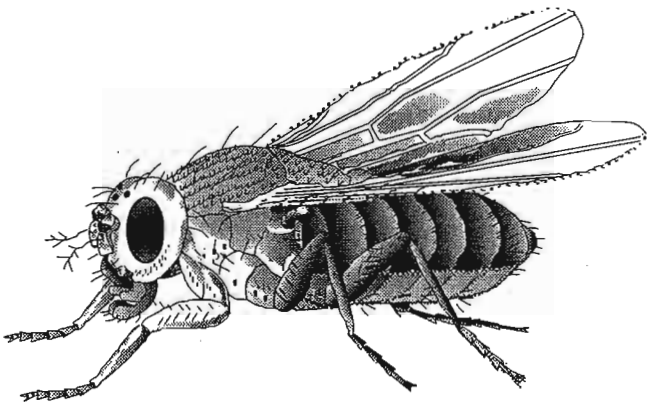
Drosophila Information Service



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Prepared at the
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Preface

Drosophila Information Service was first printed in March, 1934. Material contributed by *Drosophila* workers was arranged by C.B. Bridges and M. Demerec. As noted in its preface, which is reprinted in DIS 75, Drosophila Information Service was undertaken because, "An appreciable share of credit for the fine accomplishments in *Drosophila* genetics is due to the broadmindedness of the original *Drosophila* workers who established the policy of a free exchange of material and information among all actively interested in *Drosophila* research. This policy has proved to be a great stimulus for the use of *Drosophila* material in genetic research and is directly responsible for many important contributions." During the more than 60 years following that first issue, DIS has continued to promote open communication.

The production of DIS 76 could not have been completed without the generous efforts of many people. Stanton Gray, Laurel Jordan, Merl Kardokus, Roxana Serran, April Sholl, and Eric Weaver helped prepare and proof manuscripts; Lou Ann Lansford and Shalia Newby maintained key records; and Coral McCallister advised on artwork. Any errors or omissions in presenting the contributed material are, however, the responsibility of the editor.

We are also grateful to the DIS Advisory Group: Michael Ashburner (Cambridge University), Daniel Hartl (Harvard University), Kathleen Matthews (Indiana University), and R.C. Woodruff (Bowling Green State University). The publication of *Drosophila* Information Service is supported in part by a grant from the National Science Foundation to R.C. Woodruff for the Mid-America *Drosophila melanogaster* Stock Center, Bowling Green, Ohio. We hope that you find a lot of useful information here, and we invite you to let us know what can be done to improve DIS as a source of communication among *Drosophila* researchers.

James N. Thompson, jr.

Jenna J. Hellack

Drosophila Information Service

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List of Contributions

General Announcements

Personal Announcements/Requests

Stock Lists

Mutation Notes: *Drosophila melanogaster*

Mutation Notes: Other Species

Research Notes

Technique Notes

Drosophila Information Newsletter Excerpts, Volumes 16-19

Conference Reports

Mutation Notes - Drosophila melanogaster

NOTE: For other reports of new alleles and information about mutations, please see articles in the Research Reports section.

Report of B.H. Judd.
Laboratory of Genetics, NIEHS, Research Triangle Park, NC 27709.

Df(1)Su(z)J93

This deficiency occurs at very low frequency as an ectopic exchange product from females heterozygous for $y^2\ w^{sp\text{-}2}$ and $z^1\ w^{zm}$ or $z^1\ w^{zl}$. Several strains were recovered from both types of heterozygotes. Original recombinant chromosomes contained the w^{zm} or w^{zl} alleles. These have been replaced by crossingover with w^+ from Oregon-R or with w^{65a25}.

The distal breakpoint of this deficiency is 35 to 60 kb proximal to the *w* locus and extends through *rst* and *vt* but does not include *N*. The deficiency acts as a dominant suppressor of z^1 apparently by acting on the *w* locus in *cis*. It also exhibits *rst, vt,* reduced viability and female sterility. From deletion mapping against various *rst* and *vt* deficiencies, the suppressor of z^1 element is proximal to *rst-vt*.

z^{J91}

This allele occurred spontaneously in $z^1\ w^{65a25}\ spl\ sn^3$. It causes lemon-yellow eye-color in $z^{J91}\ w^+$ males and $z^{J91}\ w^+/z^+\ w^+$ females. It acts as a dominant only in *cis*, however, having no effect on single w^+ genes in transpositions but represses paired copies. Possibly it is a double lesion in the *zeste* gene.

Reference: Judd, B.H., 1995, Genetics (submitted).

Report of E. Nitasaka, T. Yamazaki, and M.M. Green*.
Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812-81, Japan, *Section of Molecular and Cellular Biology, University of California, Davis, California 95616.

New alleles of eye color mutations isolated from natural populations in *Drosophila melanogaster*.

By inbreeding the progeny of wild caught *Drosophila melanogaster* females (Spencer, 1947), many eye color mutations were isolated from seven separate geographic sites distributed among Japan, California, Siberia and Hungary. Each mutant was crossed with tester stocks which have *bw, cd* or *ca*. One of such mutations, *bw* was analyzed molecularly (Nitasaka, *et al.*, 1995). We obtained several dark brown eye color mutants from the Ishigaki-island population, and they seem to be allelic to each other. Thus, each population has population-specific mutations such as *bw* and *cd* in Katsunuma and a dark ruby mutant in Ishigaki-island. Our molecular analysis showed that the *bw* alleles found at Katsunuma seem to have the same origin (Nitasaka, *et al.*, 1995).

Table 1. Eye color mutations derived from natural populations

Locus	Strain #	Phenotype (Eye color)	Origin
brown (bw, 2-104.5)	all strains are described in Nitasaka *et al.* (1995).		
cardinal (cd, 3-75.7)	KN5O	yellowish vermilion like *cd*^{1}	Katsunuma 1988‡
	KN 187	yellowish vermilion like *cd*^{1}	Katsunuma 1988
	KN211	yellowish vermilion like *cd*^{1}	Katsunuma 1988
	KN308	yellowish vermilion like *cd*^{1}	Katsunuma 1989§
	KN309	yellowish vermilion like *cd*^{1}	Katsunuma 1989
claret (ca, 3-100.7)	KN311	ruby like *ca*^{1}	Katsunuma 1989
Unidentified†	KN304	dark brown	Katsunuma 1989
	KN307	light brown	Katsunuma 1989
	IG45	dark ruby & small wings	Ishigaki-island 1990¶
	IG202	dark brown	Ishigaki-island 1990
	IG238	dark brown	Ishigaki-island 1990
	IG248	dark brown	Ishigaki-island 1990
	IG262	dark brown	Ishigaki-island 1990
	HS36	dark brown	Hirosaki 1990‖

†These mutants are not identified, however, they are at least not *bw* or *ca* alleles.
‡150 isofemale lines derived from Katsunuma, Yamanashi prefecture in 1988.
§ 420 isofemale lines derived from Katsunuma, Yamanashi prefecture in 1989.
¶ 220 isofemale lines derived from Ishigaki-island, Okinawa prefecture in 1990.
‖100 isofemale lines derived from Hirosaki, Awomori prefecture in 1990.

References: Nitasaka, E., T. Yamazaki, and M.M. Green 1995, The molecular analysis of brown eye color mutations isolated from geographically discrete populations of *Drosophila melanogaster*. Mol. Gen. Genet. in press; Spencer, W.P., 1947, Adv. Genet. 1: 359-402.

Mutation Notes - Other Species

Report of S.N. Hegde and M.S. Krishna.
Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore-570 006, India.
A spontaneous mutation in *Drosophila bipectinata*.

Drosophila bipectinata belongs to the *bipectinata* complex of the *ananassae* subgroup of the *melanogaster* species group. It is distributed in South East Asia including India. We report a spontaneous autosomal recessive mutation in this species.

Table 1. The normal, reciprocal and test crosses between normal and brown eyed flies in *Drosophila bipectinata*.

Class	Normal cross			Reciprocal cross		
	Number observed (a)	Number expected (mn)	χ^2	Number observed (a)	Number expected (mn)	χ^2
Wild	281	274.5	0.15	219	217.5	0.01
Brown	85	91.5	0.46	71	72.5	0.03
Total	366	366.0	0.61	290	290.0	0.04

P_{value} = Insignificant at 0.05 level.

Test cross results obtained between F_1 females crossed with brown eyed males.

Class	Number observed (a)	Number expected (mn)	χ^2
Wild	75	80	0.31
Brown	85	80	0.31
Total	160	160	0.62

P_{value} = Insignificant at 0.05 level.

A laboratory stock of *D. bipectinata* was established from a single naturally impregnated female collected from Mysore, Karnataka in 1993 and is being maintained in our laboratory. In this stock, we detected several females and males with brown eye colour. These brown-eyed flies were separated and maintained in vials with fresh food and used for making crosses. The crosses between brown-eyed males and females produced brown-eyed offspring. This shows that the culture is pure for brown-eye colour. The pattern of inheritance of brown eye was studied by crossing brown-eyed males with wild type females. Reciprocal crosses were also made using wild type bachelor males and virgin mutant females. In the F_1, all the flies of both the crosses irrespective of sex were red-eyed. This suggests that brown eye colour is due to an autosomal recessive gene. The F_2 progeny gave the wild and mutant flies in a 3:1 ratio (Table 1). These results show that the brown-eyed mutant is inherited as a normal Mendelian trait. The test cross results confirm the monofactorial inheritance of the brown eye colour gene.

Acknowledgments: The authors are grateful to the Professor and Chairman, Department of Studies in Zoology, University of Mysore, for providing facilities.

Report of H.T. Band.
Department of Zoology, Michigan State University, E. Lansing, MI 48824.

Chymomyza amoena stocks currently being maintained:

Country/State	Location	Year	Source	Designation
Switzerland	Zurich	1994	baited	Zurich
	Maggia Valley	1991	emerged, nuts	MV-E
	Maggia Valley	1991	baiting	MV-B
Virginia	Mt. Lake Biological Station	1989	emerged, acorns	ML-acorn
	Rt. 700	1992	emerged, apples	Rt. 700
Michigan	E. Lansing	1992	emerged, apples	E. L.
	Iron Mountain	1993	emerged, apples	I. M.

Report of K. Shimada[1], H. Watabe[2] and N.N. Vinokurov[3].
[1]Institute of Low Temperature Science, Hokkaido University, Sapporo, 060 Japan, [2]Biological Laboratory, Hokkaido University of Education, Sapporo, 002 Japan, and [3]Yakutsk Institute of Biology, Siberian Branch of the Russian Academy of Sciences, Yakutsk, 677891 Russia.
Wing morphology mutants isolated from an east Siberian population of *Chymomyza costata*.

In the course of genetic studies on diapause in wild drosophilids, we isolated several mutants from an east Siberian population of *Chymomyza costata*. Here, we report two wing morphology mutants that are comparable to segment polarity mutants, *costal* and *wingless* in *D. melanogaster*.

Original flies of *C. costata* were collected at Olekminsk in July, 1993 (Watabe, *et al.*, 1994). Their F_1 offspring were used to establish isofemale lines. From 2 of 14 isofemale lines, *costal* (Figure 1a) and *wingless* (Figure 1b) were isolated. The phenotype of *costal* involves mirror-image duplication of wing(s) and/or haltere(s), and/or irregular patterns of thoracic and/or abdominal segment(s). The phenotype of *wingless* involves loss of wing(s) and/or haltere(s). Both mutant genes are recessive and may be located on autosomes. However, these are not mapped yet because of the complete lack of marker genes.

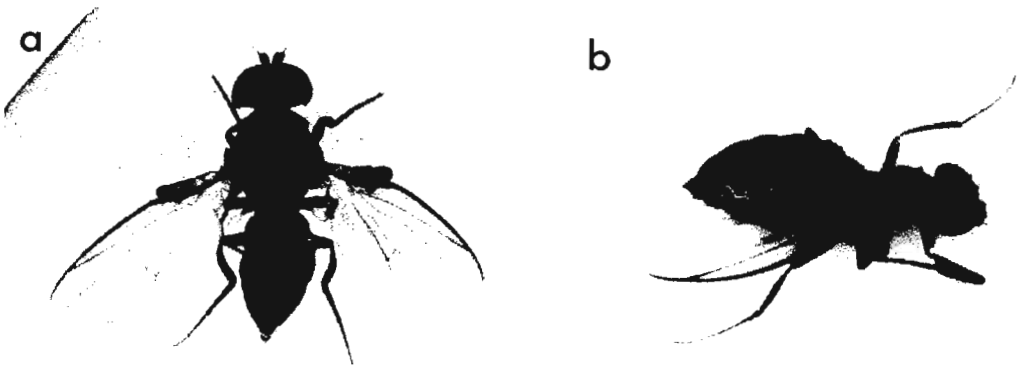

Figure 1. Phenotypes of *costal* (a) and *wingless* (b) isolated from an east Siberian population of *Chymomyza costata*.

In *D. melanogaster*, *costal* and *wingless* mutations were induced by chemical and radioactive mutagens (Sharma, 1973; Whittle, 1974) or P-element insertion (Baker, 1987). No spontaneous mutation has been known (see FlyBase, ID numbers FBgn0000353 and FBgn0004009). So, the presence of *costal* and *wingless* mutants in natural population of *C. costata* arouses much interest.

References: Baker, N.E. 1987, EMBO J. 6:1765-1773; Sharma, R.P. 1973, Dros. Inf. Serv. 50:134; Watabe, H., M.J. Toda and N.N. Vinokurov 1994, Dros. Inf. Serv. 75:145-147; Whittle, J.R.S. 1974, Heredity 33:139.

Report of S. Sugaya and Y. Fuyama.
Department of Biology, Tokyo Metropolitan University, Tokyo, Japan.
plum of *D. simulans* is homologous to *Punch* of *D. melanogaster*.

The *plum* (2-103) mutation in *D. simulans* was discovered by Sturtevant in 1919. He tested various eye color mutants of *D. melanogaster* for homology, without success (Sturtevant, 1929). Here we report that *pm* is homologous to *Punch*(*Pu*; 2-97) in *D. melanogaster*.

The basis for the homology is established by the following observations. First, crosses between *D. simulans* males homozygous for *pm* and *D. melanogaster* females carrying either *Df(2R)AA21* or *Df(2R)PuD17* both of which were known to include the *Pu* locus (57C5-6; O'Donnell, *et al.*, 1989), produced hybrid progeny with purplish eyes. Second, *pm* failed to complement a recessive allele of *Pu*, Pu^{r1}, in the hybrids.

References: Sturtevant, A.H., 1929, Carnegie Inst. Wash. Pub. 399: 1-62; O'Donnell, J., R. Boswell, T. Reynolds and W. Mackay 1989, Genetics 121: 273-280.

Report of M. Pascual and F. Mestres.
Dept. de Genètica, Fac. de Biologia, Universitat de Barcelona, Spain.
Sex comb mutation in *D. subobscura*. *scc*, sex comb curved.

Half of the male offspring of a female captured in Gilroy (California) presented the proximal sex comb curved (Figure 1). This is a spontaneous mutation, which occurred in natural conditions. The number of teeth is seldom reduced. It ranges from 8-10 while the range reported for the proximal sex comb of normal *D. subobscura* males in North America is 9-13 (Beckenbach and Prevosti, 1986). The sex combs of normal males are nearly parallel to the proximal-distal axis of the tarsus (Figure 2) while the proximal sex comb of the *scc* mutant is twisted and curved 45 degrees or more with respect to this axis (Figure 3). The trait presents variable expressivity; two individuals without sex comb on the first tarsal segment were observed out of 460 (Figure 4). The number of teeth of the distal sex comb is normal (10-13), although a few individuals present one or two teeth of their proximal extreme bent.

Figure 1. Proximal sex comb of the mutant.

Figure 2. Sex combs of a normal *D. subobscura* male.

Figure 3. Sex combs of a mutant male.

Figure 4. Almost complete disappearance of the proximal sex comb in a mutant male.

Genetic analysis has shown that the mutation is recessive and located on the A (sexual) chromosome. Hemizygous males and homozygous females have normal viability and fertility.

References: Beckenbach, A., and A. Prevosti 1986, Am. Midl. Nat., 115(1): 10-18.

Report of B.N. Singh, Seema Sisodia, and Rakhee Banerjee.
Department of Zoology, Banaras Hindu University, Varanasi, India.
Spontaneous mutations in *Drosophila bipectinata*.

Drosophila bipectinata which was described by Duda from Darjeeling, India, in 1923, belongs to the *bipectinata* species complex of the *ananassae* subgroup of the *melanogaster* species group (Bock and Wheeler, 1972). This species has wide geographical distribution and is of common occurrence in the Indian subcontinent. It shows incomplete sexual isolation with other species of the *bipectinata* species complex (Bock, 1978; Singh, *et al.*, 1981, 1982; Singh and Chatterjee, 1991a). Evidence for incipient sexual isolation within *D. bipectinata* has also been presented (Singh and Chatterjee, 1991b). It is characterized by genic and chromosomal polymorphisms in its natural populations (Yang, et al., 1972; Bock, 1971; Gupta and Panigrahy, 1990; Singh and Das, 1991; Banerjee and Singh, 1994). Certain aspects of behaviour of this species, such as larval pupation behaviour, oviposition site preference and sexual activity, have also been studied by Singh and coworkers (Singh and Pandey, 1991; Srivastava and Singh, 1993a, b; Pandey and Singh, 1993; Singh and Sisodia, 1994).

A large number of stocks of *D. bipectinata* established from Flies collected from different geographical localities are being maintained in our laboratory. This note describes two spontaneous mutations which were detected in laboratory stocks of *D. bipectinata*.

1. sepia eye colour (*se*): One male with sepia eye colour was detected from TV stock of *D. bipectinata* which was raised from flies collected from Trivandrum, Kerala, in June 1994. A sepia eye male was crossed with a red eye female, and in the F_1 all the flies were red-eyed. Nearly a 3:1 ratio between red-eyed and sepia-eyed flies was found in the F_2 generation which shows that it is a recessive autosomal mutation in *D. bipectinata*. A separate stock of *se* mutants is being maintained in our laboratory.

Figure 1. Cut wing phenotype in *Drosophila bipectinata*.

2. cut wing (*ct*): Four males showing cut wings were detected from AD stock of *D. bipectinata* which was initiated from the flies collected from Alipur Dwar, West Bengal, in July 1993. Cut wing phenotype is shown in Figure 1. Cut wing males were crossed with wild type females. In the F_1, all flies were wild type. In the F_2 generation, a 2:1:1 ratio was observed among wild type females, wild type males, and *ct* males, which shows that it is a recessive sex-linked mutation in *D. bipectinata*. A separate stock of *ct* wing mutants is being maintained in our laboratory.

Acknowledgments: The financial support from the UGC, New Delhi, in the form of a research project to BNS and a JRF to RB is gratefully acknowledged.

References: Banerjee, R., and B.N. Singh 1994, Proc. Zool. Soc. (Calcutta): submitted; Bock, I.R., 1971, Chromosoma 34: 206-229; Bock, I.R., 1978, Aust. J. Biol. Sci. 31: 197-208; Bock, I.R., and M.R. Wheeler 1972, Univ.

Texas Publ. 7213: 1-102; Duda, O., 1923, Ann. Hist. Nat. Mus. Natl. Hung. 20: 24-59; Gupta, J.P., and K.K. Panigrahy 1990, Genetica 82: 45-49; Pandey, M.B., and B.N. Singh 1993, Ind. J. Exp. Biol. 31: 912-917; Singh, B.N., and S. Chatterjee 1991a, Genome 34: 849-852; Singh, B.N., and S. Chatterjee 1991b, Evol. Biol. 5: 105-113; Singh, B.N., and A. Das 1991, Biol. Zent. bl. 110: 157-162; Singh, B.N., Y.N. Dwivedi, and J.P. Gupta 1981, Ind. J. Exp. Biol. 19: 898-900; Singh, B.N., and M. Pandey 1991, Ind. J. Exp. Biol. 29: 926-929; Singh, B.N., O.P. Singh, and J.P. Gupta 1982, Experientia 38: 237-238; Singh, B.N., and S. Sisodia, 1994, Biol. Zent. bl. 114: in press; Srivastava, T., and B.N. Singh 1993a, Ind. J. Exp. Biol. 31: 460-462; Srivastava, T., and B.N. Singh 1993b, Evol. Biol. 7: 193-205; Yang, S.Y., L. Wheeler, and I.R. Bock 1972, Univ. Texas Publ. 7213: 213-227.

Report of Tulika Srivastava and B.N. Singh.
Department of Zoology, Banaras Hindu University, Varanasi, India.
A spontaneous mutation in *Drosophila malerkotliana*.

Drosophila malerkotliana was described by Prasad and Paika from Punjab, India, in 1964. This species belongs to the *bipectinata* complex of the *ananassae* subgroup of the *melanogaster* species group (Bock and Wheeler, 1972) and shows incomplete sexual isolation with other members of the *bipectinata* complex (Bock, 1978; Singh, *et al.*, 1981, 1982; Singh and Chatterjee, 1991). Intraspecific sexual isolation has also been examined within *D. malerkotliana* and two geographic strains showed incipient sexual isolation (Singh and Chatterjee, 1992). Chromosomal polymorphism has also been studied and inversions are known to occur in natural populations of *D. malerkotliana* (Bock, 1971; Rahman and Jha, 1973).

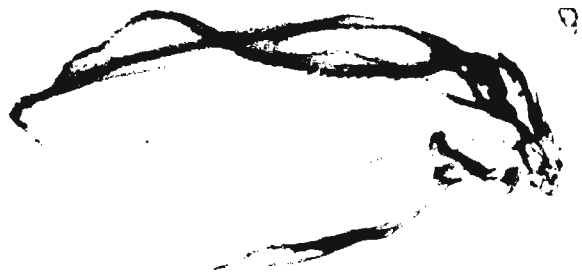

Figure 1. Crumpled wing phenotype in *Drosophila malerkotliana*.

Several stocks of *D. malerkotliana* established from flies collected from different geographic localities are being maintained in our laboratory. A laboratory stock of *D. malerkotliana* was raised from the flies collected from Baripada, Orissa, in October 1987. In this stock, we observed several flies (both females and males) showing shortened and crumpled wings (Figure 1). Legs of these flies were also irregularly shortened. Due to poor viability of flies, the stock could not be maintained, and the mode of inheritance of crumpled wings could not be studied.

References: Bock, I.R., 1971, Chromosoma 34: 206-229; Bock, I.R., 1978, Aust. J. Biol. Sci. 31: 197-208; Bock, I.R., and M.R. Wheeler 1972, Univ. Texas Publ. 7213: 1-102; Prasad, R., and I.J. Paika 1964, Res. Bull. Punjab Univ. 15: 225-252; Rahman, S.M.Z., and A.P. Jha 1973, Egypt. J. Genet. Cytol. 2: 265-273; Singh, B.N., and S. Chatterjee 1991, Genome 34: 849-852; Singh, B.N., and S. Chatterjee 1992, Ind. J. Exp. Biol. 30: 260-263; Singh, B.N., Y.N. Dwivedi, and J.P. Gupta 1981, Ind. J. Exp. Biol. 19: 898-900; Singh, B.N., O.P. Singh, and J.P. Gupta 1982, Experientia 38: 237-238.

Research Notes

Kekić, Vladimir[1], and Gerhard Bächli[2]. [1]Institute of Zoology, Faculty of Science, University of Belgrade, 11000 Beograd, Studentski trg 16, Yugoslavia; [2]Zoologisches Museum, Universität Zürich-Irchel, Switzerland. List of *Drosophila* species recorded in ex-Yugoslavia.

The first information about species of *Drosophila* found on the territory of the former Yugoslavia (SFRJ) was given by Strobl who recorded, between 1883 and 1910, eight species, including the doubtful records of *D. nigricolor* (for references see Bächli and Rocha Pité, 1982). Soos (1945) added *D. limbata*, Coe (1959) eight species more, Aradi (1959) *D. transversa*, Hackman (1965) *D. lundstroemi*, and Bächli and Burla (1985) *D. buzzatii*.

Using modern methods of collecting by baits, the total number of species was brought up to 25 (Kekic and Markinkovic, 1979; Kekić and Bächli, 1981, 1983, 1990, 1991; Bachli and Kekic, 1983 a, b; Kekić et al., 1983, 1984, 1992; Kekić, 1990).

Table 1 presents *Drosophila* ssp. collected on 47 different localities, on different altitudes, from 5 m to 1500 m above sea level, ranging from Adriatic islands and coast in the west to the Pannonia Plain in the east, and from Porec in the North to Ohrid Lake in the south. According to their ecological conditions, we characterize the habitats as "wild", "semi domestic" and "domestic". "Wild" habitats are forests mainly, situated near settlements or tourist spots, visited occasionally or seasonally. "Semi domestic" habitats are locations "constantly" under human influence (orchards, vineyards, surroundings of seaside beaches, city parks, including the Botanical Garden in the center of Belgrade). "Domestic" habitats are balconies, house interiors, spots near barrels (in orchards or vineyards) with fermenting fruit prepared for distillation of the home made brandy slivovitz.

A short comment on three species found in "domestic" habitat is necessary. In the forest cabin, in the National Park "Durmitor", in the bucket with fermenting fruit used for bait, six specimens of *D. obscura* and two specimens of *D. subobscura* were collected; in the similar habitat on Jastrebac Mountain we found one *D. subobscura* and one *D. ambigua*; two specimens of *D. subobscura* were collected on a balcony in Belgrade, shaded by trees.

	Species	Habitats "wild"	"Semi Domestic"	"Domestic"	T
1.	*D. alpina*	245	30	—	275
2.	*D. ambigua*	688	186	1	875
3.	*D. andalusiaca**	9	64	—	73
4.	*D. bifasciata*	16	11	—	27
5.	***D. busckii***	10	18	132	160
6.	*D. buzzatii*		X		X
7.	*D. cameraria**	59	249	—	308
8.	*D. confusa**	1755	10	—	1765
9.	*D. deflexa*	17	3	—	20
10.	*D. fenestrarum**	149	—	—	149
11.	***D. funebris****	106	318	162	586
12.	*D. helvetica*	1551	64	—	1615
13.	*D. histrio**	273	16	—	289
14.	***D. hydei***	50	229	81	360
15.	***D. immigrans***	801	1576	55	2432
16.	*D. kuntzei**	3254	215	—	3469
17.	*D. limbata**	12	5	—	17
18.	*D. littoralis**	42	118	—	160
19.	*D. lundstroemi*				X
20.	***D. melanogaster****	230	16974	12761	29965
21.	*D. nigricolor*				X
22.	*D. nigrosparsa*	20	—	—	20
23.	*D. obscura**	4405	186	7	4598
24.	*D. phalerata**	6856	1469	—	8325
25.	***D. repleta***	—	10	12	22
26.	*D. rufifrons**	1	82	—	83
27.	*D. schmidti*	1	—	—	1
28.	***D. simulans***	11	16154	105	16270
29.	*D. subobscura**	23636	33627	5	57268
30.	*D. subsilvestris*	282	3	—	285
31.	*D. testacea**	3508	1079	—	4587
32.	*D. transversa**	1547	149	—	1696
33.	*D. tristis*	317	22	—	339
34.	*D. trivittata**	1	—	—	1
35.	*D. unimaculata*	131	—	—	131
	Total	49983	72867	13321	136171

*= Fruit flies recorded before our investigations on the territory of the former Yugoslavia.
Bold letters- synantropic (domestic) and cosmopolitan species.

References: Aeadi, M.P., 1959, Lozlem., Ser.Nov., 12:409-426; Bächli, G. and H. Burla 1985, Diptera Drosophilidae. Insecta Helvetica, Fauna, Bd. 7. Zurich; Bächli, G. and M.T. Rocha Pité 1982, Beitr. Int. 32:303-392; Bächli, G. and V. Kekić 1983a, Drugi simpoz. o fauni SR Srbije. Zbornik 111-114; Bächli, G. and V. Kekić 1983b, Biosistematika 9:109-118; Coe, R.L., 1959, Gals. prir. muz. Beograd., Ser. B 12:181-206; Hackman, W., 1965, Notul. ent. 45:61-64; Kekić, V., 1990, Biosistematika 16:81-88; Kekić, V., M. Andjelkovic, and G. Bächli, 1984, Dros. Inf. Serv. 60:128-129; Kekić, V. and G. Bächli 1981, Glas. Republ. Zavoda Zast. Prirode - Prir. muz. Titograd 14:85-88; Kekić, V. and G. Bächli 1983, Drugi simpoz. o fauni SR Srbije. Zbornik 107-110; Kekić, V. and G. Bächli 1990, Glas. zem. muz (PN), NS 29:65-71; Kekić, V. and G. Bächli 1991, Posebna izd. knjiga 24., PN.15:207-220; Kekić, V., R. Hadziselimovic. and Z. Smit 1983, Dros. Inf. Serv. 59:61-62; Kekić, V. and D. Marinkovic 1979, Aquilo Ser. Zool. 20:119-128; Kekić, V.,

N. Popovic, and N.J. Milosevic 1992, Glas. prir. muz. Beograd, B 47:175-186; Soos, A., 1945, Fragm. faunist. hung. 8:18-23.

Hoenigsberg, H.F., and E. Bustos. Instituto de Genética, Universidad de los Andes, Santafé de Bogotá, D.C., Colombia. Results of comparative fitness of *Drosophila starmeri* (Repleta group) from the Guajiran deserts in Maicao in the Colombian Caribbean.

One can simply state that there is an impressive amount of folk literature and of laboratory hearsay surrounding laboratory culturing of *Drosophila*. Ever since the Morgans, Bridges and Muller cultured in the "fly room" at Columbia University the first *Drosophila melanogaster*, the ways of how to culture *Drosophila* in general have varied enormously. *Drosophila's* natural populations have received considerable attention as far as culturing conditions go. For instance Wagner (1944) found important differences in the way *D. mulleri* and *D. aldrichi* utilize eight species of yeast isolated from natural cactus fruits. Buzzati-Traverso (1949), Da Cunha (1951), Lindsay (1958), and Begon (1973) discovered species of yeast that differentially screen various *Drosophila* species. Richardson and Kambysellis (1968) had to use a cactus-supplemented banana food for cultures of the Repleta group of *Drosophila* in order to produce them in the laboratory. Sang (1949a,b,c, 1950, 1956) in his ecological requirements for population growth of *Drosophila* hinted at the considerable amount of genetic variability that supposedly exists in *Drosophila* to be able to cope with such wide nutritional requirements. The same conclusions can be on hand to rationalize the many media that exist among different laboratories (Wheeler, 1967; Offermann and Schmidt, 1936; Lewis, 1942; Lewis, 1960; Li, 1931; Godbole *et al.*, 1971; Sang, 1956; etc.). The same can be said for difficult species when brought to the laboratory. For example, rye or barley malt (Lakovaara, 1969) as supplement to corn meal medium has been successfully used for some of them. Hoenigsberg (1971) has described three new media quite successful for field studies in tropical forests of South America to support growth of many species in the *saltans, willistoni* and *melanogaster* groups. The following is not a complete list of authors that have made important media to cultivate the *quinaria* group (Jacger, 1957) or the *willistoni* group (Hinton *et al.*, 1951; Spieth, 1974) and of course the various media for the Hawaiian Drosophilidae made with a wide variety of substrates like leaves, stems, fruits, flowers and fungi (see Heed, 1968).

Table 1.*

Banana-Agar		Corn-meal	
Water	5000 ml	Water	10,000 ml
Agar	100 g	Corn	300 g
Tegocept	100 ml	Dry Yeast	100 g
Dry Yeast	85 g	Salt	20 g
Bananas	60	Agar	120 g
Propionic Acid	43 ml	Tegocept	160 ml
		Propionic Acid	50 ml

* even when the quantities are halved the result is the same.

Table 2.

D. starmeri	Banana-Agar	Corn-meal
1) Egg produced in mass culture of 10 pairs	1019	436
2) Pupae	60	200
3) Adults	21	184

In the following two tables. I show that unless specified it is profoundly erroneous to claim highest fitness for a population of *Drosophila starmeri* recently brought from the deserts with just egg laying to deal with.

References: Buzzati-Traverso, A., 1949, Dros. Inf. Serv. 25:88; Da Cunha, A.B., 1951, Evolution 5:395-404; Godbole, N.N., R.M. Kothari, and V.G. Vaidya 1971, Dros. Inf. Serv. 46:58-59; Heed, W.B., 1968, Univ. Texas Publ. 6818:387-419; Hinton, T., D.T. Noyes, and J. Ellis 1951, Physiol. Zool. 24:335-353; Hoenigsberg, H.F., 1971, Dros. Inf. Serv,. 47:77; Jaeger, C.P., and E.C. Jaeger 1957, Dros. Inf. Serv. 31:176; Lakovaara, S., 1969, Dros. Inf. Serv. 44:128; Lewis, M.T., 1942, Science 96:282; Lewis, E.B., 1960, Dros. Inf. Serv. 34:117-118; Li, J.C., 1931, Peking Nat. Hist. Bull. 5:29-31; Offermann, C.A., and I.K. Schmidt 1936, Dros. Inf. Serv. 6:54-65; Richardson, R.H., and M.P. Kambysellis 1968, Dros. Inf. Serv. 43:187; Sang, J.H., 1979a, Physiol. Zool. 22:183-202; Sang, J.H., 1949b, Physiol. Zool. 22:202-210; Sang, J.H., 1949c, Physiol. Zool. 22:210-223; Sang, J.H., 1950, Biol. Rev. 25:188-219; Sang, J.H., 1956, J. Exp. Biol. 33:45-72; Spieth, H.T., 1974, Dros. Inf. Serv. 51:146; Wagner, R.P., 1944, Univ. Texas Publ. 4445:109-128; Wheeler, M.R., 1967, *Handbook: The Care and Management of Laboratory Animals*, 3d ed., E. and S. Livingstone Ltd., Edinburgh.

Hoenigsberg, H.F. Instituto de Genética, Universidad de los Andes, Santafé de Bogotá, D.C., Colombia. Collecting *Drosophila* species in natural surroundings.

One of our research notes in this volume presented some useful hints on how to use various baits to collect *Drosophila*. Moreover, the success in field work was shown to depend on different know-hows displayed in high mountain field work and in the lowland and tropical rain forest collection. In this brief but, I hope, equally helpful suggestion, I will show how the attraction of *Drosophila* species depend on the kind of bait used.

Table 1. Drosophila species attracted to figs in the Sierra Nevada of Sta. Marta, Colombia at 1000 m. of altitude. The following species were found flying and surrounding a fig tree with many rotten figs on the ground. This collection was found in the rain forest far away from human habitation.

Species	No. individuals (♀♀ & ♂♂)
D. nigricincta	20
D. willistoni	49
D. paulistorum	21
D. capricorni	12
D. mediostriata	7
D. tripunctata	6
D. crocina	10
D. calloptera	1
D. lumiformis	15

Table 2. Collection done near human dwellings but also around fig trees, no other bait was used.

Species	No. individuals (♀♀ & ♂♂)
D. willistoni group	74
D. capricorni	29
D. fumipennis	115
D. tripunctata	61
D. nigricincta	75
D. angustibucca	28
D. shiri	38
D. unipunctata	5
D. medianotata	3
D. parabocainensis	2
D. angustibucca var. A	2
D. castanea like	15
D. nebulosa	1
D. tripunctata B	2
D. albicans	1
D. paulistorum like	1
D. paulistorum like A	1
D. paulistorum like B	2

Table 3. Collection made on avocadoes left on the ground with *Oenothera* flowers.

Species	No. individuals (♀♀ & ♂♂)
D. tripunctata	8
D. tripunctata like	2
D. fumipennis	2
unknown browns sp.	4

Table 4. Collection of *Drosophila* species using *P. quayada* (*quayaba*) only.

Species	No. individuals (♀♀ & ♂♂)
D. melanogaster	11
D. willistoni	200
D. cardini	1
D. capricorni	2
D. sturtevanti	2
D. subsigmoides	2
D. latifasciaeformis	3

Table 5. Collection done over fallen flowers near human dewellings

Species	No. individuals (♀♀ & ♂♂)
D. tripunctata type 3	8
D. castanea	1
D. unipunctata	1
D. tripunctata type 11	1
D. angustibucca	1

Table 6. Over mangoes.

Species	No. individuals (♀♀ & ♂♂)
D. willistoni group	50
D. capricorni	22
D. melanogaster	23
D. ananassae	1
D. mediostriata	2
D. castanea	2
D. black fascioloides	1
D. gibberosa	2
D. emarginata	12
D. nebulosa	1
D. prosaltans	1

Hoenigsberg, H.F., and D.A. Montaño. Instituto de Genética, Universidad de los Andes, Santafé de Bogotá, D.C., Colombia. Patterns of gene variation in *Drosophila starmeri* from the northern deserts of Colombia.

While studying population genetic structures of the *martensis* cluster we have endeavored to illustrate the different genetic patterns that emerge in populations of some of them in the northern deserts of Colombia, (Hoenigsberg *et al.*, 1990, 1991). The ecological setting and the nutritional specificities of *D. starmeri* in those northern deserts of Colombia characterize certain gene-protein systems. It is very important to understand the biochemical genetics of enzymes. To do population-biochemical-genetical analysis of their presence in nature it is advisable to distinguish between isozymes and allozyme electrophoretic variation particularly when their frequencies are used comparatively to discover their diagnostic value. Such is the case that we want to discuss in this brief note for beta-esterase, peptidase-1 and peptidase-2, aldehyde oxidase. Isozymes in its broadest operational sense are electrophoretically and/or chromatographically separable enzymes which catalyse the same reaction but they are *not* due to allelic variants of a structural gene. In fact, isozyme variation has several possible origins such as histochemical and developmental regulation (O'Brien and Macintyre, 1978). No doubt that the underlying molecular differences between multiple enzyme forms are the same from species to species, but there are also basic ways by which multiple forms are produced *in vivo*. Thus, isozymes frequently have characteristic biochemical properties, tissue distributions and developmental profiles. Although, we realize that the factors that contribute to isozyme production reflect various genetic and developmental controls which interact, in

various and as yet unknown ways, to form a functional organism, we will only be directing our interest in characterizing the population genetic structure through allelic frequencies of true allozymes. We will remember that allozymes are electrophoretic variants which are allelic products of the same genetic locus (Prakash, *et al.*, 1969). We will not distinguish isozyme variation now, for to do that, we would have to do genetic analysis.

In the Table that follows we show that there is a surprising allozymic polymorphism. The great amount of allelic variation within demes (Camarones) and between populations of the northern Guajiran deserts of Colombia reveal very large differences in expected (Hardy-Weinberg) and observed heterozygotes, very low average in the number of alleles per locus, and moderately large fixation indices (F_{IS}).

Table 1. Males of *D. starmeri* sampled in Riohacha (Rio), Camarones (Ca), Malcao (Mai), Uribia (Ur), Barrancas (Ba), Numbers 1, 2, 3, 4 refer to different baits supporting different samples in each population. All baits had the same ingredients i.e, bananas, beer, dry yeast, the night before sweeping. Collections were done and recorded after four consecutive sweepings in the early morning (6:30 am) and late afternoons (5:30-6:30 pm).

	♀♀ *					♂♂ *				
	1	2	3	4	5	1	2	3	4	5
Rio =	30	13	23	30	29	78	24	30	47	40
Ca =	75	217	20	108	—	92	279	61	175	—
Mai =	168	8	5	15	—	153	12	24	34	—
Ur =	46	43	97	113	—	44	69	91	127	—
Ba =	33	11	37	—	—	43	17	52	—	—

* Several collections were poor as far as sample size.

Table 2. Allelic frequencies of *D. starmeri* between populations. Ho = observed heterozygotes, He = expected(H.W.), Fis = fixation index, ne = average No. alleles per locus.

	Uribia N = 167	Camarone N = 262	Riohacha N = 86
β- Esterase			
Alleles			
99	0.015	0.038	0.032
100	0.173	0.542	0.677
101	0.037	0.248	0.108
102	0.775	0.166	0.183
103	0.000	0.006	0.000
Ho	0.043	0.214	0.304
He	0.368	0.616	0.495
Fis	0.883	0.653	0.386
ne	1.045	1.272	1.437
Peptidase-1			
Alleles			
100	0.009	0.002	0.000
101	0.041	0.415	0.233
102	0.950	0.583	0.767
Ho	0.063	0.120	0.067
He	0.096	0.488	0.358
Fis	0.344	0.754	0.813
ne	1.067	1.126	1.072
Peptidase-2			
Alleles			
100	0.000	0.016	0.000
101	0.095	0.430	0.086
102	0.905	0.528	0.914
103	0.000	0.026	0.000
Ho	0.086	0.158	0.143
He	0.172	0.536	0.157
Fis	0.500	0.705	0.089
ne	1.094	1.188	1.167
Aldehyde-oxidase			
Alleles			
100	0.671	0.769	0.700
101	0.329	0.231	0.300
Ho	0.263	0.269	0.250
He	0.441	0.356	0.420
Fis	0.404	0.244	0.405
ne	1.357	1.368	1.333

Table 3. Allelic frequencies of *D. starmeri* within subpopulation Camarones.

	Ca - 1 N = 76	Ca - 2 N = 82	Ca - 3 N = 67	Ca - 4 N = 37
β- Esterase				
Alleles				
99	0.053	0.018	0.007	0.095
100	0.599	0.482	0.545	0.392
101	0.289	0.214	0.336	0.297
102	0.059	0.268	0.112	0.216
103	0.000	0.018	0.000	0.000
Ho	0.355	0.110	0.343	0.146
He	0.551	0.633	0.578	0.627
Fis	0.356	0.826	0.407	0.767
ne	1.555	1.136	1.522	1.171
Peptidase-1				
Alleles				
100	0.000	0.130	0.000	0.000
101	0.276	0.390	0.434	0.447
102	0.724	0.480	0.566	0.553
Ho	0.000	0.040	0.073	0.074
He	0.399	0.600	0.491	0.491
Fis	1.000	0.933	0.851	0.849
ne	1.000	1.042	1.078	1.079
Peptidase-2				
Alleles				
100	0.000	0.068	0.000	0.000
101	0.537	0.370	0.446	0.423
102	0.426	0.519	0.541	0.563
103	0.037	0.043	0.013	0.014
Ho	0.111	0.148	0.189	0.158
He	0.529	0.587	0.509	0.536
Fis	0.790	0.748	0.629	0.705
ne	1.125	1.174	1.013	1.014

Conclusions: These results of pure allozymic polymorphism in structural loci in the northern Colombian deserts suggest (following infinite allele model) a tendency to develop heterozygosity in most loci. However, for more conclusive evidence on the organismic role of beta-esterase and aldehyde oxidase within the subpopulations (= demes) of Camarones, a study of isozyme variation should be forthcoming for notwithstanding inbreeding several alleles are maintained in these loci.

References: Hoenigsberg, H.F., D.A. Montaño, and M. Sanz de la Rosa 1990, Evol. Biol. 4:141-178; Hoenigsberg, H.F., D.A. Montaño, A. Moreno, and M. Sanz de la Rosa 1991, Evol. Biol. 5:285-319; O'Brien, S.J., and R.J. Macintyre 1978, In: *The Genetics and Biology of Drosophila*, vol. 2, pp. 395-551; Prakash, S., R.C. Lewontin and J.L. Hubby 1969, Genetics 61:841-858.

Narise, Sumiko, and Hiroko Tominaga. Laboratory of Biochemistry, Department of Chemistry, Faculty of Science, Josai University, Sakado, Saitama, 350-02 Japan. Purification and partial amino acid sequencing of alpha-esterase from *Drosophila virilis*.

Two esterase loci, *alpha-* and *beta-Est*, have been usually found in *Drosophila* species. Alpha-Esterase encoded by the former locus hydrolyzes to the same extent both alpha- and beta-naphthyl acetates, while beta-esterase by the latter prefers the beta-ester to the alpha-ester. Recently, the *Est-6* gene of *D. melanogaster* (Oakeshott *et al.*, 1987) and *Est-5* of *D. pseudoobscura* (Brady *et al.*, 1990) were cloned and sequenced. Both loci specify beta-esterase protein. The cloning of the *alpha-Est* gene has not been reported so far. This report describes purification of alpha-esterase from *D. virilis* and its partial amino acid sequences. The strain used was homozygous for the *alpha-Est*^{5} allele. Purification procedures included the following consecutive steps; ammonium sulfate precipitation, column chromatography of DE-52, gel filtration by Sephadex G-150 and hydroxyapatite gel chromatography. Preparative electrophoresis using Biophoresis III, ATTO Corporation, Japan was conducted for the final step. Purity of the final preparation was monitored by PAGE (Figure 1). As shown in Figure 1, while the sample before Biophoresis contained two active bands (A and B) for alpha-naphthyl acetate and other inactive, faint protein bands, several fractions from Biophoresis contained only each of these bands. A fraction having A or B band was subjected to SDS-PAGE to estimate the molecular weight of the subunit. The results indicated that the molecular weight of A band was about 55,000 and that of B band, 23,000. Early studies showed that alpha-esterase in a monomer and its native molecular weight is about 50,000 (Narise, 1973; Sasaki and Narise, 1978). On the basis of these, the A protein was estimated to be *Est-alpha*^{5}. The amino terminus of *Est-alpha*^{5} protein was blocked. Carboxymethylated *Est-alpha*^{5} was cleaved by cyanogen bromide treatment, and the fragments were separated on HPLC reverse-phase column. Two of the several peaks were applied to a sequence analyzer (Shimadzu, PQS-1). The one was assigned to be the N-terminal fragment. Amino acid sequence of the other was as follows, Met-Val-Tyr-Gln-Trp-Arg-Gln-Leu-Leu-Asp-Asp-Tyr-Lys-Arg-Ile-.

The amino acid sequences of the other fragments will be determined and used for making a probe for DNA sequencing of the *alpha-Est* gene. It was troublesome for purification of alpha-esterase from *D. virilis* to exclude contaminated proteins having very similar chemical structure to the alpha-esterase. Moreover, the esterase content was very low. A preparative electrophoresis by Biophoresis III proved to be useful because dilution of sample did not occur, unlike an ordinary preparative electrophoresis.

References: Brady, J.P. *et al.*, 1990, Mol. Biol. Evol. 7:525-546; Narise, S., 1973, Japn. J. Genet. 48:119-132; Oakeshott, J.G., *et al.*, 1987, Proc. Natl. Acad. Sci. 84:3359-3363; Saski, M. and S. Narise 1978, Dros. Inf. Serv. 53:123-124.

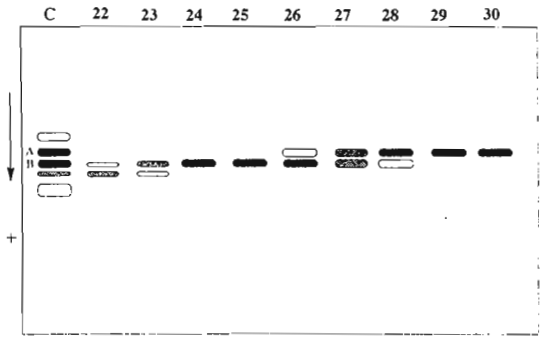

Figure 1. Schematic pattern of PAGE of Est-alpha^5 in fractions after preparative electrophoresis. C indicates sample before Biophoresis and numerals, fraction numbers. Intensity of protein bands decreases in order of black, shaded and white.

Mathew, S., and B.N. Singh. Zoology Department, Banaras Hindu University, Varanasi, India. Chromosomal variability in a base population employed in directional and stabilizing selection for sternopleural bristle number in *Drosophila ananassae*.

In order to test the effect of directional and stabilizing selection on the number of sternopleural bristles in *Drosophila ananassae*, a base population was constructed. Five different mass culture wild stocks originating from different localities in India (Jammu, Varanasi, Baripada, Madras and Kanniyakumari) were crossed with each other, and hybrids were mixed to establish the base population. It was maintained for five generations before starting the selection experiments. Directional and stabilizing selection experiments for sternopleural bristle number have been initiated by using this stock.

Since chromosome inversions often persist in laboratory stocks of *D. ananassae* (Singh, 1982a), chromosomal analysis of the base population was made by squashing larvae taken randomly from the culture bottles. Temporary squash preparations of the polytene chromosomes were made by using lacto-aceto-orcein stain, and polytene chromosomes of 100 larvae were examined.

Table 1. Observed and expected numbers of different karyotypes and frequency (in percent) of different gene arrangements in 2L (AL), 2R (ZE) and 3L (DE) of *D. ananassae*.

Chromosome arm	Total no. of larvae analysed		karyotypes			Gene arrangements		Total no. of chromosomes examined
			ST/ST	ST/AL	AL/AL	ST	AL	
2L	100	obs.	24	63	13	55.5	44.5	200
		expt.	30.8	49.4	19.8			
			$\chi^2 = 7.59$		df = 1	P < 0.01*		
			ST/ST	ST/ZE	DE/ZE	ST	ZE	
2R		obs.	63	37	0	81.5	18.5	
		expt.	66.42	30.2	3.4			
			$\chi^2 = 5.15$		df = 1	P < 0.025*		
			ST/ST	ST/DE	DE/DE	ST	DE	
3L		obs.	75	23	2	86.5	13.5	
		expt.	74.8	23.4	1.8			
			$\chi^2 = 0.023$		df = 1	P > 0.05		

* = Significant

Table 2. Observed and expected numbers of different intra- and inter-chromosomal combinations.

		2L			
		ST/ST	ST/AL	AL/AL	Total
2R	ST/ST	23 (15.12)	36 (39.69)	4 (8.19)	63
	ST/ZE	1 (8.88)	27 (23.31)	9 (4.81)	37
	Total	24	63	13	
		$\chi^2 = 17.84$	df = 2	P < 0.001*	
		2L			
		ST/ST	ST/AL	AL/AL	Total
3L	ST/ST	19 (18.00)	47 (47.25)	9 (9.75)	75
	ST/DE	4 (5.52)	16 (14.49)	3 (2.99)	23
	DE/DE	1 (0.48)	0 (1.26)	1 (0.26)	2
	Total	24	63	13	
		$\chi^2 = 4.62$	df = 4	P > 0.05	
		3L			
		ST/ST	ST/DE	DE/DE	Total
2R	ST/ST	47 (47.25)	14 (14.49)	2 (1.26)	63
	ST/ZE	28 (27.75)	9 (8.51)	0 (0.74)	37
	Total	75	23	2	
		$\chi^2 = 1.22$	df = 2	P > 0.05	

* = Significant. Expected numbers are given in parentheses.

Chromosomal analysis of the base population revealed the presence of three inversions viz., AL (alpha) in 2L, ZE (zeta) in 2R, and DE (delta) in 3L. The AL and DE are the cosmopolitan inversions in *D. ananassae* (Singh, 1983a). The ZE (2R) inversion was described for the first time by Singh, A.K. and B.N. Singh (1991) from a laboratory stock established from a female collected from Madurai, Tamil Nadu, India. The observed and expected (via Hardy-Weinberg proportions) numbers of different karyotypes in 2L, 2R and 3L are given in Table 1. The deviation from Hardy-Weinberg expectation is significant for 2L and 2R karyotypes. However, there is no significant deviation from Hardy-Weinberg expectation for 3L karyotypes. The mean number of heterozygous inversions per individual is 1.23.

In *D. ananassae*, data on intra- and interchromosomal associations have been reported earlier (Singh, 1982b, 1983b, 1984; Singh, B.N. and A.K. Singh, 1990, 1991; Singh, A.K. and B.N. Singh, 1989). It has been found that interchromosomal associations occur randomly which suggests that interchromosomal interaction does not occur in *D. ananassae*. However, linked inversions often show non-random association (linkage disequilibrium) in laboratory stocks. During the analysis of the base population, three inversions, two of the second chromosome and one of the third chromosome have been found. From the number of different karyotypes in 2L, 3L and 2R, the data on various intra- and interchromosomal associations have been obtained. The expected numbers of different intra- and interchromosomal combinations have been calculated from the marginal totals of an RxC contingency table under the assumption of random combinations in the base population of *D. ananassae* are presented in Table 2. Due to the absence of ZE/ZE homozygotes, there are only six combinations between 2L and 2R karyotypes. The X^2 value (17.84) shows highly significant deviation from expectation which indicates non-random association (linkage disequilibrium) between AL and ZE inversions of the second chromosome of *D. ananassae*. However, various interchromosomal associations (between 2L and 3L; and 2R and 3L) occur randomly as the deviation from randomness is not significant. Thus unlinked inversions in *D. ananassae* are associated randomly which provides no evidence for interchromosomal interactions in *D. ananassae*. This is in agreement with the previous findings of Singh and others.

In certain cases, the correlation between inversion polymorphism and morphometric variations has been shown (see Singh and Das, 1991). Selection experiments using the base population are in progress, and after completion of the

experiments, different lines will be examined for chromosome inversions in order to test whether there is any relationship between inversion polymorphism and the number of sternopleural bristles in *D. ananassae*.

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Zivanović, G., M. Milanović and M. Andjelković. Institute for Biological Research "Sinisa Stankovic", 11060 Belgrade, Yugoslavia. Inversion polymorphism in *Drosophila subobscura* from Jastrebac Mountain and localization of the gene arrangement O_{3+4+1} distribution.

Similar to some other species of the genus *Drosophila, D. subobscura* species is characterized by a high degree of inversion polymorphism which was observed in most of the populations analyzed so far (Krimbas, 1992). The existence of such an extremely high degree of this kind of polymorphism raises the question on the level of its adaptive significance. The phenomenon of cline variability and frequency of the gene arrangements dependent on latitude was taken as the evidence for the adaptive significance of the inversion polymorphism in *D. subobscura*. This phenomenon was registered earlier in European populations (Prevosti, 1964), and after the colonization of the American continents by this species in the American populations, as well (Prevosti *et al.*, 1987). In addition to some complex gene arrangements, this phenomenon is the most expressed in standard gene arrangements at all chromosomes. However, some gene arrangements do not express a gradual distribution of the North-South type. Gene arrangements characterized by a localized kind of distribution were found at all five acrocentric chromosomes. The highest number of inversions and gene arrangements characterizing this type of distribution were found to occur in the O chromosome, and the regions involved in it were more seldom found in the northern than in the southern parts of Europe (Krimbas, 1992). In the region of the Balkan Peninsula representing the state of Serbia, analysis of the inversion polymorphism was performed so far only on the population of *D. subobscura* from Fruska Gora Mt. (Andjelkovic and Sperlich, 1973). The data obtained revealed a localized distribution of the gene arrangement O_{3+4+2} (34%) in this area.

The aim of the present work was to get a better insight into localization of distribution of individual gene arrangements of *D. subobscura* from two ecologically different habitats (beach and oak forest).

Wild males from "isofemale" lines were crossed with virgin females of a standard "*Kusnacht*" line which is homozygous for the standard gene arrangements at all chromosomes. Polytene chromosomes were prepared by squash technique. Eight larvae from the progeny of each male were analyzed. The gene arrangements were identified using a chromosome map of Kunze-Muhli and Muller (1958).

In Table 1, the frequencies of the gene arrangements of the O chromosome are presented. They are characterized by a localized distribution in the European part of *D. subobscura* (O_{3+4+1}, O_{3+4+2}) range of distribution, including the two analyzed populations from Jastrebac Mt. Gene arrangement O_{3+4+2} was recorded with very low frequency in the two analyzed populations, and its frequency was 2% in individuals from both habitats. However, the results on the gene arrangement O_{3+4+1} showed its extremely increased frequency in both Jastrebac Mt. populations in comparison with other European populations (Table 1). Frequencies of this gene arrangement were 24% and 27% for the "beech" and "oak" habitats, respectively, while it was not observed in the population from the nearest locality, Fruska Gora Mt. In other European localities, the frequency of this gene arrangement varied between 0.54% and 6.40%. Average frequency of the gene arrangement O_{3+4+1} in some ten analyzed populations from Greece was 23% (Krimbas, 1965). Based on a comparative analysis of the frequency of this gene arrangement in our populations from Jastrebac Mt. and the others shown in Table 1, it can be concluded that the populations from Jastrebac Mt. have the highest frequency,

Table 1. Frequency of the gene arrangements O_{3+4+1} and O_{3+4+2} in several European populations of *Drosophila subobscura*.

Locality	GENE ARRANGEMENTS (%) O_{3+4+1}	O_{3+4+2}
Edinburgh*	/	/
The Netherlands*	/	/
Zurich ****	0.54	0.78
Vienna *	1.60	/
Central Italy *	6.40	8.90
South Italy *	3.00	7.90
Spain ***	0.95	5.71
Fruška gora Mt. **	/	34.00
JASTREBAC		
a) beech forest	**24.00**	**2.00**
b) oak forest	**27.00**	**2.00**
Greece*	23.00	3.00
Crete*	6.00	/
Israel*	/	91.00

The data obtained throughout the present study are given in bold characters. * Krimbas (1965); ** Andjelkovic and Sperlich (1973); *** De Frutos and Prevosti (1984); **** Gosteli (1990).

and the maximum frequency of this gene arrangement with localized distribution occurs just in this part of Balkan Peninsula.

Explanations for the localized distribution of the gene arrangements of O, as well as the other chromosomes, were based on historical factors. According to Krimbas and Loukas (1980), localized distribution of the gene arrangements can be interpreted by an increased action of drift as well.

