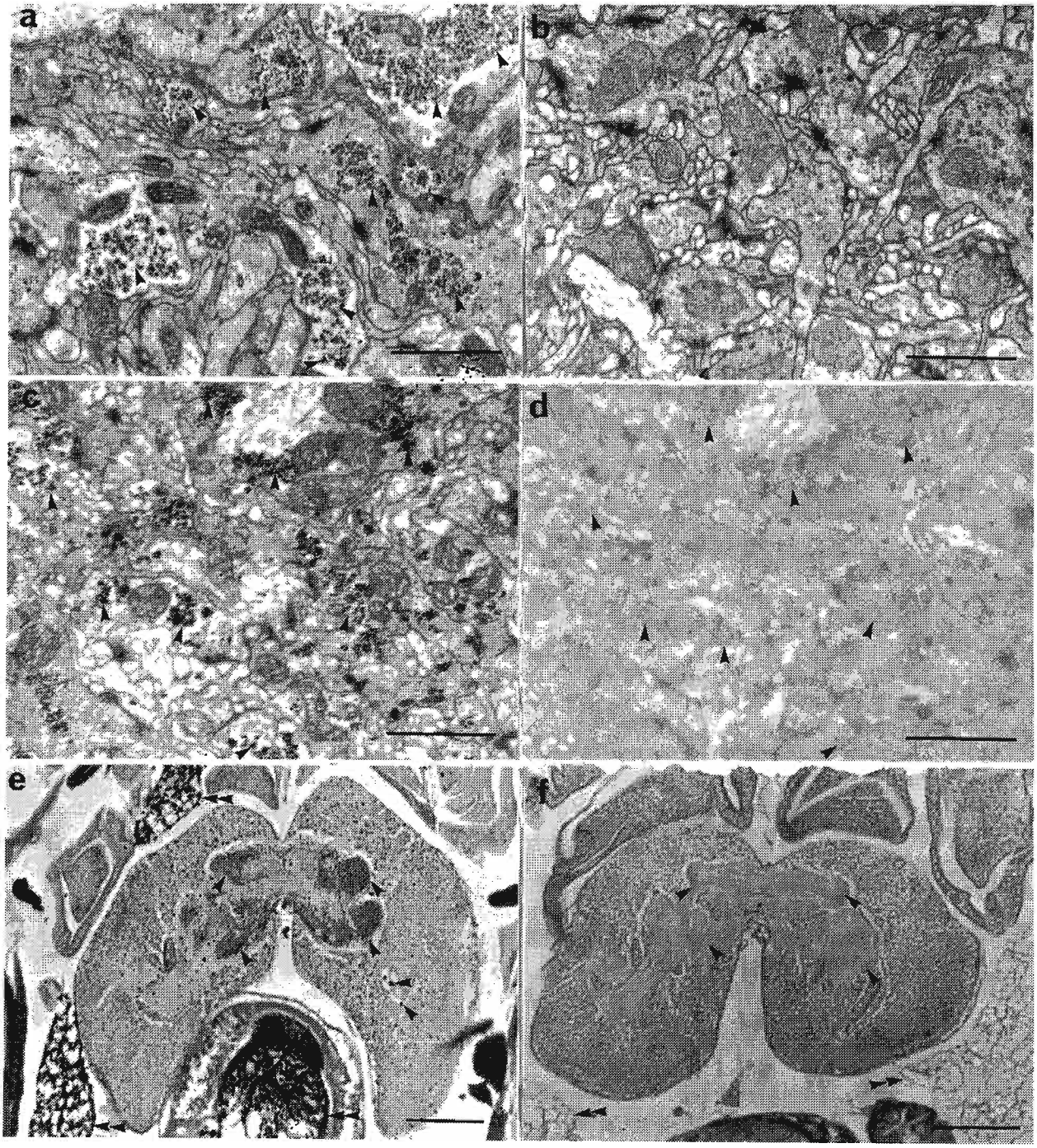
Electron microscopic examination of the ultrathin sections of larval CNS, which were stained with uranyl acetate followed by Reynold's lead citrate (Reynolds, 1963) showed the presence of aggregates of electron dense particles in the neuropils (Figure 1, arrow marked). The typical size of an individual particle in the aggregation was approximately 20nm. In appearance, the electron dense particles were similar to granular precipitates, which were usually surrounded by a region translucent to electrons (Figure 1a). These electron dense particles were present in the neuropils of the larval CNS, including the brain hemispheres and the ventral ganglia. Even one hour after hatching, the larvae were found to contain these particles in the neuropils. These particles were found to be present also in the neuropils of *Drosophila* pupae until they were white. In contrast, neuropils of the adult brain and thoracic ganglia of *Drosophila* are devoid of these electron dense particles (Figure 1b). Our studies with the third instar larvae of *Musca domestica* also showed the presence of electron dense particles, while neuropils of adult housefly were found to be devoid of such particles.

For the characterization of the aggregates of eletron dense particles observed by transmission electron microscopy, ultrathin sections of the larval CNS were collected individually on 'Formvar' coated copper slots with 2 x 1 mm window. Alternate sections were stained with 4% uranyl acetate aqueous solution at 60°C for 10 minutes, followed by staining with lead citrate (Reynolds, 1963) for five minutes at room temperature. The remaining sections were stained only with 4% uranyl acetate aqueous solution at 60°C for 10 minutes.

The Figure 1c is an electron micrograph of a section of the larval neuropil, stained with uranyl acetate followed by lead citrate, while Figure 1d is an electron micrograph of a neighboring section stained only with uranyl acetate. It was observed that these particles were not significantly stained with uranyl acetate alone and were stained well when followed by lead citrate staining (Figure 1c).

Characterization of the aggregates of the electron dense particles was also done by light microscopy. Eight um thick sections from paraffin embedded blocks when stained according to periodic acid Schiff (PAS) staining technique (Hotchkiss, 1948; Pearse, 1972), showed many purple-red deposits in the neuropils as well as some in the cortex of the larval CNS (Figure 1e). Celestin blue B, which stains nuclei dark-blue and is optional was omitted (Pearse, 1972).

Figure 1. (a) Electron micrograph of neuropil of third instar larval brain of *D. melanogaster* showing aggregates of electron dense particles (arrowheads). (b) Electron micrograph of neuropil of adult *D. melanogaster* brain devoid of aggregates of electron dense particles. (c) Electron micrograph of an ultrathin section of the neuropil of third instar larval brain, stained with uranyl acetate followed by lead citrate. Lead citrate specifically stains these particles, indicating them to be glycogen or glycogen-like deposits (arrowheads). (d) Control of Figure c, where lead citrate staining was omitted showing that the particles are not significantly stained by uranyl acetate. Magnification bar in (a) - (d) = 1 um. (e) Photomicrograph of 8 um thick section of third instar larval brain stained by PAS technique. Arrowheads show some of the many glycogen deposits in the neuropil and cortex. Fat bodies and proventriculus known to be bulk deposits of glycogen also show intense positive staining with PAS technique (double arrowheads). (f) Control of Figure e, where section was treated with alpha-amylase which abolishes subsequent PAS staining of aggregates of electron dense particles (arrowheads) and other known bulk deposits of glycogen such as fat body (double arrowheads) and proventriculus. Magnification bar in (e) and (f) = 50 um.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Alternate ribbons used as controls were first treated with 0.5% alpha-amylase (Sigma, type II A) solution in 0.1 M sodium phosphate buffer pH 6.9 at 37°C for 30 minutes and then stained by PAS technique (Hotchkiss, 1948; Pearse, 1972). The treatment with alpha-amylase abolished the PAS response of eletron dense particles (Figure 1f). Fat bodies and proventriculus which are known to have the bulk of glucogen in mosquito larva (Wigglesworth, 1942), also showed intense PAS positive reaction in *Drosophila* larvae (Figure 1e, double arrowheads). Serving as an internal control this staining is also abolished by pretreatment with alpha-amylase (Figure 1f, double arrowheads).

The following morphological and histochemical properties may be attributed to the aggregates of electron dense particles: (1) In ultrathin sections, these particles were specifically stained by lead citrate and not by uranyl acetate and they appear as granular deposits. (2) Individual particles in the aggregation were about 20 nm in size and devoid of any membrane boundary. (3) The aggregates of these electron dense particles were positively stained in light microscopy with PAS technique and subsequent to the treatment with alpha-amylase their staining was abolished. The PAS technique is known to give positive color reaction with starch, glycogen, mucin, hyaluronic acid, reticulin, fibrin, hyalin, and most basement membranes. Of these starch is usually present in plant cells, reticulin and fibrin are proteins which would not become PAS reagent negative on pretreatment with alpha-amylase. In addition, mucin, hyaluronic acid and hyalin are either extra-cellular or membrane associated. Whereas, these particles are intracellular PAS stained positive deposits and digestible with alpha-amylase (Lehninger, 1983). On the basis of the above criteria, these aggregates of electron dense particles resemble known glycogen. These deposits are very similar in appearance and size to the glycogen deposits found in vertebrate liver cells--hepatocytes (Porter and Bonneville, 1968; Rhodin, 1963).

Wigglesworth (1949) studied the distribution of glycogen in dissected specimens and paraffin/celloidin embedded tissue sections of adult *Drosophila*. The bulk of the glycogen was found in the fat body of the abdomen, halteres, proventriculus, throughout the indirect flight muscles as minute granules and in small amounts in the cells of the mid-gut. Only traces of glycogen were found in the cells of the CNS (Wigglesworth, 1949). No glycogen was detected in the neuropil of the adult *Drosophila*.

Presence of glycogen was also noted and studied in *Drosophila* in relation to changes associated with ageing (Takahashi et al., 1970; Miquel, 1971), or senescence (Burch et al., 1970). Butterworth et al., (1965) found deposits of lipid and glycogen in the adipose tissue of second and third instar larvae and the adult female of *D. melanogaster*. These studies and our finding that glycogen is present in the larval neuropils but not in the neuropils of adult *Drosophila* are in agreement. It is interesting to note that in the study with mosquito larva, Wigglesworth (1942) observed that glycogen occurs throughout the ganglia and nerve cord of the CNS in the form of granular deposits and small vacuoles.

Glycogen is known to be degraded to trehalose -- a disaccharide -- and serves as a major source of energy in adult *Drosophila* (Clegg and Evans, 1962). It is conceivable that the larval neuropil of *Drosophila* during the development and metamorphosis of adult neuropil, meets part of its energy requirement from the glycogen stored in the neuropil. The energy reserve in the form of glycogen in the larval neuropil is mostly consumed by the time the formation of the adult CNS takes shape in *Drosophila*.

Acknowledgments: We thank Ms. Shubha Shanbhag for helpful suggestions.

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Smith, M.T. and R.B. Huey. University of Washington, Seattle, Washington USA. Ether and CO_2 affect heat tolerance in *Drosophila melanogaster.*

We examined the effects of anesthetics (CO_2 and ether) on the ability of *Drosophila melanogaster* to survive heat shock. Two-week old flies (raised at 25°C) were anesthetized for about 1 min. with CO_2 or with ether. A control group was untreated. Approximatley 20 hours later, all flies were heat shocked (under high humidity) for 30 min. at 39.3°C, quickly cooled, returned to vials that had a high humidity, and maintained at 25°C for 18 hours (see Huey et al., 1991). Vials (N=12/ group) were scored for the percentage that were alive ("alive" flies were able to move their limbs when touched). In Experiments 1 and 2, we compared survival in CO_2, ether, and control groups. In Experiment 3, we compared survival in CO_2 and control groups only.

Table 1: Percentage of flies surviving a heat shock.

		Percentage alive ± Standard error		
		Males	Females	Pooled
Experiment 1:				
	Control	14 ± 6	29 ± 5	14 ± 11
	CO_2	2 ± 2	14 ± 11	6 ± 8
	Ether	16 ± 9	29 ± 14	19 ± 20
Experiment 2:				
	Control	4 ± 2	36 ± 3	10 ± 10
	CO_2	2 ± 1	17 ± 7	4 ± 4
	Ether	21 ± 9	38 ± 18	21 ± 15
Experiment 3:				
	Control	9 ± 9	33 ± 18	21 ± 18
	CO_2	3 ± 3	17 ± 11	10 ± 10

Anesthesia seems to effect the heat tolerance of flies 20 hours after exposure. CO_2 reduces heat tolerance, whereas ether may even improve it. For pooled data (males and females, Table 1), the three treatment groups showed a marginally significant heterogeneity in survival for Experiment 1 (Kruskal-Wallis test, $P < .10$) and significant heterogeneity in Experiment 2 ($P < .025$). For data with the sexes separated, heterogeneity was significant for males (Table 1, P's < .05), but not for females (P's < .25). Males and females showed similar trends. In paired comparisons (Wilcoxon-U test) in which probabilities were combined from male and female comparisons (Fisher combined probablity test), CO_2 reduced heat tolerance ($P < .01$; Experiments 1-3), but ether may improve survival ($P < .10$; Experiments 1-2).

Anesthesia has diverse behavioral and physiological effects on *Drosophila* (Dijken, Sambeek, and Scharloo, 1977; Hooper, 1970; Huey, Partridge, and Fowler, 1991; Nicolas and Sillans, 1989; Stark, 1972). Our data show that anesthesia can also confound measurements of heat tolerance even one day after exposure.

References: Dijken, F.R., M.J.P.W. Sambeek, and W. Scharloo 1977, Experientia 33:1360-1361; Hooper, G.H.S. 1970, J. Econ. Entomol. 63:1962-1963; Huey, R.B., L. Partridge, and K. Fowler 1991, Evolution in press; Nicolas, G. and D. Sillans 1989, Ann. Rev. Entomol. 34:97-116; Stark, W. 1972, DIS 48:82.

Sneddon, A. and A.J. Flavell. The University of Dundee, Scotland. *Copia* transcript levels in cultured *Drosophila* cells are unresponsive to heat shock.

The retrotransposon *copia* is present in about 100 to 200 copies in cultured cells. It encodes two major RNAs (5kb and 2kb RNAs) and several minor RNAs which together comprise several percent of the total polyadenylated RNA in cultured cells and somewhat less in flies. Transcript levels of *copia* in flies have been reported to be induced approximately six-fold under a variety of environmental stresses including 37° heat shock (Strand and McDonald, 1985). During our studies of the *copia* promoter elements we have observed that this enhancement of transcription is not exhibited by the two cultured *Droposphila* cells lines which we have studied.

We purified total RNA from *D. melanogaster* Kc cells which had either been incubated at 25° or heat shocked at 37° for 30 or 60 min (Figure 1A). Dilution series of each preparation were electrophoresed on a 1.2% formaldehyde-agarose gel containing ethidium bromide. The gel was photographed (Figure 1A) and then blotted to nitrocellulose. Probing this Northern blot with *copia* shows that heat shock induced no increase of *copia* transcripts relative to ribosomal RNA, or the total amount of RNA loaded.

We have also tested the messenger ability of a *copia*-CAT fusion RNA following transfection onto similar cells (Gorman *et al.*, 1982; Figure 1B) or DH 33 *Drosophila hydei* cells (not shown). No enhancement of CAT activity was seen.

These results do not directly contradict those of Strand and McDonald (1985) because these authors studied transcript levels in whole flies. However, it is surprising that a promoter responds differently to heat shock in different cell types of the same species when the heat shock promoter is expressed efficiently in both cultured cells and flies (our

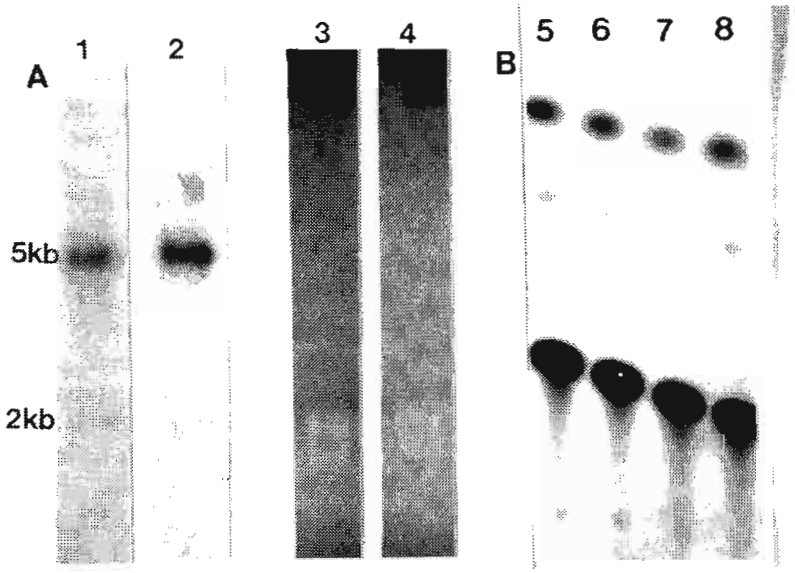


Figure 1. A. Total RNA from 10^5 Kc cells, grown at 25° then harvested (lanes 1 and 3) or heat shocked at 37° for 60 min (lanes 2 and 4), was electrophoresed on a 1.2% formaldehyde-agarose gel. The gel was photographed (lanes 3 and 4) then blotted and probed with *copia* (lanes 1 and 2). B. CAT assays on transiently expressed pKS-copCAT (containing the CAT gene fused to *copia* at nucleotide 820 [Sneddon and Flavell, 1989], *copia*-CAT1 [Di Nocera and Dawid, 1983] fuses the CAT gene to nucleotide 283 of *copia*) in Kc cells. Cells were heat shocked at 37° for 30 min then allowed to recover at 25° for 60 min before assay (4). Two dishes were used for each assay, both results are shown. Lanes 5 and 7: minus heat shock, lanes 6 and 8: heat shocked.

unpublished results).

Braude-Zolotarjova and Schuppe (1987) reported that the *copia*-CAT 1 recombinant plasmid produces higher levels of CAT enzyme activity after heat shock when transiently expressed in 67j25D cultured *D. melanogaster* cells or 79fDv3g cultured *D. virilis* cells. This plasmid contains a smaller fragment of *copia* than ours (see Figure legend) and perhaps importantly, lacks the majority of the 5' untranslated region of the *copia* RNAs. This untranslated region also contains a transcriptional enhancer (Sneddon and Flavell, 1989). It is possible that the heat shock response can only be detected when the *copia* enhancer is missing or not functioning.

Another possibility is that this is a post-transcriptional effect. The untranslated leader of heat shock genes strongly affects post transcriptional expression of these genes under heat shock conditions (McGarry and Lindquist, 1985; Hultmark, Klemenz and Gehring, 1986). It may be that the difference between our results and those of Braude-Zolotarjova and Schuppe is due to a similar phenomenon for *copia* gene expression.

References: Braude-Zolotarjova, T.Y. and N.G. Schuppe 1987, Dros. Inf. Serv. 66:33; Gorman, C.H., LF. Moffat and B.H. Howard 1982, Mol. Cell Biol. 2:1044-1051; Hultmark, D., R. Klemenz and W.J. Gehring 1986, Cell 44:429-438; McGarry, T.J. and S. Lindquist 1985, Cell 42:903-911; Sneddon, A. and A.J. Flavell 1989, Nucleic Acids Res. 17:4025-4035; Strand, D.J. and J.F. McDonald 1985, Nucleic Acids Res. 13:4401-4409.

Sparrow, J.C. Department of Biology, University of York, U.K. *veinlet* is a recessive flightless mutation affecting wing function.

The flight ability of a number of strains which carry recessive markers has been tested by releasing flies singly from 20 cm above the bench and recording whether they fly up (U), horizontally (H), down and to the side (D) or do not fly at all (N). Among 25 inbred strains tested two proved flightless. One of these was *w; ve e ca* (Table 1).

When males from either reciprocal cross between the *Canton-S* and *w; ve e ca* strains were flight-tested they proved not to be flightless (data from one set of males is included in Table 1) indicating an autosomal recessive inheritance of the trait. The F_1 females from these crosses were backcrossed to the *w; ve e ca* strain. The recombinant progeny were scored and flight-tested. While 96.8% of *ve* recombinants (n = 150) were flightless, only 9.7% of ve^+ recombinants (n = 152) were flightless. This indicates either that flightlessness is closely linked to *ve*, or that the phenotype is due to the *veinlet* mutation and the apparent cross-overs are a consequence of the flight-testing system. The latter explanation seems more likely for three reasons: 1) of the *ve* flies which flew none flew 'up'. They all were judged capable of some horizontal flight; 2) in Table 1 the flighted *Canton-S* wild-type strain has some flies which, for unknown reasons, failed to fly, suggesting that the flightless ve^+ flies might have a similar origin; and 3) *ve* homozygotes produced by crossing *w; ve e ca* to a *w; ve h TE 39 e bar-3/In(3LR)D, ru h* D^3 are flightless. These are two inbred strains obtained from different sources.

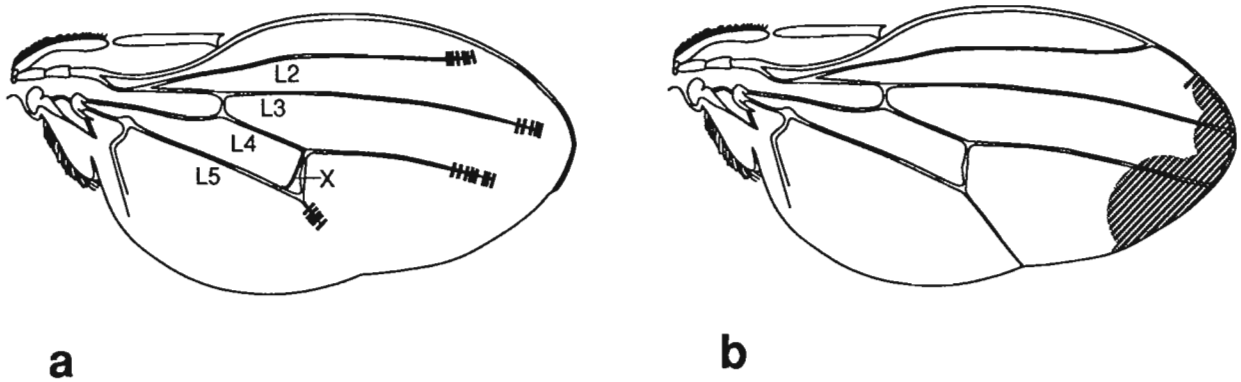


Figure 1. Composite diagrams of a) *ve* wings and b) *Ser* wings. (sample size 20 wings. In a) each vertical line indicates the terminus of at least one wing vein in one or more wing; the bold line (X) shows the position of the crossvein in the absence of any extension of L5: in b) the shaded area is a composite of all the areas missing in the sample of *Ser*/+ wings from sibs of those flight-tested (Table 1).

It seems likely that the *veinlet* mutation and its effects on venation are responsible for the flightlessness; *ve* flies do attempt flight, performing both jumping, wing raising and wing lowering but the wing-beat cycle is not initiated. The visible effects of *ve* on wing structure are not large, particularly in the strain used. The phenotype (Figure 1a) is less extreme than that shown in Lindsley and Grell (1968). Given that flies with defects of the wing-blade can fly (e.g. *Ser*/+ flies), and fly as as well as wild-type, (Table 1) the extreme flightlessness of *ve* with such a slight effect on venation was surprising. The defects produced by these mutants are very different and cannot be directly compared. Some conclusions are possible. Neither the absence of the wing-tip to varying degrees in *Ser*/+ flies (Figure 1b) nor the absence of part of the thickened wing margin and the attachment of wing-veins L3 and L4 appear critical for flight. At a simple level this suggests that the reduced length of veins L3 and L4 in *ve* flies is not what makes flies of this genotype flightless. Possible explanations for *ve* flightlessness come from the recent work of Ennos (1988a,b) on the importance of the different parts of the wing structure in enabling the wing to perform passively the figure-of-eight movement during the wing-beat cycle. An important feature of his interpretations is that the posterior part of the wing has a major role in changing the direction of wing movement. Since vein L5 is the only stiffening of this part of the wing in *Drosophila*, its absence may cause the *veinlet* flightlessness.

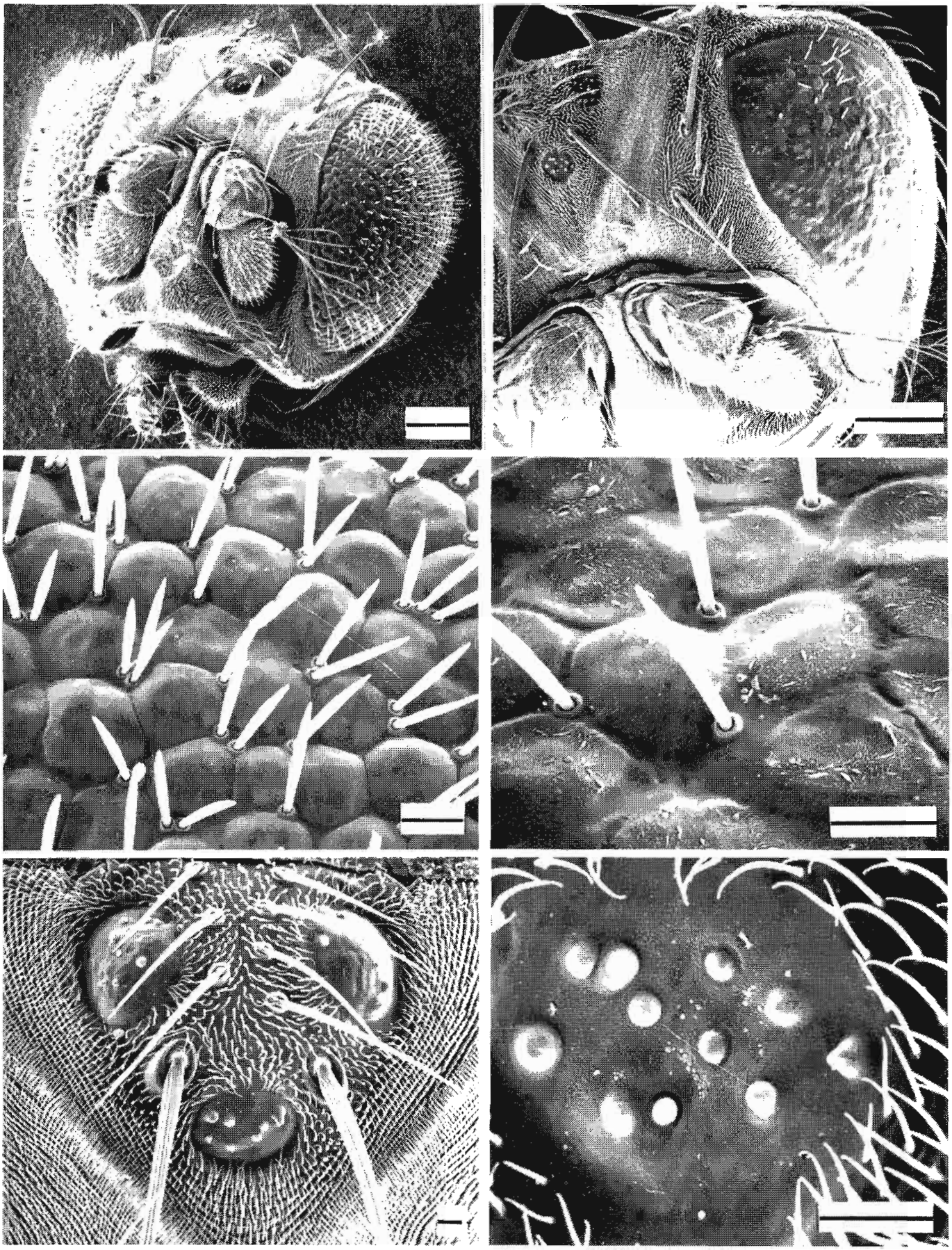
Table 1. Flight-testing of 2-5 day old adults of different genotypes.

Genotype	U	H	D	N	%Fli⁻ (= [D + N]/Total x 100%)
Canton-S	35	5	4	0	9.1
w; ve e ca	0	0	0	31	100.0
w/Y; +/ve e ca	32	9	4	5	18.0
TM3,Ser Sb/+	24	4	1	0	3.4

References: Lindsley, D.L. and E.H. Grell 1968, Genetic variations of *Drosophila melanogaster*; Ennos, A.R. 1988a, J. exp. Biol. 140:137-160; _____ 1988b, J. exp. Biol. 140:161-169.

Stark, W.S. and S.D. Carlson. University of Missouri, Columbia and University of Wisconsin, Madison. Comparison of the surfaces of gl^{none}'s ocelli and compound eyes with those of several *glass* alleles.

Drosophila mutants with structural abnormalities of the compound eyes and the simple eyes (ocelli) continue to be very useful in determining the properties underlying normal development. Among the genes which, if mutant, cause such abnormalities is *glass* (*gl*) which has a long history of study (Csik, 1929; Garen and Kankel, 1983; Johannsen, 1924; Meyerowitz and Kankel, 1978; Pak et al., 1969). Recent interest in this gene has intensified since it has been determined that it codes for a certain type of DNA-binding protein important in gene regulation, a protein having zinc-fingers (Moses et al., 1989). We had reported the structure of the ocelli and compound eyes of a mutant [*none* = no ocelli narrow eyes, (Stark et al., 1984, 1989)] similar in morphology to a mild *glass* allele [gl^3]. The purpose of this note is to report that *none* is indeed an allele of *glass* (and hence we propose



to tentatively rename it gl^{none}) and also to present scanning electron micrographs (SEM's) of the simple and compound eyes of weak and strong *glass* alleles for comparison with our earlier reports on gl^{none}. These micrographs are particularly important because views of the ocellar remnants in *glass* mutants are lacking from the literature.

This work was initiated after discussion with Kevin Moses of the University of California - Berkeley (Moses et al., 1989) who then sent us 4 *glass* stocks [gl^1, gl^2 e^4 {e = *ebony*}, gl^3 and gl^{60j}]. We set up the appropriate crosses between these 4 stocks and our gl^{none} stock. All the progeny showed the mutant phenotype indicating that gl^{none} is an allele of *glass*.

The accompanying plate shows the relevant surface morphology of the weak allele (gl^3 - left) which is like that of gl^{none} and of a representative strong allele (gl^{60j} - right) which is like that of gl^1 and gl^2. Heads were cut off, dehydrated in alcohol, critical point dried, sputter coated with gold and viewed and photographed with a Hitachi S-570 microscope. The top shows the head (calibration = 100 um) while the middle and bottom show higher magnifications of the compound eye and the ocelli respectively (calibrations = 10 um). The compound eye of gl^3 is disorganized and looks just like that of gl^{none}. Underneath gl^{none}'s slightly disarrayed cornea and distal optics, there is a retina of mostly pigment cells with no rhabdomeres much like what was observed for gl^3 earlier (Johannsen, 1924; Csik, 1929; Garen and Kankel, 1983). However retinula cell axons can be seen to cross the basement membrane in gl^{none}'s compound eye, and some synapses are present in its lamina ganglionaris (Stark et al., 1989) in agreement with (Garen and Kankel, 1983)'s observations on gl^3. The compound eye of gl^{60j} is much less ordered. For instance, there are corneal hairs only in the center. Inside, based on pilot transmission electron microscopy, there are red eye pigment granules only in the center, these being absent in the rim, and the disarray is more pervasive. Ocellar lenses in all 3 *glass* alleles are the typical size, but lack wild type's bulge; gl^3, gl^{60j} and gl^{none} have small protuberances in the ocellar lens which had been described before but not shown. There are no ocellar retinula cells under this lens in gl^3 (Moses et al., 1989) and gl^{none} (Stark et al., 1989). In all cases, the lenses of the compound eye and ocelli are roughened with "corneal nipples," (best seen in the highest magnifications of the middle and bottom right) an adaptation against reflection most likely laid down as a replica of the microvilli as the corneagenous cells start their job.

Is gl^{none} one and the same with gl^3? Kevin Moses (personal communication) has evidence for too much molecular similarity for coincidence, suggesting that the two alleles are identical. However, the gl^{none} stock originated in Allen Shearn's lab, and, at the time, it seemed like a new mutant. It cropped up in a cross involving a chromosome with *Glued* (from the Caltech stock center around 1968) and a compound chromosome. Although gl^3 should not be on these chromosomes, it is possible that gl^3 was present but masked on the former chromosome or was present but released by a double crossover from the latter chromosome. If either of these possibilities is the case, then gl^{none} is really gl^3.

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The phenotype produced by two dominant *apterous* mutations, $apterous^{ID}$ and $apterous^{Xa}$.

Mutations at the *apterous (ap;* 2-55.2) locus produce phenotypes in the adult wing that range from severely deficient wings to wings with pattern repetitions and transformations (Butterworth and King, 1965; Stevens and Bryant, 1985, 1986). Several temperature sensitive alleles have been characterized (Wilson, 1981; Stevens and Bryant, 1986) and produce temperature sensitive periods ranging from the late second to the mid third larval instar; the length of the temperature sensitive period is dependent on the severity of the allele. A dominant temperature-sensitive allele, ap^{ID}, when heterozygous with ap^+, produces a strap wing phenotype at 17°; at higher temperatures, the wing becomes progressively more reduced. A second non-temperature sensitive mutation, ap^{Xa}, produces a mitten-shaped wing when heterozygous with ap^+. Animals heterozygous for both ap^{ID} and ap^{Xa} frequently show duplications of the notum accompanied by loss of wing tissue. The temperature sensitive period of the duplicated thorax phenotype is shown in Figure 1 and is consistent with that reported for other *apterous* mutations (Wilson, 1981; Stevens and Bryant, 1986). The tsp ranges from the early-third to the mid-third larval instar.

The duplications produced by ap^{ID}/ap^{Xa} heterozygotes resemble the notal duplications produced by the *wingless*

Table 1. Wing phenotypes of apterous heterozygotes and wingless apterous compound heterozygotes.

°C	Parental Cross	F1 Genotype	Mitten	2 Strap	1 Strap 1 Duplication	2 Duplication	N
17°	ap^{ID}/ap^{Xa} x ap^{ID}/ap^{Xa}	ap^{ID}/ap^{Xa}	0.0	99.5	0.5	0.0	198
17°	wg^1 x ap^{ID}/ap^{Xa}	wg^1 ap^+/wg^+ap^{ID}	0.0	32.0	38.3	29.7	128
17°	wg^1 x ap^{ID}/ap^{Xa}	wg^1 ap^+/wg^+ap^{Xa}	100.0	0.0	0.0	0.0	464
27°	cn x ap^{ID}/ap^{Xa}	ap^+ $cn/ap^{ID}cn^+$	0.0	99.3	0.7	0.0	307
27°	cn x ap^{ID}/ap^{Xa}	$ap^+cn/ap^{Xa}cn^+$	100.0	0.0	0.0	0.0	473
27°	ap^{ID}/ap^{Xa} x ap^{ID}/ap^{Xa}	ap^{ID}/ap^{Xa}	0.0	81.3	12.1	6.5	1780
27°	wg^1 x ap^{ID}/ap^{Xa}	wg^1 ap^+/wg^+ap^{ID}	0.0	14.0	28.7	57.3	422
27°	wg^1 x ap^{ID}/ap^{Xa}	wg^1ap^+/wg^+ap^{Xa}	100.0	0.0	0.0	0.0	1346

Wild type animals were crossed with wg^1 at both 17°C and 27°C. All F1 progeny had normal wings.

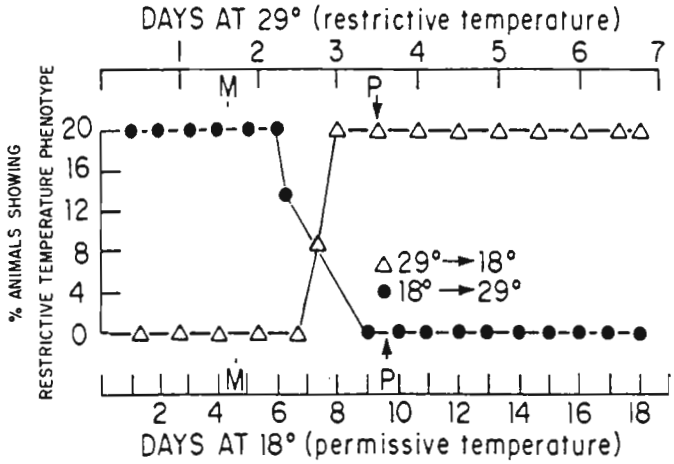

Figure 1. Ts period determination for the duplicated thorax phenotype in ap^{ID}/ap^{Xa} animals. closed circle = time of upshift from 18 to 29°; open triangle = time of downshift from 29 to 18°. M indicates the time of the second to third-instar molt, and P indicates the time of puparium formation. Phenotype was assessed by measuring the percentage of survivors showing the "restrictive-temperature phenotype" (notal duplications).

(wg) mutation (Sharma and Chopra, 1976; Morata and Lawrence, 1977). The *wingless* gene has been cloned and the pattern of expression in embryos and imaginal discs has been characterized (Baker, 1987, 1988), showing that the *wingless* gene product is involved in both embryonic and adult development. The mechanism by which *wingless* produces notal duplications is not understood.

Pattern abnormalities in certain *apterous* alleles may be due to disruption of positional fields in the imaginal wing disc (Stevens and Brower, 1986). If the mechanism producing notal duplications in ap^{ID}/ap^{Xa} animals is similar to that in *wingless* animals, it is possible that the two genes may have related functions. To test for any interaction between these two mutations, double heterozygotes ($wg\ ap^+/wg^+ap^{ID}$) were constructed (Table 1). These double heterozygotes showed a much higher frequency of duplications that either wg^1/wg^+ or ap^{ID}/ap^+, showing that the two gene products are interacting to some degree. It will be of interest to examine *apterous* mutants for embryonic effects similar to those described for *wingless* mutants.

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Sushmita, Maitra, Mita Ghosh and A.S. Mukherjee. University of Calcutta, India. Characterization of haemolymph tissue protein from the wild type and the mutant M^{m} [In(1)BM^{2} (rv)] of *Drosophila melanogaster.*

Haemolymph was collected from late third instar larvae, in 0.2M sucrose, 0.001M PMSF and phenylthiourea (PTU), from males and females of Oregon R^{+} and In(1)BM^{2} (rv) *Drosophila melanogaster.* Haemolymph tissue protein was isolated and fractionated in a 7.5% polyacrylamide slab gel at 2mA/lane constant current, and also on 13% SDS-PAGE at 2 mA/lane. Proteins were stained with Coomassie Brilliant Blue R-250 and preserved in 10% acetic acid. Photographs were taken with Ilford 50 ASA film.

Proteins run on native polyacrylamide gel show four main bands in all four samples [Oregon R^{+} male, Oregon R^{+} female, In(1)BM^{2} (rv) male, In(1)BM^{2} (rv) female] (Figure 1). SDS-PAGE gel banding profiles are similar for the four types of larvae. However, a low molecular weight protein of about 26.5 kd is present in greater quantity in both the In(1)BM^{2} (rv) males and females than in the Oregon R^{+} male and female [arrow]. Molecular weight was estimated using the standard Dalton 7 molecular weight marker (Figure 2).

Thus it appears that the presence of this protein fraction in greater amount in the In(1)BM^{2} (rv) strain is not only present in whole larval extracts as revealed by Ghosh et al. (1987) but is also present at tissue levels.

References: Ghosh, M., S. Maitra and A.S. Mukherjee 1987, Abs. VIth All Ind. Congress on Cytology and Genetics.

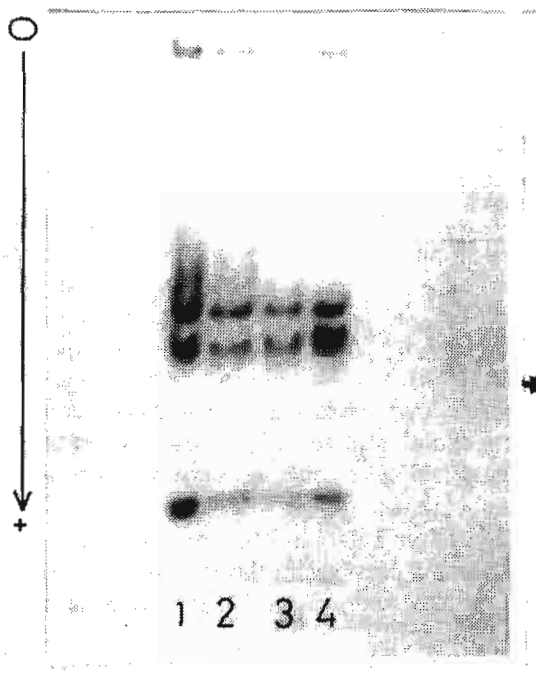

Figure 1. Electrophorogram (7.5% PAGE) of haemolymph protein. Oregon R^{+} male (lane 1), Oregon R^{+} female (lane 2), In(1)BM^{2} (rv) male (lane 3), In(1)BM^{2} (rv) female (lane 4).

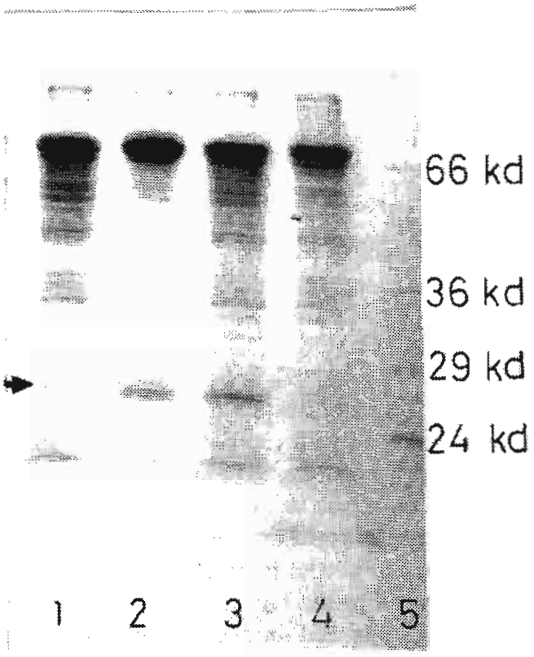

Figure 2. Electrophorogram (13% SDS-PAGE) of haemolymph protein. Oregon R^{+} female (lane 1), In(1)BM^{2} (rv) male (lane 2), In(1)BM^{2} (rv) female (lane 3), Oregon R+ male (lane 4), Dalton protein marker (lane 5).

Tripathy, N.K., K. Patnaik, L. Day, B. Majhi and C.C. Das. Department of Zoology, Berhampur University, Berhampur-760007, Orissa, India. Sevin is genotoxic in the *Drosophila* female germ line.

Drosophila, with its elaborately studied genetics, constitutes a model system for genotoxic screening assays. Chemically-induced alterations in its genotype will manifest phenotypically provided suitable recessive genetic markers are used in its chromosomes. Appropriate genetic markers are available for the identification of female germ line mutations in *Drosophila melanogaster* (Wieschaus and Szabad, 1979).

Sevin, carbaryl (1-Naphthyl-N-methyl Carbamate, CAS No. 63-35-2), is a carbamate insecticide effectively used against various agricultural insect pests. Sevin is reported to be nonmutagenic in bacterial reversion assay (Moriya *et al.*,

1983). This compound was demonstrated to induce sex-linked recessive lethals in *Drosophila* after larval feeding (Sinha and Sinha, 1984; Dey *et al.*, 1987). Sevin is also mutagenic in the wing and eye primordial cells of *Drosophila* (Dey *et al.*, 1986). This insecticide, however did not induce partial or total chromosome loss in this organism (Woodruff *et al.*, 1983). Further, it did not induce chromosome aberrations in the mouse bone marrow cells (Degraeve *et al.*, 1984). The authors were inclined to test the genotoxicity of sevin in an easily demonstrable system, the female germ line mosaic assay in *Drosophila melanogaster.*

The larvae to be treated with sevin came from the matings of w^{co}/w^{co}; flr^3 *se*/TM2, *ubx se* females and *fs(1)K10 w*/Y; *mwh se/mwh se* males. The allele *fs(1)K10 (=K10)* is a recessive genetic marker, located on the chromosome 1 (1-0.5) causing abnormal egg shape (Wieschaus *et al.*, 1978), is used in the present experiments to detect the induction of mosaicism in the female germ line cells. The *K10* eggs differ from the normal wild type eggs by the absence of the two dorsal filaments but, instead, contain a large mass of chorionic material extending from the anterior end of the egg on the ventral and lateral sides.

Table 1. Data on the frequency of mosaic induction in the female germ line of *Drosophila* after larval exposures to sevIn.

Treat-ment	Conc. (%)	Females tested	Mosaics No.	Mosaics %	Concl.[a]	Total eggs examined	Total K10 eggs observed	Average daily egg production
Pooled	control	517	6	1.16		201613	8	27.8
48 h	1.0×10^{-2}	99	11	11.11	POS	34096	38	24.6
	5.0×10^{-3}	103	7	6.80	POS	30715	24	21.3
72 h	5.0×10^{-3}	107	16	14.95	POS	31610	48	21.1
	2.5×10^{-3}	104	7	6.73	POS	29899	29	20.5

[a]Conclusion on the basis of x^2 test, POS = positive, level of significance $P < 0.001$.

Eggs were collected for 4 h on standard *Drosophila* food. Larvae, 72 h and 48 h, corresponding to the 3rd and 2nd instar larval stages, were collected by floating them in 50% glycerine. About 100 larvae of each instar were exposed to different concentrations of sevin, manufactured by the Union Carbide India Ltd., Bhopal, with 50% active ingredient, homogeneously mixed in the food to determine the LD_{50}. The LD_{50}, were nearly 50% larvae hatched to the adult stage, was $1.0X10^{-2}$% (w/w) for 72 h and $5.0X10^{-3}$% for 48 h larvae. In this experiment the larvae were exposed to the LD_{50} and 50% of this dose for 48 h and 72 h respectively (Graf *et al.*, 1984). One-day old individual *K10* heterozygous females, grown as larvae on normal and sevin-supplemented food, were allowed to lay eggs in small plastic vials with normal food of which surface darkened with charcoal powder to facilitate counting of eggs. The flies were transferred to fresh vials every day for 14 days and the number of normal and *K10* eggs laid were counted. Statistical analysis of the data on the frequency of mosaic induction was done with the help of chi-square test.

The data on female germ line mosaic test are represented in Table 1. The frequency of mosaic induction was significantly increased in all the treatments. However, there was a decrease in the average daily egg production in the treated series compared to the control. It is observed that the frequency of mosaic induction in the female germ line depended both on the dose and duration of larval treatment with sevin.

The female germ line mosaic assay involves the exposure of a population of *K10* heterozygous cells of the female germ line to a chemical substance and the mosaic females are recognized as the ones laying *K10* eggs occasionally (Wieschaus and Szabad, 1979). Mitotic recombination in the female germ line cells and/or a mutation in the $K10^+$ gene or its deletion through chromosome breakage may lead to *K10* homozygosity (Mollet and Szabad, 1978). These *K10* homozygous cells divide and ultimately give rise to *K10* eggs. In this experiment, since there is a significant increase in the frequency of mosaic females after larval treatments, it is concluded that sevin is mutagenic/recombinogenic in the female germ line cells of *Drosophila melanogaster.*

Acknowledgments: We thank Dr. Janos Szabad of the Institute of Genetics, BRC, Szeged, Hungary, for providing the stocks. The award of Research Fellowships to KP, LD and BM by the CSIR, New Delhi is thankfully acknowledged.

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Turelli, M., S. H. Bryant, and J.A. Coyne. University of California, Davis; California State Polytechnic University; and University of Chicago.

Long-distance migration of *Drosophila*. 4. Effect of experimental design on estimating the movement of flies near a desert oasis.

In a previous paper (Coyne et al. 1987), we attempted to determine the distance from which flies are attracted to a favorable habitat. Large samples of *D. melanogaster, D. simulans,* and *D. pseudoobscura* were trapped in a date grove in Furnace Creek oasis, Death Valley, California. These flies were marked with fluorescent dust and released in the desert at various distances from the edge of the oasis. We recaptured the flies along two traplines parallel to the edge of the oasis and equidistant from the release point (see Figure 4 in Coyne et al. 1987). The "oasis" trapline was between the release point and the oasis, the "desert" trapline was on the opposite side of the release point. Releases were made before sunup, and we counted the number of marked flies captured on the oasis and desert traplines. Our measure of the attractiveness of the oasis to the flies was the proportion of recaptured flies of a given species caught on the oasis trapline. This proportion was denoted *P*. Values greater than 0.5 indicated that flies moved preferentially toward the oasis.

Our main finding was that the estimated "attractive radius" of the oasis was small: the proportion of flies recaptured on the oasis trapline exceeded 0.5 only when they were released within 100 m of the oasis. Many non-released wild flies were found beyond 100 m. These results supported the idea that flies regularly become lost in the desert when they wander beyond the attractive radius of an oasis. This can lead to long-distance movement between oases (and genetic homogeneity of apparently isolated populations) as the flies attempt to find new feeding and breeding sites (Jones et al. 1981; Coyne et al. 1982).

Our interpretation of the *P* values assumes that the proportion of flies caught on the two traplines accurately reflects the proportion of flies moving in those directions. This assumption may be wrong. The probability of attraction to a bait may differ for flies moving toward or away from the oasis. If, for example, flies moving toward the oasis are less likely to come to a bait because they are heading toward an observed landmark, the resulting *P* values would tend to be below 0.5, even with uniform random movement. The use of *P* could, therefore, underestimate the attractive radius of the oasis. This conjecture is plausible because Coyne et al. (1987) reported *P* values less than 0.5 for all releases beyond 100 m.

To determine whether direction of movement affects probability of capture, we performed a series of nine releases during March, 1988 in the location used for our previous experiment. Our protocol was similar to that used previously: we captured flies in the Furnace Creek oasis, marked them, and released them at various distances from the south edge of the oasis (release point is X in Figure 1, at a distance A from the edge of the oasis). Flies were then captured at traplines on either side of the release point. In contrast to our previous experiment, we used two traplines instead of one on each side of the release point. We used three buckets on the two inner traplines (a distance B on either side of the release point), and four buckets on the two outer traplines (a distance C on either side of the release point). Buckets were a distance D from each other along the traplines.

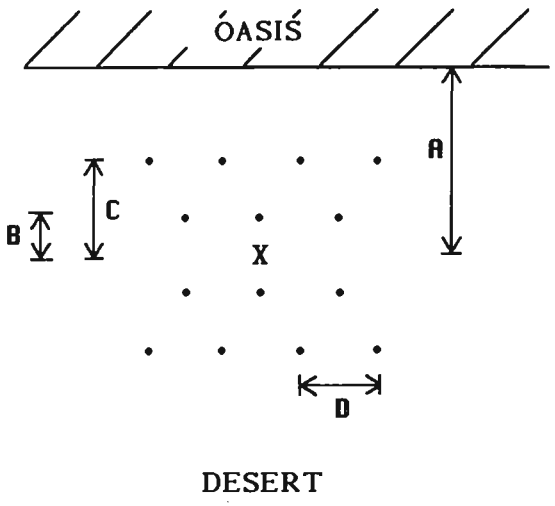

Figure 1. Arrangement of the trapline at the south edge of Furnace Creek oasis (see text for description).

This design was intended to determine whether perception of the desert or oasis affects the probability of recapture. If it does not, then the proportion of marked flies caught on the oasis side should be the same for the outer and inner pairs of traplines. If flies perceiving the oasis are less likely to respond to the baits, we would expect the *P* value for the outer pair of traplines to be smaller than the *P* value for the inner pair. This assumes that flies closer to the oasis are more likely to perceive it, as indicated by the results of Coyne et al. (1987). A consistent difference between these simultaneously estimated *P* values would suggest bias in our previous estimates of the attractive radius. If $P_{\text{in-ner}} > P_{\text{outer}}$, the attractive radius was underestimated; if $P_{\text{inner}} < P_{\text{outer}}$, it was overestimated.

Table 1 gives the *P* values for the inner and outer traplines and numbers of recaptured flies (N) for nine releases,

made at both 100 and 300 m. For the six 100-m releases, the distances indicated in Figure 1 were B=25 m, C=75 m, and D=20 m; for the 300-m releases, B=50 m, C=150 m, and D=20 m. Three of the six 100-m releases were made in the afternoon to determine if time of day greatly affects the estimated attractive radius. *P* values were estimated for both *D. pseudoobscura* and *D. melanogaster/simulans*. We recorded the sex and species of the recaptured flies, except that we could not distinguish *D. melanogaster* from *D. simulans* females. However, to simplify the presentation in Table 1, we have pooled the data from males and females and pooled all *D. melanogaster* and *D. simulans* counts. As in Coyne et al. (1987), the heterogeneities within these classes tend to be much smaller than the differences reported in Table 1. Because of this pooling, the significance values indicated, which are based on chi-square tests, are only approximate. More accurate values could be derived from log-linear analyses without pooling across sexes and species (data available on request).

Table 1. Summary of the recaptures from March 1988 releases.

				D. pseudoobscura			*D. melanogaster / D. simulans*		
Rel.	Date	Dist.	Time	P_{inner}(N[a])	P_{outer}(N)	diff[b]	P_{inner}(N)	P_{outer}(N)	diff
1	16	100	am	0.418 (67)	0.194 (36)	0.224*	0.584 (214)	0.378 (37)	0.206*
2	16	100	pm	0.214 (14)	0.688 (16)	-0.473**	0.324 (219)	0.862 (130)	-0.537***
3	17	100	am	0.308 (13)	0.091 (11)	0.217	0.170 (94)	0.360 (25)	-0.190*
4	17	100	pm	.	.	.[c]	0.757 (502)	0.715 (144)	0.042
5	18	100	am	0.550 (40)	0.806 (31)	-0.256*	0.408 (260)	0.850 (80)	-0.442***
6	18	100	pm	.	.	.[c]	0.713 (390)	0.860 (286)	-0.147***
7	19	300	am	0.367 (49)	0.611 (18)	-0.244	0.358 (586)	0.437 (71)	-0.078
8	20	300	am	0.786 (56)	0.500 (18)	0.286*	0.653 (513)	0.591 (66)	0.062
9	21	300	am	0.645 (31)	0.545 (11)	0.100	0.637 (778)	0.527 (74)	0.110

[a] N is the number of recaptured flies used to estimate the P value given.
[b] diff = P_{inner} - P_{outer}, approximate significance levels for these differences are based on chi-square tests.
[c] Fewer than 15 flies were recaptured in total.
* P<0.05, **P<0.01, ***P<0.001.

Although some of the inner and outer *P* values do differ significantly, the statistically significant differences, calculated as P_{inner}-P_{outer}, are not consistent in sign: three are positive and six are negative. This suggests that the probability of recapture is not consistently affected by the flies' direction of movement. The data support the qualitative conclusion of Coyne et al. (1987) that the attractive radius is small; but there is a notable quantitative difference in the behavior of flies released at 300 m. As in our previous study, the two classes of flies, *D. melanogaster/simulans* and *D. pseudoobscura*, behave similarly, so we will pool the results. At 100 m, the average of the 20 *P* values reported in Table 1 is 0.513. This is quite close to the average value of 0.543 found by Coyne et al. (1987). Both sets of 100-m releases indicate that once flies are 100 m from the oasis, they are likely to disperse farther into the desert. At 300 m, the average of the 12 *P* values is 0.555. Like the 100-m results, this is near 0.5, as expected for flies moving uniformly at random; but it differs from the 300-m releases reported in Coyne et al. (1987), which gave an average *P* value of 0.276. Applying the Mann-Whitney U test to the two sets of *P* values (7 from 1987 reported in Table 3 of Coyne et al. (1987) and 12 from 1988 reported in Table 1) indicates that the difference between the results from these two years is highly statistically significant (P<0.001). We do not understand this discrepancy, nor do we understand the 1987 results suggesting that flies at 300 m preferentially moved away from the oasis. Despite this ambiguity, our new results support our previous conclusion that the attractive radius of an oasis is surprisingly small.

For *D. melanogaster/simulans*, we can compare the behavior found in three morning and three afternoon releases at 100 m. The average probabilities of recapture near the oasis are somewhat larger in the afternoon (the average *P* value is 0.705 for the pm releases versus 0.458 for the am releases). However, a Mann-Whitney U comparison of the six *P* values from morning (am) releases versus the six values from afternoon (pm) releases (see Table 1) indicates that the difference is not statistically significant. Nevertheless, the differences observed suggest that it may be interesting to perform additional releases to determine whether flies are more strongly attracted to the oasis in the afternoon, when it is much hotter and flies are probably more stressed.

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Conditioned inhibition of the proboscis extension reflex in lines of *D. melanogaster* selectively bred divergently for conditioned excitation: The stability of genetic differences across behavioral measures.

Since the early selection studies with rats by Tolman (1924) and Tryon (1929, 1940), there have been only a few studies demonstrating quantitatively the influence of genes on learning capacity. Médioni *et al.* (1978) have successfully bred divergent lines of *D. melanogaster* for inhibitory conditioning, i.e., speed of acquisition of a conditioned suppression of the proboscis extension reflex (PER). Using a somewhat similar but modified experimental preparation (Holliday and Hirsch, 1984; Holliday, Vargo and Hirsch, 1983), Hirsch and Holliday (1991) were successful in two attempts to breed selectively for conditioning: The first, based on acquisition alone produced only more or less excited flies. The second attempt included extinction and bred only for a "good" line, but was lost to infertility. In the third attempt, which also included extinction of the PER in the selection criterion, as well as its acquisition, divergent selection succeeded in creating the lines whose inhibitory conditioning is described here.

The lines have been derived by selection from the control line (Champaign wild-type) and have been maintained throughout at 25° and 50% R.H. at the University of Illinois. Samples of both lines and the control (H_{24}, L_{23}, C_{24}) were sent to Toulouse late June, 1988, where they have been maintained by mass transfer without selection under conditions of controlled temperature (at 25°C ± 0.5°C, but at 19°C ± 0.5°C during summer), humidity (80% ± 5%) and light exposure (light:dark 12:12 hr, 100 lx from a fluorescent tube, light beginning at 0800 hr). The observations we report now were made from 25 December 1988 through 5 January 1989, i.e., after 6 months of relaxed selection.

The apparatus permits testing 3-4 flies at once yielding scores for identified individuals. The conditioning procedure is that used by Vaysse *et al.* (1988; also by Vaysse and Corbière, 1987), involving a half blind test (in a given session the experimenter knows that the flies belong to the same line but is unable to identify to which line). At a 2-min intertrial interval (ITI), 4-5 day old males, 22 ± 2 hr deprived, were given 16 presentations of a molar concentration of sucrose stimulation followed immediately by an aversive unconditioned stimulus of quinine chloride (10^{-4} mol/L^{-1}). On each trial the occurrence and intensity of the PER were scored on a behavioral scale. But here, we report only the number of inhibitions on 16 trials.

Because 49 flies were conditioned, the analysis was done for 15 flies per line (after a random elimination of two flies from each the H_{24} and the L_{23} lines). For the 3 lines, Figure 1 presents acquisition curves (in blocks of 4 trials) showing the mean number of suppressions over 16 trials. As shown in Table 1, however, they differ significantly in their response to

Table 1. Analysis of variance of data. Mean of inhibitions for blocks of 4 trials.

Source	DF	Mean Square	F value	
Between subjects	29	170.042		
A (Groups)	1	72.075	20.600	p < .001
Subj.within groups	28	97.967		
Within subjects	90	63.75		
B (Trials)	3	6.092	3.321	n.s.
Inter. A*B	3	6.292	3.430	n.s.
Inter. B*Subj(wg)	84	51.367		

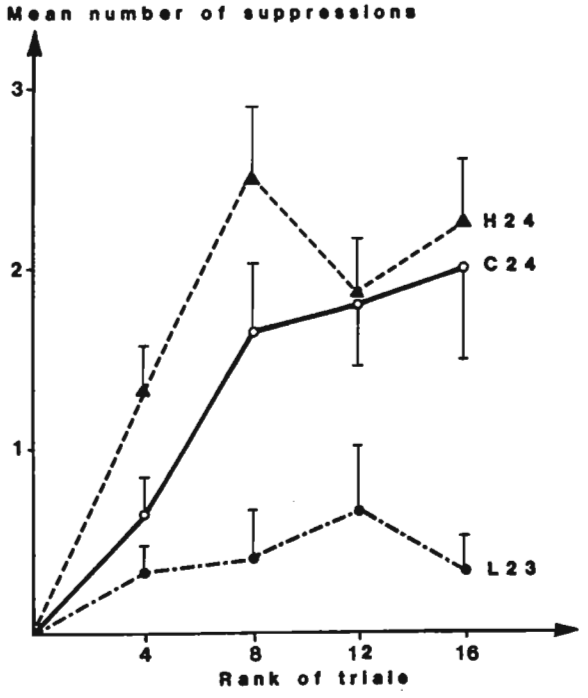

Figure 1. Acquisition of inhibition of PER (means and standard errors in blocks of 4 trials) in selected and control lines (H_{24}, L_{23}, C_{24}).

the test procedure, exhibiting the same rank of performance level for inhibitory conditioning as that for which the two divergent extremes have been selected for excitatory conditioning. It is of interest to note in the figure that the high line remains above the control during the first 8 or 9 trials, after which they converge, i.e., selection pressure for excitatory conditioning was based on a 9-trial acquisition criterion prior to extinction with a 6-min ITI (Hirsch and Holliday, 1991).

The results are interesting because they show the selected lines to be sufficiently stable to be different after 6 months of relaxation of selection pressure, through about 10 mass transfers. Especially since they were selected for excitatory, not the inhibitory, conditioning measurements reported here.

1. The genetic differences affect the threshold of the response to sucrose (or the effect of deprivation on the threshold response), because the levels of the first response to a molar sucrose stimulation are significantly different between the two selected lines (Mann-Whitney test: $U = 53$, $Z = 2.468$, $p < .05$).
2. The genetic differences are affecting either or both, central nervous system activity or the conditioning process, because the dopamine level is lower in L_{23} than in H_{24}.
3. The genetic differences might have produced an asymmetrical divergence from the control line on three measures, with the low line appearing to be more extreme with respect to sucrose response threshold, number of conditioned suppressions and dopamine level.

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Vianen, A. van and R. Bijlsma. University of Groningen, Haren, The Netherlands. The effect of male mating status on remating of females mated to these males in *D. melanogaster.*

D. melanogaster males are able to perform a high number of fertile copulations in a short period. During one day up to ten females can be inseminated by a single male (Duncan, 1930; Stenveld and Bijlsma, 1988). However, when a male is given the opportunity to remate within short time intervals the number of sperm transmitted decreases after the second or third consecutive mating (Levefre and Jonsson, 1962) resulting in a decrease in the number of progeny from these later matings (Kaufman and Demeric, 1942; Kvelland, 1965). During a few days after mating females remain unreceptive due to the so-called sperm effect (Manning, 1962; Scott, 1987). It is thought that the number of sperm in storage influences the degree of receptivity in a previously mated female (Gromko et al., 1984).

In the present experiment the effect of the mating status of the male, meaning the number of consecutive matings it has performed, on the willingness to remate of females to which they have been mated, is examined.

The flies used for the experiment originated from the Groningen 83 population that had been established in 1983 with flies caught at a local fruit market. All tests were done at 25°C and 50-70% RH on standard food (sucrose/dead yeast/agar medium).

Table 1. Mean offspring numbers (± SE) produced by females mated to males with different mating status. Means followed by the same letter do not differ significantly at the 5% probability level (Wilcoxon Matched-pairs Signed-rank test).

Male mating status	Progeny numbers
1	107.6 ± 12.5 a
2	108.7 ± 11.9 a
3	85.0 ± 12.7 b
4	56.3 ± 9.7 c

Table 2. Percentage remating of females five days after their first mating with males of different mating status together with the mating percentage of ten-day-old virgin females. Remating percentages followed by the same letter do not differ significantly at the 5% probability level (Contingency chi-square test).

Female type	Number Tested	Percentage (re)mating
1	60	40.0 a
2	43	41.9 a
3	40	72.5 b
4	29	86.2 b,c
Virgins	60	92.1 c

Single five-day-old males were allowed to mate to four females of the same age consecutively. Each female was offered for 45 minutes to a male. When no copulation took place within this period, the flies were discarded. In this way four different types of females were obtained: type 1 females were mated to males that had never mated before (mating status 1), type 2 females mated to males that had mated to males that had previously mated to one other female (mating status 2), type 3 females mated to males that had mated twice previously (mating status 3) and type 4 females mated to males that had mated three times previously

(mating status 4). From these females 20 complete series of four females mated to the same male were allowed to lay eggs in vials. They were transferred to new vials every day during the first five days after the mating and thereafter twice a week. Their total offspring numbers were counted individually. All other mated females were kept isolated for five more days after the mating and then a five-day-old virgin male was offered to each of these females for 45 minutes. For each female type the number of females that remated during this period was established. For comparison the mating of virgin ten-day-old females to five-day-old virgin males was also measured.

The total offspring numbers produced by the different types of females are shown in Table 1. Males of mating status 1 and 2 show significantly higher offspring numbers than males of mating status 3 and 4. Especially males of mating status 4 show a very marked reduction in offspring of almost 50% compared to males of mating status 1 and 2. This clearly indicates that males after the second and third consecutive mating produce lower amounts of, or less effective, sperm.

The results of the remating experiment are shown in Table 2. Females of type 1 and 2 are still very reluctant and show a remating percentage of around 40% which is significantly lower than found for types 3 and 4. Females of type 4 show the same amount of remating as virgin females of the same age, indicating that they have returned to full receptivity.

In conclusion the results show that receptivity of females is affected by the mating status of their mates. This is most probably due to the fact that males after a number of consecutive matings transfer less sperm or sperm of lesser quality and that the females mated to these males therefore deplete their sperm supply sooner than females mated to virgin males.

References: Duncan, F.N. 1930, Am. Nat. 64:545-551; Gromko, M.H., M.E.A. Newport and M.G. Kortier 1984, Evol. 38:1273-1282; Kaufman, B.P. and M. Demerec 1942, Am. Nat. 76:445-449; Kvelland, I. 1965, Hereditas 53:281-306; Levefre, G. and U.B. Jonsson 1962, Genetics 47:1719-1736; Manning, A. 1962, Nature 194:252-253; Scott, D. 1987, Anim. Behav. 35:142-149; Stenveld, H. and R. Bijlsma 1988, DIS 60:138.

Villar, B., E. Garcia-Vazquez, A. Gutierrez, and J. Izquierdo. Dpto. Biologia Funcional (Area Genetica), Facultad Medicina, Oviedo, Spain. Analysis of chromosomal polymorphism in *D. melanogaster* populations: study by isofemale lines.

In this work, the cosmopolitan inversions of an Asturian population of *D. melanogaster* were analysed by isofemale lines. Usually, populations are studied by analysing inversions of one larvae from each isofemale line (i.e., Knibb et al., 1981). But meaning this method, perhaps the obtained inversion frequencies are not representative of the population, if the number of isofemale lines is low. Studying one single larva in each progeny, easily heterozygote females cannot be detected as inversion carriers. In this work we will try to determine if one larva is enough, or if it is necessary to study more larvae for obtaining inversion frequencies representative of a sample of isofemale lines.

A sample of 56 females was captured in a cellar of Villaviciosa (Asturias, Spain) by means of banana and yeast traps. An isofemale line was founded with the progeny of each female. In the first generation in the laboratory, the inversions of 5 larvae were analysed and noted in order of proceeding. The inversions were studied in the salivary gland chromosomes (Levine and Schwartz, 1970), by direct analysis of the chromosomal pattern (following Lefevre, 1976). We study the cosmopolitan inversions In(2L)t, In(2R)NS, In(3L)P, In(3R)P and In(3R)C.

Results are presented as the frequency of chromosome arms carrying each inversion. We consider for the test the inversion frequencies obtained with the first analysed larva, and the inversion frequencies obtained with the five larvae of each line. In Table 1, we can see that there are not differences between the frequencies obtained through analysing one or five larvae per line.

Table 1. Frequencies (in percent) of chromosome arms carrying each inversion, analysing one or five larvae per line. Test of differences between the two frequencies for each inversion. Sample of 56 lines.

Inversion	Larvae Number Per Line 1	5	E Value	Statistical Significance
2Lt	8.03%	7.50%	0.136	N.S.
2RNS	7.14%	7.32%	0.047	N.S.
3LP	1.78%	1.96%	0.089	N.S.
3RP	0.89%	1.43%	0.320	N.S.
3RC	15.18%	15.18%	0.000	N.S.

We can conclude that, in a large sample, it is enough to analyse one single larva per line. But perhaps this sentence is not valuable if the sample is shorter. Taking only 20 lines, the inversion frequencies are shown in Table 2. There are not significative differences between the frequencies obtained by both methods; this is, one single larva of each isofemale line is representative of population inversion frequencies.

Authors as Ashburner and Lemeunier (1976) consider a

Table 2. Frequencies (in percent) of chromosome arms carrying each inversion, analysing one or five larvae perline. Test of differences between the two frequencies for each inversion. Sample of 20 lines.

Inversion	Larvae Number Per Line 1	5	E value	Statistical Significance
2Lt	5.00%	4.00%	0.064	N.S.
2RNS	5.00%	6.00%	0.055	N.S.
3LP	0.00%	1.00%	0.142	N.S.
3RP	0.00%	3.00%	0.248	N.S.
3RC	20.00%	13.50%	0.237	N.S.

Table 3. Frequencies (in percent) of chromosome arms carrying each inversion, analysing one larva per line. Test of differences between the two frequencies for each inversion analysing 20 and 56 isofemale lines.

Inversion	Number of Individuals 20	56	E value	Statistical Significance
2Lt	5.00%	8.03%	0.635	N.S.
2RNS	5.00%	7.14%	0.468	N.S.
3LP	0.00%	1.78%	0.849	N.S.
3RP	0.00%	0.89%	0.601	N.S.
3RC	20.00%	15.18%	0.706	N.S.

sample of 20 chromosome sets representative of the inversions of a wild population. In Table 3 we can see that the frequencies obtained with 20 larvae are not statistically different from those obtained with 56 larvae; our results confirm this method as correct for evaluating inversion variability of a population.

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Vlassova, I.E., A.S. Graphodatsky, E.S. Belyaeva, and I.F. Zhimulev. Institute of Cytology and Genetics, Novosibirsk, 630090, USSR.

Constitutive heterochromatin in early embrygenosis of *Drosophila melanogaster.*

For better understanding of mechanisms of gene inactivation as a result of position effect, when a gene is transferred in a proximity to heterochromatin, of special interest is an early embryogenesis of *Drosophila.* At precisely this time compaction ("heterochromatization") of the inactivated euchromatin chromosomal regions evoked by its position close to heterochromatin takes place. The temperature-sensitive period for such compaction was found to be the first 3-6 hours of embryonic development (Hartmann-Goldstein, 1967; Zhimulev et al., 1988). It may be assumed that during this critical period inactivating influence of heterochromatin expands to the neighboring euchromatin drawing it in the process of compaction, which results in the changes of DNP packing. A question arises - are these events due to the changes in the state of heterochromatin in early *Drosophila* embryogenesis?

Studies on various objects (pisces, amphibia, mammals) carried out by A.A. Prokofjeva-Belgovskaya (1982) led her to a conclusion that morphology of metaphase chromosomes in early development differs drastically from those at later developmental stages: they are thread-like, rather decondensed and lack heterochromatin blocks. She coined them "juvenile".

Morphology of *Drosophila* chromosomes in early embryogenesis has been studied insufficiently. In early works (Huettner, 1933; Sonnenblick, 1950) and in later electron-microscopic studies (Mahowald and Hardy, 1985) it was noted that interphase nuclei did not have a chromocenter and metaphase chromosomes - heterochromatin. For all this, nonspecific techniques of chromosomal staining were used to distinguish between eu- and heterochromatin. It seems interesting to obtain C-banding of "juvenile" *Drosophila* chromosomes since this staining technique is known (Pimpinelli, 1975) to detect regions with positive heteropicnosis at any stage of condensation. In the present study an attempt was made to establish at what time in *Drosophila* embryogenesis can one detect constitutive heterochromatin by means of the C-banding technique.

The Batumi-L wild strain of *Drosophila melanogaster* has been used. Egg synchronization was made as follows. By 1000 pairs of males and virgin females were collected for 6-7 days, fed well, and placed in the population box. The embryos were processed and cytological preparations were made following commonly employed techniques. After 4 hours the eggs laid during 15 min were moved to a sticky tape where they were dechorionated and put in octane for 10 min to improve the permeability of the vitteline membrane.

Despite the precise egg aging we could not exclude the presence of more mature eggs. Therefore the eggs were checked in octane under a dissecting microscope. Octane was rinsed with physiological saline and the eggs were put in 0.04% colchicine for 15-20 min. After that the eggs were fixed in methanol:acetic acid (3:1) for 12 h at 4-6°C. The embryos were removed from vitteline membrane prior to their squashing in 45% acetic acid. The cover slips were removed by means of liquid nitrogen freezing, then the preparations were air-dried. C-bands were obtained by the method of Sumner. Some 200 metaphase plates from 30 embryos have been analysed.

Neural ganglia of third-instar larvae were incubated 30-40 min in 0.04% colchicine, fixed in methanol:acetic acid

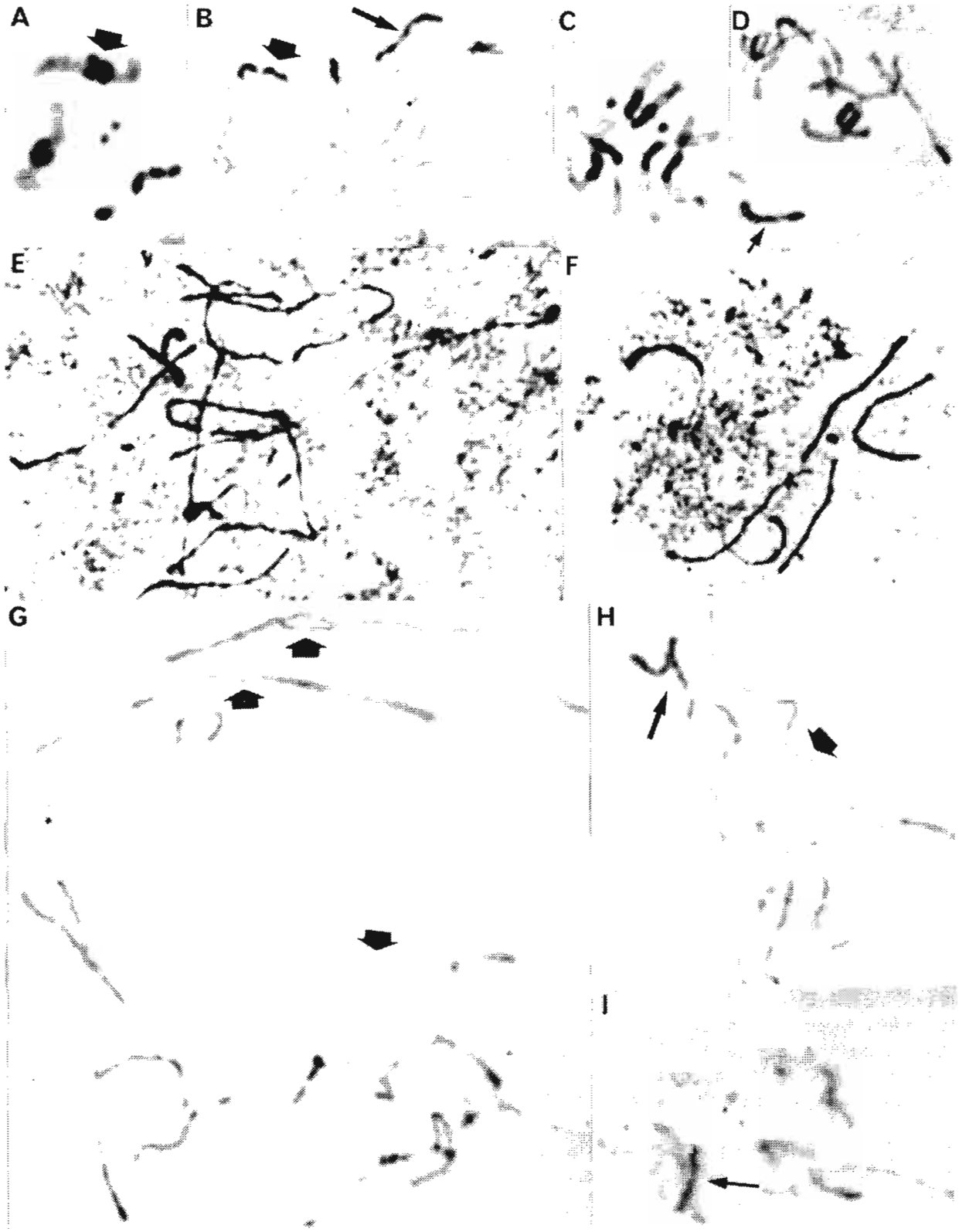

Figure 1. C-banding of metaphase (a) and prometaphase (b) chromosomes of *D. melanogaster* neuroblasts (male), metaphase (c) and prometaphase (d) chromosomes at the stage of a blastoderm (female and male), metaphase chromosomes at early embryonic stages (e-h), e,f - Giemsa staining, g-i - C-banding (female and male). Thick arrows indicate the pericentric regions, thin - Y chromosome.

(3:1) and embedded in 45% acetic acid. After thawing and air-drying the preparations were stained.

C-banding patterns of the neuroblast chromosomes obtained as a control (Fig. 1a, b) were in good agreement with those of other authors (Pimpinelli et al., 1975). In autosomes of neuroblasts (Fig. 1a) heterochromatin is represented by two large pericentric blocks, and the X by a short arm, whereas the Y chromosome appears almost completely heterochromatic. The same pattern typical of metaphase chromosomes was also observed in the decondensed prometaphase chromosomes of neuroblasts (Fig. 1b). C-bands of metaphase and prometaphase chromosomes at the stage of blastoderm (Fig. 1c, d) were similar to those of neuroblasts.

The metaphase chromosomes at the first stage of cleavage division (30-60 min after egg laying) differ from those at the stage of blastoderm. They are seen as long, thin, weakly condensed threads (Fig. 1e, f). Such chromosomes are characteristic of early development and display a specific C-banding pattern (Fig. 1g-i). The Y chromosome is quite intensively-stained, the centomeric regions of the autosomes in the X chromosome arms show only "traces" of stain. It may be assumed that metaphase chromosomes at early stages of embryogenesis are not only weakly condensed but have some other morphological differences. For instance, sometimes we could observe loop-like formations in individual chromosome regions (Fig. 1g, thick arrows).

The period of weak chromosome condensation at early developmental stages is transient. Our observations of metaphase chromosomes show that after 4-5 divisions they develop their usual morphology and C-banding pattern characteristic of the somatic cells.

Thus, our experiments on C-banding of *Drosophila* chromosomes at early developmental stages did not show differential heterochromatin staining. The specific chromosomal structure of heterochromatin may not be due to different DNA amount but to its specific packing caused by some chromosomal proteins. Some data indicate that there are so-called juvenile lysine-rich histones which are substituted by "mature" forms up to 10-11 cleavage division (Das et al., 1964). Such shift of histones in the course of embryogenesis has been shown for chromosomes of sea urchin (Weinberg et al., 1979) and helix (Bloch and Hew, 1960).

Peculiarities of the organization of juvenile chromosomes at early developmental stages are evidently associated with the specificity of their function. It is known that early embryonic development is provided by maternal RNA stored in the egg. The nuclei become transcriptionally active only in about 1.5 hours after fertilization (Undewood and Lengyel, 1988) - after the completion of the 13th division (Wieshaus and Sweeton, 1988), i.e. juvenile chromosomes are not transcribed. Additionally, the presence of differentially compacted regions (in particular, dense heterochromatic zones) might serve as a factor to provide a super-rapid replication of chromosomes - mitotic divisions at early cleavage stages occur at 9.6 min interval (Rabinowitz, 1941), lengthening profoundly only after the 12-13th divisions.