However, the discovery of a gradual distribution of the gene arrangements in North American populations of *D. subobscura* (Prevosti *et al.*, 1987) along different ecological, and first of all temperature gradients, as well as a high correlation level of a complex of ecological factors and variation of the gene arrangements frequency in *D. subobscura* (Menozzi and Krimbas, 1992) do not support previously reported assertions.

Based on the fact of high efficient number of individuals checked during some ten years at "beech" habitat of Jastrebac Mt. (to be published), the localized distribution of the gene arrangement O_{3+4+1} is impossible to explain by the influence of genetic drift. Taking into account the data quoted, it could be supposed that the temperature as an ecological factor may have much higher influence on the localized distribution of the gene arrangement O_{3+4+1}.

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Kaiser, M. Zoologisches Institut, Universitaet Basel, Rheinsprung 9, CH-4051, Basel, Switzerland. Influence of anaesthesia by carbon dioxide on hatching time and weight at eclosion in *Drosophila melanogaster*.

Introduction: An ideal anesthetic should have no side effects. Unfortunately, the anaesthetics (diethylether, triethylamine, chilling and CO_2) commonly used with flies do often influence behavior and various fitness components (Ashburner and Thompson, 1978; Dijken, *et al.*, 1977; Gimelfarb and Willis, 1988; Gilbert, 1981; Smith and Huey, 1991). For example, ether shortens viability and reproductive capacity. CO_2 (reviewed in Nicolas and Sillans, 1989) markedly reduces survival and fertility of adults if administered within 3 hours of eclosion but is apparently non-toxic if it is used 5 hours after eclosion or later.

Stearns, *et al.* (1987) developed a machine to fractionate flies by their age at eclosion. Pupae are put into the machine about one day before the first flies should eclose, and then CO_2 is sprayed into each vial for 30 seconds at timed intervals (*e.g.*, every 6 h) until all the flies have eclosed. The pupae (especially late-eclosing ones) are, therefore, repeatedly exposed to CO_2.

Because CO_2 has diverse effects on physiology (Nicolas and Sillans, 1989), repeated exposure of pupae to CO_2 might influence development times or body mass of flies. Consequently, I designed an experiment to test for these effects. Further I determined whether any such effects could be reduced by flushing the vials with fresh air immediately after CO_2 exposure.

Materials and Methods: I used heterozygous flies produced by crossing two homozygous lines of *D. melanogaster*. Fifty pairs were allowed to lay eggs for four hours. The following day, sets of 12 first-instar larvae were collected and put in plastic vials (50 x 18 mm, N = 33), each with 2 ml medium, and kept at 25°C. When all larvae had pupated, and about one day before eclosion, the vials were equally divided into three groups. The 11 vials from group A (CO_2 exposure, no flushing) were placed into the machine at randomised places, and CO_2 was sprayed into the vials for 30 seconds every 4 hours. Vials for group B, (CO_2 exposure, air flushing) were treated as above, but fresh air was sprayed into each vial for 5 seconds immediately following each CO_2 exposure, to flush the vials of any residual CO_2. Group C served as control: all hatched flies were collected manually (*i.e.*, without exposure to CO_2 or fresh air) every 4 hours. All vials were kept at 25°C. After collection the flies were killed by freezing, dried at 50°C for 3 hours and weighed to 0.01 mg.

Results:

Age at eclosion: CO_2 anaesthesia appeared to prolong development time for both females and males (Figure 1). A Kruskal-Wallis test indicated significant differences between the groups at the $p < 0.1$ level (females $p = 0.078$ and males $p = 0.058$) (Table 1). Similar results were obtained from an ANOVA and two survival analyses (Lifetest and Cox-regression in SAS). Therefore, I calculated a combined probability from independent tests of significance (Sokal and Rohlf, 1969). I found $p < 0.025$ for both females and males. This means that CO_2 anaesthesia delayed eclosion.

Dry weight at eclosion: Females and males from groups B and C were significantly heavier than flies from group A (Table 2), but group B and C did not differ significantly.

Table 1. Mean time to eclosion in days at 25° C. The deviation is one standard error of the mean.

	Females	Males
Hatching machine	9.68 ± 0.05	9.76 ± 0.06
with CO_2 and fresh air	9.52 ± 0.05	9.60 ± 0.06
without CO_2	9.57 ± 0.06	9.68 ± 0.05
p - value Kruskal - Wallis	0.0583	0.0748
combined p - value	< 0.025	< 0.025

Table 2. Mean dry body weight (mg) at eclosion at 25°C. The deviation is one standard error of the mean. Groups marked with the same letter did not differ significantly (SNK).

	Females	Males
Hatching machine	0.28 ± 0.004 B	0.21 ± 0.003B
with CO_2 and fresh air	0.30 ± 0.004A	0.23 ± 0.004A
without CO_2	0.29 ± 0.005A	0.22 ± 0.004A
p - value ANOVA	0.0061	0.0001

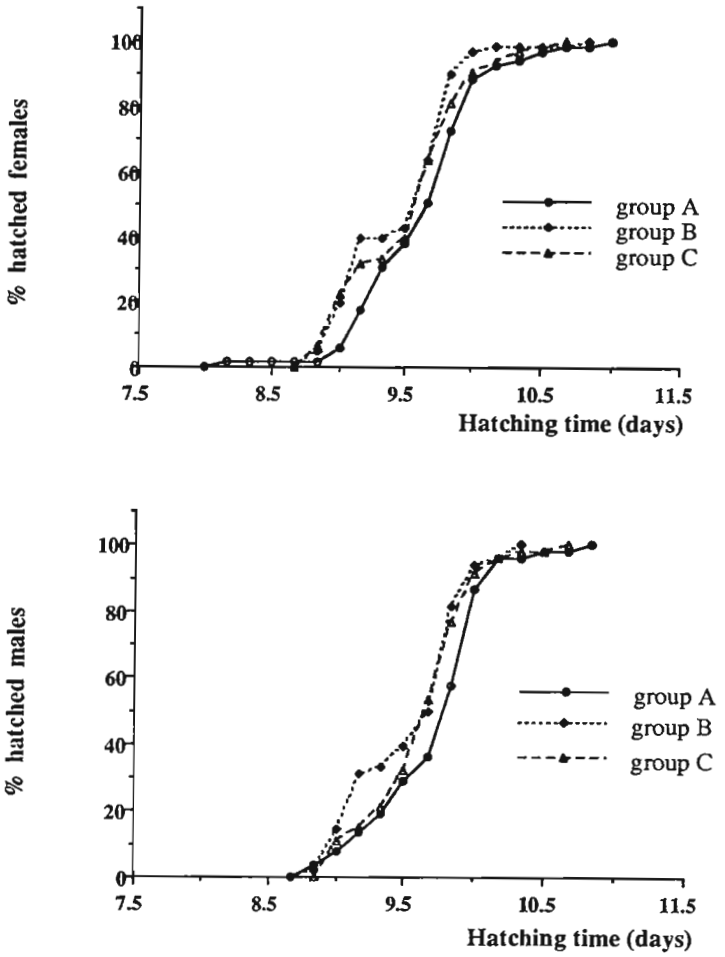

Figure 1. Eclosion curve for (A) females and (B) males at 25°C.

Discussion: CO_2 anaesthesia tends to prolong hatching time and significantly reduces dry mass at eclosion. These results agree with Nicolas and Sillans (1989) in their review. Interestingly, this effect occurred despite the fact that CO_2 exposure was restricted to the last day prior to eclosion, when pupae and newly eclosed flies from groups A and B were exposed to CO_2. During this time carbon dioxide had an unknown physiological influence, making the flies lighter and retarding eclosion.

Both results for group C lay unexpectedly between those for groups A and B, suggesting poor air circulation in the vials of group C. In this group the flies were collected every 4 hours without spraying either CO_2 or fresh air into the vials.

Conclusion: Exposure to CO_2 during late pupation influences both weight at eclosion and hatching time. However, flushing the vials immediately after CO_2 exposure reduces these effects. Consequently, if pupae are repeatedly exposed to CO_2 (either in the hatching machine, or simply to anaesthetize newly-eclosed adults in a vial), vials should be flushed with fresh air immediately following CO_2 exposure. The machine described in Stearns *et al.* (1987) has been reconstructed, so that CO_2 is now flushed from the vials after collecting newly-eclosed flies.

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Sidorenko, V.S. Institute of Biology and Pedology, Far Eastern Division of the Russian Academy of Science, Vladivostok-22, Russia. Drosophilidae of Saghalien, Russia.

Russian Far East (especially in the southern part) has a relatively rich but rather little known fauna of Drosophilidae. Several interesting areas of Russian Far East still wait exploration. One such area is Saghalien I. Up to present, only 8 species were recorded from this island, namely *Lordiphosa collinella, L. magnipectinata, L. stackelbergi, Scaptomyza okadai, Sc. consimilis, Drosophila testacea, D. nigromaculata,* and *D. virilis* (Kikkawa and Peng, 1938; Sidorenko, 1992, in press).

Mr. S. Kholin, during his stay in Saghalien, collected a small number of Drosophilidae fro Kholmsk (25, 08, 1992) and Pionery, 22 km N Kholmsk (27 and 30, 08. 1992). The results of determination are reported here.

1. *Drosophila (Dorsilopha) busckii* Coquillett, 1901
   25.08 - 10 males, 9 females; 27.08 - 1 female; 30.08 - 1 male, 1 female
2. *Drosophila (Drosophila) funebris* (Fabricius, 1787)
   27.08 - 1 male
3. *Drosophila (D.) histrio* Meigen, 1830
   27.08 - 3 females; 30.08 - 5 females
4. *Drosophila (D.) nigromaculata* Kikkawa and Peng, 1938
   30.08 - 1 female
5. *Drosophila (D.) testacea* Roser, 1840
   27.08 - 1 female; 30.08 - 2 males, 2 females

Acknowledgment: I thank Mr. Sergey Kholin for providing the *Drosophila* collection from Saghalien.

References: Peng, H., and F.T. Kikkawa 1938, Jpn. J. Zool. 7:507-552; Sidorenko, V., 1992, Entomofauna, in press.

Sidorenko, V.S. Institute of Biology and Pedology, Vladivostok-22, Russia. Some early spring Drosophilidae from Ussuri-region, Russia.

The present report deals with the results of my collections of early spring drosophilids carried out in the vicinity of Ussurian Nature Reservation, Ussuri-region, Russia. Flies were caught in mixed coniferous-broad-leaved forest on tree sap from 11th to 19th April, 1990.

Subfamily Steganinae

Amiota (Phortica) conifera Okada, 1977
male, 3 females; April 19
Gitona distigma Meigen, 1830
6 males, 8 females; April 11-13, 15, 18-19
Leucophenga (Leucophenga) quadripunctata (Meijere, 1908)
female; April 18
Leucophenga (Neoleucophenga) quinquemaculipennis Okada, 1956
2 females; April 18-19

Subfamily Drosophilinae

Drosophila (Drosophila) funebris (Fabricius, 1787)
2 females; April 12-13
Drosophila (Drosophila) moriwakii Okada et Kurokawa, 1957
7 males, 2 females; April 18-19
Drosophila (Sophophora) bifasciata Pomini, 1940
98 males, 57 females; April 11-13, 17-19
Hirtodrosophila trivittata (Strobl, 1893)
female; April 13
Hirtodrosophila ussurica (Duda, 1935)
female; April 13
Lordiphosa collinella (Okada, 1968)
female; April 17
*Scaptodrosophila sp.**
3 males, 6 males; April 19

* species yet to be identified

Bennett, H.J.[1,2], **L.S. Jenkinson**[1], **and A.J. Davis**[1]. [1]Drosophila Unit, Pure and Applied Biology, and [2]Department of Genetics, The University, Leeds, West Yorkshire, LS2 9JT UK. *Drosophila mercatorum* (Patterson & Wheeler 1942) (Diptera: Drosophilidae): first record for the British Isles.

On 19 October 1994 HJB caught an adult *Drosophila* on banana-pulp bait that had been exposed on 30 September 1994 at Hillingdon [OS grid reference TQ 083 843, 51 33' N 0 26' W], in the outer suburbs of west London. The baits were exposed in a conservatory, open to the outside, adjacent to a garden mostly of lawn with a few flower beds but also containing fruit-bearing trees and soft-fruit bushes. The weather at the time of bait exposure was changeable but warm.

The individual, a female, ran to *Drosophila repleta* Woolaston 1858 in the key of Baechli and Burla (1985) but differed in being yellower and lacking the darkened distal costal segment. Closer examination using Beuk (1993)

indicated that the individual might be *D. mercatorum* and this identification was later confirmed (P. Beuk and B. Pitkin, pers. comm.). *Drosophila funebris, D. melanogaster, D. phalerata* and *D. subobscura* were caught on the same bait at the same time as *D. mercatorum*.

This record is the first for *D. mercatorum* in the United Kingdom and for the British Isles. The species has only once before been recorded from north-western Europe, in the Netherlands at a latitude similar to that of Hillingdon (Kraaijeveld, 1992). Currently, therefore, three *repleta*-group species, *D. hydei, D. mercatorum* and *D. repleta*, are known from the British Isles.

Drosophila mercatorum can develop on a wide range of decaying and fermenting plant material and, in the Netherlands, was reared out of fermenting fruit and maintained on a cereal-based yeast-rich culture medium (A.R. Kraaijeveld, pers. comm.). It has not been found in the north temperate zone and, apart from the specimens mentioned here, is restricted in western Europe to the western Mediterranean basin (Baechli and Rocha Pite 1981). It is also found in Hawaii and eastern Europe, but is predominantly an American species (Wheeler, 1981a), probably originating in the neotropics (Wheeler, 1981b). The two records from north-western Europe might result from the import of fruit carrying *D. mercatorum* eggs, larvae or puparia. In the case reported here, however, this is unlikely since our baits were of unbruised bananas which were peeled before use. The baiting site is also distant from likely sources of imported fruit that might carry *D. mercatorum*. Our record may therefore indicate the presence, at least during the summer, of a small population of free-living *D. mercatorum* in the most southerly part of the British Isles.

It is possible that *D. mercatorum* is more common in the British Isles than the lack of records suggests since it is superficially similar to both *D. repleta* and the very common and widespread *D. hydei*. We recommend therefore that specimens of these two species are carefully checked to ensure that individual *D. mercatorum* are identified and the distribution of this rarity correctly determined.

Acknowledgments: We thank A.D. Bennett for the use of her conservatory and to P. Beuk (Naturlijke Historische Museum, Leiden, Netherlands) and B. Pitkin (British Museum - Natural History, London, UK) for confirming our identification.

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Banerjee, Rakhee, and B.N. Singh. Department of Zoology, Banaras Hindu University, Varanasi, India. Persistence of chromosome inversions in laboratory stocks of *Drosophila bipectinata* and *D. malerkotliana*.

In *Drosophila*, chromosomal polymorphism due to paracentric inversions is very common and is an adaptive character (Dobzhansky, 1970). It is often maintained in natural populations as well as in laboratory populations by balancing selection due to heterosis. Chromosome inversions have been found to persist in laboratory stocks of various *Drosophila* species (Levene and Dobzhansky, 1958; Singh, 1982; Singh and Das, 1991, 1992). Since the laboratory stocks are maintained by transferring a smaller number of flies than the total number of flies hatch in the bottles, the frequency of inversions may change considerably and a particular arrangement may be eliminated also due to genetic drift (Singh, 1982, 1987).

In this note, we report the results of our investigations on inversion polymorphism in laboratory stocks of two closely related species, *Drosophila bipectinata* and *D. malerkotliana* of the *bipectinata* complex of the *ananassae* subgroup of the *melanogaster* species group. Chromosome inversions are known to occur in natural populations and laboratory stocks of both these species (Bock, 1971; Rahman and Jha, 1973; Gupta and Panigrahy, 1990;

Table 1. Karyotype frequencies.

	Karyotypes				Gene arrangements (%)	
	ST/ST	ST/In	In/In	χ^2	ST	In
Observed	69	30	1	1.34	84.00	16.00
Expected	70.56	26.88	2.56			

Das and Singh, 1992; Singh and Das, 1992; Banerjee and Singh, 1994). A mass culture stock of *D. bipectinata* was established from flies collected from Trivandrum (Kerala) in June 1994. It was maintained for five generations in bottles. Chromosomal analysis of this stock was made by squashing 100 larvae taken randomly from culture bottles by lacto-aceto-orcein method. Only one inversion *viz.*, *In C in2R*, was detected (Figure 1). All the three karyotypes, ST/ST, ST/In and In/In, could be identified. The observed and expected (via Hardy-Weinberg proportions) numbers of different karyotypes and the frequency of ST and In gene arrangements are shown in Table 1.

Table 2. The number of homokaryotypes and heterokaryotypes.

	1. Isofemale line 49			2. Isofemale line 12	
	Homokaryotype	Heterokaryotype		Homokaryotype	Heterokaryotype
In IILA	52	48		53	47
			In IIRA	63	37

Observed and expected numbers of different karyotypic combinations between IIL and IIR

		IIL		
		Homo.	Hetero.	Total
IIR	Homo.	38(33.39)	25(29.61)	63
	Hetero.	15(19.61)	22(17.39)	37
	Total	53	47	100
		$\chi^2 = 3.64$	df =1	P > 0.05

Although the deviation from Hardy-Weinberg expectation is not significant, this inversion has been found to persist in the laboratory stock. The same inversion has been found to persist in other laboratory stocks for several generations and significant deviation from the equilibrium has also been found in these stocks (Das and Singh, 1992; Banerjee and Singh, 1994).

Two isofemale lines of *D. malerkotliana* raised from females collected from Botanical Garden, Banaras Hindu University, in August 1994 were also examined for chromosome inversions in the fourth generation. The *In 2LA* inversion was detected in the isofemale line 49 and two inversions, *In 2LA* and *In 2RA*, were found in the isofemale line 12. Microphotographs of these two inversions in heterozygous condition are depicted in Figure 2. The standard and inversion homokaryotypes could not be identified separately at both inversion loci in *D. malerkotliana*. The numbers of homo- and heterokaryotypes in both the lines and observed and expected numbers of various intrachromosomal associations between 2L and 2R in isofemale line 12 are shown in Table 2.

Figure 1 (above). Microphotograph of a heterozygous inversion *In C* in IIR of *D. bipectinata*.

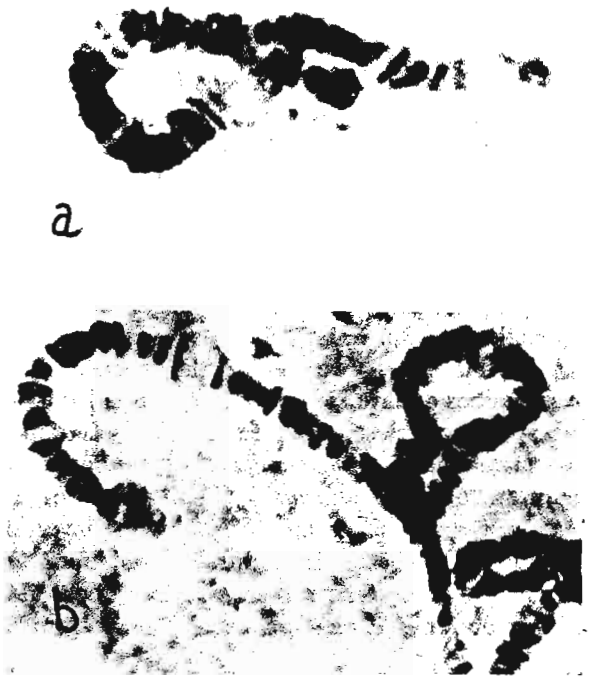

Figure 2 (right). Microphotographs of two heterozygous inversions, *IILA* (Figure a) and *IIRA* (Figure b) in *D. malerkotliana*.

In both the isofemale lines of *D. malerkotliana*, inversion heterozygotes occur in considerable frequency and show persistence under laboratory conditions. Since two types of homozygotes could not be distinguished, deviation from genetic equilibrium could not be tested. In isofemale live 12, two inversions of the second chromosome have been found. To test whether these two inversion show linkage disequilibrium, deviation from randomness has been tested. The X^2 value (3.64; $P > 0.05$) shows that two inversions are associated randomly and there is no evidence for linkage disequilibrium between inversions in *D. malerkotliana*. The inversions of *D. bipectinata* and *D. malerkotliana* detected in the laboratory stocks during the present study have been reported earlier in natural populations and laboratory stocks and occur at considerable frequency in the stocks analysed during the present study. This indicates that chromosome inversions in *D. bipectinata* and *D. malerkotliana* are adaptive.

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Asha Devi, J.S., and S.R. Ramesh. *Drosophila* Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri MYSORE-570 006, India.
Analysis of pre-adult parameters in three strains of *Drosophila nasuta nasuta*.

Analysis of pre-adult parameters such as fecundity, rate of development and viability of the mutant strains of *Drosophila nasuta nasuta* has not been carried out so far. These are important adaptive traits which determine the rate of increase or decrease of a population (Andrewartha and Birch, 1954). It is an outcome of the interaction of the genotype and the environmental factors (Bonnier, 1960). Present note reports the results of our investigations on the fecundity, rate of development and viability in two eye colour mutants, namely white and sepia of *D. n. nasuta* in comparison at constant temperature of 25±1°C.

To determine the fecundity, virgins were isolated, aged for 5 days, and pair matings were conducted in 3" X 1" culture vials containing wheat cream agar medium. 30 such replicates were set up for each strain. These pairs were transferred to fresh culture vials once every 24 hours. The number of eggs laid per vial per day were counted for a period of 15 days.

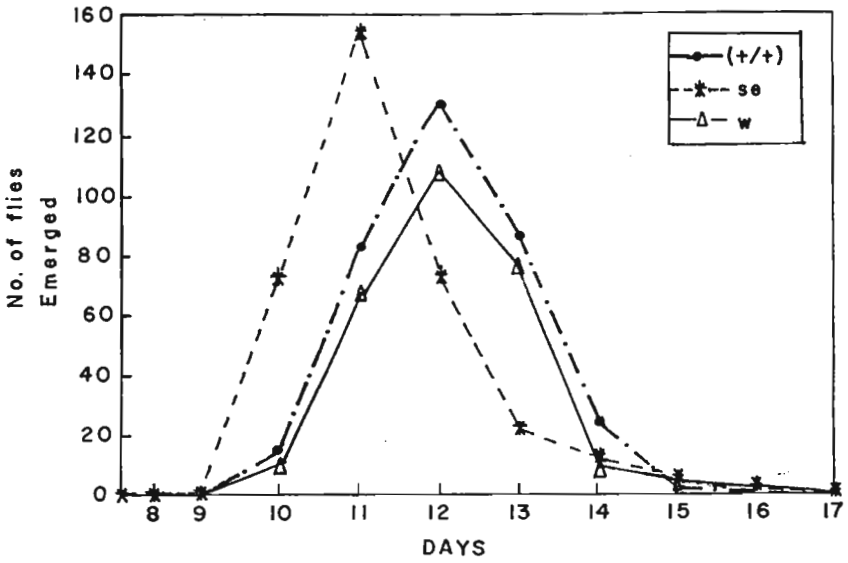

Figure 1. Pattern of adult emergence in three strains of *Drosophila nasuta nasuta* at constant temperature.

To find out egg to adult rate of development, synchronized eggs were collected by using the modified procedure of Delcour (Ramachandra and Ranganath, 1988) to maintain the uniformity with regard to age of the eggs. 12 replicates raised with 50 eggs per culture vial were maintained for each strain. The total number of flies that emerged were counted every day to determine the rate of development and viability.

The test for determination of fecundity revealed that the mutant sepia produces a higher number of eggs as compared with that of wild type and white eye mutant. All three strains show statistically significant variation in fecundity.

The analysis of egg to adult rate of development revealed that the peak of

emergence of adults in case of mutant strain sepia was found to be on the 11th day, while in wild type and white eye strains the peak of emergence was on the 12th day (Figure 1). The mean rate of development in case of sepia strain was 11.32 ± 0.53 days, while it was 12.13 ± 0.62 and 12.10 ± 0.55 days for white and wild type strains, respectively. Thus sepia develops faster than the other two strains tested. Analysis of the data by student t-test revealed that the differences in rate of development in the three strains are statistically insignificant.

Analysis of egg to adult viability revealed that the viability was least being 46% in white eye strain as compared with the other two strains, wherein the percentage of viability was 56.83% in case of wild type and 57% in case of sepia strain. Computation of the data by analysis of variance (ANOVA) for all the three strains showed significant differences in viability.

Our findings on the fitness parameters in wild type strain of *D. n. nasuta* are in conformity with the results of Ranganath and Krishnamurthy (1972), Ramachandra and Ranganath (1986) and Ramachandra *et al.*, (1987). Batabyal and Sidhu (1972) have found that *D. melanogaster* mutant strains show lower egg production and fertility as compared with that of wild type. In the present investigations, it is evident that such a trend with a superiority of fitness of wild type strain is absent. Instead, the sepia mutant strain which has better egg production, fecundity and rate of development is superior in its fitness as compared with that of the wild type strain. The same is not true for the white eye strain though it is also an eye colour mutant. Similar analyses of fitness parameters involving all the three strains are being made to examine the possible superiority of sepia mutant under varying population densities and temperature.

Acknowledgment: We thank the Chairman of the department and Prof. H.A. Ranganath, *Drosophila* Stock Centre and also the Department of Biotechnology, Govt. of India, for providing the facilities. We are grateful to Prof. Dr. W.-E. Kalisch, Institut fur Genetik, Ruhr Universitat Bochum, Bochum, Germany, for his constant encouragement and for providing the mutant stocks.

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Kekić, V.[1], Sofija Pavkovic-Lucic[1], and N.J. Milosevic[2]. [1]Institute of Zoology, Faculty of Biology, University of Belgrade, Belgrade, Yugoslavia; [2]Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Belgrade, Yugoslavia. Sampling methods and wing length in *Drosophila melanogaster*.

During July 1994 working on sexual selection in *D. melanogaster*, in the same locality where we conducted our previous researches (Taylor and Kekic, 1988), in orchard near a small rural village Sremska Kamenica, Serbia, Yugoslavia, we collected uncoupled flies, beside pairs *in copulo*, in order to compare their body size. Flies were collected in two barrels, set one beside the other, containing fermenting fruit (sweet cherry, plum and apricot) prepared for the homemade brandy distillation. Both barrels (200 l content) contained the same quantity of substrate (about 30 cm from the top of the barrel) in the "last stage of fermentation", *i.e.*, immediately before the distillation. At that moment about 10,000 individuals were in both barrels. After the collecting flies *in copulo*, at 9 a.m., we sampled flies in two ways: by aspirator (in all elevations uniformly, from substrate surface to the barrel top); later (at 9:30 a.m.), after we scared flies by striking both barrels, we captured them by net at about 50 cm above barrels. There are two important remarks related to sampling: first, all flies were available in a way they could be all captured by aspirator, and second, flies that first flew out from barrels were captured by net. In all flies a right wing length was measured taken from the intersection of the anterior cross

Table 1. Mean wing length ($\bar{x}$) and coefficient of variation (CV) in *D. melanogaster* sampled in two different ways, by aspirator or by net

Sampling	MALES				FEMALES			
method	n	$\bar{x}$	S.E.	CV(%)	n	$\bar{x}$	S.E.	CV(%)
aspirator	141	93.106	± 0.550	7.016	174	108.983	± 0.499	6.045
net	87	95.218	± 0.646	6.329	108	110.853	± 0.605	5.669

Table 2. ANOVA of wing length in *D. melanogaster* sampled in two different ways, by aspirator or by net

Source of variation	d.f.	SS	MS	F	P
Males					
Between groups	1	239.99	239.99	5.963	0.0154
Within groups	226	9096.25	40.25		
Females					
Between groups	1	254.02	254.02	6.062	0.0144
Within groups	280	11733.49	41.91		

vein and longitudinal vein 3 (L3) to the intersection of L3 with the distal wing margin given in ocular units (x 0.014 = 1 mm). Table 1 presents mean values of wing lengths in males and females sampled in two ways mentioned above, and Table 2 presents results obtained by ANOVA. Significant differences of wing length between groups compared lead to the conclusion that flies with longer wings, although larger and heavier (since these characteristics are phenotypically correlated; Reeve and Robertson, 1953; Anderson, 1966), compared to flies with shorter wings, fly faster and can escape faster when disturbed.

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Johnson, Norman A. Section for Evolutionary Genetics, Department of Biology, The University of Texas at Arlington, Arlington, Texas 76019. Sex ratios of F_1 hybrids between Zimbabwe and non-Zimbabwe *Drosophila melanogaster*.

Until very recently, *Drosophila melanogaster* has been thought to consist of one worldwide panmitic population with little or no geographic differentiation at neutral sites (Singh and Rhomberg, 1987) and little within-species mating isolation (Henderson and Lambert, 1982; but see Cohet and David, 1980). Two years ago, Begun and Aquadro (1993) found that Zimbabwe populations of *D. melanogaster* were very distinct from other populations at the DNA level, with F_{ST} values ranging from 0.25 (in regions of high recombination) to 0.6 (in regions of restricted recombination).

Wu *et al.* (1995) found strong but asymmetrical sexual isolation between Zimbabwe and other populations of *D. melanogaster*. Females from most of the collected Zimbabwe lines mate almost exclusively with Zimbabwe males in double-choice experiments. Even in no-choice experiments, these females are reluctant to mate with non-Zimbabwe males. (These lines are henceforth designated Z-lines). In the reciprocal cross, while non-Zimbabwe females generally prefer males from their own line to Zimbabwe males, they will readily mate both types of males. (These lines are designated C-lines). Wu *et al.* (1995) also found polymorphism within the Zimbabwe population: in some lines, females would accept C-line males (though they still prefer Z males) but their males would be accepted by Z-females. (These lines are designated Z'-lines). The mating phenotypes appear to be robust to a variety of environmental conditions and perturbations.

Wu *et al.* (1995) noted that no overt hybrid sterility or inviability had been found between Z (or Z') and C lines. Tests for more subtle forms of hybrid fitness reduction, however, have not been performed. According to Haldane's rule (1922) if there are sex-biased differences of fitness of the F_1 hybrids, the heterogametic sex (male in the case of *Drosophila*) will be more severely affected. Thus if Haldane's rule for viability is followed, there will be female-biased sex ratios in the F_1. In this experiment, I examined the sex ratios of hybrids from single pair crosses of the following general types: Z x Z, Z x Z', Z x C, Z' x Z, Z' x C, C x Z, C x Z', C x C. (the female of each pair is listed first followed by the male).

Table 1. Progeny sex ratios for F_1 crosses

Female parent	Male parent	# vials est.	# vials producing	# offspring	% males	Comments
CAM	FR	16	12	690	48.55	first vial
LCCA	CAM	16	16	798	49.67	first vial
CAM	Z29(Z')	16	11	1,142	48.42	
CAM	Z34	8	5	533	51.22	
CAM	Z56	14	12	1,146	45.2	G = 10.575, p = 0.001
FR	Z34	16	16	1,152	47.66	
LCCA	Z53	16	12	1,076	38.85	G = 53.985, p <0.0001
OR-R	Z53	9	6	447	40.26	G = 17.041, p < 0.0001
Z29 (Z')	Z30	9	4	309	45.63	
Z34	Z56	5	2	122	35.25	G = 10.783, p = 0.001
Z53	Z29(Z')	14	11	1,176	49.57	
Z56	Z29(Z')	11	4	225	48.89	
Z29 (Z')	CAM	16	14	594	48.48	first vial
Z29(Z')	OR-R	10	10	995	48.44	
Z30	FR	7	1	64	51.56	
Z34	CAM	10	0	0	—	
Z53	FR	8	1	91	48.35	

first vial = only the progeny from the first vial (first 6 days of egg laying) were scored.
G and p values listed only for significant ($p < 0.05$) results. All others are not significant. Listed p values have not been corrected for multiple tests.

Four different Z-lines (Z30, Z34, Z53, and Z56), one Z' line (Z29), and four C-lines (CAM 108 NC from North Carolina, FR V3-1b from France, LCCA from Lemon Cove, California, and Oregon-R from Oregon) were used. Table 1 lists the crosses that were established. Virgin 2-4 day old females were mated individually to 2-4 day old males by placing them in a 8 dram vial with Ward's instant *Drosophila* media-Blue. After 6 days, the pairs of flies were

transferred to new vials where they remained for an additional 6 days. The adult progeny emerging from each vial were scored and sexed 19-21 days after the vial was established. Flies were maintained at 24±1°C with a 14 h light/10 h dark schedule.

Table 1 displays the data on the proportion of single pair matings producing progeny, the numbers of progeny produced, and the sex ratios of those progeny of the various crosses. As expected, most of the Z x C crosses failed to produce any progeny whereas most of the Z' x C, C x Z, and C x Z' crosses were rather productive. This is consistent with the patterns of premating reproductive isolation documented by Wu *et al.* (1995).

The progeny resulting from some of the crosses between Zimbabwe and non-Zimbabwe populations have female-biased sex ratios. For example, the cross of LCCA x Z53 produced 658 females and 418 males, clearly different from the expectation of 50% males ($G = 53.985$ $p << 0.00001$). This is consistent with Haldane's rule and combined with the molecular divergence and strong premating isolation between these two groups, suggests incipient speciation within *D. melanogaster*. There also appears to be polymorphism in this trait as many C x Z crosses do not produce biased progeny sex ratios. In addition, there may be some isolation between different Zimbabwe lines (those involving line Z56, see Table 1). Further examination of the variation within Zimbabwe for traits revealed in the hybrids with non-Zimbabwe *D. melanogaster* is planned.

Biased sex ratios in the F_1 may actually be a weak test of hybrid fitness reduction. In some cases where the F_1 progeny of both sexes are viable and fertile, the F_2 and backcross progeny often suffer severe reductions in fertility and/or viability (*e.g.*, Hennig, 1977). This phenomenon is known as hybrid breakdown. In addition, hybrid sterility appears to evolve at a much faster rate than hybrid inviability (Wu and Davis, 1993; Johnson and Wu, 1993). Examinations of hybrid fertility reduction and hybrid breakdown are forthcoming.

Acknowledgment: I thank Chung-I Wu for flies and Sean Griffith for laboratory assistance.

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Kozhemiakina, T.A., and D.P. Furman. Institute of Cytology and Genetics, Siberian Department of the Russian Academy of Sciences, Novosibirsk, 630090, Russia. Increased transposition rates of copia-like TEs while deriving isogenic lines of *Drosophila melanogaster*.

It is known that certain factors enhance transpositional activity of transposable genetic elements in *Drosophila*. Transposition frequencies rise under hybrid dysgenesis, disturbances of chromosome balance, or following experimental treatments (Gerasimova *et al.*, 1984; Kidwell, 1985; Yannopoulos *et al.*, 1987; Finnegan, 1988; Bashkirov *et al.*, 1992; Anikeeva *et al.*, 1994).

That the TE location would change following genetic manipulations with chromosome balancers was also noted (Pasyukova *et al.*, 1987; Kaidanov *et al.*, 1991), without, however, evaluation of the frequencies of the events.

We wished to know how often insertions and excisions of mdg1, Dm412, copia, and B104 take place in isogenizing crosses to the *Cy/Pm;D/Sb* balancer stock.

The transposable elements were localized by routine *in situ* hybridization on salivary gland polytene chromosomes (Pardue and Gall, 1975).

To reduce experimental errors on account of polymorphism with respect to the harbor sites of the transposable elements in the initial genomes, two isogenic lines were taken in the experiment. These lines (hereinafter called first order isogenic lines) were derived after a routine technique from a cross to the M5; *Cy/Pm;D/Sb* line that was bearing inversions, lethals and phenotypic markers (Lindsley and Grell, 1968). The positions of mdg1, Dm412, copia, and B104 in the genomes of the lines were determined twice: once when the line was in the making, once after two and a half years of maintaining under laboratory conditions (see Table 1 for the total numbers of TE harbor sites in the lines). By that time, the first order isogenic lines had been past 65 generations of mass mating. Transpositions of the mobile elements that had taken place over the time elapsed were regarded as spontaneous.

Then the first order isogenic lines were subject to isogenization again, now with respect to autosomes, via the *Cy/Pm;D/Sb* line. Each initial line gave birth to 9 derivatives, or second order isogenic lines, each of which was founded by one female randomly sampled from the initial line (Figure 1). The chromosomal locations of mdg1, Dm412, copia, and B104 in the second order isogenic lines were determined immediately upon establishment.

For each of the four mobile elements, 2 to 4 preparations in the first order isogenic lines and not less than 4

preparations in each second order isogenic lines and balancer line were analyzed. TE-occupied sites were determined after Bridges' maps (Lefevre, 1976). Not less than 10 nuclei were examined in each preparation. We searched the second order isogenic lines, and whenever a labeled site that had not been present at either of the parental lines was present at the second order isogenic lines, we acknowledged an insertion following isogenization; whenever a site that had been present in the parental lines was missing from the second order isogenic lines, we acknowledged an excision.

All maintaining and isogenizing crosses were performed on a standard medium at 25±1°C.

Statistical significance was checked after Student's criterion.

The first order isogenic lines were analyzed again after two and a half years of maintaining under laboratory conditions. The second order isogenic lines were checked immediately upon isogenization. That is why not only do the results of the analyses allow us to definitely say whether TE transpositions occurred spontaneously or they did so under the isogenization procedure, but these results allow us to calculate the frequencies of the events.

Table 1. Overall number of positions occupied by mdg1, Dm412, copia, B104 in $sc^{147P-14}$ and sc^{59P-5} lines. N_1 is the number of sites at the instant of establishment of the lines, N_2 is the number of sites after 65 generations of laboratory maintenance.

Mobile element	N_1 Lines		N_2 Lines	
	$sc^{147P-14}$	sc^{59P-5}	$sc^{147P-14}$	sc^{59P-5}
mdg1	20	16	21 (3)	17 (4)
Dm412	24	21	25 (2)	21 (2)
copia	23	18	23 (2)	19 (3)
B104	40	37	41 (2)	37 (2)

While counting for N_2, a site was acknowledged to be occupied, if it was labeled on at least one preparation following hybridization. Number of preparations is given in parentheses.

Insertion of B104 into the site 78E exemplifies a spontaneous transposition during maintenance. The site was registered labeled in the $sc^{147P-14}$ line after two years and a half of breeding under laboratory conditions (Table 2).

Analysis of data on all registered sites for the four mobile elements in question provides evidence that there are both spontaneous insertions and spontaneous excisions, but their contribution into variation for TE location does not seem to be great: evaluations set the frequencies of these events at about 10^{-4}, which does not exceed literature values (Table 3) (Finnegan and Fawcett, 1986; Charlesworth *et al.*, 1989; Harada *et al.*, 1990).

Note that excisions are somewhat outnumbered by insertions, which is consistent with literature data, too (Harada *et al.*, 1990).

The process itself of isogenization appears to have more influence upon change of the TE pattern. Examples of insertions and excisions of B104 during isogenization are given in Table 2 (sites 66C, 61DE, 64A, etc.).

Table 2. Distribution of sites occupied by B104 on 3L chromosome in the initial line, $sc^{147P-14}$ (column 1); on the preparations of the same line after two and a half years of laboratory maintenance (column 2); in isogenic derivatives of $sc^{147P-14}$ (columns 3-11); in the balancer line, *Cy/Pm;D/Sb.*

	$sc^{147P-14}$		2-order isogenic lines, $sc^{147P-14}$ -n									*Cy/Pm;*
sites	initial	21/2 yr.	-2	-9	-19	-5	-7	-4	-6	-13	-16	*D/Sb.*
65AB	+	+	+	+	+	+	+	+	+	+	+	+
68C	+	+	+	+			+	+	+		+	+
75A	+	+	+	+			+	+	+	+	+	+
62C					+	+	+					*
66C								+				
67A	+	+	+	+			+	+	+	+	+	
78E		*	+	+			+	+		+		*
61DE	+	+	+	+	+	+	+		+	+	+	+
63A	+	+	+	+	+	+	+	+	+	+	+	+
64A	+	+		+	+	+	+	+	+	+	+	+
70A	+	+	+				+		+		+	*
75C	+	+	+				+	+	+	+	+	
79CD	+	+	+	+			+	+	+	+	+	+

* - sites having occurred not on all preparations assayed.

Table 3. Spontaneous insertion and excision rates for mdg1, Dm412, copia, B104 in $sc^{147P-14}$ and sc^{59P-5} lines.

Mobile element	Insertion rate Lines		Excision rate Lines	
	$sc^{147P-14}$	sc^{59P-5}	$sc^{147P-14}$	sc^{59P-5}
mdg1	2.6×10^{-4} (1/3900)	2.4×10^{-4} (1/4160)	0	0
Dm412	3.0×10^{-4} (1/3120)	0	3.0×10^{-4} (1/3120)	0
copia	0	2.8×10^{-4} (1/3510)	0	0
B104	1.9×10^{-4} (1/5200)	0	0	2.0×10^{-4} (1/4810)

Insertion: a hybridization site in F_{65}, yet not present in F_0. Excision: a hybridization site in F_0, yet not present in F_{65}. The frequency of an event was calculated as the ratio of number of events to (number of sites in the initial line) x 65 (number of generations) x (number of preparations assayed).

As was shown by comparison of the TE localization patterns in the first and second order isogenic lines with due account of how the TE were distributed in the balancer line, the label appeared in 66 new sites in the second order isogenic lines of sc^{59P-5} (13 for mdg1, 23 for copia, 23 for Dm412, and 7 for B104) and in 86 sites in the $sc^{147P-14}$ derivatives (21, 32, 21, 12 for mdg1, Dm412, copia and B104, respectively).

The label was missing from 21 sites in the $^{59P-5}$ derivatives (4, 8, 6, 3 for mdg1, Dm412, copia and B104, respectively) and from 14 sites in the second order isogenic lines of $sc^{147P-14}$ (2. 7, 2, 3 for mdg1, Dm412, copia, B104).

All the second order isogenic lines have nearly the same number of "missing" sites, which is on the average 1.6 per line for $sc^{147P-14}$ derivatives and 2.3 per line for sc^{59P-5} derivatives

Table 4. Insertion and excision rates for mdg1, Dm412, copia, B104 in $sc^{147P-14}$ and sc^{59P-5} lines following isogenization.

Mobile element	Insertion rate Lines		Excision rate Lines	
	$sc^{147P-14}$	sc^{59P-5}	$sc^{147P-14}$	sc^{59P-5}
mdg1	3.7×10^{-2} (21/ 567)	2.8×10^{-2} (13/459)	5.3×10^{-3} (3/567)	8.7×10^{-3} (4/459)
Dm412	4.7×10^{-2} (32/675)	4.0×10^{-2} (23/567)	1.0×10^{-2} (7/675)	1.4×10^{-2} (8/567)
copia	3.4×10^{-2} (21/621)	4.5×10^{-2} (23/513)	3.0×10^{-3} (2/621)	1.2×10^{-2} (6/513)
B104	1.1×10^{-2} (12/1107)	0.7×10^{-3} (7/999)	2.7×10^{-3} (3/1107)	3.0×10^{-3} (3/729)

Insertion rates and excision rates for each element were calculated per position per genome per generation. The frequency of an event was calculated as the ratio of the number of events to (number of sites in the initial line) x 3 (number of generations) x 9 (number of second order isogenic lines)

(differences fall short of significance).

Transposition frequencies under the second isogenization procedure are presented in Table 4. The values are higher by 1-2 orders of magnitude than the corresponding figures in Table 3 (*e.g.*, insertion rates in $sc^{147P-14}$ are 2.6×10^{-2} and 2.8×10^{-4}, respectively). Differences are highly significant ($P < 0.01$).

Note that in 50% of the situations the insertion rates significantly ($P < 0.05$) exceed the excision rates (so is for mdg1 under isogenization of both lines and for copia and B104 under isogenization of $sc^{147P-14}$). In four cases (for Dm412 under isogenization of both lines and for copia and B104 under isogenization of sc^{59P-5}) the differences fall short of significance.

Thus, the observed rates of TE transpositions following crosses to the balancer line compare, with respect to the order of magnitude, to those endowed by such a strong stimulus as heat shock (Anikeeva *et al.*, 1994). This is evidence that in itself the process of mating to the balancer line is, too, a strong stimulus of TE transpositions in the genomes of the lines under isogenization. The mechanisms underlying this phenomenon are unclear so far.

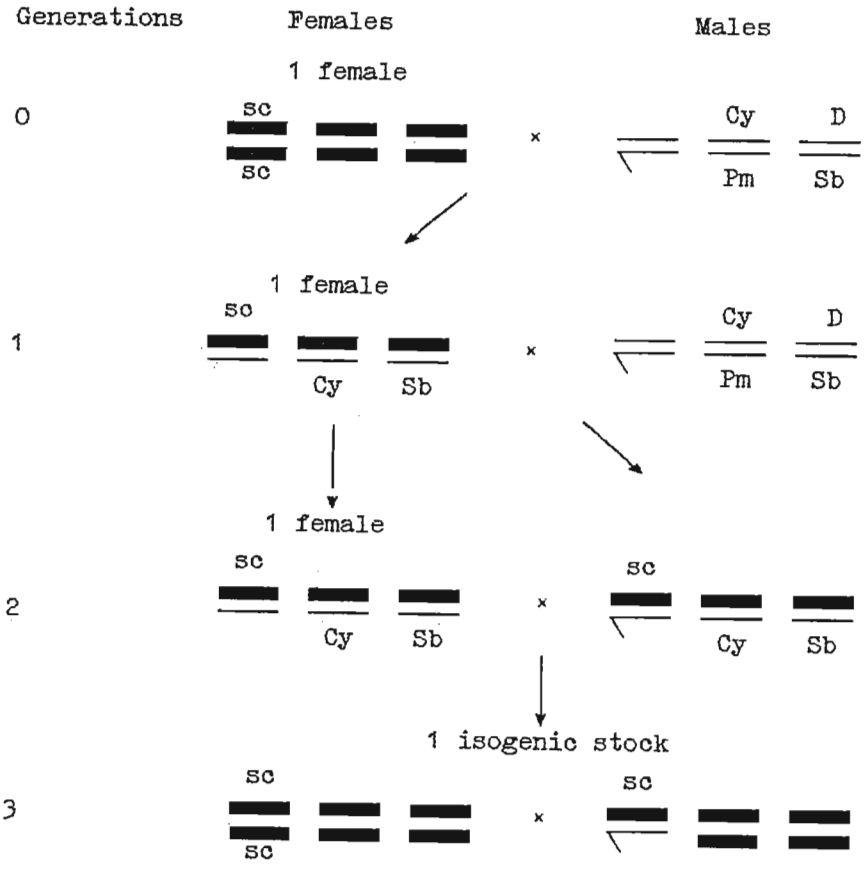

Figure 1. Crossing scheme to generate a set of isogenic derivatives from different stocks. Each isogenic stock was derived from one female, randomly sampled from the initial line. Crosses 1 and 2 involve the same female. (There are less non-zero values for excision rates than for insertion rates in Table 3).

The experimental conditions were set up so as to exclude any rise in the mobility of the transposable elements as might be due to heat treatment (the flies were kept in a thermostat at 25°C).

Some investigators believe that hybrid dysgenesis accounts for increased transposition rates of copia-like transposable elements under interline crosses (Gerasimova *et al.*, 1984; Lewis and Brookfield, 1987). If so, hybrid dysgenesis might have been the case in the situations in questions. However, it should not be associated with the P-M system, as neither *Cy/Pm;D/Sb* nor sc^{59P-5} has a site of hybridization of P-element, the $sc^{147P-14}$ has the only such site (Furman *et al.*, 1993), while the experiment had been set up so as to avoid dysgenesis: of the flies that were potential carriers of P-elements, only females were involved in the crosses that might be dysgenic. Typification of hybrid dysgenesis, if it took place, requires further study. Perhaps, it could be the H-E type linked with hobo, of which a carrier can be one of the lines. It was H-E hybrid dysgenesis and, concurrently, activation of copia-like element transpositions that Kaidanov *et al.* (1991) observed in crosses with the *Cy L/Pm* balancer line. Whilst others report that neither P-M nor H-E dysgenesis are stimuli for mobilization of copia-like transposable elements and that the transposition rates of each elements are

controlled independently (Eggleston *et al.*, 1988; Harada *et al.*, 1990), therefore the phenomenon that we observe should be interpreted in a different way.

Balancer chromosomes are used when it is necessary to keep a homologous chromosome intact. However, the data reported show that it is not always guaranteed that use of balancers warrant the full preservation of the properties and structure of the homologous chromosome, that can have been changed following TE transpositions.

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Weisman, Natalya Ya, Dmitry E. Koryakov, and Ilya K. Zakharov. Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Division, Novosibirsk, 630090, Russia. New geographical allelic variants of the *Drosophila melanogaster* genes *rotund* and *roughened eye*.

The purpose of the given research is devoted to studies in dynamics the genetic variability of the gene pool in the natural *Drosophila melanogaster* populations (Zakharov, *et al.*, 1991). Out of two geographically remote natural populations of Eurasian *Drosophila melanogaster*, the mutant phenotypes were isolated that are characterized by the wide spectrum of the similar phenotypic alterations, namely, the strains #89300 and #89386 from the Uman population (Ukraina) in 1989 and #920017, #921503, #921314 from the Gorno-Altaisk population (Republic of Gorny Altai) in 1992. In the Uman population of 1989 and the Gorno-Altaisk population of 1992, 1068 and 2360, respectively, haploid genomes were analysed, the frequencies of the mutant chromosome in these populations equal to 0.19% and 0.17% per haploid genome, respectively. The similar mutant phenotype, the strain #i13, was originated from gamma-irradiated laboratory strain *Canton-S*.

Chromosome #89386. #89386 is a recessive mutation which modifies different organs of the fly (Figure 1). In the chromosome #89386 homozygotes, legs are shortened with 1-3 tarsal segments, instead of 5 segments in the norm, which are often fused. In males, sex combs are absent or poorly expressed. The wing blade is shortened - the fore part of the wing is reduced. The wing width is almost normal and the wing is oval. In some mutant homozygotes, L2, L5 veins and posterior crossvein are interrupted. The wings are slightly raised and moved apart. The fourth antennal segment is missing.

The eye is rough because of irregular faceting and changes in eye texture. The number of facets is reduced, thus, the eye is reduced in size and the shape is changed. The body size is reduced. The life time of the imago is sharply shortened. Homozygous males and females are totally sterile. Outer components of the genitalia are morphologically undistinguished from the normal. However, in females, the internal components of genitalia are reduced in size. Some small eggs are generated there, but they are not laid.

Chromosome #89300. The mutation #89300 is recessive. In homozygotes of the chromosome #89300 the tarsal part of legs is shortened, the segmentation is disturbed, and the number of segments is reduced. The wings are of irregular shape; they are shortened, but to a lesser extent than in mutation #89386. The wings are slightly moved apart and raised. The L2 vein is broken in some mutant homozygotes. The fourth antennal segment is reduced. The eyes of ruby colour with mat touch are normal in size. The body size is less than in normals. The chromosome #89300 homozygotes are sterile as a rule. Mutant females lay a small amount of eggs, out of which either none or single flies enclose. Some individual males are fertile as in normal flies. The outer components of genitalia in males and in females both look perfect. The ovaries are large enough and the eggs are mature. At 25°C, the life time of adults does not differ noticeably from the normal.

The eyes of heterozygotes #89300/89386 are normal, but the other mutant characters of homozygotes

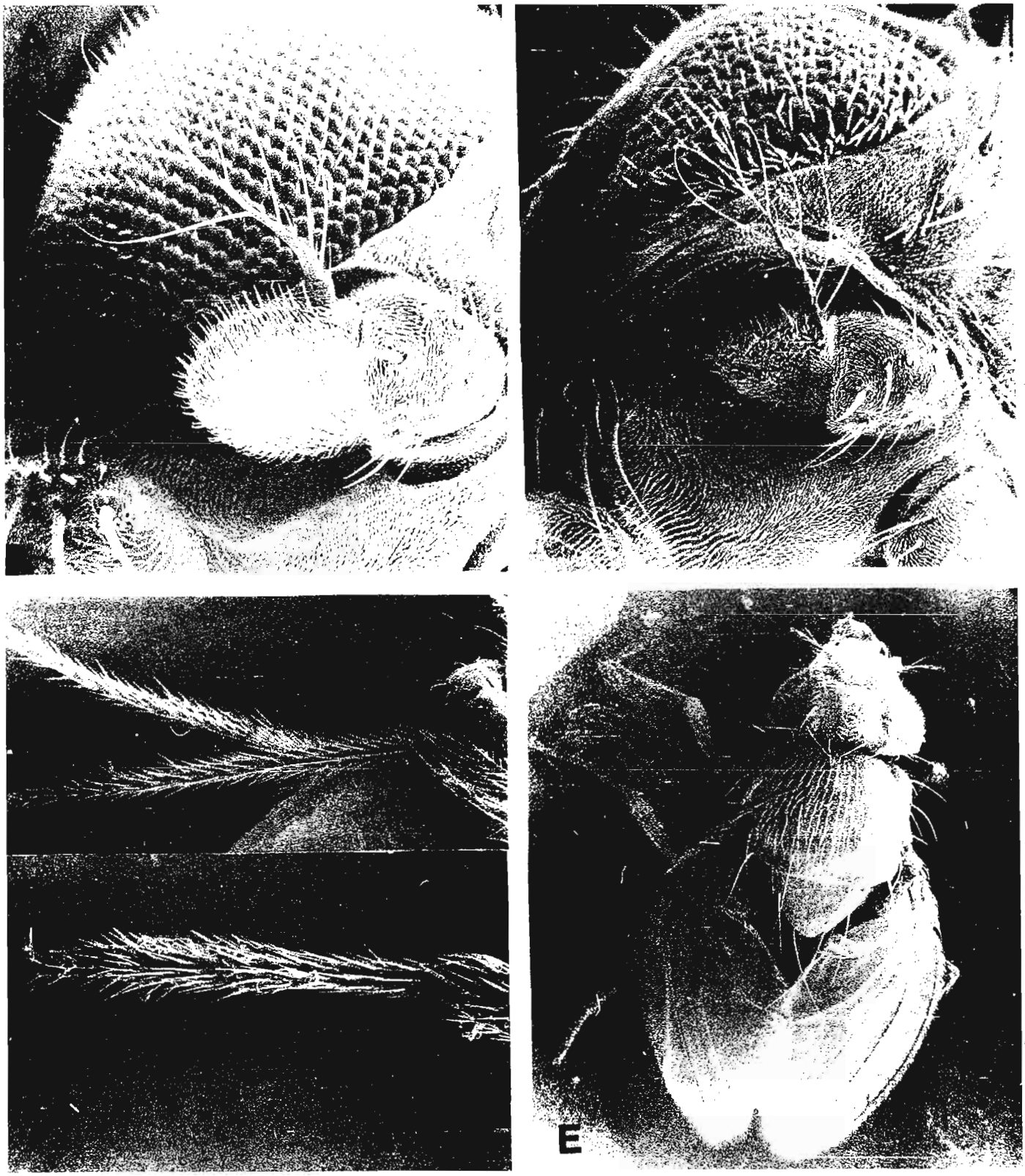

Figure 1. Scanning electron micrographs of the wild type and mutant #89386. A, Eye and antennae of the wild type; B, Reduced roughened eye and antennae with reduced 4th segments in the mutant #89386[b]; C, 5 tarsal leg segments in the normal; D, 3 tarsal leg segments in the mutant #89386; E, External appearance of the mutant #89386.

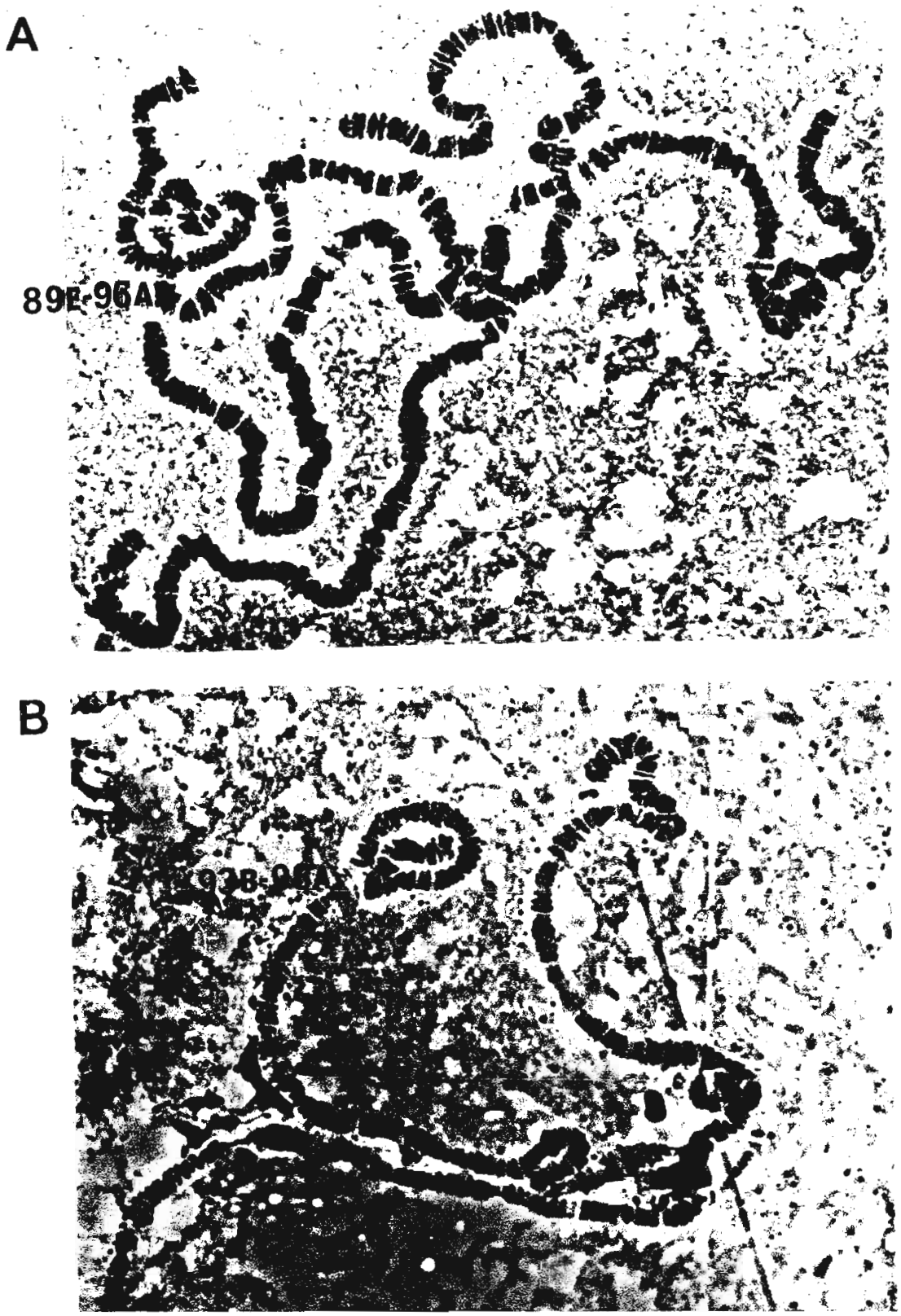

Figure 2. Cytogenetic analysis of mutants. A, Inversion *In(3R)89E-96A* in the chromosome #89386; B, Inversion *In(3R)93B-98A* in the chromosome #89300.

#89386/89386 are present. So, the mutations dealing with appendages are allelic in both chromosomes, #89300 and #89386. The phenotype #89386 is caused by two mutations, namely, #89386-1, which modifies the eye size and shape,

and #89386-2, which controls the alterations in wing, antennae and tarsal leg segments. The mutations never occurred separately among more than 7,000 F_2 progeny in the crosses of the heterozygotes #89386/*D*, carrying the marker gene Dichaete (*D*; 3-40.7), to the wild type flies (Lindsley and Zimm, 1992).

The mutant phenotype #89300 is also under the control of two genes. The first, #89300-1, controls the eye colour; the second, #89300-2, controls the alterations in appendages.

Out of the progeny of gamma-irradiated female from the normal laboratory stock *Canton-S*, the mutant individuals (of the chromosome #i13) were isolated. The phenotype of these flies exactly coincides with that of heterozygotes #89300/#89386, which are characterized by shortened oval wings, irregular legs and antennae, incorrect sex combs, and normal eyes. The phenotype of the hybrid #i13/#89300 to #i13/89386 is similar to that in homozygotes #i13/#i13, which points to the allelism of the mutation #i13 to mutations #89300-2 and #89386-2.

As homozygotes of the chromosomes #921314, #921503, #920017, as heterozygotes (#921314/#89386, #921503/#89386 and #920017/#89386) are phenotypically similar to each other and to the homozygote #89386/#89386. This proves the allelism of the mutation discussed.

By genetic analysis, the mutations #89300 and #89386 belong to the 3rd linkage group. Mutations #89300 and #89386 in crosses with the markers of the right arm of the 3rd chromosome, Curl-3 (*Cu*; 3-66.0), Delta (*Dl*; 3-66.2), and Prickly (*Pr*, 3-90.0), are characterized by the equally low percent of crossing-over. By cytogenetic analysis of the studied chromosomes, the #89300 and #89386 ones were found to carry inversions, *In(3R)93D-98F* and *In(3R)89E-96A*, respectively, which prevent the normal crossing-over (Figure 2).

By the allelism test, in which the heterozygotes #89386/*D* were crossed with the strains having the eye phenotype similar to the mutation #89386, namely, the recessive mutations of the 3rd chromosome - roughoid (*ru*; 3-0.0), almond eye (*ale*; 3-47.5) and *kidney* (*k*; 3-64.0), no allelism was observed. The mutation #89386 in compound with mutations which concern the wing venation, veinlet (*ve*; 3-0.2) and *radius incompletus* (*ri*; 3-47.0), led to the normal wing venation.

The offspring from the cross among the substrain #89300-1, characterized by the modified eye colour and normal appendages, and the phenotypically similar eye mutations sepia (*se*; 3-26.7) and rosy (*ry*; 3-52.0) were undistinguished from the normal. However, heterozygotes of the claret (*ca*; 3-100.7), #89300-1/*ca*, had the mutant phenotype "claret". The allelism test applied to the mutation #89300-2 against *ve* and *ri* was negative. Nevertheless, in compound #89300-2/*ve vn ri*, some individuals had interrupted or incompletely developed L2 and L5 veins. This fact may be explained by the interaction of mutations #89300 and vein (*vn*; 3-16.0).

Homozygotes of the chromosome #89386 originating from different families differed in the size and shape of the eye. In some strain derivatives the eyes were slightly reduced and oval (substrain #89386^a), in the other they were stripy and strongly reduced in size (the substrain #89386^b). These morphological differences are stable and inherited. The cross of two substrains with the different eye shape gives the mutants with oval eye. In crosses of the balanced strain #89386^b/*D* to the wild type flies, the flies with the oval eyes can be found among F_2 homozygotes. The share of such males and females is equal. In the offspring of the line #89386^a/*D* and the wild type flies no segregation was observed; all the mutants had oval eyes. The substitution of the chromosome 2 in the line #89386^b/*D* did not lead to the changes in eye shape. The oval eyes phenotype appeared only as the result of substitution of the chromosome *Dichaete* carrying the vast inversion - suppressor of crossing-over. It may be concluded that the phenotype "striped eyes" originated in the interaction of mutation #89386 with some unknown factor located on the chromosome 3.

The presence of the inversions and disruption of crossing-over in the chromosomes #89300 and #89386 prohibited the concrete localization of mutations on the chromosome 3. The analysis of numerous literature data devoted to eye and wing mutations enables us to conclude that mutations #89386-1 and #89386-2 (#89300-2) correspond to the mutant phenotypes of two genes, roughened eye (*roe*; 3-47.6) and rotund (*rn*; 3-47.6) (Cavener, *et al.*, 1986). Due to our assumption, the chromosomes #89386, #920017, #921314, and #921503 carry new geographical allelic variants of the gene with pleiotropic effect *rn* and the gene *roe*, by analogy, the chromosome #i13 carries an allele of the gene *rn*, and the chromosome #89300 carries alleles of the genes *rn* and *ca*.

It is interesting that the alleles of the genes *roe* and *rn* were found in relatively high concentrations in the geographically remote populations of Ukraina and Altai. During the previous decades these mutations were not found in our research of population genetics in different points of the area of *Drosophila melanogaster*. The almost simultaneous appearance of the genes *roe* and *rn* in the geographically distinct regions may be associated with the phenomenon "vogue of mutations."

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Ivannikov, Andrey V., and Ilya K. Zakharov. Institute of Cytology and Genetics of the Russian Academy of Sciences, Siberian Department, Novosibirsk, 630090, Russia. Distribution of *Drosophila mercatorum* into the Eurasian continent.

Antropogenic landscape is a unique ecological niche all over the World. Among synantropic species of *Drosophila* one can observe real cosmopolitans, for instance *D. melanogaster*, that are distributed everywhere. The other species of *Drosophila* occur in some regions and are absent in the others. For some of them, the periodical enlargement of the breeding area in the antropogenic landscape is characteristic (Dubinin, *et al.*, 1937; Komai, 1937; Moriwaki, 1937).

From 1988 to 1994 we have performed the study of species content of synantropic *Drosophila* in the Ukraine, Siberia, and Middle Asia (Ivannikov and Zakharov, 1994). On the territory of the former Soviet Union, *D. mercatorum* was not observed 15-20 years ago (Stackelberg, 1970; Mitrofanov, 1977; Ivannikov, 1991). The place this species was first found by us was the Ukraine. Some individuals of the species were caught in 1990 in Uman. In 1990, the population of *D. mercatorum* was found also in West Siberia - Novosibirsk, and its neighbourhoods. The Novosibirsk population is stable. The Novosibirsk autumn collections of synantropic *Drosophila* in 1991-1994 contained (sometimes in a great majority) *D. mercatorum*. The Novosibirsk population is local, since in the other towns of West Siberia located to the South, Rubtsovsk, Pospelikha, Zmeinogorsk, Biisk and Gorno-Altaisk, *D. mercatorum* is absent. It was also not found in the East of Siberia (Watanabe, *et al.*, 1994). In 1994 *D. mercatorum* appeared in Tajikistan. Among about 2,000 *Drosophila* flies, caught in November in Dushanbe, together with *D. melanogaster* and *D. immigrans*, three males of *D. mercatorum* were found. This is of interest, because Tajikistan is much more isolated territory for the ingress of new synantropic species of *Drosophila* than the Ukraine or Siberia. This Republic does not import, but only exports, the agricultural production. Nevertheless, during the last 5 years (1990-1994), two new species of *Drosophila*, *D. lebanonensis* and *D. mercatorum*, were registered at Tajikistan. As concerns *D. mercatorum*, it is obvious that during the recent years (the exact date of appearing in the Ukraine is doubtful) this species distributed into the inner regions of the Eurasian continent, where it was not observed earlier. In some regions, where *D. mercatorum* settled only recently, their populations are stable.

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Pauli, D., Brian Oliver and Anthony P. Mahowald*. Department of Zoology and Animal Biology, University of Geneva, CH-1224, Chene-Bougeries, Switzerland. *Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago IL 60637, USA. Dose dependent enhancers and suppressors of *ovo*^{D2}.

The ovarian phenotypes of *ovo*^{D}/+ *Drosophila melanogaster* females can be modified by mutations in genes involved in sex determination or sexual differentiation (Oliver, *et al.*, 1990; Pauli, *et al.*, 1993). These observations prompted us to search for other genes interacting with *ovo*^{D} mutations by systematically testing the *Drosophila melanogaster* genome with cytologically visible deletions (Pauli, *et al.*, 1995). The *ovo*^{D2} allele was extensively used due to its intermediate phenotype and its sensitivity to genetic background. Using 300 different deficiencies, we have analyzed about 58% of the euchromatic genome (Tables 1 to 5). These tables summarize the full data set discussed at length, but not shown, in Pauli, *et al.* (1995). The format of each Table is the same and as follows: Females of the indicated genotypes were crossed to *ovo*^{D2} *v*^{24}/Y males. A star after a given balancer chromosome indicates that the stock was outcrossed for at least four generations prior to the experiment. For example, all the stocks with a CyO* balancer have the same genetic background except for the deficiency chromosomes. The mean number of egg chambers/ovary at stage 10 or more mature is indicated. T1 is maximal distance between the cumulative distribution of egg chambers/ovary for the deficiency females vs. the balancer females. P is the level of significance. NS is not significant. The rank of the particular chromosome was compared to all the other chromosomes in the particular data set (for example a given first chromosome was ranked against all first chromosomes in the study). Wtr indicates that the rank was between the 10th and 90th percentile, chromosomes falling above or below this level are indicated. The 10th to 90th percentiles for the various chromosomes are: 0.4 and 11.3 oocytes/ovary for chromosome 1; 0.1 and 6.6 oocytes/ovary for chromosome 2; 0.1 and 5.7 oocytes/ovary for chromosome 3 (the chromosome three value was used arbitrarily for chromosome 4 as well).

References: Oliver, B., D. Pauli, and A.P. Mahowald 1990, *Genetics* 125:535-550 (Corrigendum 126:477); Pauli, D., Oliver and A.P. Mahowald 1993, *Development* 119:897-908; Pauli, D., B. Oliver and A.P. Mahowald 1995, Identification of regions interacting with *ovo*^{D} mutations: Potential new genes involved in germline sex determination of differentiation in *Drosophila melanogaster*, *Genetics* in press.

Table 1. *ovo*^{D2} heterozygotes with reduced doses of regions on chromosome 1.

Deficiency/ Balancer	Cytology	Progeny	oocytes/ovary mean	oocytes/ovary No	Statistics T1	Statistics P	Rank
*ac / FM7a**	tip of X	*ovoD2/Df*	0.6	53	0.175	NS	wtr
		ovoD2/Bal	1.5	56			wtr
260-1 / FM4	1A1;1B4-6	*ovoD2/Df*	0.6	64	0.120	NS	wtr
		ovoD2/Bal	1.4	54			wtr
*y74k24 / FM7a**	1A1;1B5-6	*ovoD2/Df*	2.2	90	0.127	NS	wtr
	or B9-10	*ovoD2/Bal*	1.1	93			wtr
su83 / FM3	1B10;1D6-E1	*ovoD2/Df*	2.3	40	0.520	<0.01	wtr
		ovoD2/Bal	7.1	78			wtr
sta / FM6	1D3-E1;2B3-4	*ovoD2/Df*	9.0	89	0.192	NS	wtr
		ovoD2/Bal	6.7	86			wtr
*sta / FM7a**		*ovoD2/Df*	12.4	83	0.511	<0.01	90%
		ovoD2/Bal	6.2	65			wtr
S39 / FM6	1E1-2;2B5-6	*ovoD2/Df*	18.6	40	0.704	<0.01	90%
		ovoD2/Bal	5.8	48			wtr
*S39 / FM7a**		*ovoD2/Df*	11.3	80	0.800	<0.01	90%
		ovoD2/Bal	1.9	80			wtr
*S39 / FM7c**		*ovoD2/Df*	7.3	70	0.608	<0.01	wtr
		ovoD2/Bal	2.0	65			wtr
A94 / FM6	1E3-4;2B9-10	*ovoD2/Df*	22.1	43	0.629	<0.01	90%
		ovoD2/Bal	9.4	66			wtr
*A94 / FM7a**		*ovoD2/Df*	12.8	68	0.690	<0.01	90%
		ovoD2/Bal	4.4	72			wtr
*A94 / FM7c**		*ovoD2/Df*	8.5	61	0.529	<0.01	wtr
		ovoD2/Bal	2.3	59			wtr
RA19 / FM6	1E3-4;2B9-10	*ovoD2/Df*	8.1	48	0.333	<0.01	wtr
		ovoD2/Bal	4.8	64			wtr
*RA19 / FM7a**		*ovoD2/Df*	11.3	84	0.661	<0.01	90%
		ovoD2/Bal	3.1	82			wtr
dor2T / FM6	2B6;2E1-2	*ovoD2/Df*	0.8	59	0.966	<0.01	wtr
		ovoD2/Bal	14.5	58			90%
Pgd35,pn/FM7	2C2-4;2E2-F1	*ovoD2/Df*	9.8	76	0.469	<0.01	wtr
	or F5	*ovoD2/Bal*	4.6	70			wtr
*Pgd35, pn / FM7c**		*ovoD2/Df*	0.6	86	0.018	NS	wtr
		ovoD2/Bal	0.6	68			wtr
Pgd-kz / FM6	2D3-4;2F5	*ovoD2/Df*	4.8	66	0.769	<0.01	wtr
		ovoD2/Bal	15.1	47			90%
2F1-3A4 / FM6	2F1;3A4	*ovoD2/Df*	0.3	61	0.179	NS	10%
		ovoD2/Bal	0.6	64			wtr
64c18 / FM7a	2E1-2;3C2	*ovoD2/Df*	2.6	16	0.210	NS	wtr
		ovoD2/Bal	3.2	98			wtr

X12 / FM6	2F5-3A1; 3B5-C1	*ovoD2/Df*	52	1.4	0.288	NS	wtr
		ovoD2/Bal	68	3.0			wtr
JC19 / FM7c	2F6;3C5	*ovoD2/Df*	0.323	10.4	49	NS	wtr
		ovoD2/Bal		14.3	42		90%
*JC19 / FM6**		*ovoD2/Df*	0.561	1.4	78	<0.01	wtr
		ovoD2/Bal		7.6	58		wtr
HC194 / FM7	3A1;3C3-4	*ovoD2/Df*	0.179	9.7	30	NS	wtr
		ovoD2/Bal		11.9	57		90%
N-8 / In(1)dl49, Hw	3C2-3;3E3-4	*ovoD2/Df*	0.357	1.3	78	<0.01	wtr
		ovoD2/Bal		4.3	69		wtr
*N-71h / FM7a**	3C4;3D5	*ovoD2/Df*	0.321	7.8	77	<0.01	wtr
		ovoD2/Bal		4.0	96		wtr
*N-69h9 / FM7a**	3C6;3D1 or D4	*ovoD2/Df*	0.698	11.5	56	<0.01	90%
		ovoD2/Bal		3.3	57		wtr
biDL5 / FM6	3C7-12;4E1-2	*ovoD2/Df*	0.375	4.7	46	<0.01	wtr
		ovoD2/Bal		3.0	57		wtr
*dm75e19 / FM7c**	3C11;3E4	*ovoD2/Df*	0.449	0.5	42	<0.01	wtr
		ovoD2/Bal		2.3	45		wtr
GA102 / FM7c	3D4-5;3F7-8	*ovoD2/Df*	0.333	1.5	66	<0.01	wtr
		ovoD2/Bal		3.1	54		wtr
*A113 / FM7a**	3D6-E1;4F5	*ovoD2/Df*	0.711	0.0	28	<0.01	10%
		ovoD2/Bal		5.5	97		wtr
rb33 / FM6	3F4;4C15	*ovoD2/Df*	0.079	0.8	68	NS	wtr
		ovoD2/Bal		1.2	72		wtr
rb1 / FM6	3F6-4A1;4C7-8	*ovoD2/Df*	0.229	10.3	37	NS	wtr
		ovoD2/Bal		12.5	41		90%
rb46 / FM7	4A3-6;4C6-7	*ovoD2/Df*	0.026	0.4	66	NS	10%
		ovoD2/Bal		0.4	43		10%
RC40 / FM7	4B1;4F1	*ovoD2/Df*	0.542	0.0	24	<0.01	10%
		ovoD2/Bal		1.2	24		wtr
biD2 / FM7c	4B6-C1;4D7-E1	*ovoD2/Df*	0.322	9.0	87	<0.01	wtr
		ovoD2/Bal		5.8	99		wtr
GA56 / FM7c	4C5-6;4D1	*ovoD2/Df*	0.038	1.8	60	NS	wtr
		ovoD2/Bal		2.4	41		wtr
rb13 / FM6	4C5-6;4D3-E1	*ovoD2/Df*	0.537	3.6	76	<0.01	wtr
		ovoD2/Bal		8.3	86		wtr
C149 / FM6	5A8-9;5C5-6	*ovoD2/Df*	0.324	9.3	80	<0.01	wtr
		ovoD2/Bal		14.0	83		90%
*C149 / FM6**		*ovoD2/Df*	0.803	1.0	68	<0.01	wtr
		ovoD2/Bal		9.4	73		wtr
N73 / FM6	5C2;5D5-6	*ovoD2/Df*	0.667	3.1	110	<0.01	wtr
		ovoD2/Bal		11.6	113		90%
*N73 / FM6**		*ovoD2/Df*	0.885	0.4	53	<0.01	wtr
		ovoD2/Bal		10.5	52		wtr
JF5 / FM7	5E3-5;5E8	*ovoD2/Df*	0.216	2.2	50	NS	wtr
		ovoD2/Bal		1.0	52		wtr
*JF5 / FM7c**		*ovoD2/Df*	0.160	0.1	61	NS	10%

		ovoD2/Bal		0.6	62		wtr
G4e[L]H24i[R]/ FM7	5E3-8;6B	*ovoD2/Df*	0.433	1.3	65	<0.01	wtr
		ovoD2/Bal		5.2	60		wtr
Sxl-bt/Binsinscy	6E2;7A6	*ovoD2/Df*	0.354	10.9	104	<0.01	90%
		ovoD2/Bal		6.5	103		wtr
*Sxl-bt / FM7a**		*ovoD2/Df*	0.478	9.6	77	<0.01	wtr
		ovoD2/Bal		4.2	62		wtr
HA32 / FM7c	6E4-5;7A6	*ovoD2/Df*	0.146	0.6	41	NS	wtr
		ovoD2/Bal		1.1	44		wtr
*HA32 / FM6**		*ovoD2/Df*	0.233	8.6	58	NS	wtr
		ovoD2/Bal		10.7	53		wtr
*ct-J4 / FM7a**	7A2-3;7C1	*ovoD2/Df*	0.102	4.9	58	NS	wtr
		ovoD2/Bal		5.0	85		wtr
ct268-42/FM4	7A5-6;7B8-C1	*ovoD2/Df*	0.029	1.2	68	NS	wtr
		ovoD2/Bal		1.4	98		wtr
ct4b1/Binsn	7B2-4;7C3-4	*ovoD2/Df*	0.096	0.3	47	NS	10%
		ovoD2/Bal		0.1	63		10%
*C128 / FM6**	7D1;7D5-6	*ovoD2/Df*	0.437	6.4	103	<0.01	wtr
		ovoD2/Bal		12.1	65		90%
*C128 / FM7a**		*ovoD2/Df*	0.297	6.3	68	<0.01	wtr
		ovoD2/Bal		3.9	66		wtr
HA11 / FM7	7D13-14;7D22	*ovoD2/Df*	0.068	0.5	90	NS	wtr
		ovoD2/Bal		0.8	78		wtr
RA2 / FM7c	7D10;8A4-5	*ovoD2/Df*	0.393	0.0	112	<0.01	10%
		ovoD2/Bal		1.0	107		wtr
KA14 / FM7c	7F1-2;8C6	*ovoD2/Df*	0.945	0.03	89	<0.01	10%
		ovoD2/Bal		9.9	91		wtr
*KA14 / FM6**		*ovoD2/Df*	0.900	0.0	60	<0.01	10%
		ovoD2/Bal		7.7	50		wtr
C52 / FM6	8E;9C-D	*ovoD2/Df*	0.199	9.6	30	NS	wtr
		ovoD2/Bal		12.3	64		90%
ras217 / FM6	9A;9E7-8	*ovoD2/Df*	0.855	0.2	69	<0.01	10%
		ovoD2/Bal		10.1	69		wtr
*ras217 / FM7a**		*ovoD2/Df*	0.501	0.2	88	<0.01	10%
		ovoD2/Bal		3.5	78		wtr
v-L15 / FM6	9B1-2;10A1-2	*ovoD2/Df*	0.385	4.9	102	<0.01	wtr
		ovoD2/Bal		9.8	119		wtr
N110 / FM6	9B3-4;9D1-2	*ovoD2/Df*	0.210	7.5	62	NS	wtr
		ovoD2/Bal		9.6	62		wtr
HC133 / FM7c	9B9-10;9E-F	*ovoD2/Df*	0.590	3.8	79	<0.01	wtr
		ovoD2/Bal		9.4	72		wtr
sbr1,B/FM6,v	9B9-10;9F13-A1	*ovoD2/Df*	0.935	0.4	60	<0.01	10%
		ovoD2/Bal		13.9	62		90%
sbr1,B/FM7a,v*		*ovoD2/Df*	0.374	0.3	87	<0.01	10%
		ovoD2/Bal		1.9	84		wtr
v-L11 / ? B	9C4;10A1-2	*ovoD2/Df*	0.724	1.1	44	<0.01	wtr
		ovoD2/Bal		6.9	43		wtr
v-M1 / FM6	9D3;10A1-2	*ovoD2/Df*	0.711	0.5	54	<0.01	wtr

		ovoD2/Bal		6.7	59		wtr
ras59 / FM6	9E1;9F10-11	*ovoD2/Df*	0.780	0.0	66	<0.01	10%
		ovoD2/Bal		3.6	50		wtr
*ras59 / FM7a**		*ovoD2/Df*	0.301	0.9	66	<0.01	wtr
		ovoD2/Bal		2.6	64		wtr
ras-P14 / FM6	9E1-2;9F3-4	*ovoD2/Df*	0.541	5.5	62	<0.01	wtr
		ovoD2/Bal		11.7	60		90%
*ras-P14 / FM7a**		*ovoD2/Df*	0.117	1.1	64	NS	wtr
		ovoD2/Bal		0.9	71		wtr
ras203 / FM6	9E1-2;9F13	*ovoD2/Df*	0.899	0.3	61	<0.01	10%
		ovoD2/Bal		8.9	59		wtr
*ras203 / FM7a**		*ovoD2/Df*	0.147	0.5	82	NS	wtr
		ovoD2/Bal		0.8	69		wtr
*v-L3 / FM7a**	9F10;10A7-8	*ovoD2/Df*	0.099	5.3	205	NS	wtr
		ovoD2/Bal		4.4	156		wtr
*v-L2 / FM7a**	9F13;10A1	*ovoD2/Df*	0.129	5.2	62	NS	wtr
		ovoD2/Bal		5.6	62		wtr
RA37 / FM7c	10A6;10B15-17	*ovoD2/Df*	0.128	4.1	58	NS	wtr
		ovoD2/Bal		4.8	60		wtr
*KA7 / FM7a**	10A9;10F6-7	*ovoD2/Df*	0.114	2.0	45	NS	wtr
		ovoD2/Bal		1.8	65		wtr
*N71 / FM7a**	10B3 or B2-8; 10D3-8	*ovoD2/Df*	0.310	1.0	21	NS	wtr
		ovoD2/Bal		1.6	46		wtr
*N71 / FM6**		*ovoD2/Df*	0.296	7.2	39	NS	wtr
		ovoD2/Bal		10.5	67		wtr
HA85 / FM7c	10C1-2;11A1-2	*ovoD2/Df*	0.317	5.8	48	<0.01	wtr
		ovoD2/Bal		4.1	65		wtr
m259-4 / FM4	10C2-3;10E1-2	*ovoD2/Df*	0.040	0.6	75	NS	wtr
		ovoD2/Bal		0.5	75		wtr
M-13 / FM7c	10D;11A3-5	*ovoD2/Df*	0.216	1.9	54	NS	wtr
		ovoD2/Bal		0.9	62		wtr
KA6 / FM7c	10E1;11A7-8	*ovoD2/Df*	0.168	8.2	61	NS	wtr
		ovoD2/Bal		6.5	63		wtr
*RA47 / FM7a**	10F1;10F9-10	*ovoD2/Df*	0.513	7.5	67	<0.01	wtr
		ovoD2/Bal		3.2	63		wtr
N105 / FM6	10F7;11D1	*ovoD2/Df*	0.732	2.0	131	<0.01	wtr
		ovoD2/Bal		9.8	129		wtr
KA10 / FM7c	11A1;11A7-8	*ovoD2/Df*	0.270	5.8	69	<0.05	wtr
		ovoD2/Bal		7.9	68		wtr
JA26 / FM7c	11A1;11D-E	*ovoD2/Df*	0.329	3.0	50	<0.01	wtr
		ovoD2/Bal		1.0	55		wtr
HF368 / FM7c	11A2;11B9	*ovoD2/Df*	0.519	8.3	79	<0.01	wtr
		ovoD2/Bal		4.1	82		wtr
*HF368 / FM7a**		*ovoD2/Df*	0.580	6.9	68	<0.01	wtr
		ovoD2/Bal		2.3	66		wtr
wy26 / FM7	11B17-C1; 11E9-10	*ovoD2/Df*	0.940	0.1	84	<0.01	10%
		ovoD2/Bal		8.6	84		wtr
N12, ras v/FM6	11D1-2;11F1-2	*ovoD2/Df*	0.115	6.7	77	NS	wtr
		ovoD2/Bal		7.2	70		wtr
C246 / FM6	11D-E;12A1-2	*ovoD2/Df*	0.223	12.7	40	NS	90%
		ovoD2/Bal		15.3	93		90%
g-l,f B/In(1)AM	11F10;12F1	*ovoD2/Df*	0.371	1.4	39	<0.01	wtr
		ovoD2/Bal		0.2	43		10%
g-l,f B/FM7c,v*		*ovoD2/Df*	0.083	1.0	24	NS	wtr
		ovoD2/Bal		0.7	19		wtr

KA9 / FM7c	12E2-3; 12F5-13A1	*ovoD2/Df*	0.164	7.4	184	NS	wtr
		ovoD2/Bal		8.1	119		wtr
*KA9 / FM7c**		*ovoD2/Df*	0.179	0.4	103	NS	10%
		ovoD2/Bal		1.1	92		wtr
*KA9 / FM6**		*ovoD2/Df*	0.534	4.7	71	<0.01	wtr
		ovoD2/Bal		10.9	67		90%
RK3 / FM7	12E2-6;13A6-11	*ovoD2/Df*	0.298	2.9	62	<0.05	wtr
		ovoD2/Bal		5.1	61		wtr
RK5 / FM7	12E9-11; 13A9-B1	*ovoD2/Df*	0.034	1.5	40	NS	wtr
		ovoD2/Bal		1.4	31		wtr
RK4 / FM7	12F5-6;13A9-B1	*ovoD2/Df*	0.432	1.4	59	<0.01	wtr
		ovoD2/Bal		6.0	63		wtr
sd72b / FM7c	13F1;14B1	*ovoD2/Df*	0.097	2.7	48	NS	wtr
		ovoD2/Bal		2.6	53		wtr
*l9 / FM7a**	13F;14E-F	*ovoD2/Df*	0.371	0.0	100	<0.01	10%
		ovoD2/Bal		0.9	70		wtr
*r-D1 / FM7a**	14B6;15A2 or 14C2-4;15B2	*ovoD2/Df*	0.095	6.0	70	NS	wtr
		ovoD2/Bal		5.1	88		wtr
*B / FM6**	15F9;16A7	*ovoD2/Df*	0.233	5.9	60	NS	wtr
		ovoD2/Bal		9.4	60		wtr
N19 / FM6	17A1;18A2	*ovoD2/Df*	0.617	2.0	28	<0.01	wtr
		ovoD2/Bal		7.6	45		wtr
*N19 / FM6**		*ovoD2/Df*	0.402	3.4	37	<0.01	wtr
		ovoD2/Bal		7.9	67		wtr
E160.2 / FM7	17B2-C1;18A	*ovoD2/Df*	0.416	3.2	77	<0.01	wtr
		ovoD2/Bal		8.8	77		wtr
E128 / FM7	17C;18A	*ovoD2/Df*	0.646	0.7	138	<0.01	wtr
		ovoD2/Bal		6.8	153		wtr
JA27 / FM7c	18A5;20A	*ovoD2/Df*	0.846	0.9	39	<0.01	wtr
		ovoD2/Bal		8.7	39		wtr
*JA27 / FM6**		*ovoD2/Df*	0.925	0.7	46	<0.01	wtr
		ovoD2/Bal		11.4	67		90%
HF396 / FM7c	18E1-2;20	*ovoD2/Df*	0.362	4.5	55	<0.01	wtr
		ovoD2/Bal		2.7	68		wtr
*mal3 / Fm7a**	19A1-2;20E-F	*ovoD2/Df*	0.617	11.8	126	<0.01	90%
		ovoD2/Bal		4.9	196		wtr
mal3 / FM7c		*ovoD2/Df*	0.379	7.3	54	<0.01	wtr
		ovoD2/Bal		3.1	67		wtr
16-3-22 / FM6	19D1;20A2	*ovoD2/Df*	0.155	9.6	59	NS	wtr
		ovoD2/Bal		10.7	70		wtr
B57 / FM6	19E1-2;19F1	*ovoD2/Df*	0.049	0.3	81	NS	10%
		ovoD2/Bal		0.2	55		10%
GA37 / FM7	19E2;19F6	*ovoD2/Df*	0.136	10.5	64	NS	wtr
		ovoD2/Bal		9.4	66		wtr
JA21 / FM7c	19E5-6;20	*ovoD2/Df*	0.091	1.9	70	NS	wtr
		ovoD2/Bal		1.6	63		wtr
DCB1-35b / FM6	19F1-2;20E-F	*ovoD2/Df*	0.366	4.7	74	<0.01	wtr
		ovoD2/Bal		8.5	72		
JC4 / FM7c	20A1;20E-F	*ovoD2/Df*	0.296	1.8	42	NS	wtr
		ovoD2/Bal		4.8	41		wtr
*JC4 / FM6**		*ovoD2/Df*	0.532	4.1	56	<0.01	wtr
		ovoD2/Bal		11.0	63		90%

Table 2. *ovo*^{D2} heterozygotes with reduced doses of regions on the left arm of chromosome 2.

Deficiency/ Balancer	Cytology	Progeny	oocytes/ovary mean	oocytes/ovary No	Statistics T1	Statistics P	Rank
TE75w+ / CyO	tip;21B4-6	*ovoD2/+;Df/+*	4.5	67	0.565	<0.01	wtr
		ovoD2/+;Bal/+	1.3	66			wtr
*TE75w+ / Gla**		*ovoD2/+;Df/+*	4.2	38	0.092	NS	wtr
		ovoD2/+;Bal/+	3.2	48			wtr
*TE75w+ / CyO**		*ovoD2/+;Df/+*	2.8	81	0.301	<0.01	wtr
		ovoD2/+;Bal/+	0.7	61			wtr
al / In(2L)Cy	21B8-C1; 21C8-D1	*ovoD2/+;Df/+*	2.8	48	0.447	<0.01	wtr
		ovoD2/+;Bal/+	5.6	59			wtr
S2 / In(2L+2R)Cy	21C6-D1; 22A6-B1	*ovoD2/+;Df/+*	1.6	51	0.259	NS	wtr
		ovoD2/+;Bal/+	1.2	74			wtr
ast1 / Cy	21C7-8;23A1-2	*ovoD2/+;Df/+*	1.8	36	0.069	NS	wtr
		ovoD2/+;Bal/+	1.9	48		wtr	
ast-2 / Cy	21D1-2;22B2-3	*ovoD2/+;Df/+*	1.7	66	0.340	<0.01	wtr
		ovoD2/+;Bal/+	0.5	49			wtr
S3 / SM1	21D2-3; 21F2-22A1	*ovoD2/+;Df/+*	0.4	76	0.462	<0.01	wtr
		ovoD2/+;Bal/+	3.5	78			wtr
*S3 / In(2LR)Cy**		*ovoD2/+;Df/+*	0.7	62	0.479	<0.01	wtr
		ovoD2/+;Bal/+	2.8	65			wtr
*S3 / CyO***		*ovoD2/+;Df/+*	0.1	50	0.020	NS	10%
		ovoD2/+;Bal/+	0.1	50			wtr
dpp59 / CyO	22A;23A(?)	*ovoD2/+;Df/+*	0.4	52	0.236	NS	wtr
		ovoD2/+;Bal/+	1.8	35			wtr
DTD2 Dp DTD48/ Gla	22D4-5;22E2-4 23A1;23B1	*ovoD2/+;Df/+*	1.1	21	0.301	NS	wtr
		ovoD2/+;Bal/+	1.0	41			wtr
edSZ / Cy	24A3-4;24D3-4	*ovoD2/+;Df/+*	4.1	62	0.504	<0.01	wtr
		ovoD2/+;Bal/+	1.0	54			wtr
*edSZ / CyO***		*ovoD2/+;Df/+*	1.4	60	0.402	<0.01	wtr
		ovoD2/+;Bal/+	0.1	62			10%
ed-dp-h1/SM1	24C1-3; 25A1-4	*ovoD2/+;Df/+*	3.7	80	0.570	<0.01	wtr
		ovoD2/+;Bal/+	0.3	60			wtr
dp-h28 /CyRoi	24D8;24F6-7	*ovoD2/+;Df/+*	1.2	36	0.449	<0.01	wtr
		ovoD2/+;Bal/+	0.1	79			10%
dp-h25 / CyRoi	24E2-4;25B2-5	*ovoD2/+;Df/+*	0.8	56	0.107	NS	wtr
		ovoD2/+;Bal/+	0.9	64			wtr
M-zB / SM1	24E2-F1;24F6-7	*ovoD2/+;Df/+*	0.6	43	0.277	NS	wtr
		ovoD2/+;Bal/+	1.6	76			wtr
dp-h19 / CyRoi	24F1-2;24F6-7	*ovoD2/+;Df/+*	0.2	71	0.168	NS	wtr
		ovoD2/+;Bal/+	0.4	75			wtr
dp-h24 / CyRoi	24F4;25A1-4	*ovoD2/+;Df/+*	1.7	52	0.121	NS	wtr
		ovoD2/+;Bal/+	1.6	53			wtr
tkvSz-2 / SM5	25D2-4;25D6-E1	*ovoD2/+;Df/+*	10.5	61	0.584	<0.01	90%
		ovoD2/+;Bal/+	3.8	26			wtr

cl-h3 / CyRoi	25D2-4;25F1-2	*ovoD2/+;Df/+*	0.7	60	0.297	<0.05	wtr
		ovoD2/+;Bal/+	0.1	58			10%
cl-h2 / CyRoi	25D6;25E4-5	*ovoD2/+;Df/+*	0.7	53	0.262	NS	wtr
		ovoD2/+;Bal/+	1.1	48			wtr
cl1 / Cy	25D7-E1;	*ovoD2/+;Df/+*	0.1	122	0.905	<0.01	10%
	25E6-F3	*ovoD2/+;Bal/+*	8.5	63			90%
cl7 / Cy	25D7-E1;26A7-8	*ovoD2/+;Df/+*	7.8	70	0.377	<0.01	90%
		ovoD2/+;Bal/+	4.2	71			wtr
*cl7 / CyO***		*ovoD2/+;Df/+*	1.1	49	0.371	<0.01	wtr
		ovoD2/+;Bal/+	0.2	50			wtr
GpdhA / CyO	25D7-E1;26A8-9	*ovoD2/+;Df/+*	0.3	69	0.033	NS	wtr
		ovoD2/+;Bal/+	0.3	85			wtr
*GpdhA/In(2LR)Cy**		*ovoD2/+;Df/+*	2.4	52	0.281	NS	wtr
		ovoD2/+;Bal/+	4.4	62			wtr
2802 / CyO	25F2-3;	*ovoD2/+;Df/+*	8.8	60	0.704	<0.01	90%
	25F4-26A1	*ovoD2/+;Bal/+*	0.9	71			wtr
*2802 / In(2LR)Cy**		*ovoD2/+;Df/+*	6.4	60	0.150	NS	wtr
		ovoD2/+;Bal/+	5.4	60			wtr
*2802 / CyO***		*ovoD2/+;Df/+*	0.4	60	0.100	NS	wtr
		ovoD2/+;Bal/+	0.1	60			wtr
spdX4 / CyO	27E;28C	*ovoD2/+;Df/+*	3.0	73	0.438	<0.01	wtr
		ovoD2/+;Bal/+	0.6	73			wtr
wgCX3 / CyO	28A?-?	*ovoD2/+;Df/+*	8.8	59	0.743	<0.01	90%
		ovoD2/+;Bal/+	0.2	58			wtr
*wgCX3 / In(2LR)Cy**		*ovoD2/+;Df/+*	6.9	60	0.108	NS	90%
		ovoD2/+;Bal/+	9.4	24			90%
30A;C / Cy	30A;30C	*ovoD2/+;Df/+*	10.5	64	0.603	<0.01	90%
		ovoD2/+;Bal/+	3.7	70			wtr
*30A;C / In(2LR)Cy**		*ovoD2/+;Df/+*	5.3	57	0.354	<0.01	wtr
		ovoD2/+;Bal/+	3.2	51			wtr
*30A;C / CyO***		*ovoD2/+;Df/+*	2.2	52	0.315	<0.05	wtr
		ovoD2/+;Bal/+	1.2	50			wtr
J-der 2 / Cy	31B;32A1-2	*ovoD2/+;Df/+*	4.0	54	0.138	NS	wtr
		ovoD2/+;Bal/+	3.3	55			wtr
J-der 27 / Cy	31D;31F3	*ovoD2/+;Df/+*	6.1	66	0.245	NS	wtr
		ovoD2/+;Bal/+	3.8	58			wtr
*J-der 27 / CyO***		*ovoD2/+;Df/+*	2.3	58	0.474	<0.01	wtr
		ovoD2/+;Bal/+	0.5	48			wtr
Prl / Cy	32F1-3;33F1-2	*ovoD2/+;Df/+*	0.4	46	0.117	NS	wtr
		ovoD2/+;Bal/+	0.8	45			wtr
escP3-0/CyRoi	33A1-2;33B1-2	*ovoD2/+;Df/+*	1.6	58	0.340	<0.01	wtr
		ovoD2/+;Bal/+	3.5	62			wtr
escP2-0/CyRoi	33A1-2;33E	*ovoD2/+;Df/+*	0.2	63	0.032	NS	wtr
		ovoD2/+;Bal/+	0.2	63			wtr
esc10 / CyO	33A8-B1;33B2-3	*ovoD2/+;Df/+*	1.4	63	0.250	NS	wtr
		ovoD2/+;Bal/+	0.7	66			wtr

prd1.7 / Cy	33B3-7;34A1-2	*ovoD2/+;Df/+*	8.0	53	0.858	<0.01	90%
		ovoD2/+;Bal/+	0.6	60			wtr
*prd1.7/ In(2LR)Cy**		*ovoD2/+;Df/+*	6.5	60	0.117	NS	wtr
		ovoD2/+;Bal/+	7.3	60			90%
64j / Cy	34D1-2;35B9-C1	*ovoD2/+;Df/+*	2.1	42	0.221	NS	wtr
		ovoD2/+;Bal/+	2.8	62			wtr
TE35A-5 / CyO	34D2;35C1	*ovoD2/+;Df/+*	12.4	71	0.164	NS	90%
		ovoD2/+;Bal/+	11.2	64			90%
b75 / Cy	34D4-6;35E5-6	*ovoD2/+;Df/+*	12.8	69	0.807	<0.01	90%
		ovoD2/+;Bal/+	2.8	62			wtr
el80f1 / CyO	34E3;35D7	*ovoD2/+;Df/+*	0.2	68	0.779	<0.01	wtr
		ovoD2/+;Bal/+	5.3	68			wtr
75C / Cy	35A1-2;35D4-7	*ovoD2/+;Df/+*	0.3	57	0.503	<0.01	wtr
		ovoD2/+;Bal/+	2.4	56			wtr
C75RL / CyO	35A2;35B3 + Dp 26D2;27D1	*ovoD2/+;Df/+*	0.2	74	0.697	<0.01	wtr
		ovoD2/+;Bal/+	2.7	77			wtr
*W / In(2LR)Cy**	35A2-3;35B3-5	*ovoD2/+;Df/+*	10.1	81	0.396	<0.01	90%
		ovoD2/+;Bal/+	5.3	70			wtr
*W / CyO***		*ovoD2/+;Df/+*	2.1	55	0.345	<0.01	wtr
		ovoD2/+;Bal/+	0.6	55			wtr
do1 / Gla	35B1-2;35D1-2	*ovoD2/+;Df/+*	1.6	74	0.410	<0.01	wtr
		ovoD2/+;Bal/+	0.1	67			wtr
A446 / Cy	35B1-3;35E6-F2	*ovoD2/+;Df/+*	0.3	53	0.058	NS	wtr
		ovoD2/+;Bal/+	0.3	60			wtr
osp29 / Cy	35B2-3;35E6	*ovoD2/+;Df/+*	6.4	63	0.493	<0.01	wtr
		ovoD2/+;Bal/+	2.9	66			wtr
*osp29 / CyO***		*ovoD2/+;Df/+*	0.7	60	0.300	<0.05	wtr
		ovoD2/+;Bal/+	0.1	60			wtr
H20 / Cy	36A8-9;36E1-2	*ovoD2/+;Df/+*	3.8	122	0.142	NS	wtr
		ovoD2/+;Bal/+	5.0	130			wtr
TW137 / Cy	36C2-4;37B9-C1	*ovoD2/+;Df/+*	3.8	86	0.286	<0.01	wtr
		ovoD2/+;Bal/+	5.2	96			wtr
TW50 / Cy	36E4-F1;38A6-7	*ovoD2/+;Df/+*	0.7	47	0.354	<0.01	wtr
		ovoD2/+;Bal/+	0.1	60			10%
*TW50 / CyO***		*ovoD2/+;Df/+*	0.5	64	0.228	NS	wtr
		ovoD2/+;Bal/+	0.1	95			10%
*TW50 / Gla**		*ovoD2/+;Df/+*	0.7	88	0.076	NS	wtr
		ovoD2/+;Bal/+	1.1	73			wtr
E71 / CyO	36F2-6;37C6-D1	*ovoD2/+;Df/+*	0.1	102	0.559	<0.01	10%
		ovoD2/+;Bal/+	1.8	130			wtr
TW158 / CyO	37B2-8;37E2-F4	*ovoD2/+;Df/+*	0.4	64	0.248	NS	wtr
		ovoD2/+;Bal/+	1.5	69			wtr
pr-A16 / CyO	37B2-12;38D2-5	*ovoD2/+;Df/+*	2.3	66	0.079	NS	wtr
		ovoD2/+;Bal/+	1.6	68			wtr
TW130 / CyO	37B9-C1;37D1-2	*ovoD2/+;Df/+*	0.4	60	0.619	<0.01	wtr
		ovoD2/+;Bal/+	3.3	61			wtr
*TW130 / CyO**		*ovoD2/+;Df/+*	0.0	88	0.198	NS	10%
		ovoD2/+;Bal/+	0.4	86			wtr

*TW130 / Gla**		*ovoD2/+;Df/+*	0.0	120	0.089	NS	10%
		ovoD2/+;Bal/+	0.2	101			wtr
VA16 / CyO	37B9-C1; 37F5-38A1	*ovoD2/+;Df/+*	0.1	80	0.313	<0.01	10%
		ovoD2/+;Bal/+	0.7	80			wtr
VA12 / CyO	37C2-5;38B2-C1	*ovoD2/+;Df/+*	1.7	60	0.333	<0.01	wtr
		ovoD2/+;Bal/+	0.3	60			wtr
Sd77 / CyO	37D1-2;38C1-2	*ovoD2/+;Df/+*	7.0	60	0.698	<0.01	90%
		ovoD2/+;Bal/+	0.6	58			wtr
pr76, Sco / CyO	37D;38E	*ovoD2/+;Df/+*	12.1	60	0.983	<0.01	90%
		ovoD2/+;Bal/+	0.4	60			wtr
E55 / Cy	37D2-E1; 37F5-38A1	*ovoD2/+;Df/+*	12.1	67	0.821	<0.01	90%
		ovoD2/+;Bal/+	2.7	73			wtr
*E55 / In(2LR)Cy**		*ovoD2/+;Df/+*	9.6	60	0.402	<0.01	90%
		ovoD2/+;Bal/+	5.6	52			wtr
*E55 / CyO***		*ovoD2/+;Df/+*	3.0	60	0.650	<0.01	wtr
		ovoD2/+;Bal/+	0.1	60			wtr
TW2 / Cy	37D2-E1;38E6-9	*ovoD2/+;Df/+*	0.1	61	0.005	NS	10%
		ovoD2/+;Bal/+	0.1	80			wtr
TW9 / CyO	37E2-F4; 38A6-C1	*ovoD2/+;Df/+*	9.6	62	0.907	<0.01	90%
		ovoD2/+;Bal/+	1.0	71			wtr
*TW9 / CyO**		*ovoD2/+;Df/+*	8.9	36	0.849	<0.01	90%
		ovoD2/+;Bal/+	0.2	82			wtr
*TW9 / Gla**		*ovoD2/+;Df/+*	3.6	83	0.608	<0.01	wtr
		ovoD2/+;Bal/+	0.3	80			wtr
TW150 / CyO	37F5-38A1; 38B2-C1	*ovoD2/+;Df/+*	9.3	66	0.923	<0.01	90%
		ovoD2/+;Bal/+	0.6	65			wtr
*TW150 / Gla**		*ovoD2/+;Df/+*	9.3	77	0.548	<0.01	90%
		ovoD2/+;Bal/+	2.0	72			wtr
*TW150 / CyO**		*ovoD2/+;Df/+*	4.1	87	0.639	<0.01	wtr
		ovoD2/+;Bal/+	0.2	65			wtr
TW84 / CyO	37F5-38A1; 39D3-E1	*ovoD2/+;Df/+*	6.6	65	0.923	<0.01	wtr
		ovoD2/+;Bal/+	0.1	79			wtr
*TW84 / Gla**		*ovoD2/+;Df/+*	7.4	66	0.804	<0.01	90%
		ovoD2/+;Bal/+	0.2	70			wtr
*TW84 / CyO**		*ovoD2/+;Df/+*	2.7	89	0.434	<0.01	wtr
		ovoD2/+;Bal/+	0.5	75			wtr
TW65 / Cy	37F5-38A1 39E2-F1	*ovoD2/+;Df/+*	4.1	63	0.508	<0.01	wtr
		ovoD2/+;Bal/+	1.9	58			wtr
TW161 / CyO	38A6-B1; 40A4-B1	*ovoD2/+;Df/+*	7.9	114	0.757	<0.01	90%
		ovoD2/+;Bal/+	1.1	107			wtr
TW1 / CyO	38A7-B1; 39C2-3	*ovoD2/+;Df/+*	0.8	132	0.369	NS	wtr
		ovoD2/+;Bal/+	3.3	125			wtr
DS6 /CyO or SM6a	38F5; 39E7-F1	*ovoD2/+;Df/+*	3.4	50	0.146	NS	wtr
		ovoD2/+;Bal/+	2.3	45			wtr
PR31 / Cy	2L hetero-chromatin	*ovoD2/+;Df/+*	5.6	66	0.333	<0.01	wtr
		ovoD2/+;Bal/+	9.1	47			90%

Table 3. *ovo*^{D2} heterozygotes with reduced doses of regions on the right arm of chromosome 2.

Deficiency / Balancer	Cytology	Progeny	oocytes/ovary mean	oocytes/ovary No	Statistics T1	Statistics P	Rank
M-S2-4 / SM1	41A	*ovoD2/+;Df/+*	0.4	39	0.885	<0.01	wtr
		ovoD2/+;Bal/+	5.8	52			wtr
M-S2-8 / SM1	41A	*ovoD2/+;Df/+*	0.1	92	0.473	<0.01	10%
		ovoD2/+;Bal/+	1.7	91			wtr
M-S2-10 / SM5	41A	*ovoD2/+;Df/+*	0.4	48	0.164	NS	wtr
		ovoD2/+;Bal/+	0.1	70			10%
rl10a/ In(2LR)Cy	41A	*ovoD2/+;Df/+*	1.0	47	0.506	<0.01	wtr
		ovoD2/+;Bal/+	0.04	78			10%
rl10b/ In(2L)Cy	41A	*ovoD2/+;Df/+*	3.1	69	0.668	<0.01	wtr
		ovoD2/+;Bal/+	0.2	61			wtr
*rl10b/ In(2LR)Cy**		*ovoD2/+;Df/+*	10.0	59	0.428	<0.01	90%
		ovoD2/+;Bal/+	6.3	20			wtr
cn88b / SM5	42A;42E	*ovoD2/+;Df/+*	0.3	68	0.750	<0.01	wtr
		ovoD2/+;Bal/+	6.0	72			wtr
pk78s / Cy	42C1-7; 43F5-8	*ovoD2/+;Df/+*	0.6	58	0.154	NS	wtr
		ovoD2/+;Bal/+	0.4	24			wtr
cn9 / CyRoi	42E;44C	*ovoD2/+;Df/+*	1.1	68	0.144	NS	wtr
		ovoD2/+;Bal/+	2.1	62			wtr
pk78k / Cy	42E3;43C3	*ovoD2/+;Df/+*	7.4	85	0.547	<0.01	90%
		ovoD2/+;Bal/+	1.6	78			wtr
*pk78k / In(2LR)Cy**		*ovoD2/+;Df/+*	5.7	60	0.200	NS	wtr
		ovoD2/+;Bal/+	4.1	60			wtr
*pk78k / CyO***		*ovoD2/+;Df/+*	1.7	50	0.160	NS	wtr
		ovoD2/+;Bal/+	0.5	50			wtr
P32 / Cy	43A3;43F6	*ovoD2/+;Df/+*	0.3	48	0.183	NS	wtr
		ovoD2/+;Bal/+	0.8	51			wtr
ST1 / Cy	43B3-5; 43E1-8	*ovoD2/+;Df/+*	6.1	75	0.237	NS	wtr
		ovoD2/+;Bal/+	3.8	84			wtr
cn83c / SM5	43C5-D1; 44B6-C1	*ovoD2/+;Df/+*	2.2	63	0.441	<0.01	wtr
		ovoD2/+;Bal/+	0.2	54			wtr
CA53 / CyO	43E6;44B6	*ovoD2/+;Df/+*	9.1	62	0.233	NS	90%
		ovoD2/+;Bal/+	7.2	62			90%
B5 / CyO	46A;46C	*ovoD2/+;Df/+*	9.4	70	0.886	<0.01	90%
		ovoD2/+;Bal/+	0.4	70			wtr
eve1.27 / CyO	46C3-4; 46C9-11	*ovoD2/+;Df/+*	5.9	91	0.669	<0.01	wtr
		ovoD2/+;Bal/+	0.6	89			wtr
*eve1.27 / CyO**		*ovoD2/+;Df/+*	1.9	85	0.253	<0.01	wtr
		ovoD2/+;Bal/+	0.3	102			wtr
*eve1.27 / CyO***		*ovoD2/+;Df/+*	3.4	60	0.416	<0.01	wtr
		ovoD2/+;Bal/+	0.4	60			wtr
X1 / CyO	46C;46E-F	*ovoD2/+;Df/+*	6.3	62	0.768	<0.01	wtr
		ovoD2/+;Bal/+	0.6	70			wtr
en-A / CyO	47D3;48A5-6	*ovoD2/+;Df/+*	0.8	59	0.223	NS	wtr

		ovoD2/+;Bal/+	0.3	60			wtr
en-B / CyO	47E3-6; 48A4-B2	*ovoD2/+;Df/+*	0.1	27	0.003	NS	10%
		ovoD2/+;Bal/+	0.1	26			10%
en30 / SM5; Dp(2;3)en28	48A3-4;4 8C6-8	*ovoD2/+;Df/+*	0.8	54	0.162	NS	wtr
		ovoD2/+;Bal/+	0.3	82			wtr
vg135 / CyO	49A;49D-E	*ovoD2/+;Df/+*	0.0	88	0.387	<0.01	10%
		ovoD2/+;Bal/+	0.7	62			wtr
vgC / SM5	49A4-13; 49E7-F1	*ovoD2/+;Df/+*	0.0	80	0.667	<0.01	10%
		ovoD2/+;Bal/+	2.0	51			wtr
vgD / Cy	49C1-2; 49E2-6	*ovoD2/+;Df/+*	1.4	86	0.608	<0.01	wtr
		ovoD2/+;Bal/+	6.4	62			wtr
*vgD / CyO***		*ovoD2/+;Df/+*	1.6	50	0.320	<0.05	wtr
		ovoD2/+;Bal/+	0.5	50			wtr
vg104 / SM5	49C4;49F13	*ovoD2/+;Df/+*	0.4	65	0.265	NS	wtr
		ovoD2/+;Bal/+	1.7	59			wtr
vg107 / SM5	<49Da-49Ea	*ovoD2/+;Df/+*	0.2	70	0.586	<0.01	wtr
		ovoD2/+;Bal/+	1.9	70			wtr
vg133 / SM5	<49Da-49Dc	*ovoD2/+;Df/+*	0.8	60	0.020	NS	wtr
		ovoD2/+;Bal/+	0.8	39			wtr
vg33 / SM5	49D;50A	*ovoD2/+;Df/+*	1.1	59	0.103	NS	wtr
		ovoD2/+;Bal/+	1.7	64			wtr
vgB / SM5	49D3-4; 49F15-50A3	*ovoD2/+;Df/+*	1.2	97	0.235	NS	wtr
		ovoD2/+;Bal/+	1.7	44			wtr
vg136 / SM5	vg-49Ea	*ovoD2/+;Df/+*	0.8	66	0.153	NS	wtr
		ovoD2/+;Bal/+	0.8	63			wtr
L-R+48 / Cy	50F-51A1; 51B	*ovoD2/+;Df/+*	2.9	76	0.175	NS	wtr
		ovoD2/+;Bal/+	3.4	79			wtr
*L-R+48 / CyO**		*ovoD2/+;Df/+*	0.6	50	0.140	NS	wtr
		ovoD2/+;Bal/+	0.3	50			wtr
trix / Cy	51A1-2;51B6	*ovoD2/+;Df/+*	1.2	48	0.615	<0.01	wtr
		ovoD2/+;Bal/+	5.1	32			wtr
JP1 / CyO	51C3;52F5-9	*ovoD2/+;Df/+*	0.9	68	0.142	NS	wtr
		ovoD2/+;Bal/+	1.7	62			wtr
XTE18 / Cy	51E3; 52C9-D1	*ovoD2/+;Df/+*	0.3	112	0.071	NS	wtr
		ovoD2/+;Bal/+	0.1	92			10%
WMG / Gla	52A;52D	*ovoD2/+;Df/+*	0.3	44	0.024	NS	wtr
		ovoD2/+;Bal/+	0.3	48			wtr
JP5 / CyO	52A13-B3; 52F10-11	*ovoD2/+;Df/+*	1.1	36	0.367	<0.01	wtr
		ovoD2/+;Bal/+	0.1	60			10%
JP8 / CyO	52F5-9; 52F10-53A1	*ovoD2/+;Df/+*	11.8	72	0.427	<0.01	90%
		ovoD2/+;Bal/+	6.3	77			wtr
Pcl7B / CyO	54E8-F1; 55B9-C1	*ovoD2/+;Df/+*	0.0	90	0.897	<0.01	10%
		ovoD2/+;Bal/+	4.1	58			wtr
Pcl11B / CyO	54F6-55A1;	*ovoD2/+;Df/+*	0.0	73	0.278	<0.01	10%

	55C1-3	*ovoD2/+;Bal/+*	0.4	72			wtr
Pc4 / CyO	55A;55F	*ovoD2/+;Df/+*	0.0	144	0.202	<0.01	10%
		ovoD2/+;Bal/+	0.4	134			wtr
*Pc4 / In(2LR)Cy**		*ovoD2/+;Df/+*	0.0	78	0.783	<0.01	10%
		ovoD2/+;Bal/+	6.6	60			90%
PuD17 / SM1	57B5;58B1-2	*ovoD2/+;Df/+*	0.5	76	0.097	NS	wtr
		ovoD2/+;Bal/+	0.8	105			wtr
Pl3 / Cy	57B20;	*ovoD2/+;Df/+*	0.6	46	0.092	NS	wtr
	57D8-9	*ovoD2/+;Bal/+*	0.3	47			wtr
bwD23 / Cy	59D4-5;	*ovoD2/+;Df/+*	6.6	95	0.346	<0.01	90%
	60A1-2	*ovoD2/+;Bal/+*	3.4	34			wtr
*bwD23 / CyO**		*ovoD2/+;Df/+*	0.3	54	0.067	NS	wtr
		ovoD2/+;Bal/+	0.2	60			wtr
*bwD23 / Gla**		*ovoD2/+;Df/+*	1.9	60	0.170	NS	wtr
		ovoD2/+;Bal/+	0.9	68			wtr
bwS46 / Cy	59D8-11;	*ovoD2/+;Df/+*	11.6	99	0.592	<0.01	90%
	60A7	*ovoD2/+;Bal/+*	4.8	72			wtr
*bwS46 CyO**		*ovoD2/+;Df/+*	2.5	63	0.566	<0.01	wtr
		ovoD2/+;Bal/+	0.2	60			wtr
*bwS46 / Gla**		*ovoD2/+;Df/+*	1.6	64	0.369	<0.01	wtr
		ovoD2/+;Bal/+	0.2	60			wtr
bw5/In(2LR)Cy	59D10-E1;	*ovoD2/+;Df/+*	1.4	67	0.304	<0.01	wtr
	59E4-F1	*ovoD2/+;Bal/+*	0.7	62			wtr
Px,S Sp Bl	60B8-10;	*ovoD2/+;Df/+*	0.2	48	0.075	NS	wtr
*L^2 Px/CyO***	60D1-2	*ovoD2/+;Bal/+*	0.04	104			10%
Px, S Sp Bl L^2 Px/		*ovoD2/+;Df/+*	0.1	64	0.313	<0.01	wtr
*Gla**		*ovoD2/+;Bal/+*	0.7	64			wtr
Px2 / SM5	60C5-6;	*ovoD2/+;Df/+*	0.7	40	0.079	NS	wtr
	60D9-10	*ovoD2/+;Bal/+*	0.8	62			wtr
D11-MP / SM6a	60E1-2;	*ovoD2/+;Df/+*	0.5	70	0.392	<0.01	wtr
	60E5-6	*ovoD2/+;Bal/+*	2.8	69			wtr
M-c33a / SM5	60E2-3;	*ovoD2/+;Df/+*	0.5	106	0.765	<0.01	wtr
	60E11-12	*ovoD2/+;Bal/+*	5.7	90			wtr
Kr10 / SM1	60E10-11;	*ovoD2/+;Df/+*	1.3	51	0.307	<0.05	wtr
	60F5	*ovoD2/+;Bal/+*	0.5	60			wtr

Table 4. *ovo^{D2}* heterozygotes with reduced doses of regions on the left arm of chromosome 3.