The time when chromosomal structure becomes heterogeneous correlates with the initiation of transcription and elongation of replication period. Cytologically it can be detected by differential C-banding and is likely to be due to some general changes in the organization of chromosomes at this period - differential compaction as grounds for differential gene activity. This period is also critical for compaction for euchromatic regions relocated to heterochromatin under position effect (see above). It is very likely that this process is conditioned by the same factors (for instance, proteins - compactizators) that form heterochromatin as specifically detectable dense structure. At this stage in the nucleus there appears a protein specific for pericentric and to some extent for intercalary heterochromatin in the structural gene of which is a suppressor of position effect (James et al., 1989). In this respect studies on the changes in heterochromatin structure during an early embryogenesis may serve as a key to explain processes underlying variegated inactivation as a result of position effect.

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Vlassova, I.E., T.A. Kozhemyakina and M.O. Protopopov. Institute of Cytology and Genetics, Novosibirsk 630090, USSR. Investigation of transcriptional activity of the X chromosome 2B region in *Drosophila melanogaster* under position effect variegation using DNA:RNA hybridization *in situ* technique.

DNA: RNA hybridization *in situ* technique was used to investigate the transcriptional activity of *Drosophila melanogaster* X chromosome 2B region in the T(1;2)dor^{var7} rearrangement that evoked position effect variegation. Earlier (Zhimulev et al., 1986) it was shown that juxtaposition of the short translocated element 1A-2B7-8 to the 40F region of the centromeric heterochromatin resulted in the compaction of chromosomal material and formation of the so called "block" in the 2B region. The block formation was accompanied by the genetic inactivation of the loci of this region.

Studying of the ^{3}H-uridine incorporation in the salivary glands *in vitro* revealed lowering of transcriptional activity in the 2B region with compact morphology in T(1;2)dor^{var7}/FM6 females. The reduction of ^{3}H-uridine incorporation was 90% (Zhimulev et al., 1988). It is known that ^{3}H-uridine incorporation leads to the labelling of the whole chromosome, thus complicating the calculation of silver grains in the block. Besides, in the blocks of the short translocated elements joined to chromocenter the calculation of labels is impossible because of the labelling of the chromocenter itself, that limits the sample greatly. Therefore DNA:RNA hybridization *in situ* technique was applied in the present study. By means of this method RNA complementary to the given clone could be revealed (Livak et al., 1978). It was interesting to compare the results obtained by the two independent techniques. The DmP205 clone coding a sequence from the 2B3-5 puff was used for hybridization (Belyaeva et al, 1987). Various hybridization conditions were tested and those described by Livak et al. (1978) were found to be optimal ones. The preparations treated with RNAase A (100 ug/ml in 2 x SSC, 2 h, 37°C) were used as a control. Pretreatment of the salivary gland chromosomes with RNAase A reduced (80-90%) but did not entirely eliminate hybridization in the puff 2B. Two alternative explanations are possible for this latter fact: a) the label remains because RNAase A does not remove the nascent RNA from DNA:RNA hybrids, b) DNA:DNA hybridization proceeds in the 2B puffs under the conditions used, although at a low level. After the treatment with RNAases A and RNAase H (5 ug/ml, 1 h, 37°C) the "remaining" label was still observed, that may provide evidence for low intensity of DNA:DNA hybridization. Similar observations were reported by Artavanis-Tsakonas et al. (1979) though according to data by Izquiredo et al. (1981) DNA:DNA hybridization did not occur under similar conditions. One can suppose that in regions with large puffs, such as heat shock puffs, DNA structure undergoes certain alterations which allow partial DNA:DNA hybridization under nondenaturing conditions (Artavanis-Tsakonas et al., 1979).

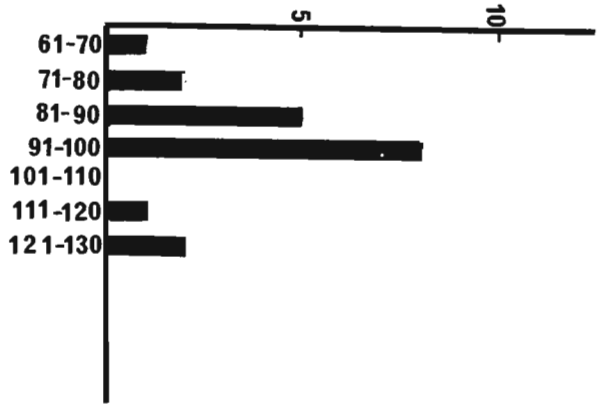

Figure 1. Distribution of nuclei as a function of the transcriptional activity in the 2B region of the short translocated element (state - "block") in comparison with the 2B puff of the FM6 homologue. Abscissa - ratio of number of silver grains over the 2B region of the short translocated element (state - "block") to that over the 2B puff in the FM6 homologue (%). Ordinate - number of nuclei.

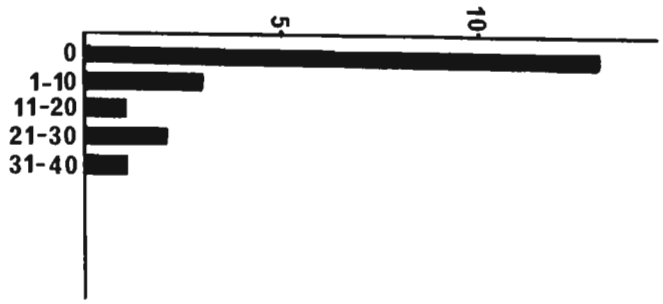

Figure 2. Distribution of nuclei as a function of the transcriptional activity in the 2B region of the short translocated element (state - "puff") in comparison with the 2B puff in the FM6 homologue. Abscissa - ratio of number of silver grains over the 2B puff of the short translocated element to that over the 2B puff of the FM6 homologue (%). Ordinate - number of nuclei.

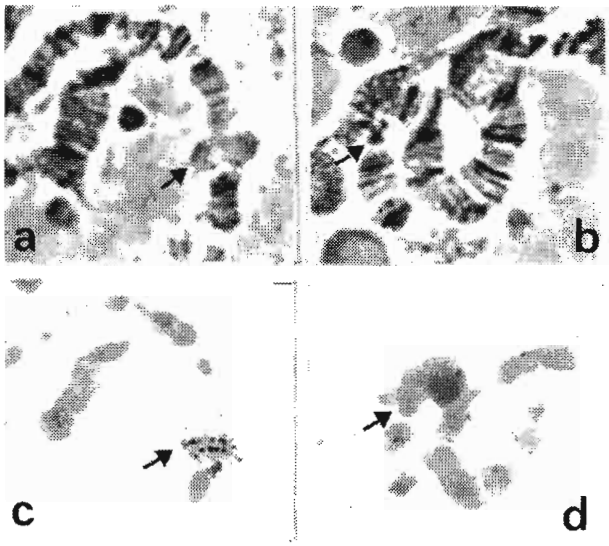

Figure 3. Hybridization of the DmP205 clone with RNA in the block (shown by an arrow) of the compact chromatin (b,d) and in the puff (shown by an arrow) of the FM6 homologue (a,c) in the same nucleus. a, b) phase contrast before the autoradiography; c, d) the same chromosomes after autoradiography.

Correction was made for DNA:DNA hybridization as follows: the average number of silver grains in the 2B puff on the preparations treated with RNAase A was calculated. Then this number was subtracted from the number of silver grains in every 2B puff on the preparations without RNAase. Figs. 1, 2 and 3 show the data on the distribution of nuclei according to labelling level of the 2B region in the short translocated element with different morphology (block or puff) in comparison with a puff in the balancer chromosome FM6 after the hybridization of the DmP205 clone with chromosomal RNA. When a compact block of chromatin appeared in the 2B region, the transcriptional activity in this region was 6% on the average when compared with the FM6 homologue (the average was calculated according to data of the histogram presented in Fig. 1). If there appeared a puff in the 2B region of the translocated homologue, the transcriptional activity of 2B was on the average 96%, i.e., it practically did not differ from the control (see histogram in Fig. 2). The data on the suppression of the transcriptional process in the 2B region in the short translocated element with compact morphology obtained by either ^{3}H-uridine incorporation technique or by means of hybridization *in situ* of clone from 2B with chromosomal RNA demonstrated strict correlation.

References: Artavanis-Tsakonas, S., L.P. Schedl, M.E. Mirault, L. Moran, and J.T. Lis 1979, Cell 17:9-18; Belyaeva, E.S., M.O. Protopopov, E.M. Baricheva, V.F. Semeshin, M. Izquierdo and I.F. Zhimulev 1987, Chromosoma 95:295-310; Izquierdo, M., C. Arribas and C. Alonso 1981, Chromosoma 83:353-366; Livak, K.J., R. Freund, M. Schweber, P.C. Wensink, and M. Meselson 1978, PNAS USA 75:5613-5617; Zhimulev, I.F., E.S. Belyaeva, O.V. Fomina, M.O. Protopopov, and V.N. Bolshakov 1986, Chromosoma 94:492-504; Zhimulev, I.F., E.S. Belyaeva, A.V. Bgatov, E.M. Baricheva, and I.E. Vlassova 1988, Chromosoma 96:255-261.

Waddle, F., H. Monk and W. Williams.
Fayetteville State University, Fayetteville, North Carolina. Linkage data.

Attempts to make up a stock containing the sex-linked mutants vibrissae (vb), scalloped (sd) and forked (f) indicated that the present placement of vb to the left of sd (Lindsley and Grell, 1968) is incorrect. In order to determine the actual location of vb, a crossover test was conducted. Of 3369 male offspring from sd vb +/+ + f females, 189 contained crossovers in the sd - vb region and 110 contained crossovers in the vb - f region of the X chromosome (total 299). Thus, vb is located 189/299 or 63.2% of the distance between sd (51.5) and f (56.7) at approximately 54.8.

Acknowledgment: This work was supported by NIH Grant #S06 RR08206-03.

Reference: Lindsley, D.L. and E.H. Grell, 1968, Genetic Variations of *Drosophila melanogaster*, Carnegie Inst. Wash. Publ. No. 627.

Wappner, P., F. Manso, J. Cladera and L.A. Quesada-Allué. I.I.B. "Fundación Campomar",1405 Buenos Aires and CICA-INTA, 1712 Castelar, Argentina. Dark cuticle mutants of *Drosophila* and *Ceratitis* might be related.

We report here that two dark puparium mutations of the Mediterranean fruitfly *Ceratitis capitata* might be related to *black* (b) and *ebony* (e) *Drosophila* mutants.

Drosophila mutants showing black or darker-than-normal cuticle pigmentation have been studied extensively (Bridges and Morgan, 1923; Ashburner et al., 1980). Within the past two decades, the biochemical aspects of these mutations have also been examined (Jacobs, 1980, 1985; Wright, 1987). The amino acid B-alanine (B-ALA) is an important component of sclerotized wild type cuticles from *Drosophila* and others insect species whereas it is almost undetectable in melanic mutants (Roseland et al., 1987). B-alanyldopamine apparently participates in cuticle sclerotization (Hopkins et al., 1982). In the absence of B-alanine, dopamine metabolism is shifted to melanin production.

Drosophila melanogaster black(b) mutants fail to synthesize B-ALA (Hodgetts, 1972) and the normal phenotype can be restored by feeding or injecting B-ALA (Jacobs, 1974; Hodgetts and Choi, 1974). *Drosophila ebony* (e) mutants have undetectable levels of B-alanyldopamine synthetase activity (Wright, 1987), thus being unable to utilize B-ALA in the tanning of cuticular structures (Jacobs and Brubaker, 1963). In other insect species, melanic mutants containing low haemolymph levels of B-ALA have been reported and probably are related to *Drosophila black* mutants. No mutations eventually related to *Drosophila ebony* have been reported.

Ceratitis c. Black pupa (B) is a semi-dominant autosomic mutation showing an extremely dark puparium (Lifschitz, 1985). *Ceratitis c. niger* (nig) is a recessive autosomic mutation that produces darker than normal posterior larval spiracles, puparia and adult cuticle (Manso and Lifschitz, 1979). In order to know whether these mutants are related to *Drosophila* melanic mutants, we performed microinjections of tanning precursors. Complete restoration of w.t. phenotype was obtained by injection of 18 ug/animal of B-ALA to *Black pupa* pre-pupae. No phenotype reversion was observed when *niger* pre-pupae were injected with up to 214 ug/fly of B-ALA.

When [^{14}C]B-ALA was injected as tracer, incorporation of radioactivity into *niger* puparia was found to be 15 times lower than that in wild type or *Black pupa* puparial cases. From the above preliminary results it can be inferred that *Ceratitis* (B) and (nig) mutations might be related respectively to *Drosophila* (b) and (e) mutations. However, different developmentally regulated gene expression seems to occur in both flies since *Ceratitis c.* mutants show almost black puparia and darker than normal adults, whereas *Drosophila m.* mutants have relatively pale puparia and dark adults. No dark puparia have been described ever in *Drosophila m.* These differences might be due to the fact that this species has lower haemolymph levels of dopamine at the beginning of pupariation than other dipterans (Hodgetts and Konopka, 1973).

Acknowledgment: supported in part by CONICET and University of Buenos Aires grants; P.W. is a graduate fellow of the latter institution.

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Yadav, J.P. and R. Parkash. M.D. University, Rohtak, India. *Drosophila* collection from northeastern part of India.

Table 1 reveals data on the male and female individuals of seven *Drosophila* species which were bait-trapped from four sites -- Bagdogra and Hasimara (North Bengal); Phuntsholing (Bhutan); and Dhulabari (Nepal) in northeastern Himalayan region of the Indian subcontinent. These sites are characterised by heavy rain fall and humid climate. The collections were made in the months of Jan.-Feb., 1988. Out of seven *Drosophila* species, *D. immigrans* (41%) was the most abundant species; three species (*D. takahashii, D. ananassae* and *D. nepalensis*) occurred in the range of 11% to 19%; while the other three (*D. melanogaster, D. bipectinata* and *D. kikkawai*) constitute the least frequent species (2.5% to 5%). The sex-wise distribution of these species revealed significant deviation in case of *D. nepalensis* (where males constitute twice than the females) and in *D. ananassae* (with females 1.5 times more than males). Such observations were made on these two species in other north Indian populations also. The present observations concur with Bock (1980) who argued that the majority of the species of the *melanogaster* group are fruit niche species and occur in the tropical rain forests of southeast Asia. Except *D.*

immigrans which belongs to subgenus *Drosophila*, all other six species belong to the *melanogaster* group.

Reference: Bock, I.R. 1980, Syst. Entomol. 5:341-356.

Table 1. Data on *Drosophila* species individuals collected from four northeastern sites of India.

Species	Bagdogra		Dhulabari		Hasimara		Phuntsholing		Total ratio		χ^2	Total	%
	M	F	M	F	M	F	M	F	M	F	Sex	(N)	
D. immigrans	55	61	105	110	65	78	50	55	275	304	1.45	579	40.89
D. takahashii	49	53	42	46	32	34	5	8	128	141	0.63	269	19.00
D. ananassae	15	22	5	9	40	56	35	50	95	137	7.60*	232	16.38
D. nepalensis	36	18	29	8	30	15	22	10	117	51	25.93*	168	11.86
D. melanogaster	3	5	0	1	10	16	14	22	27	44	4.07	71	5.01
D. bipectinata	4	7	—	—	13	19	7	12	24	38	3.16	62	4.38
D. kikkawai	—	—	—	—	8	14	4	9	12	23	3.46	35	2.47

Collection sites represent heavy rainfall and humid climate. M & F represent males & females.
(Hasimara = 26°42'N & 89°22'E; Bagdogra = 26°41'N & 88°19'E).
* Significant at 1% level.

Zhimuley, I.F.[1] and J. Szidonya[2]. [1]Institute of Cytology and Genetics, Siberian Branch, USSR Acad. Sci.; [2]Institute of Genetics, Biological Res. Ctr., Hung. Acad. Sci. ***In vivo* and *in vitro* puffing of lethal *ft* mutations of *D. melanogaster*.**

Lethal mutations of the *G-ft* complex locus were obtained after EMS and X-ray mutagenesis as well as in dysgenic crosses, and were localized in the 24D5-7 region of chromosome 2. Homozygous larvae have a greatly extended larval life during which cell proliferation in the imaginal discs is continued. The discs retain their single layered structure and their ability to metamorphose (Bryant et al., 1988).

The salivary gland chromosome puffing pattern of 10 day-old homozygous larvae of two lethal alleles, $l(2)ft^{a13}$ (EMS induced) and $l(2)ft^{fd}$ (dysgenic) growing at 25°C were analysed. The salivary glands of these mutants are smaller than those of wild type and do not contain the special vacuoles described by Slizynski (1964) for larvae carrying the ft^1 viable allele. Few of the 10 day-old $l(2)ft^{fd}$ homozygous larvae formed prepupae. Puffing analysis was undertaken from both larvae (7 larvae of each allele) and 0 h prepupae. All larvae but one [$l(2)ft^{fd}$] showed a puffing pattern characteristic for PS1 with two exceptions, i.e. the 25B and 68C puffs were absent. In one *fd* homozygote the lumen of the salivary gland was filled with glue secretion and its puffing pattern corresponded to PS7 with 78C, 74E and 75B puffs typical for this developmental stage. The 0 h prepupa (*fd*) showed PS10 (63E, 62E, 71EF) marker puffs (see Ashburner, 1975 for references of PS).

The PS1 salivary glands of homozygotes for both alleles were incubated *in vitro* with ecdysterone (see Belyaeva et al., 1981 for experimental details). After 4 hour incubation at 22°C in all the salivary glands (10 larvae of each genotype) the puffing pattern corresponded to PS5-7.

These data indicate that the failure of these larvae to pupariate is associated with a delay in secretion or low level of ecdysone but that the cells still can react upon its appearance.

References: Ashburner, M. 1975, in: Handbook of Genetics (R.C. King, ed.) vol. 3, pp. 793-811; Belyaeva, E.S., I.E. Vlassova, Z.M. Biyasheva, V.T. Kakpakov, G. Richards and I.F. Zhimulev 1981, Chromosoma 84:207-219; Bryant, P.J., B. Huettner, L.I. Held jr., J. Ryerse and J. Szidonya 1988, Dev. Biol. 129:541-554; Slizynski, B.M. 1964, Cytologia 29:330-336.

Zhuchenko, A.A., V.P. Gorodetsky, L.P. Kovtyukh, and A.B. Korol. Institute of Ecological Genetics, Kishinev, USSR. **The experimental analysis of the evolution of recombination in fluctuating environment.**

The regularities of microevolutionary changes in the rec-system is one of the most complicated problems in population genetics. Notwithstanding the abundance of theoretical models (see Maynard Smith, 1978) the possibility of changing the recombination level within a population of a sufficiently large size exposed to selection for increased adaptivity, has not been given any experimental effort. The exception is the one obtained by us on large *Drosophila* cage populations with size variations from 1 to 6 thousand flies.

It has been shown that the selection for resistance to daily temperature fluctuations with the amplitude increasing in generations leads to elevation of the crossing-over frequency (rf) in zone *b cn vg* of chromosome 2 (Zhuchenko et al., 1983, 1985).

This paper presents the results of repeated evaluation of changes in the recombination level due to selection for resistance to temperature fluctuation (from 22°C up to 27°C at the beginning of the experiments and from 11°C up to 32°C at the end), obtained from the other hybrid population (*b cn vg bw* x Domodedovo-18). Control was carried out at a constant temperature of 25 ± 1°C. Considering the possibility of direct temperature influence upon crossing-over, rf evaluation for F_1 as well as for compared populations F_{10} was done both in the optimum and extreme conditions. Significant differences between the experimental and control populations were observed only in $rf_{b\text{-}cn}$ while comparing variants under climachamber conditions, $rf_{b\text{-}cn}$ increased at a rate of 0.43% per generation. These results were confirmed in F_{30} as well. To expose heterozygotes for marker loci in F_{30} we tested individually over 100 phenotypically normal females from the experiment and about 400 females from the control. The developmental cycle took place under the conditions created for whole the population. Males of the *b cn vg bw* line in the test cross (1 female F_{30} ++++/ *b cn vg bw* x 2 male *b cn vg bw*). The progeny of the above cross has been cultivated at 25 ± 1°C. The results given in the table are the evidence of a very high efficiency of the induced (indirect) selection for rf. The population differences are highly significant in all cases.

	Control			Experiment		
Segment	No. of families	Amount of progeny	rf,%	No. of families	Amount of progeny	rf,%
b—cn	14	5542	7.65 ± 0.65	12	5367	19.78 ± 1.87***
cn—vg	9	3964	9.97 ± 0.75	11	4282	16.11 ± 1.62**
b—vg	5	1990	13.83 ± 1.83	7	2638	33.20 ± 2.45***
vg—bw	5	1865	29.42 ± 1.82	11	4114	35.72 ± 1.36*

*, **, ***, differences from the control are significant at P< 0.02, 0.01, 0.001

Judging from the data obtained the rate of $rf_{b\text{-}cn}$ change, beginning with F_{11} did not decrease and was 0.46% on the average per generation (the average for 30 generations). It should be noted, however, that the differences between the populations in F_{30} in all tested zones have been caused not only by the growth of rf in the experimental variant but by the true decrease in control as well.

These data could be considered as the first experiment proving Fisher's (1930) hypothesis of a possible rf decrease in generations in a constant environment. We have observed analogous, though less significant differences in testing another population derived from a free cross of heterozygotes which were obtained by hybridization of *b cn vg bw* and D-18, D-32, Algeria, and Canton-S lines. In this experiment significant differences in rf between the control and experimental populations of F_{30} were found only for b-cn zone (5.93 ± 0.85 and 12.5 ± 1.07%). It should be noted that the obtained value of $rf_{b\text{-}cn}$ in F_{30} of the control population coincides well with the weighted mean for initial heterozygotes (5.89%) and is nearly twice lower than in the experiment.

In our opinion, the cause of differences between the two- and five-component populations concerning the rate of rf changes depends first of all on a larger genotypic variability of the five-component population. Since the reserve of variability available for selection is higher in a five-component population than in a two-component one, then, naturally one can suppose that to adapt it to temperature flucuations a lower level of recombination is sufficient and that is why the adaptation to the same conditions has "induced" better advantages for high crossing-over genotypes in a two-component population than in a five-component one.

On the whole, the results presented in this work, as well as our previous research, attest to the fact that selection for the increased ecological resistance may lead to recombination increase in population.

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Technique Notes

Barnes, P.T. and V.C. Courreges. Connecticut College, New London, Connecticut USA. A technique for rapid collection of large numbers of replicate egg sets from multiple sources in *Drosophila*.

A technique was devised for the rapid collection of replicate egg sets from multiple sources over a specified period of time. Such a technique is useful in population and quantitative genetic experiments where numerous genotypes, isofemale lines or random samples of individuals populations are being used. The technique avoids virtually any direct contact with or manipulation of the eggs, thus avoiding any potential damage to individual eggs. As a result, normal fertility or egg hatchability levels (specific for each stock, of course) are achieved.

The round bottoms of small polystyrene plastic test tubes (12 mm x 75 mm) are removed with a coping saw. The bottoms are then plugged with rubber stoppers (size 00). A 1.5% agar in distilled water solution (w/v), with food coloring added to provide greater visibility of eggs, is prepared, transferred to the tubes and allowed to cool completely to room temperature (Fig. 1a). The agar plug from one tube is transferred onto one of two available plain glass microscope slides (25 mm x 75 mm). The transfer is accomplished by removing the rubber stopper and pushing the agar plug out of the tube with the flat end of a wooden dowel (or the flat end of a grease marking pencil, in a pinch). The plug is sliced in half longitudinally with a fine, sharp scalpel (#3 handles with removable #10 or #11 blades) and each half is placed flat side down on the separate slides (Fig. 1b). The plugs can then be sculpted with the scalpel to provide maximum visibility from above and/or a flat surface area for placement of a medium for inducement of egg-laying. Best visibility seems to be achieved by beveling the ends from top to bottom and removing a narrow longitudinal slice from

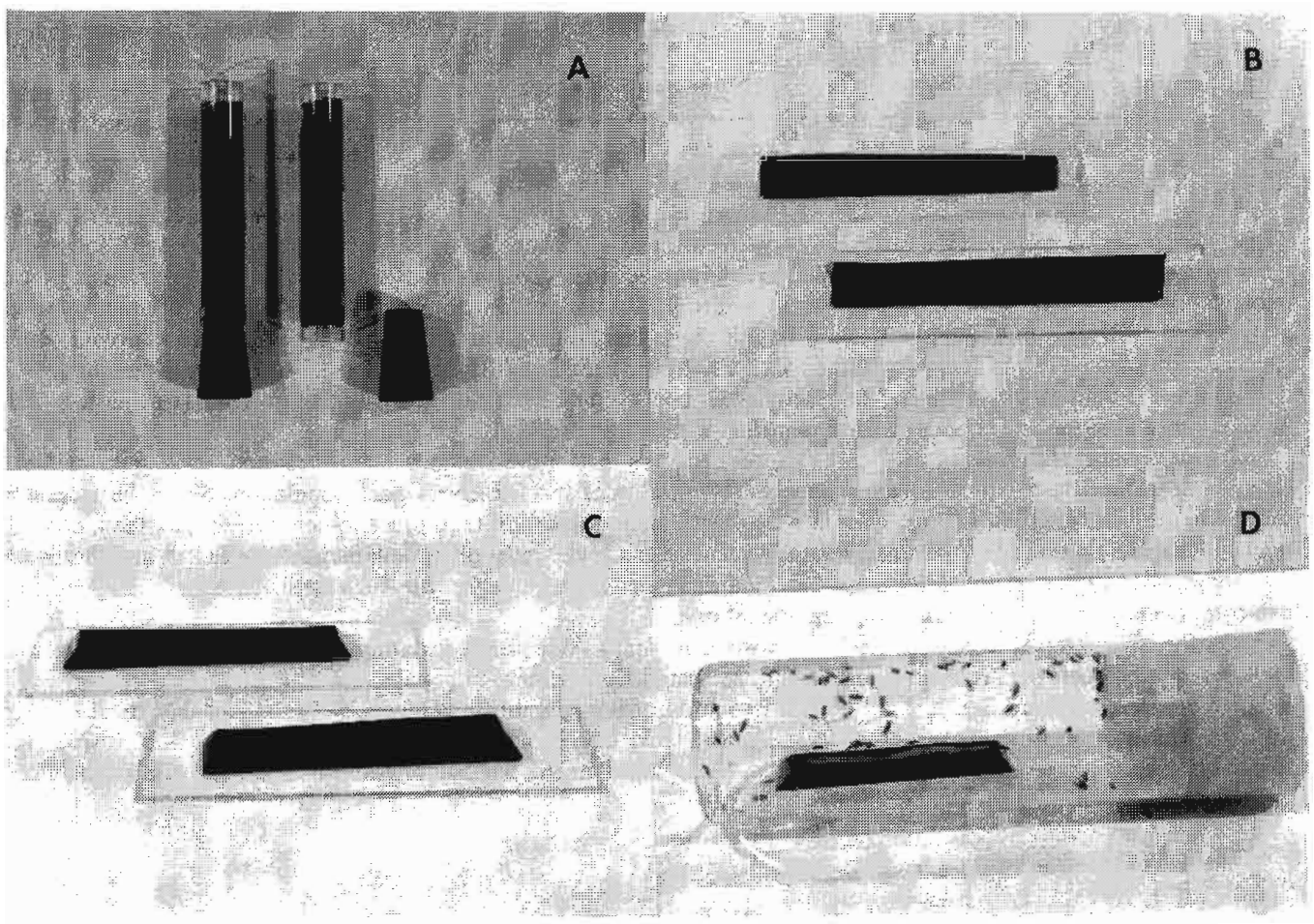

Figure 1. Technique for preparing agar strips for egg collections. (a) Food colored agar solidfied in upright tubes; (b) One agar plug sliced in half providing two agar strips on glass slides; (c) Sculpted agar strips providing maximum visibility from above; (d) Agar strip and glass slide inside tube for egg collecting.

the rounded top edge (Fig. 1c). This arrangment allows the entire exposed surface of the agar to be seen from above and allows for easy egg counting with a dissecting microscope. Each slide is then placed in a large, 16 dram vial (plastic culture vials with foam plugs from Carolina Biological Supply Co. work well), which is then kept on its side (Fig. 1d). Flies may be added to the tubes either before the slides and agar, with mass transfer, or afterwards with an aspirator. It is difficult to tap the flies to the bottom of the tubes once the slides and agar are added, as the agar may slide and break into pieces. If a precise number of adults is not critical, but rapid set up and collection is, then adding the adults through mass transfer prior to the slides and agar works best. A few flies may be lost through escape or crushing when adding the slides and agar to the tube. However, if sufficient numbers are initially added (approximatley 25 pairs or more) then the slight mortality has little effect on the number of eggs subsequently available. For extended egg collection periods (12 hrs or more) it is necesary to maintain as high a relative humidity as possible to prevent dessication of the agar and flies. Groups of 24 tubes on their sides can be maintained in plastic shoe boxes.

The slide and agar are removed from the tube by encircling the plugged end of the horizontal tube with one hand, driving the flies toward the bottom, lighted portion of the tube. The plug is quickly removed, the slide grasp with slide forceps and removed with gentle shaking to dislodge any flies from the slide and agar. The slide and agar are then placed immediately under a dissecting microscope for egg counting and collecting. If the agar was scuplted as previously described, there are five "surfaces" available to help in organizing the counting (two short ends, two long sides and one long flat top). Groups of specified numbers of eggs can be removed by slicing the agar with the fine scalpel and transferring the agar plus eggs en masse to a vial containing *Drosophila* medium. The transfer can be done with the scalpel or a microspatula. The number of eggs available can be controlled to a certain extent by varying the number of adults present and/or the length of the egg collecting period. Also, the same set of flies can be used for a second and third round of egg collecting if a food source (i.e., egg-laying inducement medium) is spread lightly on the flat top surface of the agar.

This method has been used in a recent experiment with *Drosophila melanogaster* (Barnes et al., 1989) to collect 108 sets of 50 eggs per day, requiring a two-hour period per day with two workers for the actual counting and distributing of the eggs. Three sets of 50 eggs (150 total) were collected from each of 36 vials (groups of flies) for distribution among three temperatures for subsequent developmental time and absolute viability measurements. A 12 hr egg-laying period was used with 20-25 pairs of flies in each vial. An egg-laying inducement medium was used: dry baker's yeast is slowly added to a 1:1 mix of apple cider vinegar and pineapple juice (also darkened with food coloring) until a thick paste is formed (B.W. Geer, personal communication). This paste is then spread sparingly along the flat top surface of the agar strips.

Acknowledgments: We thank P. Fell for help with the photographs. Supported in part by a Bristol-Myers Company Grant of Research Corporation.

References: Barnes, P.T., B. Holland and V.C. Courreges 1989, Genetics 122:859-868.

Bel, Y.[1], E. Wilkerson[2], and K.B. Jacobson[2].
[1]Department of Genetics, University of Valencia, Spain, and [2]Oak Ridge National Laboratory, Oak Ridge, Tennessee. A method to collect large quantities of *Drosophila* larvae or pupae.

Sometimes, for biochemical or genetic studies, it is necessary to handle or obtain a large number of synchronized *Drosophila* larvae or pupae. We describe herein a quick and easy method to obtain high amounts of these individuals free of food debris, based on the method of E.C. Travaglini and D. Tartof, slightly modified (Travaglini and Tartof, 1972).

Drosophila embryos are obtained allowing adult flies to lay eggs on trays which contain a paste-like mixture of water and live yeast. After 1-2 hours, eggs are collected on a fine sieve by washing the trays under tap water. Then, the eggs are transferred to a plastic "shoe box" containing 6 to 8 layers of pharmaceutical cotton gauze soaked with liquid medium composed of 288 g baker's yeast, 144 g sugar, 0.28% phosphoric acid and 0.06% propionic acid in 1 l water. The boxes are covered with a plastic lid (in which a ventilation opening has been cut out in the center and covered up with a fine mesh acrylic gauze secured with tape), and placed in a culture chamber at the desired temperature. At the appropriate time, larvae or pupae can be collected on a sieve by washing the boxes under tap water.

With the wild type of *Drosophila melanogaster*, developmental times under this treatment are as in standard media, and insect fertility and general development after it are completely normal. However, we have observed that this different diet modifies the amino acid pool in the organism: we have measured phenylalanine (an essential amino acid) and tyrosine by High Performance Liquid Chromatography, and the results showed that the amount of these amino acids in third instar larvae were increased (26% and 57%, respectively) with respect to their levels in the same individuals grown in standard media. This fact must be taken into account if this method has to be applied to the study

of amino acid metabolism in this insect.

Acknowledgments: Work supported by a grant from the Conselleria D'Educacio i Ciencia de la Generalitat Valenciana.

References: Travaglini, E.C. and D. Tartof 1972, DIS 48:157.

Bixler, A. and L. Tompkins, Temple University, Pennsylvania, USA. Sucrose density separation of *Drosophila* larvae from instant medium.

As noted by Nothiger (1970), *Drosophila* larvae that are cultured on cooked cornmeal media can be easily separated from the substrate by suspending the contents of the culture bottles in 20% sucrose; after a few minutes, the larvae float on the surface of the sucrose solution and the medium sinks to the bottom. However, we have observed that particles of Caroline 4-24 Instant Drosophila Medium, which is less dense than cornmeal media, float on the surface of 20% sucrose solutions. Hence, larvae cultured on this medium cannot be efficiently separated from the substrate with 20% sucrose. Although 30% sucrose is also ineffective, we have found that larvae grown on Instant Medium can be separated from the substrate by suspending the contents of culture bottles in 40% sucrose.

Acknowledgments: Support was provided by a National Science Foundation Undergraduate Research Project Grant, awarded to Temple University (A.B.) and grant BNS-8615554 from the National Science Foundation (awarded to L.T.).

Reference: Nothiger, R. 1970, DIS 45:177.

Boccaccio, G. and L.A. Quesada-Allué, Instituto de Investigaciones Bioquímicas "Fundación Campomar", Buenos Aires, Argentina. A convenient micromethod for protein and lipid extraction from *Drosophila* and *Ceratitis* tissues.

This technical note describes an easy procedure to isolate lipid-free proteins as required for electrophoresis in polyacrylamide gels. Lipids and soluble substances are simultaneously isolated in different fractions.

Conditions were optimized to avoid phenoloxidases and proteases activity.

This method is specially suitable for studies on glycoconjugates (glycoproteins and glycolipids) from small samples like single whole flies, integument or cuticle pieces, imaginal discs, fat bodies, etc.

The following protocol is routinely used in our laboratory to process either one to four whole *Drosophila* flies or a single *Ceratitis capitata* pupa or fly.

1. Homogenization: Four frozen *Drosophila* flies (liquid N_2) are usually homogenized in a micro (1 ml) teflon/glass or glass/glass homogenizer. Alternatively a home-made homogenizer of a microfuge glass tube can be used (Marcus 1985). For scaling down to one or two flies, a smaller homogenizer and exactly half the volumes should be used.

a) Four hundred microliters of methanol containing 1-phenol 2-thiourea (PTU) 10 mg/ml and butyl-hydroxytoluene (BHT) 7 mg/ml are added to the flies in the homogenizer.

b) Five microliters of a proteolysis inhibitors cocktail (containing EDTA, DFP, PMSF in 10% DMSO) are also added.

c) The flies are then completely homogenized. The piston should be motor-driven and the homogenizer maintained ice cold.

d) The piston is then twice carefully washed with 100 ul of methanol/antioxidants in order to avoid loss of material (total volume 600 ul).

2. Protein delipidation:

a) Nine hundred microliters of chloroform are added to the homogenate and carefully mixed. After ten minutes, the homogenizer is centrifugated at low speed (using an adapter). Alternatively, the extraction mixture can be left for 30 minutes up to complete precipitation of the protein.

b) The chloroform/methanol supernatant is decanted (or transferred with a Pasteur pipette) to a glass, pencil labeled, Kimax tube.

c) The pellet is washed once with 250 ul of chloroform/methanol 3/2 (v/v) and processed as in a), b). The washed pellet, containing most of the denatured protein is allowed to air dry (just to dryness) and resuspended in an aqueous buffer to be further processed or directly in the sample buffer of Laemmli to be electrophoresed in SDS-PAGE. In some cases, like homogenization of *Ceratitis* pupae, disruption of the pellet by sonication with a micro-tip may be required.

3. Lipids and water-soluble substances:

a) For lipid separation, 350 ul of a 4 mM $MgCl_2$ solution are added to the (1.75 ml) chloroform/methanol pooled extract (total volume: 2.1 ml).

b) After centrifugation, a perfectly clean upper (aqueous) phase becomes separated from a lower (organic) phase by a tiny interphase. This is a classical Folch's partition (Folch et al., 1957). The upper phase contains most of the water soluble substances and polar glycolipids. It must be carefully transferred to another tube with a Pasteur pipette. A meniscus is left in order not to disturb the interphase.

c) One ml of theoretical upper phase (chloroform/methanol/water, 1/16/16) is carefully added to the meniscus, then removed and pooled with the previous upper phase.

d) The interphase is now removed together with the new aqueous meniscus by suction with a Pasteur pipette or a plastic micro-tip. This fraction contains some lipophilic proteins.

e) The lower lipidic phase is washed twice with 1.0 ml of theoretical upper phase by mixing the phases which are then separated by centrifugation, the washed lower phase contains most of the lipids, including those belonging to the glycosylating machinery (dolichyl derivatives).

For most of the purposes and samples these procedures give quite clean protein and lipid fractions suitable for quantitative and/or qualitative analysis. However, for special radiolabeled (glyco)proteins analysis further delipidation might be required (Quesada-Allué, 1980; 1982).

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Bouletreau-Merle, J. and O. Terrier. Universite Lyon I, F-69622 Villeurbanne, France. Punching flies.

Oviposition behavior is an important factor in the fitness of *Drosophila* and there are many studies related to the choice of oviposition sites by *D. melanogaster* (Mainardi, 1968; David and Van Herrewege, 1970; Takamura and Fuyama, 1980, etc.). The possibility of choice implies that the female be able to appreciate the characteristics of the substrate and control egg laying efficiently. The latter condition is met by the existence of two different genetically determined controls acting respectively on ovulation and oviposition processes (Bouletreau-Merle and Terrier, 1986). Chemical and physiological characteristics of the substrate greatly influence the oviposition rate (see review Grossfield, 1978) and we present here a behavior which seems to correspond to a testing activity.

This behavior was pointed out first in isofemale lines originating from a natural French population, studied for retention capacity. The females exhibiting this behavior explore the substrate with the distal part of the abdomen, making holes by protrusion of the vulva (Fig. 1). No egg is laid in these cavities and, as the behavior is presented both by inseminated flies with high fecundity and by virgin females without any egg deposition, it seems that this punching behavior is related rather to substrate characterization than to egg insertion behavior. No significant difference was noted between these flies and those which do not exhibit punching behavior, in the tendency to insert eggs, as defined by Alboronoz and Dominguez (1987).

The punching behavior is not shown by males. In females, the expression of the trait is highly variable. It varies in frequency and intensity with the physical characteristics of the substrate, but also

Figure 1. Nutritive medium surface pitted by the punching activity of one female.

with time, with the developmental temperature of the flies or with the physiological state of the female. Although it occurs without egg deposition, it is probably related to egg-laying release, since its intensity of behavioral expression is higher in inseminated flies than in virgin ones and increases in virgin females during the last days of the preoviposition period.

This testing activity was present in the various temperate populations tested. This behavior is genetically determined and the intensity in the expression of the character (density and depth of the holes) was susceptible to artificial selection. A genetic analysis is in progress.

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Cockburn, A.F., H. Meier, and M.Q. Benedict. USDA/ARS/MAVERL, 1700 SW 23rd Dr., Gainesville, Florida 32604. A simplified procedure for embryo injection.

Preparation of fly embryos for injection is a tedious procedure, involving dechorionation and mounting on tape. We have found that both of these steps are unneccessary and can be eliminated.

The following technique was originally developed for injection of housefly embryos, but it appears to work well for *Drosophila* embryos as well. Eggs are collected on agar plates as usual. A wet paintbrush is used to transfer the embryos to a glass slide and the moist embryos are arranged in a line. The embryos are allowed to air dry or dried for a few minutes in a dessicator. After covering the embryos with mineral oil, they are injected using a short, sharply tapering needle (which can have a larger diameter than those used to inject dechorionated embryos). A few hours after injection embryos can be washed off the slide with water and collected on filter paper or cloth, or the slide can simply be inserted into a vial of food and the oil allowed to drain off.

Cockburn, A.F., H. Meier, J. Cibrian-Tovar, and D.A. Carlson. USDA/ARS/MAVERL, 1700 SW 23rd Dr., Gainesville, Florida 32604. Monitoring of embryo injection using a fluorescent dye.

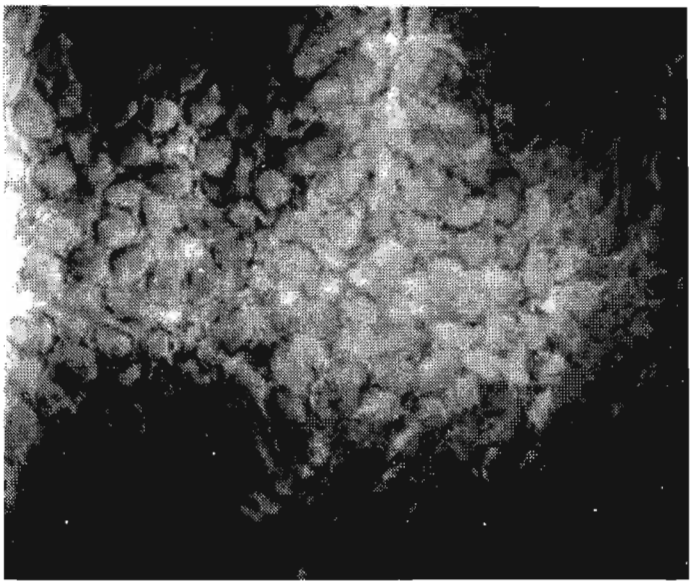
We have recently been attempting to mass-inject fly embryos by microparticle bombardment (the "DNA shot-gun" approach). Because of the difficulty of seeing particles in embryos, we investigated the suitability of various food dyes for monitoring injection. The required properties of the dye would be water solubility, low toxicity, and lack of binding to the chorion and vitelline membrane. A large selection of dyes was obtained (courtesy of Dr. Richard Brenner) and evaluated. D&C Green #8 was the best dye evaluated: it is extremely soluble, can be entirely washed off the exterior of the embryos with water, can be detected in very low amounts because it is fluorescent, and exhibits low toxicity when injected in easily detectable concentrations.

Dye was prepared at a concentration of 0.1% (w/v). Embryos containing dye could generally be seen to be green under visible light, and illumination with long-wave UV resulted in intense green fluorescence. At this concentration development of the embryos appeared to be the same as embryos injected with water alone, but hatching was reduced. Therefore we used dye in injection solutions as a training tool, because the injection process is easily visible and the fraction of injected and viable embryos can be determined afterwards.

Dyby, S. and Kalthoff, K. Center for Developmental Biology, Dept. of Zoology, University of Texas, USA. Observing cell movements in live *Drosophila* embryos with scattered light contrast (SLC).

We have developed an easy method for viewing surface details in live *Drosophila* embryos with scattered light contrast (SLC) under the compound microscope. SLC makes cellular and nuclear contours clearly visible without fixation or staining (Fig. 1). We use SLC for tracing the movements and shape changes of individual cells in videotaped recordings. SLC may also be useful for scoring cuticular abnormalities in hatched larvae.

To prepare embyros for viewing, they are dechorionated, lined up on a microscope slide with double-stick tape, and covered with a drop of insect Ringer's. To adjust our microscope (Zeiss Universal II) for SLC, we begin with normal Kohler illumination. We fully close the field diaphragm at the base of the microscope, so that its image in the focal plane is as small as possible. To further reduce the size of the image, we use a high-power condenser (Phako IV Z7 achr. apl. 1.4). Any auxiliary condenser lenses that increase the image of the field diaphragm should be swung out of the



optical pathway. We also close the condenser diaphragm almost fully for best contrast. With a low power objective, we center an embryo over the image of the field diaphragm. To observe and videotape embryos we use a 25X/0.8 Plan Neofluar immersion objective.

The beam of light that enters the embryo is scattered by the yolk sac and other egg components. We assume that the light scattering generates the contrast that brings forth the surface contours of the embryo. DIC prisms or polarizing filters are not necessary to obtain SLC, although they may further enhance the image contrast. The major drawback of SLC is the uneven light intensity, which is high over the image of the field diaphragm and falls off quickly to the sides. This problem can be reduced by opening the condenser diaphragm. However, there is a trade-off between even illumination and the degree of contrast.

Figure 1. Dorsal aspect of a *Drosophila* embyro during germ band elongation, observed with a 25X Plan Neofuar immersion of objective and scattered light contrast.

Eisses, K. Th. Department of Plant Ecology and Evolutionary Biology, Rijksuniversiteit Utrecht, The Netherlands. **A multiple choice device for measuring larval and adult food preference.**

A number of studies have been reported in which petri dishes filled with two kinds of food medium, divided over the two halves, have been used for measuring larval food preference (Sokolowski et al., 1983; Gelfand and Mc Donald, 1983). This experimental design has only a restricted application. We developed a Multiple Choice Device (MCD) as shown in Figure 1, which include large improvements. Disc A (9cmØ) consists of two rings of evenly spaced holes, six and twelve respectively, with the diameter of small tablet vials (14mm Ø) which are placed in a vial holder (Fig. 1b-B). After having placed the Choice Dial (A) in a petri dish with attached tin-foil at the bottom, the holes can be filled with food media of different kinds in various patterns, e.g. alternately or in half circles (Fig. 1a). The center well is filled with 1.9% agar to keep eggs or larvae moist. Eggs or larvae of one of the strains to be examined are placed in the center. The closed petri dish is put in a culture room for a certain period of time to let the larvae choose their preferred medium. Thereafter, the medium including the larvae or pupae can be pushed gently with a sterile plastic straw (C) in the tablet vials as indicated in Fig. 1b. If only larval food preference has to be measured, the vials with added medium discs are given a mild heat treatment (40°C) or a 10% glycerol treatment, which presses the larvae out of the medium. Otherwise the vials have to be stoppered appropriately (Fig. 2). The MCD (disc A) can also be used for measurements of female oviposition site preference and larval choice thereafter. In pilot experiments very reproducible results were gained (Eisses and Bets, 1991).

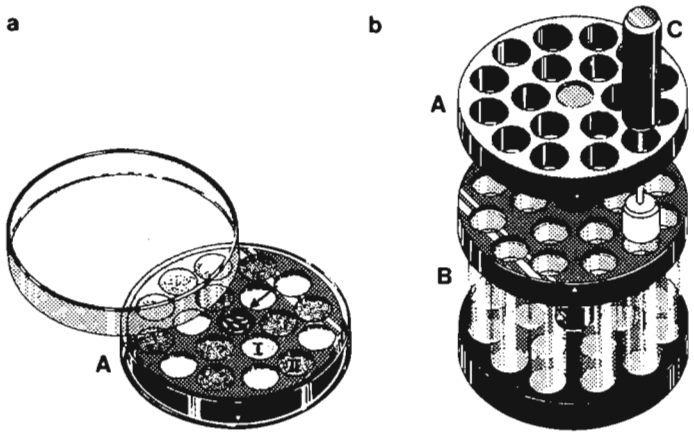


Figure 1. Multiple Choice Device: Discs A are made of plexiglass with two rings of holes, which fit precisely to the holes in the vial holder B.

Acknowledgments: I thank Mr. John Spierenburg for making the apparatus better than I could design, and Mr. Dick Pater for the beautiful drawings.

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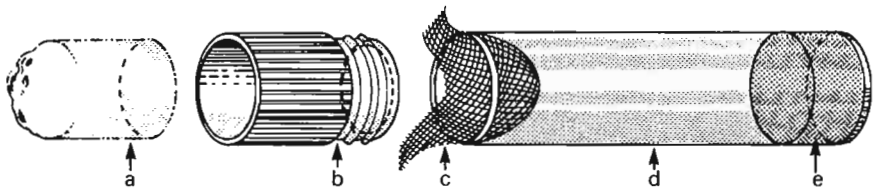


J.F. McDonald 1983, Behav. Genet. 13:281-293; Sokolowski, M.B., R.I.C. Hansell, and D. Rotin 1983, Behav. Genet. 13:169-177.

Figure 2. Tablet vial. a: cotton wool plug; b: open plastic stopper for crasping c: fine meshed cheese cloth; d: glass tablet vial (52 x 14 mm Ø); e: food medium (1 ml).

Factor, J.R., L. Ehrman, and B. Inocencio. State University of New York, Purchase, New York, USA. A microinjection technique for drosophilids.

A series of investigations into the reproductive effects of a microbial symbiont (a streptococcal L-form) have made use of a variety of microinjection techniques to transfer tissue preparations and cultured microbes into larval and and adult drosophilids. Such studies include those by Ehrman and Williamson (1965, 1969); Somerson, Ehrman and Kocka (1984); Somerson, Ehrman, Kocka and Gottlieb (1984); Williamson and Ehrman (1967); Williamson, Ehrman and Kernaghan (1971). At various times, a variety of needles have been used--steel, hand sharpened steel, microcapillary glass, and pulled glass needles. In one study, steel needles were used to inject third-instar larvae of *Drosophila paulistorum* and glass microcapillary needles were used to inject adults of *Ephestia kuehniella* (Gottlieb, Goitein, Ehrman and Inocencio, 1977).

The purpose of the current paper is to describe a microinjection technique that is the outgrowth of earlier methods and is in current use in studies similar to those described above (Ehrman, Factor, Somerson and Manzo, 1989; Ehrman, Somerson and Kocka, 1989). This technique allows carefully controlled injections of microliter volumes into larval and adult drosophilids with minimal damage to the integrity of the individual insect. It has been used successfully in the small drosophilid, *Drosophila paulistorum*.

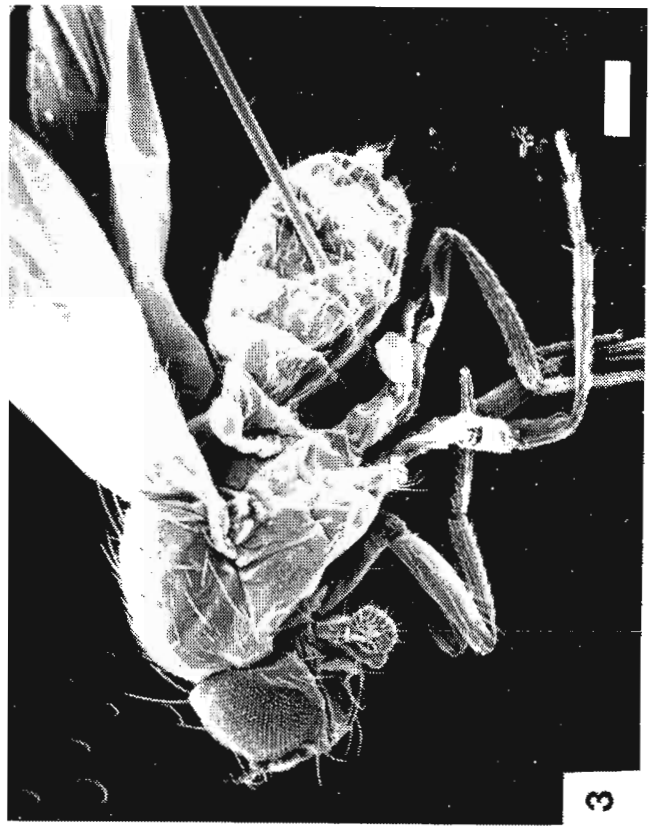
Microinjection apparatus: A fluid filled 10ul syringe (Hamilton #701, Hamilton Co., P.O. Box 10030, Reno Nev. 89510) is fitted with a 30cm length of silicon tubing (Cole Parmer #6411-6, ID 1/32 in, OD 3/32 in, Cole Parmer Instrument Co., 7425 N. Oak Park Ave., Chicago, IL 60648). The syringe is positioned in a repeating dispenser (Hamilton #PB600) and placed onto a Hamilton Electric Thumb (Model EPB-600, Hamilton #76710) equipped with a foot pedal switch. The pulled glass injection needle is inserted into the end of the silicon tubing.

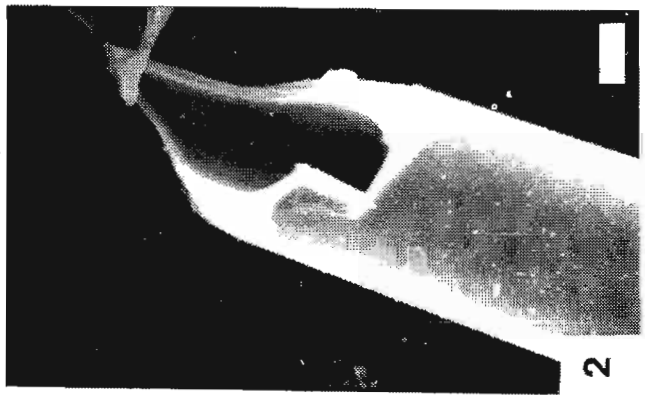
Glass needles are produced manually by pulling 50ul Accu-fill glass microcapillary pipettes (Clay Adams Division of Becton Dickinson and Co., Parsippany, NJ 07054). The pipette is held by the ends and the middle is positioned over the pilot light of a Touch-O-Matic gas burner (Hanau Engineering Co., Buffalo, NY). The glass is pulled in the usual way, by rotating the pipette over the flame until it will yield to a gentle tug; it is then pulled apart quickly and firmly and broken. The resulting needles are checked with a dissecting microscope and selected for uniformity. Two typical examples of needle tips prepared in this way are illustrated in Figures 1 and 2. Although automatic needle pullers have been tried, manually pulled needles are more quickly and easily prepared and yield excellent results.

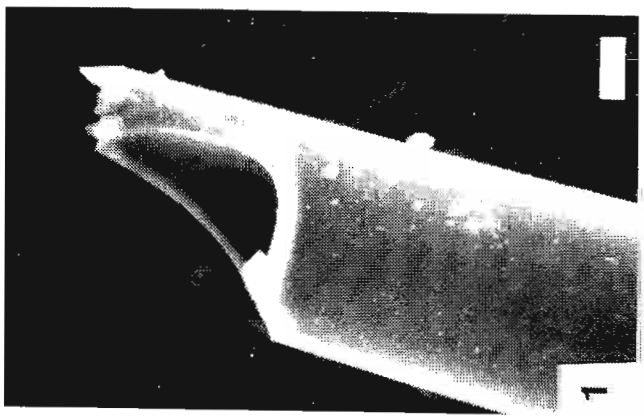
Microinjection technique: The 10ul syringe is filled with sterile mineral oil; a tuberculin syringe is used to fill the silicon tubing with mineral oil, which is then fitted over the end of the needle of the delivery syringe. The tip of the pulled glass injection needle is placed directly in inoculum and filled by capillary action; it is then inserted into the free end of the silicon tubing. Sterile technique is observed throughout.

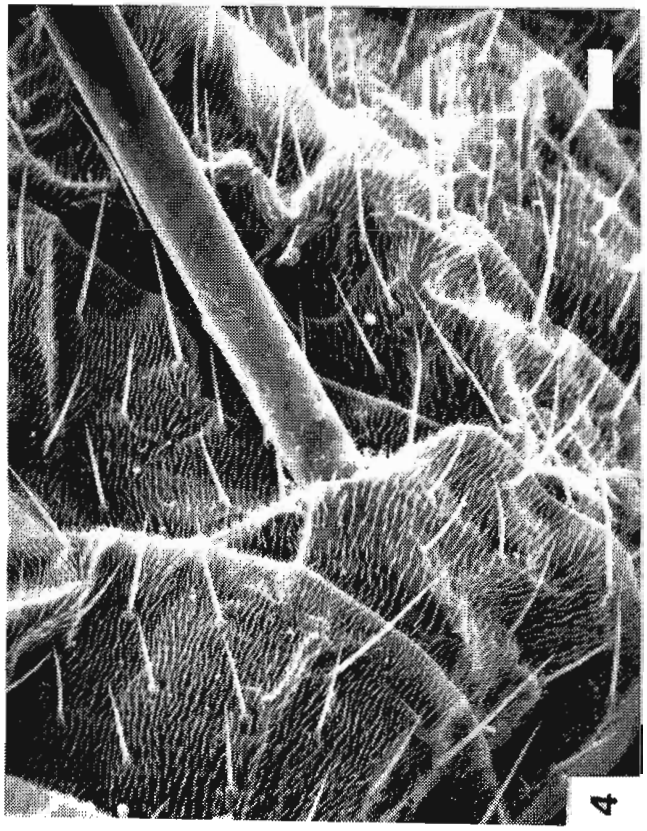
Alternatively, the Hamilton syringe with the tubing attached can be filled with mineral oil as a single unit. This is a quick method that works well. First, remove the plunger from the barrel of the Hamilton syringe, then position the syringe with the tubing attached onto the repeating dispenser. Next, fill a tuberculin syringe (needle gauge 18) with

Figures (opposite page). **1 and 2:** Examples of the tips of typical injection needles. Both scanning electron micrographs are to the same scale; diameters near tips are approximately 40 um. Scale bar represents 4.0 um. **3:** An adult *Drosophila paulistorum* female with injection needle still in place. The fly is approximately 2.1 mm long. Scanning electron micrograph. Scale bar represents 250 um. **4:** Abdomen of the same adult female in Figure 3, with injection needle still in place in the third abdominal sternite. Scanning electron micrograph. Scale bar represents 40.0 um. **5:** A wound in the third abdominal sternite of an adult *Drosophila paulistorum* female one hour after injection. Scanning electron micrograph. Scale bar represents 40.0 um.









mineral oil and insert the needle into the free end of the tubing. Depress the plunger of the tuberculin syringe and observe the oil until it fills the Hamilton syringe barrel completely. Cut the tubing near the tuberculin needle with a scalpel. Carefully return the plunger into the barrel of the Hamilton syringe and screw tighten the plunger into position on the dispenser. Insert your inoculum-filled capillary needle when you are ready to inject.

Adult flies are lightly etherized and placed on a ceramic tile under a dissecting microscope. Each fly is gently positioned against the tip of a camel-hair brush and held steady. The injection needle is shallowly inserted into the abdomen at a narrow angle and pointing anteriorly. It can be placed under the fold between the third and fourth abdominal sternites, but it sometimes pierces the cuticle in the third segment. Figures 3 and 4 illustrate a fly that was injected in this manner and was fixed with the needle still in place. Figure 5 shows a wound one hour after injection. Although micromanipulators have been tried, injection by hand is faster and simpler and yields excellent results after some practice. Delivery of the preset volume is initiated by pressing the foot pedal switch controlling the Electric Thumb. Neither antibiotics nor other special procedures to inhibit infection of the wound seem necessary.

The volume of inoculum injected is controlled by the repeating dispenser. In the studies currently underway, 0.2 ul of inoculum is routinely injected; at that point, the *Drosophila paulistorum* proboscis is extended and contracted, a routinely reliable indication that as much volume as can be tolerated has already been delivered.

Following injection, flies are immediately transferred to a clean, dry vial and held until they recover from etherization. They are then transferred to a fresh food vial or culture.

The results of this technique are excellent. Flies typically recover from the anaesthesia in 3 to 5 minutes. There is no discernible mortality due to injection. In each experiment, a sterile insect saline solution can be injected into a control group of flies to assess any possible effects of the injection procedure.

When *Drosophila paulistorum* larvae are injected, they pupate immediately, regardless of the instar injected. Such forced, early pupation is often inappropriate and results in death.

Acknowledgments: This work was made possible by funding from the National Institutes of Health and the Whitehall Foundation.

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Farkas, R. Institute of Experimental Biology and Ecology, Slovak Academy of Sciences, Czechoslovakia. Simple method for high efficiency pulse labelling of proteins in *Drosophila* larval salivary glands cultured *in vitro*.

Because of the possibility of combining cytological, genetical and biochemical approaches, *Drosophila* salivary glands are being used increasingly in the study of the regulation of eucaryotic gene expression during the development of an organism. An important and often first step in defining the mechanisms of gene regulation in cell differentiation is the biochemical elucidation of patterns of the proteins synthesized in vivo. The work reported here describes a simple and reliable method for highly-efficient pulse labelling of proteins in salivary glands dissected from *Drosophila melanogaster* larvae.

The pairs of salivary glands were dissected from larvae of the last instar. One lobe was used for protein labelling and the sister lobe was used as a control for determining the exact developmental age of the gland according to puff stage (PS) in polytene chromosomes (Ashburner, 1972). Proteins were labelled either with ^{14}C-leucine (240 mCi/mmol; UVVVR, Prague, Czechoslovakia), ^{14}C-amino acid mixture (80-240 mCi/mmol; UVVVR, Prague), ^{3}H-leucine (120-160 Ci/mmol; Amersham International plc, England) or with ^{35}S-methionine (1200 Ci/mmol; Amersham International plc.) for 20 min, 40 min, 60 min, 90 min or 120 min. Dissected salivary glands were washed 2 times in *Drosophila* saline (Ephrussi and Beadle, 1936) and transferred carefully to a drop of the same saline containing 0.1% $MgSO_4 . 7 H_2O$, 0.15% sucrose and 0.15% trehalose. Twenty gland lobes were cultured in 20 ul-drop of this saline. A particularly important fact to note is that this drop is placed onto a plastic material like polystyrene (Falcon Petri dish or Gibco serological plate cover). This allows the salivary glands to float on the surface of the drop. Then a 50 - 100 uCi amount of radiolabelled amino acid(s) is added to the drop, and glands are incubated at 24°C for the times indicated above. Proteins were extracted with sample buffer (125 mM Tris-HCl pH 6.8, 2% SDS, 5% B-mercaptoethanol, 10% glycerol). Radioactivity incorporated into proteins was measured after spotting 1 ul of protein extract onto Whatman

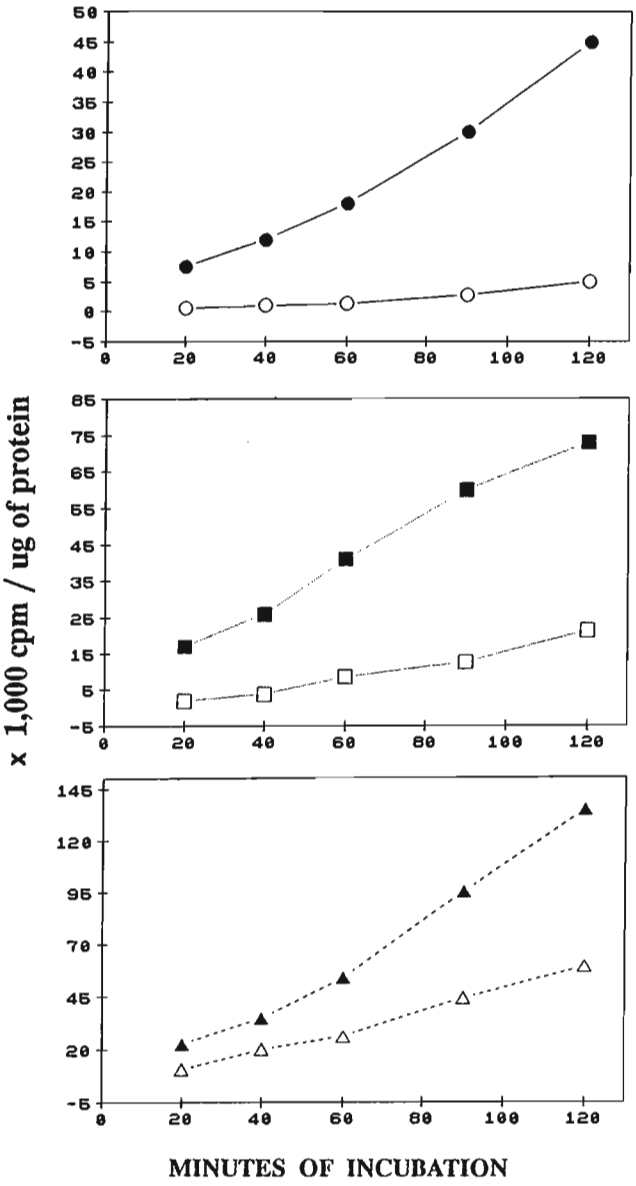

Figure 1. Graphic illustration of the incorporation of radiolabelled amino acids into proteins of larval salivary glands under different conditions. Open symbols represent rate of incorporation using submerged incubation in an amino acid medium. Filled symbols represent incorporation under complete aeration of salivary glands floating on the drop of amino acid-free medium. Circles are for ^{14}C-leucine, squares are for ^{3}H-leucine and triangles are for ^{35}S-methionine.

GF/C filter followed by trichloroacetic acid precipitation. To evaluate incorporated radioactivity as counts per minute (cpm) per ug of protein, the amount of protein was quantified from 1 ul of protein extract using the colorimetric method of Bradford (1976). The proteins were subsequently analysed by SDS-electrophoresis according to Laemmli (1970) and visualized by fluorography (Bonner and Laskey, 1974).

As shown in Figure 1, floating incubation of salivary glands in the amino acid-free medium increases incorporation of radiolabelled amino acid(s) into the proteins by a factor of 3 - 10 times in comparison to submerged incubation in a medium containing 19 amino acids (lacking the amino acid substituted for by the radioactive one). The method described here is based on two physiological facts. Firstly, the intracellular pool of amino acids in *Drosophila* cells is large enough to be utilised during short-term pulse labelling without the external support of additional amino acids (House, 1974; Chen, 1985). Secondly, floating the tissue at the surface of the drop facilitates good gaseous exchange and optimal cellular respiration.

The methods for protein labelling in salivary glands used to date have utilised complete *Drosophila* culture medium containing amino acids and a submersion of the tissue (Lewis et al., 1974; Zhimulev et al., 1980; Poeting et al., 1982). The yields of radiolabelled protein under such conditions were in the range 500 - 2,000 cpm/ug protein for ^{14}C-leucine, 10,000 - 15,000 cpm/ug protein for ^{3}H-leucine and 20,000 - 30,000 cpm/ug protein for ^{35}S-methionine (see Figure 1). In the method described here, one exogenous (radioactive) amino acid preferentially penetrates into the salivary gland cells and is incorporated into newly synthesized proteins. This increased incorporation is significantly potentiated by aeration of the salivary glands at the surface of incubation medium. When salivary glands (or any other tissue) are incubated in submerged manner, incorporation of radiolabelled amino acids is significantly reduced. In the insect body, oxygen is actively transported by the tracheal system to the closest vicinity of particular organs and tissues (Keister and Buck, 1974; Mill, 1985). Since insect blood does not contain any specific oxygen transporting system, the distance between tracheoli and tissues is about 0.2 um or less. This small distance facilitates oxygen transport by diffusion through the haemolymph (Chauvin, 1956; Miller, 1974). In the case of submerged incubation, the distance between tissue and air is more than 0.2 um (often more than 1 mm), and thus, respiratory activity of the cells is significantly reduced. This situation results in diminishing of biosynthetic activity.

In order to create optimal conditions for unlimited respiration of salivary glands, the incubation was carried out in the medium which was previously dropped onto a plastic surface. The molecules of water have a greater attraction for

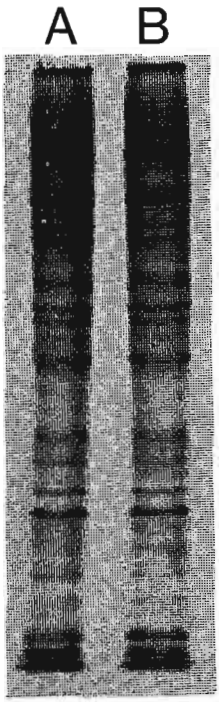

Figure 2. Autofluorogram of ^{3}H-leucine labelled proteins synthesized by salivary glands incubated by (A) the old technique and (B) the new high-efficiency method. Note that there is no difference in the protein patterns between these two samples. This indicates that the new technique has no unwanted effect on gene expression. These two samples were generated in the PS-4 stage of salivary glands for 20 min pulse. Lanes A and B contain 50,000 cpm; however, lane A was loaded with 14 ug of protein, while lane B was loaded with 3 ug of protein.

each other than for the plastic surface. This leads to a strong water surface tension which allows tissue to float at the surface of the drop. By contrast, on glass, molecules of water have a greater attraction for the glass surface than for each other and the water drop will collapse in time. Because of the weak water surface tension on the glass surface, the tissues will not float and will be completely immersed in the medium.

Figure 2 shows that there is no qualitative difference between proteins synthesized by salivary glands submersed in the medium containing 19 nonradioactive amino acids and proteins from glands floated on the drop of saline. This simple procedure for high efficiency labelling of proteins in salivary glands can be easily implemented for other *Drosophila* tissues or organs and can help in the analysis of low-concentration proteins (e.g. regulatory proteins) or proteins in low-level protein synthesizing cells (e.g. haemocytes).

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Frölich, A. and F.E. Würgler. Swiss Federal Institute of Technology and University of Zurich, CH-8603 Schwerzenbach, Switzerland.

The "High Bioactivation" cross for the SMART assay with the wing.

The *S*omatic *M*utation *A*nd *R*ecombination *T*ests (SMART) with the wing and with the eye have been developed to screen chemicals for their genotoxic potential (reviewed in Würgler and Vogel, 1986). In order to improve the usefulness of the wing SMART assay for detecting promutagens depending on cytochrome P450 dependent metabolic activation, a set of tester strains was constructed carrying chromosomes 1 and 2 from the DDT-resistant Oregon R(R) strain (Dapkus and Merell, 1977) conferring the chromosome substitution strains with a high constitutive level of cytochrome P450 (Hallstrom and Blanck, 1985; Frölich and Würgler, 1990d). Using this "high bioactivation HB" cross females from a ORR(1), ORR(2), mwh strain are mated to males from the strain ORR(1); ORR(2); flr^{3}/TM3, ri P^{p} e bx^{34e} e^{8} Ser (Frölich, 1989; Frölich and Würgler, 1989). When larvae from this cross are exposed to a number of promutagens they show an increased response compared to the standard cross. The sensitivity has been shown to be increased for compounds such as: diethylnitrosamine, benzo[a]pyrene, benz[a]anthracene, 7,12-dimethylbenz[a]anthracene, aflatoxin B1, urethan (ethylcarbamate) and N-nitrosopyrrolidine (Frölich, 1989; Frölich and Würgler, 1989, 1990a,b,c,d).

This technical note pursues two aims: (1) to make *Drosophila* workers interested in Genetic Toxicology aware of the new possibilities and (2) to document some specific technical aspects that have to be considered if one is working with the HB cross. In the HB cross, in contrast to the standard cross, we often find certain areas of the wing in which the hairs (trichomes) are arranged in a whirl-like fashion and/or are irregularly arranged, sometimes forming groups of juxtaposed hairs. This is particularly the case in the proximal part of field C (for a description of the different wing areas see Garcia-Bellido and Merriam, 1971). Photographs demonstrating these peculiarities encountered in analyzing the wings of flies from the HB cross may be obtained by writing to F.E. Würgler (Institute of Toxicology, Schorenstrasse 16,

CH-8603 Schwerzenbach, Switzerland). The morphological peculiarities of the wings in the progeny of the HB cross demand care in the analysis of wing-hair patterns. We trust, however, that experienced workers, being aware of the configurations shown on the photographs, will be able to identify wing spots on HB wings correctly.

We would like to stress a second technical point of importance: If any laboratory interested in using the HB cross obtains cultures from any source, it should check for the integrity of the stocks. We recommend that this is best done by checking the sensitivity of the larvae of the HB cross for high sensitivity to urethane or diethylnitrosamine. Among other sources urethane (ethylcarbamate) and diethylnitrosamine can be obtained from Fluka AG (Buchs, Switzerland). When doing these checks it should be kept in mind that for reasons not yet understood in detail the quantitative results from repeated experiments might vary up to a factor of two. Therefore, if discrepancies are encountered, a repeat of the check should be performed and results from both experiments should be considered in judging the status of the tester strains.

Acknowledgment: Supported by the Swiss Cancer League (Grant FOR.311.85) and the Foundation Finanz-Pool 3R (project no. 4/87).

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Graf, U., N. van Schaik, and R. Pacella. Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, Switzerland and Department of Genetics, University of the Witwatersrand, South Africa. Improved "High Bioactivation" cross for the SMART wing assay.

The *S*omatic *M*utation *A*nd *R*ecombination *T*est (SMART) in the wing of *Drosophila melanogaster* using larvae trans-heterozygous for two recessive third chromosome markers (multiple wing hairs (mwh) and flare-3 (flr^3)) has proved to be an easy and inexpensive test for mutagenicity screening in a eukaryotic organism *in vivo* (Graf et al., 1984, 1989; van Schaik and Graf, 1991). *Drosophila* is capable of bioactivation of promutagens, and recently Frölich and Würgler have produced additional test strains in which chromosomes 1 and 2 from a DDT-resistant Oregon R line (ORR) were incorporated into the standard mwh and flr^3 lines. These lines (ORR mwh and ORR flr^3) constitutively overproduce enzymes active in bioactivation and give increased sensitivity to a number of promutagens (Frölich, 1989; Frölich and Würgler, 1989, 1990a,b).

The "High Bioactivation" (HB) cross as used by Frölich and Würgler, however, presents a number of difficulties as pointed out in their DIS Technical Note. These are: (1) The presence of an irregular whorling in the pattern of wing hairs making spot classification difficult especially for inexperienced scorers. (2) An undesirably high variation in results from repeated experiments. (3) The low egg production of the ORR mwh females (this is also a problem in the standard cross when mwh is used as the female parent).

We now report that further studies indicate that all of these problems can be overcome by using the cross ORR flr^3 females x mwh (standard) males instead of the ORR mwh x ORR flr^3 cross used originally. The genetic determinant(s) responsible for the whorling pattern are located on chromosome 2 and are thus linked to the gene(s) responsible for the high bioactivation, but whorling is recessive whereas the high bioactivation present in the ORR flr^3 line is dominant. The ORR/+; mwh +/+ flr^3 hybrids exhibit high bioactivation with no complicating whorling and give more reproducible results than the ORR/ORR; mwh +/+ flr^3 hybrids. This has been shown so far with diethylnitrosamine, 7, 12-dimethylbenz [a] anthracene, urethane (ethyl carbamate), and N-nitrosopyrrolidine (Graf and van Schaik, 1991). The use of the strain carrying the flr^3 marker as the female parent assures good fertility. Our laboratories are now using the flr^3 line as female parent in the standard cross as well.

An additional improvement of the technique for both crosses is in the collection of larvae according to the method suggested by Magnusson and Ramel (1990). We use a solid agar base made of 5% agar-agar in tap water and cover it completely with an approx. 5 mm layer of a thick paste of fresh baker's yeast with a little sucrose added. This is allowed to dry under a gauze cover before the introduction of the flies for the egg laying. Later on, the larvae can be washed out of these yeast-agar bottles with tap water through a fine-meshed stainless steel strainer or through nylon gauze. This eliminates the use of a separation funnel and of sodium chloride or sucrose solution. The agar base bottles without yeast keep well for up to three weeks covered with parafilm and kept in a refrigerator.

This improved HB cross (ORR flr^3 females x mwh males) and the technical improvements make a useful addition to the versatility of the wing SMART in *Drosophila*.

References: Frölich, A. 1989, Thesis no. 8850, Swiss Federal Institute of Technology, Zurich, Switzerland; Frölich, A. and F.E. Würgler 1989, Mutation Res. 216:179-187; ____ 1990a, Mutation Res. 234:71-80; ____ 1990b, Mutation Res. 244:201-208; Graf, U., and N. van Schaik 1991, Mutation Res., submitted; Graf, U. et al. 1984, Environ. Mutagen. 6:153-188; ____ 1989, Mutation Res. 222:359-373; Magnusson, J. and C. Ramel 1990, Mutagenesis 5:511-514; Van Schaik, N. and U. Graf 1991, Mutation Res., in press.

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A system for rapid morphometry of wings and quick selection for wing traits.

Experimental directional selection for some quantitative morphological character is generally slow and difficult. With the "wingmorphometer" system the image of a live fly can be projected onto a digital pad, greatly increasing its size and permitting a perfect measurement of several morphometrical characters. Thus, flies can be measured and selected quickly and efficiently. The fly's visible morphology is reduced to a single plane and measured in two dimensions. Visibility of surface detail depends on local opacity. The resolution of surface detail of wings is very clear, making them therefore the best organ for morphometrical analysis with this system. Those parts which are less translucent, such as legs, antenae, halteres, head, thorax and abdomen can only be measured along their outlines.

The system includes the following elements: a metacrilate box with a CO_2 diffuser supply which secures an anaesthetized fly for subsequent measurement. Slides covered with a strip of double sided adhesive tape so that the slide has an adhesive surface. Here, the fly is carefully placed in a supine position, with its wings fixed to the slide so that the wing's visible morphology is reduced to a single plane and presents a clear projected image. The projection system is based on a microprojector composed of a microscope; two mirrors to transfer the projected image and a digitizer pad linked to a computer to record and process the measurements. A holding device secures the elements of the projection system and gives the local opacity to digital pad.

In order to measure the wings, flies are loaded and positioned in the slide as follows: firstly the flies are anaesthetized and placed on the slide's adhesive surface in a supine position, secondly the wings are lightly fixed to the slide. The arrangement of the wings is determined by a paint-brush in accord to the desired viewing orientation. The slides with the flies are kept in the CO_2-saturated metacrilate box until their subsequent measuring. The projection system produces a great increase in the wing's real size. (Wings are currently being measured in all orientations). The image of the fly is reflected by the first mirror, placed upon the microscope with an inclination of 45 degrees, onto a second opposed with the same inclination upon the pad, and then down onto the digital pad. The image beam passes from the first mirror to the second through an aperture in the holding device. The wing's final size depends on the augmentation of microscope lenses and the distances between elements of the projection system. The visibility of surface detail depends on the perfect local opacity of the wingmorphometer, since it removes the light from the projection system, the computer, and any external light which could distort the projection. An infra-red-absorbing glass is interposed between the condenser and the slide to prevent any damage to the flies.

The rapidity of the selection process is due to the ease of handling of the system. With the digital pad the wings are measured with a digital pencil and data are stored in the computer. The SELALAS original program indicates which flies must be selected, depending on the intensity of selection applied; the selected flies are separated from the adhesive surface with the aid of a fine paint-brush. All measuring and selection is done by hand, using the SELALAS program during selection. With this system about 5000 flies can be measured and selected in 30 hours.

Harisanova, N.T. and K.H. Ralchev. University of Sofia, Bulgaria.
Differential extraction of *Drosophila* histone fractions from polyacrylamide gels with dodecylbenzolsulfonic acid.

Histones were isolated from chromatin of *Drosophila hydei* embryos and analyzed by acetic acid/urea (Oliver and Chalkley, 1972) and acetic acid/urea/ Triton DF-16 (Alfageme et al., 1974) gel electrophoresis. The gels were stained with 0.1% Coomassie Blue and destained with methanol - acetic acid. The electrophoregramms were then treated with dodecylbenzolsulfonic acid, neutralized with ethanolamine and diluted with 7% acetic acid (1:40) until the gels were fully destained. In order to examine whether this fact is due to the extraction of the dye or/and of the protein itself the gels were stained and destained again.

It is evident from Figure 1 that the dodecylbenzolsulfonic acid extracts the dye from all histone fractions but not the protein with exception of that in H2A fraction. We used these results to identify H2A histone in polyacrylamide gels.

References: Oliver, D. and R. Chalkley 1972, Exp. Cell Res. 73:295-302; Alfageme, C.R., A. Zweidler, A. Mahovald

and L.H. Cohen 1974, J. Biol. Chem. 249:3729-3736.