Deficiency / Balancer	Cytology	Progeny	oocytes/ovary mean	oocytes/ovary No	Statistics T1	Statistics P	Rank
emcE12 / TM2	61A;61D3-4	*ovoD2/+;Df/+*	0.03	96	0.010	NS	10%
		ovoD2/+;Bal/+	0.05	96			10%
Ar12-1 / TM2	61C;61F3	*ovoD2/+;Df/+*	0.03	100	0.209	<0.05	10%
		ovoD2/+;Bal/+	0.4	118			wtr
Ar14 / TM2	61C3-4;62A	*ovoD2/+;Df/+*	0.0	116	0.020	NS	10%
		ovoD2/+;Bal/+	0.03	102			10%

RG5 / TM6B	62A10-13; 63C3-5	*ovoD2/+;Df/+*	2.1	60	0.669	<0.01	wtr
		ovoD2/+;Bal/+	0.1	62			wtr
RG7 / TM6B	62B2-8;62F2-5	*ovoD2/+;Df/+*	0.1	80	0.023	NS	wtr
		ovoD2/+;Bal/+	0.04	74			10%
R / TM6C	62B7;62B12	*ovoD2/+;Df/+*	2.2	60	0.483	<0.01	wtr
		ovoD2/+;Bal/+	0.2	60			wtr
GN19 / TM6B	63E6-9;64B2-4	*ovoD2/+;Df/+*	1.7	90	0.346	<0.01	wtr
		ovoD2/+;Bal/+	0.2	68			wtr
X37 / TM6B	63E6-9; 64B14-17	*ovoD2/+;Df/+*	0.4	76	0.048	NS	wtr
		ovoD2/+;Bal/+	0.5	66			wtr
ems13 / TM6B	64B2-4;64E	*ovoD2/+;Df/+*	0.2	58	0.073	NS	wtr
		ovoD2/+;Bal/+	0.1	62			wtr
V65c / TM3	64E;65C-D	*ovoD2/+;Df/+*	0.02	84	0.090	NS	10%
		ovoD2/+;Bal/+	0.2	88			wtr
h-i22 / TM3	66D10-11; 66E1-2	*ovoD2/+;Df/+*	2.5	66	0.085	NS	wtr
		ovoD2/+;Bal/+	1.7	60			wtr
29A6 / TM3	66F5;67B1	*ovoD2/+;Df/+*	7.5	83	0.398	<0.01	90%
		ovoD2/+;Bal/+	2.6	69			wtr
AC1 / TM3	67A;67D	*ovoD2/+;Df/+*	0.3	63	0.098	NS	wtr
		ovoD2/+;Bal/+	0.7	69			wtr
vin2 / TM3	67F2-3;68D6	*ovoD2/+;Df/+*	0.0	48	0.318	NS	10%
		ovoD2/+;Bal/+	0.6	44			wtr
vin5 / TM3	68A2-3;69A1-3	*ovoD2/+;Df/+*	0.2	54	0.616	<0.01	wtr
		ovoD2/+;Bal/+	2.3	55			wtr
vin4 / TM3	68B1-3;68F3-6	*ovoD2/+;Df/+*	6.0	74	0.269	<0.01	90%
		ovoD2/+;Bal/+	8.8	76			90%
vin6 / TM3	68C8-11;69A4-5	*ovoD2/+;Df/+*	8.1	128	0.182	NS	90%
		ovoD2/+;Bal/+	6.7	145			90%
vin7 / TM3	68C8-11;69B4-5	*ovoD2/+;Df/+*	0.8	60	0.050	NS	wtr
		ovoD2/+;Bal/+	0.8	63			wtr
BK9 / TM3	68E;69A1	*ovoD2/+;Df/+*	1.8	70	0.629	<0.01	wtr
		ovoD2/+;Bal/+	0.04	72			10%
fzGF3b / TM6B	70B?;70D6	*ovoD2/+;Df/+*	0.3	70	0.072	NS	wtr
		ovoD2/+;Bal/+	0.1	59			wtr
fzGS1a / TM3	70C6-15;70E4-6	*ovoD2/+;Df/+*	3.8	126	0.265	<0.01	wtr
		ovoD2/+;Bal/+	1.6	132			wtr
fzM21 / TM6	70D2-3;71E4-5	*ovoD2/+;Df/+*	0.02	87	0.090	NS	10%
		ovoD2/+;Bal/+	0.2	79			wtr
fzD21 / TM6B	70D;71F	*ovoD2/+;Df/+*	0.9	73	0.123	NS	wtr
		ovoD2/+;Bal/+	0.2	69			wtr
st-f13 / TM6B	71B1-2;73A3-4	*ovoD2/+;Df/+*	0.4	50	0.104	NS	wtr
		ovoD2/+;Bal/+	0.7	74			wtr

BK10 / TM3	71C;71F	*ovoD2/+;Df/+*	0.03	49	0.013	NS	10%
		ovoD2/+;Bal/+	0.1	77			wtr
th102 / TM3	72B1;72D12	*ovoD2/+;Df/+*	1.1	69	0.237	NS	wtr
		ovoD2/+;Bal/+	2.1	63			wtr
st8P / TM3	72E4;73B4	*ovoD2/+;Df/+*	1.8	61	0.502	<0.01	wtr
		ovoD2/+;Bal/+	0.3	53			wtr
st4 / TM6B	72E5-F1;73B5-7	*ovoD2/+;Df/+*	0.2	60	0.067	NS	wtr
		ovoD2/+;Bal/+	0.1	60			wtr
st7 / TM3	72F3-4;74C3-4	*ovoD2/+;Df/+*	0.2	69	0.424	<0.01	wtr
		ovoD2/+;Bal/+	1.0	59			wtr
81K19 / TM6B	73A3;74F	*ovoD2/+;Df/+*	5.2	44	0.841	<0.01	wtr
		ovoD2/+;Bal/+	0.2	88			wtr
W10 / TM6B	75B3-6;75C1-2	*ovoD2/+;Df/+*	3.4	73	0.711	<0.01	wtr
		ovoD2/+;Bal/+	0.1	54			wtr
w[+R4] / TM6B	75B8-11;75C5-7	*ovoD2/+;Df/+*	9.6	70	0.824	<0.01	90%
		ovoD2/+;Bal/+	0.7	48			wtr
Cat / TM6	75C1-2;75F1	*ovoD2/+;Df/+*	0.4	49	0.142	NS	wtr
		ovoD2/+;Bal/+	0.2	78			wtr
VW3 / TM3	76A3;76B2	*ovoD2/+;Df/+*	0.4	64	0.266	NS	wtr
		ovoD2/+;Bal/+	1.2	54			wtr
in61 / TM1	76F;77D	*ovoD2/+;Df/+*	0.2	71	0.444	<0.01	wtr
		ovoD2/+;Bal/+	1.3	42			wtr
rdgC / TM6C	77A1;77D1	*ovoD2/+;Df/+*	5.6	75	0.447	<0.01	wtr
		ovoD2/+;Bal/+	2.0	77			wtr
ri79C / TM3	77B-C;77F-78A	*ovoD2/+;Df/+*	0.4	62	0.140	NS	wtr
		ovoD2/+;Bal/+	1.2	57			wtr
Pc-MK / TM3	78A3;79E1-2	*ovoD2/+;Df/+*	10.1	97	0.584	<0.01	90%
		ovoD2/+;Bal/+	3.1	108			wtr
Pc / TM3	78D1-2;79A4-C1	*ovoD2/+;Df/+*	16.6	73	0.501	<0.01	90%
		ovoD2/+;Bal/+	10.6	70			90%
Pc / TM3 other stock		*ovoD2/+;Df/+*	14.2	88	0.960	<0.01	90%
		ovoD2/+;Bal/+	1.1	58			wtr
Pc23937-30A / TM3	78D	*ovoD2/+;Df/+*	16.5	70	1.000	<0.01	90%
		ovoD2/+;Bal/+	0.2	70			wtr
Pc-Cp1 / TM3	78D3-6;78E-F	*ovoD2/+;Df/+*	13.0	68	1.000	<0.01	90%
		ovoD2/+;Bal/+	0.5	74			wtr
Pc-T7 / TM3	78E1-2;79E4	*ovoD2/+;Df/+*	0.5	71	0.082	NS	wtr
		ovoD2/+;Bal/+	0.3	70			wtr
1-16 / TM3	80Fa-g	*ovoD2/+;Df/+*	0.3	60	0.169	NS	wtr
		ovoD2/+;Bal/+	0.1	64			wtr
10-26 / TM3	80Ffg + 81Fa	*ovoD2/+;Df/+*	0.5	64	0.165	NS	wtr
		ovoD2/+;Bal/+	0.1	59			wtr

Table 5. *ovo*^{D2} heterozygotes with reduced doses of regions on the right arm of chromosome 3 or chromosome 4.

Deficiency/ Balancer	Cytology	Progeny	oocytes/ovary mean	oocytes/ovary No	Statistics T1	Statistics P	Rank
Z / TM3	82A;82E3-4	*ovoD2/+;Df/+*	1.9	65	0.317	<0.01	wtr
		ovoD2/+;Bal/+	0.4	76			wtr
110 / TM3	82C;82F	*ovoD2/+;Df/+*	0.4	58	0.426	<0.01	wtr
		ovoD2/+;Bal/+	2.6	86			wtr
6-7 / TM3	82D3-8;82F3-6	*ovoD2/+;Df/+*	5.7	68	0.775	<0.01	90%
		ovoD2/+;Bal/+	0.7	54			wtr
3-4 / TM3	82F1-2;82F10-11	*ovoD2/+;Df/+*	11.7	66	1.000	<0.01	90%
		ovoD2/+;Bal/+	0.3	66			wtr
Tp110, Dp(3;3) Dfd[rvX1]/TM6B	83C1-2;83D4-5 and 84A4-5;84B1	*ovoD2/+;Df/+*	0.2	64	0.031	NS	wtr
		ovoD2/+;Bal/+	0.1	51			wtr
Tp16 / TM3	83D1-2;84A4-5	*ovoD2/+;Df/+*	2.5	55	0.120	NS	wtr
		ovoD2/+;Bal/+	3.4	57			wtr
Win11 / TM3	83E1-2;84A4-5	*ovoD2/+;Df/+*	2.0	66	0.379	<0.01	wtr
		ovoD2/+;Bal/+	0.7	58			wtr
Dfd13 / TM3	83E3;84A4-5	*ovoD2/+;Df/+*	0.2	83	0.561	<0.01	wtr
		ovoD2/+;Bal/+	2.2	70			wtr
9A99 / TM1	83F2-84A1; 84B1-2	*ovoD2/+;Df/+*	7.0	110	0.785	<0.01	90%
		ovoD2/+;Bal/+	0.2	102			wtr
Scr / TM3	84A1-2;84B1-2	*ovoD2/+;Df/+*	2.4	65	0.310	<0.01	wtr
		ovoD2/+;Bal/+	0.9	75			wtr
MAP11 / TM3	84A1-2;84B1-2	*ovoD2/+;Df/+*	0.3	74	0.375	<0.01	wtr
		ovoD2/+;Bal/+	1.4	78			wtr
MAP2 / TM3	84A1-2;84A3	*ovoD2/+;Df/+*	5.0	68	0.235	NS	wtr
		ovoD2/+;Bal/+	2.9	68			wtr
pbX2 / TM6B	84A4-5;84B1-2	*ovoD2/+;Df/+*	1.1	66	0.265	NS	wtr
		ovoD2/+;Bal/+	0.3	60			wtr
Antp3 / TM3	84A4-5;84C2-3	*ovoD2/+;Df/+*	4.7	46	0.451	<0.01	wtr
		ovoD2/+;Bal/+	1.0	24			wtr
Hu / TM3	84A6-B1;84D4-5	*ovoD2/+;Df/+*	0.1	122	0.255	<0.01	wtr
		ovoD2/+;Bal/+	0.6	132			wtr
Antp17 / TM3	84B1-2;84D11-12 or 84A6;84D14	*ovoD2/+;Df/+*	5.9	60	0.692	<0.01	wtr
		ovoD2/+;Bal/+	0.7	67			wtr
A41 / TM3	84B1-2;84D1-2	*ovoD2/+;Df/+*	2.4	62	0.460	<0.01	wtr
		ovoD2/+;Bal/+	0.3	59			wtr
D6 / TM1	84D2-3; 84F13-16	*ovoD2/+;Df/+*	2.1	69	0.130	NS	wtr
		ovoD2/+;Bal/+	1.6	56			wtr
dsx28 / TM2	84D13-E1; 85A4-5	*ovoD2/+;Df/+*	0.2	64	0.066	NS	wtr
		ovoD2/+;Bal/+	0.2	50			wtr
dsx5 / TM3	84E1-2;84F11-12	*ovoD2/+;Df/+*	0.1	54	0.074	NS	wtr

		ovoD2/+;Bal/+	0.0	88			10%
p40, In(3LR), T(2;3),red e/TM3	84E8-9;85B6	*ovoD2/+;Df/+*	0.5	42	0.247	NS	wtr
		ovoD2/+;Bal/+	1.1	66			wtr
by10 / TM3	85D8-12;85E7-F1	*ovoD2/+;Df/+*	0.0	55	0.278	<0.05	10%
		ovoD2/+;Bal/+	0.6	90			wtr
by416 / TM3	85D10-12; 85E1-3	*ovoD2/+;Df/+*	0.01	148	0.193	NS	10%
		ovoD2/+;Bal/+	0.5	100			wtr
by62,T(2;3)by62, red e / TM1	85D11-14;85F6	*ovoD2/+;Df/+*	0.9	67	0.221	NS	wtr
		ovoD2/+;Bal/+	2.0	57			wtr
GB104 / TM3	85D12;85E10	*ovoD2/+;Df/+*	0.3	62	0.258	NS	wtr
		ovoD2/+;Bal/+	1.2	62			wtr
M-Kx1 / TM3	86C1;87B1-5	*ovoD2/+;Df/+*	0.8	62	0.200	NS	wtr
		ovoD2/+;Bal/+	0.3	64			wtr
cu40 / TM6	86C1-2;86D8	*ovoD2/+;Df/+*	0.3	43	0.112	NS	wtr
		ovoD2/+;Bal/+	0.04	72			10%
TE32 / TM3	86E2-4;87C6-7	*ovoD2/+;Df/+*	4.3	65	0.440	<0.01	wtr
		ovoD2/+;Bal/+	2.0	82			wtr
TE10 / TM3	86F1-2;87C5-7	*ovoD2/+;Df/+*	5.1	70	0.680	<0.01	wtr
		ovoD2/+;Bal/+	0.5	64			wtr
kar1W / TM3	87A6-7; 87D12-13	*ovoD2/+;Df/+*	0.1	77	0.065	NS	wtr
		ovoD2/+;Bal/+	0.0	36			10%
ry615 / TM3	87B11-13; 87E8-11	*ovoD2/+;Df/+*	1.2	68	0.502	<0.01	wtr
		ovoD2/+;Bal/+	0.1	70			wtr
ry27 / MKRS	87D1-2;87F1-2	*ovoD2/+;Df/+*	1.9	36	0.023	NS	wtr
		ovoD2/+;Bal/+	1.8	68			wtr
red3l / MKRS	87F12-14;88C1-3	*ovoD2/+;Df/+*	0.2	42	0.254	NS	wtr
		ovoD2/+;Bal/+	0.7	67			wtr
red3l / TM3		*ovoD2/+;Df/+*	0.3	78	0.283	<0.01	wtr
		ovoD2/+;Bal/+	1.0	80			wtr
su(Hw)7 / TM6B	88A9;88B2	*ovoD2/+;Df/+*	0.0	189	0.092	NS	10%
		ovoD2/+;Bal/+	0.2	130			wtr
red-P93, Sb / In(3L)P In(3R)P18	88A10-B1;88C2-3	*ovoD2/+;Df/+*	2.1	111	0.292	NS	wtr
		ovoD2/+;In/+	0.4	13			wtr
red1 / TM1	88B1;88D3-4	*ovoD2/+;Df/+*	0.04	108	0.086	NS	10%
		ovoD2/+;Bal/+	0.2	70			wtr
sbd105 / T(2;3)apXa	88F9-89A1; 89B9-10	*ovoD2/+;Df/+*	0.0	50	0.000	NS	10%
		ovoD2/+;Bal/+	0.0	63			10%
sbd104 / T(2;3)apXa	89B5;89C	*ovoD2/+;Df/+*	0.2	69	0.016	NS	wtr
		ovoD2/+;Bal/+	0.2	70			wtr
bxd100 / Dp(3;3)P5 Sb	89B5-6;89E2-3	*ovoD2/+;Df/+*	0.8	36	0.278	NS	wtr
		ovoD2/+;Dp/+	1.4	62			wtr
P10 / TM3	89C1-2;89E1-2	*ovoD2/+;Df/+*	0.5	64	0.029	NS	wtr
		ovoD2/+;Bal/+	0.4	61			wtr
P10, In(3LR)Sta /		*ovoD2/+;Df/+*	6.9	58	0.754	<0.01	90%

TM1		*ovoD2/+;Bal/+*	0.4	50			wtr
P10 / TM1		*ovoD2/+;Df/+*	1.4	64	0.170	NS	wtr
		ovoD2/+;Bal/+	0.5	60			wtr
P2 / TM1	89D9-E1;89E2-3	*ovoD2/+;Df/+*	0.4	63	0.183	NS	wtr
		ovoD2/+;Bal/+	0.9	64			wtr
C4/Dp(3;3)P5,Sb	89E;90A	*ovoD2/+;Df/+*	6.8	65	0.795	<0.01	90%
		ovoD2/+;Dp/+	0.8	62			wtr
P14/T(2;3)apXa	90C2-D1;91A1-2	*ovoD2/+;Df/+*	6.6	69	0.813	<0.01	90%
		ovoD2/+;Bal/+	0.4	72			wtr
ChaM7 / TM6B	91A;91F5	*ovoD2/+;Df/+*	0.6	41	0.089	NS	wtr
		ovoD2/+;Bal/+	0.7	57			wtr
DlBX12 / TM6B	91F1-2;92D2-6	*ovoD2/+;Df/+*	0.2	63	0.041	NS	wtr
		ovoD2/+;Bal/+	0.1	71			wtr
e-N19 /TM2	93B;94	*ovoD2/+;Df/+*	1.5	60	0.108	NS	wtr
		ovoD2/+;Bal/+	1.5	56			wtr
eBS2 / TM3	93C3;93F	*ovoD2/+;Df/+*	2.9	73	0.305	<0.01	wtr
		ovoD2/+;Bal/+	1.5	65			wtr
Tl-P / TM3	97A;98A1-2	*ovoD2/+;Df/+*	1.5	69	0.328	<0.01	wtr
		ovoD2/+;Bal/+	0.7	53			wtr
Tl-X / TM3	97B;97D1-2	*ovoD2/+;Df/+*	0.9	60	0.071	NS	wtr
		ovoD2/+;Bal/+	0.8	48			wtr
tllG / TM6b	99F1-2;100B4-5	*ovoD2/+;Df/+*	1.1	75	0.317	<0.01	wtr
		ovoD2/+;Bal/+	0.2	66			wtr
Df(4)M62f/ey[D]	101E;102B10-17	*ovoD2/+;Df/+*	5.1	52	0.596	<0.01	wtr
		ovoD2/+;Bal/+	1.5	52			wtr
Df(4)M63a/ci[D];	101F2-102A1	*ovoD2/+;Df/+*	5.8	81	0.395	<0.01	wtr
	102A2-5	*ovoD2/+;Bal/+*	2.6	109			wtr
Df(4)G / ci[D]	102E2; tip	*ovoD2/+;Df/+*	0.6	60	0.146	NS	wtr
		ovoD2/+;Bal/+	1.0	64			wtr

Ehrman, L., and Y.-K. Kim. Division of Natural Sciences, State University of New York, Purchase, NY 10577, (914) 251-6671, FAX (914) 251-6635. Influence of developmental isolation on *Drosophila paulistorum* rare male mating advantages.

Since 1991 (Ehrman, *et al.*, 1991), I (LE) have not published on rare male mating advantages; we (see Kim and Ehrman in this issue) have instead investigated effects of alternative rearing environments on subsequent adult mating choices. These surveys have involved the sibling species of the useful *willistoni* species group. In the *D. willistoni* species group, *D. willistoni*, *D. equinoxialis*, and *D. tropicalis* exhibit mating advantages of less-frequent types of males (Ehrman and Petit, 1968). Therefore frequency-dependent mating trials were attempted with their sibling species, *D. paulistorum*, isolated at 3 different developmental stages.

Two types of differently-cultured flies, both belonging to the same semispecies, were directly observed in Elens-Wattiaux chambers (Elens and Wattiaux, 1964) at three different ratios. When the rare males had been isolated at egg or first instar larval stages, they were more successful in mating. (These mating advantages disappeared when they were the more-frequent males, except for the Santa Marta egg stage.) Developmental isolation at pupal stages absolutely never resulted in the rare male advantages.

In intersemispecific crosses, enhanced mating success was apparent when the isolated Mesitas strain was less-frequent compared to the controls (which had been communally raised, $p < 0.001$).

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References: Ehrman, L., and C. Petit 1968, Evolution 22:649-658; Ehrman, L., M.M. White, and B. Wallace 1991, In: *Evolutionary Biology* (Hecht, M.K., B. Wallace, and R.J. MacIntyre, eds.), Vol. 25, Plenum Press, New York, pp. 175-209; Elens, A.A., and J.M. Wattiaux 1964, Dros. Inf. Serv. 39:118-119.

Table 1. Santa Marta strain (Transitional semispecies): Effects of isolated vs. communal rearing.

Stage at isolation	Ratio (C:S)	Mating Combination				I ± SE	X^2
		C♀ x C♂	C♀ x S♂	S♀ x C♂	S♀ x S♂		
Egg	20: 5	60	20	12	8	0.36 ± 0.09	4.00*
	12:12	46	14	49	11	-0.05 ± 0.09	40.83***
	5: 20	9	11	20	60	0.38 ± 0.09	5.06*
1st	20: 5	56	24	14	6	0.24 ± 0.10	6.25**
	12: 12	46	14	37	23	0.15 ± 0.09	19.23***
	5: 20	7	13	10	70	0.54 ± 0.08	0.56
Pupa	20: 5	70	10	9	11	0.62 ± 0.08	0.06
	12: 12	37	23	19	41	0.07 ± 0.09	0.53
	5: 20	9	11	10	70	0.58 ± 0.08	0.06

C = communally-raised; S = secluded (isolated) from consemispecifics at given stages; I = isolation index; SE = standard error; X^2 test was utilized to see deviations from random mating expected for each male ratio. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. Mesitas strain (Andean-South Brazilian semispecies): Effects of isolated vs. communal rearing.

Stage at isolation	Ratio (C:S)	Mating Combination				I ± SE	X^2
		C♀ x C♂	C♀ x S♂	S♀ x C♂	S♀ x S♂		
Egg	20: 5	59	21	12	8	0.34 ± 0.09	5.06*
	12:12	45	15	35	25	0.17 ± 0.09	13.33***
	5: 20	4	16	18	62	0.32 ± 0.09	0.25
1st	20: 5	60	20	9	11	0.42 ± 0.09	7.56**
	12: 12	42	18	29	31	0.22 ± 0.09	4.03*
	5: 20	4	16	15	65	0.38 ± 0.09	0.06
Pupa	20: 5	66	14	12	8	0.48 ± 0.09	0.25
	12: 12	37	23	21	39	0.27 ± 0.09	0.13
	5: 20	6	14	18	62	0.36 ± 0.09	1.00

C = communally-raised; S = secluded (isolated) from consemispecifics at given stages; I = isolation index; SE = standard error; X^2 test was utilized to see deviations from random mating expected for each male ratio. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 3. Intersemispecific crosses: Effects of isolated vs. communal rearing.

Stage at isolation	Ratio (C:S)	Mating Combination SM♀ x SM♂	SM♀ x M♂	M♀ x SM♂	M♀ x M♂	I ± SE	X^2
SM	20: 5	80	0	0	20	1.00 ± 0.00	0.00
	12:12	60	0	0	60	1.00 ± 0.00	0.00
	5: 20	15	5	1	79	0.88 ± 0.05	1.00
M	20: 5	15	5	3	77	0.84 ± 0.05	0.25
	12: 12	58	2	0	60	0.97 ± 0.02	0.13
	5: 20	58	22	4	16	0.48 ± 0.09	20.25***
Controls (SM:M)	20: 5	80	0	1	19	0.98 ± 0.02	0.06
	12: 12	59	1	1	59	0.97 ± 0.02	0.00
	5: 20	20	0	0	80	1.00 ± 0.00	0.00

SM = Santa Marta strain (Transitional semispecies); M = Mesitas strain (Andean-South Brazilian semispecies); Controls = communally-raised; I = isolation index; SE = standard error; *** $p < 0.001$.

Kim, Y.-K., and L. Ehrman. Division of Natural Sciences, State University of New York, Purchase, NY 10577, (914) 251-6671, FAX (914) 251-6635. Influence of developmental isolation on courtship behavior of *Drosophila paulistorum*.

Courtship behavior in *Drosophila* is species-specific, and is a major component of ethological isolation which leads to reproductive isolation between closely related species (Mayr, 1963; Spiess, 1987). This is desirable in that ill-adapted sterile hybrids are then not routinely produced. In a series of investigations into the development of discriminatory behavior in *D. paulistorum* semispecies (Kim, *et al.*, 1992, 1994a, b), discriminatory abilities were reduced when individuals were totally isolated from their consemispecifics: The earlier the developmental stage at which isolation was initiated, the more frequent the heterogamic sterile-progeny producing matings. In contrast, the discriminatory abilities significantly increased when two intersterile semispecies were brought together in an artificially sympatric setting. Identification of specific types of damage to the mate recognition system from developmental isolation should be studied.

Differences in courtship between communally-raised males and socially-isolated males (from first instar stage on) were observed in small chambers where two types of males and one communally-raised virgin female were contained at one time. The observation continued until copulation occurred or over a period of one hour, but about 90% of copulation occurred in 10 minutes after flies had entered chambers. Responses of females toward courtship from each male were recorded under a dissecting microscope. When given mate choices, the socially-isolated males displayed courtship toward moving individuals regardless of sex. In five out of eight elements observed, their sexual activities were significantly higher than those of communally-raised males.

At the beginning of mate choice, females displayed more rejection behavior (decamping and kicking) toward the disorganized, socially-isolated males than communally-raised ones. The females, however, were more receptive to the physically active males and thus the copulation ratio was significantly higher for the isolated males than for the communally-raised males: The greater the courtship displays, the higher the mating

Table 1. Mean numbers of courtship elements from an isolated male plus a communally-raised male toward a virgin female of Mesitas strain (Andean-Brazilian semispecies), in female choice test (2 ♂♂, 1 ♀; replicates = 50). Total number of each element observed is shown in parentheses.

courtship elements	Males I		C		Prob.*
Orientation	2.06	(103)	1.06	(53)	<0.05
Tapping	4.62	(231)	2.86	(143)	0.0618
Circling	0.76	(38)	0.30	(15)	<0.05
Scissoring	0.46	(23)	0.28	(14)	0.3372
Vibration	4.28	(214)	1.06	(53)	<0.001
Licking	2.92	(139)	0.60	(31)	<0.001
Attempted copulation	0.32	(16)	0.08	(4)	0.1863
Attempted copulation toward the other male	0.28	(14)	0.00	(0)	<0.05

I = isolated at first instar larval stage from consemispecifics; C = communally-raised; * approximate t test was utilized because of considerable heterogeneity of variances between two samples.

Table 2. Responses of females toward courtship from isolated males and communally-raised males of Mesitas strain (Andean-South Brazilian semispecies), in 50 female choice tests (2 ♂♂, 1 ♀). Mean numbers of each response are shown with total numbers in parenthesis.

Responses of females	Males I		C		Prob.*
Decamping	6.04	(320)	3.74	(187)	<0.05
Kicking	0.84	(42)	0.28	(14)	<0.05
Extrusion	0.40	(20)	0.12	(6)	0.0540
Standing	2.46	(123)	1.14	(57)	<0.05
Wing-spreading	0.96	(48)	0.18	(9)	<0.001
Copulation	0.82	(41)	0.18	(9)	<0.001

I = isolated at first instar larval stage from consemispecifics; C = communally-raised; * approximate t test results.

success. Therefore, the socially-isolated males increased their Darwinian fitness.

Acknowledgment: This work was supported by the Whitehall Foundation.

References: Kim, Y.-K., L. Ehrman, and H.R. Koepfer 1992, Behav. Genet. 22:545-556; Kim, Y.-K., L. Ehrman, and H.R. Koepfer 1994a, Developmental isolation and subsequent adult behavior of *D. paulistorum*. III. Alternative rearing (submitted); Mayr, E., 1963, *Animal Species and Evolution*, Belknap Press, Cambridge; Spiess, E.B., 1987, In: *Kin Recognition in Animals* (Fletcher, D.J.C, and C.D. Michener, eds.), pp. 75-119, Wiley, New York.

Orr, H. Allen. Department of Biology, University of Rochester, Rochester, N.Y. A new linkage map of the *D. pseudoobscura* X chromosome.

While attempting to map loci causing sterility of *D. pseudoobscura* USA ("Mainland")-*D. pseudoobscura* Bogota hybrids, I found that the standard X chromosome linkage map (summarized in Anderson and Norman, 1980) is partly incorrect. The standard map is largely based on results from Sturtevant and Tan (1937) and Sturtevant and Novitski (1941), who, in turn, primarily relied on data from Lancefield (1922). The standard map suffers from two problems. First, the reported recombination rates in one region appear to be incorrect (see below). Second, the standard map is constructed from raw recombination rates that have not been corrected by any mapping function (indeed much of Lancefield's map was built before Haldane introduced the first mapping function in 1919). Because some of the distances between markers on the *D. pseudoobscura* X are quite large, such corrections have a substantial effect on the map. I present a new map here that corrects these problems.

G. Cobbs kindly provided a new recessive visible marker, "scalloped" (*sd*) which closely resembles *sd* of *D. melanogaster* (in *D. pseudoobscura*, the mutant phenotype is much stronger in males than females). Preliminary data (not shown) showed that *sd* is loosely linked to yellow and thus resides on the left arm of the large X chromosome (X^L). Complementation tests showed that *sd* is not an allele of cut (*ct*) another X^L-linked gene with a somewhat similar mutant phenotype.

To locate *sd*, *sd* males were crossed to *y sn v co sh* females (see Anderson and Norman, 1977) and the F_1 females were backcrossed to *y sn v co sh* males. The *y*, *v* and *sd* markers were scored among 815 backcross males. The percent recombination between these markers were: *sd-y*, 27.9%; *y-v*, 10.8%; *sd-v*, 36.0%. Thus, *sd* is located to the left of *y* (see Fig. 1).

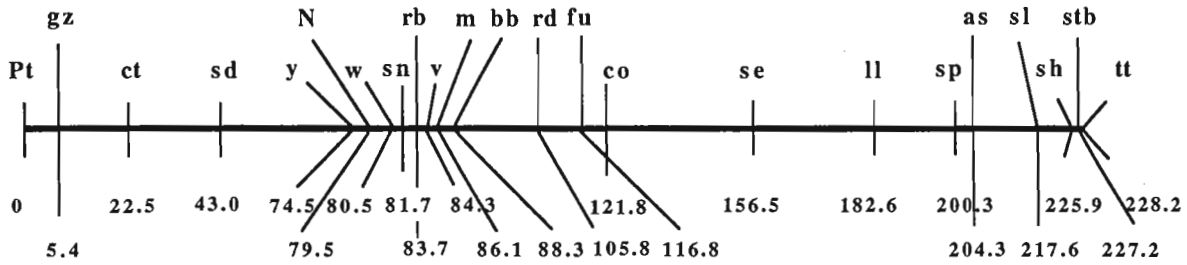

Figure 1. Revised map of the *D. pseudoobscura* X chromosome. See text for details.

In another experiment, *sd/sd* females were crossed to *ct* males, and F_1 females were backcrossed to *sd* males. (With practice, *sd* and *ct* are easily distinguished; moreover, the simultaneous presence of *sd* and *ct* in males causes a much more extreme wing phenotype than seen with either mutant separately.) Data from 468 backcross males showed that *sd* and *ct* recombine at a frequency of 19.4%. Thus the estimated distance between *ct* and *y* is 47.3 cM (= 19.4 + 27.9), compared to 37.4 cM in the standard map (Anderson and Norman, 1980). The standard map thus underestimates the size of this region of X^L by approximately 10 cM. A new linkage map, taking this error into account, is shown in Figure 1. Except for the region studied above, data on percent recombination between adjacent markers were taken from Sturtevant and Tan (1937) and Sturtevant and Novitski (1941). These values were then corrected using the Kosambi (1944) mapping function. The resulting corrected map position for each marker is shown below the chromosome. Markers that are still extant are shown close to the chromosome; those that apparently have been lost are shown raised far above the chromosome. As Figure 1 shows, these corrections have substantially increased the estimated length of the X: while the old map showed that the X chromosome was at least 204.2 cM long, the new map shows that

the X is at least 228.2 map units long.

Acknowledgments: I thank G. Cobbs for kindly supplying the *sd* marker. This work was partly supported by NIH grant GM51932.

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Thompson, D. Bruce and W. W. Doane. Department of Zoology, Arizona State University, Tempe, AZ, U.S.A. 85287-1501. *Grt*, a new glucose-repressible gene in the *Amy* region of *Drosophila melanogaster*.

In *Drosophila melanogaster*, the duplicate genes for alpha-amylase, *Amy-p* (centromere-proximal) and *Amy-d* (distal), lie tightly linked within the *Amy* locus (Lindsley and Zimm, 1992). Repression of *Amy* gene expression by dietary glucose (Hickey and Benkel, 1982; Benkel and Hickey, 1986; Thompson, *et al.*, 1992) is mediated through *cis*-acting promoter sequences located near the transcriptional start site of the gene (Benkel and Hickey, 1987; Magoulas, *et al.*, 1993). Tissue-specific *Amy* expression is controlled by less well defined promoter sequences (Doane, *et al.*, 1990; Hawley, *et al.*, 1992; Thompson, *et al.*, 1992; Grunder, *et al.*, 1993) which presumably bind transcription factors produced by *trans*-acting regulatory genes such as *mapP* (see Lindsley and Zimm, 1992).

Hickey, *et al.* (1994) recently showed that *Saccharomyces cerevisiae* can be transformed by *Amy* genes from *D. melanogaster*. They also showed that the promoter of the proximal *Amy* gene from an Oregon-R strain of flies could mediate glucose repression of a heterologous reporter gene in transformed yeast cells, utilizing the yeast machinery for glucose repression of amylase expression in transformed cells, whether of fly or yeast origin (Magoulas, *et al.*, 1993; Hickey, *et al.*, 1994). Two sequence elements upstream of the transcription start site of this gene have been implicated in glucose repression: the 5'-GTGGGG-3' motif at position -42 (Hickey, *et al.*, 1987, 1994) and a 36-bp element at -109 which contains a 5'-CAAAT-3' motif at its 3'-end (Magoulas, *et al.*, 1993). Although Hickey, *et al.* (1994) have likened glucose repression in yeast and *Drosophila* to catabolite repression in bacteria, the mechanism in prokaryotes is clearly different from glucose repression in yeast (Ronne, 1995) and, presumably, in *Drosophila* as well.

We have identified a second gene in *D. melanogaster* whose expression is repressed by dietary glucose at the RNA level. Preliminary evidence for this gene has appeared (Doane, *et al.*, 1990) but, until now, it has remained unnamed. Northern analysis revealed that it produces two RNA transcripts, one large (about 4.0 kb in length) and the other small (about 1.5 kb). The 4.0 kb transcript is less abundant than the 1.5 kb transcript, whether isolated from flies reared on a glucose-yeast diet (10% glucose, 5% brewers yeast, 1.5% agar, 0.15% buffered propionic acid) or on a yeast diet (same diet, but glucose-free). Abundance of both transcripts is reduced in extracts from flies raised on the glucose-yeast diet in comparison to those reared on the yeast diet. We have named the gene producing these transcripts *Grt: Glucose repressible transcripts*, based on its RNA phenotype. The protein products of *Grt* have not yet been identified.

Densitometric scans of northern dot blots were used to quantify the levels of *Grt* and *Amy* RNAs isolated from flies raised on the above yeast vs. glucose-yeast diets. RNA was isolated from an inbred strain of flies expressing the $Amy^{1,6}$ haplotype. Results indicate that *Amy* RNA levels were repressed approximately 2-fold on the glucose-yeast diet, while the 1.5 kb *Grt* RNA levels were repressed 7-fold. Image analysis also revealed that the low abundance, 4.0 kb *Grt* RNA species is not repressed by dietary glucose to the same extent as the 1.5 kb *Grt* RNA, suggesting differential regulation of the two *Grt* transcripts.

Grt is closely linked to the proximal end of the *Amy* locus, which is located in region 54A of the polytene map for chromosome 2R (Gemmill, *et al.*, 1985). The restriction map in Figure 1 shows this relationship and is based on Southern analysis. Above the map are given the *Eco*RI fragments which were subcloned from our original *Amy* clone, lambda-Dm65 (Levy, *et al.*, 1985), or from lambda-Dmc14.5 (Doane, *et al.*, 1987) into pBluescript vectors.

Southern analysis revealed no sequence homology between *Grt* and the two *Amy* genes within the *Amy* locus (Doane, *et al.*, 1990). This has been confirmed by sequencing 2,884 bp of genomic DNA which spans roughly half of the upstream portion of the coding region for the 4.0 kb *Grt* transcript and the overlapping coding region of the smaller, 1.5 kb *Grt* transcript, plus some 5' promoter sequences (Thompson, D.B., W.W. Doane and H. Chen, unpublished). The sequenced region includes the entire 2.8 kb *Eco*RI fragment from lambda-Dm65, the 0.2 kb *Eco*RI fragment just distal to it, and 206 bp from the centromere-proximal end of the 3.8 kb *Eco*RI fragment in lambda-Dm65, which contains an $Amy\text{-}p^1$ allele (Figure 1). We have detected in our *Grt* sequencing data the two sequence motifs alluded to earlier which apparently play a role in glucose repression of *Amy-p*, namely, the 5'-GTGGGG-3' and 5'-CAAAT-3' motifs, and their spacing is comparable to that in the *Amy-p* promoter region.

When single-stranded DNA molecules with opposite orientations were made from the insert in subclones

containing the 2.8 kb *Eco*RI fragment shown above the map in Figure 1 and hybridized to poly-A+ RNA immobilized on nylon membranes, the direction of transcription could be effectively assessed for *Grt*. Both *Grt* transcripts appear to be transcribed in the same direction as *Amy-p*[1]. The same single-stranded DNA from the 2.8 kb *Eco*RI fragment also was hybridized to RNA isolated from flies reared on the yeast diet, and the resulting RNA-DNA hybrids treated with S1 nuclease. The residual products were resolved on agarose gels, transferred to nylon membranes and probed with the 2.8 kb *Eco*RI fragment. A 1.5 kb protected fragment was detected, indicating that the 1.5 kb *Grt* transcript is not interrupted by introns.

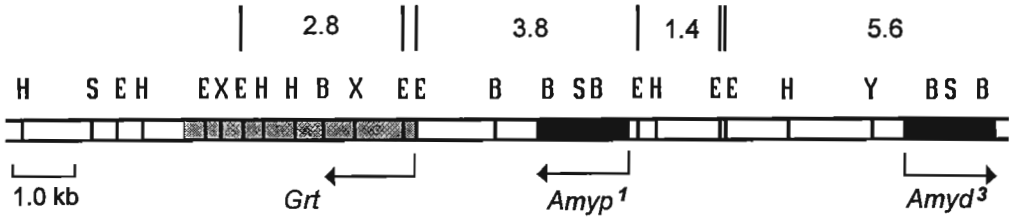

Figure 1. Composite restriction map of the *Grt-Amy* region in 54A of chromosome 2R. Restriction sites include: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal*I; X, *Xho*I; and Y, *Xba*I. The divergently transcribed *Amy* genes are shown as black rectangles; *Grt* is depicted by hatch marks. Arrows indicate the direction of transcription for each gene. All nucleic acid manipulations used to identify *Grt* and construct this map were performed according to Maniatis, *et al.* (1982).

We have isolated a *Grt* cDNA clone from a lambda-gt10 library prepared by T. Kornberg from adult female RNA of *D. melanogaster*. The insert of this clone, which is 4.0 kb in length, hybridizes to both the 1.5 kb and 4.0 kb *Grt* RNA species, suggesting it carries most or all of the base sequence for the larger *Grt* transcript. Once the insert of this cDNA clone has been sequenced and its predicted amino acid sequence determined, we hope to be able to identify the two protein products of the *Grt* gene.

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Singh, B.N., T. Srivastava, and A.K. Sundaran. Department of Zoology, Banaras Hindu University, Varanasi, India. Presence of apical black patch on the wings of males and females of *Drosophila biarmipes*.

Drosophila biarmipes was described by Malloch in 1924 from Coimbatore, India. This species belongs to the suzukii subgroup of the *melanogaster* species group. *D. biarmipes* males possess an apical dark black patch on their wings. Variation in male apical wing patch has been observed and the males without patch have also been found (Singh and Chatterjee, 1987). Males possessing dark wing patch are more successful in mating than those without patch (Singh and Chatterjee, 1987). The purple eye colour mutation which was detected in a laboratory stock of *D. biarmipes* (Singh and Pandey, 1992) has been found to affect the sexual activity of both sexes (Singh and Pandey, 1993, 1994a). Larval pupation behaviour and oviposition site preference in *D. biarmipes* have also been studied (Pandey

and Singh, 1993; Srivastava and Singh, 1993a, b). Different geographic strains of *D. biarmipes* do not show sexual isolation (Singh and Pandey, 1994b).

In two stocks of *D. biarmipes* (MY - Mysore; RC - Raichur, Karnataka), we observed apical black patch on the wings of both sexes. In males the patch is darker and touches the margin of wings. However, in females the patch is lighter and does not touch the margin of wings. Wings of a female and a male showing the apical patch are depicted in Figure 1. In both the stocks, flies without a wing patch have also been observed. All the flies have been found to possess apical wing patch in a stock which was isolated from the RC stock of *D. biarmipes*.

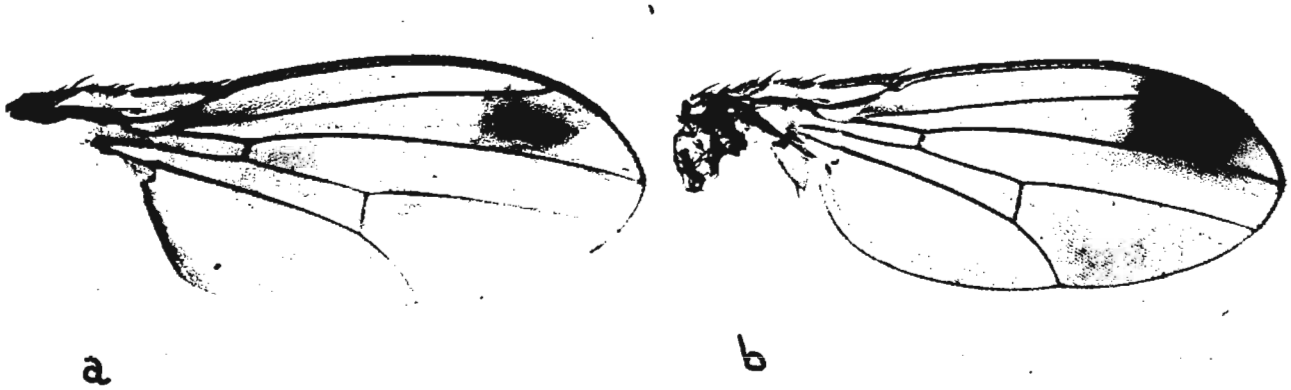

Figure 1. Wings of *Drosophila biarmipes* showing apical black patch: a, female; b, male.

References: Pandey, M.B., and B.N. Singh 1993, Ind. J. Exp. Biol. 31:912-916; Singh, B.N., and S. Chatterjee 1987, Ethology 75: 81-83; Singh, B.N., and M.B. Pandey 1992, Dros. Inf. Serv. 71: 158; Singh, B.N., and M.B. Pandey 1993, Ind. J. Exp. Biol. 31: 932-933; Singh, B.N., and M.B. Pandey 1994a, Ind. J. Exp. Biol. 32: 482-485; Singh, B.N., and M.B. Pandey 1994b, Dros. Inf. Serv. 75: 58-59; Srivastava, T., and B.N. Singh 1993a, Ind. J. Exp. Biol. 31: 460-462; Srivastava, T., and B.N. Singh 1993b, Evol. Biol. 7: 193-205.

Davis, Patricia S., and Burke H. Judd. Laboratory of Genetics, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709. Molecular characterization of the 3C region between *white* and *roughest* loci of *Drosophila melanogaster*.

The 3C region of the X chromosome between the *white* (*w*) and *split* (*spl*) loci has been extensively studied cytogenetically, particularly by Lefevre and Green (1972) who demonstrated that deficiencies for the region survive except those that remove all of the chromomeres between 3C2 and 3C7. Mutational and deletion analyses have identified only two mutants in that interval, namely, *roughest* (*rst*) and *verticals* (*vt*).

Molecular characterization of the region has been limited to a chromosomal walk extending about 120 kb proximal to *w* (Goldberg *et al.*, 1983) and the recent cloning and molecular analysis of the *roughest* locus (*irreC-roughest*) by Ramos *et al.* (1993). This note describes a chromosomal walk that spans the region between *w* and *rst* from *white* locus coordinates +100 to +163, connecting the two loci. We have identified and sequenced a gene of unknown function in this interval.

A P1 clone, 138-66, which is reported to hybridize to the 3C region of the X chromosome, was obtained from the laboratory of D. Hartl (Smoller, *et al.*, 1991). A lambda library was made by partially digesting 138-66 DNA with *Sau*3A and cloning into lambdaGEM12-*Xho*I half-site arms (Promega). A walk was initiated by screening the library with the 3.7-kb most proximal *Eco*RI fragment of lambdaC52 of Goldberg, *et al.* (1983); the approximate coordinates of that fragment, relative to *white*, are +101.3 to +105. Each subsequent step in the walk was made by re-probing the library with the proximal-most restriction fragment of the last isolated lambda clone. This generated a set of six overlapping clones, lambdaPD.A5, lambdaPD.B8, lambdaPD.C6, lambdaPD.D8, lambdaPD.E6 and lambdaPD.F15,* which span the region of +100 to +163 (Figure 1).

The proximal-most clone, lambdaPD.F15, was partially sequenced to locate the insert-vector junction. It was found that the proximal end of the *Drosophila* insert, coordinate +163.4, is homologous to exon C of the *irreC-roughest*

HB3-cDNA (Ramos, *et al.*, 1993); this corresponds to coordinate -15.9 of the *roughest* map on which the P-element insertion is designated coordinate zero. This would indicate that the distance between *white* and *roughest* is approximately 179 kb.

The overlapping clones described above were used as probes in Northern analyses of Canton-S poly (A+) RNAs (Clontech). Approximately 6-7 RNAs were detected, ranging in size from about 1.6 to 5 kb. This indicated that it might be possible to isolate a cDNA from the region, and indeed screening an eye imaginal disc library obtained from the laboratory of G. Rubin (Gaul, *et al.*, 1992) yielded a cDNA with homology to both lambdaPD.C6 and lambdaPD.D8. This cDNA, 2 kb in length, was sequenced (accession number U22295) and found to be a partial cDNA that encodes a total of 622 amino acids followed by a short non-coding region and a poly A tail of 45 nucleotides. This would seem to indicate that there is at least one gene in the region between *white* and *roughest*, even though extensive mutation screening of the region has failed to identify any mutants except *rst* and *vt*. Transformants carrying plasmid pPD.C6 were obtained by J.K. Lim. The transformed lines fail to rescue either *rst* or *vt* mutations.

A small deletion has been recovered that includes *rst* and *vt* and extends proximal to those loci. It causes female sterility and reduced viability and acts as a dominant suppressor of *zeste*. None of these mutant phenotypes are modified in transformed lines. The deletion is described elsewhere in this issue.

In an effort to determine the potential 5' end of the cDNA, sequencing of the corresponding genomic DNA region, *i.e.*, plasmid pPD.D8, was undertaken. A total of 4,358 bases encompassing the region +134.5 to +138.8 was sequenced (accession number U22182). Starting with base 1,974, an open reading frame (ORF) was found that encodes a 791 amino acid peptide homologous with that of the cDNA. Upstream were found two more ORFs that encode, respectively, a 56- and a 67 amino acid peptide. Whether two or all of these ORFs belong to the same gene is unknown.