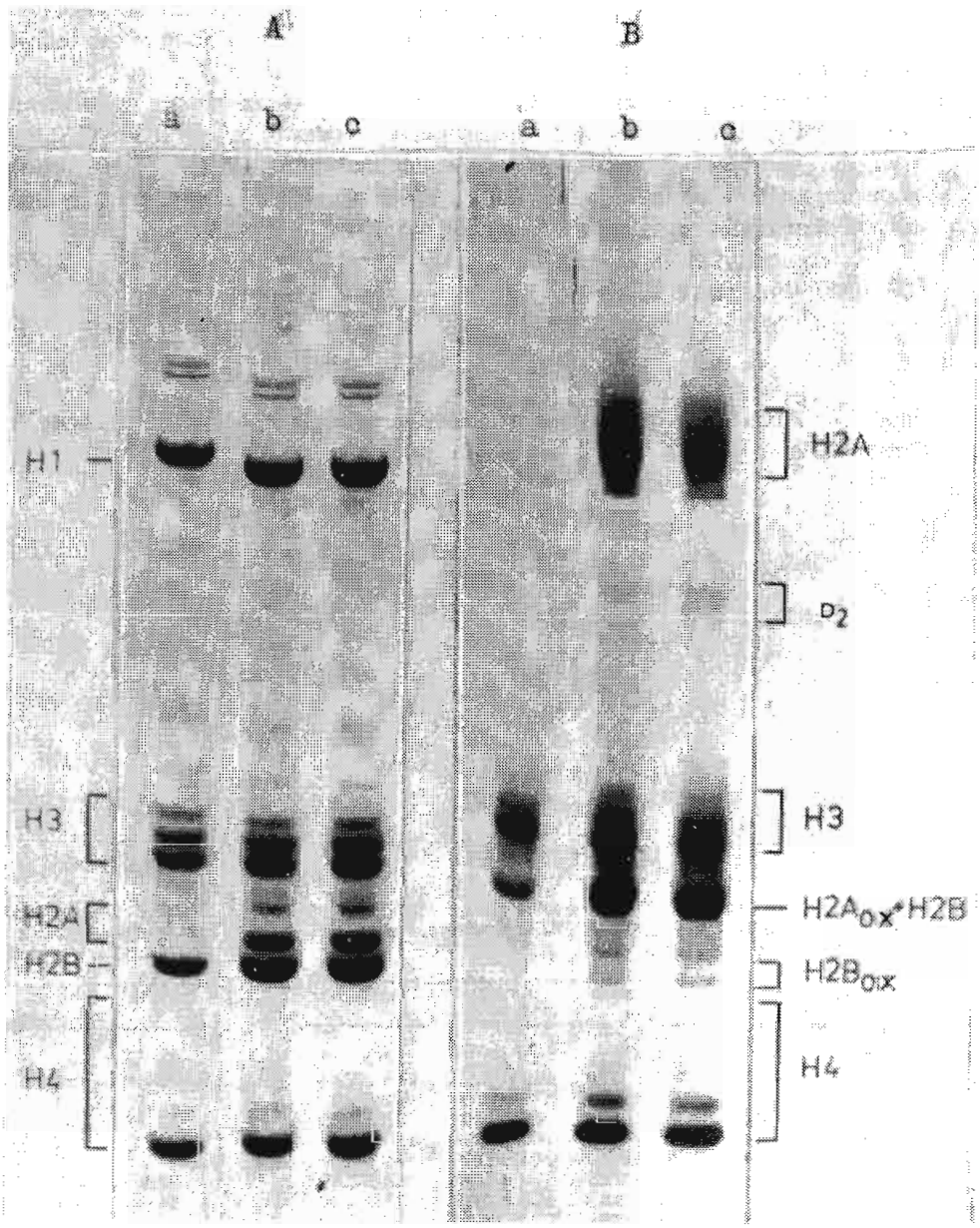


Figure 1. Differential extraction of histone H2A with dodecylbenzolsulfonic acid from acid/urea (A) and acid/urea/Triton DF-16 (B) polyacrylamide gels. a, gels stained with Coomassie Blue after being extracted with dodecylbenzolsulfonic acid. b and c, non-extracted gels.

Harisanova, N.T., K.H. Ralchev and M.I. Simeonovska. University of Sofia, Bulgaria.

Quantitative determination of *Drosophila* histones using dodecylbenzolsulfonic acid.

Histones were isolated from *Drosophila hydie* embryos and separated in acid/urea polyacrylamide gel (Oliver and Chalkley, 1972). Electrophoregramms were stained with Coomassie Blue, Amido Black or Fast Green. Figure 1 shows the electrophoretic pattern of histones separated on 25 cm long slab gel.

The stained gels were scanned photometrically at 600 nm and quantitated by weighing the paper enclosed by the peaks. The results are presented in Table 1.

We propose a new method for quantification of the histone fractions using dodecylbenzolsulfonic acid. For that purpose the gel pieces containing the individual histone fractions were extracted with 3 ml 2.5% dodecylbenzolsulfonic acid neutralized with ethanolamine in 7% acetic acid for 48 hr. The absorbance of the extracts was measured photometrically at 600 nm. The relative amounts of the histone fractions expressed as a percentage of the total histones are presented in Table 2.

It is evident from Table 1 and 2 that there is no essential difference between both methods used. But we observed

some when comparing the results obtained using different dyes. Most probably this fact is to be explained with the difference in the absorption affinity of the individual histones to the examined dyes.

So it is possible to determine the relative amounts of the histone fractions either by quantitative densitometry of the electrophoregramms or by extraction of the stain from the individual fractions with dodecylbenzolsulfonic acid. The proposed extraction method has the advantage of being not harmful as compared to the existing method of extraction with dimethylsulfoxid (Johns, 1967).

References: Oliver, D. and R. Chalkley 1972, Exp. Cell Res. 73:295-302; Johns, E.W. 1967, Biochem. J. 104:78-82.

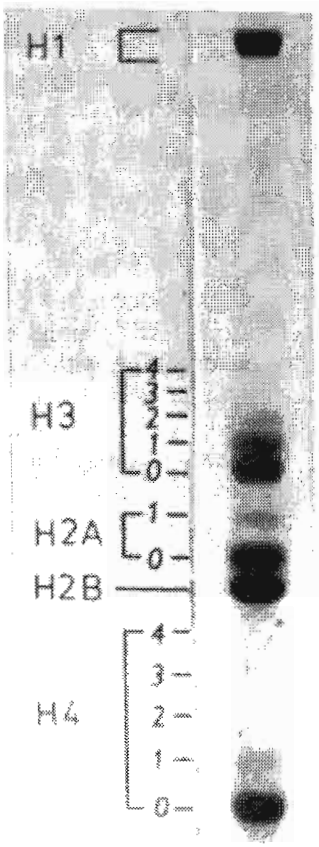


Fig. 1. Acetic acid/urea polyacrylamide gel electrophoresis of histones from *Drosophila* embryos.

Table 1. Relative amounts of histone fractions determined by the densitometer scans of polyacrylamide gels and expressed as a percentage of the total histone.

Histone	No. of acetylation	Coomassie Blue	Amido Black	Fast Green
H1		18.9 ± 0.41	16.8 ± 1.27	15.0 ± 1.46
H3	0	11.7 ± 0.20	14.0 ± 2.59	11.4 ± 0.53
	1	7.0 ± 0.11	7.7 ± 0.04	8.2 ± 0.34
	2	3.3 ± 0.20	3.7 ± 0.08	5.2 ± 0.85
H2A	0	11.2 ± 0.55	14.8 ± 0.54	14.0 ± 0.24
	1	3.1 ± 0.09	4.3 ± 0.29	5.0 ± 0.50
H2B		27.8 ± 0.45	21.7 ± 1.13	22.7 ± 0.09
H4	0	14.2 ± 0.09	15.1 ± 0.79	16.1 ± 0.50
	1	2.9 ± 0.06	2.2 ± 0.73	2.5 ± 0.09

The data are mean values of 3 different histone preparations.

Table 2. Relative amounts of histone fractions determined by dodecylbenzolsulfonic acid extraction of the gel pieces containing the fractions and expressed as a percentage of the total histones.

Histone	No. of acetylation	Coomassie Blue	Amido Black	Fast Green
H1		20.2 ± 3.38	14.4 ± 0.34	12.5 ± 0.63
H3	0	8.9 ± 0.81	10.6 ± 0.23	10.1 ± 0.96
	1	4.5 ± 0.53	7.1 ± 0.60	7.0 ± 0.56
	2	3.1 ± 0.34	4.3 ± 0.41	3.4 ± 0.27
H2A	0	11.5 ± 0.40	14.9 ± 0.87	15.3 ± 0.79
	1	3.9 ± 0.71	6.8 ± 0.84	5.2 ± 0.86
H2B		29.9 ± 0.45	24.7 ± 0.64	25.3 ± 1.06
H4	0	12.0 ± 1.55	13.7 ± 0.63	16.5 ± 0.90
	1	6.3 ± 0.06	3.9 ± 0.35	4.4 ± 0.87

The data are mean values of 7 different histone preparations.

Hovemann, Bernhard T. Zentrum für Molekulare Biologie Heidelberg, Universität Heidelberg, D-6900 Heidelberg, Im Neuenheimer Feld 282, F.R.G. Construction of a random primed embryonic *Drosophila* cDNA expression library cloned into phage lambda-gt11.

The formation, structure and function of a specific type of RNP particle that accumulates at heat shock puff 93D after induction is still not completely understood. This RNP contains the P11 and Q18 proteins that were named after the monoclonal antibodies that specifically detect them on polytene chromosomes (Dangli and Bautz, 1983). Since both proteins are also present at regular growth temperature and during embryonic development (Dequin, Saumweber and Sedat, 1984), I constructed an expression library from embryonic RNA for their subsequent cloning and characterization.

RNA was extracted from frozen *Drosophila melanogaster-CS* embryos of up to 16 hrs of age using the guanidinium thiocyanate extraction method (Chirgwin, Przybla, Mac Donald and Rutter, 1977). The preparation was enriched for poly $(A)^+$ containing RNA by oligo (dT) cellulose chromatography (Aviv and Leder, 1972) and as judged by

glyoxal-RNA gel electrophoresis (McMaster and Carmichael, 1977) the poly $(A)^+$ RNA fraction was about 50% pure. 15 g of this RNA was used for cDNA preparation according to Gubler and Hoffmann (1983). To assure an equal distribution of expression of all possible epitopes that is necessary for monoclonal antibody screening, a mixture of hexameric oligonucleotide primers has been used for random priming of the first cDNA strand.

In short: First strand cDNA synthesis was performed with AMV reverse transcriptase; DNA polymerase I, RNase H and DNA ligase were added for second strand synthesis. Double stranded cDNA was then treated with EcoRI methylase and blunt ends were obtained with T_4 DNA polymerase. After addition of EcoRI linkers and digestion with EcoRI the cDNA was ligated to EcoRI digested dephosphorylated phage lambda-gt11 DNA (Young and Davis, 1983). The ligation products were packaged in vitro and plated on NZYDT agar. More than 98% of the obtained plagues contained cDNA inserts. 10^6 recombinants (insert sizes: 1-2kb) were collected; this number corresponds to the number of independent cDNA clones in this library.

References: Dangli, A. and E.K.F. Bautz 1983, Chromosoma 88:201-207; Dequin, R., H. Saumweber, and J.W. Sedat 1984, Dev. Biol. 104:37-48; Chirgwin, J.D., A.E. Przybla, R.J. Mac Donald, and W.J. Rutter 1977, Biochemistry 8:5294-5295; Aviv, H. and P. Leder 1972, Proc. Natl. Acad. Sci. 69:1408-1412; McMaster, G.K. and G.G. Carmichael 1977, Proc. Natl. Acad. Sci. 74:4835-4838; Gubler, U. and B.J. Hoffman 1983, Gene 25:263-269; Young, R.A. and R.W. Davis 1983, Proc. Natl. Acad. Sci. 80:1194-1198.

Hughes, K.A. and J.G. Rux. University of Chicago, Chicago, Illinois. A new device for assessing mating ability in male *Drosophila*.

Various techniques have been used to measure competitive mating ability in male *Drosophila*. Such assessments usually involve allowing two types of males to compete for matings with a limited number of females. We have designed a simple apparatus and technique for conducting such trials. The device is easily assembled from inexpensive materials, most of which can be found on the shelves of a *Drosophila* lab.

The primary motivation for designing this device was that we wished to avoid confounding real differences in mating behavior with differential response to anesthetization. So, we wanted to conduct mating trials with flies that had not been anesthetized in the previous 24 hours. We also wished to be very precise in the numbers of flies used in the trials. When we attempted to combine unanesthetized flies from two vials by the normal transferring methods, we often lost some flies, and it was very difficult to determine which flies had been lost. Our device allows us to set up the experiments in advance and to allow sufficient time for the flies to recover from anesthetization.

We found that standard glass cover slips (Gold Seal cover glass, 22 x 60 mm) will fit neatly inside the 8-dram shell vials typically used for stock maintenance. The cover slip is inserted so that it divides the space within the vial into approximately equal halves and is pushed a few millimeters into the medium. The fit is snug, so that a fly placed on one side of the cover slip cannot crawl between the vial and the slip to get to the other side. (This was tested by placing flies on one side of the cover slip and noting that none had made it to the other side after 24 hours.) A metal-rim tag is taped to the cover slip before insertion to make removal easier (see Figure). We then take foam vial plugs (Scientific Products disPo Plugs 22-28 mm) and cut them in half along the vertical axis. One half of a plug can then be used to seal each half of the vial.

Flies to be used in the trials are anesthetized on the day prior to that on which the trial is to be run. Males are anesthetized and then transferred into one side of a divided vial. That side of the vial is then stoppered with a half foam plug. Next we anesthetize the females that are to be used in the trial, transfer them to the unoccupied half of the vial and stopper that with another foam plug.

We allow 24 hours to elapse so that the effects of anesthetization are diminished. Then we initiate the trials by simply removing the cover slips separating the two chambers within each vial. The tags attached to the slips make this very easy. Usually the vial is held upside down during the removal because the flies will tend to move upward, away from the plugs, and are therefore less likely to get caught and crushed between the slip and the plugs. The slip is removed far enough so that it no longer projects past the plugs. When the trial is complete, all flies in a vial are anesthetized and removed from the mating chamber.

Jacobson, J.W. Department of Biology, University of Houston, Houston, Texas 77204-5513. Estimation of transposable element copy number by relative hybridization: *mariner* in *Drosophila mauritiana*.

Typically, estimates of transposon copy number and genomic distribution in *D. melanogaster* have been obtained through *in situ* hybridization of labeled transposon probes to salivary gland polytene chromosomes (Pardue, 1986). However, this useful technique is not readily applied to all other *Drosophila* species. For example in many strains of *D. mauritiana* the salivary gland polytene chromosomes exhibit reduced polyteny and appear to undergo extensive ectopic pairing (J. Lim, pers. comm.) making the localization and quantification of hybridizing sites difficult. What is often needed to address questions concerning transposon population dynamics is a relative measure of copy number, rather than an absolute copy number estimate.

For example, in *D. mauritiana* a dominant genetic factor, *Mos* has been identified that enhances the rate of excision of the *mariner* transposable element. If *mariner* copy number in the presence of *Mos* differs from that in *Mos*-free strains, then *Mos* may well contribute to the overall population dynamics of this transposable element and additional experiments can be designed. Alternatively, if *mariner* copy number appears to be independent of *Mos* genotype, experimental efforts can be concentrated on other factors. In order to examine intra-specific variability in *mariner* copy number in *D. mauritiana*, I have utilized a method that allows comparison of transposon copy number to a single-copy sequence that serves as an internal control and allows reliable inter-strain comparisons to be made without the technical difficulties and obstacles presented by *in situ* hybridization in non-*melanogaster* species.

Materials and Methods:

Strains: The strains used in these studies are described in detail elsewhere (Jacobson and Hartl, 1985; Haymer and Marsh, 1986; Bryan *et al.*, 1987; Hartl, 1989; Jacobson, 1990). Briefly, the original *white-peach* (w^{pch}) mutation arose spontaneously in a wild-type laboratory strain of *D. mauritiana* and was recovered by backcrossing to the wild-type strain to replicate the mutant chromosome. Ultimately, a true-breeding w^{pch} strain was established. The w^{pch} strain and the w^{+} strain from which it was derived have since been in continuous laboratory culture and are the strains used in the present study. In a series of genetic experiments employing the w^{pch} strain (Jacobson and Hartl, 1985), a mutation occurred that produced a heritable somatic eye color mosaicism and this new mutation was recovered and established by mating among siblings to produce the E25H strain that has been the subject of genetic and molecular analyses (Bryan *et al.*, 1987; Jacobson, 1990) and is the E25H strain used in the present study. E25H carries a dominant genetic factor, *Mos*, on the third chromosome that is known to promote a tenfold increase in the reversion rate of w^{pch} and this reversion is due to excision of the *mariner* element (Jacobsen *et al.*, 1986). The *Mos* factor is a particular copy of the *mariner* transposon (Medhora *et al.*, 1988) although its mode of action is not known.

The *D. mauritiana* wild-type isofemale strains: G27, G102, G122, G206 and a strain (BGW) carrying a chemically-induced *white* mutation (w^{1} in Jacobson and Hartl, 1985) were obtained from R.C. Woodruff and R.F. Lyman. In a genetic background containing the w^{pch} allele, the presence of *Mos* is readily detected by the production of eye-color mosaics (Bryan *et al.*, 1987; Medhora *et al.*, 1988). In separate crosses with the w^{pch} strain, none of these strains (except E25H) produced eye-color mosaics (Jacobson and Hartl, 1985; Jacobson, unpublished) and are used herein to represent *Mos*-free strains.

All *Drosophila* strains and crosses were reared at 25° on Formula 4-24 Instant Drosophila Medium (Carolina Biological Supply, Burlington, NC).

Sample preparation and estimation of copy number

Intra-specific variation in *mariner* copy number was estimated in *D. mauritiana* by comparing the hybridization signal corresponding to the transposon with a single-copy control from the *D. mauritiana white* gene as shown in Figure 1. Hybridization signals representing two restriction fragments were used to obtain copy number estimates. The first (A) corresponds to *mariner* and is a 893 bp *RsaI/RsaI* fragment that is conserved in each copy in the genome (Jacobson *et al.*, 1986). Consequently, digestion of total genomic DNA with *Rsa*I produces a single such fragment from each copy of *mariner*. The second restriction fragment (B) is a 1151 bp *RsaI/Bam*HI fragment from the *D. mauritiana white* locus that serves as a single-copy control hybridization signal. In these analyses the hybridization probe contains both *mariner* and *white* sequences, therefore, both hybridization signals can be detected in the same lane on the gel, using the same restriction digest and the same probe and labeling reaction. To estimate transposon copy number in each experiment, the total radioactivity (disintegrations per minute: dpm) corresponding to *mariner* hybridization is divided by the total dpm for the *white* locus single copy control in the same lane. The resulting value is an estimate of *mariner* hybridization signal relative to the signal from a single copy DNA sequence in the same preparation of genomic DNA.

Preparation of genomic DNA from adult flies, digestion of DNA with restriction endonucleases, agarose gel electrophoresis, labeling of radioactive probes with ^{32}p and hybridization of filter-bound DNA were all carried out using routine procedures (Jacobson *et al.*, 1986; Maniatis *et al.*, 1982). DNA samples corresponding to 10 flies (about 3 ug;

females:males = 1:1) were digested with an excess of the restriction endonucleases *Bam*HI and *Rsa*I and the restriction fragments were fractionated by agarose gel electrophoresis. Both between and within sample replication was carried out for each strain to ensure reproducibility and replicates of individual samples were separated in random order with respect to each other on the electrophoretic gels to avoid artifacts due to position on the gel. Transfer to nylon membranes (Hybond-N, Amersham Corp., Arlington Heights, IL) was accomplished by vacuum transfer on a "Vacublot" transfer apparatus (American Bionetics, Emeryville, CA). The hybridization probe was the entire 4300 bp *Bam*HI/*Bam*HI restriction fragment shown in Figure 1 which contains both *mariner* and *D. mauritiana white* sequences (Jacobson *et al.*, 1986). Following autoradiography, the exposed X-ray film was aligned with radioactive ink orientation marks on the hybridization membrane, taped in place and pieces of the membrane corresponding to the autoradiographic signals were excised using a standard paper punch (about 0.39 cm^2) and placed in mini-scintillation vials. The probe was stripped from the filter pieces by incubation in 0.2N NaOH for 30 minutes at 45°C. Scintillation cocktail was added to the vials and the samples were counted in an LKB Model 1209 liquid scintillation counter (Pharmacia LKB Biotechnology, Inc., Gaithersburg, MD).

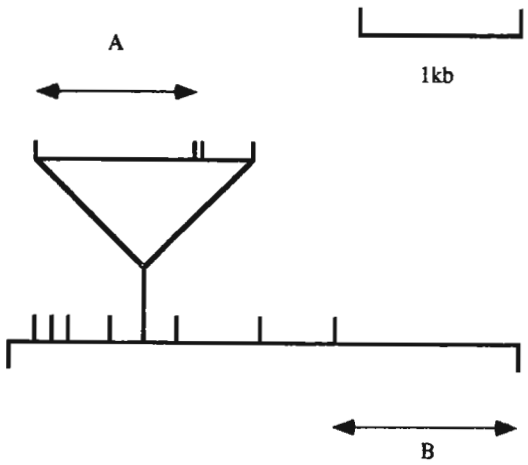


Figure 1. Restriction map of a portion of the *D. mauritiana* w^{pch} allele (Jacobson *et al.*, 1986). The site of *mariner* insertion is designated by the triangle. Vertical lines above the horizontal represent sites cleaved by the *Rsa*I restriction endonuclease, while vertical lines below the horizontal represent sites cleaved by the *Bam*HI restriction endonuclease. The *mariner* restriction fragment denoted by (A) is an 893 bp *Rsa*I/*Rsa*I fragment that is conserved in each copy of *mariner* in the genome and the restriction fragment denoted by (B) is an 1151 bp *Rsa*I/*Bam*HI fragment from the *D. mauritiana white* gene.

Statistical Analyses: Data analyses were carried out using JMP software (SAS Institute, Box 8000, Cary, NC 27512) and standard statistical testing procedures as found in Sokal and Rolf (1987).

Results and Discussion:

To acquire data on intra-specific variation in *mariner* copy number in the absence of *Mos*, estimates were obtained from several strains of *D. mauritiana* *Mos*-free strains that are not closely related and the results are presented in Table 1. As can be seen, *mariner* copy number in these six strains ranges from 9.7 copies to 16.5 copies with an overall mean of 12.4 $\pm$ 2.4 (mean $\pm$ s.e.). A one-way ANOVA of the data reveals significant heterogeneity in *mariner* copy number among strains (5 degrees of freedom, $p < 0.0005$) suggesting that, although the range is not dramatic, genetic variation for *mariner* copy number probably exists among the *D. mauritiana* strains employed in this study and, perhaps, in *D. mauritiana* in general.

To examine the possible consequences of the *Mos* factor on *mariner* population dynamics, estimates of copy number in the related *D. mauritiana* w^+, w^{pch} and E25H strains were obtained. Since only one of these related strains contains *Mos*, any affect on *mariner* population dynamics should be most evident in comparisons between the two *Mos* genotypes, *Mos* (E25H) versus Mos^+ (w^+ or w^{pch}). A lower copy number in E25H is expected if the only effect of *Mos* is to promote *mariner* excision. Other possible outcomes of higher or equal *mariner* copy number would imply that *Mos* also has an impact on *mariner* transposition or no observable effect. Finally, all three strains may differ in *mariner* copy number without regard to genotype.

The copy number data for these three strains are presented in Table 1 and demonstrate that whereas *mariner* copy number in the w^{pch} and w^+ strains appears to be the same, E25H contains more copies of *mariner* than either of the other two strains. A one-way ANOVA with *a priori* contrasts reveals that genotype (*Mos* or Mos^+) is the sole significant source of *mariner* copy number variation among these three strains (one degree of freedom, $p < 0.0250$). Although the *Mos* factor is known to promote transposon excision, *mariner* is almost twice as abundant in E25H than in the w^{pch} strain from which it was derived, a result not readily explained if enhancement of transposon excision is the only effect of *Mos* in the genome. Rather, *Mos* appears to influence *mariner* transposition rate as well, since copy number in the presence of *Mos* is greater than in its absence. E25H and w^{pch} had been maintained separately for approximately 120 generations at the time of this analysis. Whether these copy number differences have arisen gradually over this period of time or are the result of one or several rounds of increased transposition cannot be discerned from these observations.

The identification of a genetic factor that enhances the processes of *mariner* excision and transposition and that is itself a particular copy of this transposable element is intriguing and merits additional study. Genetic factors like *Mos* segregating in natural populations can have profound effects on the population dynamics of transposable elements and might lead to the dramatic differences in transposon abundance and distribution observed among even very closely related strains and species (Young, 1979; Dowsett and Young, 1982; Dowsett, 1983; Martin *et al.*, 1983). Additional data on other *Mos* containing strains and species are required to elucidate the interaction between *Mos* and *mariner* and determine the overall impact on *mariner* population dynamics. Relative hybridization comparisons provide an internally controlled approach that allows the rapid collection of copy number data from a variety of strains and species.

Acknowledgments: I wish to thank D.C. Wiernasz and C.R. Hughes for statistical advice and comments on the manuscript. This work was partially supported by a grant from the Houston Coastal Center, and by BRSG S07 RR 07147-16 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

Table 1. Mariner copy number in strains of *D. mauritiana*.

Experiment	Strain	n	Copy Number	Standard Error
A	G27	4	13.52	1.32
	G102	4	9.69	0.38
	G122	4	10.55	0.89
	G206	4	11.65	0.38
	BGW	4	12.24	0.83
	Wild-type	4	16.54	0.89
B	Wild-type	6	17.80	0.50
	White-peach	6	17.20	1.00
	E25H	6	31.10	1.10

Table footnote: For each experiment (A and B), genomic DNA was extracted from (n) replicates of 25 adult flies from each strain. From each replicate, two copy number estimates were obtained and averaged to give the copy number estimate for each replicate. Experiment B presents the analysis of two subsequent generations (Blocks). Copy number data for these two generations do not differ significantly from one another (t test, df=8, p=0.255) and are pooled to provide estimates of mean copy number for each of the three strains.

References: Bryan, G.J., J.W. Jacobson and D.L. Hartl 1987, Science USA 235:1636-1638; Dowsett, A.P. 1983, Chromosoma 88:104-108; Dowsett, A.P. and M.W. Young 1982, Proc. Natl. Acad. Sci. USA 79:4570-4574; Hartl, D.L. 1989, In: Mobile DNA (Berg and Howe, eds.), 531-536; Haymer, D.S. and J.L. Marsh 1986, Develop. Genetics 6:281-291; Jacobson, J.W. 1990, Genetical Research 55:153-158; Jacobson, J.W. and D.L. Hartl 1985, Genetics 111:57-65; Jacobson, J.W., M.M. Medhora and D.L. Hartl 1986, Proc. Natl. Acad. Sci. USA 83:8684-8688; Maniatis, T., E.F. Fritsch and J. Sambrook 1982, Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory; Martin, G., D. Wiernasz and P. Schedl 1983, J. Mol. Evol. 19:203-213; Medhora, M.M., A.H. MacPeek and D.L. Hartl 1988, The EMBO J. 7:2185-2189; Pardue, M.L. 1986, in: Drosophila: A Practical Approach, (Roberts, ed.), 111-137; Sokal, R.R. and F.J. Rohlf 1987, Biometry, W.H. Freeman and Co.; Young, M.W. 1979, Proc. Natl. Sci. USA 76:6274-6278.

McEvey, S.F. Labo. Biol. et Genetique Evolutives, Gif-sur-Yvette Cedex, France. TRACE - a method to analyse sexual interactions in mixed groups of unmarked flies.

A method has been developed for examining the sexual behavior of cryptic species or strains in intermixed groups. The method does not require physical marking. It involves the tracking of individual flies on a video film and can be carried out either manually or with computer assistance. The sequence of behavior before or after any event of interest can be determined as well as the identity of the flies involved.

The technique called TRACE (Tracking by Replay And Computer Eduction) involves the following steps:

(1) Flies are inserted into an 8cm-diameter Elens-Wattiaux (DIS 39:118) observation chamber while a camera, mounted directly above, monitors the sequence of introduction. The 'Panasonic/National 1/2 inch Time Lapse Video Recorder Model NV-8050' with a built in 'Panasonic/National Time Date Generator, Model NV-F85' is suitable. Flies of a certain genotype, sex, species, etc. are 'labelled' by virtue of their recorded sequence of delivery into the arena. For example, type A females may be introduced 1st and 2nd and type B females 3rd and 4th (as shown in Table 1). The TRACE technique effectively identifies individuals by linking them to their 'queue-position' at insertion. A clear view of every fly must be maintained from the beginning until the end of the trial and for this reason 20 flies per trial is maximal, the method works best with good resolution. A digital time display is fed simultaneously to the video recorder during a trial.

(2) After the trial the film is replayed as many times as there are flies in the chamber. Each replay a different fly is

tracked on the screen by continuous observation from insertion (where it is identified) until the end. Flies spend much time walking or preening; during these periods, playback can be speeded up. However, when the fly becomes sexually active, details must be noted manually or electronically. During a 30 min trial several hundred bouts of courtship may occur among the flies.

(3) Three coordinates are recorded when a fly does something of interest: x (time, s), y (position, ca 1.0 cm^2 units) and z (behavior). Although this has been done effectively without a computer, a 'mouse' with an attached bull's-eye can be used to register a position coordinate electronically. For this the monitor is mounted horizontally beneath a flat transparent table. Time can be registered automatically when a position is signalled via the 'mouse'. A programmed key-pad can record behavior. At the end of one playback one list of coordinate sets (x,y,z) - a history of times, places and activities - is available (equivalent to one column in Table 1).

(4) The identity of another fly involved in any particular sexual interaction can be determined after all flies have been processed. Points of intersection in the three-dimensional matrix of all coordinates x, y and z indicate a match. If at x-time and y-position a fly does z to another fly, then that other fly must have a matching set of coordinates which will be x, y and the reciprocal of z. A correct match for the z-coordinate is the reciprocal of ittt because if one fly is courted (z), for example, the other must be a male and he is courting (represented in Table 1 by uppercase letters). Position and time errors will occur but will rarely be significant.

Table 1. A hypothetical TRACE data matrix. Each column is a 'history' of activity for the individuals (01-08); compare z/y in one column with z/y in another on the same line (+/- 1 s) to find the partner. B or b, orientation begins; C or c, wing-extension; E or e, orientation terminates; i, insertion into arena; X or x, copulation; 1-8, position coordinates.

	Females (01 - 04)				Males (05 - 08)			
Times	A 01	A 02	B 03	B 04	A 05	B 06	A 07	B 08
00:01	8/i	-	-	-	-	-	-	-
00:05	-	8/i	-	-	-	-	-	-
00:10	-	-	8/i	-	-	-	-	-
00:15	-	-	-	8/i	-	-	-	-
00:20	-	-	-	-	8/i	-	-	-
10:01	6/b	-	4/c	-	4/C	6/B	-	-
10:02	6/c	1/b	-	2/b	-	6/C	-	2/B
10:03	7/c	-	-	2/c	-	7/C	1/B	2/C
10:04	-	-	-	3/c	5/E	-	-	3/C
10:05	7/c	1/e	5/e	3/x	-	7/C	1/E	3/X
x	y/z	y/z	y/z	y/z	y/Z	y/Z	y/Z	y/Z

Table 1 shows hypothetical "histories" of 8 flies during insertion and in a 5 s interval after 10 min. A computer can be programmed to search such an array for matches although this can be done manually. Through matching it is possible to determine which flies interacted together. In the time interval 10:01-05, male 05 courted female 03, paused for 2 s then terminated orientation; male 06 oriented to and courted female 01; male 07 oriented to - then moved away from - female 02 without courting her; male 08 oriented, then courted and mated with female 04.

During mating flies are, of course, not involved in other sexual interactions. TRACE has the additional advantage of yielding precise information about the times individual flies are able to be active and consequently the ratio of sexually active to inactive time can be calculated with precision. Various aspects of sexual interaction can be readily quantified. A 20 to 30 min film yields an enormous amount of information.

The method has been successful in analysing the behavior of mixed groups of *Drosophila nasuta, D. kepulauana* and their hybrids - morphologically indistinguishable flies. It was possible to demonstrate that F_1 *nasuta* x *kepulauana* hybrid males were more active sexually, they oriented and courted more females more often and mated significantly less often than non-hybrids which tended to mate positive assortatively.

Mukherjee, J. and R.N. Chatterjee. University of Calcutta, India. A technique for quick fixation of *Drosophila* for scanning electron microscopy.

The scanning electron microscope is one of the most useful tools for analyzing the detailed structure of many of the pattern elements at higher magnification. Attempts have been made in the past to improve the resolution of the micrographs in various ways and means (see Hodgkin and Bryant, 1978). This report presents a much simplified and rapid method for fixation of adult *Drosophila* for scanning electron microscopy.

An adult fly was etherized and was placed in the groove of a depression slide by keeping the ventral side down. Thereafter, a square coverglass was placed over the fly covering half of the groove and touching the thorax of the fly. When the fly regained some sense, the coverglass was held firmly in place with the help of the index finger and the fly was quickly drenched with a few drops of ether. The ether was then allowed to evaporate. Then the fly was directly transferred to a vial containing 30% ethanol with the help of a fine brush. It was then further processed for scanning electron microscopy by passing through ascending grades of ethanol. Parallel preparation was also processed by

conventional fixation technique (Hodgkin and Bryant, 1978). The examples of the electron micrographs processed by the two methods described, are presented in Figure 1a-b. As it appears from the electron micrograph presented in Figure 1a, the technique of ether fixation does not seem to introduce any distortion of the external cuticular morphology. On the other hand, if acetone is used as the dehydration fluid instead of ethyl alcohol, the fly often collapses under the vacuum, even when the specimen was dried immediately by the critical-point drying method (Figure 1b). It is therefore, suggested that the ether fixation of *Drosophila* is far better for studying the external cuticular morphology of the adult fly under the scanning electron microscope.

Acknowledgments: This work was supported by the UGC pre-doctoral fellowship to Jaba Mukherjee.

Reference: Hodgkin, N.M. and P.J. Bryant 1978, in: "The Genetics and Biology of *Drosophila*", (M. Ashburner and T.R.F. Wright, Eds.), Academic Press, London, Vol. 2C: 337-358.

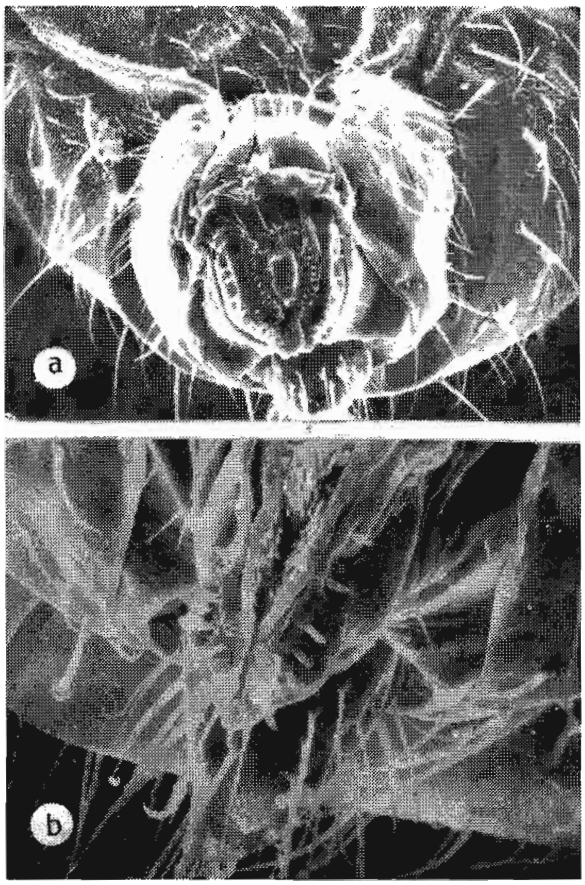


Figure 1. Terminalia of the adult female (ventral view) of *Drosophila melanogaster*: a) Processed by ether fixation (200x) and b) Processed by conventional method (400x). No alteration in ultrastructure was noticed after fixation in ether.

Osgood, C., B. Powell and J. Wagner. Department of Biological Sciences and Science Shop, Old Dominion University, Norfolk, Virginia. An accurate and inexpensive *Drosophila* counter.

A sophisticated and accurate *Drosophila* counter was described by Barr and Søndergaard (1984) which was, for a time, manufactured in Denmark. The manufacturer no longer produces these units and we have found it difficult to find compatible replacement parts in the U.S. We describe here a simple, accurate and inexpensive unit, assembled by the Science Shop at Old Dominion University, that other workers may wish to consider. The unit can be assembled for under $100 and matches more expensive units in counting performance.

Shown in the Figure is a schematic of the *Drosophila* counter. An infrared transmitter (IR LED) and detector monitor the passage of a fly through the transmitter/detector housing and triggers a transistor (2N2222A). This signal, in turn, activates half of the 556 IC, which is set up as a monostable oscillator. The pulse from the oscillator turns on a transistor, in the 2N2222A, causing a pulse which increments the counter CUB III.

Power to the circuit is supplied by a voltage regulator circuit using a 7805 regulator. The counter also has its own internal power supply consisting of two "N" batteries. The other half of the 556 IC is set up as a stable oscillator to test the counter (see schematic in the lower portion of the Figure).

The IR transmitter/detector are housed within a section of wooden dowel and spaced such that a piece of glass tubing with a 3/32 ID passes between them. In our experience, the glass tubing is easier to clean than plastic tubing.

Other options, such as an audible cue, could easily be incorporated into this unit, but we have found that functions beyond counting and display are not routinely used and have therefore opted for the simple unit described here.

The single most expensive component of the apparatus is the CUB III counter which costs $24 and is manufactured by Digi-Key Corp., P.O. Box 677, Thief River Falls, MN 56701-0677. Other components were purchased from Radio Shack (listed in Figure legend) and could easily be obtained from other electronics suppliers. Assembled units may be purchased from Old Dominion University for the cost of the components and $10 per hour labor.

References: Barr, C. and L. Søndergaard 1984, DIS 60:214-215.

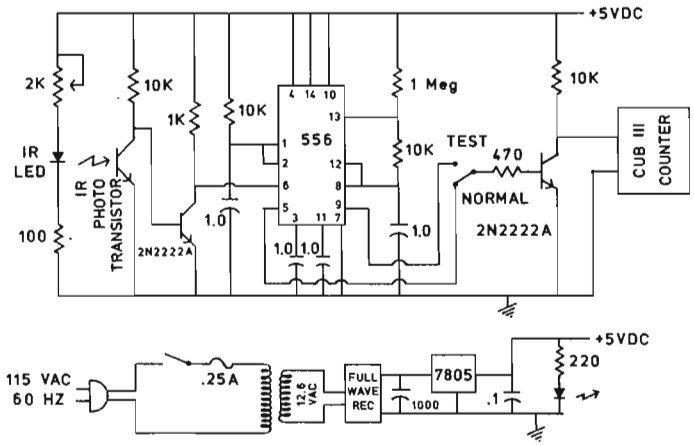


Figure 1. *Drosophila* counter schematic. IR LED = Radio Shack 276-143; IR Phototransistor = Radio Shack 276-145; Cub III Counter = Digi-Key RLCO3-ND; 556 = Radio Shack Timer 276-1728; 7805 = Radio Shack Voltage Regulator 276-1770.

Singh, O.P. and W.-E. Kalisch. Ruhr-Universität Bochum. F.R. of Germany. SSP technique applied for EM genome analysis and photo mapping in *Drosophila nasuta*.

Electron microscopic (EM) studies of polytene chromosomes in *Drosophila* species have contributed additional information of previously determined LM band and puffing patterns (cf. Zhimulev *et al.*, 1981; Kalisch *et al.*, 1985; Saura, 1986). Two different chromosome preparation techniques are commonly used for EM analysis: The squash-thin sectioning method (Sorsa and Sorsa, 1967) and the surface spread polytene (SSP) chromosome preparation technique (Kalisch and Whitmore, 1986).

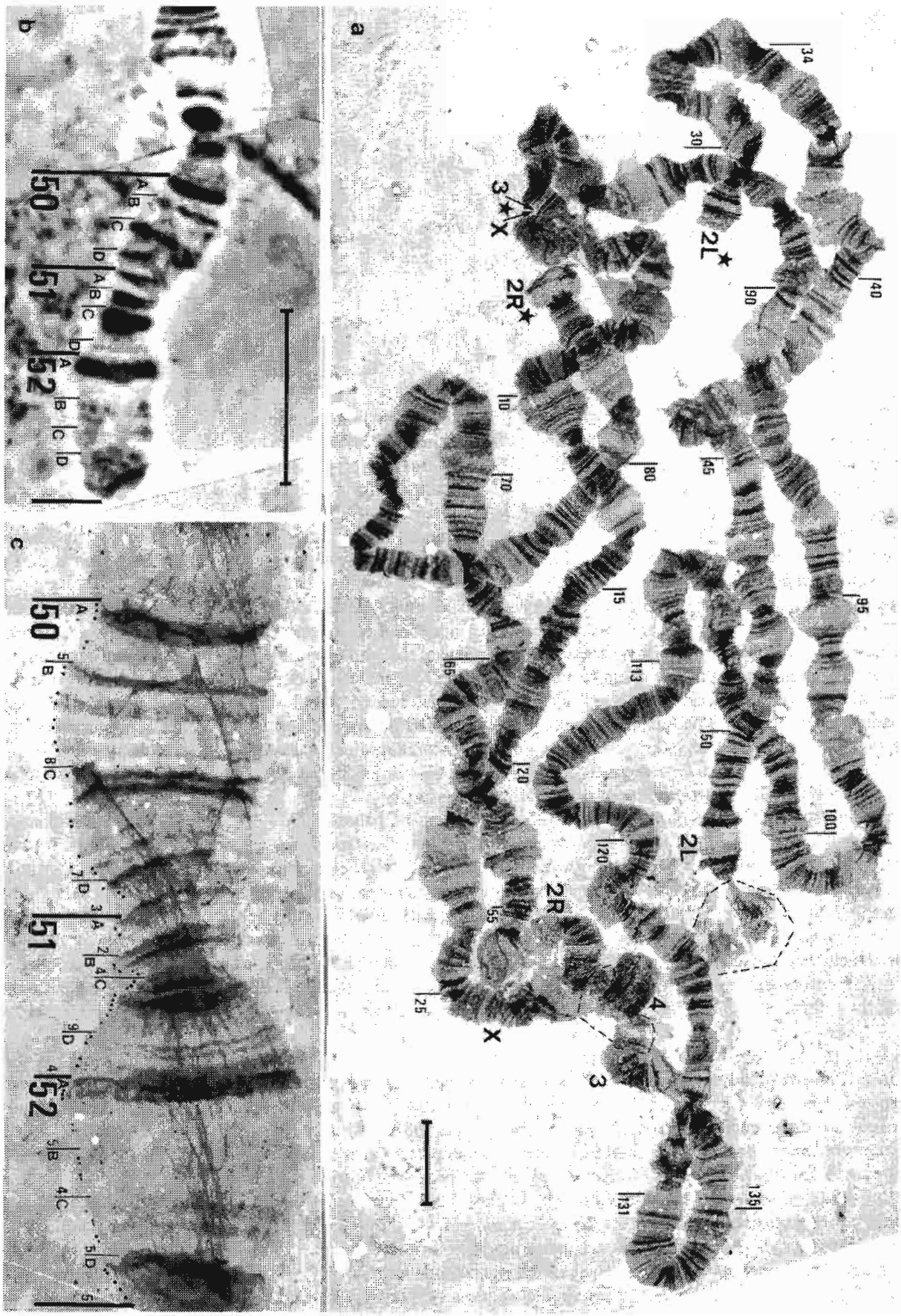
The squash-thin sectioning method is an excellent technique for detailed EM banding pattern analysis, however, it has been found to be a time-consuming procedure for the analysis of entire chromosomes (Saura, 1986). Moreover, this method can hardly be used for studies where an EM overview of the whole genome is required. To the contrary, the SSP chromosome preparation technique could be used for routine EM analysis of the entire genome. SSP chromosomes are observed directly (without thin-sectioning) which is possible due to the lateral and longitudinal spreading after chromosome pretreatment. The technique is applicable for different species and larval tissues of diptera as well as for various types of staining, hybridization techniques, and microscopic studies (cf. Kalisch *et al.*, 1986).

In this short note, we want to focus interest to the application of SSP chromosome preparations for routine EM genome overviews of different members of the *D. nasuta* subgroup.

Our interest in EM genome analysis and photo mapping of polytene chromosomes in *D. n. nasuta* and *D. n. albomicans* originated as result of recent data concerning cyto-, biochemical-, and evolutionary genetics (cf. Ramesh and Kalisch, 1989). The relationship between both members (as well as other members) of the *D. nasuta* subgroup is still controversial. On one hand, the band-interband patterns indicate no considerable differences at the LM level, but on the other hand, there are great differences on the molecular genetic level. Further EM analyses of *D. n. nasuta/D. n. albomicans* hybrid chromosomes would indicate to what extent genetic divergence can exist without changing the polytene structure of the chromosomes. However, so far there are only LM photo maps of *D. n. nasuta* available.

The SSP chromosome preparation technique as described by Kalisch and Whitmore (1986) for *D. melanogaster* was used with the following modifications: 3.74 M citric acid (12 g dissolved in 15 ml of aqua bidist.) was used instead of a

Figure 1a-c. Salivary gland chromosomes of *D. nasuta*. **(a)** EM micrograph of one SSP chromosome preparation in *D. n. albomicans*. Bar equals 20 um. Sectioning according to the LM photo map of Ranganath and Krishnamurthy (1974). Asterics indicate distal ends of the chromosome arms. Fusion of chromosome-X and -3 tips is common. Dotted lines enclose the centromeric region. Note that the band-interband pattern at the chromosome superpositions is stretched and cannot be used for analysis. **(b)** LM micrograph of the proximal portion of chromosome-2L (50-52) in a squash preparation of *D. n. nasuta* (Leibold-Kaufmann, unpublished). Bar equals 10 um. **(c)** EM micrograph of one SSP chromosome preparation of region 50-52 (2L) in *D. n. albomicans*. Same chromosome preparation technique used as in (a). Chromosome bands identified on the original print are individually labeled by dots. The maximum number of bands from all preparations analysed so far, are given at the right-handed border of each subdivision. Chromosomes in (b) and (c) show the same magnification and comparable degrees of polyteny (average degrees of SSP spreading: 2,6-fold lateral; 2.0-fold longitudinal).


a
b
c
2L
2R
3
X
50
51
52

3.18 M concentration. For the final composition of the salivary gland pretreatment solution, we mixed 15 ml of citric acid (stock solution of 3.74 M) and 30 ml of propionic acid (8.82 M. i.e. 19.8 ml propionic acid + 10.2 ml aqua bidist.).

Optimal spreading could be achieved after 5-7 min of salivary gland pretreatment. A 2-3 fold longitudinal and lateral spreading degree throughout the entire genome could be obtained without loosing basic structural details. Separation of each chromosome arm (except common fusion of chromosome-X and -3 tips as well as loop-like fusion of the proximal region of 2R) can be achieved by gentle stiring the salivary glands with a needle during the pretreatment process.

Grids were coated with a 0.35% Formvar film which was found strong enough to observe the genome with an EM magnification of up to 3000X. Well-spread full compliments of the genomes were picked up on copper grids with a central 500 x 1000 um mesh (No. B8010 01 197, diameter 3.05 mm. BALZERS FRG).

Fig. 1a shows an EM overview of the *D. n. albomicans* genome in a routine SSP chromosome preparation. Fig. 1c indicates at a higher magnification the resolution of the EM band-interband pattern in a comparable SSP chromosome preparation. Chromosome arm identification and sectioning have been done according to the *D. n. nasuta* LM photo map of Ranganath and Krishnamurthy (1974). Subsectioning includes the prerequisites for computer plotting of chromosome maps (Kalisch *et al.*, 1984, 1986). Fig. 1c is part of an EM chromosome-2 photo and computer designed chromosome map of *D. n. albomicans* (in preparation). The LM resolution of a selected squash preparation (Fig. 1b) and the EM resolution of a routine SSP chromosome preparation (Fig. 1c) can be compared.

Preliminary comparison between the LM photo maps and the EM data indicates that on the average about a 100% increase in additional bands can be noted in individual SSP chromosome preparations.

Finally, SSP chromosomes offer an opportunity for EM puffing pattern analysis of the entire genome in one preparation. However, one has to consider that in SSP chromosomes puffs are comparatively more prominent than in squash preparations in accordance with the spreading process itself.

For those who do not have access to an EM equipment, SSP chromosome preparation together with LM analysis is recommended. This still enables a better resolution of the band-interband pattern in comparison with the pattern resolution of squash preparations (Kalisch, 1982).

Acknowledgments: We thank Mrs. Leibold-Kaufmann for using her unpublished LM photo map of *D. nasuta*, Mrs. Chr. Plehn for technical assistance, and Mr. T. Whitmore for linguistic aid, OPS is grateful to the Univ. Grants Commission, New Delhi, India for sponsoring and to the Deutscher Akademischer Austauschdienst. F.R. of Germany, for the award of a scholarship.

References: Kalisch, W.-E. 1982, DIS 58:85-87; Kalisch, W.-E., T. Whitmore and H. Reiling 1984, Cytobios 41:47-62; Kalisch, W.-E., T. Whitmore and G. Schwitalla 1985, Chromosoma 92:265-272; Kalisch, W.-E., G. Schwitalla and T. Whitmore 1986, Cytobios 45:185-194; Kalisch, W.-E. and T. Whitmore 1986, DIS 63:142-146; Ramesh, S.R. and W.-E. Kalisch 1989, Genetica 78:62-72; Ranganath, H.A., and N.B. Krishnamurthy 1974, J. Mysore Univ. 26B:65-69; Saura, A.O. 1986, Ph.D. thesis, Univ. Helsinki, Finland; Sorsa, M. and V. Sorsa 1967, J. Ultrastruct. Res. 20:302; Zhimulev, I.F., E.S. Belyaeva and V.F. Semeshin 1981, Chromosoma 82:9-23.

Sved, J.A. Biology A12, Sydney University, NSW 2006, Australia. Pushing flies on the PC.

DROSIM is a teaching program which allows students to design and run a crossing program on microcomputers. It is derived from an earlier program running on main-frame computers, which was described in DIS 55:171 (1980). The philosophy of the program is that each student is given an unknown mutant lying somewhere in the genome, and is asked to map it as accurately as possible. A standard set of around 30 markers spanning the genome is provided for the mapping. No multiple marker stocks are provided, so the student must build up any stocks required for the mapping, which constitutes the testing part of the exercise. The program is probably too difficult for beginning students unless a reasonable amount of tutorial help is available. However the program also has a more elementary mode of use in which the instructor rather than the student is in charge of setting up crosses. In this case the program is directed to setting up problems in which each student in the class gets a different set of data.

The program is provided on a disk, along with a program which allows the instructor to tailor the exercise for a particular class. A separate disk must be made up for each student. On completion of the exercise this disk is returned along with the student's report, and the instructor's program enables the correct answer to be read from the disk for marking the report.

The program should run on all IBM and IBM-compatible machines with a minimum of 256K capacity. A Macintosh version is also available, although this version makes no use of the Mac interface. The program is available on request, but a donation of $10 to cover the cost of disk, printing and postage would be appreciated.

Tompkins, L. Temple University, Philadelphia, Pennsylvania, USA. A culture medium for adult Hawaiian *Drosophila* species.

Because of their extraordinary evolutionary history and their unique behavior patterns, the flies from picture-winged Hawaiian *Drosophila* species are of interest to many mainland investigators. In addition, the flies' large size should make them attractive to physiologists and others whose ideal fly would be a blowfly-sized drosophilid. Since Hawaiian *Drosophila* species are more difficult to maintain than *D. melanogaster*, some investigators may choose to obtain adult flies from the National Species Resource Center in Bowling Green, Ohio, or the Hawaiian Drosophila Stock Center at the University of Hawaii in Honolulu, which maintains many species in culture and periodically collects flies from their native habitat. If the flies are to be subjected to analysis or frozen within a few days of the time that they are received from a stock center, they can be maintained at 17-20°C in the vials in which they were shipped. However, in some circumstances, it may be necessary or simply more convenient to maintain live flies for some time after they arrive in the laboratory, in which case they must be transferred to fresh medium periodically.

To facilitate the efforts of those who may be interested in working with Hawaiian *Drosophila* in mainland laboratories, I have developed a recipe for an adult fly maintenance medium, modified from that of Wheeler and Clayton (1960). The advantage of this recipe is that small batches of the medium can be made quickly, using readily available equipment and ingredients.

A cereal powder (32g of Kretschmer's wheat germ, 32g of Kellogg's Special-K cereal, and 32g of Gerber's baby cereal, pulverized in a blender or food processor) is prepared in advance, then stored in a refrigerator in a tightly closed container. This mixture keeps for several months. To make the medium, add 1g of agar (Sigma) to 90ml of distilled water in a 200ml beaker. Microwave the solution until the water boils vigorously. Stir in 3g of the cereal powder and 24g of strained banana baby food (with or without tapioca). Microwave the mixture again until it boils vigorously and starts to thicken. Cool it for a few minutes, then stir in 375ul propionic acid and 375ul 95% ethanol. Pour the medium into recently autoclaved glass shell vials (for vials that are 33mm in diameter, use ca. 3ml of medium per vial), then cap the vials with cotton or foam plugs. If they are not used immediately, the filled vials may be refrigerated for up to 7-10 days; allow them to warm up to incubator temperature before use.

By maintaining the flies at 18-19°C and transferring them to fresh vials every 4-5 days, I have been able to maintain *D. adiastola* adults on this medium for several weeks. In spite of the fact that I do not provide the aqueous extract of *Clermontia* leaves that is routinely added to culture vials at the Hawaiian Drosophila Stock Center, the sexual behavior of the flies that are maintained in my laboratory is normal and they appear to be otherwise healthy.

Acknowledgments: This work was supported by a grant from the Temple University Research Incentive Fund to the author.

Reference: Wheeler, M.R. and F. Clayton 1965, DIS 40:98.

Waddle, F. Fayetteville State University Fayetteville, North Carolina. A *"Drosophila* Kit" for the genetics teaching lab.

I have developed a *"Drosophila* Kit" which consists of a 3 x 6 x 15 inch plastic tray containing the following items:

1. Instant *Drosophila* medium in 220 ml container.
2. Tegosepted cotton (0.1%) in 4 1/2 oz. container.
3. Tegosepted water (0.1%) in squeeze bottle.
4. Yeast in small plastic vials (2).
5. Foam plugs (for 20mm test tube) in large container.
6. Scoopula (small stainless steel scoop).
7. Microspatula.
8. Labels.
9. Test tube brush.
10. Etherizer.
11. Ether bottle (with medicine dropper held in attached test tube).
12. Sorting plate.
13. Sorting brush (fine pointed artist's brush).
14. Lens paper.
15. Dissecting needles (2).
16. Forceps, very fine pointed.
17. Glass slides (in container, not loose).
18. #1 Cover slips.
19. Siliconed lens paper (for making squashes, not for lenses).
20. Razor blade.

Each student is given a kit. Each kit is numbered. The containers and equipment within the kit are numbered the same as the kit. This discourages students from "borrowing" from one another. Ideally, the student should have a cabinet with lock in which to keep the kit and a stereomicroscope.

To go with the kit, the student has ready access (either directly or on request) to:

1. Test tubes, 20 X 150mm.

2. Test tube racks.
3. Salivary gland stains in dropper bottles.
4. Refills for fly food, plugs, etc.

The 20 X 150mm test tubes require less food than bottles or vials and are excellent for single pair matings. Moreover, breakage is considerably less than for glass vials. Carolina Biological Supply Company sells an inexpensive 50 tube rack that works well. Tegosepted water is used to prepare food in the tubes and the tubes are slanted. The tegosepted cotton is for use at the bottom of the slant. Fisher's etherizer is far superior to Carolina Biological's, provided the cotton in the etherizer is replaced with a larger piece and tightly packed. To minimize the ether hazard, we use small, corked (not rubber), ether bottles and put the dropper for it in a small test tube rubber banded to the bottle.

With the student thus equipped, the faculty work load can be reduced while the student learns the basics of *Drosophila* research from making food, collecting virgins and making crosses to washing the glassware afterwards. Since batch matings are avoided, fewer virgins are required for equivalent result while even the unintentional use of a nonvirgin in a particular vial can provide a learning experience without disastrous result.

Waddle, F. Fayetteville State University, Fayetteville, North Carolina USNA. Quick'n' simple *Drosophila* medium.

When problems developed in keeping flies alive on molasses medium, the following formula was developed. The food appears to be superior to molasses medium especially when used fresh. After a day or more of refrigeration, it seems somewhat risky to use for low vigor stocks, for single female matings or holding virgins (but still superior to molasses).

20g	Brewer's yeast, debittered
10g	Agar
100g	Cornmeal
100g	Dextrose
1g	Tegosept (mold inhibitor)
1 drop	Benzyl benzoate (mite inhibitor)
100ml	Water
	Live yeast

Since the only liquid to be added to the water is benzyl benzoate, making the food is simplified. The dry materials can be weighed out and mixed several batches at a time and stored (preferably in a refigerator to minimize loss of nutrient in the brewer's yeast) for future use. The benzyl benzoate can be added to the dry materials during batch preparation or stirred into the food after cooking. The medium can be cooked in a microwave oven in 15 min provided it is stirred at 2-4 min intervals and care is taken to avoid boiling. Any extra medium left after pouring can be refrigerated and remelted later.

This lab uses 0.1% tegosepted cotton to pad the bottom of slanted vials (unnecessary if several vigorous, reasonably fertile and unetherized females are to be added as in transferring stocks). The cotton is prepared by the addition of 200ml of 0.05% tegosept per 100g sterile cotton followed by oven drying. Before plugging, the vials are sprinkled lightly with live yeast.

This work was supported by NIH Grant #S06 RR08206-03.

Waddle, F. Fayetteville State University, Fayetteville, North Carolina USNA. Moldy instant media.

The amount of mold inhibitor (Tegosept-M) in Fisher Scientific and Carolina Biological Supply Company instant *Drosophila* medium is adequate for batch matings (as in the large plastic vials these companies supply). But single female matings do not produce enough larvae to prevent mold growth. For these matings, additional mold inhibitor should be added to the water (0.1%) and to the cotton padding at the bottom of the slant (also 0.1%).

Acknowledgments: This work was supported by NIH Grant #S06 RR08206-03.

Waddle, F. Fayetteville State University, Fayetteville, North Carolina USNA.
Some stocks for the teaching lab.

I use the following "unknowns" in the teaching lab: w^a (sc^6), w^e, ras^2, g^F, car, 1t (stw), or^{45a} (sp^2), se (h), p^p (ri), red. The following stocks are used for tests with the unknowns: Ore-R, b, e, cv f, Cy0/Sp bw; In(3LR)DcxF, D/Sb e.

The eye color mutations, including the garnet allele, are all easily distinguishable from wild type. Sex linked mutations were chosen such that any competent mapping test with cv and f can distinguish one locus from the others. Genes in parentheses are "teacher markers". They are mostly rank 2 or 3 mutations not obvious to the student but which allow the instructor to distinguish among stocks of similar eye color.

Each student is given one sex linked and one autosomal unknown plus the stocks to test them with. No two students have the same combination of unknowns. Depending on the autosomal unknown, either b or e is given, without identification, so that the student can do dihybrid crosses. The CyO/Sp bw; In (3LR)DcxF,D/Sb e stock is excellent for determining which chromosome an autosomal unknown is on. If the student chooses to make chromosome squashes of Ore-R heterozygotes, he/she has the opportunity to observe that one of the bands near the tip of 2R is heterozygous for a deletion.

Welbergen, Ph. Justus-Liebig University, Giessen, Federal Republic of Germany.
A controlled mating chamber for video observation of *Drosophila* courtship behaviour.

Courtship behaviour and mating success of *Drosophila melanogaster* and *D. simulans* are influenced by temperature, relative humidity, and light intensity (Grossfield, 1966; Parsons, 1977; Krause et al., 1984). Hence, in studies of describing and comparing behavioural variables one should standardize the experiments for these factors. The mating chamber described here is especially constructed to fulfill these requirements (Figure 1).

The chamber-stand contains in the middle a notch of 25mm diameter and a groove of 1mm thick at a distance of 35mm from the midpoint. The groove should hold the diffused light cylinder (Figure 2). Holes of 5mm length are bored at three sides of the courting chamber (Figure 3). Two of them have a diameter of 3mm and are closed by the perspex stoppers. The third hole has a diameter of 4mm and is used to hold the perspex partition. Exactly at the other side there should be a notch of 1mm deep with a diameter of 4mm in order to hold the partition firmly during the introduction of the flies. The same three holes are also present in the sand-blasted circular diffused light cylinder.

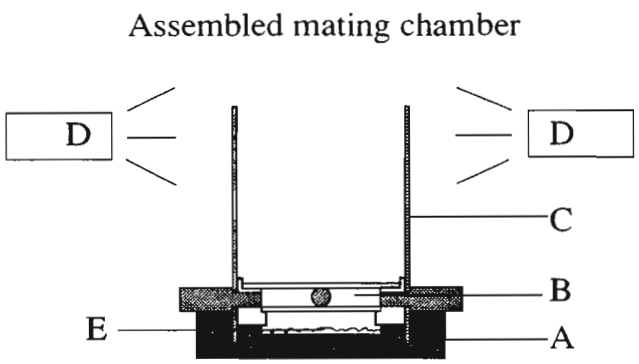


Figure 1. The assembled mating chamber. A. Sand-blasted circular perspex chamber-stand (Ø = 51mm, h = 10mm); B. Sand-blasted circular perspex courting chamber (inside: Ø = 25mm, h = 4mm; outside: Ø = 35mm, h = 10mm); C. Sand-blasted circular perspex diffused light cylinder (Ø = 37mm, h = 50mm); D. Cold light sources; E. Absorption paper.

To assemble the pieces of the mating chamber, one starts by placing the grid floor on the brim of the courting chamber. On the grid floor is put a replaceable but dry white piece of absorption paper. The next step is to put the diffused light cylinder around the courting chamber in such a way that the holes are connected. The partition is pushed gently through the hole by holding the absorption paper with a pair of tweezers. The other two holes are closed by the stoppers. Later, these holes are used to introduce the sexes separatedly. The courting chamber as a whole is to be closed by the transparent perspex top. Then the courting chamber with the diffused light cylinder is put on the chamber-stand which contains a wet paper in the notch. The mating chamber as a whole should be placed in cold light. Four light sources have to be directed on the cylinder.

The males and females can be introduced separatedly by using an aspirator. It is preferable to wait for five minutes after the introduction, in order to let the flies adapt and to be sure that the relative humidity in the chamber is 100% at the onset of recording. The temperature in the surrounding of the mating chamber should be held constant. Light intensity can be measured by using a meter with a remote opening, that is placed under the chamber-stand. The video camera with a macro lens is to be positioned above the chamber.

References: Grossfield, J. 1966, Univ. Tex. Publ. 6615:147; Parsons, P.A. 1977, Adv. Genet. 19:1; Krause, J., B. Köbke and W. Köhler 1984, Verh. Dtsch. Zool. Ges.

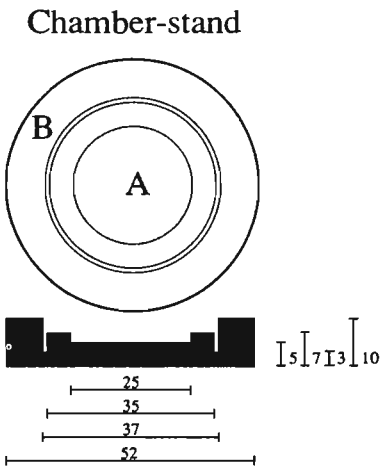


Figure 2. Chamber-stand (view from the top and from the side of a section). A. Notch to hold the wet absorption paper; B. Groove. The measures are in millimeters.

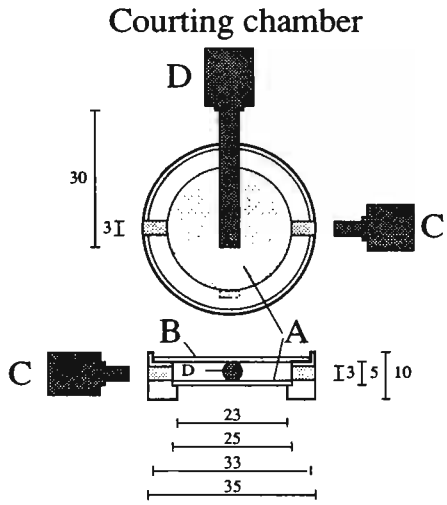


Figure 3. Courting chamber (view from the top and from the side of a section). A. Rustless grid floor (Ø = 25mm); B. Transparent perspex cover (Ø = 33mm); C. Perspex stoppers (Ø = 3mm, l = 16mm) with rectangle hilts); D. Perspex partition (Ø = 4mm, l = 36mm) with a rectangle hilt. The measures are in millimeters.

Welbergen, Ph.[1] **and P. Lankinen.**[2] [1]University of Utrecht, The Netherlands, and [2]University of Oulu, Finland. A practical device for sound recording of *Drosophila*.

A conspicuous element of male courtship behaviour of *Drosophila melanogaster* and *D. simulans* is the vibration of a wing. By vibrating the wing, the male produces a species-specific "love song". The female receives the song with their antennal aristae by responding to the particle displacement in the sound wave (Bennet-Clark, 1972).

The song is a very small acoustic source. For the purpose of recording the song, Bennet-Clark (1984) developed a highly sensitive particle velocity microphone. Other types of microphones have been used, but the particle velocity microphone has the advantages of low cost, small size, robustness and a broad frequency response.

According to Bennet-Clark high quality recordings can be made with minimal sound insulation. However, to achieve high quality recordings with the particle velocity microphone, one has to consider two points. First, sound insulation is still a necessity because of the relatively high noise/sound ratio which is produced by environmental noise (e.g. heating/cooling system, observer) and alternating currents (e.g. light sources, recording equipment). The INSECTAVOX of Gorczyca and Hall (1987) fulfills to a high degree the requirement of dampening the environmental noise and currents. It is, however, unclear how they transfer the flies into the enclosed mating chamber. Second, real comparisons between songs of different *Drosophila* species, strains, or lines profit when temperature, relative humidity and light intensity are standardized in an experiment.

We developed a device with which these requisites are met up to the mark. It consists of different parts (Figures 1 and 2). The device should still be used in a relatively quiet room. The influences of alternating currents, mostly 50-60 Hz, can be much reduced by using Faraday enclosures around the inner chamber and microphone-head (Figure 1).

The difficulty with temperature control is the inevitable noise of the heating/cooling instrument. For efficient functioning, the instrument should be placed within the outer chamber, but then recording is impossible. Our solution for this problem is to use the heating/cooling system of the building as a rough regulator. Because of the insulation by the outer chamber the confounding influence of the noise of the system on the recording is much reduced. Fine adjustment of the temperature in the outer chamber and, therefore, also in the inner chamber can be achieved just before recording by warming it up with a light bulb or cooling it down with ice. It sounds rather primitive, but since the temperature can be checked on every moment with a thermometer, it is remarkably efficient. Within one recording the fluctuation is only a few decimal centigrades.

The relative humidity is held constant near to 100% through a wet towel that is placed on the bottom of the outer chamber. The level of humidity can be checked also on every moment by the indicator that is built in the lid of the outer

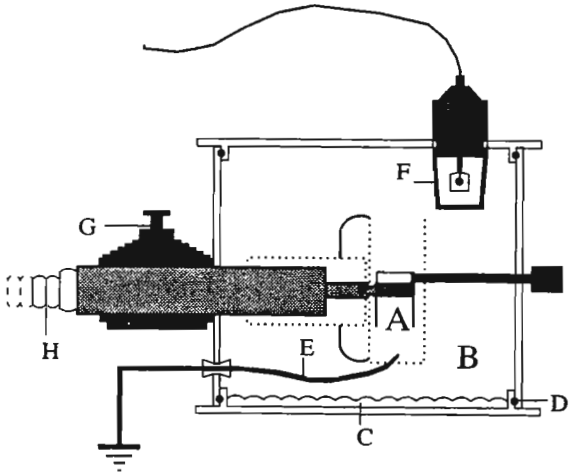


Figure 1. Device for recording of *Drosophila* sound. A. inner chamber built upon an electret microphone (for details, see Fig. 2); B. sand-blasted outer perspex chamber, resting on the microphone head; C. wet towel; D. circular rubber ring; E. earth cable connected with the Faraday enclosure; F. Temperature and Humidity indicator HMI12 (Vaisala, Finland); G. screw with holder to stabilize the position of the outer chamber; H. goose neck containing the output lead and supply cables (according to Fig. 2c of Bennet-Clark, 1984), and connecting the microphone with the amplifier, which, on its turn, should be connected to a tape recorder.

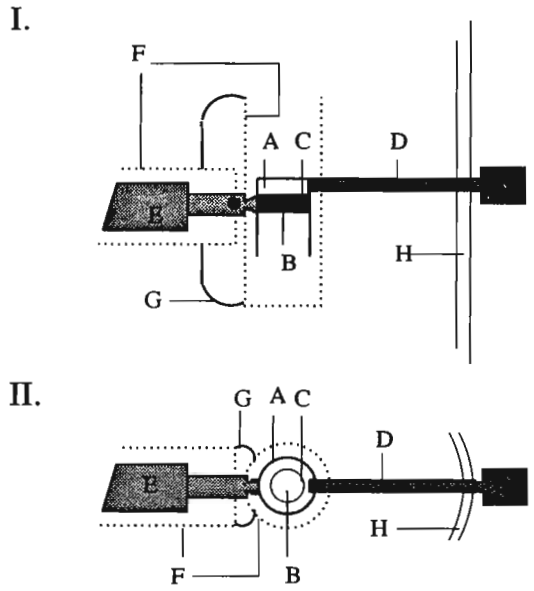


Figure 2. Close-up of a part of the device inside the outer chamber taken from the side (I.) and from above (II.). A. inner perspex chamber (Ø = 10mm, h = 3mm); B. electret microphone (built according to Figure 2a and 2b of Bennet-Clark, 1984); C. silk cover; D. permanently fixed perspex tube (Ø = 3mm); E. microphone head (built according to Fig. 2c of Bennet-Clark, 1984); F. Faraday enclosures; G. connecting cables; H. outer perspex chamber wall; I. perspex stopper (Ø = 3mm).

chamber.

The intensity of light is standardized by making use of cold light sources. The wall of the outer chamber should be sandblasted in order to spread the light in a diffused way. Before the recording session is started, the light intensity should be measured with an intensity meter temporarily placed at the inside of the outer chamber. The light intensity can be adjusted if necessary through the dimmers of the light sources.

The flies are transferred with an aspirator from a vial to the inner chamber through the permanently fixed tube (Figure 2). After transference the tube is closed by a stopper. Removing the flies happens through sucking up with an aspirator.

Acknowledgments: Ph. W. was supported by a NWO Grant from the Netherlands.

References: Bennet-Clark, H.C. 1972, DIS 49:127; Bennet-Clark, H.C. 1984, J. Exp. Biol. 108:459; Gorczyca, M. and J.H. Hall 1987, DIS 66:157.

New Mutants

New Mutants: D. melanogaster

Report of Aguado-Rodriguez, P., F. Galan-Estella and J. Gonzalez-Julian.
University of Salamanca, Spain.

The "uniungula" gene of *Drosophila melanogaster*.

The recessive and sex-linked "uniungula" mutant of *Drosophila melanogaster* appeared spontaneously in a culture flask of our collection. The morphological characteristics of the specimens belonging to this new phenotype involve two different morphological structures: the eyes and the claws of the feet. The eyes of these specimens exhibit chromatic variegation, there being red and white ommatidia. The red ommatidia are arranged in a branched fashion which converges in the centre of the eye. The other morphological characteristic of this new class of specimen is the possession of a single claw on the central part of each of the legs.

These morphological characteristics are related to a pleiotropic gene for which the symbol "*ung*" is proposed.

The "uniungula" locus is situated in the first linkage group between the "yellow" (y,1,0.0) and "white" (w, 1, 1.5) loci, the calculated genetic distance between the "yellow-uniungula" loci being 1.1159 centimorgans.

Report of Artjomova, E.V.
Institute of Cytology and Genetics, USSR Academy of Science, Siberian Division, Novosibirsk 630090, USSR.

New Mutants.

Oocytes of the 7th and 14th stage of development in S Px^2/SM5, al^2 Cy lt^v sp^2 females were irradiated by gamma-rays(3000R), capacity 150 R/min). The females were crossed to F(2L), 1(2)gl pr; F(2R), bw 1(2)ax/SM5, al^2 Cy lt^v sp^2 males. In individual crosses F(2L), 1(2)gl pr; F(2R), bw 1(2)ax/ S Px^2 daughters were tested for the presence of 2R rearrangement by means of "the nondisjunction test" (see Chadova, this issue). Cytological analysis of polytene chromosomes showed the following mutations in the selected cultures:

In(2R)12 = In(2R) 46A; 57F
In(2R)24 = In(2R) 58F; 59D
In(2R)40 = In(2R) 42A; 57F

The Px^2 marker is present in inverted chromosomes of number 12 and 24.

Report of Artjomova, E.V. and G.N. Buzykanova.
Institute of Cytology and Genetics, USSR Academy of Sciences, Siberian Division, Novosibirsk 630090, USSR.

New Mutants.

S Px^2/+ males were irradiated by gamma-rays (3000R, capacity 150 r/min) and crossed to SM5, Cy/F(2L), 1(2)gl pr; F(2R), 1(2)ax females. S Px^2/F(2L), 1(2)gl pr; F(2R), 1(2)ax daughters were tested in individual crosses for the presence of 2R rearrangement in S Px^2 autosomes (see Chadov, this issue). Cytological analysis of polytene chromosomes showed the following rearrangements: (Note: the following list is presented in separate columns to save space).

In(2R)A1 = In(2R) 47A; 550
In(2R)B208 = In(2R) 41F; 52C
In(2R)B1 = In(2R) 43C; 56F
In(2R)B119 = In(2R) 41; 49F
In(2R)B2 = In(2R) 41; 53D
In(2R)B3 = In(2R) 41F; 49D
In(2R)C92 = In(2R) 41; 57A
In(2R)C1 = In(2R) 42A; 46A-B
In(2LR)A187 = In(2LR) 39E-F; 56F
In(2LR)B7 = In(2LR) 36E; 59D
In(2LR)B8 = In(2LR) 26D; 51A
In(2LR)B11 = In(2LR) 37C; 48E
In(2LR)C2 = In(2LR) 39F; 51D
In(2LR)A92 = In(2LR) 22B; 29C; 34D; 35B; 36D-E; (40-41); 42A; 53C; 57F; 58F; 60

New order:
21-22B/36E-(40-41)/35B-34D/60B-60F/22B-29C/35B--36D/60B-58F/57F-58F/53C-42A/57F-53C/42A-(41-40)/29C/
35B-36D/60B-58F/42C-42A/57F-53C/42A-(41-40)/29C-32D/60F
T(1;2) A3 = T(1;2) 7D; 51F
T(2;3) A7 = T(2;3) 45F; 87C
T(2;3) A8 = T(2;3) 48B; 85E
T(2;3) A9 = T(2;3) 48B; 85E
T(2;3) B4 = T(2;3) 52E; 82F-82A
T(2;3) C5 = T(2;3) 42A; 96E
T(2;3) C8 = T(2;3) 56C; 81F-82A
T(2;3) C9 = T(2;3) 33E; 41; 62B; 89D

New order:
21-33E/89D-62B/41-60;61-62B/41-33E/89D-100
Tp(2)A170bw^{v} = Tp(2) 21D; (39-40); 59D; 60B
New order:
21-21D/60B-59D/(39-40)-59D/(39-40)-21D/60B-60

Tp(2;3)A6 = Tp(2;3)41; 58C; 77E-F
New order: 21-41/58C-60; 61-77E/41-58C/77F-100
Tp(2) A10 = Tp(2) 23D; 41; 58D
New order: 21-23D/41-58D/23D-41/58D-60

Report of Beckman, C.
Department of Biology, Concordia University, Montreal, Quebec, Canada H3G 1M8
Sh^{C}: *shaker Concordia*

Lethal in homozygous and hemizygous condition. Heterozygous females show more pronounced shaking under light etherization than either homozygous *Sh*^{3} or homozygous *Sh*^{5} females. More violent shaking can be induced by jarring or touching the flies. X-ray induced.

Report of Cicchetti, R., G. Argentin, C. Idili and B. Nicoletti.
II University of Rome, Italy.
Pgm$^{1.20,tr,5.9}$: phosphoglucomutase$^{1.20,tr,5.9}$.

A new isoelectrophoretic allele at the phosphoglucomutase (PGM) locus has been found in a natural population collected near Rome, by using the isoelectric-focusing method (Cicchetti et al., Atti A.G.I., 1986; Cicchetti et al., Biochem. Genet. 28: 247-255, 1990). This allele, recovered in heterozygosis with the *Pgm*$^{6.1}$ and *Pgm*$^{6.4}$ alleles, has displayed an isoelectric point equal to pH 5.9 and is designated as *Pgm*$^{5.9}$. At the same time, this variant has also been tested with the electrophoresis and heat denaturation methods (see the Research Notes section of the present issue), revealing itself to be an *Pgm*$^{1.20,tr}$ allele.

Report of Eisenberg, J.C. and J.S. Ryerse.
St. Louis University Medical School, St. Louis, MO.
ey-2: a recessive eyeless mutation on the second chromosome of *Drosophila melanogaster*

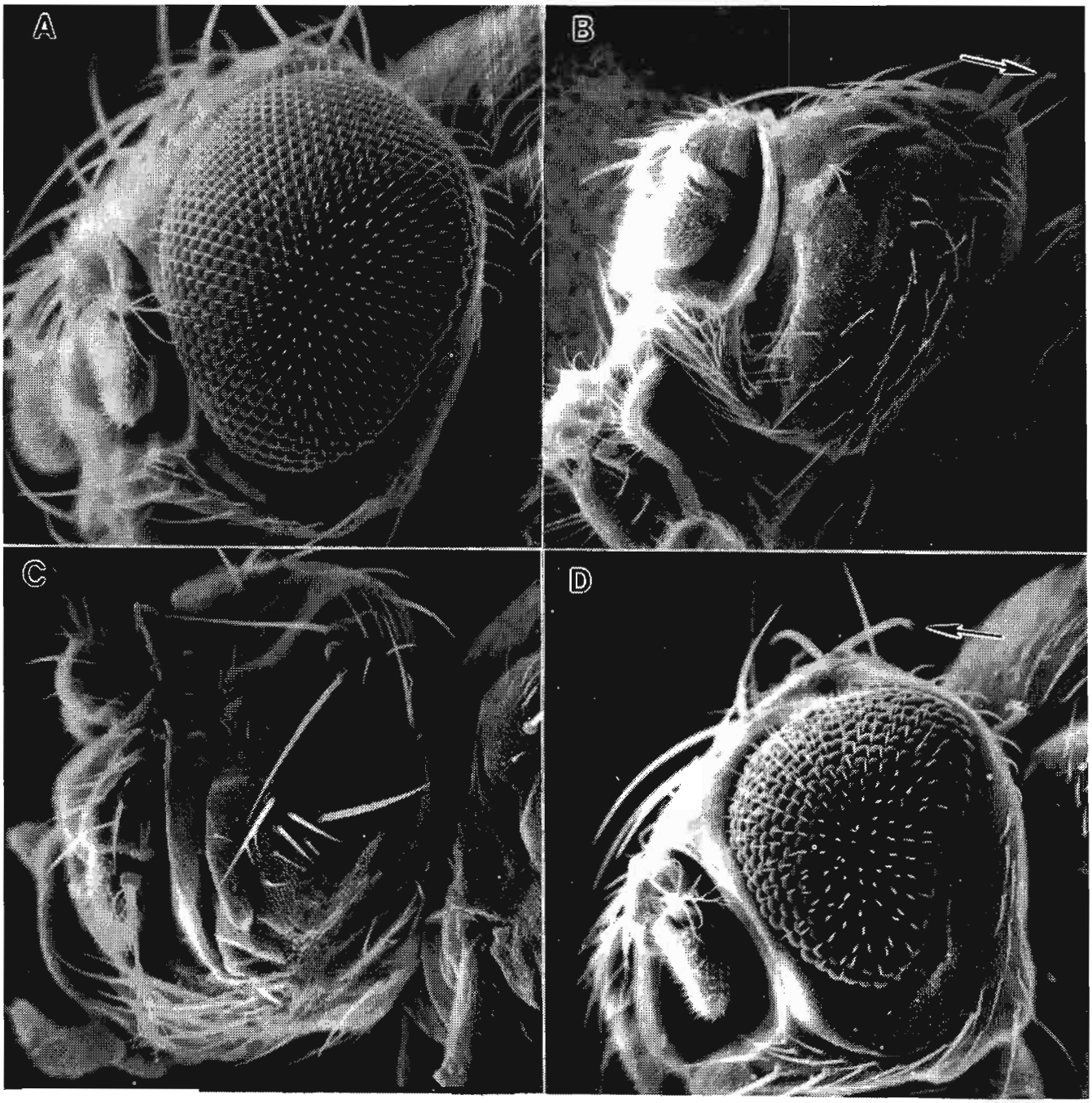
Flies with severely reduced or absent eyes were recovered in an Oregon R stock contaminated with P elements from which a viable and fertile homozygous stock was established. While the eye phenotype is variable (see below), ocelli are missing and the posterior vertical bristles of the head are truncated with frayed ends (Fig. 1B). No other defects, such as duplication of head structures, were observed. The mutation is fully recessive. Crude recombination mapping places the locus on the second chromosome between *dumpy* (13.0) and *black* (48.5). Pending assignment as an allele of a previously described gene, we propose the name "*eyeless-2*" (*ey-2*).