The genomic sequence is as follows:

```
   1  AAGCCTATTTTGGAAATAGCATTTAAATGCGCCTAAAATTGGCTGTAAAA   50
  51  GTGTAAATGTATTGGGCCAAAAGAGTTTTGCTATAGAGAACCTTTAAATT  100
 101  TAAATCACAGTCCGCCTGCAAAATAATGCAAGATCGTAAGAAACAACTAT  150
 151  CTGAATTAAAATAAATTGCAATTGCTTTTGCATTTTCCTTCAAATATTAC  200
 201  CATTATTTTCGGCTTGCAAAACAATTGTAAAGAACAATAATTTTCACAGA  250
 251  GCGGGTACATCGAGCTATTCCCGATATTAAATGTTAATTGAATGCCTTCA  300
 301  ATAGTGGCTGAAATTGGAGACCAATCGATTTCCTCAGCCCTACTCAATCC  350
 351  GAATTTGGCCACTGACCACGGGTGGTATGATTAATTTATGTGCTGTATTT  400
 401  TTTTCGAACTCCATGTTTTTTTTGCCAGCCTCTCCGATGGTTTATCTAAT  450
 451  TTATGTTATTGCCGCGGCAATTATTTAAATTTTCCACAGCTACCGTTGGC  500
 501  AGTTCTTTCTAATGACAAGGGCGGACTGGGGGATTTAAAAAGGAAAACAT  550
 551  ATTTGCCGCCCATTATGGCGACATAAACAAATGTTTTAATAACCAAATTA  600
 601  TGCGAAAAAGAACGCAATGTATTCATAAGCTCGAAGAGCTGGGCGAGCAA  650
 651  AAGAGATCCCAGCCAAGTTATTTGATGCTAATGGAAATGCATAAAAGATC  700
 701  GTAATACTCATTAGCAAAGTGTGTGGTTCTAGCTATGTATATGGCAAAGA  750
 751  AAGTTGCCCCCATGGCGGGGCAGCACATCTCACCCAATCATTTGCGGGGT  800
 801  CAACATATTTTGGAAAGTTACTGTTGTAGGGAAAGTTCTATTTTTGGAGC  850
 851  GCGTCTAAGAAATATTCCCAAAAATTAGCACACTGATCCACATTCGATGA  900
 901  AAAGAAATCGCAGAGCGGAAATTACTAGTGAATTTCGGGGAATTCACTTG  950
 951  ACTTGCAATCAATCTATATCTCTTTGATATTCATTACTAATTTATGCACG 1000
1001  TGTACAAAATGATACGCAATCAGGAAATCCGATTGATTAATTAAGCTAAT 1050
1051  GGTTCTTTTGCATAACAAGCACAATGGTATTGTAAGCTATTTGAAGAAAT 1100
1101  CCTGTCTATCCTGCGTAAGAAATTATCAACTTATTGTTAATCTAAAAAAT 1150
1151  AAGTTTTAATAATATGTAGTTTTCTTTCTAAAACAGTTCATTAAATTAGC 1200
1201  GTATGTCATATTTTGCAAAGTATTTCCTAATTAAGTGAGTCAAGTAAGTA 1250
1251  AATTTCCTAACAAGTAAGTCAAACCAAAGTTACCCATCAGGCGTGGTCAT 1300
1301  TTAAACCATAATATGTACGGCTCTCTACACCCGAACTAGTTTTTAGAAAA 1350
1351  TATTTCGAACCGTTTAGAATCGCGACCTGCGACCCGATCTCTTTTGGCCA 1400
1401  ACGGTCAACAAACCGCAAACTGATTTACAGCCCCCAGTGGCAAAGGCAAA 1450
1451  AAAAAAAATGGAGAGAGCTTTGCCATTCCGAATGGCTGGCTCTCTTTCTC 1500
1501  TCTCTCTCTCTCTCCGGATCGTTACGCTCTTTTTGGCAGAACATGGGCTT 1550
1551  TTGGCCAAAACCGTTTGTAGACGGCACATAAAAAGCTCGGGTCGCAATGC 1600
1601  TCCCCCAACAGTTCGGCAAACGAAACCTCTGTCTGTGGACCCCCAGAAAT 1650
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1651  CGGGAGCTTGATTAAGAACCAGAGCGATACAAGTCGAGAATCCCAGTGCG  1700
1701  TGCGTGAAATTAGAAACCCGAAGAAAATCCACAAAAAAACCATGACCAAG  1750
1751  TTCGTGGTGGTGGGCATGTGCCTGCTGATGGCCTTCGGCCATATGGCTTC  1800
1801  CGTTTCCGCCGTGACCGAGGCTCCGGAGGTGGAGCAGTCAACGGAGTCGC  1850
1851  CCATCGCCGACATGGCGCTGATTGGTAGGCCCACGGATCCAATGTCTTCC  1900
1901  CCTAACCCCTAGAATTTCCATTAATTTATACCTGTGTTTCTCTTTTTTTT  1950
1951  TTCATGTGCCGTGTGTTTTGTTTATGTGTGCATTTTTGTCTGTGATTTTT  2000
2001  GTGCGGGTGGCGCAGAGGATGCGCCAATGGCTCCCAAAACTCCCACGGAG  2050
2051  AAGCCGCCCATTGTCCAGGAAGCCGCAATGGTGGTGGACGCTCAGGAGAA  2100
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2151  CACCCGCTGATCGCCTTCAAGCCACGCCAGGCCTCCCTAGAGGAGATCCA  2200
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2551  AGCGACCTAACCGCTTCAATAACCGACTGGACTCCTTCGAGGACGACTAC  2600
2601  TACGAGGACGATTACTTCCAGGGCAACCGATTCGATGACGAGTTCGACGA  2650
2651  CGATGAGGACGAGCAAAGCCTGGAGCAATTCGAGGATGACGATTCCCTGG  2700
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2751  CAAAATCAGCGCAGGAAGTTCCAGCAAGAGGAGCAGGTGATCAGCCAGAA  2800
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3001  CCTCGTCGACTAGGCGCAGCCAAAACCAGCCCAATTTCATCCAGCGCGGC  3050
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3151  GCTCTTCGAAGACCCGTCGGCCGCAGCAGACTACGCTCCTGGTAAACCGC  3200
3201  AATGGACAGACCGTTTACGTGGCTCCCGAGCTGCTGGGCCTGAACTCTCC  3250
3251  CTATCCCTACGCTTATGTCCAGGGTCAGACGAAGAAACAAAGGGTTCCCA  3300
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3551  GGCGGTTCCCCTTTTGGCTGACGCCTCACTTATGGACATCTTCCAGCCTC  3600
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3851  TTCTGCGCCACAAACGGCAGCCAATGGAACCGAGCAGCAGGAGTTCATCT  3900
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4101  GAGAAGCCAAAGGAGGAGGAGCAGAAGGAGAAGCTTCCAAAGGAGGAAGT  4150
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4201  AGGAGGAGCCACAAAAGGAGGAGCCCCAGAAGGAGGAGCCACGAAAGGAG  4250
4251  GAGCCACAAAAGGAGGAGCCACAAAAGGAGGAGCCACAAAAGGAGGAACC  4300
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4351  TTTGTAAA        4358
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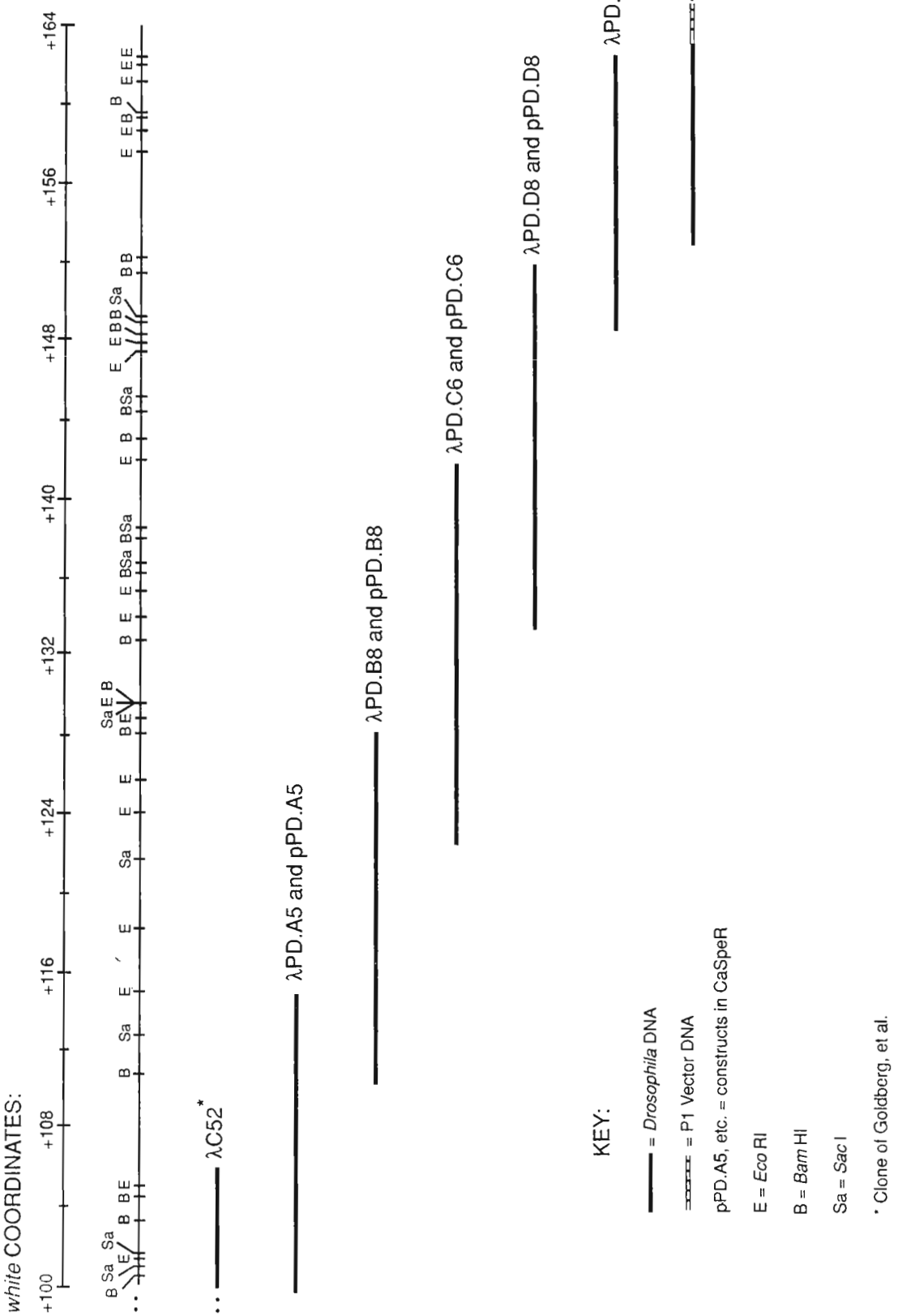
white COORDINATES:
+100
+108
+116
+124
+132
+140
+148
+156
+164
λC52*
λPD.A5 and pPD.A5
λPD.B8 and pPD.B8
λPD.C6 and pPD.C6
λPD.D8 and pPD.D8
λPD.E6 and pPD.E6
λPD.F15
KEY:
= Drosophila DNA
= P1 Vector DNA
pPD.A5, etc. = constructs in CaSpeR
E = Eco RI
B = Bam HI
Sa = Sac I
* Clone of Goldberg, et al.

*The lambda series of clones is not available for distribution; however, CaSpeR plasmid constructs containing the *Drosophila* DNA inserts of these clones are available.

References: Gaul, U., G. Mardon and G.M. Rubin 1992, Cell 68: 1007-1019; Goldberg, M.L., J-Y. Sheen, W.J. Gehring and M.M. Green 1983, Proc. Natl. Acad. Sci., USA 80: 5017-5021; Goldberg, M.L., 1985, personal communication; Lefevre, G., Jr., and M.M. Green 1972, Chromosoma 36: 391-412; Ramos, R.G.P., G. L. Igloi, B. Lichte, U. Baumann, D. Maier, T. Schneider, J.H. Brandstaetter, A. Froehlich and K-F. Fischbach 1993, Genes and Development 7: 2533-2547; Smoller, D.A., D. Petrov and D.L. Hartl 1991, Chromosoma 100: 487-494.

Davis, Patricia S., and Burke H. Judd. Laboratory of Genetics, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709. Nucleotide sequence of the transposable element, BEL, of *Drosophila melanogaster*.

The transposable element, BEL, whose nucleotide sequence is presented here, was first described as an insertion sequence associated with the *white* mutation, w^{a4} (Goldberg, *et al.*, 1983) and is the same as or closely related to the 3S18 element described by Bell, *et al.* (1985). It exists as 15-25 copies distributed throughout the genome, as indicated by *in situ* hybridization, and the locations of its insertion vary among strains. The BEL element insertion is also responsible for the *white-zeste mottled* (w^{zm}) mutation discovered by Green (1967); the position of that insertion into the first intron of *white* was determined by O'Hare, *et al.* (1984). The 5' end of the BEL sequence below is proximal in w^{zm}. An analysis of a family of w^{zm} derivatives and their effect on the determination and stability of *white* expression was made by Peterson, *et al.* (1994).

The BEL element, a copia-like retrotransposon, is 6,126 nucleotides in length. It has direct repeats at the ends; these LTRs are 361 nucleotides long. There is one long open reading frame (ORF); bases 919 - 5,739 encode a 1,607 amino acid peptide, within which are found motifs associated with putative *gag* and *pol* proteins; *env* motifs are not found. In a search of the BEL hypothetical protein using the Wisconsin Sequence Analysis Package there was found another motif, that of prokaryotic membrane lipoprotein lipid attachment site; this is an 11 amino acid peptide encoded by nucleotides 3,883 - 3,915. The significance, if any, of this latter motif in this *Drosophila* transposable element is unknown.

Both strands of BEL were sequenced and the sequence is as follows:

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   1  TGTTTGGGAACGAGACACCCTGTATACGCGAACAAGTCACCCTTTATCTT   50
  51  TATTTACATTCTTATTTGTCTGCAGCTTCATCGGAGCTTATCAGCGGAAT  100
 101  CAATGTAAGCATCGCACCGCTGTAATTGTCCGCGAGCTTGCCCAGTACTT  150
 151  TTCCAAACTTCTAACTCCCTTCTAACTGTAACTTGTTTACGTCTTATGCT  200
 201  AGACTAATCGTATGGCGTGATTACAGCCAAAGCTGAAGTCAGTCACAATT  250
 251  TTGATCTGCGAGAAAACGTACGCATCGGTGTCGAAATAATTAATATTAAG  300
 301  TGTCTGAACTTAACCAATAAATGAAAATTAACAGTAACACTGGCGGTTTT  350
 351  ATTTATAAACATAAAAATTGGTCCTTCGAGCCGGATAACCGGAAGTGCGT  400
 401  TTCGTTCGGGCATTTGATTTTGATTATTGGCCTTTTGGCAAACGATAATC  450
 451  TATAGATTCCTACATCGTGTAGAATCGTTCCCTTCTTTCGACCACCATGC  500
 501  GGAGTGTGATTCAACAACGGGGCTTCTGCAAAAGCCAAATTACTCGTGCG  550
 551  CATAATAATGCCTTAAAATTTGTTGATGACATTCACTCAGTGCAAACAAT  600
 601  AGTTGTCCGCCTGGCGCAACTACAGGAAAATTATTTGCGGTTCGTACGGC  650
 651  TCTCGGAAGAGCTGTATGCATTTCAATCGGAAGCCGATTGGGAGAACCCT  700
 701  GACGAGGATTTTGACGCATATGAGGACAAACATTATGCTACACACGCTAT  750
 751  TCTCAGCAATACTTTGGAGGAGTTGAGACGGGATGTCACCTCAAACAGTA  800
 801  TTGATGCCACAGTTCAAGCGCAGGCACACCCCAGAGAAGTCATGTCGATT  850
 851  TTCAGTTCGAGAGAATTAAACTTCCGACTTTTTCTGGAAATTATGAGGAC  900
 901  TGGAAACATTTTTCGGACATGTTTATTGGATCGATTGCTTCCAATTCGAG  950
 951  CCTGACGGATTGCCAACGATTTCATTATTTAAAATCGTACCTTGCCGGAG 1000
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1051  GAAGCATGGGAGCGGCTGGAACAGCGATATAACAAACAATCGCTAATTAT 1100
1101  TCGATCGTTCTTAAACAGTTTCATGAGCCTTCCGAGTGCTATAAATTCAA 1150
1151  ATATCGGCACAGTGCGGAAAATTGCCGATGGTGCAGACGAAGTTATTCGT 1200
1201  GGTCTACGAGCTCTTAATTGCGAAGAGAGGGATCCCTGGCTAATTTTCAT 1250
1251  TCTACTTTCAAAATTAGATAGCGATACCCGCCAAGCCTGGGCTCAGTGCG 1300
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1351  ACATCACGCTGCGATACGTTGGAGGCTTTTGAATTAACTCGATCAACCCA  1400
1401  AGCTCGACGCGCAGCTACCACGCACCACGCAGACACGCATCCAAGACGGG  1450
1451  AAGAGCCGAAGTGCACATCGTGCCAGCAGAATCACCAACTGTTTAAGTGT  1500
1501  CCTCAATTCATCGCACTCGACATTGCATCTCGCCGAGACTTCCTCAAATC  1550
1551  AAGAAAGCTCTGTTTCAATTGCCTCAGCCCGGCTCATATGGTGGGCAACT  1600
1601  GTACATCGAGGCATACTTGTCGGATCTGCCGCCGCAAGCATCATACTTTG  1650
1651  GTTCATGGCTCGTCGCAGCCAATTCAAAATGGCAACAACATTGACACAGC  1700
1701  AAGTGTTGACAGCCGCGATCGACCAGCAGTCTCACATGCGGGATCTACAA  1750
1751  TTGGCCACAATCAACCGCTAGCTCGAGAAGGTCATCGCTTGGGAAGCGAG  1800
1801  ACTCCCGCGGAAAACAACTTTACGCATCATACTCTGGAGAATATTCCGGC  1850
1851  GGCTGGTTCTCAGACTCTGTTGCCAACCATCCTTGCTGACGTCATCGACG  1900
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1951  ATAACCTTGGCATCGGAATCATTTGTTCAGCGAATAGGCGTGCGTCGAAC  2000
2001  GCACGCACGGATTTCTATTCTCGGTCTCGCCGCCAACAGCGCGGGCGTTA  2050
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2101  GTCGAATTGGTCTCGTTCATTCTCACCTCGCTGACGTCATCACTTCCTGC  2150
2151  CCAAGTTATTGACACCTCATCCTCTACGTGGAGGCAAATCTGCGAGCTTC  2200
2201  CTTTGGCAGACCCAACGTTCTGCACACCTGGAGCAATCGATGTCATTGTT  2250
2251  GGATCGGATCAACTTTGGTCTCTATACACAGGAGATCGGAAACACTTTGG  2300
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GenBank accession number U23420.

References: Bell, J.R., A.M. Bogardus, T. Schmidt and M. Pellegrini 1985, Nuc. Acids Res. 13: 3861-3871; Goldberg, M.M., J-Y. Sheen, W.J. Gehring and M.M. Green 1983, Proc. Natl. Acad. Sci., USA 80: 5017-5021; Green, M.M. 1967, Genetics 56: 467-482; O'Hare, K., C. Murphy, R. Levis and G. Rubin 1984, J. Mol. Biol. 180: 437-455; Peterson, K.M., P.S. Davis and B.H. Judd 1994, Mol. Gen. Genet. 242: 717-726.

Munjal, A.K., R. Parkash and Dev Karan. M.D. University, Rohtak, India. Starvation and desiccation tolerance in *D. bipectinata* and *D. malerkotliana*.

The Indian sub-continent represents a diverse array of climatically variable habitats, and there is little information on starvation and desiccation tolerance of various drosophilids. In the present studies, three Indian geographical populations of *D. bipectinata* and *D. malerkotliana* living under very different climatic conditions were studied for their desiccation and starvation tolerance by measuring the adult survival under dry and humid conditions, respectively. The population samples of *D. bipectinata* and *D. malerkotliana* were bait-trapped from three latitudinally varying sites of the Indian subcontinent (Cochin, 9°.58'N; Hyderabad, 17°.20'N and Rohtak, 28°.54'N). Starvation tolerance (mean survival time with water only) and desiccation tolerance (mean survival time with no food and zero percent humidity) were studied by measuring the survival duration in hours at 22°C in different isofemale lines by following the method of Da Lage et al. (1989).

The mean survival duration under desiccation conditions of three Indian geographical populations of *D. bipectinata* and *D. malerkotliana* is shown in Table 1. Life duration was found to be shorter under desiccating condition and thus indicated the specific effects of desiccating stress. The difference between the two successive generations was not significant and hence the desiccation tolerance trait seemed to be genetically controlled. In the Rohtak (North) population, the survival time was higher under desiccation conditions than that of the other two Indian populations of *D. bipectinata* as well as *D. malerkotliana*. Thus, all the three populations of *D. bipectinata* and *D. malerkotliana* revealed a gradient of survival hours with respect to latitude (Table 1). The ranking order for the survival time under desiccation conditions in three populations of *D. bipectinata* and *D. malerkotliana* was found as Rohtak > Hyderabad > Cochin. The mean survival time under desiccation conditions was significantly different among three Indian geographical populations of *D. bipectinata* (8 hours to 16 hours) and of *D. malerkotliana* (10.5 hours to 17.5 hours). The survival time for southern populations was significantly lower than those of the northern populations of *D. bipectinata* and *D. malerkotliana*. The statistical analysis of desiccation tolerance of G_1 and G_2 generations on the basis of student's "t" test revealed an insignificant difference. The desiccation tolerance correlations between two generations of *D. malerkotliana* and *D. bipectinata* populations were found to be insignificant except for the Cochin populations of *D. malerkotliana* and thus revealed genetic control of this ecological trait.

Table 1. Data on desiccation tolerance and starvation tolerance in three Indian geographical populations *of D. bipectinata* and *D. malerkotliana*.

Population / latitude	Species	Generation	Desiccation tolerance					Starvation tolerance				
			n	m	cv	sd	t*	n	m	cv	sd	t*
Rohtak (28° .54'N)	*D. bipectinata*	G_1	4	17	9.89	1.58	1.72	4	57	2.68	1.58	2.80
		G_2	4	15	8.13	1.22		4	61	3.17	1.87	
		M	4	16				4	59			
	D. malerkotliana	G_1	4	17	8.29	1.41	.82	4	65	2.88	1.88	1.39
		G_2	4	18	8.78	1.58		4	67	1.82	1.22	
		M	4	17.5				4	66			
Hyderabad (17° .20'N)	*D. bipectinata*	G_1	4	14	13.35	1.87	2.20	4	73	3.12	2.24	1.63
		G_2	4	11	12.64	1.58		4	70	3.27	2.34	
		M	4	12.5				4	71.5			
	D. malerkotliana	G_1	4	14	11.29	1.58	2.0	4	79	2.82	1.58	2.50
		G_2	4	12	5.95	.71		4	83	2.69	2.23	
		M	4	13				4	81			
Cochin (9° .58'N)	*D. bipectinata*	G_1	4	9	19.75	1.58	1.53	4	89	3.46	3.16	2.14
		G_2	4	7	19.75	1.58		4	94	2.68	2.44	
		M	4	8				4	51.5			
	D. malerkotliana	G_1	4	10	12.0	1.22	1.08	4	103	1.22	1.0	1.53
		G_2	4	11	6.42	.71		4	105	1.18	1.58	
		M	4	10.5				4	104			

n = number of lines; m = mean of four isofemale lines; G_1 = 1st generation; G_2 = 2nd generation; M = mean of two generations; t = Student's 't' test between the isofemale lines of the G_1 and G_2 generations; * significant at 1%.

The populations from the southern peninsula (Hyderabad and Cochin) revealed significantly higher starvation tolerance as compared with Rohtak populations of *D. bipectinata* and *D. malerkotliana*, i.e. Cochin > Hyderabad > Rohtak. The statistical analysis of G_1 and G_2 data of starvation tolerance revealed a higher correlation between the Hyderabad population of *D. bipectinata* and Rohtak and Cochin populations of *D. malerkotliana*. The three populations of *D. bipectinata* and *D. malerkotliana* did not reveal significant statistical differences between G_1 and G_2 generations on the basis of student's "t" test (Table 1). Such data indicated that the starvation tolerance trait is genetically controlled.

In the present studies, the observed higher starvation tolerance in southern populations of *D. bipectinata* (59 hours to 91.5 hours) and *D. malerkotliana* (66 hours to 104 hours) seems to be adaptively maintained due to a higher metabolic rate under continuously humid conditions and stable temperature conditions. However, the northern populations of *D. bipectinata* and *D. malerkotliana* are characterised by life under the least humid conditions and by variable metabolic rates under changing seasonal climatic conditions, and thus revealed significantly lower starvation tolerance. On the contrary, the desiccation tolerance potential of northern populations of *D. bipectinata* and *D. malerkotliana* (16 hours and 17.5 hours) was found to be higher than that of the southern populations (8 hours and 10.5 hours), respectively. Thus, under dry conditions during the experiment, the southern populations revealed lower tolerance due to their adaptation to hot and humid climatic conditions. The northern populations of *D. bipectinata* and *D. malerkotliana* (16 hours and 17.5 hours) was found to be higher than that of the southern populations (8 hours and 10.5 hours), respectively. Thus, under dry conditions during the experiment, the southern populations revealed lower tolerance due to their adaptation to hot and humid climatic conditions. The northern populations of *D. bipectinata* and *D. malerkotliana* had been adapted to a drier and variable temperature range due to seasons, and thus the higher desiccation tolerance could be due to a relatively lower metabolic rate under such subtropical climatic conditions. Thus, Indian populations of *D. bipectinata* and *D. malerkotliana* revealed contrasting patterns of starvation and desiccation tolerance along the north-south transect.

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Ivannikov, Andrey V. Institute of Cytology and Genetics of the Russian Academy of Sciences, Siberian Department, Novosibirsk, 630090, Russia. *Drosophila* species from forest mushrooms in Novosibirsk vicinity.

Table 1. *Drosophila* species from mushrooms nearby Novosibirsk in 1990

Species	Number of individuals collected
Drosophila (*Drosophila*) *histrio*	8
Drosophila (*Drosophila*) *limbata*	189
Drosophila (*Drosophila*) *phalerata*	1623
Drosophila (*Drosophila*) *transversa*	3015
Drosophila (*Sophophora*) sp. (*obscura* gr.)	1
Total	4836

Flies living in forest mushrooms were collected in August-September 1990. Collections were performed in the vicinity of Novosibirsk-city (Western Siberia), 8 km away towards the East of Akademgorodok, in the forest near Kluchi-village.

In the collections, the genus *Drosophila* was represented by five species from two subgenera (Table 1). Two species, *D. phalerata* and *D. transversa*, were clearly superior by number over other species. The species of the "*obscura*" group represented in the collections by a single female were not identified.

Milosevic, N.J.[1] and V. Kekić[2]. [1]Institute for Biological Research, University of Belgrade, Yugoslavia. [2]Institute of Zoology, Faculty of Biology, University of Belgrade, Yugoslavia. Ethanol experience and survival in *Drosophila melanogaster.*

Smell of ethanol, the main fermentation product of yeast (flourishing on decaying fruit), is not merely an information of potential food (or oviposition site, etc.) for *D. melanogaster*, but also a source of energy capable of producing a significant increase in life duration on low concentrations (Van Herrewege and David, 1974, 1978). However, a large concentration of ethanol, when used as a "food", has a toxic effect with different toxic threshold in different species, populations and genotypes (Libon-Mannaert, *et al.*, 1976; Parsons and Spence, 1981; David and Herrewege, 1983; David, *et al.*, 1986).

On the other hand, it is well known that *D. melanogaster* has a highly developed ability to modify its behavior in response to sensory stimulation as a consequence of previous experience (see for review McGuire, 1984; Tully, 1984).

In an experiment presented flies were reinforced by 10% ethanol solution for the response to the smell of ethanol. According to our results ethanol has an effect as a positive reinforcement to some flies and negative to some others. We wanted to see if flies, after the experience (alcohol consumption), in repeated situations react adaptively and select an environment more suitable for surviving.

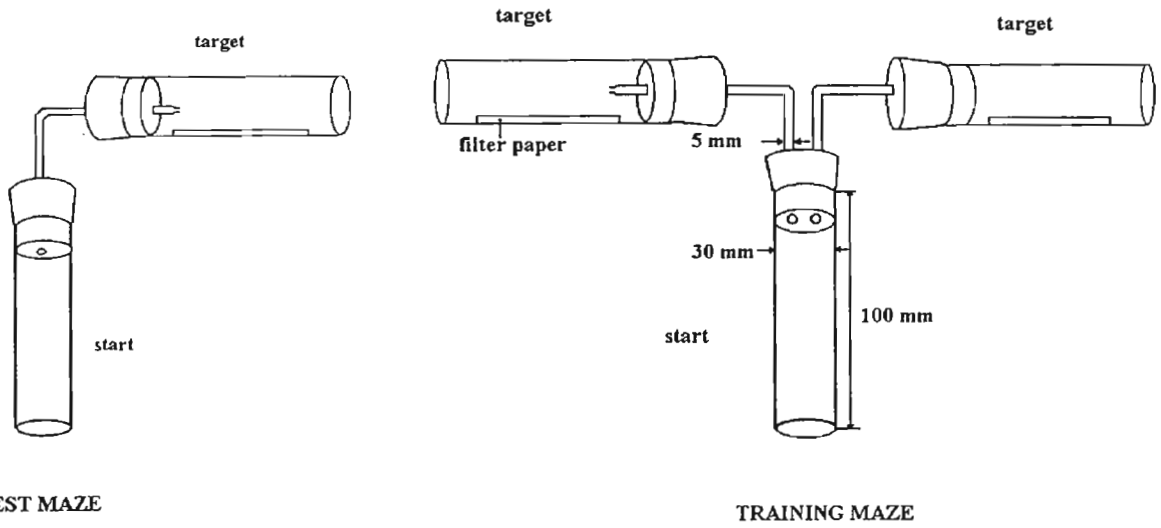

Figure 1. T-mazes for conditioning and testing some behavioral patterns in *Drosophila*.

Flies: *D. melanogaster* flies from 47th laboratory generation (raised on corn medium in mass culture at 25°C) without sexing and 3-6 days old were used in the experiment. All flies were starved 24 hours before training (they were in vials containing a piece of filter paper with 1 ml distilled water).

Maze: Mazes made of glass vials and tubes and rubber stoppers were used in training and testing procedures (Figure 1).

Training: Training maze was set horizontally with a neon source of lights in a front of the target vial (light intensity 1,400 lux). At the beginning about one hundred flies were placed in start vial, and after 5 minutes rest a start vial was connected to a target vial containing a piece of filter paper with 0.5 ml of 10% ethanol solution. Solution was colored in order to confirm if flies consumed alcohol (red color for food) - which all flies did after crossing to a target vial (they have red abdomens). After 30 minutes of the training procedure, almost all individuals crossed to the target vial (about 5% remain in start vial). Flies collected from the target vial were transferred again into the clean target vial and after 15 minutes rest, the training procedure was repeated.

Testing: After the second training procedure, flies were transferred into the clean target vial where they rested for 30 minutes when the vial was connected to the rest of the test maze, two target vials: one with the 10% ethanol and the other with the same quantity of distilled water. Different to the training procedure testing was performed with the light source (1,400 lux) above the test device.

Conditions and manipulations: Training and testing was performed at 20°C. Flies were transferred without anesthesia (by aspirator or putting vial to vial and by using a positive phototaxis). 45 minutes before use, mazes were prepared: ethanol or distilled water were put in target vials. Clean mazes were used always. 10% ethanol solution was used in our experiment on the basis of the experience of Soliman and Knight (1981, 1984) who used a practically identical T-maze in their experiments. This concentration, even higher, is frequent in natural environments of fruit flies, especially in man-made fermentation (McKenzie and McKechnie, 1979). On the other hand, this concentration is still below an average threshold value (for European populations, particularly) after which a longevity is severely reduced in laboratory experiments (Parsons and Spence, 1981; David and Herrewege, 1983; David, *et al.*, 1986).

Choice: A total of 845 individuals were tested (8 replicas). After two training cycles an average of 0.21 $\pm$ 0.02% of individuals tested went to the target vial of the test maize with the solution of 10% ethanol (these flies were signed with the acronym ALC+), into the vial with distilled water 0.63 $\pm$ 0.02% (ALC-), while in the start vial 0.16 $\pm$ 0.00% of individuals stayed (STn -- these flies were not used in the experiment). The outcome of an ANOVA, after angular transformation (Sokal and Rohlf, 1981), revealed that trained flies in a larger degree choose distilled water.

Surviving: From each group (ALC+ and ALC-) 120 flies were taken and put in 6 vials (10 males and 10 females)

containing filter paper with 10% ethanol as the only source of food and water. Every morning 1 ml of 10% ethanol solution was added in each vial, and every morning and evening a number of living flies was recorded. Surviving was monitored at 25°C.

Survival curves of the ALC+ and ALC- groups of flies (Figure 2) compared by Kolmogorov-Smirnov test (Sokal and Rohlf, 1981) showed that ALC+ flies have significantly higher survival on the solution of 10% ethanol than the ALC- flies ($D = 0.217 >> D_{0.05} = 0.175$).

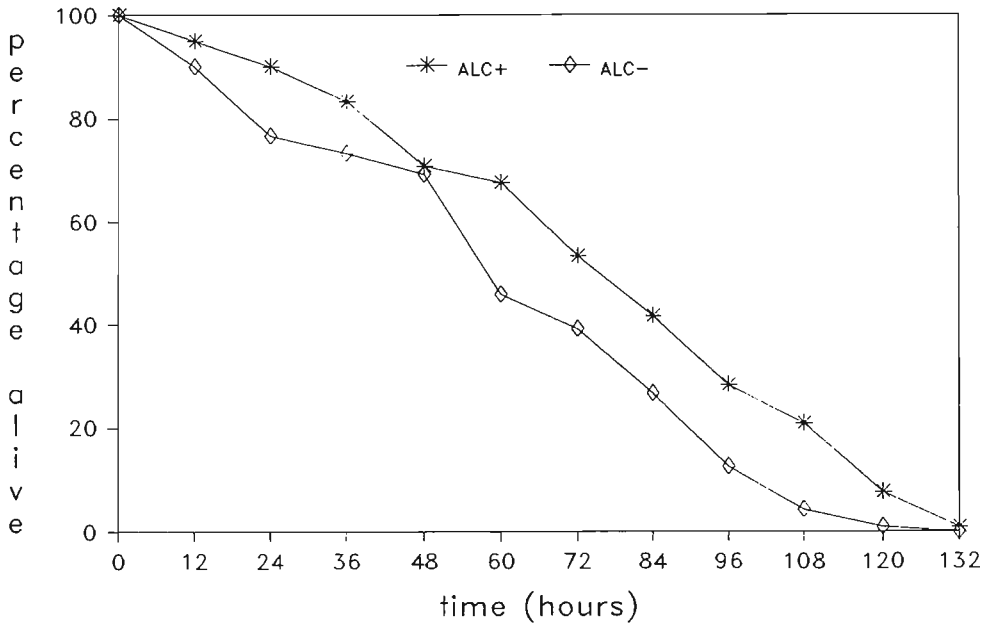

Figure 2. Survival curves of flies on 10% ethanol. ALC+, trained flies that choose ethanol; ALC-, trained flies that choose distilled water.

At first our results seemed to be discordant to Hoffmann and Cohan (1987) who examined the relationship between ethanol tolerance and attraction by characterizing the olfactory responses of *D. melanogaster* lines previously selected for increased knockdown resistance to ethanol fumes (5% concentration): "The present findings indicate that increased tolerance of ethanol is not necessarily associated with increased attraction and suggest that behavioral responses may even change in a 'nonadaptive' manner as a consequence of selection; i.e., flies which are better at utilizing/tolerating ethanol are less attracted to it".

Attractibility to certain ethanol concentrations, especially for naive flies, those that did not consume alcohol, need not to be in connection with their ability to use these concentrations in a diet -- aren't they always attracted by a glass of brandy?

Our results indicate that flies really use experience, because they react adaptively immediately after it, depending on how they "experienced" it, positively or negatively. After the alcohol consumption (training) a group of flies (ALC+) choose again (during the testing) an environment with the alcohol where they survive better than the other group of flies (ALC-), that respond also adaptively, because they avoided an alcohol environment during the testing.

References: David, J.R., and J. Van Herrewege 1983, Comp. Biochem. Physiol. 74A:283-288; David, J.R., H. Mercot, P. Capy, S.F. McEvery, and Jeanine Van Herrewege 1986, Genet. Sel. Evol. 18:405-416; Hoffmann, A.A., and F.M. Cohan 1987, Behav. Genet. 17:307-312; Libion-Mannaert, M., J. Delcour, M.C. Deltombe-Lietaert, N. Lenelle-Montfort, and A. Elens 1976, Experientia, 32:22-23; McGuire, T.R., 1984, Behav. Genet. 14:479-526; McKenzie, J.A., and S.W. McKenchnie 1979, Oecologia, 40:299-309; Parsons, P.A., and G.E. Spence 1984, Aust. J. Zool. 29:671-678; Sokal, R.R., and F.J. Rohlf 1981, *Biometry*. W.H. Freeman and Company, San Francisco; Soliman, M.H., and M-L. Knight 1981, In: *Genetic Studies of* Drosophila *Populations* (Eds., Gibson, J.B., and J.G. Oakeshott), Australian National University Press, Canberra, pp.95-102; Soliman, M.H. and M-L. Knight 1984, Behav. Genet. 14:295-313; Tully, T., 1984, Behav. Genet. 14:527-557; Van Herrewege, J., and J.R. David 1974, C.R. Acad. Sci., Paris 279:335-338; Van Herrewege, J., and J.R. David 1978, Experientia 34:163-164.

Karan, Dev, R. Parkash and A.K. Munjal. M.D. University, Rohtak, India. Allozymic similarity in *D. nepalensis*.

The *takahashii* species subgroup comprised of twelve described species which occurred largely in the oriental region and most of the species except *D. takahashii* were known to be endemic. Seven species of this subgroup had been reported from India and four were endemic, *i.e.*, *D. nepalensis* and *D. kurseongensis* occurring in the northern part of the Indian sub-continent as well as Nepal while *D. giriensis* and *D. jagri* were reported from some hilly sites of southern India. Except a single study on the salivary gland chromosomes map of *D. nepalensis* (Parshad and Gandhi, 1971), there are no reports on the genetic structure of *D. nepalensis*. Thus, the present studies were carried out to analyse allozymic polymorphism in ten Indian populations of *D. nepalensis*. *D. nepalensis* is an endemic cold adapted species and occurs only in India and Nepal (Bock, 1980). *D. nepalensis* occurs mainly in north Indian regions during winter months, *i.e.*, December to February. Isofemale lines were established for populations sampled from ten different sites (Hasimara, 26°.40'N; Darjeeling, 27°.03'N; Rohtak, 28°.94'N; Roorkee, 29°.52'N; Saharanpur, 29°.46'N; Risikesh, 29°.60'N; Dehradun, 30°.19'N, Chandigarh, 30°.43'N; Jammu, 32°.42'N; and Kathua, 33°.43'N).

Table 1. Distribution of allelic frequencies at seven polymorphic loci in ten Indian natural populations of *D. nepalensis*.

Locus	Electromorph	Hasimara	Darjeeling	Rohtak	Roorkee	Saharanpur	Risikesh	Dehradun	Chandigarh	Jammu	Kathua
Est-7	F	.29	.37	.31	.33	.33	.31	.34	.35	.39	.38
	S	.71	.63	.69	.67	.67	.69	.66	.65	.61	.62
	N	112	96	100	120	62	86	80	102	103	75
Acph	F′	-	.02	-	-	-	-	-	-	.01	-
	F	.87	.87	.96	.90	.88	.92	.91	.93	.90	.89
	M	.08	.07	.04	.06	.04	.06	.07	.07	.07	.08
	S	.05	.04	-	.04	.05	.02	.02	-	.02	.03
	S′	-	-	-	-	.03	-	-	-	-	-
	N	90	92	81	142	76	64	73	90	134	106
Adh	F	1.00	.98	1.00	1.00	.98	1.00	.97	1.00	1.00	1.00
	S	-	.02	-	-	.02	-	.03	-	-	-
	N	80	93	101	106	84	98	127	80	95	80
Ao	F′	.09	.10	.04	.08	.04	.06	.07	.06	.13	.12
	F	.36	.38	.36	.38	.38	.32	.31	.35	.32	.33
	S	.55	.52	.58	.54	.58	.60	.61	.59	.55	.55
	S′	-	-	.02	-	-	.02	.01	-	-	-
	N	98	100	126	88	96	134	97	88	94	72
Odh	F	.76	.74	.79	.73	.73	.75	.73	.77	.78	.75
	S	.24	.26	.21	.27	.27	.25	.27	.23	.22	.25
	N	93	75	110	97	79	118	110	121	104	118
α-Gpdh	F	.80	.85	.85	.82	.81	.81	.82	.85	.78	.79
	S	.20	.15	.15	.18	.19	.19	.18	.15	.22	.21
	N	98	90	100	126	96	130	112	117	90	120
Mdh	F	.90	.87	.92	.90	.89	.88	.87	.91	.88	.85
	S	.10	.13	.08	.10	.11	.12	.12	.09	.12	.15
	N	117	100	120	90	90	105	90	96	84	92

Table 2. Contingency χ^2 analysis of allelic frequency heterogeneity and genotypic frequency heterogeneity and Wright's F_{ST} values at seven polymorphic loci in ten Indian natural populations of *D. nepalensis*.

	Contingency χ^2 analysis				Wright's F_{ST} analysis		
Locus	allelic freq.	d.f.	genotypic freq.	d.f.	H_T	H_S	F_{ST}
Est-7	5.33	9	60.76*	10	.456	.447	.023
Acph	177.76*	27	72.25*	35	.348	.346	.006
Adh	39.54*	9	3.37	3	-	-	-
Ao	138.95*	27	248.93*	39	.275	.270	.017
Odh	4.94	9	25.68*	10	.372	.368	.012
α-Gpdh	62.98*	9	65.87*	10	.297	.296	.004
Mdh	5.86	9	52.51*	10	.198	.195	.006

* significant at 5% level. d.f. = degree of freedom.

Data on genetic indices (number of alleles and allelic frequencies) for diallelic loci (*Acph, Est-7, Adh, Ao, Odh, alpha-Gpdh* and *Mdh-1*) revealed similarity in genic diversity patterns in ten populations of *D. nepalensis* (Table 1). The most common allelic variant as well as frequency patterns at the various polymorphic loci were found to be almost similar in all the ten natural populations of *D. nepalensis*. The data on inter-populational heterogeneity in terms of genotypes were represented in Table 2. The distribution patterns of genotypes at polymorphic loci in ten natural populations were analysed on the basis of contingency chi-square test, and maximum deviation was observed at the *Ao* locus

and lesser deviations at *Est-7, Acph, Odh, alpha-Gpdh* and *Mdh* loci (Table 2). Similarly, the allelic frequency distribution pattern at polymorphic loci in ten populations of *D. nepalensis* was analysed on the basis of contingency chi-square test to reveal inter-population heterogeneity of allelic frequencies. Significant allelic frequency heterogeneity was observed at *Acph, Adh, Ao* and *alpha-Gpdh* loci.

The data on the amount of genetic differentiation at six polymorphic loci in ten natural populations of *D. nepalensis* were calculated in terms of Wright's Fixation Index (F_{ST}) and such values were represented in Table 2. The heterozygosity at the polymorphic loci was partitioned into within population as well as between population components, and the observed F_{ST} values at six polymorphic loci in ten natural populations of *D. nepalensis* were found to be quite low (.004 to .023) and thus indicated little genetic differentiation.

The electrophoretic analysis of ten Indian natural populations of *D. nepalensis* revealed homogeneity of allozymic frequencies, *i.e.*, the most common allele was found to be the same everywhere, and its frequency was almost similar at most of the loci in different populations. The data on Wright's Fixation Index (F_{ST}) also revealed that there was very little genic differentiation. The homogeneity patterns of genic variation at most polymorphic loci in ten Indian natural populations of *D. nepalensis* can be explained on the basis of gene flow which is expected to affect all the polymorphic loci similarly.

References: Bock, I.R., 1980, Systematic Entomology 5:341-356; Parshad, R., and S.C. Gandhi 1971, Res. Bull. Punjab Univ. Zool. 22:5-11.

Parkash, R., Dev Karan and A.K. Munjal. M.D. University, Rohtak, India. Resource utilisation in five colonising drosophilids.

The fermentation by-products produced in the environment depend on the type of micro flora (yeasts and other microbes) involved and the types of organic matter undergoing decomposition. Thus, it can be predicted that diverse types of drosophilids could reflect interspecific differences in tolerance of different alcoholic resources. *D. melanogaster* and *D. lebanonensis* have been reported as highly ethanol tolerant while many species were found to be ethanol sensitive (David and Van Herrewege, 1983). The objective of the present study was to analyse alcoholic utilisation in five colonising drosophilids (*D. melanogaster, Z. indianus, D. repleta, D. kikkawai* and *D. immigrans*) from Bhuntar in Himachal Pradesh.

Table 1. Comparison of ethanol and acetic acid tolerance indices (increase in longevity LT_{50} hrs, LT_{50}max / LT_{50} control, adult threshold concentrations and LC_{50} at 4th day) in five sympatric species (*D. melanogaster, Z. indianus, D. repleta, D. kikkawai* and *D. immigrans*) from Bhuntar (Himachal Pradesh).

Species	Ethanol tolerance				Acetic acid tolerance			
	LT_{50}hrs	LT_{50}max/ LT_{50} control	Threshold conc.(%)	LC_{50}	LT_{50}hrs	LT_{50}max/ LT_{50} control	Threshold conc.(%)	LC_{50}
D. melanogaster	240	3.28	13.8	13.0	130	2.0	9.7	9.0
Z. indianus	250	2.08	7.1	8.0	150	1.25	6.1	7.3
D. repleta	285	2.0	6.3	7.0	180	1.28	6.2	7.6
D. kikkawai	117	1.56	3.8	4.0	100	1.33	4.0	4.8
D. immigrans	105	1.50	2.6	3.0	98	1.40	3.5	3.5

Table 2. Data on primary (n-propanol and n-butanol) and secondary alcohols (2-propanol and 2-butanol) tolerance indices [increase in longevity LT_{50} hrs, and LC_{50} (%)] in five sympatric species from Bhuntar (Himachal Pradesh).

Species	n-propanol		2-propanol		n-butanol		2-butanol	
	LT_{50}hrs	LC_{50}(%)	LT_{50}hrs	LC_{50}(%)	LT_{50}hrs	LC_{50}(%)	LT_{50}hrs	LC_{50}(%)
D. melanogaster	141	4.0	88	2.50	135	3.0	85	1.0
Z indianus	156	1.80	137	1.20	186	1.70	127	0.68
D. repleta	198	2.0	152	2.12	224	1.82	202	1.05
D. kikkawai	113	1.40	78	0.8	120	0.80	106	0.42
D. immigrans	90	0.80	72	0.65	84	0.60	72	0.42

The adult ethanol, acetic acid, primary and secondary alcohol tolerance patterns were assessed following Starmer *et al.* (1977). Four replicates were performed for each concentration for all the experiments. The effects of metabolic alcoholic vapours were assessed from the number of flies alive after various time intervals, and LT_{50} values were expressed as the number of hours at which 50% of the flies died and were estimated by linear interpolation. The alcoholic threshold values were used as indices, *i.e.*, if the vapours were utilised as resource then LT_{50} maximum/LT_{50} control was found to be >1, if this ratio was <1, then it acted as stress, and the threshold values were determined when

LT_{50} maximum/LT_{50} control = 1.

The increased longevity data due to ethanol revealed lesser effects in *D. immigrans* (105 hr) and *D. kikkawai* (117 hr) as compared with *D. melanogaster* (240 hr), *Z. indianus* (250 hr) and *D. repleta* (185 hr). The adult threshold values were found to be lesser in *D. immigrans* (2.60%), *D. kikkawai* (3.80%), but higher for *D. repleta* (6.30%), *Z. indianus* (7.10%) and *D. melanogaster* (13.80%). The LC_{50} ethanol concentrations were calculated from mortality data of adults after four days in all the five species, and LC_{50} values were found as 3.0% in *D. immigrans*, 4.0% in *D. kikkawai*, 7.0% in *D. repleta*, 8.0% in *Z. indianus* and 13.0% in *D. melanogaster*. The present results revealed that acetic acid and ethanol constitute parallel resources. n-propanol and 2-propanol were also metabolised by all the five drosophilids (Table 2). n-butanol was metabolised more than that of 2-butanol, and the LT_{50} hours for n-butanol revealed significant increase in longevity. Lower concentrations of n-propanol and 2-propanol are non-toxic to *D. repleta, Z. indianus* and *D. melanogaster.* However, 2-butanol (>1.2%) revealed a toxic effect for *D. melanogaster, Z. indianus, D. repleta, D. kikkawai* and *D. immigrans* (Table 2).

The ethanol and acetic acid utilisation in five drosophilids revealed that (a) *D. melanogaster* was found to be highly ethanol tolerant (13.8%) and also utilised acetic acid up to 9.5%, (b) *Z. indianus* and *D. repleta* revealed parallel utilisation of ethanol and acetic acid up to 7.0%, (c) *D. kikkawai* and *D. immigrans* showed lesser utilisation of acetic acid and ethanol up to 4.0% as compared with three other species. Thus, *D. melanogaster, Z. indianus* and *D. repleta* revealed lesser utilisation of alcohol resources. The data on longevity revealed that *D. repleta* could significantly utilise primary and secondary alcohols as compared with the other four drosophilids. In *D. melanogaster*, acetic acid resulted in two fold and ethanol revealed three fold increase in longevity while secondary alcohols showed lesser effect on longevity. Thus, the patterns of resource utilisation are species specific.

References: Starmer, W.T., W.B. Heed and E.S. Rockwood Sluss 1977, Proc. Natl. Acad. Sci. USA 74:387-391; David, J.R., and J. Van Herrewege 1983, Comp. Biochem. Physiol. 74A:283-288.

Noor, M.A., and J.A. Coyne. Department of Ecology and Evolution, University of Chicago, Chicago, IL, U.S.A. (e-mail: mnoo@midway.uchicago.edu). Sterility in *D. pseudoobscura/D. p. bogotana* hybrid males.

Drosophila pseudoobscura bogotana has been considered a paradigm of incipient speciation. Hybrid male offspring with *D. pseudoobscura bogotana* mothers and North American *D. pseudoobscura* fathers were found to be sterile (Prakash, 1972). More recently, Powell (1982) produced evidence that the observed sterility was not innate in the naturally-occurring flies, but it evolved in response to laboratory culturing. He treated the rearing medium with tetracycline and found that it increased the fertility of hybrid males. Hybrid males from *D. p. bogotana* strains that were cultured for 2-5 generations were not completely sterile, but other strains that had been kept in culture for five years were sterile. The finding that tetracycline increased hybrid male fertility implies that a microorganism might be responsible for the sterility. The supposed role of a microorganism, or anything else associated with laboratory culturing, in producing the sterility is questionable in light of the consistent genetic mapping of the hybrid sterility to the X-chromosome in different laboratory strains of *D. p. bogotana* (Prakash, 1972; Orr, 1989).

D. Weinreich provided us with a *D. p. bogotana* strain that had been in culture for only 7 generations, and we collected three isofemale lines of *D. pseudoobscura* from Mather, CA, 10 generations before the experiment. The Mather *pseudoobscura* lines were intercrossed immediately to form a single, outbred stock. Five *D. p. bogotana* females were mated to 5 *D. pseudoobscura* males and reared at 21°C on standard corn medium. The same combination was also reared on 0.03% tetracycline-treated medium (Hoffmann, *et al.*, 1986).

Hybrid male offspring were tested for fertility 7 days after eclosion by squashing the testes on a slide with Ringers solution and looking for sperm. Twenty hybrid male offspring were tested from each medium, and no sperm were seen. This result may indicate that the hybrid sterility observed in these subspecies evolves in the laboratory in less than 8 generations. Given the consistency among the genetic studies of hybrid sterility, it is more likely that the strains used in the study of microorganism-induced sterility in these subspecies were anomalous.

References: Hoffmann, A.A., M. Turelli, and G.M. Simmons 1986, Evolution 40: 692-701; Orr, H.A., 1989, Evolution 43: 180-189; Powell, J.R., 1982, *Mechanisms of Speciation*, pp. 67-74, Alan R. Liss, Inc., New York; Prakash, S., 1972, Genetics 72: 143-155.

Ali, Ismat Ara, and M.A. Hossain. University of Rajshahi, Rajshahi, Bangladesh. Assessment of mutagenic damage following treatment with ethyl methanesulfonate and caffeine in *Drosophila melanogaster*.

Evidence for the mutagenic activity of chemicals as well as for ionizing radiation was established with experiments using *Drosophila melanogaster* as the test system. Most of the mutagenicity studies of ethyl methanesulfonate (EMS) in insects have been done using this species. EMS is a very potent mutagen in *Drosophila*, inducing dominant and recessive lethal mutations (Sram, 1970; Ali, *et al.*, 1993a, b).

Caffeine is a plant alkaloid (1,3,7-trimethyl xanthine). It occurs in many widely distributed drinks and medical drugs. Since caffeine is a purine analogue, it has been extensively tested for mutagenic activity. Although there is now good evidence that caffeine is mutagenic in many animals, a different response has been reported in different test systems.

In *Drosophila*, it was reported that caffeine is mutagenic (Andrew, 1959; Fillippova and Jurkov, 1973), but neither of these findings has found confirmation in subsequent experiments with *Drosophila* by other workers (Yanders and Seaton, 1962; Mittler and Callaghan, 1969).

The present study was undertaken to determine the effect of caffeine on the yield of EMS-induced dominant and sex-linked recessive lethal mutations in *Drosophila melanogaster*.

For the experiment, normal Oregon-R and Muller-5 strains of *D. melanogaster* were used. Tester flies were starved in empty vials for six hours to assure immediate uptake of the test solution by flies at the beginning of the treatment. Pieces of filter paper soaked with 1 ml of EMS solution of 6.0 mM concentration was placed in vials containing 1-2 day old flies. For the treatment with caffeine, a piece of filter paper soaked with 1 ml solution of 0.2% caffeine in 10% sucrose solution was kept in the vials containing starved flies. Duration of treatment for both EMS and caffeine was restricted to 24 hr.

At the beginning of the first mating, the age of flies of both sexes was restricted to 3-4 days, and later one, this age was maintained for females only and the age of males were changed following succession of brood intervals. The testes of adult males

Table 1. Dominant lethal mutations (DLM) following treatment with ethyl methanesulfonate (EMS) (6.0 mM) and caffeine in *D. melanogaster*.

Cross *	Brood **	No. of eggs	No. of unhatched eggs	% of unhatched eggs ± S.E.	% of DLM
	I	2554	308	12.02 ± 0.91	—
Control ♂	II	3248	465	14.28 ± 0.69	—
X	III	2747	419	15.22 ± 0.86	—
Control ♀	IV	2908	432	14.76 ± 1.29	—
	I - IV	11457	1624	14.07 ± 0.71	—
	I	2756	691	25.02 ± 0.80	14.78^{l}
EMS ♂	II	3055	736	24.07 ± 0.37	11.42^{m}
X	III	2875	629	21.80 ± 1.06	7.76^{n}
Control ♀	IV	3047	640	20.90 ± 1.24	7.20^{n}
	I - IV	11733	2696	22.95 ± 0.96	10.29 ± 1.76^{a}
	I	2059	393	17.30 ± 0.98	6.00^{l}
Caffeine ♂	II	2953	498	16.80 ± 0.69	2.94^{m}
X	III	2473	416	16.80 ± 0.86	1.86^{m}
Control ♀	IV	2588	425	16.36 ± 1.04	1.88^{m}
	I - IV	10073	1732	16.81± 0.19	3.17 ± 0.98^{b}
	I	2790	737	26.32 ± 1.32	16.25^{l}
EMS + Caffeine ♂	II	2945	762	25.83 ± 0.57	13.47^{m}
X	III	2357	522	22.12 ± 0.77	8.14^{n}
Control ♀	IV	3206	700	21.78 ± 1.08	8.23^{n}
	I - IV	11298	2721	24.01 ± 1.20	11.52 ± 2.01^{a}
	I	2851	716	25.06 ± 1.20	14.82^{l}
EMS ♂	II	2243	562	25.00 ± 0.58	12.50^{l}
X	III	2922	647	22.05 ± 1.17	8.06^{m}
Caffeine ♀	IV	2725	596	21.83 ±1.21	8.29^{m}
	I - IV	10741	2521	23.48 ± 0.89	10.92 ± 1.65^{a}
	I	2684	673	25.05 ± 0.40	14.81^{l}
Caffeine ♂	II	1948	485	24.71 ± 1.67	12.17^{l}
X	III	2455	542	22.01 ± 1.63	8.01^{m}
EMS ♀	IV	3060	671	21.80 ± 1.67	8.26^{m}
	I - IV	10147	2371	23.39 ± 0.86	10.81 ± 1.64^{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 ethyl methanesulfonate (EMS) (6.0 mM) and caffeine in *D. melanogaster*.

Cross *	Brood **	No. of chromosomes tested	No. of lethals	% of SLRLM ± S.E.
	I	953	1	0.08 ± 0.08
Control ♂	II	876	2	0.23 ± 0.11
X	III	852	1	0.09 ± 0.09
Control ♀	IV	878	1	0.10 ± 0.10
	I - IV	3559	5	0.12 ± 0.03[a]
	I	796	138	17.34 ± 0.14[l]
EMS ♂	II	893	127	14.24 ± 0.11[l]
X	III	820	52	6.34 ± 0.11[m]
Control ♀	IV	836	31	3.70± 0.16[m]
	I - IV	3345	348	10.40± 3.22[b]
	I	847	4	0.46 ± 0.08[l]
Caffeine ♂	II	865	3	0.35 ± 0.003[l]
X	III	870	2	0.21 ± 0.10[l]
Control ♀	IV	932	2	0.20 ± 0.10[l]
	I - IV	3514	11	0.30 ± 0.06[a]
	I	846	144	17.03± 0.11[l]
EMS + Caffeine ♂	II	821	110	13.41± 0.13[l]
X	III	952	56	5.11± 0.10[m]
Control ♀	IV	976	29	2.97± 0.06[m]
	I - IV	3595	339	9.63± 3.34[b]
	I	947	158	16.69± 0.07[l]
EMS ♂	II	846	116	13.69± 0.10[l]
X	III	830	50	6.03± 0.14[m]
Caffeine ♀	IV	866	27	3.16± 0.10[m]
	I - IV	3489	351	9.89± 3.17[b]
	I	864	146	16.87± 0.14[l]
Caffeine ♂	II	872	120	13.74± 0.11[l]
X	III	897	52	5.79± 0.11[m]
EMS ♀	IV	839	24	2.86± 0.04[m]
	I - IV	3472	342	9.80± 3.30[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.

contain all germ cell stages, viz., spermatozoa, spermatids, spermatocytes, and spermatogonia. The amount of genetic damage induced in cells of various stages was tested by transferring the treated males every three days to fresh virgin females. Each transfer constituted a brood and four such broods were studied. In this manner a succession of four 3-day broods were produced, permitting subsequent identification of induced mutations at stage-specific times of spermatogenesis as designed by Fahmy and Fahmy (1961).

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

The effects of EMS 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 brood IV followed by the spermatocytes and spermatogonia. The effects of caffeine on the spermatogonic stages revealed that although the mean percentages of lethals were higher in broods I and II, it did not show any appreciable differences with other broods. Caffeine also did not play any role to modify the EMS-induced mutations.

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

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Krebs, R.A., V. Loeschcke, and C. Håkansson. Department of Ecology and Genetics, University of Aarhus, Ny Munkegade, Bldg. 540, 8000 Aarhus C, Denmark. Resistance and acclimation to thermal stress in adult *D. buzzatii* following collection under ether and CO_2.

Genetic variation for resistance to thermal extremes is present within and among populations of *Drosophila* (Hosgood and Parsons, 1968; Parsons, 1979; Morrison and Milkman, 1978; Kilias and Alahiotis, 1985; Quintana and Prevosti, 1990; Loeschcke *et al.*, 1994; Krebs and Loeschcke, 1994a), although genetic variation for acclimation to thermal extremes has not been found in natural populations (Hoffmann and Watson, 1993; Loeschcke *et al.*, 1994). However, lines of *Drosophila* maintained at lower constant temperatures for many years may acclimate to temperature extremes at lower temperatures than lines maintained at higher temperatures (Cavicchi *et al.*, in press).

We examined genetic variation for acclimation to heat stress in populations known to vary in their resistance, using conditions known to induce the heat-shock response in *Drosophila* cells (DiDomenico *et al.*, 1982a, 1982b). This work contributes to earlier analyses of variation within and among populations of *D. buzzatii* (Loeschcke *et al.*, 1994; Krebs and Loeschcke, 1994a), and asks how resistance to thermal stress may be affected by conditioning adults with a different potential stress, anesthetization, either by ether or CO_2, with and without conditioning by high temperature.

Mass populations of *D. buzzatii* originated from Teneriffe in the Canary Islands (CI), Cordoba, Spain (COR), Oxford Downs, Queensland, Australia (OD) and El Chanar, Tucuman province, Argentina (ARG). Collection sites, maintenance of lines and general methods for analyzing heat-stress resistance are described in Krebs and Loeschcke (1995). Virgin adults emerging from 10 bottles for each population were pooled and anesthetized either by light ether anesthesia or by CO_2 anesthesia to separate males and females to vials (20 per vial). The following day, one vial of males and females of each population and anesthesia group either was placed at 38°C for 75 min or was maintained at 25°C, and 24 hr later those flies from the same collection day were heat stressed for 100 min at 41.5°C as 4-day-old adults. This procedure was repeated over five collection days (which were considered statistically as separate blocks of replicates).

Table 1. Analyses of variance for the comparison of survival to heat stress for *D. buzzatii* that were conditioned or not by heat (separate ANOVAs due to block x conditioning treatment interactions in combined data), among males and females from four localities collected either by ether or CO_2 anesthesia.

	Not conditioned		Conditioned	
Source	df	MS	df	MS
Block	4	0.28***	4	2.20***
Locality	3	0.23***	3	0.62***
Anesthesia	1	0.32**	1	0.16 P< 0.10
Sex	1	0.23**	1	0.61***
Loc x Anes	3	0.03	3	0.03
Loc x Sex	3	0.10*	3	0.17*
Anes x Sex	1	0.15*	1	0.02
Loc x Anes x Sex	3	0.04	3	0.05
Error	145	0.031	146	0.044

* P < 0.05, ** P < 0.01, *** P < 0.001

Data were arcsine-square-root transformed in analyses of variance. For these, a general linear models procedure (SAS Institute, 1989) was used to test significance of the effects, locality, sex, conditioning by heat and anesthesia treatment, and to test interactions among these effects. All effects were treated as fixed. The large differences in survival between treatments, whereby flies either were heat conditioned before exposure to stress or were not (Fig. 1a,b), led to unequal variances between groups, and a highly significant block interaction with treatment. Therefore, data were separated for final analysis, and other interactions with block were left as components of the error variance. Block effects occurred (Table 1), probably due to day-to-day variation in the temperature or perhaps the heat-up time within the incubator, either of which would affect the stress level imposed on flies for any particular block of replicates.

Survival of males and females of all populations was much higher where they were conditioned by heat 24 hr before exposure to the heat stress than where no heat conditioning treatment was given, and significant differences in survival after heat stress were present among localities under both of these treatments (Table 1). Survival of flies from Oxford Downs and Canary Islands was highest (Figure 1), followed by that for the Argentina and Cordoba localities, but only the difference between Cordoba and all other localities was significant (Tukey's multiple comparisons test, $P < 0.05$). These differences among populations were similar to those found previously (Krebs and Loeschcke, 1995). As the rank order for survival of flies among populations was similar with and without conditioning by heat, and as the locality x anesthesia and locality x anesthesia x sex interaction effects were not significant, we found no evidence for genetic variation in acclimation to heat stress among these populations.

The method of anesthesia affected survival significantly for flies that were not conditioned with heat, and at a level approaching significance for the heat acclimation group (Table 1). Survival of individuals collected 48 hr before exposure to stress was higher where ether was used as the anesthesia than CO_2 (Figure 1). Flies from the different localities were not affected differently by the anesthesia treatments (Table 1), but for non-conditioned flies, the sex x anesthesia interaction was significant due to a greater difference in males than females for survival to stress between the ether and CO_2 anesthesia methods (Figure 1). Survival of males was greater than that of females in all populations but Cordoba, the locality with the lowest mean survival, which may have led to a significant locality x sex interaction (Table 1). Survival of males generally is higher than that of females in *D. buzzatii* (Loeschcke *et al.*, 1994), and the significant locality by sex interactions may be explained by increased female survival relative to that of males where stress levels cause very high mortality. Although the mechanism is unknown, this sex by stress level effect is consistent across numerous experiments and populations (Krebs and Loeschcke, 1994a; Krebs and Loeschcke, 1995; Dahlgaard *et al.*, 1995).

The comparison of thermal stress resistance between individuals anesthetized with ether or CO_2 supports observations that mild exposure to chemical stress also may improve resistance to heat stress (Tanguay, 1983), although

interaction effects between males and females were found. Resistance to different types of stress, such as heat, desiccation and ethanol, are correlated (Hoffmann and Parsons, 1989). The link may be through changes in metabolic rate (Service *et al.*, 1988; Hoffmann and Parsons, 1991, ch. 6; Parsons, 1992), especially where the stress agent causes anoxia (Parsons, 1974). Recovery time from ether is much longer than that from CO_2 anesthesia suggesting that ether may be a greater stress than CO_2, but the duration for recovering, about 10-15 min at most, is unlikely to cause anoxia. An alternative possibility for ether-induced acclimation to thermal stress is induction of the heat-shock proteins. Ether has been shown to induce heat shock proteins in rat tissue that were exposed directly to ether, but has not led to a general response in the body (Holbrook and Udelsman, 1994).

The increase in survival to heat stress with exposure to ether did not approach the change caused by conditioning individuals with high temperature, but marked improvement was found in comparison with CO_2. Males, however, showed much greater improvement than females. Similarly, survival after heat stress in *D. melanogaster* was higher for flies previously exposed to ether than to CO_2, with differences in males greater than those for females (Smith and Huey, 1991). An explanation could be that sex differences occur in the stress level that induces an acclimation response. Costs of activating the stress response may be greater in female than in male *D. melanogaster* (Krebs and Loeschcke, 1994b). If males activate heat-shock proteins after treatments where females do not, for example following a mild chemical conditioning treatment, male survival would be greater than that of females under these experimental conditions.

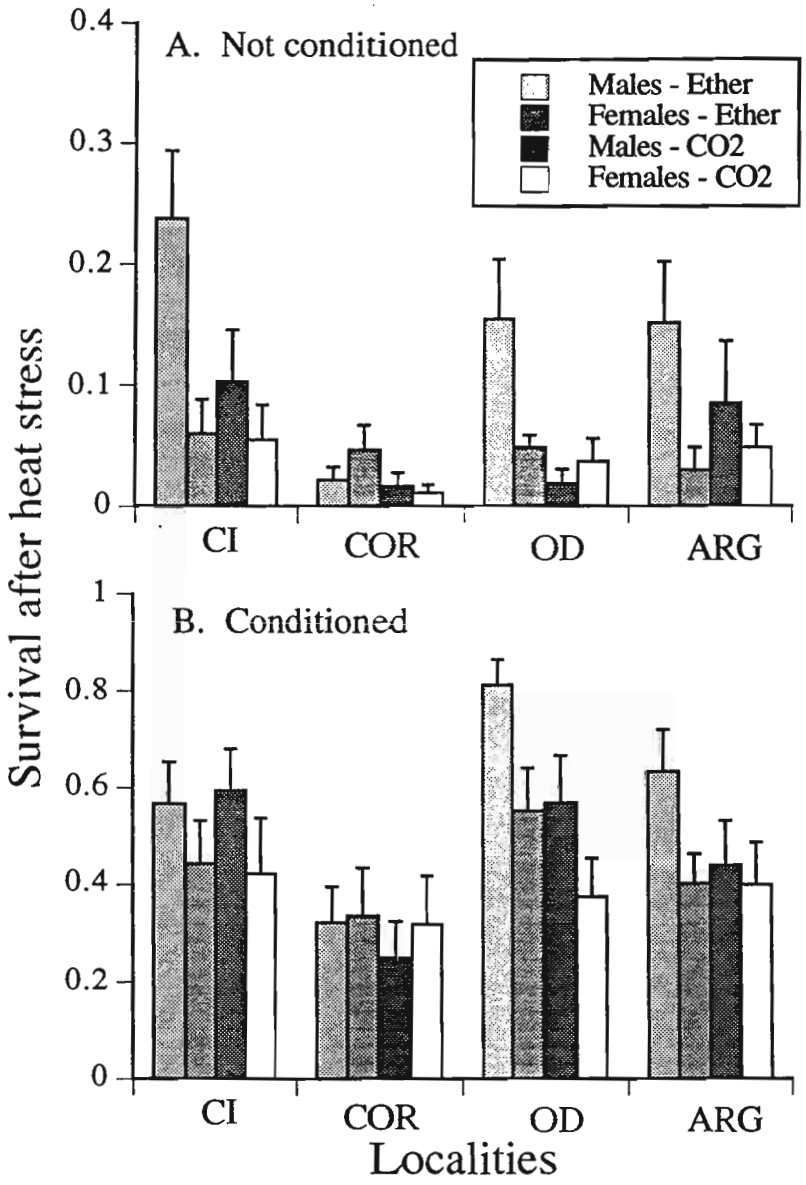

Figure 1. Mean survival (±se) after thermal stress for males and females from four mass populations of *D. buzzatii*: the Canary Islands, CI, Cordoba, Spain, COR, Oxford Downs, Australia, OD, and El Chanar, Argentina, ARG. Flies were collected under either ether or CO_2 anesthesia and either (A) not conditioned or (B) conditioned 24 hr before exposure to heat stress.

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Rabossi, A., and L.A. Quesada-Allué. I.I.B. Fundacion Campomar, Antonio Machado 151, Buenos Aires (1405), Argentina. Temporal correlation of metamorphosis events in *Drosophila melanogaster* and *Ceratitis capitata*.

Comparing two evolutionarily distant dipteran species, *Ceratitis capitata* and *Drosophila melanogaster*, the span in hours between any two well-characterized developmental markers is equivalent to a similar portion of the life cycle. For example, in both species, the three larval stages represent approximately 50% of the total life cycle. Here, published data on *D. melanogaster* development (Bodenstein, 1951; Hadorn and Mitchell, 1951; Bainbridge and Bownes, 1981; Ashburner, 1989) were correlated with our recent and previous Medfly observations (Boccaccio and Quesada-Allué, 1989; Rabossi, *et al.*, 1991, 1992; Rabossi and Quesada-Allué, 1993).

Table 1. Comparison of *Drosophila melanogaster* and *Ceratitis capitata* metamorphosis

Metamorphosis markers	*D. melanogaster* hours	*D. melanogaster* cumulative* percentages	*C. capitata* hours	*C. capitata* cumulative* percentages
1- Onset of pupariation, "Time zero". The shaping of the puparium is completed, the larval segmentation is obliterated, the cuticle is still white and rough. a-b-c-f	0	**0**	0	**0**
2- Puparium fully colored. c-g	3.5	**3.6**	16	**5.1**
3- Larval- pupal apolysis. b-c The insect positively buoyant. b-g	6	**6.2**	20.5	**6.6**
4- Evertion of the head, wings and legs. c-g	13	**13.5**	48	**15**
5- Pupal - adult apolysis b. Adult cuticle proteins start to be deposited. e	50	**52**	160	**51.3**
6- The red eye pigment (Drosopterin-like) appears. d-h	60	**62.5**	192	**61.5**
7- Bristle pigmentation begins dorsally on head and thorax. b-c-g	70	**72.9**	216	**69**
8- The tip of the folded wing becomes gray. Bristle pigmentation begins in first and second abdominal segments. b-c-g	76	**79.2**	240	**77**
9- Pigmented bristles are visible on legs. b-c-g	78	**81.2**	240	**77**
10- Wings are fully gray. b-c-g	78	**81.2**	264	**84.6**
11- Tarsal bristles darken and claws become dark. b-c-g	81.9	**85.3**	264	**84.6**
12- Wings darken to black. b-c-g	86	**89.6**	288	**92.3**
13- Eclosion of the imago. b-c-g	96	**100**	312	**100**

* *Drosophila* and *Ceratitis* were grown respectively at 25 °C and 23 °C in the conditions as indicated below in references b, c and f. The progress of metamorphosis is indicated in terms of cumulative percentage of time. To compare both flies, the described timing of events in *Drosophila* (references b and c) were correlated as accurately as possible with our observations in *Ceratitis*; in some cases recording the end of the event.
Letters correspond to citations below: a) Ashburner, 1989; b) Bainbridge and Bownes, 1981; c) Bodenstein, 1951; d) - Hadorn and Mitchell, 1951; e) Boccaccio and Quesada-Allué, 1989; f) Rabossi *et al.*, 1991; g) Rabossi *et al.*, 1992; h) Rabossi and Quesada Allué, 1993

After egg eclosion, *D. melanogaster*, when grown at 25°C, needs approximately 96 h to initiate metamorphosis and 192 h for the imago to emerge from the puparium as compared to the 288 h and 600 h, respectively, required by *C. capitata* at 23°C. To compare both flies, the times of occurrence of developmental events are expressed as cumulative percentages of the total metamorphosis span, defining "time zero" as the beginning of pupariation (Bodenstein, 1951; Rabossi, *et al.*, 1991). The data show that, except in the case of puparium tanning, the normalized timing of events as shown in Table 1 never differ more than 10%. Therefore, the time of occurrence of a given developmental landmark in one fly provides an accurate estimation of the occurrence of the same one in the other fly. Moreover, a preliminary review of data scattered through the literature suggest that this seems to be valid for many cychloraphous dipterans.

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Wismar, J.,* O. Vef, and E. Gateff. Institut fur Genetik, Johannes Gutenberg-Universitat, Saarstrasse 21a,, 55099 Mainz, Germany. *corresponding author (Tel: 06131/395343; Fax: 06131/395845). Genetic and molecular analysis of five deficiencies in the region 97F.

The tumor suppressor gene *lethal(3)malignant brain tumor* [*l(3)mbt*] was mapped to the region 97F3-11 with the help of the P-element induced deficiency *Df(3R)mbt*^{p} (Gateff, *et al.*, 1993). To identify the *l(3)mbt* gene within the 52 kb genomic walk encompassing the breakpoints of *Df(3R)mbt*^{p} we needed overlapping deficiencies, since the EMS induced *l(3)mbt* alleles did not show any significant differences in Southern blot analysis (Wismar, 1994). Five deletions established as TM6B or TM3 balanced stocks were generated in a P-element excision mutagenesis. For the excision mutagenesis we used two stocks with a P-element insertion in the region 97F3-5. The P-elements were mobilized with the help of the transposase producing stock *w/w*; *Sb*, delta2-3/TM3 *Ser* (Robertson, *et al.*, 1988). Table 1 presents the five resulting deficiencies including their designation, cytology, size, phenotype and the complementation results with *l(3)mbt*^{tsl}.

The deletion breakpoints located within the 52 kb genomic walk were determined by Southern blot analysis excepting the centromeric breakpoint of *Df(3R)7-213* and the telomeric breakpoint of *Df(3R)27-278* which are located outside of the cloned genomic region. *In situ* hybridization analysis with probes derived from different parts of the 52 kb genomic walk showed that this region represents approximately the chromosome bands 97F3-11 (Gateff, *et al.*, 1993). However, the salivary gland chromosome squashes of *Df(3R)7-213* and *Df(3R)27-278* revealed that the neighboring bands 97F1-2 and 98A1-2 are still present. Since these both bands are more or less strong we assume

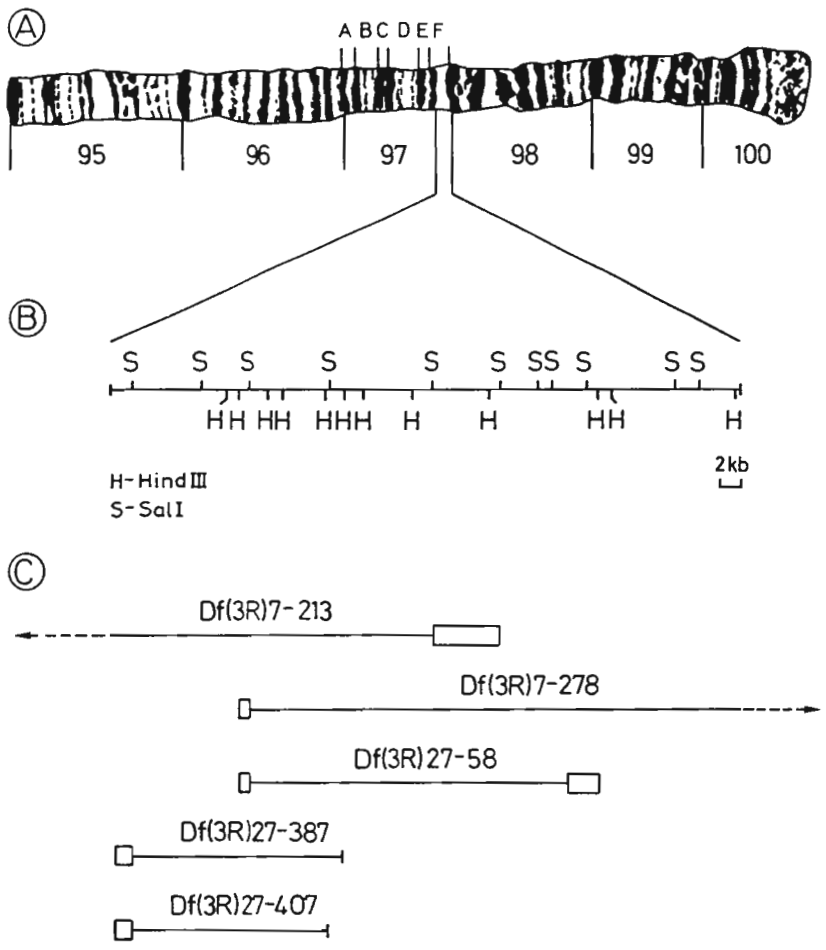

Figure 1. Localization of five deficiencies in the region 97F. A shows a schematic drawing of the chromosome bands 95-100 on the right arm of the third chromosome. The restriction map of the cloned genomic region in 97F3-11 is presented in B. The localization and breakpoints of the five deficiencies are shown is C. The lines indicate the deleted region while the open bars represent the fragments to which the breakpoints could be determined. The arrows point out that the centromeric breakpoint of *Df(3R)7-213* and the telomeric breakpoint of *Df(3R)27-278* are located outside the cloned region.

that the breakpoints of the two deficiencies may be located within these bands without causing a visible rearrangement in the chromosome banding pattern.

Table 1. Genetic and molecular analysis of five deficiencies.

Designation	Cytology	Size	Phenotype	Complementation with *l(3)mbt*^{ts}
Df(3R)7-213	97F1-2; 97F6-9	> 30 kb	embryonic lethal	no
Df(3R)7-278	97F3-5; 97F11-98A1	> 40 kb	embryonic lethal	no
Df(3R)27-58	97F3-5; 97F9-11	28 kb	embryonic lethal	no
Df(3R)27-387	97F3-5; 97F6-9	18 kb	embryonic lethal	yes
Df(3R)27-407	97F3-5; 97F6-9	17 kb	embryonic lethal	yes

Figure 1 shows the five deficiencies aligned to the restriction map of the 52 kb genomic walk. This walk represents most probably the bands 97F3-11 as indicated in A, B. The extents of the five deficiencies and their breakpoints are presented in C. For some deficiencies the breakpoints could not be determined to a defined point but to a genomic fragment (open bars). The arrows represents the breakpoints located outside the cloned region.

The deficiencies described above could be useful for analyzing other genes located in the region 97F besides *l(3)mbt*. In addition the fact that out of 1,150 P-element excision stocks five deficiencies of at least 17 kb could be isolated is interesting. In contrast to other observations, that P-element excision leads to deletions of flanking genomic regions larger than 7 kb only rarely (Engels, 1989), our results demonstrate that at least for some P-element insertions large deletions could be induced by P-element excision comparatively frequently.

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Drosophila albomicans, a new member of parthenogenetic *Drosophila*.

Since Stalker's (1951) initial discovery, a total of 10 species have been reported to be capable of parthenogenesis in the genus *Drosophila* (Templeton, 1983; Fuyama, 1984). All but one species (*D. mangabeirai*; Carson, *et al.*, 1957) are known to be tychoparthenogenetic (facultative parthenogenesis). In only four species, *D. mercatorum* (Carson, 1962), *D. ananassae, D. pallidosa* (Futch, 1973) and *D. melanogaster* (Fuyama, 1986), parthenogenetically reproducing strains have successfully been established in the laboratory. Here, we add another species, *D. albomicans*, to the list. This species belongs to the *D. nasuta* subgroup of the *immigrans* species group in which no parthenogenetic species had been known.

Virgin females of an isofemale strain (KKU119) of *D. albomicans* originally collected at Kiikatsuura, Japan in 1990, unexpectedly, produced a few progeny and all were female. We established a parthenogenetically reproducing strain from these progeny. The strain has been maintained to date for 39 generations only by parthenogenesis.

Table 1. Parthenogenetic ability and productivity of impaternate progeny.

Generations	No. of mothers tested (a)	No. of mothers produced progeny (b)	% of mothers reproduced (b/a)	Impaternates/ mother (± S.E.)
11th to 15th	266*	216	81.2	10.8 ± 0.6
25th	60	54	90.0	10.8 ± 1.3
30th	40	32	80.0	11.5 ± 2.0

Table 1 shows the parthenogenetic ability of this strain at the 11th to 15th, 25th, and 30th generations. In these generations, more than 80% of females reproduced parthenogenetically and each female produced about 10 impaternate progeny on the average. In the parthenogenetic species previously reported, some XO males emerged together with the female progeny. None of 3,980 progeny emerged in the parthenogenetic strain of *D. albomicans* were male. A possible explanation of the lack of males is that in *D. albomicans* the third chromosomes fused to the sex chromosomes (Wilson, *et al.*, 1969), therefore, XO males must have haplo 3 karyotypes that are most likely not viable.

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Noor, M.A. and J.A. Coyne. University of Chicago, Chicago, IL, U.S.A. A factor causing distorted sex ratios in *Drosophila simulans*.

We report here an X-linked factor causing a biassed sex ratio in *Drosophila simulans*. The sex-ratio disturbance conveniently originated, either by mutation or infection, in a stock with a visible mutant marker on every major chromosome: garnet (X-42), cinnabar (2-54), and ebony (3-61). The stock produces an excess of females every generation (70%, on average, but varying between 50% and 99%). A similar sex-ratio disturbance was reported by Faulhaber (1967) and mapped to a region of third chromosome. This factor produced an even greater excess of females at lower temperatures. Our experiments show that the factor we observed is not the one that characterized by Faulhaber.

All experiments were carried out at 23°C unless otherwise specified. We first determined whether the skewed sex ratio was produced by the male or the female parent. Reciprocal F_1 crosses were made between the *g;cn;e* distorting stock and a wild stock from Florida City, Florida (FC) with a normal sex ratio. All vials with fathers from the skewed sex ratio stock produced an excess of female offspring (85%). All vials with mothers from the skewed sex ratio stock produced close to 50% females. Thus, the fathers were responsible for the skewed sex ratio of their offspring.

Next, we identified which, if any, chromosome was responsible for the excess of female offspring. In November 1992, we crossed the male offspring from the reciprocal F_1 crosses above to FC females. The male offspring with mothers from the skewed sex ratio stock, and hence carrying an X-chromosome and cytoplasm from that stock, produced an excess of female offspring (91.9% female, N = 815), whereas those with FC mothers produced offspring with a normal sex ratio (50.1% female, N = 611). Since these males differed only in their X-chromosome and cytoplasm, we conclude that the skewed sex ratio is produced by a factor or factors on the X-chromosome or cytoplasmic effects.

Cytoplasmic microorganisms can sometimes cause the production of an excess of female offspring. As one test of this possibility, we reared *g;cn;e* skewed sex ratio flies on regular medium and on tetracycline-treated medium simultaneously (concentration as given by Hoffmann, *et al.*, 1986). After one generation, both groups produced on average 73% (N = 197 normal and 95 TC-treated) female offspring. After a second generation on the same media types, the TC-treated flies produced approximately 74% (N = 163) female offspring, and the normal flies produced 71% (N = 628). Thus, there is no effect of treating the flies with tetracycline, unlike that observed in flies infected with *Wolbachia*.

To determine whether temperature affected the sex ratio phenotype, a result reported by Faulhaber, we reared flies at 18°C. After the first generation, the offspring sex ratio was 77% (N = 60), not significantly different from the flies reared at 23°C. After the second generation, though, the offspring sex ratio dropped to 64% (N = 611). This response to colder temperatures is opposite of that observed by Faulhaber (1967).

Next, to further localize the mutation, we crossed the males from the *g;cn;e* skewed sex ratio stock to females from a stock with the mutant markers cinnabar and ebony. The F_1 offspring were intercrossed to form an F_2 generation. In the F_2 generation, half of the males had the X-linked mutant marker garnet. Both the *cn;e* and *g;cn;e* males were then crossed to the FC strain to determine whether there was an association of the skewed sex ratio with the X-linked mutation garnet.

Since the skewed sex ratio phenotype overlaps with the normal phenotype, as mentioned above, it was difficult to classify males that produced only a slight excess of 50% female offspring. We looked at all males that produced fewer than 50% females, assuming that all of these males possess the normal phenotype. We assumed that the distribution of offspring sex ratio produced by the normal males was similar but reversed for proportions greater than 50% female. Hence, we made a mirror image of the distribution of offspring sex ratio less than 50% to estimate a distribution of normal male offspring with sex ratio greater than 50%. If the offspring sex ratio was more than two standard deviations above 0.50, the male parent was scored as having a skewed sex ratio phenotype. This scoring underestimates the number of skewed sex ratio males.

Using this criterion, we observed 18 out of 83 putative recombinant males between garnet and the sex ratio factor. Hence, the approximate map distance between the sex ratio factor and the garnet mutation is 21 map units, though again, this is probably an overestimate. This association with the X-chromosome also further demonstrates that the skewed sex ratio phenotype is not completely cytoplasmically transmitted.

The *g;cn;e* skewed sex ratio males were then crossed to females with the X-linked mutations yellow (0.0), forked (56.0), and Beadex (62.2) to further localize the sex ratio factor(s). The recombinational distances of the skewed sex ratio "factor" from these markers were as follows: yellow, 42 map units (N = 55); forked, 20 map units (N = 55); and Beadex, 20 map units (N = 46). The recombinational distances are inaccurate because of the small sample sizes and overlap of sex ratios between genotypes, but the factor(s) producing the skewed sex ratio phenotype are clearly associated with the proximal tip of the X-chromosome.

The sex ratio bias could result from either a meiotically-driven X-chromosome, or from a greater mortality of males possessing this X-chromosome. In 1995, we produced F_1 males from both reciprocal crosses between the skewed sex ratio stock and FC. These males were crossed to FC females. We scored the egg-hatch and sex ratio of these crosses. 50 eggs were placed in each vial and scored for hatchability after 48 hours. Of the offspring of fathers with the X-chromosome from the skewed sex ratio stock, 91.6% of the eggs hatched (N = 1250), whereas for the reciprocal cross, 76% hatched (N = 1200). The sex-ratio for the offspring of fathers with the skewed sex ratio X-chromosome was now found to have dropped to 61.3% (N = 877). Thus, the reduction in egg-hatch could account for the sex ratio bias, and we cannot conclude that the skew results from meiotic drive.

In sum, the skewed sex ratio in our stock is caused by a gene or genes at the proximal end of the X-chromosome of the fathers. The skew in the sex ratio has deteriorated significantly over the three years we have maintained it, probably due to the accumulation of modifiers. The cause of the skew in the sex ratio is unknown, but it is associated with increased egg lethality. The stock has been sent to the Indiana stock center at Bloomington for distribution.

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Sur, Ruplekha, Sudipa Basu, Saswati Ghosh and A.S. Mukherjee. Genetics Research Unit, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta, 700 019 India. Epigenetic interaction between Scutoid (*Sco*) and Tufted (*Tft*) of *Drosophila melanogaster*.

The *Sco*/+ flies exhibit the loss of 10-15 bristles; scutellars, notopleurals, upper humerals and anterior postalars being most frequently affected (Figure 2b), (DIS 68:191). Tufted is a dominant mutation characterised by an increased number of bristles in the postalar (13-18), dorso-central (5-10) and scutellar (7-10) regions (Figure 2d); the normal number being 4-5, 3-4 and 4-5, respectively, in Oregon R+ (Figure 2a) (DIS 68:271). When *Sco*/+ flies were crossed with *Tft*/+ flies, the resultant progeny *Sco/Tft*, expressed an intermediate status, with the bristle numbers being significantly higher than Scutoid (*Sco*/+) but lower than Tufted (*Tft*/+) heterozygotes (Figure 2c), particularly in the scutellar (1-4), postalar (5-11) and dorso-central (4-6) regions (Table 1; Figure 1). The bristles of *Sco/Tft* flies were often thin and reduced in size. However, the *Sco/Tft* flies were fertile, fully viable and no abnormality was detected in either the abdominal pigmentation or in the external genitalia. These results suggest that *Sco* and *Tft* exhibit epigenetic interaction in the developmental hierarchy.

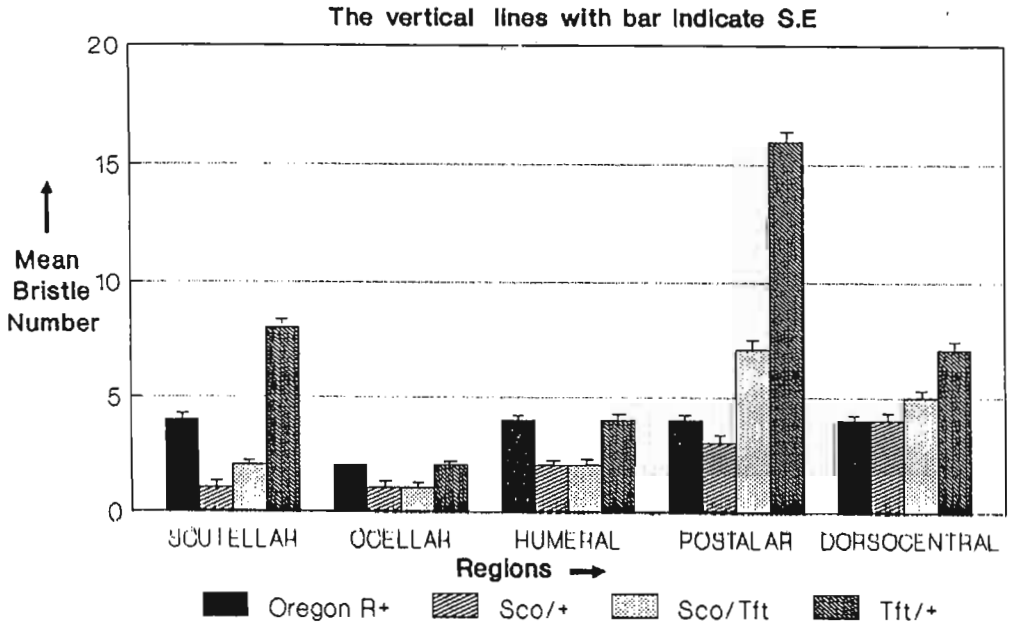

Figure 1. Histogram showing the mean bristle number ± S.E. in different strains of *Drosophila melanogaster*.

Table 1. Mean bristle number ± standard error.

Strain	Scutellar	Ocellar	Humeral	Postalar	Dorsocentral
Oregon R$^+$	4 ± 0.08	2 ± 0.00	4 ± 0.10	4 ± 0.08	4 ± 0.07
Sco / +	1 ± 0.15	1 ± 0.14	2 ± 0.14	3 ± 0.23	4 ± 0.25
Sco / Tft	2 ± 0.01	1 ± 0.14	2 ± 0.11	7 ± 0.37	5 ± 0.20
Tft / +	8 ± 0.25	2 ± 0.07	4 ± 0.11	16 ± 0.36	7 ± 0.26

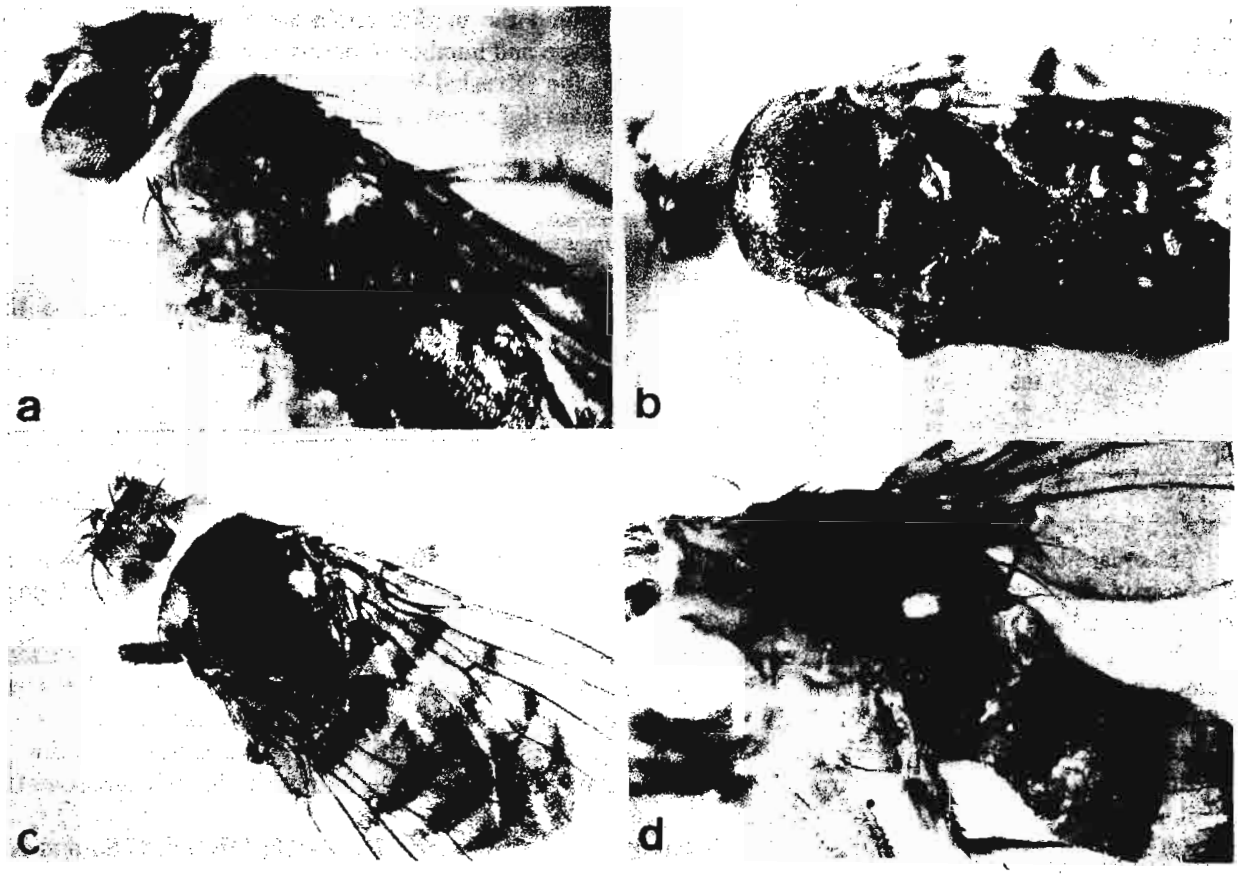

Figure 2. Bristle pattern of scutellar, postalar, and dorsocentral regions in (a) Oregon-R$^+$, (b) *Sco/+*, (c) *Sco/Tft*, and (d) *Tft/+*.

Kamping, Albert, and Wilke van Delden. Department of Genetics, University of Groningen, P. O. Box 14, 9750 AA Haren, The Netherlands. Interchromosomal effects of the *In(2L)t* inversion on recombination rates in *Drosophila melanogaster.*

One of the main roles of crossing-over at meiosis is generating genetic variability. Genetic control of recombination is rather complicated, and particular parts of the genome or the whole genome may be affected by single genes or polygenes, respectively. The rate of recombination may be influenced by both environmental and genomic stresses, such as high temperature, radiation, stress media, transposable elements and inversions. In most of these cases, recombination rates are positively affected. However, the presence of inversions in heterozygous state will protect genomic regions on the chromosome involved from recombination and may positively influence recombination on other chromosomes (Schultz-Redfield effect).

In this study we investigated the influence of inversion *In(2L)t* heterokaryotypes on recombination rates of the third chromosome in *Drosophila melanogaster*. *Inversion (2L)t* (22D3-E1; 34A8-9) is located on the left arm of the second

chromosome.

Strains homozygous for *In(2L)t* and Standard (St) karyotypes were extracted from a Dutch population (Gron) as described in Van Delden and Kamping (1989). The same procedure was followed for a Spanish population which was captured in Vernet Les Bains, Pyrenees Orientales (Vernet). For each karyotype and population, three strains were used for recombination analysis. All strains were inversion-free for the X and third chromosomes. As a consequence of the presence of *In(2L)t*, effective recombination in heterozygotes is absent in the *In(2L)t* region and in surrounding regions recombination is suppressed.

Table 1. Average number of observed cross-overs per individual on chromosome 3 for different second chromosome karyotypes of two populations.

Origin/karyotype	1	2	3	mean	S.E.
Gron St	0.922	1.068	1.010	1.000	0.042
Gron In (2L) t	1.340	1.213	1.205	1.253	0.044
Vernet St	1.049	1.095	1.066	1.070	0.013
Vernet In (2L) t	1.391	1.450	1.419	1.420	0.017

The homosequential multiple recessive chromosome marker strain *ru h th st cu str* e^s *ca*, kindly supplied by Bowling Green Stock Center, was used for recombination analysis of the third chromosome. F_2 individuals of crosses between the various strains and the marker strain were classified according to their phenotype and number of observed cross-overs on chromosome 3. With the Kruskal-Wallis One-way nonparametric test, no significant differences in the number of observed cross-overs per individual among strains of the same karyotype and population were observed.

Table 2. Number of individuals with different numbers of observed third chromosome cross-overs for Standard and Inversion (2L) t strains of two populations (strains combined).

No. of observed	Gron		Vernet		Total	
cross-overs	St	In (2L) t	St	In (2L) t	St	In (2L) t
0	75	62	72	35	147	97
1	103	108	112	75	215	183
2	44	83	52	60	96	143
>2	14	23	17	25	31	48
total	236	276	253	195	489	471

The Vernet population possesses a significantly higher ($p < 0.01$) overall recombination rate and a lower variance among strains compared to the Gron population, possibly due to fixation of a gene which influences recombination. For both population F_1 females heterozygous for *In(2L)t* and St show significantly ($p < 0.001$) more third chromosome cross-overs as compared to homozygous Standard (Table 1).

Chi-square tests for heterogeneity were highly significant when Standard and Inversion karyotypes were compared for observed number of cross-overs per individual: $p < 0.01$ and $p < 0.005$ in the Gron and Vernet populations, respectively, and $p < 0.001$ for the totals (Table 2).

The increase in recombination rate on the third chromosome is not restricted to particular genomic regions, but all chromosome segments between the marker used are positively affected in both populations. The mechanisms underlying the increase of recombination on other chromosomes remain speculative. Genes with positive effects on recombination located within inversions, increase in pairing time in meiosis, or a general compensation effect of reduction in recombination on the chromosome with the inversion may account for positive interchromosomal effects of recombination.

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Pandey, M.B. Department of Entomology and Agricultural Zoology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. Path length and pupation height relationship of *Drosophila* spp.

Drosophila larvae spend most of their lives foraging for food. A *Drosophila* larva feeds by shoveling food with its mouth hooks and moves by alternately extending is anterior end and retracting its posterior end. Foraging behaviour reflects the relative amount of feeding and locomotion. Sokolowski (1980) identified a behavioral polymorphism in *Drosophila melanogaster*. Depending upon the locomotory component of larval foraging behaviour, she had identified two types of larvae; 'rover' and 'sitter'. A rover larva traversed a large area while foraging on a yeast-covered food in a petri dish, whereas a sitter larva covered a relatively small area. A positive correlation was found between two prepupal behaviours; path length (length of trail a foraging larva leaves on the yeast paste during a 5 min test period) and pupation height (distance between the anterior spiracle of pupa and surface of food medium in a

vial) by Sokolowski and Hansell (1983) and Sokolowski (1985). Sokolowski (1982) found a non-significant positive correlation between these two behaviours of larva. The present note describes the result of studies on 'path length' and 'pupation height' in four species of *Drosophila* which are very common in India to know the relationship between these two prepupal behaviours.

Table 1. Correlation between path length and pupation height of *Drosophila* spp. (r stated for correlation coefficient).

Strain	Mean pupation height ± S.E. (mm)	t-value	p	mean path length ± S.E. (mm)	t-value	p	% of rovers	% of sitters	r value
D. ananassae									
Orissa (LN)	8.72 ± 2.02			71.06 ± 2.98			78	22	0.21
		2.25	<0.05*		1.78	>0.05			
Earnakulum (EK)	4.19 ± 0.60			64.63 ± 1.26			76	24	0.11
D. bipectinata									
Unchwa Lodge	0.75 ± 0.07			54.29 ± 2.19			80	20	0.03
		4.85	<0.001*		2.00	>0.05			
Townsville (Tv)	0.41 ± 0.04			48.18 ± 2.65			81	19	0.40
D. malerkotliana									
Unchwa Lodge	1.33 ± 0.11			60.38 ± 5.62			81	19	0.47
		3.33	<0.01*		0.73	>0.50			
Mirzapur (Mirza)	0.97 ± 0.10			55.44 ± 3.62			76	24	0.64*
D. biarmipes									
Raichus (RC)	1.18 ± 0.16			67.03 ± 3.04			79	21	0.18
		1.56	>0.10		1.29	>0.10			
Nagpur (NG)	0.94 ± 0.10			62.42 ± 1.82			75	25	0.54

* Significant; For t-values, df = 18, For r values, df = 8

The material of the present study comprises four Indian species of *Drosophila*; *D. ananassae*, *D. bipectinata*, *D. malerkotliana* and *D. biarmipes*. Two stocks of each species with significantly different pupation height and from different localities of India were selected (data has been taken from my Ph.D. dissertation) for experiment. For each stock of each species, virgin females and males were collected and aged for 4-5 days separately. Ten females and ten males were selected and placed for mating and oviposition in a petri dish for about 48 hr. Then adult flies were discarded. After 24 hr about 200 1st instar larvae of about the same age were removed and transferred in another petri dish filled with a 2.5 cm thick layer of artificial food material. After about 72 hr a third instar larva was removed and length of trail, left on yeast paste during a 5 min test period, was measured (in mm). Like this 'path length ' of 100 larvae selected from same petri dish was measured for each stock of each species. A larva which had a path length of greater than 35 mm was classified as 'rover', and a larva with 35 mm or less than 35 mm was classified as 'sitter'. Mean path length was calculated for each stock.

Table 1 shows the mean pupation height and mean path length of various stocks of four *Drosophila* species, which clearly indicate the significant interstrain variation in pupation height of all the species except *D. biarmipes*. Similarly interstrain variation for path length was also evident for each species but without any significance. It is further inferred through this table that a stock with higher pupation height also shows greater path length. Accordingly there existed non-significant positive correlation between path length and pupation height in each stock of each of the four *Drosophila* species, except the 'Mirzapur' stock of *D. malerkotliana* which shows significant positive correlation between these two larval behaviours. Further study of path length manifested the presence of 'rover' and 'sitter' larvae in all the stocks at more or less relatively constant frequency (75-81% rovers and 19-25% sitters).

Hence, it is concluded that strains showing significant differences in pupation height also show differences in their path length and remained positively correlated.

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van 't Land, Jan, Pim van Putten, Haroldo Villarroel[@], Albert Kamping, and Wilke van Delden. Department of Population Genetics, University of Groningen, P.O. Box 14, 9750 AA Haren, the Netherlands and [@] Department of Biology, University of Playa Ancha, Av. Gran Bretana 40, Valparaiso, Chile. Latitudinal variation in wing length and allele frequencies for *Adh* and *alpha-Gpdh* in populations of *Drosophila melanogaster* from Ecuador and Chile.

Little is known about the occurrence and distribution of different alleles for *Adh* and *Gpdh* in Latin-American populations of *Drosophila melanogaster* (Pipkin, *et al.*, 1976; Charles-Palabost, *et al.*, 1984). In an earlier report we described high frequencies for the *S* allele of *Adh* and *F* allele of *Gpdh*, accompanied by relatively high percentages of the inversion *In(2L)t*, in five wild populations from Panama, collected at latitudes between 7.45°N and 9.19°N (van 't Land, *et al.*, 1993). Here we present some preliminary data concerning fieldwork performed in Ecuador (Guayaquil) and Chile (all other locations) during February and March 1995. Wild *D. melanogaster* flies were collected on 10 locations with latitudes between 2.13°S and 41.30°S and with a longitudinal range of less than 10°W. Almost all flies were caught by sweeping a net over boxes with rotten fruits in fruit stores or large fruit markets. Only two populations (Linares and Copiapo) were collected at locations with an altitude higher than 50 meters above sea level. After allowing the females to produce eggs, and measuring (left-)wing length of male flies (anterior crossvein to wing tip), horizontal polyacrylamide gel electrophoresis was performed on male and female flies. Frequencies of the two alleles of *Adh* and the two *alpha-Gpdh* alleles were calculated. The results are summarised in Table 1.

Table 1. Locations of populations sampled, together with some characteristics measured on wild caught flies.

Population	latitude (South)	longitude (West)	altitude (mtr.)	# collected flies	allele frequencies		♂ wing length mm. (N, s.d.)
					Adh^S	$\alpha Gpdh^F$	
Guayaquil	2°13'	79°54'	< 50	130	.99	.62	1.08 (38, 0.04)
Arica	18°28'	70°19'	< 50	49	.25	.66	1.30 (17, 0.07)
Iquique	20°13'	70°10'	<50	28	.34	.62	1.31 (21, 0.10)
Antofagasta	23°38'	70°24'	< 50	72	.26	.66	1.27 (23, 0.09)
Copiapó	27°20'	70°21'	350	90	.17	.46	1.32 (56, 0.08)
Coquimbo	29°56'	71°24'	<50	22	.16	.45	1.38 (15, 0.14)
Valparaíso	33°05'	71°40'	< 50	334	.14	.69	1.37 (243, 0.10)
Linares	35°48'	71°36'	140	194	.07	.43	1.41 (102, 0.08)
Valdivia	39°48'	73°14'	<50	192	.11	.47	1.45 (87, 0.10)
Puerto Montt	41°30'	72°50'	<50	43	.13	.64	1.46 (32, 0.11)

Regression analysis was performed using angularly transformed allele frequencies and log-transformed wing lengths. The frequency of Adh^S as well as wing length appeared to be highly correlated with latitude ($P < 0.001$, $r^2 = 0.783$ resp. $r^2 = 0.930$). No significant relationship was observed between latitude and *alpha-Gpdh*^{F} frequency ($P > 0.05$, $r^2 = 0.115$).

These results proving the existence of a latitudinal cline in *D. melanogaster* for Adh^S frequency and for wing length are in concordance with reports for other regions (Singh, *et al.*, 1982; Anderson, *et al.*, 1987; Capy, *et al.*, 1993; Imasheva, *et al.*, 1994). However, the fact that the frequency of *alpha-Gpdh*^{F} did **not** vary with latitude remains puzzling and is in disagreement with results from other authors (*e.g.*, Oakeshott, *et al.*, 1984). Future investigations will focus on the analysis of these results using climatic data. Our research concerning inversion polymorphism and other laboratory work on these Chilean and Ecuadorian populations will possibly provide a better understanding of the causes of latitudinal clines for several traits in *Drosophila melanogaster*.

Acknowledgments: An important part of the laboratory work (electrophoresis and wing length preparation) was carried out at the University of Playa Ancha in Valparaiso, Chile. Dr. R. Choez and her students from the University of Guayaquil, Ecuador, kindly assisted with collecting the Ecuadorian flies.

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Hogga, Ilham, and Francois Karch. Department of Zoology and Animal Biology, University of Geneva, 154 route de Malagnou, 1224 Geneva, Switzerland. Targeting a specific deletion, *a la* Engels, in the *Fab-7* boundary of the bithorax complex.

Introduction: Given the difficulty to introduce DNA into a large numbers of individuals and because of the lack of embryonic stem cells or pole cell lines, site directed mutagenesis by homologous recombination is still impossible to perform in *Drosophila*. In 1991, however, Bill Engels and his colleagues have developed an elegant technique to perform site directed mutagenesis near the site of insertion of a P-elements (Gloor, *et al.*, 1991). The procedure takes advantage of the fact that gaps created during excision of P-elements are often repaired by the sequences carried on the homologous chromosome. In the absence of allelic sequences, Gloor, *et al.* (1991) were able to repair gaps with an ectopic template that had been previously introduced by P-element transformation. The procedure has been well-tried by the Engels' laboratory using the white gene as a model. Since then, however, no conversions has been published, and there are concerns that the technique is not efficient enough with P-element localized on the autosomes. In this paper, we describe the targeting of a specific deletion of 789 nucleotides in the *Fab-7* boundary region of the bithorax complex which is localized at 89E on the right arm of the third chromosome.

The PS-specific patterns of *Abd-B* in PS10 to 12 are respectively generated by the *iab-5, iab-6* and *iab-7* parasegment-specific cis-regulatory regions, which lie on a 60 kb region of DNA 3' to the *Abd-B* transcription unit (Celniker, *et al.*, 1990; Sanchez-Herrero, *et al.*, 1991). *Fab-7* is a dominant mutation that transform PS11 into PS12. We have shown that in *Fab-7*, the deletion of a boundary element between *iab-6* and *iab-7* causes the ectopic activation of *iab-7* in PS11 where normally only *iab-6* is active (Gyurkovics, *et al.*, 1990; Galloni, *et al.*, 1993). *Fab-7* is also defined by the bluetail element (*blt*). In this line, a P element carrying a *Ubx-lacZ* reporter gene is inserted in the proximal side of *iab-7*, just to the right of the *Fab-7* boundary. By mobilizing the *blt* element we were able to generate new *Fab-7* mutations. Among them, $Fab\text{-}7^2$ is a deletion that removes 789 nucleotides on the proximal side of the insertion site of the *blt* element (Galloni, *et al.*, 1993; Karch, *et al.*, 1994). The presence of the *blt* next to *Fab-7* is an ideal tool to further dissect the boundary by gene conversion. Since we had cloned the DNA from the $Fab\text{-}7^2$ mutation and because we know the phenotype associated with this mutation, we have decided to use it as a control to test the efficiency of the procedure.

Results: Figure 1 shows the strategy utilized to target the $Fab\text{-}7^2$ specific deletion in the *Fab-7* boundary of the BX-C. The P-element used to create gaps in *Fab-7* was the bluetail transposon inserted at position 124 kb in the BX-C (Galloni, *et al.*, 1993). This element contains a *Ubx-lacZ* reporter gene and is marked by the $rosy^+$ gene. Any deletion of the *blt* element will be recognized by the loss of the $rosy^+$ eye color in the progeny. In trans to the bluetail chromosome we have used the $Fab\text{-}7^1$ chromosome. Since $Fab\text{-}7^1$ is a 4.3 kb deletion that uncovers the insertion site of the bluetail transposon (as drawn in Figure 1) there will be no available sequences carried by the homologue to repair gaps created by imprecise excisions. At cytological location 12E on the X chromosome, we had an ectopic copy of the *Fab-7* region carrying the $Fab\text{-}7^2$ deletion. The homologies were of 1,577 nucleotides on the proximal side of the *blt* element and of 1,238 nucleotides on the distal side (the gray box indicates the homology regions; the extents of the $Fab\text{-}7^1$ and $Fab\text{-}7^2$ deletions are shown by the brackets). The $Fab\text{-}7^2$ ectopic copy was introduced into flies with a P-element marked with the mini-white gene. As a source of transposase, the delta2-3 transposon of Robertson, *et al.* (1988) inserted at 99B was recombined on the $Fab\text{-}7^1$ chromosome. This chromosome was also marked with *Sb*. 74 independent crosses between a single bluetail/*Sb*, $Fab\text{-}7^1$ delta2-3 male carrying the ectopic template on the X chromosome and 4-5 females ry^{506} were established. Out of a total progeny of 14,175 individuals, 225 ry^- flies were recovered. Among them, 22 independent flies showed the *Fab-7* phenotype and were established as stocks (we made sure to get rid of the X linked $Fab\text{-}7^2$ ectopic template). In order to sort the new deletions of the boundary created by imprecise excision of the bluetail element from the conversion events, we used PCR analysis with a battery of primers derived from both sides of the insertion site of the *blt* element (see legend of Figure 1). The conversion events were detected with a specific primer derived from a foreign sequence of 26 nucleotides that is present on the distal deficiency endpoint of $Fab\text{-}7^2$ (Karch, *et al.*, 1994). Out of the 22 lines associated with *Fab-7* phenotype, 4 independent conversion events occurred. The 18 remaining were due to gaps that were not converted and that created new boundary deletions.

Discussion: We have targeted a specific deletion of 789 nucleotides in the *Fab-7* boundary region of the bithorax complex at 89E on the right arm of the third chromosome. Our conversion frequency of 0.0003% of the total progeny appears very low when compared to the frequencies obtained with the white gene that ranged from 0.1% to 10% (Gloor, *et al.*, 1991; Engels, *et al.*, 1994). However, these numbers cannot be readily compared because our gene conversion occurred on an autosome. In particular, we do not know how efficiently the ectopic template can reach its target sequence when the two homologue chromosomes can pair very near the site of the gap to be repaired (the $Fab\text{-}7^1$ deletion removes only 2,311 nucleotides on the proximal side of the *blt* element and about 2,000 nucleotides on the distal

side; see Figure 1). Among the numerous parameters that can influence the conversion efficiently is the frequency at which the P-element that creates the gap is mobilized. In our experiment we recovered 225 rosy flies giving a frequency of excision of 1.5%. The occurrence and extent of gaps were not analyzed because deletions on the distal side of the P-element (towards *iab-7*) are associated with recessive phenotype (Galloni, *et al.*, 1993) and were not kept in our experiment. We have recovered 22 lines associated with the *Fab-7* gain-of-function phenotype. All of them are due to deletions that remove at least 500 bp on the proximal side of the bluetail element. In 4 of them the gap had been repaired by the ectopic copy of the *Fab-7* region present on the X chromosome. In the experiment described here, the association of the conversion events with a dominant phenotype greatly simplified the screening procedure. However, with an occurrence of 4 independent conversions in 225 excision events and with the ease to perform PCR analysis on individual flies, we think that this procedure is a very powerful tool to further dissect the *Fab-7* boundary region.