Trans-heterozygotes of *ey-2* with *decapentaplegic-blink* (*dpp*^{blk}) and of *ey-2* with *sine oculis* (so) are wild-type. However, an interesting interaction was found between *ey-2* and the mutation "*eyes absent*" (*eya*) described by Sved (1986). Homozygous *eya* adults lack ommatidia but have normal ocelli and posterior vertical bristles (Fig. 1C). *ey-2/eya* transheterozygotes have reduced eyes with fewer facets and the facets are disorganized (Fig. 1D). Ocelli are present but the posterior vertical bristles are truncated and frayed as in *ey-2*. This interaction suggests that there may be partial complimentation between two hypomorphic alleles or that *ey-2* and *eya* are different genes which affect different aspects of a similar developmental pathway.

Unlike *eya*, the *ey-2* phenotype shows a gradient of defects when flies are raised at 18-22°C. Four general classes of eye defects, from more to less extreme, may be discerned:

1. All eye tissue absent; the space normally occupied by the ommatidia is blank cuticle containing occasional hairs or bristles (Illustrated in Fig. 1B).
2. Streaks of red-pigmented cuticle; no eye facets present.
3. Patches of red-pigmented cuticle occupying up to 10% of the wild-type eye area; 1-10 hemispherical facets present, facets rarely contact one another.
4. Patches of red-pigmented cuticle occupying 10-25% of the wild-type eye area; 10-25 hemispherical facets present, facets lack orderly spacing.

Figure 1 (opposite page). A-D: Scanning electron micrographs illustrating eye and surrounding head structures in (A) wild-type, (B) *ey-2* homozygote, (C) *eya* homozygote and (D) *ey-2/eya* transheterozygote. x 150. Arrows in (B) and (D) indicate frayed posterior vertical bristles. E: Light micrograph showing areas of dead cells (arrows) in a toluidine blue-stained plastic section of an *ey-2* eye disc. x 350.



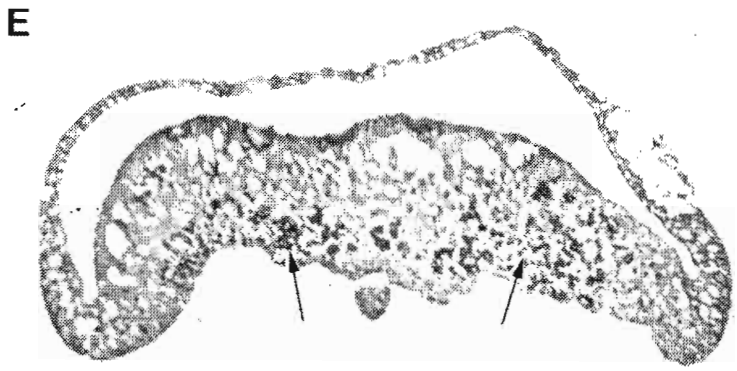


Figure 1.

Light microscopy of sections of eye discs from late third instar *ey-2* larvae revealed extensive areas of dead cells in the presumptive ommatidial region (Fig. 1E). In intact discs viewed under the dissecting microscope, these regions appeared opaque within the more translucent background of normal tissue. These morphological results show that *ey-2* belongs to the cell death class of imaginal disc mutants. Like *vestigial* (O'Brochta and Bryant, 1983), in which cell death in the presumptive wing blade of imaginal wing discs results in truncation of the adult wing blade, cell death in the presumptive ommatidial region of imaginal eye discs appears to be responsible for the reduced eye phenotype in adult *ey-2* flies.

References: O'Brochta, D.A. and P.J. Bryant 1983, Roux's Arch. Dev. Biol. 192:285-294; Sved, J. 1986, DIS 63:169.

Report of Erk, F.C., M. Mehta, and K.N. Patel.
State University of New York at Stony Brook.

A heritable phenotype showing loss of legs: *amputee*.

A serendipitous cross between two loss-of-wing mutants, vg^{x} and wg^{1A}/SM5, in *Drosophila melanogaster* yielded several flies (out of hundreds) with a single missing mesothoracic leg. When these "amputee" flies were mated, a few more flies with only five legs appeared among the offspring. Subsequent crosses between five-legged *amputee* flies (5 x 5) showed increased penetrance of the trait among the offspring, but crosses between six-legged offspring of amputee parents (6 x 6) yielded only an occasional *amputee* fly. After repeated matings between five-legged parents, a few four-legged progeny appeared in which both mesothoracic legs were missing. These four-legged *amputee* flies were usually well-balanced and upwardly mobile, but were often unable to preen their heads or wings without falling over. Occasionally flies with still fewer legs appeared among the offspring of crosses between *amputee* flies -- some three-legged and two-legged flies have emerged, but most of those had some difficulty in moving about and had low viability. On some *amputee* flies the affected legs are not missing entirely; they may be truncated abruptly or reduced to a stump, and sometimes they are shrivelled or grossly distorted in the femoral segment.

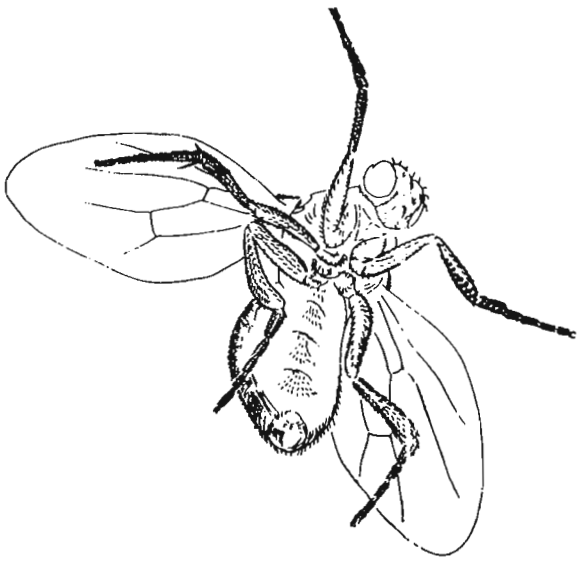


Figure 1. Five-legged *amputee* fly.

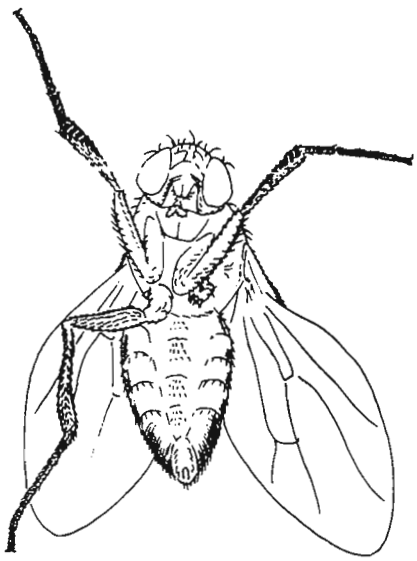


Figure 2. Three-legged *amputee* fly.

From 42 successful pair matings between parents with four or five legs (5 x 5, 4 x 5 or 4 x 4) some 1066 progeny were obtained. Of these, 658 or 61.7% were *amputee*, with a slight excess (51.4%) of males. Of the *amputee* flies obtained from these crosses, 56.7% had five normal legs, 32.7% were four-legged, 8.7% were three-legged, and 2.0% had but two legs. No flies with fewer than two legs have been recovered thus far. The genetic bases of this developmental aberration are still being sought.

References: Erk, F.C. and A. Podraza 1986, DIS 63:161; Baker, N.E. 1988, Dev. Biol. 125:96.

Report of Furman, D.P. and T.A. Kozhemyakina.
Institute of Cytology and Genetics, Novosibirsk 630090, USSR.

The *Walker*: A new homeotic mutation of *Drosophila melanogaster*.

We detected the *Walker (Wk)* mutation while breeding under laboratory conditions a line containing the sc^{53P} allele which had been earlier induced in the P-M system of hybrid disgenesis. By the instant of the event the age of the original line had been about 70 generations, and what is more, the cultivation ran through mass *intra se* crosses without any P-element "nourishing".

The abnormal phenotype appeared spontaneously, in an only male. An appropriate supposition that the mutation is localized in X chromosome was verified by crossing the *Wk* male with C(1)DX, *ywf* females. The phenotype was reproduced both in the descendant males and females, which made us assured that the mutation is dominant and autosomal (Table 1).

Table 1. Phenotypes in the *Wk* male's offspring

Offspring Phenotypes	*y w f* Females	Males	Total
Non-*Wk*	112	135	247
Wk	52	57	109
Total	164	192	356

Table 2. Mapping of Walker (Crossing: female *Cy / Pm; D / Sb* x male *Pm; Sb; Wk*)

Offspring phenotypes	non-*Wk*	*Wk*	Total
Cy ; Sb	44	53	97
Cy ; D	26	38	64
Pm ; Sb	21	118	39
Pm ; D	34	44	78
Pm / Cy ; Sb	36	18	54
Pm / Cy;D	38	10	48
Pm ; D / Sb	84	—	84
Cy ; D / Sb	76	—	76
Pm / Cy ; D / Sb	59	—	59
Total	418	281	699

To determine the linkage group we kept to the standard scheme with the use of the *Cy/Pm*; *D/Sb* test line. Basing on the results obtained we localized the actual mutation at chromosome 3 as it never occurred simultaneously with *D/Sb* markers (Table 2). When homozygous, *Wk* is lethal, that is why it was furthermore maintained as *Wk/Sb* or *Wk/D*.

Wk exhibits numerous changes in traits (Fig. 1). Abnormalities affect wings, eyes and halteres the most fatally.

Eyes reduced, monstrously formed, each often with a bundle of bristles in the centre of a caverna rushing through facets (Fig. 2). Disturbances in wing development provoke wing blade abnormalities of several types such as notches, blistering, reducing to a strap or nubbins lacking most of the wing blade material; the wing sometimes exists as a disorganized stuff (Fig. 3). An extreme manifestation is that no wing exists on either or both sides of the fly body as the wing is converted to a scutellum-like element with the adjacent part of thorax and the authentic bristles and hairs (Fig. 4). Halteres sometimes reduced, completely or partly (Fig. 5). Legs may be abnormal, too, but rarely, i.e. the contiguous segments, all shortened and bulged, meet at a random angle.

Mutant flies in these lines occur in up to 60-70 cases out of 100. However, at individual crosses with females of any genotype all the phenotypically normal males that were picked out of mutant lines under a spot check show themselves to be carriers of *Wk*. Therefore, incomplete penetrance of *Wk* is a fact.

Then penetrance was shown to depend on a cultivation temperature and, for cases of outbred matings, on the direction of the mating as well. We compared the respective phenotypical traits in the *Wk/Sb* stocks at 14°, 25 ° and 30°C and could state that the higher the temperature at which the mutants are maintained, the less cases of abnormalities are observed (i.e., the closer to normalcy is the phenotype) (Fig. 6).

Direct and reciprocal crosses between *Wk/Sb* and Oregon R were performed, the direct combination yielding a notably higher number of mutants (Fig. 7).

The mapping of *Wk* within chromosome 3 was carried out versus *st* (44.0) and *e* (70.7) after the standard scheme of calculating the frequency of recombinations between the markers and the mutation in consideration (Table 3).

It is attractive that the *Wk*-containing classes, especially *Wk* and *st*, abruptly decreases in number as compared to complementary combinations. A cause of such unbalance may be either incomplete penetrance of *Wk*, owing to which the number of flies in the complementary groups upgrades, or lower viability of mutants, or some other factors. In this connection certain problems in mapping arise. Since the correction factor (in particular, incompleteness of penetrance) is somewhat unclear, it seems appropriate to calculate the recombination frequency involving only data on crosses in *Wk*-manifesting classes, as otherwise the corresponding values

Table 3. The frequencies of recombinations between *Wk* versus *st* (44.0) and *e* (70.7). Crossing: female *Wk / st e* x males *st e / st e*)

Class	Progeny phenotype	Number	Class frequency, %
Parental	*Wk + +*	579	67.7
	+ st e	1355	57.1
Single CO	*Wk st e*	32	3.7
Wk - st	*+ + +*	410	13.1
Single CO	*Wk + e*	225	26.3
st - e	*+ st +*	598	24.3
Double CO	*Wk st +*	20	2.3
	+ + e	166	5.5
Total flies Scored		3385	

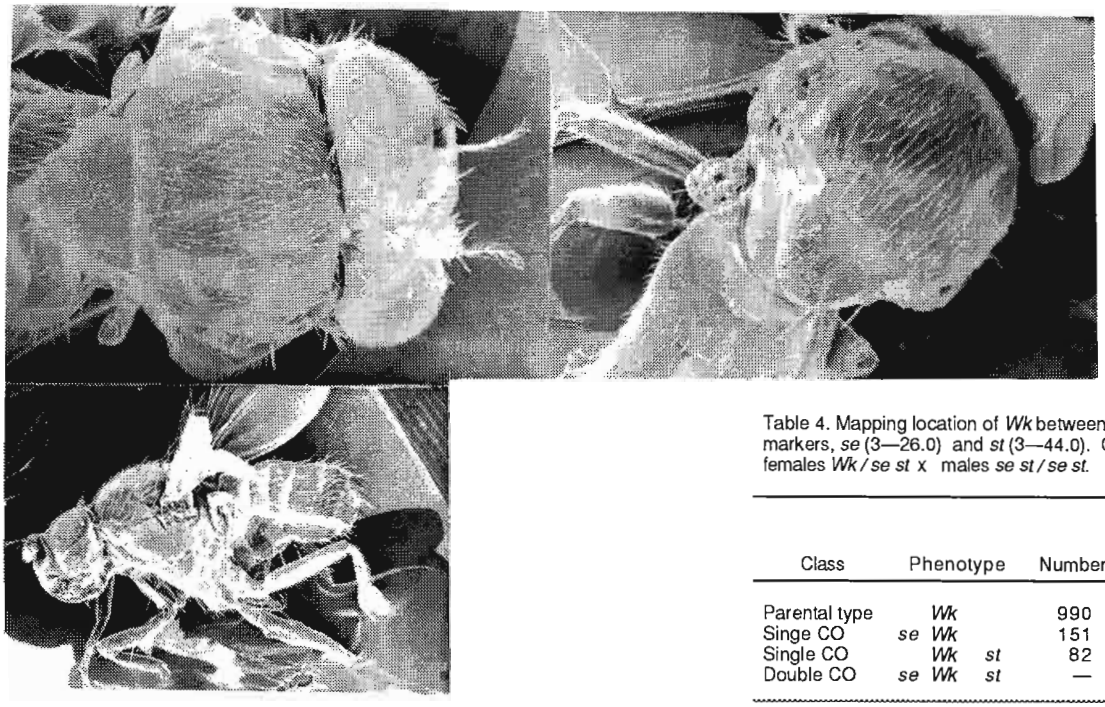
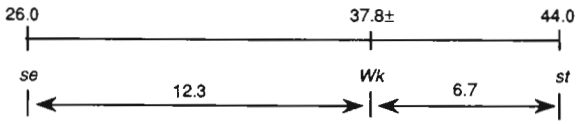


Figure 1. *Wk- Walker* (3-37.8±).

Table 4. Mapping location of *Wk* between the extreme markers, *se* (3—26.0) and *st* (3—44.0). Crossing: females *Wk / se st* x males *se st / se st*.

Class	Phenotype	Number	Class frequency, %
Parental type	*Wk*	990	81.0
Singe CO	*se Wk*	151	12.3
Single CO	*Wk st*	82	6.7
Double CO	*se Wk st*	—	—
Total		1223	100.0



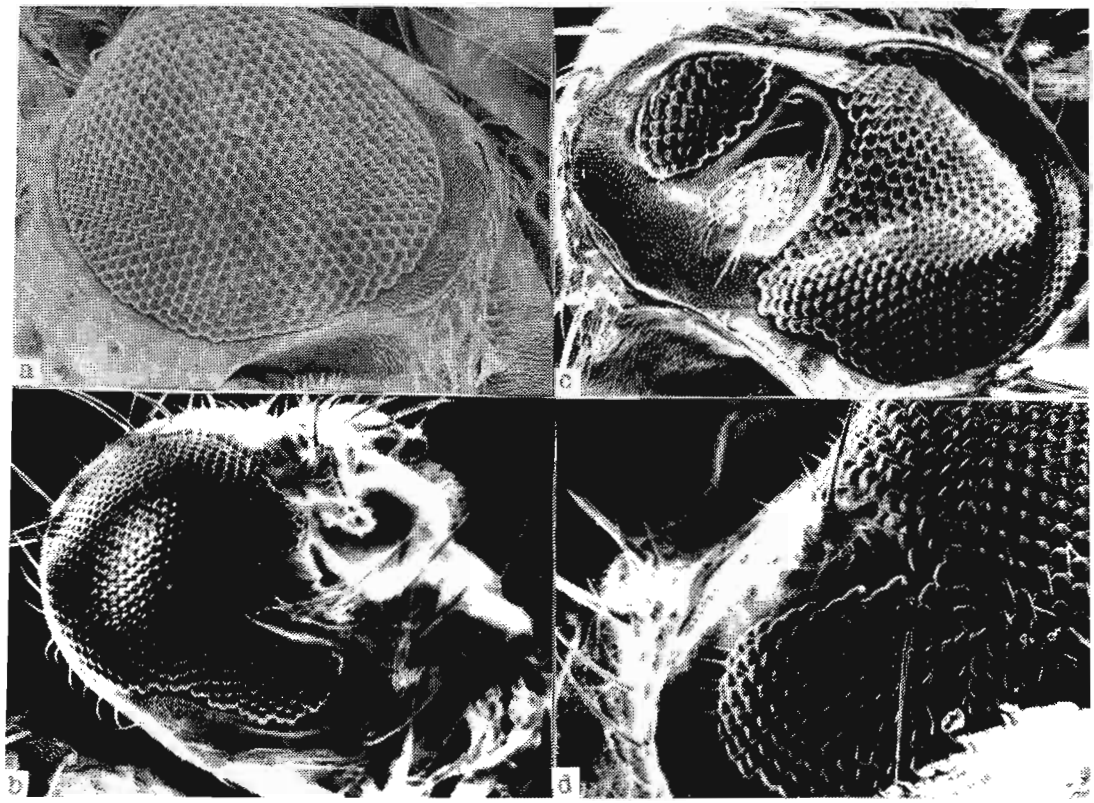


Figure 2. Morphology of the eye: a) wild type Oregon R eye, b)-d) *Wk/Sb* fly eye

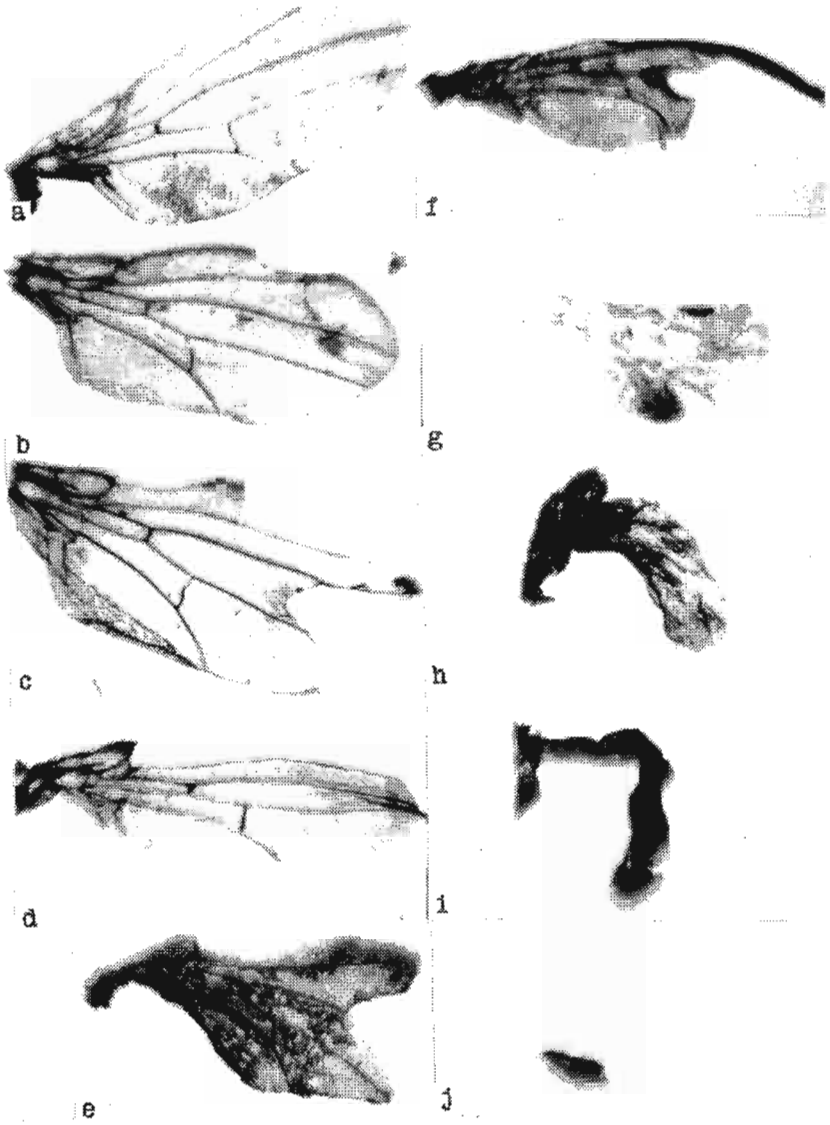


Figure 3. Wing abnormalities: a) wild type wing (Oregon R), b)-j) wing varieties in *Wk* mutant

Figure 4.

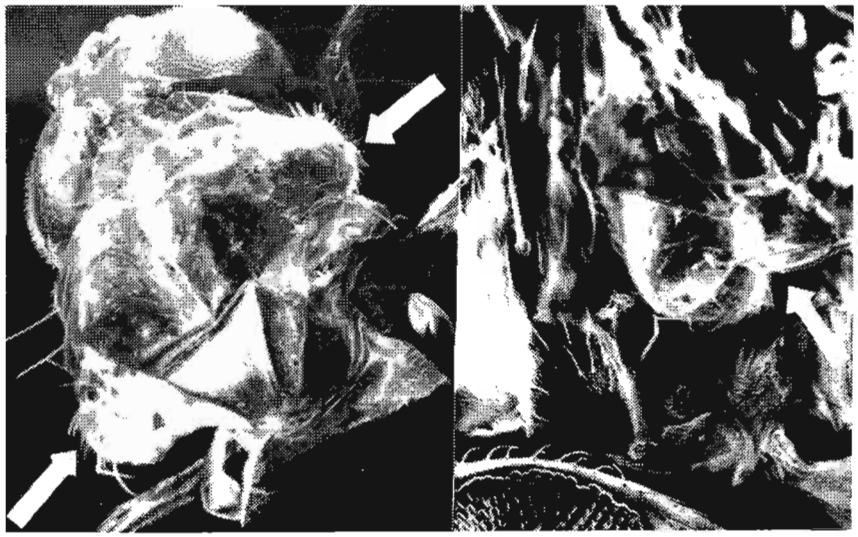


Figure 5.

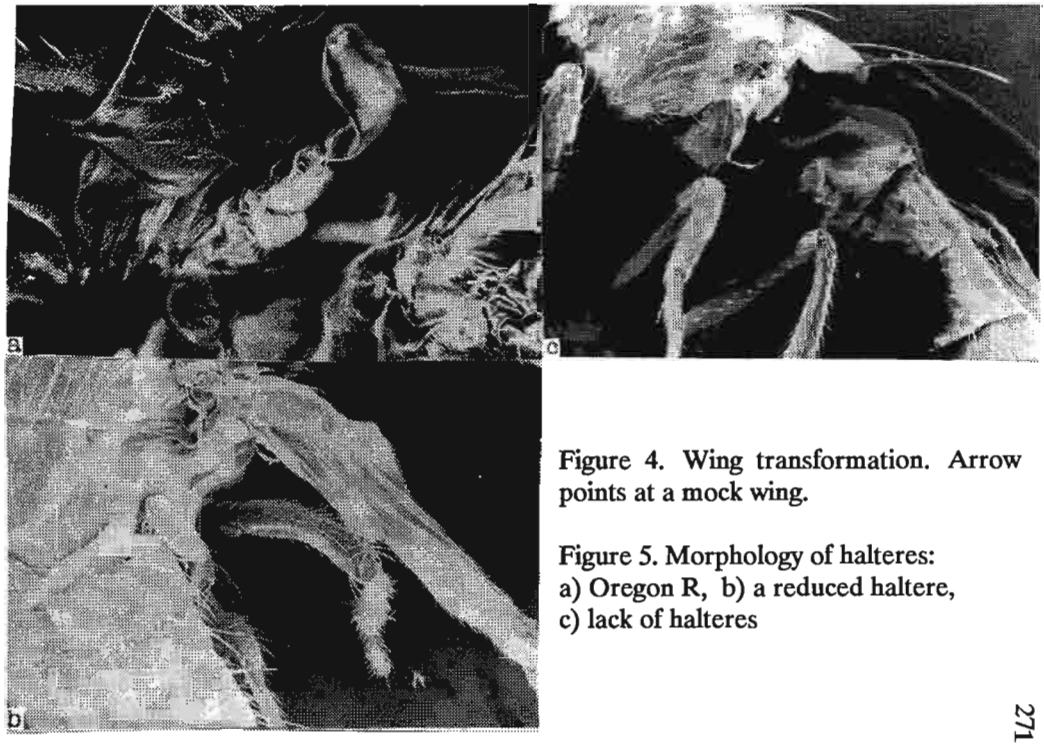


Figure 4. Wing transformation. Arrow points at a mock wing.

Figure 5. Morphology of halteres: a) Oregon R, b) a reduced haltere, c) lack of halteres

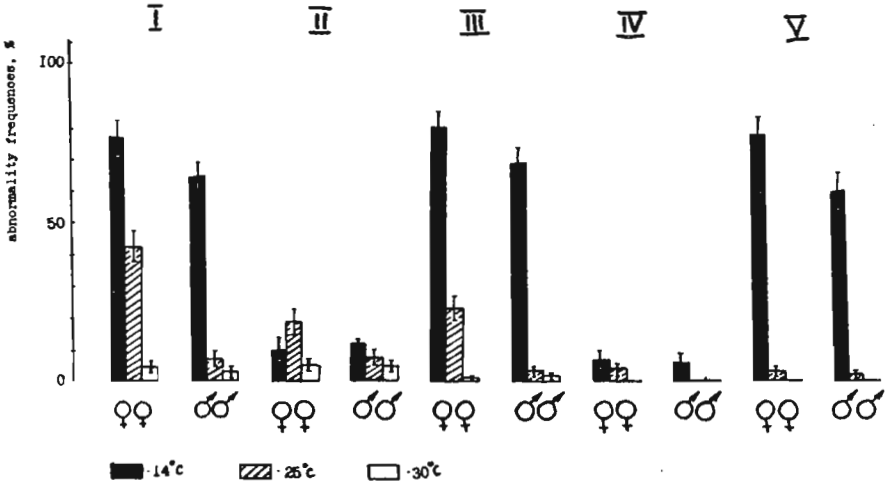


Figure 6. *Wk* manifestation at different temperature conditions. I - eye abnormalities (Fig. 2), II - wing affections (Fig. 3), III - wing transformations (Fig. 4), IV - reduction of halteres (Fig. 5a), V - lack of halteres (Fig. 5b).

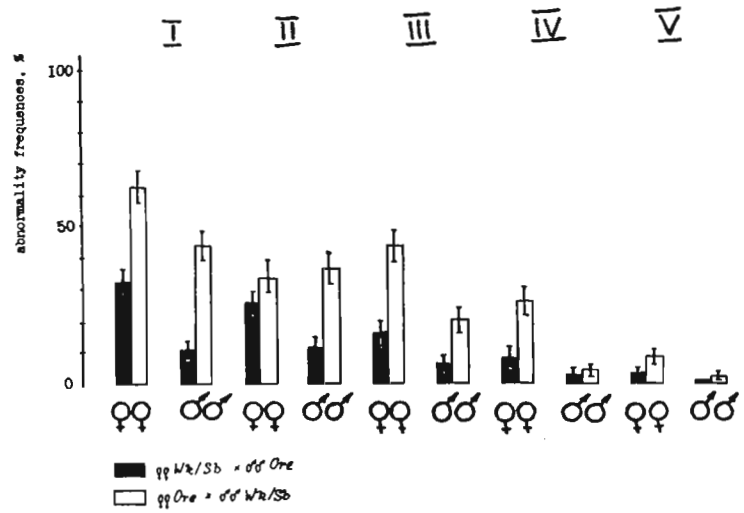


Figure 7. *Wk*/Oregon progeny phenotypes depending on cross direction. Indications as at Fig. 6.

could be overstated. The calculations yield the following distances between the genes: *Wk-st* - 6.1 cM; *st-e* - 28.6 cM; *Wk-e* - 34.7 cM. The averaging of the crossing-over values calculated versus the two markers locates *Wk* at 37.0 ± on the left arm of chromosome 3.

The localization of *Wk* was made precise by using the *se* (26.0) and *st* (44.0) genes as flanking markers, which set the mutation at ± 37.8 cM (Table 4).

A cytological analysis of salivary gland chromosomes from *Wk/Sb* third instar larvae revealed In(3L)*65E3-5; 74A1-2* inversion (Fig. 8) that was lacking in both the Sc^{53P} original line and the *Cy/Pm;D/Sb* line used in order to balance *Wk*. Moreover, no trace of this rearrangement was ever found at a homozygote stage. To verify the supposition that the inversion is a factor for the mutant phenotype we analysed the *Wk/D* line. No sign of inversion having been detected, it is reasonable to conclude that no *Wk* was its effect.

To find out about the part of mobile elements in the appearing of *Wk* we hybridized salivary gland chromosomes of *Wk/Sb, Wk/D,* sc^{53P} and *Cy/Pm;D/Sb* larvae with ^{3}H-labelled *mdg*1,2,4,B104 and P-element DNA probes *in situ*.

Among the results obtained the most interesting are those of the hybridization with *mdg*2. Fig. 9 presents the localization of labelled sites throughout chromosome 3 from *Wk/*sc^{53P} larvae. The homolog carrying the *Wk* mutation is marked by *In(3L)65E3-5;74A1-2* inversion. Five sites of *mdg*2 localization, namely 65E, 67C, 68C, 73D and 74A are fixed within the region of rearrangement. Both sc^{53P} and *Wk*-carrying lines are noted for *mdg*2-labelled 67C and 73D sites. In the intact homolog, sites 65E and 74A, breakpoints of *In(3L)65E3-5;74A1-2* harbour the label, but remain unlabelled in the altered, which appears to be because of the total or partial excision of the mobile element during the inversion formation (if partial, the residual fragments must be so small that *in situ* hybridization is not too fine to detect them). Finally, 68C showed no hybridization in both basic lines sc^{53P} and *Cy/Pm;D/Sb*, but positively did in all lines with *Wk* phenotypes (*Wk/*sc^{53P}, *Wk/Sb* and *Wk/D*) which gives us grounds to suspect that it is the insertion of *mdg*2

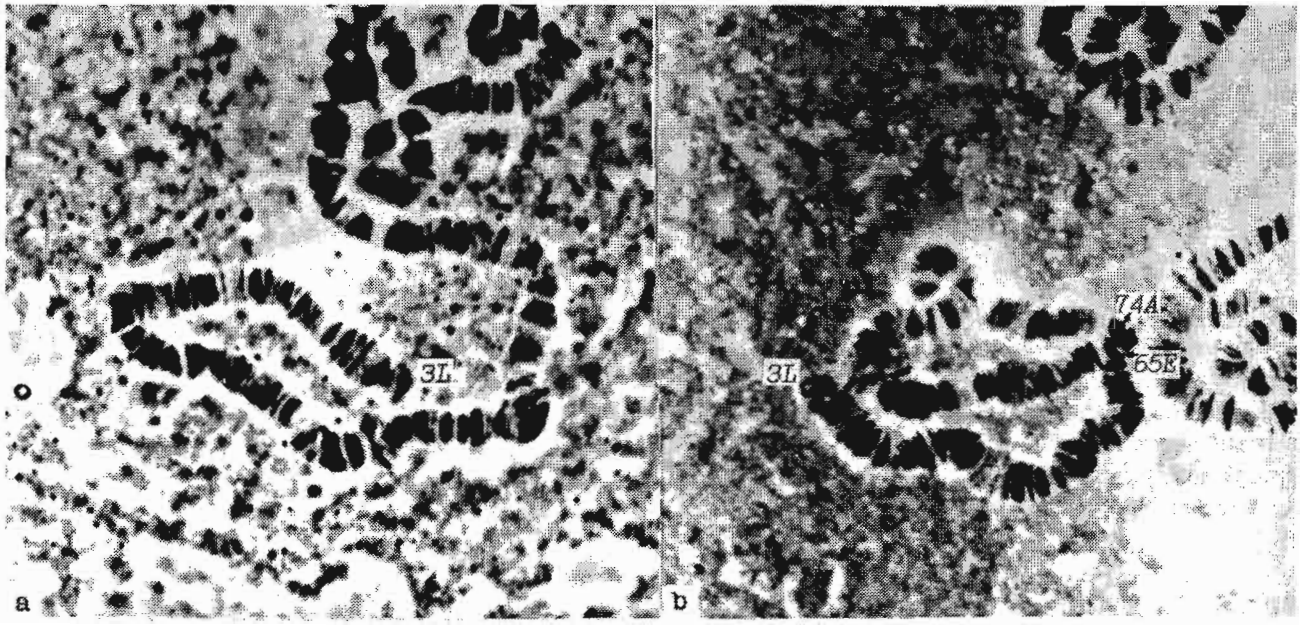
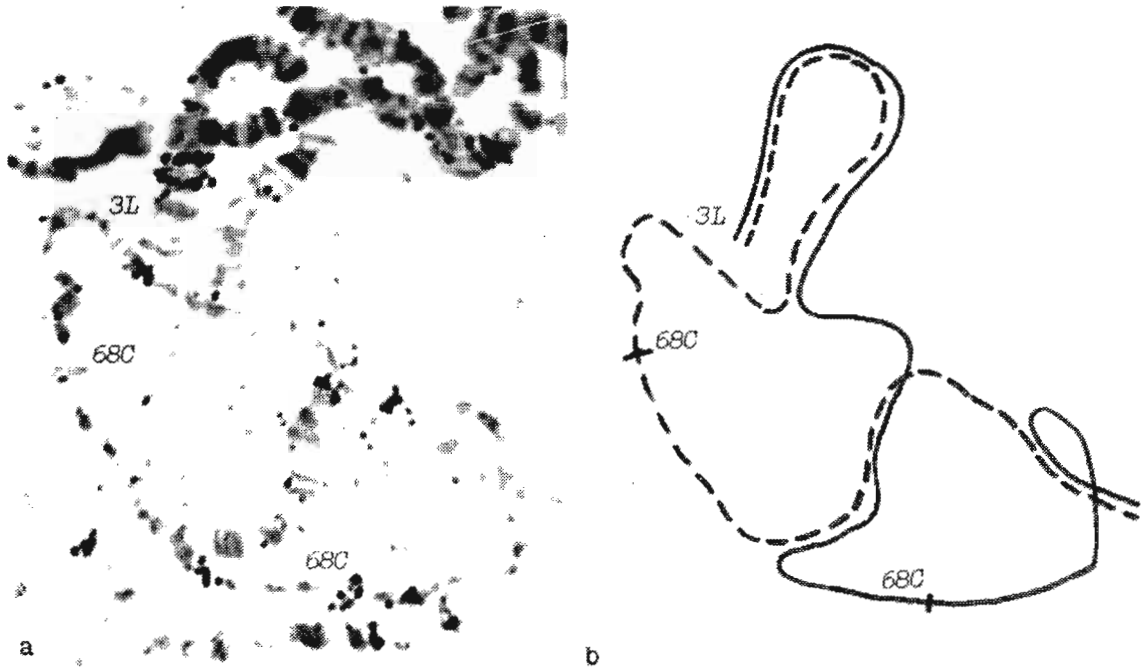


Figure 8 (above). 3*L* chromosome from the sc^{53P} (a) and *Wk/Sb* (b) lines. Arrows indicate at *In(3L)65E3-5;74A1-2* breakpoints.

Figure 9 (below). *In situ* hybridization of *mdg2* with Wk/sc^{53P} *3L* chromosome.
a) localization of the label, a microphoto; b) schematic reconstruction.



into 68C site that motivates *Wk* to arise. Note, region 68C is bound to approximately 37.8 cM of the *Wk* genetic localization at the map of salivary gland polytene chromosomes.

Thus, a new homeotic mutation, *Walker (Wk)*, has been described. Characteristics: homozygous lethal, incomplete penetrance. Inheritance: autosomal, dominant. Localization: chromosome 3, left arm, ±37.8 cM. Apparent motivation: insertion of *mdg2* into *68C*.

Acknowledgment: God save V. Filonenko, interpreter of the communication, godfather of the *Walker*.

Report of Kuhn, D.T.
Oviedo High School and Department of Biology, University of Central Florida, Orlando, Florida.
A new mutant at the *white* locus in *D. melanogaster*

w^p: white peach (1-1.5). A single male was recoved by Jack Ventimiglia after EMS treatment, which carried the recessive w^p allele. Eye color is yellowish-pink to light orange in both sexes, darkening somewhat with age. Heterozygous w^p/w females show a slightly lighter peach eye color. Adult testes sheaths and larval Malpighian tubules colorless. The position within the *white* locus was not determined. Viability is excellent. RK1.

Report of Marsh, J.L. and J. Purcell.
Developmental Biology Center, University of California, Irvine, California.
A dysgenic allele of *scalloped: sd^{jp}*.

We had occasion to revert a dysgenic allele of *hopscotch* (Lefevre's l(1)L*4*). During the course of the reversion experiment which took place under dysgenic conditions, a new mutant was recovered with severely reduced wings. Mapping and allelism tests showed that it was allelic to *sd*. It is on an X chromosome bearing *yellow* and *white* and is viable in males and females.

Report of Omelianchuk, L.V., E.V. Chadova, S.A. Kopyl, B.F. Chadova, M.L. Podoplelova and E.I. Volkova.
Institute of Cytology and Genetics, USSR Academy of Sciences, Siberian Department, Novosibirsk 630090, USSR.