Acknowledgments: Ilham Hogga was paid by a Human Frontier Science Program grant. This work was supported by the Swiss National Fund for Research and the State of Geneva. Special thanks to Laszlo Sipos for providing the *ry, Fab-7*^{1} stock.

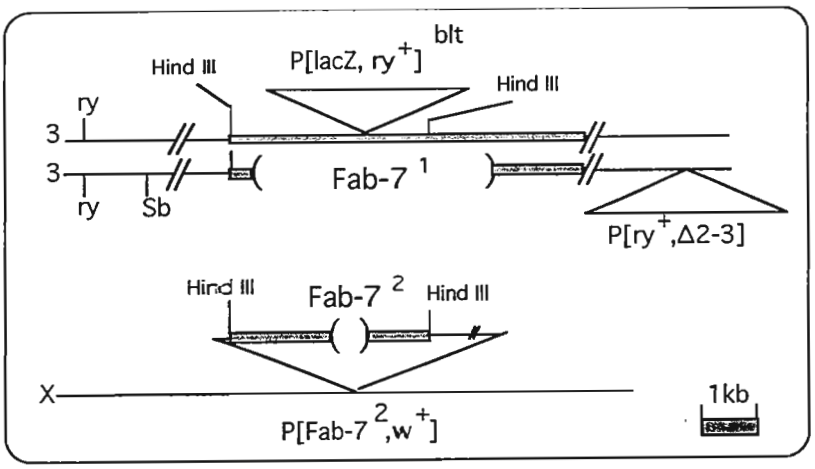

Figure 1. Illustration of chromosomes 3R and X of the jump starter male. Both copies of 3R carry the ry^{506} mutation indicated by *ry*. The chromosome carrying the delta2-3 transposase producing gene was marked with *Sb* and carry the 4.3 kb *Fab-7*^{1} deletion. The gaps were induced by mobilization of the bluetail transposon (P[*lacZ,ry*$^{+}$]^{blt}) indicated by a triangle. The gray rectangles represent the DNA covering the *Fab-7* boundary region drawn at the scale shown below the figure (see text for the strategy). The ectopic copy of the *Fab-7* boundary carry the *Fab-7*^{2} deletion which is included in a P[*white*$^{+}$] element inserted at cytological location 12E. The deletion events were analyzed by PCR with a set of three primers. The sequence data used to synthesize the different primers are reported in Karch, *et al.* (1994). The sequence of the proximal primer called BO is 5'CGAACCCCAAGGACGCA3'. The sequence of the distal primer called 928 is 5'CGTATGCGAGTTATAGA3'. The sequence of the primer called 2201, specific for the *Fab-7*^{2} deletion, is 5'CAGCCATCAGCAGGCAT3'.

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Stoltenberg, S.F., and J. Hirsch. University of Illinois, Urbana-Champaign, Illinois USNA. Further evidence that alcohol dehydrogenase is a genetic marker for geotaxis in *D. melanogaster*.

Genetic correlates of geotaxis, orientation and movement with respect to gravity, in *D. melanogaster* have been studied for more than 38 years, but are not well understood. Much of the research has focused on the chromosome level and has revealed some of the complexity underlying geotactic performance (*e.g.*, Erlenmeyer-Kimling, Hirsch and Weiss, 1962; Ricker and Hirsch, 1988).

Geotaxis has been shown to be a polygenic trait (*e.g.*, Erlenmeyer-Kimling, Hirsch and Weiss, 1962), with each of the three major chromosomes (X, II and III) contributing to differences in geotactic performance. Few studies have

been designed to detect single gene effects on geotaxis, however. Kessler and Kraemer (1975) reported an elegant study that demonstrated an effect of the white locus on geotaxis that was dependent on genetic background. McMillan and McGuire (1992) reported that flies with a certain allele of spineless aristapedia (ss^a) showed positive geotactic performance that was not wholly due to the ectopic leg tissue.

Table 1. Multiple regression analysis on geotaxis for a stratified sample of the hybrid derived lines at Generation 66 (N=719).

Variable	Coefficient	t	P
Intercept	7.01		
Sex	-1.52	9.67	.0001
ADH	-.58	2.66	.008
PGD	.13	.38	.70
AMY	-.16	.63	.53

Stoltenberg, Hirsch and Berlocher (1992a) reported an allozyme survey of high- and low-selected geotaxis lines that confirmed some earlier observations that the high- and low-lines were fixed for alternative alleles of alcohol dehydrogenase (ADH; high-S/S and low-F/F). They also found that the high- and low-lines were fixed for alternative alleles of amylase (AMY; high-2,3/2,3 and low-1/1) and 6-phosphogluconate dehydrogenase (PGD; high-A/A and low-B/B). Alleles of ADH, AMY and PGD are correlated with geotactic performance in the high- and low-selected lines. To test whether these genotype-phenotype correlations were meaningful or chance, Stoltenberg, Hirsch and Berlocher (1992b) hybridized individuals from the high- and low-lines to prepare F_2 generation individuals which were tested for geotaxis and for ADH and AMY (the PGD-geotaxis correlation was not tested). The correlation between ADH and geotaxis survived meiosis to reappear in the F_2 generation, whereas the correlation between AMY and geotaxis did not. Thus, ADH appeared to be acting as a genetic marker for some putative geotaxis locus on the second chromosome, whereas the correlation between AMY and geotaxis is probably attributable to genetic drift.

Following the collection of F_2 generation individuals for that study, the vials continued to produce progeny which were transferred to new food vials to establish free-mating "hybrid derived" lines. Two such lines were established and have since been maintained by mass transfer to fresh food vials, without anesthesia or selection, every 2-4 weeks. The LH line was derived by crossing low-line females to high-line males and the HL line by crossing high-line females to low-line males.

Sixty-six generations after the F_2, three samples of males and females from each hybrid derived line were tested for geotaxis. All testing was done in same sex groups of approximately 200 individuals as described by Stoltenberg, Hirsch and Berlocher (1995). Individuals, grouped in their respective geotaxis categories, were then frozen for later electrophoresis.

Stratified samples of approximately 180 individuals of each type (*e.g.*, HL females) were assayed for ADH, AMY and 6-phosphogluconate dehydrogenase (PGD) with cellulose acetate electrophoresis as described by Easteal and Boussy (1987). Detailed descriptions of procedures can be found in Stoltenberg (1995).

A multiple regression analysis of the stratified sample (N = 719) on geotaxis score, with Sex, ADH, AMY and PGD as the variables, indicated that Sex ($p = 0.0001$) and ADH ($p = 0.008$) are correlated with geotaxis score in the hybrid derived lines (see Table 1). PGD ($p = 0.70$) and AMY ($p = 0.53$) are not associated with geotaxis score in the hybrid derived lines. The variable "Line" was not included in this analysis because a multiple regression analysis of the larger sample (N = 2176), from which the stratified sample was drawn, detected no significant line effect on geotaxis score.

Sex effects on geotaxis have been routinely identified and may indicate effects of (1) the X chromosome, (2) the Y chromosome, (3) developmental differences, (4) other factors. We are unable to distinguish among these or other possibilities.

The maintenance of the association between ADH and geotaxis following 66 generations of possible recombination suggests that a gene very near *Adh* (2-50.1) or *Adh* itself is functionally related to differences in geotactic performance in *D. melanogaster*. This result supports the interpretation of Stoltenberg, Hirsch and Berlocher (1992b) that *Adh* is acting as a marker locus for a nearby, yet to be identified, gene related to geotaxis. It is possible that the *Adh* locus itself is pleiotropically related to geotaxis, however, at this time we are unable to address this possibility. The region around *Adh* appears to be a logical starting point for a finer scale search for genetic correlates of geotaxis.

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Miller, Andrew. S., and Martin J. Milner. School of Biological and Medical Sciences, University of St Andrews, Fife, Scotland, KY16 9TS. The ultrastructure of *Drosophila melanogaster* imaginal epithelial cells grown on human fibronectin-coated membranes *in vitro*.

Imaginal disc epithelia, as with vertebrate epithelia, exhibit apical-basal polarity (Poodry and Schneiderman, 1970). The apical surface is covered in microvilli and is specialised for the secretion of the pupal and adult cuticles whereas the basal surface faces the basement membrane and haemolymph and is more uniform in appearance (Fristrom and Fristrom, 1993). Imaginal disc cells grown *in vitro*, however, have lost this characteristic epithelial phenotype, displaying a variety of non-polarised morphologies (Currie, *et al.*, 1988; Peel and Milner, 1990). Through the use of porous membranes coated with human fibronectin, an extracellular matrix molecule implicated in promoting imaginal cell-substrate adhesion *in vitro* (Miller and Milner, unpublished observations), we attempted to recreate the appropriate cues for the re-establishment of polarity in an uncloned imaginal disc cell line, L127D6.

Cells were plated onto human fibronectin-coated cell culture inserts (Becton Dickinson) for use with 24-well plates at a density of 1×10^6 cells per insert. Cells in the inserts were either grown in 250 ul of complete sterile medium (CSM) or CSM minus fly extract and foetal bovine serum (FBS) (Cullen and Milner, 1991). The inserts themselves were bathed in 500 ul of CSM, allowing for nutrient uptake from the basal side of the cell. Feeder layers, when used, were grown in the 24 well plate itself and were seeded 1 day prior to addition of the insert at a density of 2×10^6 cells per well. The cells were then grown in the insert for a period of either one, two or three days. The membranes were then cut from the inserts and processed for visualisation by electron microscopy using an existing protocol (Milner and Muir, 1987).

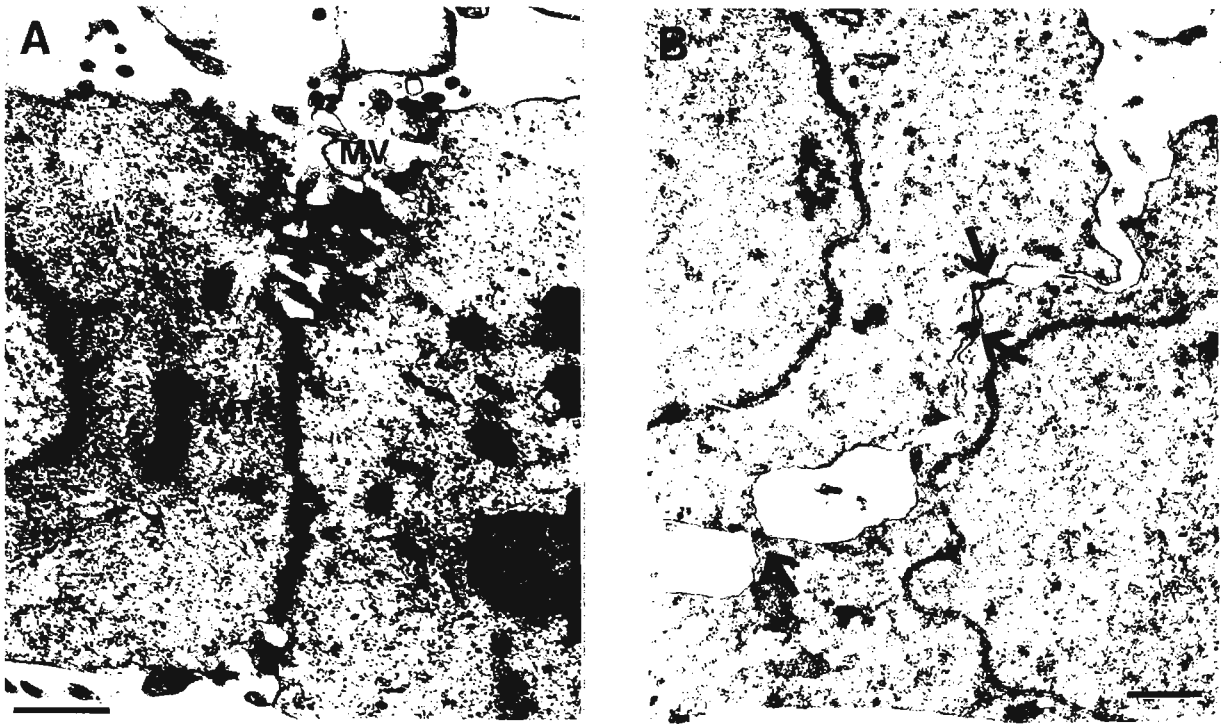
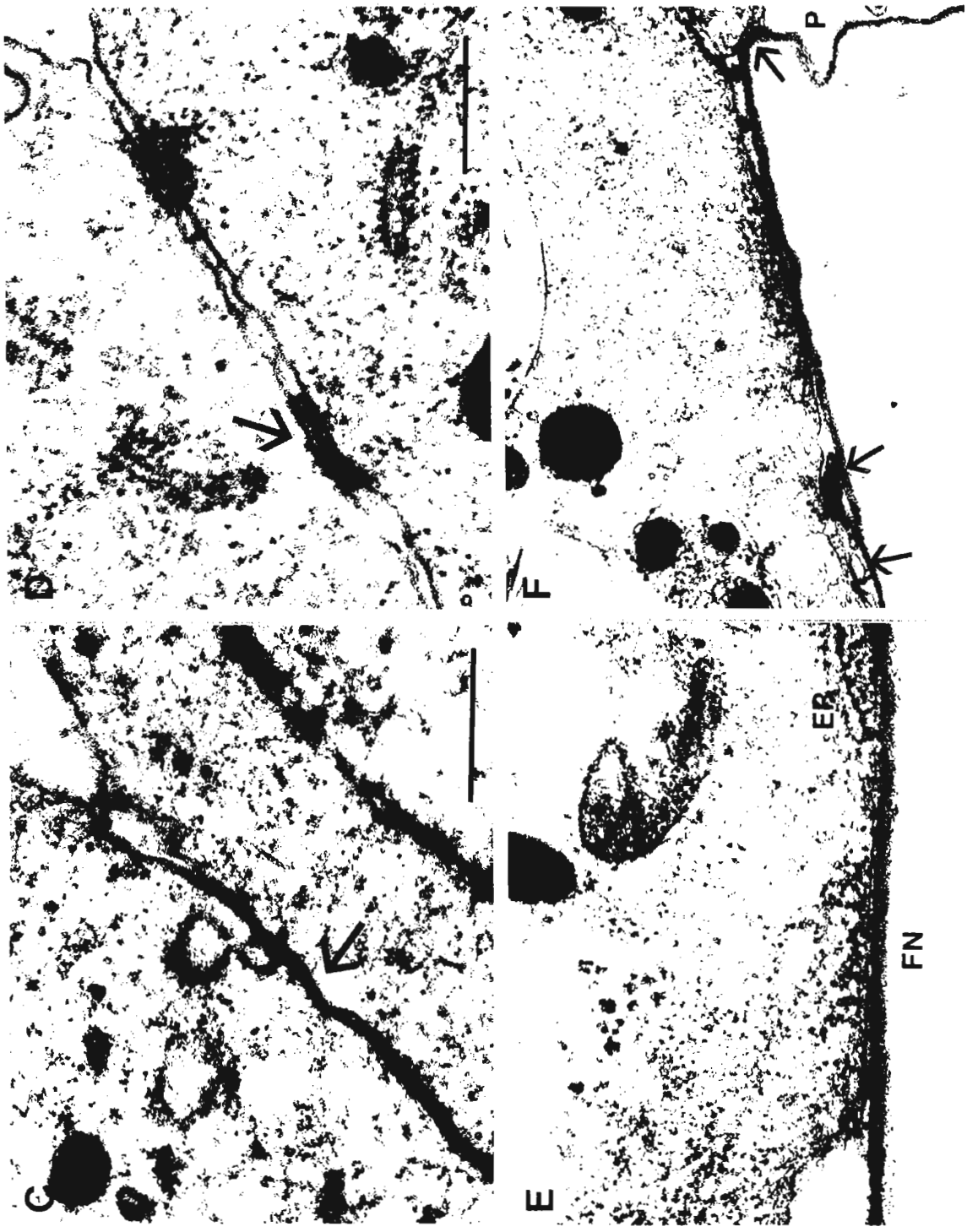


Figure 1 (continued on next page). Electron micrographs of imaginal epithelial cells from *Drosophila* grown on fibronectin-coated membranes. A. Site of adhesion between two cells grown allowing for unidirectional nutrient uptake. Towards the apical (top) end of the cells are numerous microvilli (mv) towards which some microtubules (mt) are seen to extend. B. Numerous small attachment sites (arrows) seen between two closely apposed cells. C. Aggregating cells grown allowing for bidirectional nutrient uptake adhere tightly together (arrow) but there is no evidence of any junctional differentiation. D. This micrograph illustrates a possible site of junctional differentiation (arrow), reminiscent of a septate desmosome seen *in vivo*, at the baso-lateral surface of two cells grown allowing for unidirectional nutrient uptake. E. Site of adhesion between a cell and a fibronectin coated membrane (fn). Endoplasmic reticulum (er) can be seen close to the substrate but there is no evidence for the existence of any hemidesmosomes. F. As in E. Numerous cytoplasmic extensions (arrows) or "feet" can be seen between the cell and the membrane, one of which appears to be extending into a pore (P) through which the cell is deriving nutrient. Scale bar in each represents 0.5 um.


P
F
ER
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C

Various factors are known to be important for the induction and maintenance of apical-basal polarity *in vivo*. One such factor is the direction of nutrient uptake (Simons and Fuller, 1985) which, *in vivo*, is unidirectional via the basement membrane, a cue which would be absent from cells grown directly onto tissue culture plastic. Another determining factor is the presence of suitable cell-cell and cell-extracellular matrix contact (Rodriguez-Boulan and Nelson, 1989).

Imaginal cells adhere and grow to confluence on fibronectin-coated membranes and display a partial apical-basal phenotype when a basally situated layer of feeder cells is present and serum components, in the form of FE2 and FBS, were made available from the basal side of the cells only. This phenotype was typically characterised by the presence of apically situated microvilli, differentiated junctions at the baso-lateral cell surface and basal cell extensions also known as epidermal feet (Figure 1). These cells also had a typical columnar morphology, reminiscent of the cell shape seen *in vivo* (not shown). Cells grown in the absence of unidirectional nutrient uptake, serum components being either present at or absent from both the apical and basal sides, grow much as they do on tissue culture plastic; non-polarised cells migrating into multicellular aggregates (Peel and Milner, 1990). However, the degree of apical-basal polarity achieved *in vitro* still does not reflect that which is found *in vivo*, perhaps indicating the state of dedifferentiation of this established cell line and the absence of mesodermal cells which may play an inductive role in determining polarity *in vivo*.

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Uysal, Handan.[1], **and Zafer Bahçeci**[2]. [1]Ataturk University, Faculty of Science and Art, Department of Biology, 25240, Erzurum, Turkey and [2]Gazi University, Faculty of Education, Kirsehir, Turkey. Effect of mercury chloride on the durations of developmental stages of *Drosophila melanogaster*.

The fruit fly, *Drosophila melanogaster*, is the most used insect for experiments. But, it was reported that the various external parameters, such as temperature, chemicals, radiation and so forth, affect the developmental stages of *D. melanogaster*, which is a holometabola.

In this study the effects of mercury chloride on the developmental periods in *D. melanogaster* have been examined. The flies were reared on the cornmeal-yeast-agar food medium. Traces of propionsaure were added as mold inhibitor. The doses of mercury chloride used were 0 (control), 0.05, 0.1, 0.5, 1.0 and 5.0%. The experiments were started with 7 pairs of flies, equally shared by virgin females and unmated males per population jar (size 15 cm x 5 cm). The experiments were conducted in the incubator 25$\pm$1°C.

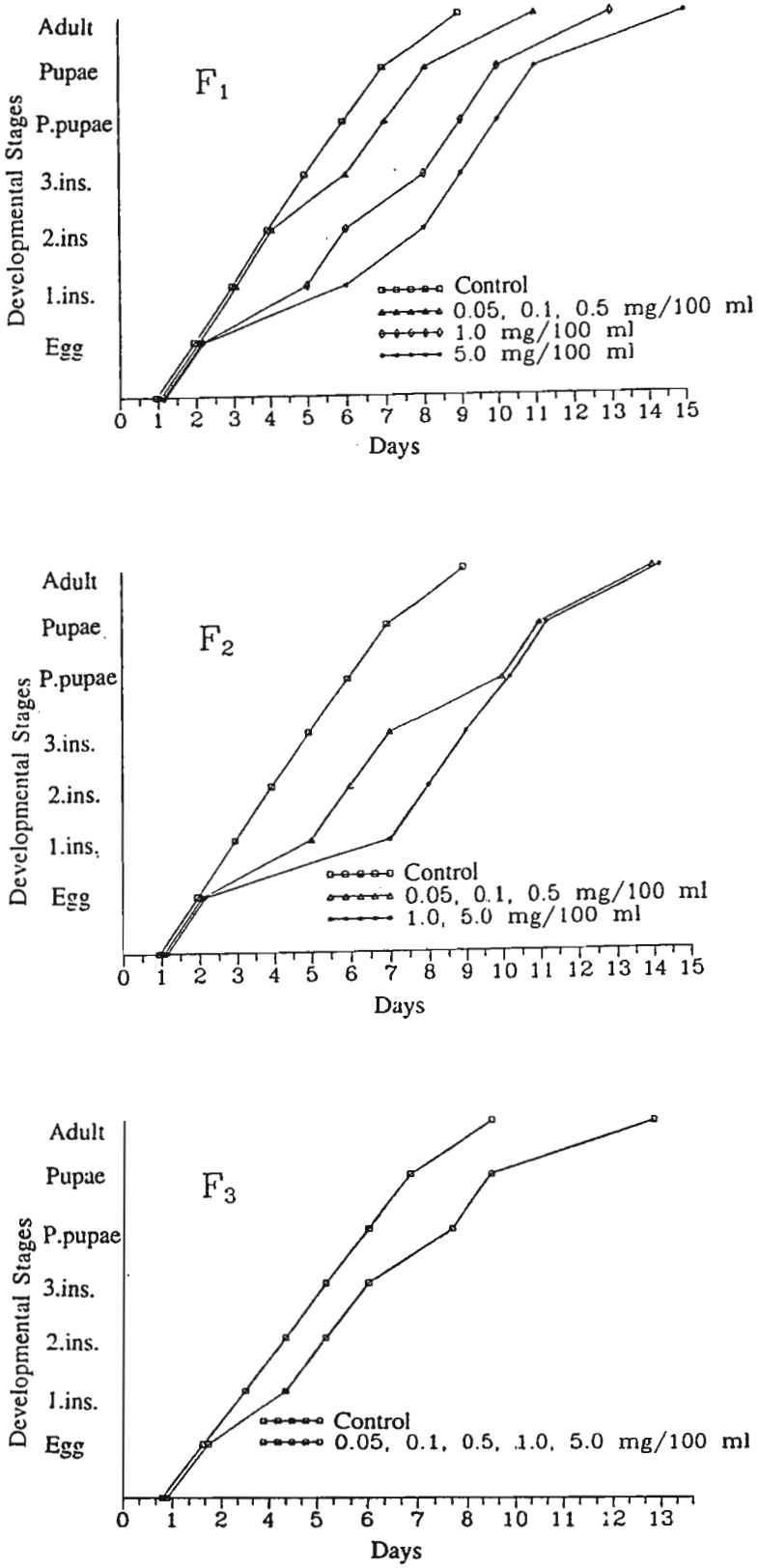
The day following the copulation, the bottles were taken to be examined. In the control group, the laid eggs were observed on the second day of crosswise and first instar larvae, second instar larvae, third instar larvae, prepupae, pupae and adult were observed at the 3, 4, 5, 6, 7 and 9th days, respectively.

However, in the F_1 generation, fed with mercury chloride at the concentrations of 0.05, 0.1 and 0.5%, the developmental stages were found to be 3, 4, 6, 7, 8 and 11th days for first instar larvae, second instar larvae, third instar larvae, prepupae, pupa and adult individuals, respectively. 1.0 and 5.0% mercury chloride has caused the lengthening of metamorphosis more than observed in the first three doses. These developmental stages were recorded to be 5, 6, 7, 9, 10 and 13th at the concentration of 1.0% and 6, 8, 9, 10, 11 and 15th days at the concentration of 5.0% for first, second, third instar larvae, prepupae, pupae and adult, respectively. As seen above, as a result of all applications the developmental stages lengthened in comparison with the control.

The F_1 generations obtained from the parents fed on different concentrations of the heavy metals were raised on normal feeding medium separately and the F_2 and F_3 generations were obtained by intra-crossing of each experimental group. In the F_2 generations, larval and pupal stages were recorded to be 5, 6, 7, 10, 11, 14; 7, 8, 9, 10, 11, 14th days at the concentrations of 0.05, 0.1 and 0.5%; 1.0 and 5.0%, respectively. But, the metamorphosis in F_3 generations were on the same days for all applications. The days of metamorphosis of F_3 were 4, 5, 6, 8, 9 and 13th. The results are given in Figure 1.

It appeared from our observations that the effect of addition of mercury chloride to the rearing medium of *D. melanogaster* has lengthened the developmental periods. Several researchers used cadmium chloride (Vasudev and Krishnamurthy, 1981), copper and ferrous sulphates (Islam, *et al.*, 1988) and found similar finding according to our results. Probably, mercury chloride inhibits the synthesis or working of some enzymes or hormones effective on the

Figure 1. Effect of mercury chloride on developmental stages of *D. melanogaster*.



metamorphosis. On the other hand, Liu (1992) showed that trivalent and hexavalent chromium inhibit root growth of *Allium cepa*. According to Ganrot, inhibitory effect of chromium resulted from the inhibiting effect of Cr^{3+} on the mitotic enzyme reactions. Furthermore, Mertz stated that Cr^{3+} is strongly bound to RNA, DNA and various proteins (Blundell and Jenkins, 1977) and the binding of Cr^{3+} to DNA occurs only on the phosphate groups (Liu, *et al.*, 1992). The lengthening of developmental stages may be due to the above reasons.

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Goñi, B., and M.E. Martinez. Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay. First record of *Drosophila subobscura* in Uruguay.

As a part of a project to survey the *Drosophila* fauna from Uruguay, we have collected drosophilids in different regions from October 1993 to March 1995. Collecting sites included both domestic and natural habitats. One of the fly collecting methods included banana traps using cardboard partially capped with plastic bags which were hanging on tree branches. Traps were placed for at least 12 hours. The specimens were identified by Dr. Carlos R. Vilela of the Department of Biology, Universidad de Sao Paulo, Brazil, and stored in the collection of the Seccion de Entomologia, Facultad de Ciencias, Universidad de la Republica, Montevideo.

A total of 6,816 individuals were collected in 17 localities. *D. subobscura* was found in two localities situated in the "Banados de Rocha" (wetlands of the Department of Rocha) around 34°S and 53°45'W: "Potrero Grande" at 10 km North of Laguna Negra lake and "Boca del Sarandi" at the western border of Laguna Negra lake. This wetland is part of a lacustral system that extends along the Atlantic coast of Uruguay and South of Brazil. A total of 6 out of 30 individuals (20%) collected from these localities were *D. subobscura*. Monthly collections from September 1994 through March 1995 in these two localities revealed seasonal variations. It was absent in the summer (December through March). *D. subobscura* was absent in collections from the nearby Castillos city locality, about 40 km from the Laguna Negra (N = 212 individuals).

D. subobscura is considered a typically paleartic species, however, field collections from Chile (Brncic and Budnik, 1979; Brncic, *et al.*, 1981; Budnik and Brncic, 1982) and later from Argentina (Prevosti, *et al.*, 1983) revealed its recent colonization in South America. More recently, this species was found in samples from Mar del Plata, a coastal city situated in the Atlantic coast (Lopez, 1985). In Uruguay, we have started field collections of *Drosophila* quite recently and do not know for how long could *D. subobscura* have been colonizing this region before 1994. Collections in two Montevideo city localities in 1990 and 1991 did not show the presence of this species in the samples. However, its presence only in samples taken from the Atlantic wetland localities may suggests either (i) its recent colonization and/or (ii) its association with a climate similar to a mild Mediterranean one. Further studies on the distribution and abundance of this species are needed to know dynamics of the colonization of *D. subobscura* in this region.

Acknowledgments: Collections in the Department of Rocha were funded by the grant "Restitucion a la Vida".

References: Brncic and Budnik 1979, Dros. Inf. Serv. 55: 20; Brncic, *et al.*, 1981 Genetica 56: 3-9; Budnik and Brncic 1982, Actas V Congreso Latinoamericano de Genetica 177-188; Lopez 1985, Dros. Inf. Serv. 61: 113; Prevosti, *et al.*, 1983, Dros. Inf. Serv. 59: 103.

Stoltenberg, S.F., and J. Hirsch. University of Illinois, Urbana-Champaign, Illinois USNA. Further evidence for Y chromosome correlates of geotaxis in *D. melanogaster*.

Polygenic correlates of continuously distributed phenotypes have long been thought to be rather common, each gene having small effects (Mather, 1944). Geotactic performance, orientation and movement with respect to gravity, in *D. melanogaster* has been shown to have such polygenic correlates, each of the three major chromosomes (X, II & III) has been identified as a correlate of geotaxis (see Ricker and Hirsch, 1988 and references therein). Y chromosome effects on geotaxis have either not been convincingly demonstrated or have been ignored by design. In this study, we report an experiment designed to have the statistical power to detect rather small Y chromosome effects on geotaxis.

In an earlier report (Stoltenberg and Hirsch, 1994) we described five generations of a backcross experiment designed to test whether Y chromosomes are correlated with geotaxis in lines that have evolved stable, extreme expressions of that behavior (Ricker and Hirsch, 1985). We interpreted the results of the backcross experiment to indicate a small Y-chromosome effect on geotaxis that was dependent on genetic and cytoplasmic background. Geotaxis score distributions from backcross sublines with a Low geotaxis-line background but Y chromosomes from different lines (*i.e.*, LxLH & LxHL) suggested a Y chromosome-effect, whereas geotaxis score distributions from backcross sublines with a High geotaxis-line background but Y chromosomes from different lines (*i.e.*, HxLH & HxHL) did not. Two additional generations of backcrossing resulted in a reduction of the Y chromosome effect in the sublines back-

Table 1. Geotaxis score summary statistics from chromosome substitution sublines.

Background		Mean	Variance	N
High				
	High Y	11.76	12.59	825
	Low Y	10.95	14.65	866
Low				
	High Y	1.43	3.40	914
	Low Y	1.06	2.52	882

Note: Within a given background, flies have cytoplasm, X chromosomes and autosomes from that geotaxis line.

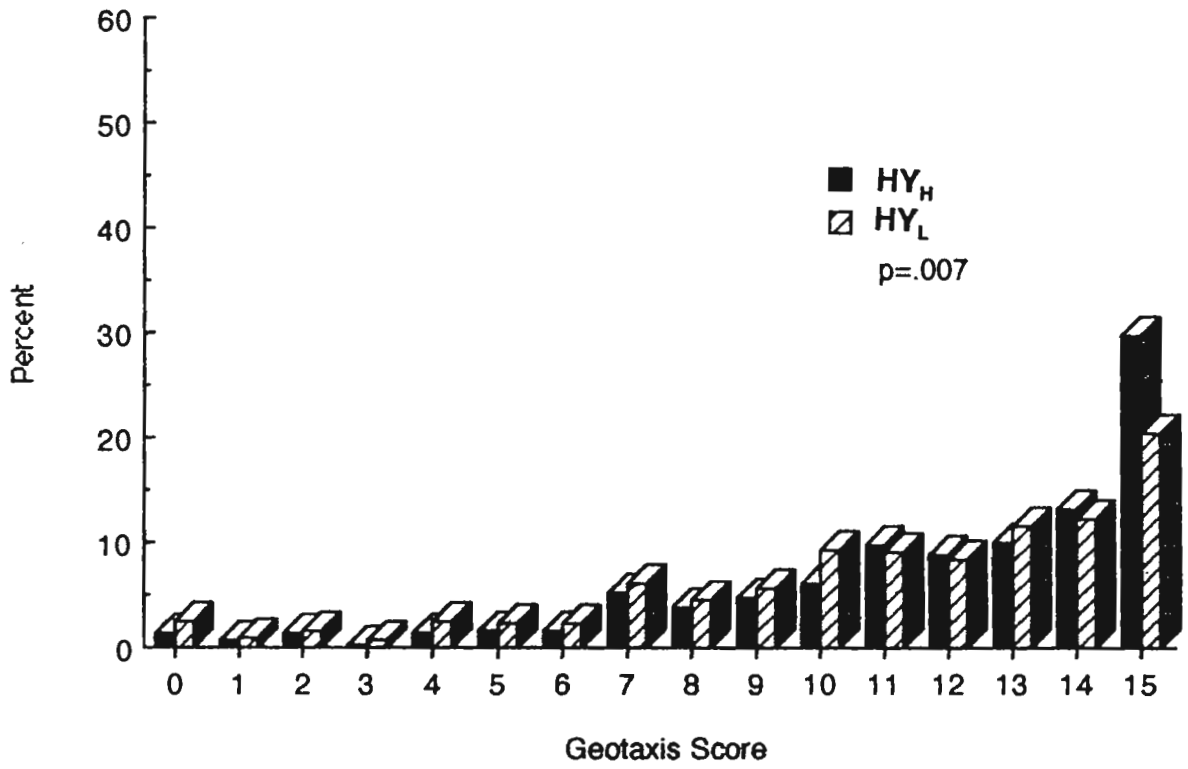

Figure 1. Geotaxis score distributions of males with genetic and cytoplasmic background from the high-geotaxis line, but Y chromosomes from the high- (HY_H) and low- (HY_L) lines.

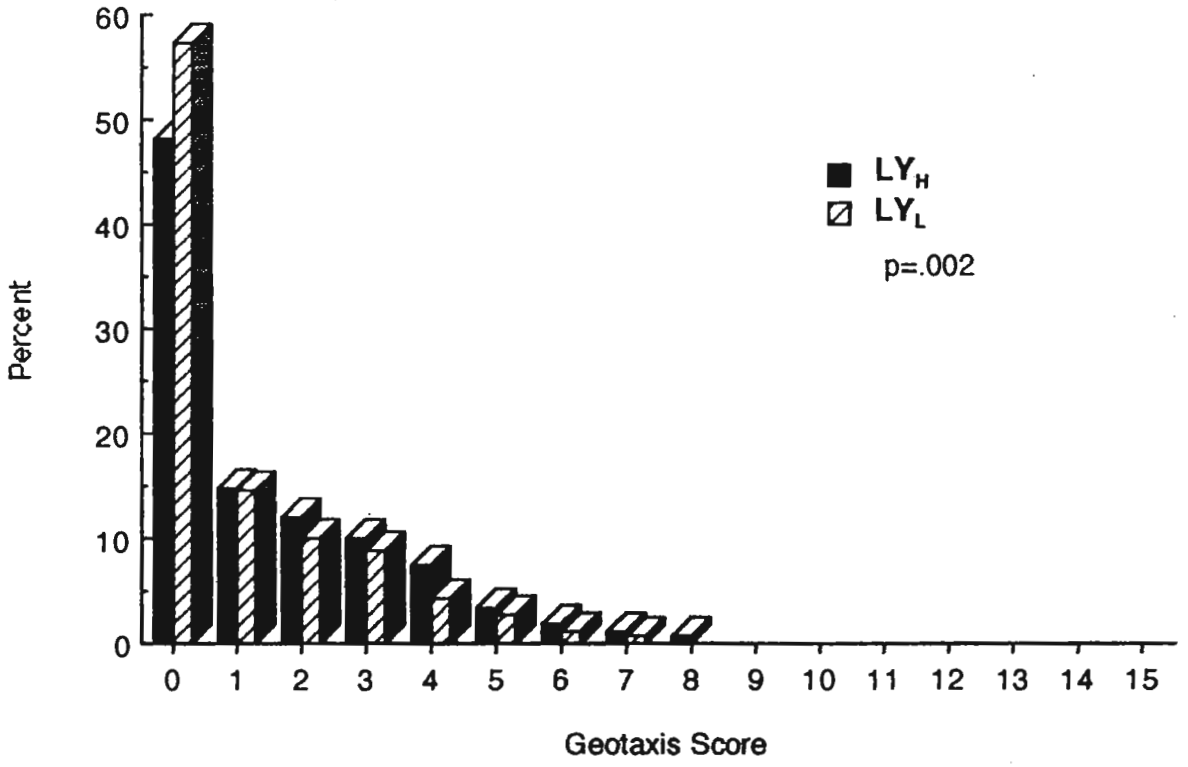

Figure 2. Geotaxis score distributions of males with genetic and cytoplasmic background from the low-geotaxis line, but Y chromosomes from the high- (LY_H) and low- (LY_L) lines.

crossed to females from the Low line. Thus, over seven successive generations of backcrossing, the evidence for a Y-chromosome effect on geotaxis was inconsistent and did not lead to an unambiguous interpretation.

Two properties of the backcross experiment reduced statistical power to detect small Y-chromosome effects: (1) residual genetic background material from the non-maternal line, (2) sample sizes for a given backcross subline were restricted to approximately 200 each generation. We performed a chromosome substitution experiment to eliminate "genetic noise" and to provide larger samples, thereby increasing the statistical power of our tests to detect possibly small Y-chromosome effects on geotaxis.

We bred flies from the high- and low-geotaxis lines at Generation 782 to flies from three balancer stocks with dominant morphological mutations that are homozygous lethal. The markers were located on the second (*CyO*), third (*Sb*) and fourth (ci^D) chromosomes (see Lindsley and Zimm, 1992 for descriptions of the mutations). Briefly, we prepared flies with all three mutations and mated them to flies from the selected geotaxis lines to create four sublines: two having X chromosomes, autosomes and cytoplasm from the high- line, but differing in their Y chromosomes (*i.e.*, HY_H & HY_L); and two having X chromosomes, autosomes and cytoplasm from the low- line, and likewise differing in their Y chromosomes (*i.e.*, LY_H & LY_L). Then, using a Monte Carlo R x C contingency table analysis (kindly provided by W. Engels, University of Wisconsin, Madison; see Lewontin and Felsenstein, 1965), we compared the geotaxis distributions of males with the same genetic and cytoplasmic backgrounds, but different Y chromosomes.

Results now support the interpretation of a Y-chromosome effect on geotaxis that is independent of genetic and cytoplasmic background. Figure 1 presents the geotaxis-score distributions of the sublines with genetic and cytoplasmic background from the high-geotaxis line, but different Y chromosomes ($p = .007$). Visual inspection indicates that more flies with a Y chromosome from the high- line (HY_H) completed the geotaxis maze in the highest category (15), making 15 up choices. Figure 2 presents the geotaxis-score distributions of the sublines with genetic and cytoplasmic background from the low-geotaxis line, but different Y chromosomes ($p = .002$). Visual inspection indicates that more flies with a Y chromosome from the low- line (LY_L) completed the geotaxis maze in the lowest category (0), making 15 down choices. Summary statistics are presented in Table 1. Mean geotaxis scores are consistent with the direction of the line of origin of the Y chromosome.

Y chromosomes are correlated with geotactic behavior in the selected geotaxis lines. In the present study we utilized techniques that provided increased statistical power relative to an earlier report (Stoltenberg and Hirsch, 1994). This study appears to be the first to demonstrate a Y chromosome effect on a non-mating behavior in *D. melanogaster.*

References: Lewontin, R.C., and J. Felsenstein 1965, Biometrics 21:19-33; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of Drosophila melanogaster*, San Diego: Academic Press; Mather, K., 1944, Proc. Roy. Soc., London B, 132:308-332; Ricker, J.P., and J. Hirsch 1988, J. Comp. Psych. 102:203-214; Stoltenberg, S.F., and J. Hirsch 1994, Dros. Inf. Serv. 75:160-161.

Hediger, M., L. Tassini, and E. Hauschteck-Jungen. Zoologisches Institut der Universitat Zurich, Winterthurerstr. 190, 8057 Zurich, Switzerland. For the expression of the Sex Ratio trait in *D. subobscura* a proximal and a distal region of the X (=A) chromosome are necessary.

The Sex Ratio trait is a meiotic drive system located on the X chromosome, known in twelve *Drosophila* species (Hurst and Pomiankowski, 1991). Males carrying the Sex Ratio X chromosome produce predominantly female offspring. In eight of nine species which express the trait, the salivary gland chromosomes were found to be protected against recombination by having inversions. Only in *D. testacea* the Sex Ratio X chromosome and a non-Sex Ratio X chromosome had the same gene arrangement (James and Jaenike, 1990). The protection against c.o. indicates that more than one gene is in charge of the expression of the Sex Ratio trait.

In *D. subobscura*. the Sex Ratio chromosome is covered by the four inversions 2, 3, 5 and 7 (Figure 1) (Jungen, 1967). In *D. persimilis* more than one region is necessary for the expression of Sex Ratio (Wu and Beckenbach, 1983). In order to localize such regions in *D. subobscura* we tried to separate the inversions of the Sex Ratio chromosome. The gene arrangement of the Sex Ratio chromosome allows only the separation of the inversions 2 and 3 from 5 and 7 because 2 and 3 overlap, and 5 and 7 have a common break point. A_2 is the only inversion which is on one hand present in the gene arrangement $A_{2+3+5+7}$, and which on the other hand occurs also independently. Males with the A_2 have offspring with a sex ratio of 1:1. Recombination between $A_{2+3+5+7}$ and A_2 results in the two gene arrangements A_{2+3} and A_{2+5+7}. The chromosome with the gene arrangement A_2 had no genetic marker. Therefore offspring of heterozygous $A_{2+3+5+7}/A_2$ females were analysed by squash preparation of the salivary gland chromosomes.

In the karyotypes of 38 dissected larvae we found both recombinant gene arrangements once, the A_{2+3} and A_{2+5+7}. Another 126 offspring of these heterozygous females were not dissected but mated to obtain lines with

recombinant gene arrangements. Five of these 126 offspring were found to have the gene arrangement A_{2+5+7}. But the gene arrangement A_{2+3} was not observed. Three of those five different recombinant gene arrangements A_{2+5+7} were used to set up lines. Twenty males from each of the three lines were mated to check whether they showed the Sex Ratio trait. In all three lines the percentage of females varied between 40 and 60% which means that none of the males with an A_{2+5+7} was a Sex Ratio male.

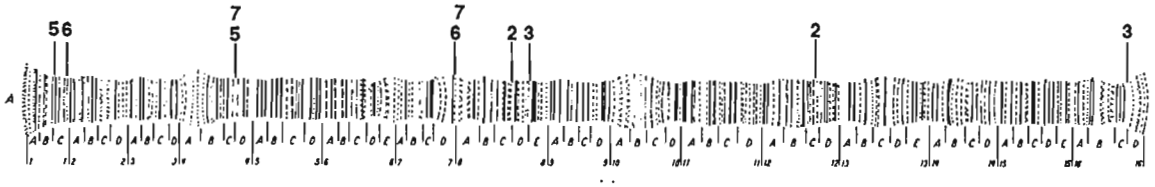

Figure 1. The $A_{standard}$ chromosome modified after Kunze-Muhl and Muller (1958). The numbers on the upper side of the chromosome indicate break points of the respective inversions. Inversion number 2 occurred before inversion number 3.

Obviously the inversion A_3 is necessary for the Sex Ratio trait in *D. subobscura*. The question was whether the A_3 is sufficient for the expression of the Sex Ratio trait. The A_3 alone was never found in *D. subobscura* but A_3 is known to form the gene arrangements A_{2+3} and A_{2+3+6}. Both are rare in natural populations. Jungen (1968) analysed eight offspring of four males with these A gene arrangements and found males as well as females. The proximal inversion A_6 is another one which was only found in combination with other inversions. A_{2+6} is an abundant gene arrangement. Males with this A chromosome produce males and females in equal ratio. Other proximal regions, not the A_6, seem to be necessary for the Sex Ratio trait. The proximal break point of A_6 is distal of the proximal break point of A_5. Therefore the short distance 1C of the A chromosome could harbour a sequence or sequences necessary for the Sex Ratio trait. Another possibility is that after the formation of the $A_{2+3+5+7}$ Sex Ratio genes appeared anywhere in the inversions 5 and/or 7.

The frequency of the recombinant gene arrangements in nature: In the natural population in Tunisia from which the $A_{2+3+5+7}$ originated (Hauschteck-Jungen, 1990; Jungen, 1968a), both the A_{2+3} and the A_{2+5+7} were extremely rare. The question arose whether the latter two gene arrangements are established in the population or whether they are recombination products which would have disappeared if we would not have trapped them.

We can compare the frequency of the A gene arrangement A_{2+5+7} in our experiments with the frequency in nature. In our experiment the recombination frequency in $A_{2+3+5+7}/A_2$ females was 4%. According to Jungen (1968b) the frequencies of $A_{2+3+5+7}$ and A_2 in nature, determined by analysis of males, were 22% and 18%. Thus 4% of females in nature could have the karyotype $A_{2+3+5+7}/A_2$. Assuming that the same recombination rate of 4% as in our experiment occurs in nature, we would suspect 0.16% recombination products which is about 1 in 600. In 552 wild trapped males the A_{2+3+5} occurred once, A_{2+3} twice (Jungen, 1968b) which fits to the assumption that the A_{2+3} and the A_{2+5+7}, observed by Jungen in wild trapped males, had been first generation products after a cross over. This means that the two gene arrangements A_{2+3} and A_{2+5+7} are not established in natural populations in Tunisia. The same seems to be true for the gene arrangement A_{2+3+6} which is a cross over product of A_{2+6} and $A_{2+3+5+7}$, and was only found twice in 552 wild trapped flies.

The elimination of cross over products is a powerful mechanism to stabilize the Sex Ratio chromosome in nature.

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Tripathy, N.K., R.K. Mishra*, U.R. Sahoo, and G.P. Sahu. Gene-Tox Laboratory, Department of Zoology, Berhampur University, Berhampur-760 007. *Department of Zoology, Stewart Science College, Cuttack-753 001, Orissa, India. Potassium bromate-genotoxicity studies in the somatic and germ line cells of *Drosophila melanogaster*.

Potassium bromate, widely used as a food additive, is reported to induce renal cell adenomas, adenosarcomas and thyroid tumours in rats following oral administration (IARC, 1986). Single dose administration, according to Kurata, *et al.* (1992), however, did not induce adenosarcoma in the same animal. According to Kimie, *et al.* (1992) this compound generates active oxygen species that help in the initiation of carcinoma.

In *Salmonella typhimurium*, potassium bromate was mutagenic in the presence of S9 mix (Ishidate, *et al.*, 1984). Kawachi, *et al.* (1980) could not, however, establish its genotoxicity in the Bacillus rec+/- assay. In the mouse bone marrow cells, following intraperitoneal injection and oral administration, the compound induced micronuclei (Awagi, *et al.*, 1992). In rat *in vivo* tests, this compound was clastogenic (Fujie, *et al.*, 1988). Its clastogenicity was also established in the Chinese hamster fibroblasts (Ishidate and Yoshikawa, 1980; Ishidate, *et al.*, 1984) both in the presence and absence of S9 mix. The present communication describes the results on the genotoxicity of potassium bromate with 95% purity, manufactured and marketed by Central Drug House Pvt. Ltd., Delhi, in the wing primordia and male germ line cells of *Drosophila melanogaster* following the wing spot and the sex-linked recessive lethal tests after chronic larval feeding.

Larvae, obtained from a cross of *mwh* female and flr^3/TM3, *Ser* males, were used in the wing spot test. The alleles *mwh* (multiple wing hairs, 3-0.3) and flr^3 (flare, 3-38.8) are recessive genetic markers on chromosome 3 and are expressed as multiple trichomes or thick and misshaped trichomes, respectively, on an otherwise normal adult wing. For details of the markers, see Lindsley and Zimm (1992). The 2nd and 3rd instar *mwh* + / + flr^3 transheterozygous larvae, obtained from the above cross, were exposed to different concentrations of potassium bromate dissolved in distilled water in instant food (Carolina Biological Supply Co., USA) and the LD_{50} was determined. The LD_{50}, where 50% larvae eclosed to adult stage, was 5 mM for both larval instars. The larvae were thus exposed to either the LD_{50} or lower doses for the rest of their larval life. The wings of the eclosing flies were mounted in Faure's solution (Graf, *et al.*, 1984) and screened under a compound microscope to record the type and size of each wing spot. The data were subjected to the conditional binomial test following Frei and Wurgler (1988).

Table 1. Summary of data obtained in the wing spot test.

Treatment	Conc. (mM)	No. of wings	Spots per wing (No. of spots) Statistical diagnoses* small singles (s = 1 - 2) m = 2.0	large singles (s > 2) m = 5.0	twins (t) m = 5.0
48 h	Control	80	0.39 (31)	0.01 (1)	0.01 (1)
	5.0	80	0.68 (54)+	0.04 (3) i	0.03 (2) i
	2.5	80	0.51 (41) i	0.03 (2) i	0.03 (2) i
	1.0	80	0.46 (37) -	0.03 (2) i	0.00 (0) i
72 h	Control	80	0.41 (33)	0.01 (1)	0.00 (0)
	5.0	80	0.80 (64)+	0.11 (9)+	0.01 (1) i
	2.5	80	0.65 (52)+	0.05 (4) i	0.01 (1) i
	1.0	80	0.50 (40)-	0.03 (2) i	0.00 (0) i

*Statistical diagnoses according to Frei and Wurgler (1988). + = positive; - = negative; i = inconclusive; m = multiplication factor. Probability level : $\alpha = \beta = 0.05$, one-sided statistical tests.

Table 2. Summary of data obtained in the sex-linked recessive lethal test.

Treatment	Conc. (mM)	Males tested	X chromosome Total	Lethal	%	Conclusion*
Pooled control	0.0	98	2021	3	0.15	
48 h	5.0	50	1054	8	0.76	POS
	2.5	52	1063	4	0.38	NS
	1.0	55	1134	3	0.26	NS
72 h	5.0	56	1205	9	0.75	POS
	2.5	51	992	5	0.50	NS
	1.0	48	936	3	0.32	NS

*Conclusion on the basis of Kastenbaum and Bowman (1970), POS = positive, NS = not significant, level of significance $P \leq 0.05$.

In the sex-linked recessive lethal test, same age larvae were treated with similar doses of the preservative as in the wing spot test. For details of the procedure refer to Tripathy, *et al.* (1994). The data obtained in the test were statistically evaluated following the conditional binomial test (Kastenbaum and Bowman, 1970).

The results obtained in the wing spot test are presented in the Table 1.

In the 48 h treatment of 3rd instar larvae, the frequency of induction of small single spots was positive only at the highest dose and the same at other doses, as well as the induction frequency of large singles

and twin spots at all the doses was either inconclusive or negative. Following 72 h exposures of 2nd instar larvae, the frequency of induction of small single spots was positive at the two highest doses and negative at the lowest dose. In addition, the frequency of large singles was also positive at the LD_{50} and inconclusive at other doses.

Twin spots with *mwh* and *flr*^{3} subclones originate following the induction of mitotic recombination in the chromosome region between the *flr*^{3} locus and the centromere (Becker, 1976). In the present experiments, since the twin spots were not induced in significantly large numbers at any of the test doses and durations of treatment, it is supposed that potassium bromate is probably nonrecombinogenic or weakly so in the wing disc cells of *Drosophila*. The single spots with *mwh* or *flr*^{3} phenotype may originate due to mutation or gene conversion in the corresponding wild type loci (Graf, *et al.*, 1984) or through the induction of segmental aneuploidy due to chromosome breakage (Haynie and Bryant, 1977). In the present experiments, since single spots have been induced in significantly higher frequencies at very high concentrations, it may be argued that probably a threshold concentration of potassium bromate is necessary for induction of such spots. Further, the frequency of large single spots has been positive following exposures of 2nd instar larvae to the highest dose of the compound. This is obvious since genetic alterations induced in the wing primordia of younger larvae will form larger clones if they survive and undergo normal division as the clone size is dependent on the number of mitotic cycles which take place between induction and metamorphosis.

Data obtained in the sex-linked recessive lethal test are summarised in the Table 2.

Since there was no difference in the lethal frequency in the two controls following exposures of 2nd and 3rd instar larvae to the solvent, the data were pooled. The lethal frequencies were positive at the highest dose and nonsignificant at the lower doses following exposures of larvae of both instars. Since this food additive is reported to be clastogenic in other eukaryotic systems and since according to Lee, *et al.* (1983) mutations and deletions in the X chromosomes of the male germ line lead to the induction of lethals, the induction of sex-linked recessive lethals was expected in the present experiments. Further, since the frequency of lethal induction was positive only at the LD_{50}, it is believed that probably a minimum concentration of this chemical is necessary for the induction of genetic changes in the germ live cells of *Drosophila*.

Thus, it is concluded that irrespective of the mechanism(s) involved, potassium bromate is genotoxic in the wing primordia and the male germ line cells of *Drosophila* at very high concentrations.

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Technique Notes

Misquitta, Leonie, and Pradip Sinha. Drosophila Stock Center, School of Life Sciences, Vigyan Bhavan, Khandwa Road Campus, Devi Ahilya Vishwavidyalaya, Indore. 452 001 India. An easy technique to obtain photographs of whole mounts of *Drosophila* adult files under compound microscopes.

Photography of the adult fly particularly in laboratories without good photomicrographic accessories attached to stereomicroscopes poses considerable difficulties. Photography of the fly is generally not possible under a compound microscope. We describe here a simple whole mount technique that can partly circumvent the problems of photography of adult flies under compound light microscopes.

Adult flies or large parts of the adult structures such as the head are placed in a warm (45°C) mountant (for preparation of mountant dissolve 7 grams of gelatin in 42 ml of water in a boiling water bath. Add 63 grams of glycerol and a crystal of phenol as a bactericide. Store at 4°C). The flies mounted in this fashion permit photography under low magnification 4X, 10X (see Figure 1). The photographs are comparable to those taken under stereomicroscopes and provide an excellent display of the adult structures. The use of a coverslip is optional. The quality of photographs can be further improved by the use of suitable filters. The mounted flies can be stored in a sealed slide box at room temperature for many months. At 4°C it can last for an indefinite period.

Figure 1. Whole mount of a fly (A) and (B) a dissected head showing the frontal view and photographed under 4X and 10X, respectively.

Misquitta, Leonie, and Pradip Sinha. Drosophila Stock Center, School of Life Sciences, Vigyan Bhavan, Khandwa Road Campus, Devi Ahilya Vishwavidyalaya, Indore. 452 001 India. A "user friendly" technique for removal of vitelline membrane in embryonic cuticular preparations and X-gal stained embryos.

The vitelline membrane of the *Drosophila* egg acts as a barrier against clear optical resolution of embryonic structures. It poses problems in localization of the cell types in immunohistochemistry or histochemistry. Removal of the vitelline membrane on the other hand by manual methods (Lohs-Schardin, *et al.*, 1979) is time consuming and the yield is not always satisfactory, while chemical devitellinization treatment (Mitchison and Sedat, 1983) demands harsh temperature shocks which along with the nature of the chemical used often destroys the staining quality. We describe here an adaptation of the chemical devitellinization technique (Panzer *et al.*, 1992) which permits retention of histochemical staining using X-gal. In addition it simplifies devitellinization of embryos for clear cuticular preparation of the first instar larvae.

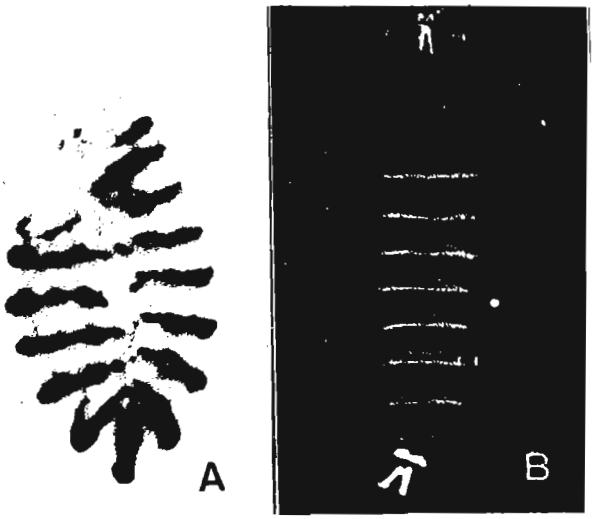

Figure 1. (A), Devitellinized X-gal stained embryo bearing a lacZ reporter gene fused to *engrailed* promotor (Hamma, *et al.*, 1990); (B), A wild type cuticular preparation of first instar larvae.

For cuticular mounts. The embryos are first dechorionated in sodium hypochlorite (bleach 3%) and washed thoroughly with water. The water is then replaced with 5 mM EDTA in 90% methanol, and an equal volume of heptane is added so that the embryos float at the interphase. The mixture is vigorously shaken for a few minutes (2-5). Most of the vitelline membranes burst in this mix and the embryos settle at the bottom of the methanol-EDTA solution. Replace the EDTA-methanol solution with methanol without drawing out the heptane and shake vigorously (5 min). The few remaining embryos too would sink into the methanol layer. If any embryos remain after the first wash, rinse again with methanol until they sink. This process normally yields 90-95% of devitellinized embryos. Once a satisfactory yield of devitellinized embryos is obtained, heptane and methanol is replaced with fixative (in this case 1:4 glycerol and acetic acid) and incubated for a period of 24 hours at room temperature. The embryos are mounted in Hoyers mountant (Ashburner, 1989) and incubated at 45°C for 1 to 2 days, to clear the soft tissues. Additional weights on the coverslip can be used for flattening the embryos during incubation wherever necessary. Better clearing of embryos can be obtained by first incubating them for 1 hour at 60°C after adding fixative followed by incubation for 24 hours at room temperature.

For X-gal stained embryos. For X-gal stained embryos, the devitellinization is carried out after staining is complete. The process is similar to the one described for devitellinization of unstained embryos with slight variations at a few steps. X-gal stained embryos (Ghysen and O'Kane, 1989) are fixed again using the fixative (*i.e.*, 1:10 formaldehyde to citric phosphate buffer) for 15 min. The fixative is then replaced by the devitellinization solution of EDTA-methanol solution and heptane, and then subsequent steps of collecting devitellinized embryos mentioned above are followed. After devitellinization, replace methanol with incubation media and mount in mountant (Ghysen and O'Kane, 1989). Complete removal of EDTA is necessary to avoid precipitation of the X-gal stain. It is only after all traces of EDTA are removed with several washes of methanol that the embryos should be processed further. These mounts can be kept indefinitely with no change in the staining quality.

The present technique is a modification of the chemical devitellinization technique of Panzer *et al.* (1992). Firstly the wash with NaCl and Triton-X has been removed (Panzer, *et al.*, 1992), because it often leads to precipitation of the stain formed during the devitellinization of X-gal stained embryos. Instead we have added an additional step of fixation after staining has been completed (post fixation) that facilitates the preservation of stain during subsequent devitellinization steps. We have also adapted this chemical devitellinization before processing the first instar embryos for cuticular mounts. We have seen that in the case of cuticular mounts the chemical devitellinization facilitates in the clearing of the embryos. We prefer to call the procedure user friendly due to its usefulness in both immunohistochemistry (Panzer, *et al.*, 1992), histochemistry and cuticular preparation reported here.

References: Ashburner, M., 1989, *Drosophila: A Laboratory Manual*, CSH Press, pp. 337; Ghysen, A., and C. O'Kane 1989, Dev. 105:35-52; Lohs-Schardin, M., C. Cremer, and C. Nusslein-Volhard 1979, Dev. Biol. 73:239-255; Panzer, S., D. Weigel, and S.K. Beckendorf 1992, Dev. 114:49-57; Mitchison, T.J., and J. Sedat 1983, Dev. Biol. 99:261-264.

Frank, J., C. Adams, and S.P. McRobert. Saint Joseph's University, Pennsylvania, U.S.A. Sucrose density separation of *Drosophila* larvae from instant medium.

It has been noted that *Drosophila* larvae can be isolated from culture media by suspending the media in a sucrose solution (Nothiger, 1970; Bixler and Tompkins, 1991). The larvae float to the surface while the medium sinks. Bixler and Tompkins (1991) reported that isolation of larvae from Carolina 4-24 Instant Drosophila Medium requires a 40% sucrose solution rather than the 20% solution typically used with cooked cornmeal media. We have found, however, that a 20% sucrose solution can be used to extract larvae from instant media provided the media is stirred vigorously with a glass stirring rod. This process not only frees the larvae from the media but breaks the media into tiny pieces, which then sink. The use of a 20% sucrose solution is preferable since it alleviates the problems associated with dissolving a 40% solution. It also alleviates concerns over creating an incredibly sticky mess by spilling a 40% solution.

Acknowledgment: Support was provided by Howard Hughes Medical Institute Grant 71194-535101, awarded to the Biology Department of Saint Joseph's University.

References: Bixler, A. and L. Tompkins 1991, Dros. Inf. Serv. 70:238; Nothiger, R. 1970, Dros. Inf. Serv. 45:177.

Di Franco, C., A. Terrinoni, D. Galuppi, and N. Junakovic. Centro per lo Studio degli Acidi Nucleici C.N.R., Dipartimento di Genetica e Biologia Molecolare, Universita "La Sapienza," P.le A. Moro 5, 00185 Roma, Italy. DNA extraction from single *Drosophila* flies.

A protocol is described which allows one to extract DNA from 40 - 50 individual flies in a day without the use of organic solvents. The procedure is similar to the one described by Jowett (1986). The major improvement is the homogenization step which makes the extraction of numerous samples simpler, faster and more reproducible.