Males of ds^{38k}/In(2L)Cy, Cy dp^2 b pr and pr pk cn genotypes were irradiated (Co^{60}, dose 3000R). Males of the first genotype were crossed to F(2L)2, pr; F(2R), + females, and those of the second genotype to F(2L)2, +; F(2R)1, In(2R), cn c sp female. The heterozygous In(2L) Cy/F(2L);F(2R) daughters were tested for the presence of rearrangement involving 2R arm of the metacentric, and pr pk cn/F(2L); F(2R) daughters for the presence of a rearrangement involving 2L arm of the metacentric. In contrast to the standard nondisjunction test (Chadova, this issue), search for the formation of the rearrangement was carried out in a background of an inversion in the opposite arm of autosome 2. In this way, we succeeded in decreasing the yield of aneuploid offspring in the females free from the newly arisen arrangement and thereby exaggerate the contrast between the progeny number of females with the new rearrangement and without it.

The cultures with high progeny number were analyzed for the presence of a new rearrangement in the preparation of the salivary gland polytene chromosomes.

The rearrangements recovered in the experiment were as follows: (Note: this list is printed in columns to save space).

I. New arrangements recovered in In(2L)Cy, Cy dp^2 b pr autosome:

In(2R)Cy5 = In(2R) 50A2-3; 57C2-10
In(2R)Cy15 = In(2R) 51D; 57B3-4
In(2R)s32 = In(2R) 44B; 41
In(2R)s37 = In(2R) 56F; 58E-F
In(2LR)Cy11 = In(2LR) 37B; 56D
In(2LR)s1 = In(2LR) 34E; 60F
*In(2LR)s2 = In(2LR) 22F2-3; 54B-C
*In(2LR)s8 = In(2LR) 36D; 45A
In(2LR)s31 = In(2LR) 26C; 49E
In(2LR)s31 = In(2LR) 26C; 49E
*In(2LR)Cy3 = In(2LR) 33C-D; 55F1-56A1
*In(2LR)Cy9 = In(2LR) 22A; 51F-52A; 57F-58A
In(2LR)p4 = In(2LR) 22C; 60C10-D1 + In(2R) 44D; 49C
*T(2;3)Cy7 = T(2;3) 52D; 71C + In(3L) 69C; 70F
T(2;3)Cy12 = T(2;3) 50C; 72F-73A1
T(2;3)p1 = T(2;3) 53D; 74A1-B1
T(2;3)s5 = T(2;3) 44F; 85D-E
T(2;3)s6 = T(2;3) 42A; 84D3-4
T(2;3)s9 = T(2;3) 46B-D; 71A-B
T(2;3)s11 = T(2;3) 49B; 61C

Rearrangements with break in chromocentral heterochromatin:

Cy4 46C; chromocenter
*Cy6 57A1-2; chromocenter
Cy10 56B; chromocenter
Cy13 47D; chromocenter
Cy16 67F; chromocenter
p3 48E-F; chromocenter
s3 47B; chromocenter

II. Rearrangements in pr pk cn autosome:

In(2L)pc2 = In(2L) 27D; 34A1-2
In(2L)pc4 = In(2L) 23E-F; 36E
In(2L)pc5 = In(2L) 27C5-D7; 33F1-34A6
In(2L)pc7 = In(2L) 26C; 34E-35A
In(2L)pc8 = In(2L) 24D; 30A
In(2L)pc17 = In(2L) 21C; 23E-F
In(2L)pc26 = In(2L) 34A-B; 36B
*In(2L)pc31 = In(2L) 22B1-5; 33A
In(2L)pc40 = In(2L) 24B; 32E-F

In(2L)pc49 = In(2L) 22D; 30A1-5
In(2L)pc57 = In(2L) 26C1-2; 29C
In(2L)pc58 = In(2L) 21A; 23E-24A; 33F
New order: 40-34A 23E-F- 21A-B 33F - 23F-24A 21A-B
In(2L)pc61 = In(2L) 28C; 34E
In(2L)pc67 = In(2L) 34A1-2; 36B-C
In(2L)pc70 = In(2L) 25E-26A1; 34E-35A1
In(2L)pc73 = In(2L) 26B; 29B
In(2L)pc80 = In(2L) 24A-C; 36B-D
In(2L)pc87 = In(2L) 34A-B; 36C-D
In(2L)pc88 = In(2L) 25A-B; 28D
*In(2LR)pc34 = In(2LR) 25A; 49B
In(2LR)pc43 = In(2LR) 27C-D; 57D
In(2LR)pc72 = In(2LR) 30B; 48C
In(2LR)pc83 = In(2LR) 24D; 58B
In(2L)pc44 = In(2L) 27D; 34A + In(2R) 55C; 59C-D
In(2LR)pc52 = In(2LR) + In(2L) 25F-26A; 30A6-9; 47F
New Order: 21-25F 47F-41 40-30A 26A-30A 47F-60
T(1;2)pc14 = T(1;2) 14D-E; 35D-F
T(1;2)pc77 = T(1;2) 4F-5A; 25D
T(2;3)pc13 = T(2;3) 23C; 98C-E
T(2;3)pc15 = T(2;3) 29F-30A; 76B
*T(2;3)pc32 = T(2;3) 40; 99B
T(2;3)pc36 = T(2;3) 28D; 66E
T(2;3)pc41 = T(2;3) 25A-B; 29F-30A; 88B-C
New Order: 21 - 25A-B 88B-C - 82; 100 - 88B-C 30A-29F - 25A-B 30A-29F - 40
T(2;3)pc45 = T(2;3) 28A-B; 89F
T(2;3)pc47 = T(2;3) 35D-F; 91D-F
T(2;3)pc48 = T(2;3) 35; 84D
T(2;3)pc50 = T(2;3) 24D-F; 75C
T(2;3)pc53 = T(2;3) 26C; 69D-E
T(2;3)pc60 = T(2;3) 27A; 70F
T(2;3)pc64 = T(2;3) 40; 93 + In(2R) 53A; 56F
T(2;3)pc68 = T(2;3) 35D-F; 98B
T(2;3)pc69 = T(2;3) 26B-27B; 89F-90A
T(2;3)pc79 = T(2;3) 25A; 80
T(2;3)pc81 = T(2;3) 29C; 69B-C
T(2;3)pc86 = T(2;3) 29D; 87D1-5
T(2;3)pc86 = T(2;3) 29D; 87D1-5
*T(2;3)pc89 = T(2;3) 30E-F; 91A
T(2;3)pc90 = T(2;3) 35B; 87F1-2
Tp(2L)pc71 = Tp(2L) 35C-F; 39E-F
New Order: 21 - 35C-F 36E-F - 39E-F 36B-F - 35C-F 39E-F 60

Rearrangements with break in chromocentral heterochromatin:
*pc13 35E-F: chromocenter
pc29 34B7-C2: chromocenter
pc37 30A1-2; chromocenter
pc39 25A; chromocenter
pc51 34D; 36C: chromocenter
pc76 26B; chromocenter

Asterisks (*) indicate the lost lines.
Rearrangements in stocks of pc15, pc17, pc67, pc68, pc76 and pc86 are viable in homozygous condition.

Preiss, Anette[1] and Spyros Artavanis-Tsakonas[2].
[1]Biozentrum, University Basel, Switzerland, and [2]Yale University, KBT, New Haven, Connecticut.
New mutants in *Drosophila melanogaster* (Meigen).

Adv: *Additional veins*. Location: 2R. Origin: X-ray induced in males with 4000R. Phenotype: First longitudinal vein split, mainly following the route of the second longitudinal vein up to the level of the anterior crossvein, where it turns toward the margin, branching twice (branches fuse frequently). Third longitudinal vein is no longer attached to L1. Homozygous lethal. Viability good. Cytology: Associated with In(2R) 48 B/C - 58 D/E.

Two-b^{100}: *Two bristles 100*. Location: 3R. Origin: X-ray induced in males with 4000R. Phenotype: Both postverticals are absent. Few extra, thick vibrissae, somewhat bushy, upper vibrissae turned towards the eyes. Homozygous lethal. Viability good. Cytology: Associated with In(3R) 84C - 87C

Report of E.I. Volkova and G.N. Buzykanova.
Institute of Cytology and Genetics, USSR Academy of Sciences, Siberian Department, Novosibirsk 630090, USSR.
New mutants.

The nondisjunction test (see Chadov, this issue) was applied to recover chromosomal rearrangements involving acrocentric autosome F(2R) (free arm). *Drosophila melanogaster* males, homozygous for free arms F(2L), pr and F(2R), cn c sp were gamma-irradiated (3000R) and crossed to Berlin wild females. The progeny consisted of +/F(2L), pr; F(2R), cn c sp heterozygotes. The recovery of paracentric inversions in F(2R) and translocation between F(2R) and non-homologous chromosomes X, 3 and 4 was carried out by the standard procedure. +/F(2L); F(2R) daughters were crossed individually to F(2L), pr; C(2R), cn males. In the control crosses of +/F(2L); F(2R) daughters with nonirradiated chromosomes, the average number of progeny per female was determined. It was 6.9. In the gamma-ray-treated series, vial with offspring number 15 (3 times the square root of the mean) or more were selected.

Among 2,644 individual cultures 90 (13.4%) were selected for cytological analysis. Among these 90, 18 paracentric inversions in F(2R) and 25 translocations between F(2R) and chromosomes of other pairs were identified.

Concomitantly, the procedure for recovery of translocations between free arms T(F2L; F2R) was performed. It was based on the reasonable expectation that heterozygotes for T(F2L; F2R) should not produce viable progeny in cross with F(2L); C(2R) line. 2,551 +/F(2L); F(2R) sons were taken from the F_1 produced by irradiated fathers, and they were individually crossed to test for sterility in crosses to F(2L), pr; C(2R), cn females. 323 males with proven sterility were crossed to al dp b pr cn sp/CyO, dp^{lv1} Cy pr cn^2 females. The majority of the males were sterile in the cross, 19 only produce progeny. Cytological analysis of the polytene chromosomes in 10 cultures established the presence of translocations between F(2L) and F(2R). The rearrangements recovered in experiment were as follows: (Note: this list is printed in columns to save space.)

I. Rearrangements in the free arm F(2R), cn c sp :

In(2R)26 = In(2R) 45B; 53 A
In(2R)29 = In(2R) 41; 50E-F
In(2R)34 = In(2R) 45D; 54F
*In(2R)39 = In(2R) 42B; 58A
In(2R)52 = In(2R) 45B; 50C
*In(2R)54 = In(2R) 41; 46A
*In(2R)153 = In(2R) 41; 58B
*In(2R)163 = In(2R) 42B; 48C
*In(2R)183 = In(2R) 44D; 51C
*In(2R)192 = In(2R) 43D; 47A + In(2R) 47A-B; 51C
*In(2R)213 = In(2R) 53A; 55E
In(2R)216 = In(2R) 41; 49F-50A
*In(2R)217 = In(2R) 41; 60A
In(2R)241 = In(2R) 41; 53E
In(2R)255 = In(2R) 48A; 52E
In(2R)282 = In(2R) 45B; 50C
In(2R)283 = In(2R) 48F; 51C
In(2R)284 = In(2R) 45C; 58A
*T(2;3) 4 = T(2;3) 36C; 96D-E
*T(2;3) 6 = T(2;3) 30B; 67E
*T(2;3) 12 = T(2;3) 28D; 44A; 75 D Combined with Df(3R) 88E; 89A
New order: 21-28D|75D-88E|89A-100; 40-28D|44A-60; 41-44A|75D-61
*T(2;3) 19 = T(2;3) 38F; 87C
*T(2;3) 23 = T(2;3) 56D; 80
*T(2;3) 20 = T(2;3) 22A; 43E; 70B; 74E-F; 94E
New order: 21-22A|70B - 74E-F|94E - 74E-F|43E-60; 40-22A|70B-61; 41-43E|94E-100
*T(2;3) 25 = T(2;3) 39F; 42B; 56F-57A; 65A
New order: 21-39F|56F-57A - 42B|65A-100; 40-39F|56F-57A - 60; 41-42B|65A-61
*T(2;3) 42 = T(2;3) 59C; 83C
T(2;3) 55 = T(2;3) 52E; 94C-D
T(2;3) 172 = T(2;3) 41; 97D
*T(2;3) 175 = T(2;3) 48D; 84D
T(2;3) 176 = T(2;3) 56B; 80A-B
*T(2;3) 193 = T(2;3) 57F; 88E Combined with In(1) 7C; 16A
*T(2;3) 194 = T(2;3) 53D-E; 80
*T(2;3) 202 = T(2;3) 57F; 83A-B
*T(2;3) 205 = T(2;3) 53D; 84D
*T(2;3) 208 = T(2;3) 46F-47A; 82B
*T(2;3) 212 = T(2;3) 57A; 67E
T(2;3) 214 = T(2;3) 51A; 83D
*T(2;3) 218 = T(2;3) 44D; 87E-F
*T(2;3) 222 = T(2;3) 44D; 92A
*T(2;3) 242 = T(2;3) 50F; 80C-D
*T(2;3) 252 = T(2;3) 56D; 80
*T(2;3) 253 = T(2;3) 54E; 82E
*T(2;3) 254 = T(2;3) 51C; 85D

Translocations between F(2R), cn c sp and F(2L), pr:

T(F2R; F2L) 10 = T(F2R; F2L) 33A; 51E
*T(F2R; F2L) 11 = T(F2R; F2L) 34D; 43D-E
T(F2R; F2L) 14 = T(F2R; F2L) 30A; 60F
T(F2R; F2L) 18 = T(F2R; F2L) 23C; 41A
T(F2R; F2L) 27 = T(F2R; F2L) 30E-F; 53D
*T(F2R; F2L) 28 = T(F2R; F2L) 36D-E; 48C
T(F2R; F2L) 30 = T(F2R; F2L) 40; 56B
T(F2R; F2L) 31 = T(F2R; F2L) 37B; 60F
T(F2R; F2L) 32 = T(F2R; F2L) 34A; 42A-B Combined with In(2R) 43C; 48C
T(F2R; F2L) 33 = T(F2R; F2L) 24A; 48C Combined with In(2L) 22A-B; 38A

Asterisks (*) indicate the lost lines. Inversions In(2R)29, 52 and 153 are viable in homozygous condition.

Report of Waddle, F.
Fayetteville State University, Fayetteville, North Carolina.

New Mutants.

lz^{85}: lozenge-85. Spontaneous in a laboratory stock. A female fertile allele of lozenge. Eyes rough in female. Rougher, smaller and more nearly oval in shape in male. Similar to but not identical to lz^k. This work was supported by NIH Grant #S06 RR08206-03.

New Mutants: Other Species

Report of Lozovskaya, E.R. and M.B. Evgen'ev.
Institute of Molecular Biology of the USSR Academy of Sciences, Moscow, Vavilov str., 32.

New mutants, obtained by means of hybrid dysgenesis in *Drosophila virilis*.

Table 1. Some of the mutations obtained in crosses between strain 9 females and strain 160 males.

Strain	Symbol	Mutant	Description
The X Chromosome:			
Ds_3,9/1-2	arc*	arch	wings bent like arch
82,Ds_3	Bx^{rec}*	Beadex-recessive	wing margins scalloped
1-2.2	cp	clipped	narrow wings with margins scalloped, broken venation,female sterile
14	dy	dusky	wings about 4/5 normal size, dusky color
82 x 44.1	jl	jelly	ovoid eyes, smaller in size, female sterile, similar to lz-mutation in *D. melanogaster*
Ds_6	mn	minikin	wings about 2/3 normal size, crossveins closer together
$Ds_{7,8}$	sn*	singed	all bristles and hairs twisted and gnarled, female partially sterile
9/1-2,Ds_{11}	w*	white	white eye color
25 x 4	y	yellow	yellow body color, hairs and bristles gray
Autosomes:			
25.2	adv	additional veins	extra veins
1-2.1,82x44x65	apt*	apterous	wings and halters reduced, female sterile
25.1	cri	crimp	inside edge of wings crimped
12.2	el	eyeless	eyes reduced partially or completely
Ds_7	fr	frigged	hairs at margins thick and rough
4	So	smooth	wings larger, veins L_4 and L_5 reduced, semidominant
44x65,Ds_7	sch*	streched	wings stretched
32	nod	nodule	wings smaller, posterior cross vein carry excrescences and nodules
32	sg	spangled	wings smaller and spangled

* mutations, obtained repeatedly from the progeny of independent dysgenic crosses.

Hybrid dysgenesis is an excellent tool for obtaining new mutations. Here we give a list of new mutants isolated in the progeny from the dysgenic crosses between strain 9 females and strain 160 males of *D. virilis* (Table 1). For phenotypes of these mutants see also Figure 1.

References: Lozovskaya, E.R. and M.B. Evgen'ev 1987, Proc. Acad. Sci. of the USSR 296:727-731.

Figure 1. Some of the mutations, obtained in the dysgenic crosses.
First page of figures:
Figure 1-1 and 1-2 (top row) - strain 9, normal eye (x240; x1200)
1-3 and 1-4 (bottom row) - strain 82 x 44.1, jl (x240; x1200)
Second page of figures:
Column 1:
Figure 1-5 and 1-6, strain 1-2.1, apt (x60; x240)
Column 2:
Figure 1-7 - strain 9, normal wing (x20)
1-8 - strain 160, wing mutations b(2) and gp-L_2(3) (x20)
1-9 - strain 4, So (x20)
1-10 - strain 4, So/+ (x20)
Column 3:
Figure 1-11 - strain Ds_6, mn (x20)
1-12 - strain 32, nod, and sg (x20)
1-13 - strain 82, Bx^{rec1} (x20)
1-14 - strain Ds_3, Bx^{rec2} (x20)

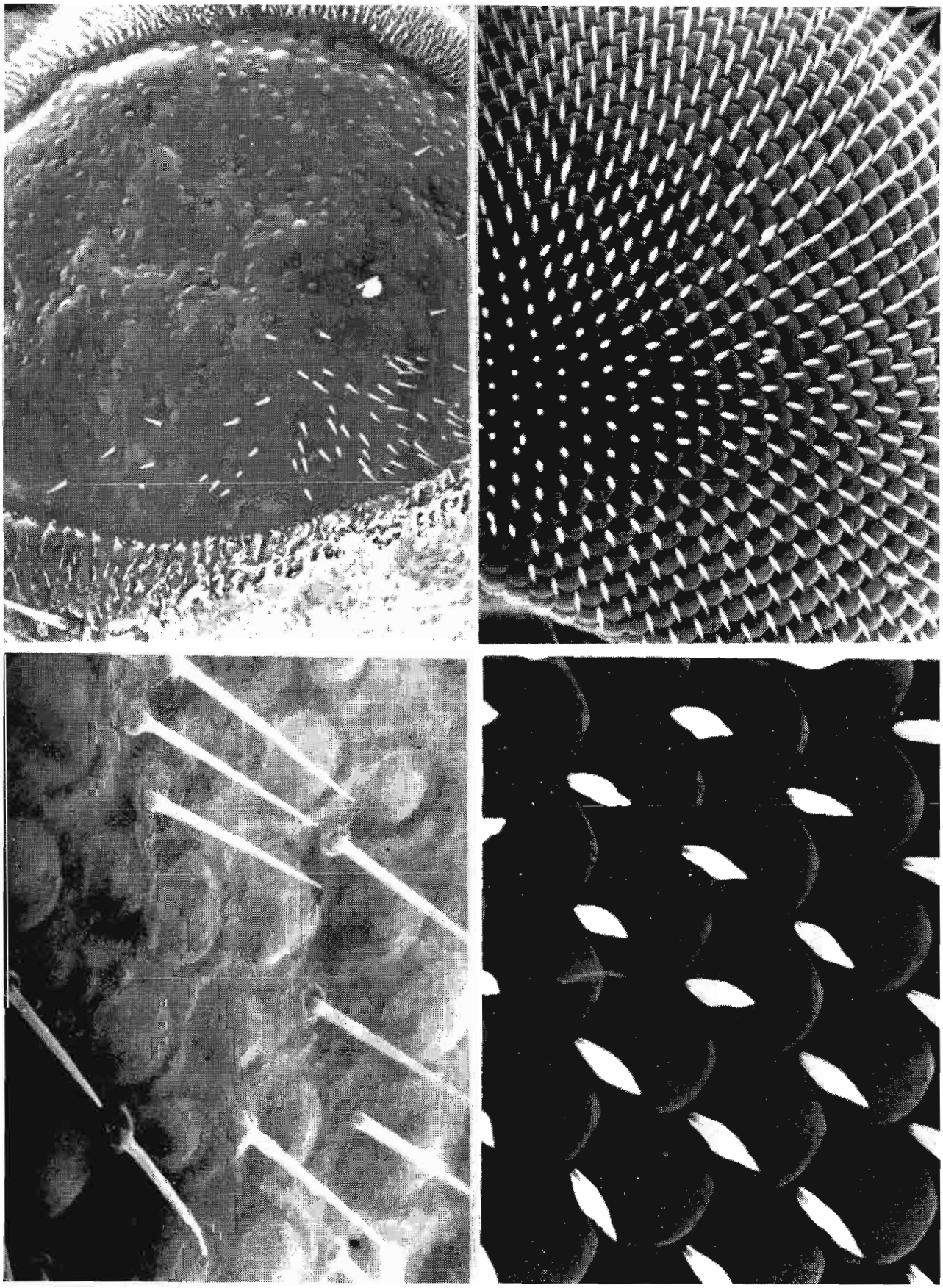
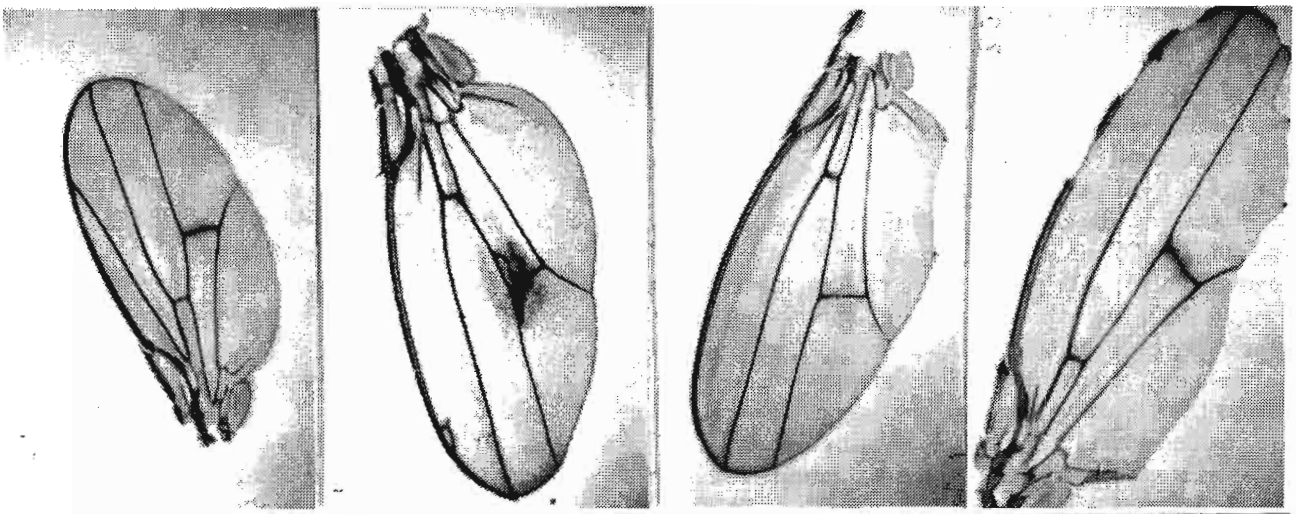
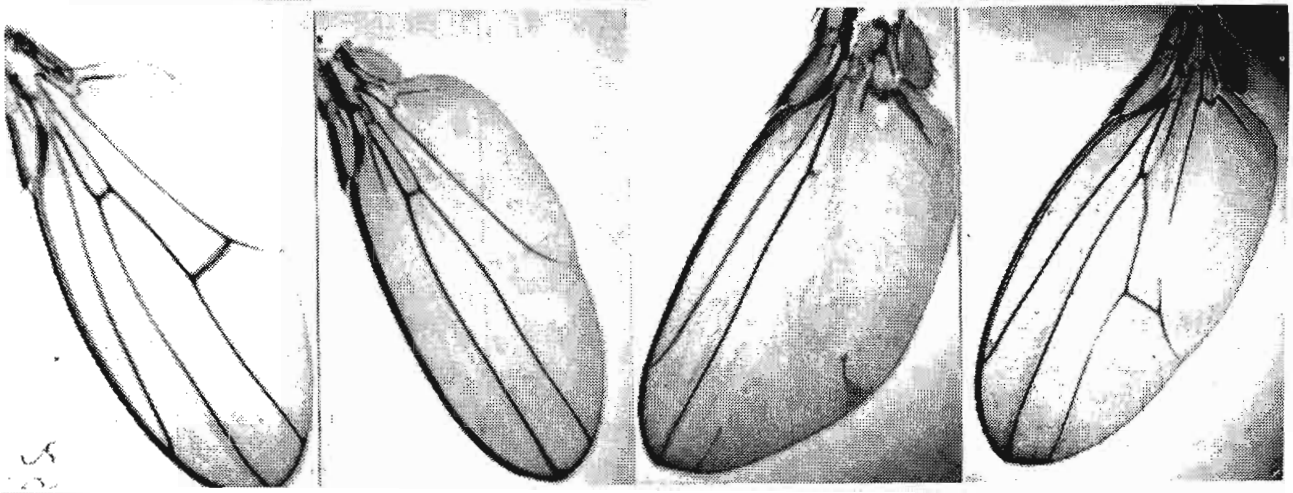


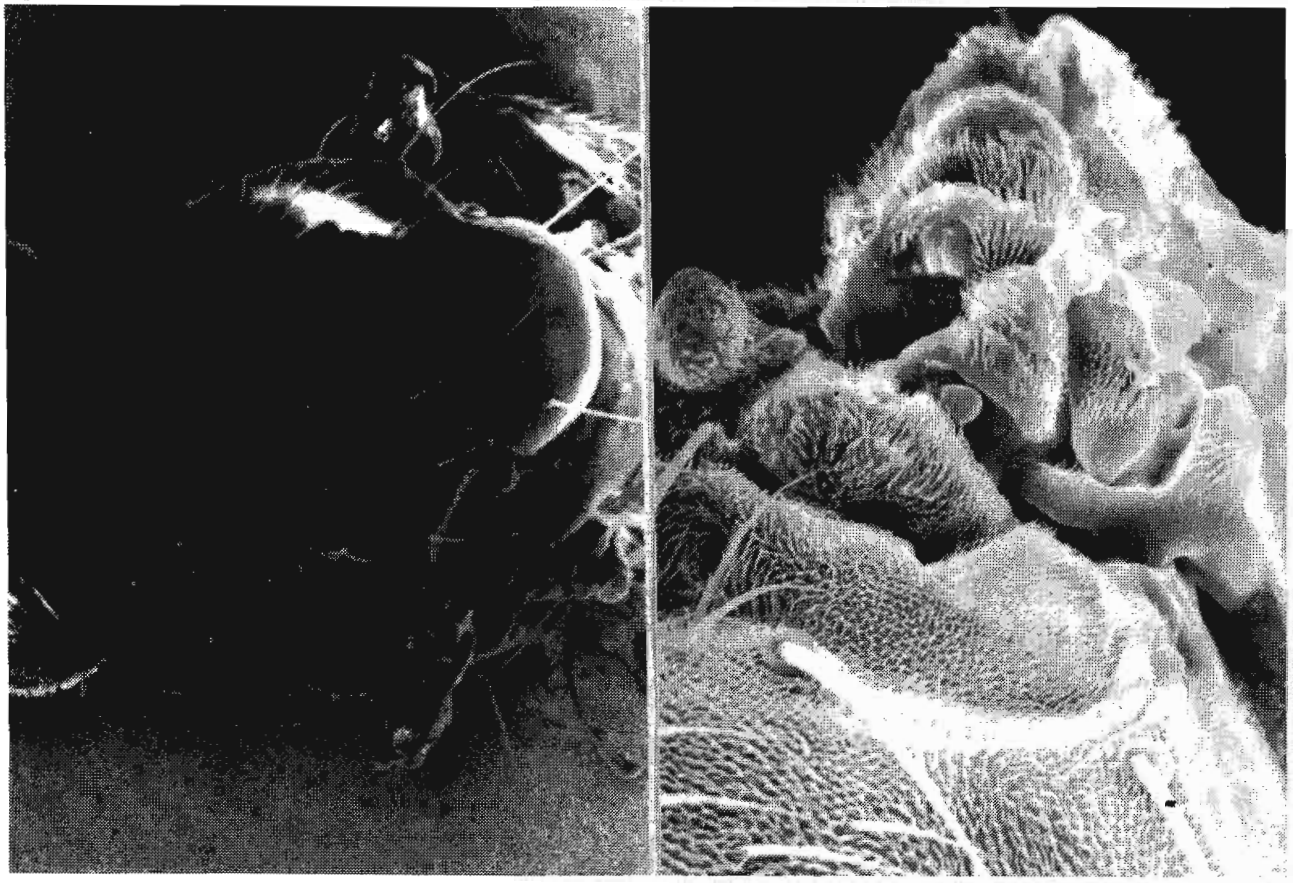
Figure 1.



Figures 1-11 to 1-14.



Figures 1-7 to 1-10.



Figures 1-5 and 1-6.

Report of Marques, H.V.S.[1], A.B. Carvalho[1], C.A. Elias[2], and L.B. Klaczko[1].
[1]Depto de Genetica, Inst.de Biologia and [2]Depto de Radiobiologia, Inst. de Biofisica. Universidade Federal do Rio de Janeiro, Brasil.

Mutants of *D. mediopunctata*.

To obtain visible mutants in *D. mediopunctata*, we used X-ray treatments (22.00 to 66.30 Gy) and inbreeding of flies coming from the field. The mutants obtained were localized through segregation tests with chromosome inversions that naturally occur in this species. Up to this moment, the following mutants have been obtained and localized:

merlot (*mt*): purplish ruby eye color, chromosome II, 3 alleles obtained by inbreeding;

coral (*cr*): eyes bright red, chromosome IV, inbreeding;

alfinete (*al*): reduced eyes, chromosome V, inbreeding;

Delta (*Dl*): veins thickened and broadened into deltas at junction with margins (Figure 1), lethal in homozygotes, chromosome II, 2 alleles obtained by X-rays;

Antennapedia (*Antp*): Antennae transformed into tarsi, with variable length (Figure 2), lethal in homozygotes, T(2;4)*Antp*, X-rays.

Acknowledgments: We are grateful to Ms. Clea Knauer da Silva and Natalicia dos Santos for their technical assistance. Financial support by CAPES, CNPq and FINEP.

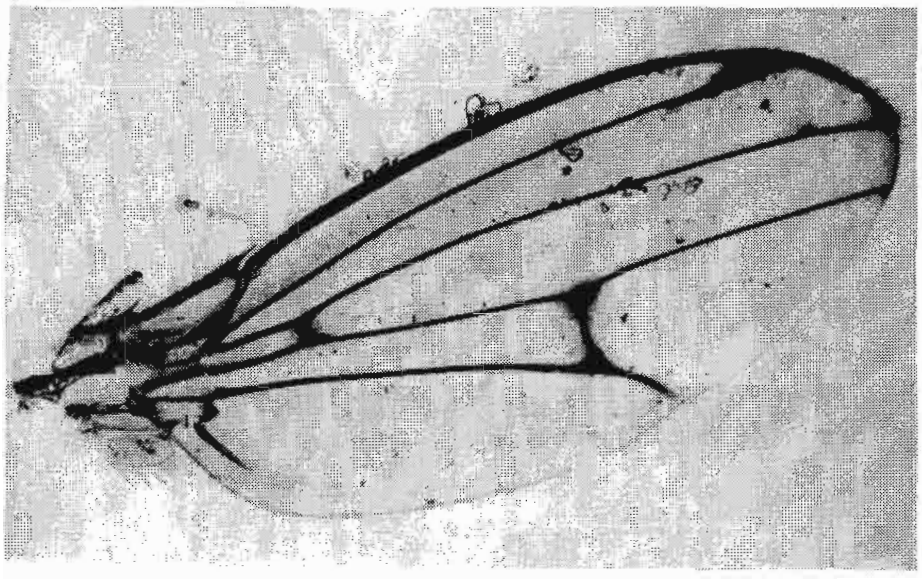


Figure 1. Wing of *Dl*.

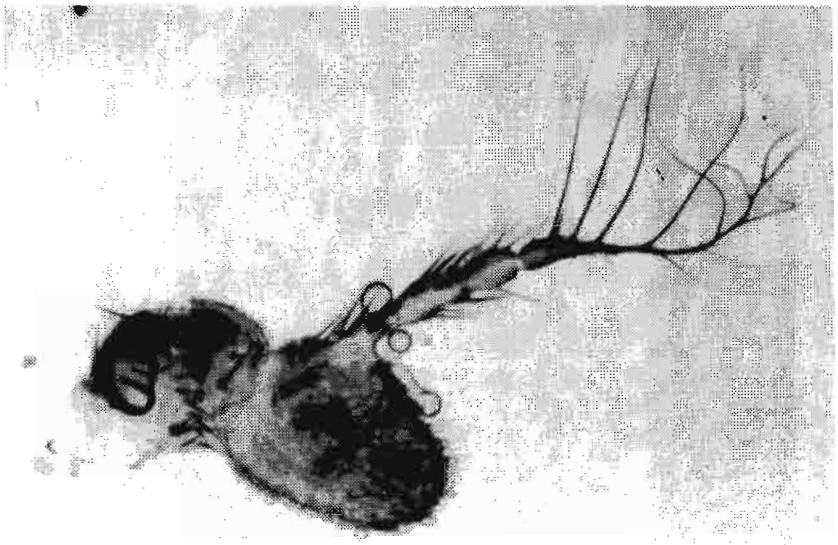


Figure 2. Antenna and arista of *Antennapedia*.

Report of Menendez, E.V. and J.R. Cardiel.
Universidad de Oviedo, Spain.

A new mutation of bristles in *D. pseudoobscura*.

This mutant arose when we were scoring for individuals with extra bristles in isofemale strains. In one of the few strains with extra dorsocentral bristles we found several males whose phenotype was clearly different from the one of their mothers, i.e., their extra bristles were in a thorax region included between the dorsocentral area of the normal positions of the supra-alar and postalar bristles (Figure 1).

The way it showed up suggested that it was a sex-linked mutant; this was subsequently checked by means of reciprocal-cross analysis. From these individuals, a mutant strain was set up and maintained at 21°C. The mean number of extra bristles in the first generations was 6.55 ± 0.13 for females and 5.49 ± 0.11 for males, measured in a sample of 500 individuals per sex, randomly taken from the strain.

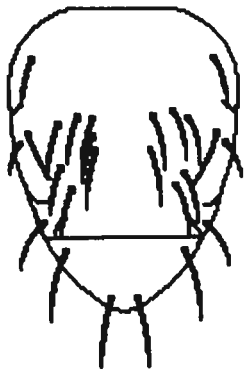


Figure 1. Phenotype of *pel* mutant.

Table 1. Recombination frequencies between marker loci and locus *pel*

Class of males	Number of individuals of each class
se sp +	230
+ + pel	234
+ + +	120
se sp pel	84
se + +	152
+ sp pel	108
+ sp +	56
se + pel	32
Total	1006

p between *sepia - pel* = 0.290 ± 0.020
p between *snapt -pel* = 0.461 ± 0.022
p between *sepia- snapt* = 0.345 ± 0.021

In order to locate precisely the site of mutation on the X chromosome, we applied the regular mapping procedure, by determining the frequency of crossing-over between the *sepia* and *snapt* markers (*se* 1-135.7; *sp* 1-176.7). The obtained recombination frequencies (Table 1) suggested that the new mutation occurred between the sites 104-107 of the X chromosome.

The main characteristic of this mutant is the position of extra-bristles, which is quite regular in all individuals both within the same generation and in successive generations. Specifically it is: a line of 2-3 bristles which runs parallel to the dorsocentral line, and it is present at least in 80% of the analyzed individuals, in every generation. Futhermore, as usual for characters expressed in both sides of the body, this mutant presents bilateral symmetry.

We propose the following term to denotate this new mutation: *presutural-extra-lateral* (*pel* 1, 104-107).

Report of Mestres, F.
Dept. Genetics, Universitat de Barcelona, Spain.

Eye colour mutation in *Drosophila subobscura*.

The study of American colonization by *D. subobscura* using lethal allelism analysis has shown that the 0 5 inversion could be very useful in elucidating certain aspects of the colonizing process, particularly the origin of the colonization (Mestres, 1988; Prevosti *et al.*, 1989; Mestres *et al.*, in press). For this reason many samples were collected in populations of the Palearctic area in order to estimate the frequency of 0 5 chromosomal arrangement and to study the lethal gene content.

An 0 5 inversion was detected in the Swedish population of Lilla - Edet. The crosses made in order to obtain this inversion in homozygous condition were carried out using the Va/Ba balanced strain (Sperlich *et al.*, 1977). This 0 5 chromosomal arrangement proved to be semilethal (with a viability of 2.316%). Many flies of normal phenotype, with regard to the Va marker of the balanced strain, showed a light brown eye colour, similar to that of white wine. Some crosses of flies with this eye colour were carried out in order to fix the mutation and to elucidate the kind of heredity. Unfortunately no offspring flies were obtained from the different crosses performed.

The mutation could be qualified as rank RK 1.

References: Mestres, F. 1988, Doctoral dissertation, University of Barcelona; Prevosti, A., L. Serra, M. Aguade, G. Ribo, F. Mestres, J. Balanya and M. Monclus 1989, in "Evolutionary Biology of transient unstable populations". Springer-Verlag: 114-129; Mestres, F., G. Pegueroles, A. Prevosti and L. Serra, in press, Evolution; Sperlich, D., H. Feuerbach-Mravlag, P. Lange, A. Michaeliditis and A. Pentzos-Daponte 1977, Genetics 86:835-848.

Report of Rux, J.G. and J.A. Coyne.
Department of Ecology and Evolution, The University of Chicago, Chicago, Illinois.

Recently-acquired or recently-arisen mutations in the sibling species *D. simulans, D. mauritiana,* and *D. sechellia.*

We report recently-acquired or recently-arisen mutations in the sibling species *D. simulans, D. mauritiana,* and *D. sechellia.* When possible, we have tried by hybridization to homologize these mutations with *melanogaster* mutations of similar phenotype and chromosomal location. (Our sources for *melanogaster* phenotypes were Braver 1956 (Carnegie Inst. Pub. 552A) and Bryant and Murnik 1980 (DIS 55:173).

D. simulans

X chromosome:

rudimentary	Identical to *rudimentary* of *D. melanogaster*. Females sterile; mutation maintained by crossing *rudimentary* males to attached-X females. Location 7 map units from *forked*.
nipped	Wings notched at the tips; variable expressivity. Located 18 map units from *white*. Not homologous with any *D. melanogaster* mutation.
prune	Identical to *prune* of *D. melanogaster*.
ruby	Identical to *ruby* of *D. melanogaster*.

Second chromosome:

brown	Eyes dull reddish-brown. Identical to *brown* of *D. melanogaster*.

D. mauritiana

X chromosome:

purplish	Eyes purplish-ruby. Located 6 map units from *yellow*. Not homologous with any *D. melanogaster* mutation.
singed	Obtained from Daniel Hartl. Identical to *singed* of *D. melanogaster*.
bordeaux	Identical to *bordeaux* of *D. melanogaster*.
vermilion	Identical to *vermilion* of *D. melanogaster*.
prune	Identical to *prune* of *D. melanogaster*.

Autosomal:

irregular	Obtained from Daniel Hartl. Eye facets irregular in arrangement. Variable expressivity, some close (but not identical) to wild-type. On third chromosome.
rose	Eyes light reddish-purple with no pseudopupil when young. Overlaps with wild-type after 3 days of age, then turns dark purple when older. On third chromosome.
jaunty	Wings curled upward. Identical to *jaunty* of *D. melanogaster*.
curled	Wings curled upward, but not as extreme as *jaunty*. Variable expressivity, with some flies approaching wild-type.

D. sechellia

purple	Eyes reddish-purple. Located on unknown autosome.
cinnabar	Identical to *cinnabar* of *D. melanogaster* (second chromosome).