Homogenization is carried out in 100 ul of 50 mM Tris, 50 mM EDTA, 1% SDS pH 9 in Eppendorf tubes. A pestle to fit the bottom of an Eppendorf tube is sold by several companies. However, by inserting the pestle into a tube containing a droplet of colored liquid (*e.g.*, Bromophenol Blue) it turns out that adhesion to the tube wall may not be uniform as not all the liquid is squeezed out. The same pestle may or may not fit Eppendorf tubes from different suppliers. At some stage, having found no satisfactory commercial pestle, one was made with dental resin which worked fine. Eventually, the pestle supplied by Applegene was found to meet the colored-droplet test with Eppendorf tubes supplied by Treff. To provide motion, a kitchen mixer is used. The two metal rods (of the mixer and of the pestle) are cut to approximately 4 cm and connected with a piece of rubber tubing leaving about 5 mm between the two metal ends. In such a way the motion is transmitted from the mixer to the pestle without the need to have the two rotating rods perfectly lined up which in turn makes the handling of dozens of samples simpler. The mixer that is used has five speeds plus the "turbo" option. The turbo option was found to provide 850 rpm which allows the homogenization to be completed in about 15-20 sec. To finish up the homogenization, the pestle is lifted out of the homogenizing mix still rotating and care is taken to touch for a second the internal wall of the tube. This helps unloading the homogenate that adheres to the pestle. To clean it between two samples, the still rotating pestle is immersed for a few seconds in a beaker containing distilled water. To dry it, the pestle is briefly brought in contact with a paper towel. Lots of about a dozen of flies at a time are homogenized at room temperature, put on ice for the few minutes needed to complete the lot and then transferred to a water bath at 65°C for 1 hr. After the first half an hour, the samples are gently mixed to resuspend the visible precipitate. Upon a few minutes at room temperature, 14 ul of K-Acetate 8 M are added and samples mixed for a few seconds until the liquid appears homogeneously denser. Half an hour on a mixture of ice and water follows. Samples are then spun at 10,000 rpm for 10 min at room temperature. Approximately 80 ul of the supernatant are transferred to fresh Eppendorf tubes taking care to avoid unloading the floating film of lipids that tends to stick to the external part of Gilson yellow tips. 80 ul of isopropanol are added and samples mixed by repeatedly inverting the rack bearing the samples. About a quarter of an hour later, nucleic acids are pelleted at 10,000 rpm for 10 min. A Pasteur pipette with a flame thinned tip is used to withdraw the supernatant. Care should be taken to avoid discarding the minute, whitish pellet with the supernatant. 200 ul of 70% ethanol are added, the tubes mixed and spun again for 5 min at 10,000 rpm. The supernatant is discarded including all visible droplets of ethanol. The tubes are then left open covered with an alu foil for 15 min. 27 ul of water

are added and the pellet left to rehydrate for about 10 min. At this stage the pellet is not visible anymore but it still may not be properly resuspended. Hence, it is important that each sample be mixed and left an additional 10 min to allow resuspension to occur. A mixture of 10 X salts plus restriction enzyme is prepared and aliquots of 3 ul containing about one unit of enzyme are added to each sample. The enzymes tested were *Hin*dIII, *Eco*RI, *Pst*I and *Bam*HI which all worked with comparable efficiency. Incubation is carried out in an oven where the tubes are at the same temperature throughout as opposed to a water bath or heat block where the lid is cooler and tends to accumulate droplets of condensed water. Incubation time is 2-3 hr but occasionally digestion is left overnight. Occasionally (in about 1 out of 100 samples) the DNA appears degraded. No correlation is observed between the time of incubation and the number of degraded samples suggesting that the extraction protocol is reasonably effective in removing or inactivating endogenous nucleases. An approximate estimate of the recovery of DNA was obtained by comparing the fluorescent bands of undigested samples to the intensity of known amounts of lambda DNA fragments. Between 200 and 500 ng of DNA could be extracted per single female and about half that much from males.

As a concluding remark it is worthwhile to comment briefly on some aspects of agarose gel electrophoresis and blotting. Usually, if unique sequences are to be detected, generous amounts of DNA per sample may compensate for less-than-optimal gel and transfer. With DNA from single flies this is obviously not practicable. One useful detail is to use slots no wider than 3-4 mm; this concentrates the autoradiographic bands over a shorter distance and enables one to load a higher number of samples per gel. To improve transfer efficiency, agarose slabs 3 mm thick are used. The gel boxes are vertical, as horizontal slabs of this thickness can accommodate very small sample volumes which in turn makes resuspension and gel loading more tricky. We use a vacuum transfer device. In about an hour 250 ml of freshly made 0.4 N NaOH efficiently denatures the DNA, transfers it and fixes it to the blotting membrane (Amersham Hybond N+). Following this protocol we were able to reprobe the same filter bearing samples from single females 15 times (Di Franco, *et al.*, 1992).

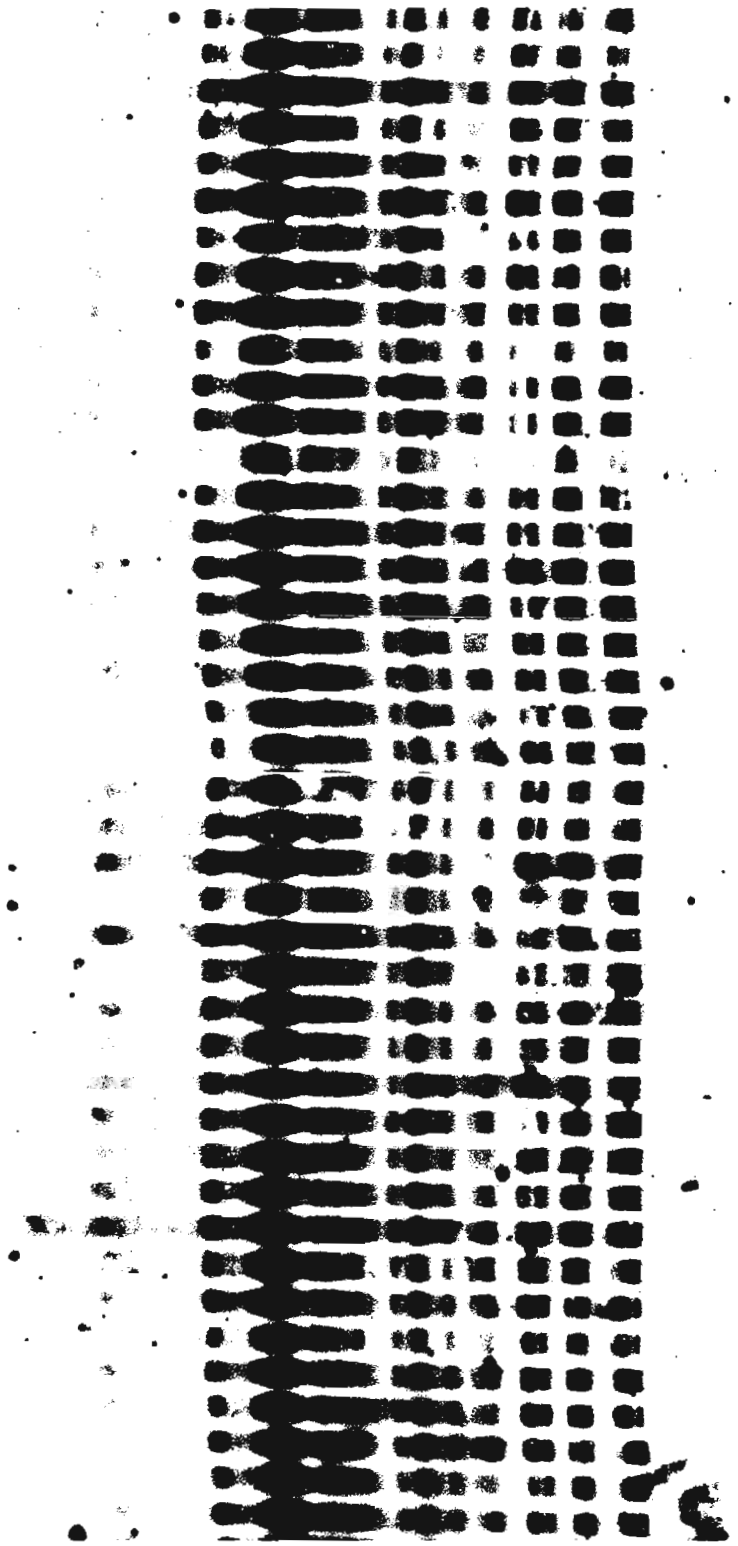

Figure 1. Autoradiograph of DNA digests from individual *Drosophila* females probed with a sequence homologous to copia transposable elements. This particular image is the outcome of the fourth round of hybridization without a detectable decrease in sensitivity.

References: Jowett, T., 1986, Preparation of Nucleic Acids. In: *Drosophila, A Practical Approach*, (Roberts, D.B., ed.), pp 275-286, IRL Press, Oxford; Di Franco, C., D. Galuppi, and N. Junakovic 1992, Genetica 86: 1-10.

Hernady, Eric. Neurobiology Research Center and Department of Biological Sciences, The University at Albany, State University of New York, 1400 Washington Avenue, Albany, NY 12222. Controlling larval density in culturing *Drosophila melanogaster.*

In *Drosophila melanogaster* larval density affects development time (Lints and Lints, 1969; Lints and Lints, 1971), viability (Budnik and Brncic, 1974), and possibly sex ratios. For developmental and/or behavioral studies it is often important to reduce variability as much as possible, so we developed a simple, rapid procedure for reliably controlling larval density.

We preselected groups of adult flies (40-50 adults/vial) to identify those that produced large numbers of eggs within 22-24 hours of being placed into fresh vials (containing approximately 5 ml of Instant *Drosophila* Medium and equal parts distilled water); our most productive groups of flies produced up to five hundred eggs/vial. Eggs were routinely harvested 14 hours after adult flies were placed into fresh vials, which were then examined under a dissecting microscope to locate the eggs (only eggs with an opaque white coating were used; if the egg casing appeared transparent and the first instar larvae could be seen within the egg, it was not used.) This insured maximum synchronization of the larvae.

Table 1.

Total Pupae	FEM	Adults	% Females
20	10	18	55.56
22	9	13	69.23
24	12	21	57.14
16	10	14	71.43
16	9	13	69.23
26	11	22	50.00
19	7	16	43.75
16	7	11	63.64
20	9	17	52.94
15	9	12	75.00
16	2	5	40.00
16	6	14	42.86
24	7	19	36.84
15	7	12	58.33
23	7	20	35.00
23	9	18	50.00
19	11	16	68.75
24	10	20	50.00
22	8	14	57.14
20	9	18	50.00
25	12	25	48.00
20	11	18	61.11
18	13	15	86.67
22	12	18	66.67
22	6	16	37.50
22	12	21	57.14
23	13	19	68.42
20	10	18	55.56
19	8	14	57.14
27	8	21	38.10
20	11	18	61.11
18	8	16	50.00
24	13	19	68.42
23	10	18	55.56
22	12	21	57.14
24	11	23	47.83
25	13	25	52.00
24	6	18	33.33
21	4	12	33.33
23	10	17	58.82
20	7	17	41.18
20	7	13	53.85
21	9	17	52.94
24	10	18	55.56

A cleaned, distilled water rinsed plastic measuring spoon (2.5 ml) was used to skim the eggs off the surface (along with some medium), which were placed into a clean, non-sterile petri dish that was filled with distilled water to a depth of one centimeter. Eggs could be collected from many vials in this fashion; we collected up to 750 eggs per day. Next the petri dish was agitated gently in order to separate the eggs from the medium. Finally, the eggs were aspirated under a dissecting microscope using a pasteur pipette; with practice eggs can be counted as they enter the pipette. Eggs were then placed into fresh vials along the vial-medium interface. In our experiments larval density was held constant by maintaining a ratio of 10 eggs/ml of medium (using 5 ml of medium in a 15 ml vial). Vials were maintained in a 12 light/12 dark cycle at 25°C.

Adults began to eclose on the ninth day and essentially all pupae eclosed by 9.5 days. This was in contrast to our stock vials in which larval density was much higher and which eclosed on the twelfth day. Viability was consistently high, averaging 21.0 adults/vial (SEM = 0.47). Male/female ratios were also quite consistent, averaging 54.4% (SEM = 1.80). By raising flies under conditions of controlled density it is possible to obtain consistent populations of flies.

Acknowledgments: This research was supported by a grant from the Whitehall Foundation to H.V.B. Hirsch.

References: Budnik, V., and D. Brncic 1974, Ecology 55:657-661; Lints, F.A., and C.V. Lints 1969, Experimental Gerontology 4:231-244; Lints, F.A., and C.V. Lints 1971, Experimental Gerontology, 6:427-445.

Hoenigsberg, H.F. Instituto de Genética, Universidad de los Andes, Santafé de Bogotá, D.C., Colombia. Collecting larvae to screen different developmental periods of *Drosophila*.

It is important to time different developmental stages since hatching is an important landmark to identify *Drosophila* species and to explore different moments of gene action in the first, second, and third instar. Eggs can be collected on various surfaces: small spoon stained with charcoal or plastic discs of 1.5% agar lightly smeared with different percentages of yeast suspension. Discs should be stored in a humid plastic box overnight. The larvae that hatch after 1 to 10 hours of copulation and that have been "held" by the female before laying can be grouped according to copulation time and to the time when they were "held" according to the larvae collected. Thus, larvae hatching from "held" eggs, in other words those eggs hatching after a certain number of hours after the mean egg collecting period, in each experiment, can be removed by gently washing the discs with a plastic squeeze bottle.

If the experimenter wants to pick out a homogeneous sample, of say first instar, he can proceed with a needle or with a slightly hardened pointed brush.

Since Nothiger (1970) developed for *D. melanogaster* larvae a sucrose density separation method, many laboratories have used this method indiscriminately for a large number of species. In fact, to collect large numbers of young larvae of *Drosophila serido*, *D. mulleri*, *D. repleta*, or *D. martensis* a small change to Nothiger's method has been found useful: a) Instead of 20%, a 30-50% sucrose with a little bit of common sugar (= panela in South America) is poured into the collecting flask that can then be strongly stirred to free the larvae from the original food. It is advisable to initiate the culture with several dozen adults for 3-4 days in order to get a very dense growth of larvae. The collecting flask can be a large (1000 ml) separatory funnel. With a little water added from the top, the larvae sink while carcasses and pupae float. The larval fraction can be filtered out and from the filter paper they can be collected gently with a needle.

Reference: Nothiger, R., 1970, Dros. Inf. Serv. 45:177.

Hepperle, B. University of Alberta, Edmonton, Alberta, Canada T6G 2N1. A simple device for preparing well flattened *Drosophila* polytene chromosomes for *in situ* hybridization.

In situ hybridization for mapping specific DNA sequences on *Drosophila* polytene chromosomes requires good spreads with well-separated chromosome arms and clearly visible, distinct bands. The chromosomes must be well flattened to preserve morphology throughout the *in situ* procedure.

Standard protocols for flattening describe squashing chromosomes by "firm thumb pressure" (Kalisch and Whitmore, 1986; Ashburner, 1989). However, it is difficult to apply sufficient pressure by thumb, or to maintain even, reproducible pressure. This method is tedious and painful, and only a few slides can be prepared at a time. Also, care must be taken while squashing to avoid lateral movement of the coverslip which can result in chromosome distortion and breakage.

Simple bench tools such as C-clamps can alleviate thumb fatigue and discomfort, but it is difficult to regulate the pressure and often the coverslip moves. A simple, inexpensive apparatus made of Plexiglas was designed that when used with a Vise-Grip locking C-clamp (Model 6SP, 150 mm) overcomes these problems. The device consists of two parts: 1) a base 100 mm long x 45 mm wide (Figure la, b) into which a standard 25 mm wide microscope slide fits and 2) an upper plate with protruding arms which interlocks with the base (Figure 1c).

To use the apparatus, the slide and cover slip are held immobile between the upper plate and the base as shown in Figure 1d. The chromosome spreads from *Drosophila* salivary glands (prepared

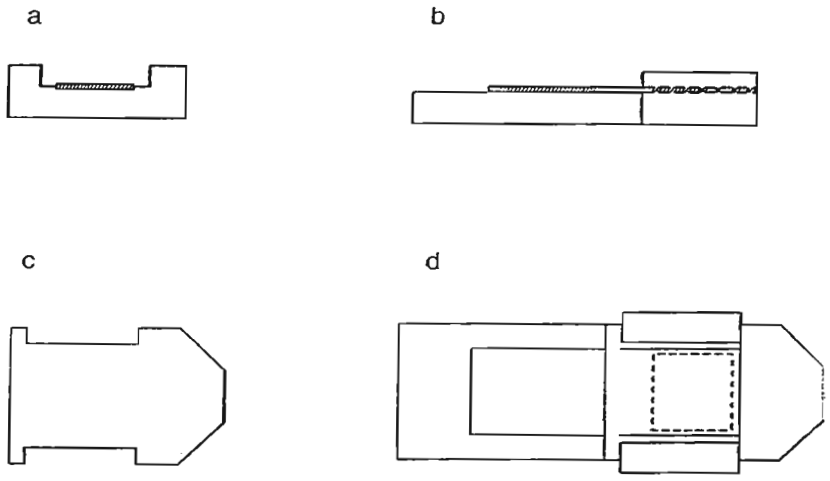

Figure 1. Component parts of the vice squash apparatus shown at 2/3 actual size. (a) end view of base and slide, (b) side view of base and slide, (c) top view of upper plate, (d) top view of assembled apparatus showing the location of the cover slip.

according to Ashburner, 1989) are then flattened by squeezing the apparatus for 1-2 min between the jaws of the Vise-Grip locking C-clamp (Figure 2). The flat swivel pads of the jaws which measure 20 x 20 mm provide a uniform pressure over a 22 x 22 mm cover slip.

The amount of pressure applied to the apparatus is regulated by rotating the end screw of the vise-grip. A consistent, reproducible pressure is obtained by securing the end screw before the handles are squeezed together locking the apparatus in place. Once the appropriate amount of pressure has been determined, well flattened chromosomes with excellent morphology can be quickly and reliably obtained.

Acknowledgments: This work is supported by a grant from the Canadian Genome Analysis and Technology program to R.B. Hodgetts. I would like to thank Mr. Nestor LaFaut for his invaluable technical assistance in designing the apparatus.

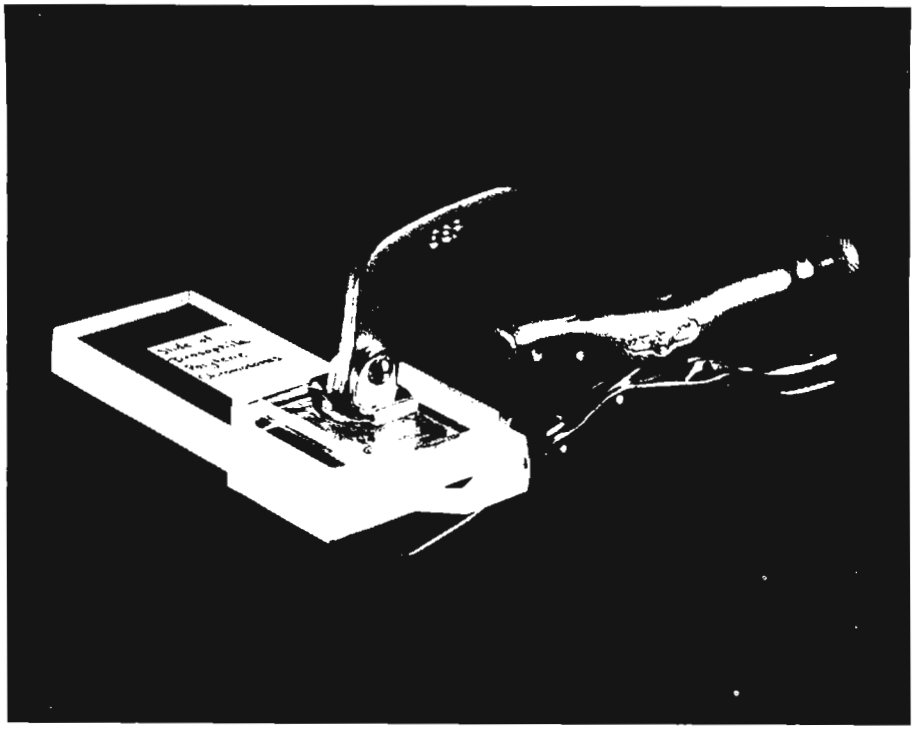

Figure 2. Assembled apparatus in vise grip.

References: Ashburner, M., 1989, *Drosophila: A Laboratory Manual*, Cold Spring Harbor, NY 11724 pp. 28; Kalish, W.-E. and T. Whitmore 1986, Dros. Inf. Serv. 63:142-146.

Hoenigsberg, H.F. Instituto de Genética, Universidad de los Andes, Santafé de Bogotá, D.C., Colombia. A simple way to get *Drosophila venezolana* 3^d instar larvae out to the surface.

To aid field workers collecting wild *Drosophila venezolana* has been quite a task! Out in the desert, Colombian and Venezuelan collections cannot be easily identified far away from lab facilities. Technicians and graduate students in Bogota or Caracas do the patient work of separating and taxonomically identifying each species.

By simply putting rotten cactus, where larvae are known to grow, in half pint bottles with the usual banana - agar medium and placing them in a refrigerator at $\pm$ 3°C for 2 hours, the larvae that crawl to the top of the bottles are all *D. venezolana*. The others, *D. mulleri, D. starmeri, D. martensis, D. serido,* and *D. melanogaster* will stay on the bottom and on the surface of the medium. Experimentally, when this method is tried in the lab, other larvae from species like *D. paulistorum, D. willistoni, D. tropicalis,* and *D. repleta* will not crawl up the top of the bottles either.

Hoenigsberg, H.F. Instituto de Genética, Universidad de los Andes, Santafé de Bogotá, D.C., Colombia. *Drosophila* baits in northern lowlands of Colombia and high altitude mountainous Andes can change according to natural fruits in the area.

Although bananas have enjoyed the greatest reputation as *Drosophila* baits among population geneticists in Tropical America, actually many fruits serve reasonably well, especially citric fruits (lemon, and varieties of oranges). Papaya, mangoes, guava, toronjas, plantain, curuba, strawberry, papayuela, custard apple, pineapple, apples, pears, and grapes have been used. Generally attrahent are prepared differently in different laboratories: Carson, Heed, Dobzhansky among the best

known Drosophilists in the U.S. have preferred Levitan's (1962) method, or small changes of it. We have preferred more natural collecting methods that have proved to be useful in Central and South American field trips. For instance we use ripe fruits including fully ripe bananas completely peeled, washed up with beer and dry yeast, when collecting at high altitudes where day temperatures are around 10°C, and night temperatures get down to freezing (0°, -5°C). In the high altitude and cold environment, the preparation is dumped in a plastic container of a few centimeters deep (about 10 cm) and about 25 cm wide. The ripe fruit should not be more than a few cm deep. It is advisable to place many of these small plastic containers separated about 20-50 m within the area that the experimenter wants to explore for population growth, migration and resources. The mash should be covered only with a loosely fit metallic grate to avoid animals visiting and destroying the bait. The mash within the container should be prepared and left on the ground preferably close to a shaded area with natural trees, and it should never be fermented before using it and certainly not at 25°C, inside a lab or a room when the collecting place is a cold ambient.

Fruits bought in markets have some important drawbacks: they may contain insecticide residues and if overripe they may contain eggs and larvae of local *Drosophila*. Generally markets have fruits already visited by parasites, mold species, and a variety of yeast. On the other hand, heat-treating fruit may reduce its attractive qualities.

When field trips are made in the lowlands where high temperatures (25° - 35°C) and high humidity are the rule in Central and South American valleys, mushrooms, decaying tomatoes, cabbage leaves left on the ground can be powerful attractants. Spencer (1950), Dorsey and Carson (1956), Kaneshiro *et al.* (1977), Dobzhansky and Hoenigsberg (1970, 1971, 1972) have employed various kinds of fungi as bait for drosophilids. In fact, in such cases the results have been strikingly different from those obtained with bananas or the above mentioned fruits. The use of flowers has also been successful (Heed *et al.*, 1960).

In Santa Marta's highlands (about 1600 m) with fruits fermented in the various valleys visited by Carson, Heed, Wassermann and Hoenigsberg and in another visit by Dobzhansky and Hoenigsberg the following unusual species were collected: *D. willistoni, D. latifasciaeformis, D. castanea, D. cardini, D. mediostriata, D. melanogaster, D. cardinoides, D. calloptera, D. limbinervis, D. albicans, D. angustibucca, D. hydei, D. capricorni, D. tripunctata, D. nigricincta, D. fumipennis, D. simulans, D. paranaensis, D. repleta, D. unipunctata, D. flavolineata, D. parabocainensis*.

References: Dobzhansky, Th., and H.F. Hoenigsberg 1970, Unpublished records; Dobzhansky, Th., and H.F. Hoenigsberg 1971, Unpublished records; Dobzhansky, Th., and H.F. Hoenigsberg 1972, Unpublished records; Dorsey, C.K., and H.L. Carson 1956, Ann. Ent. Soc. Amer. 49:177-181; Heed, W.B., H.L. Carson, and M.S. Carson 1960, Dros. Inf. Serv. 52:85; Kaneshiro, K.Y., A.T. Ohta, and H.T. Spieth 1977, Dros. Inf. Serv. 52:85; Levitan, M., 1962, Dros. Inf. Serv. 36:130; Spencer, W.P., 1950, In: *Biology of Drosophila* (M. Demerec, ed.), pp. 535-590, John Wiley and Sons, Inc., New York.

Hoenigsberg, H.F. Instituto de Genética, Universidad de los Andes, Santafé de Bogotá, D.C., Colombia.
Virginity in the genus *Drosophila*.

There are many tricks that can be used to secure *Drosophila* virgin females. Some laboratories have preferred X or Y linked genes that kill off males as adults or that kill off sperm. Others have preferred to pick out males, as they appear in several experimental stocks, in order to have just virgin females. Still others have immobilized larvae with ether, carbon dioxide or cold in order to have time to pick out virgin females selectively during working hours. When freezing or nearly freezing temperatures are used some larvae like those of *D. paulistorum* (Sta. Marta, Colombia) may remain immobilized for just 2 hours after a 2 hour freezing, others like those of *D. melanogaster* may remain immobilized for 3 hours. After the 2 hour freezing, other larvae like those of *D. serido* may stay put as many as 10 hours and the same happens to larvae of *D. venezolana*. Other members of the repleta group recover after 5 hours at 20°C room temperature. Larvae and adults of different species renew their metabolic activity at different times. In this way virgin females can be gotten whenever working conditions permit their use. Moreover, females of *D. melanogaster* at 25°C and under banana-agar culturing conditions remain virgin for about 5-7 hours after eclosion. When *D. melanogaster* larvae and pupae have received as adults a freezing shock 20 hours before they can remain virgins for 12-14 hours. It may be interesting to remember that Lutz (1911) recorded 12 hours to remain virgin. However, females of *D. repleta* from Cuba under normal culture condition at 25°C remain virgin for 4-8 hours after eclosion. On the other hand *D. paulistorum, D. willistoni, D. equinoxialis,* and *D. tropicalis* from Colombia, Brazil, Venezuela and the Caribbean can, under normal culture conditions and at 25°C, remain virgin for only 1-3 hours. Moreover, desert living species after hurricanes in northern Columbia that go through low and humid temperatures can remain virgins for as many as 10-15 hours.

Reference: Lutz, F.E., 1911, Carnegie Inst. Washington Pub. 143.

Terry, M.G.* Committee on Biopsychology, University of Chicago, Chicago, Illinois 60637. *Present address: Department of Psychology, University of Illinois (Urbana-Champaign), 603 E. Daniel St., Champaign, IL 61820. The treadmill assay of ambulatory parameters related to gravity and other conditions in individual *Drosophila melanogaster*.

The treadmill assay of ambulatory parameters related to gravity and other conditions in individual *Drosophila melanogaster* provides precise measures of behavioral components which are correlated with any of a number of highly-controlled experimental conditions. In its first experimental application, the treadmill assay consistently reveals multiple behavioral differences between individual flies from small groups of the same sex and same line (wild-type, Oregon-R sublines). In this respect, the treadmill assay compares favorably with the traditional behavior-genetic assays of the tendency of flies to move upward or downward (Carpenter, 1905; Hirsch and Tryon, 1956; Hirsch and Boudreau, 1958; Hirsch, 1959), indicating its potential utility for behavior-genetic research.

Before the assays start, each of three sublines of Oregon-R flies is cultured in a standard vial provisioned with standard medium (Instant *Drosophila* Medium with yeast). Systematic phase differences in circadian rhythms are minimized by culturing flies under constant illumination (20W, white fluorescent tube approximately 75 cm away), temperature (22-24°C), and humidity (40-50%). Adult experience with walking toward or away from earth or light is minimized by letting each adult eclose (after being transferred from a standard vial as a pupa) and mature in a narrow culture tube (50 mm long, 5 mm ID) that is stored on a horizontal shelf and illuminated from the side. The culture tubes are checked for newly-eclosed adults every 12 hours and flies are assayed the day after hatching, so age differences are reduced to less than 24 hours. Each adult is sexed under a hand lens as it walks back and forth in its culture tube, and each fly is shaken from its culture tube into the assay apparatus at its appointed time so that flies are not exposed to anesthetics at any point in their development. The physiological effects of food deprivation, dehydration, and infradian changes are minimized by handling each fly for less than half an hour before and during the assay. Except for one accidentally-crushed fly, all flies survived the assay in vigorous condition and no behavioral criteria are used to exclude flies from the population sample.

Table 1. Experimental conditions, coding variables, and derivations of coding for the regression model. Note that the longitudinal vector (LV) does not appear explicitly in the regression model because it does not vary across trials and that the related ambulatory parameter (LVA) is estimated as the constant term (Y - Intercept). (see text for description of conditions and their related ambulatory parameters).

conditions	coding	derivations
longitudinal vector	LV = + 1 on every trial	LV designates the fly's heading at trial onset
longitudinal scalar	LS = +1 if nA ≥ 0; LS = -1 if nA < 0	LS designates the fly's heading during nA
gravitational vector	GV = sin (s)	sin (s) = the proportion of headward walking that is toward (+) or
gravitational scalar	GS = GV x LS	away from (-) earth at the slope (s) presented on that trial
progressional vector	PV = BK (trial block number)	blocks are numbered from - 7 to + 7 so that their mean across the
progressional scalar	PS = PV x LS	session is zero; thus, BK is orthogonal to the other conditions
correctional vector	CV = LT (lapse time)	LT = the number of 1 - second intervals in which episodes of falling
correctional scalar	CS = CV x LS	(≥ 5 mm) occur during the 5 - second trial

In the assay, flies are tested singly in a loop of flexible tubing. The assay loop is constructed from a 36 cm length of clear tubing (Tygon R3603, 1.6 mm walls, 3.2 mm bore). The bore is rifled with the impression left by a threaded rod under steam heat and gentle compression, then the ends of the rifled tubing are spliced with solvent (cyclohexanone) to form a continuous loop. (Informal observations with test flies indicated that such rifling dramatically reduced falling at steeper slopes.) A few minutes before its assay, the fly is transferred into the loop through a short, longitudinal slit. The loop is then stretched between two pulley wheels (5 cm-diameter and 12 cm apart, center-to-center) so that the slit closes firmly. (After the assay, this process is reversed.) As the fly walks back and forth (occasionally turning around) in the assay loop, the pulleys are turned intermittently (with the right hand on the right pulley) so that the section of the loop that is holding the fly returns repeatedly to the center of one horizontal straightaway (see Figure 1). The pulleys are mounted on a swivel in the vertical plane so that a (1-second, smooth) rotation of the swivel pointer (held with the left thumb) to a specified slope indicator light (felt by the left middle finger) leads the fly to be headed up (-) or down (+) a specified slope (+0°, -0°, +30°, -30°, +60°, -60°, +90°, or -90°) at the beginning of each trial. (This procedure effectively varies the *proportion* of the fly's movement that is collinear with the vector of gravitational acceleration.)

Because the center of the treadmill is on the central axis of the swivel and the fly is centered in the treadmill when the swivel is rotated, net displacement of the fly's body during stimulus presentation is minimal. In order to allow any "agitation" effect to diminish, behavioral observation does not begin until two seconds after slope presentation. The pulleys are turned intermittently during this delay so that the fly is centered at the beginning of the behavioral

observation period. The treadmill remains stationary for five seconds while the fly's behavior is observed. At the end of the behavioral observation period, the pulleys are turned for another two seconds so that the fly is centered for the next trial.

Within each of the fifteen trial blocks in the 120-trial (20-minute) assay, the eight slopes (+0°, -0°, +30°, -30°, +60°, -60°, +90°, and -90°) are presented in a randomized sequence. Successive five-second behavioral observation periods are separated by five-second intervals in which the fly is centered, the next slope is presented, and the fly is centered again. The presentation of trials in randomized sequences on a fixed schedule with substantial inter-trial intervals is modeled on the method of constant stimuli, a parametric psychophysical technique which controls for systematic influences of previous events on current behavior.

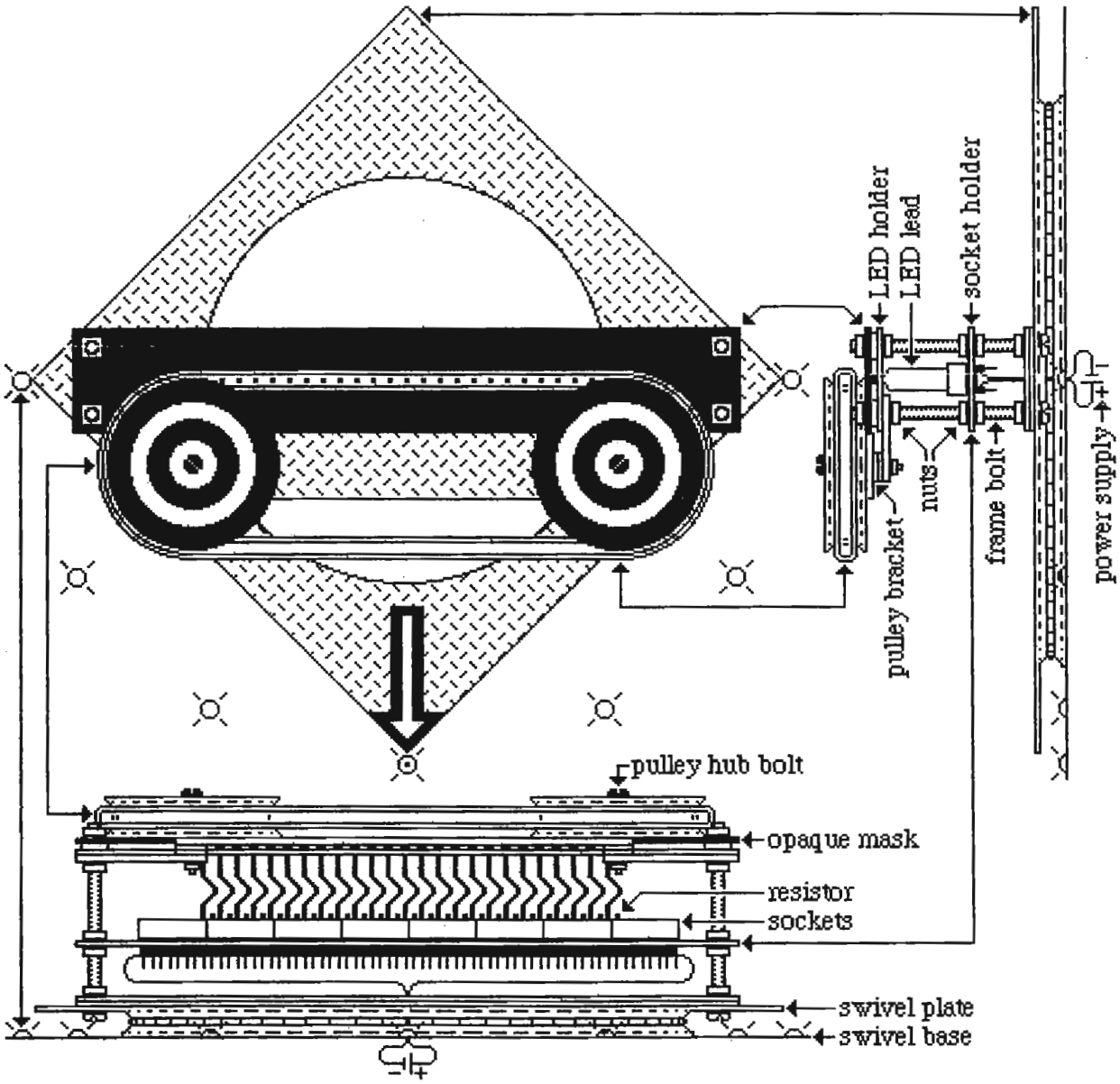

Figure 1. Orthographic projections of the treadmill assay apparatus. (double-headed arrows link different views of the same component, 1:2 scale).

Microminiature red LEDs are mounted on 5 mm centers in a row behind the treadmill to provide illumination and a measurement scale for the observer. (An opaque mask prevents the slope indicator lights from shining on the treadmill.) *Red* illumination was chosen so that any photic effects would be minimized (Gross, 1913; McEwen, 1918;

Lutz and Richtmyer, 1922; Brown and Hall, 1936; Fingerman, 1952). By sighting through the clear treadmill and past the fly to the behavior-observation lights, walking and falling is measured in 5 mm units with submillimeter accuracy. During each trial's 5-second behavioral observation period, every 5 mm increment of "headward ambulation" (*i.e.*, walking in the same direction as the fly's heading at trial onset) or "tailward ambulation" (*i.e.*, walking in the opposite direction to the fly's heading at trial onset) and every episode of falling (more than 5 mm) is narrated onto an audio tape. This "session" tape simultaneously records a prerecorded "master" audio tape, which dictates the exact timing and sequence of slope presentations, centering procedures, and behavioral observation periods. When the session tape is transcribed, net ambulation is derived from the excess of headward or tailward ambulation during each trial (nA = H - T). "Lapse time" is derived from the number of 1-second intervals in which falling occurred during each trial so that a correction can be applied for the time that is not available for walking. The narrowness of the treadmill tube prevents jumping and flying. Thus, the distortion of ambulation measurements by other forms of movement is minimized.

Each fly's ambulatory parameters are estimated by regressing its net ambulation (nA) on the values of the experimental conditions (see Table 1) across trials, according to the model:

nA = LVA + LSA(LS) + GVA(GV) + GSA(GS) + PVA(PV) + PSA(PS) + CVA(CV) + CSA(CS) + epsilon.

In other words, the regression model is used to evaluate how each fly "apportions" its walking back and forth in the treadmill according to the effects of each condition. (The dependent variable [nA] is a rate measure and the independent variables [vectors and scalars] are proportions of distance or of time, so the ambulatory parameter estimates [constant term and regression coefficients] are rate measures as well. Because the ambulatory parameter estimates are in physical units on a ratio scale, they can be compared directly to each other and to any other measures.) By simultaneously evaluating the variance in net ambulation that is related to each of these conditions, the regression analysis minimizes the influence of each ambulatory parameter on the measurement of the others. Extensive and powerful tests of the statistical assumptions underlying the regression analysis indicate that the analytic procedures are statistically appropriate and that the estimated parameters effectively account for the influences of experimental variables on each fly's ambulation.

For lack of a more appropriate terminology, each ambulatory parameter is named after its related experimental condition (see Table 1). The tendency to continue walking in the same direction as the fly was headed at trial onset was called "longitudinal vector ambulation" (LVA), the tendency to walk more or less (regardless of direction) in the treadmill was called "longitudinal scalar ambulation" (LSA), the tendency to walk more upward or downward as the slope of the treadmill increased was called "gravitational vector ambulation" (GVA), and the tendency to walk more or less (irrespective of direction) as the slope increased was called "gravitational scalar ambulation" (GSA). (The other four APs are regarded, in this context, simply as covariates whose inclusion in the regression model improves the estimation of the four APs of interest.)

The treadmill assay reveals statistically significant differences between at least two of the four APs of interest (LVA, LSA, GVA, and GSA) in every possible pair of same-sex/same-line flies from all six of the tested groups (alpha = .05, Dunn Multiple Comparisons t-Statistic for the 24 pairwise differences between flies in each group). The consistent ability of the assay to reveal multiple behavioral differences between flies within small, homogeneous groups raised and tested under highly-controlled conditions (*i.e.*, while controlling for extraneous sources of behavioral variance) indicates that the treadmill assay may be useful as a behavior-genetic research tool.

Acknowledgments: This note includes material adapted from my Master's Thesis, written under the advisorship of Dr. Paul Andronis (now at Northern Michigan University) and submitted to the Committee on Biopsychology in partial fulfillment of the requirements for the Master's Degree. The Committee and Dr. S.P. Grossman (University of Chicago) provided space and materials. Dr. William Goldstein (University of Chicago) provided valuable statistical consultations during the early development of the regression analysis. Discussions with Dr. Harry Jerison (University of California, Los Angeles) led to improvements in the regression analysis. Dr. Jerry Hirsch (University of Illinois, Urbana-Champaign) encouraged me to return to my data set in order to investigate individual differences more intensively and encouraged me to share my findings as soon as possible. Elizabeth Jerison Terry provided continual support and repeated editorial assistance.

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Yoshihara, M.,* and K. Mogami. Department of Physics, Faculty of Science, University of Tokyo, Hongo, Bunkyo, Tokyo 113 Japan. *Present address; Behavior Research Institute, Gunma University School of Medicine, Showa-machi, Maebashi 371, Japan. A simple and efficient method to screen flight defective mutants.

Flight is a highly coordinated behavior, which is presumed to involve a complex neural circuitry. Thus, mutants with defects in flight behavior give the opportunity to analyze functions of the neuromuscular system and their development at the molecular level.

We have developed a simple and efficient method that we call the "dish method" to isolate flight defective mutants of *Drosophila*. With this method we can sort out not only flightless mutants, but also poorly flying mutants. As shown in Figure 1, a large petri dish (diameter 23.5 cm, height 6.5 cm, weight 785 g) is placed on a cushion, and flies in a vial are released into the air within the dish just below the lip of the dish. The flies which fall to the bottom of the dish are stimulated by tapping the dish approximately once per second for 60 seconds. Most of the vigorous flies will fly away. The wall of the dish is coated with silicon oil to prevent flies from climbing out because it is slippery. After the agitation, only flies which are unable to fly over the wall remain in the dish. The screening procedure is done in a large plastic box to prevent the release of flies into the environment. The box has a 15W fluorescent lamp on the top at a height of 60 cm. After the screening, escaped flies can be killed by heating the box at 50°C for 3 hours or more, or by anesthetizing the escaped flies with CO_2.

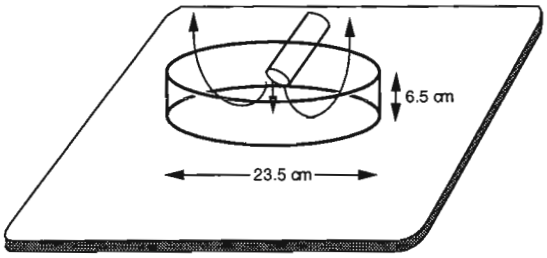


Figure 1. Flight screening apparatus.

Table 1.

	EMS treated	without EMS
Screened F1 males	2592	1333
F1 males left in the dish	173	3
Established strains	22	0

An example of the results is shown in Table 1. Canton S males were mutagenized by feeding EMS (25 mM in 5% sucrose solution), and crossed to *C(1)DX* females. The F_1 males were collected just after eclosion and kept in new vials with food (the number of flies in each vial should be smaller than 50). Two days later, the males were screened by the "dish method" at 25°C. The flies which remained in the dish were sucked out and mated singly to *C(1)DX* females, and each clone was individually screened by the dish method. As shown in Table 1, three flies (0.2%) among the progeny of untreated males failed to leave the dish. Their offspring flew normally, showing those to be false positives. From the group treated with EMS (2592 individuals), 22 strains (0.8%) were established. Eighteen of the strains were X-linked and 4 of those were found to be autosomal dominant. Of those 22 strains, only one strain was completely flightless, and others were partially flight defective.

This method is also useful for screening for completely flightless mutants. For this purpose, we released flies in the air at the level approximately 30 cm above the dish. By using this procedure many mutants with defects in the 88F actin gene had already been collected (An and Mogami, in preparation).

This method is simpler and more efficient than the ones devised previously (Benzer, 1973; Sheppard, 1974). Our method has three advantages, (1) less time-consuming, (2) fewer false positives, and (3) a larger variety of obtained phenotypes. The flight tester designed by Benzer (1973) is suitable for quantification of the flight behavior, but recoating of the cylinder with paraffin oil is time-consuming. Moreover, we obtained kinds of mutants that cannot be distinguished from wild type by the flight tester method (Yoshihara, unpublished results). Sheppard (1974) also designed a method similar to ours to isolate flightless mutants. Anesthetized flies were placed in a cylinder, and they were stimulated to fly by shaking. However, a lot of nonspecific flies were picked up in the screening. The yield of real flight mutants among selected F_1 progeny was less than 1%, whereas in our method it was more than 10%. The repetitive agitation induces the flies to fly away and reduces the number of flies that stay behind for unspecified reasons.

Acknowledgments: This work has been carried out in Professor Yoshiki Hotta's laboratory in the University of Tokyo. We are very grateful for his generous support for us. We thank Kazuo Ikeda, Yoshiaki Kidokoro, Jane Koenig, and Rodney Williamson for critical reading.

References: Benzer, S., 1973, Scientific American 229: 24-37; Sheppard, D.E., 1974, Dros. Inf. Serv. 51:150.

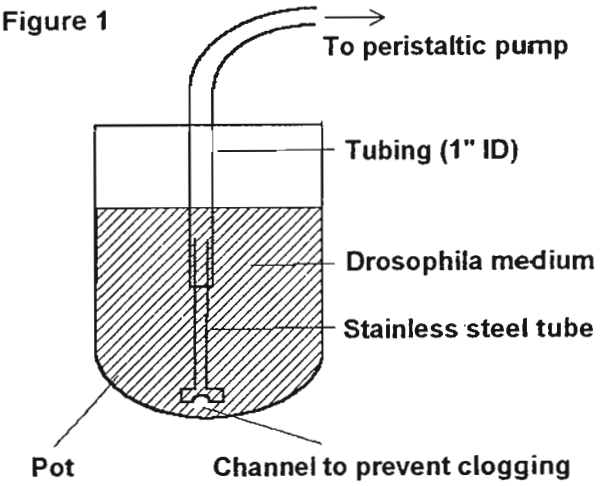
Friedlander, T.J., and W. Meltzer. 205 Pumpkin Hill Road, New Milford, CT 06776. Rockefeller University and Howard Hughes Medical Institute describes a system for automatically filling large numbers of culture vials and bottles with *Drosophila* medium.

This system requires very little user involvement for filling a large number of vials and bottles with *Drosophila* medium. Typically this type of system is used by labs which share a kitchen for preparing *Drosophila* medium.

After the *Drosophila* medium has been prepared (cooked) a weighted stainless steel tube is placed in the pot so that it is resting on the bottom (Figure 1). The weighted tube is connected to 1" ID clear tubing which runs through a computer controlled peristaltic pump (Cyberlab P-300A) to a computer controlled X-Y dispenser (Cyberlab C-100, Figure 2). The medium does **not** come in contact with any part of the pump, only the tubing, so there is **no contamination** from the pump parts. Cleanup is easily accomplished by running hot water through the tubing.

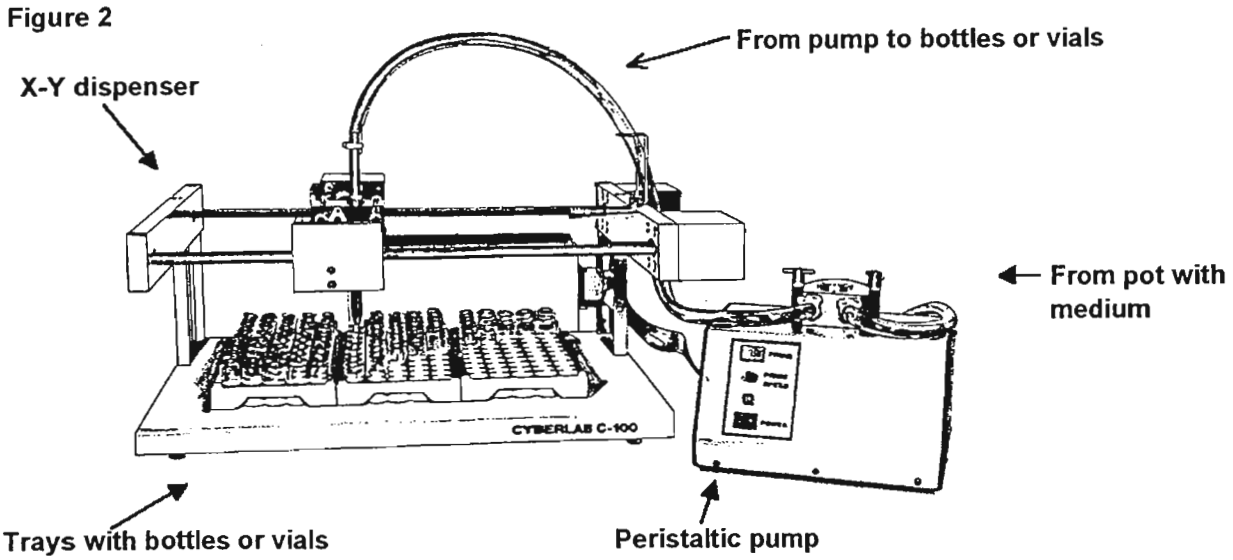
The automated X-Y dispenser moves its 5 probe manifold from left to right, pausing over the vials and bottles each time the pump is activated (all this is controlled by the computer). The pump fills the containers with an amount specified by the user.

Figure 1



The automated X-Y dispenser is capable of filling 2 trays of vials or bottles at a time. Filling takes approximately 90 seconds per tray. Since there is no reason for removing the vials and bottles from their trays they are ready to be capped or plugged right after filling. The automated X-Y dispensers' 5 probe manifold allows the filling of 5 vials or bottles simultaneously with no user intervention. The user merely places trays onto the automated X-Y dispenser's base and presses the space bar on the computer keyboard to start another cycle of filling. Depending on the consistency of the medium little or no dripping is apparent. This is due to an anti-drip feature in the peristaltic pump which causes the pump to instantly reverse direction at the end of each pumping cycle effectively "sucking back" a small amount of medium thereby virtually eliminating dripping.

Figure 2



The automated X-Y dispenser is already programmed with the most common trays and plates used in *Drosophila* medium. The user can vary the amount of medium to dispensed and the speed the medium is dispensed. In addition, the user can define a new type of trays.

The peristaltic pump is designed for highly viscous fluids and can handle any consistency medium currently being prepared.

Benefits of the system include: **fully automated; rapid filling of vials, bottles or plates (up to 3600 per hour); no dripping down the sides of the vials or bottles; fast cleanup; no contamination from pumps parts; no carpel tunnel syndrome caused by manual filling; the ability to pump medium of any viscosity; consistent volumes dispensed.**

This system was made possible by Cyberlab, Inc. which is located in New Milford, CT. The equipment is currently in use in several large *Drosophila* medium kitchens throughout the country. Cyberlab can be reached at 203-355-3171. We would like to thank Cliff Sonnenbret and Charles McCall from Howard Hughes Medical Institute for their input and support in developing this system.

Kambysellis, Michael P., and Fabio Piano. Department of Biology, New York University, New York, NY 10003.
A method for isolating eggs from museum and field collected specimens for chorionic ultrastructural analysis.

The morphology of the eggshell (chorion) in insects is a valuable character for species identification, as initially shown with sibling malaria mosquito species *Anopheles maculipennis* (Fallerion, cited by Mayr, 1963). The morphological diversity in the ultrastructure of the chorion, as revealed by scanning electron microscopy (SEM), is phenomenal not only among orders of insects (Mazzini, *et al.*, 1993) but also among members of a single genus, as exemplified by the genus *Drosophila* (Margaritis, *et al.*, 1983). However, nothing parallels the ultrastructural diversity found among the *Drosophila* species endemic to the Hawaiian Islands (Kambysellis, 1993), members of the subgenus *Drosophila*. These genetically closely related species (Carson and Yoon, 1982) exhibit extraordinary ecological diversification in their choice of substrates for egg oviposition (Heed, 1968; Montgomery, 1975) and the chorionic structures of their eggs have likewise diversified to match these ecological shifts (Kambysellis, 1993).

Routinely, *Drosophila* eggs used for SEM analysis are either oviposited or dissected from the ovaries of mature females (Kambysellis, 1974). Here we report a new method by which specimens from museum collections can be used to isolate and prepare the dry eggs for ultrastructural analyses. In order to demonstrate that chorionic structures are preserved after prolonged dry conditions, we present data from two Hawaiian *Drosophila* species: *D. silvarentis*, which has delicate lacy structures on the borders of the follicle imprints (Kambysellis, 1974), and *D. mimica* with elegant plaque-like structures in the respiratory filaments (Kambysellis, 1993).

A mature female *D. silvarentis* (K47B1) collected from Hawaii (Humuula) on January 13, 1967, by Dr. Ken Kaneshiro and a *D. mimica* female collected from Hawaii (Bird Park) on May 10, 1972, by Dr. M. Delfinado were chosen from the collections held at the University of Hawaii. First, the samples were inspected under a dissecting microscope to determine whether their ovaries were mature. This is not so difficult, although some practice is needed. If the abdomen is collapsed, then the ovary is not developed. We chose specimens with swollen abdomens in which the eggs are fairly visible through the abdominal cuticle. Once the specimen is selected, the abdomen is pinched off, making every effort not to damage the rest of the specimen (head, legs, wings, etc.), which is then returned to the collection.

The isolated abdomen is placed in a small Petri dish with insect Ringer's for about one hour for rehydration. Once softened, the cuticle, gut and other tissue can be removed with fine #5 forceps. The ovipositor, an important taxonomic structure, and one of the ovaries are placed in a microfuge tube and stored at -20° to be used later on. The other ovary is transferred to another Petri dish with Ringer's and the ovarioles are separated. The mature eggs, usually the most posterior eggs in each ovariole, are removed from the ovarioles. This is a difficult point in the procedure because the respiratory filaments can easily break and the membranes surrounding the egg are very difficult to remove. Actually, the membranes are left attached at this point. Then the isolated eggs are transferred to a new Petri dish with Ringer's and sonicated for one or two minutes (Margaritis, *et al.*, 1980) until all the membranes are removed. This is the most important part of the technique. If the sonication is not long enough, the chorion remains covered and the structures cannot be visualized under SEM. In short, you have lost the information from the specimen. (This is why it is advisable to save the other ovary in the freezer.)

We usually sonicate the eggs for 30-second intervals and track the cleaning process under the highest power of the dissecting microscope, continuing sonication until all the membranes are removed. Sonication can be considered complete when some of the eggs are ruptured, such that their yolk is removed and empty eggshells remain. The rest of the procedure is routine. The sonicated eggs are transferred to a microfuge tube with Karnofsky's fixative and fixed

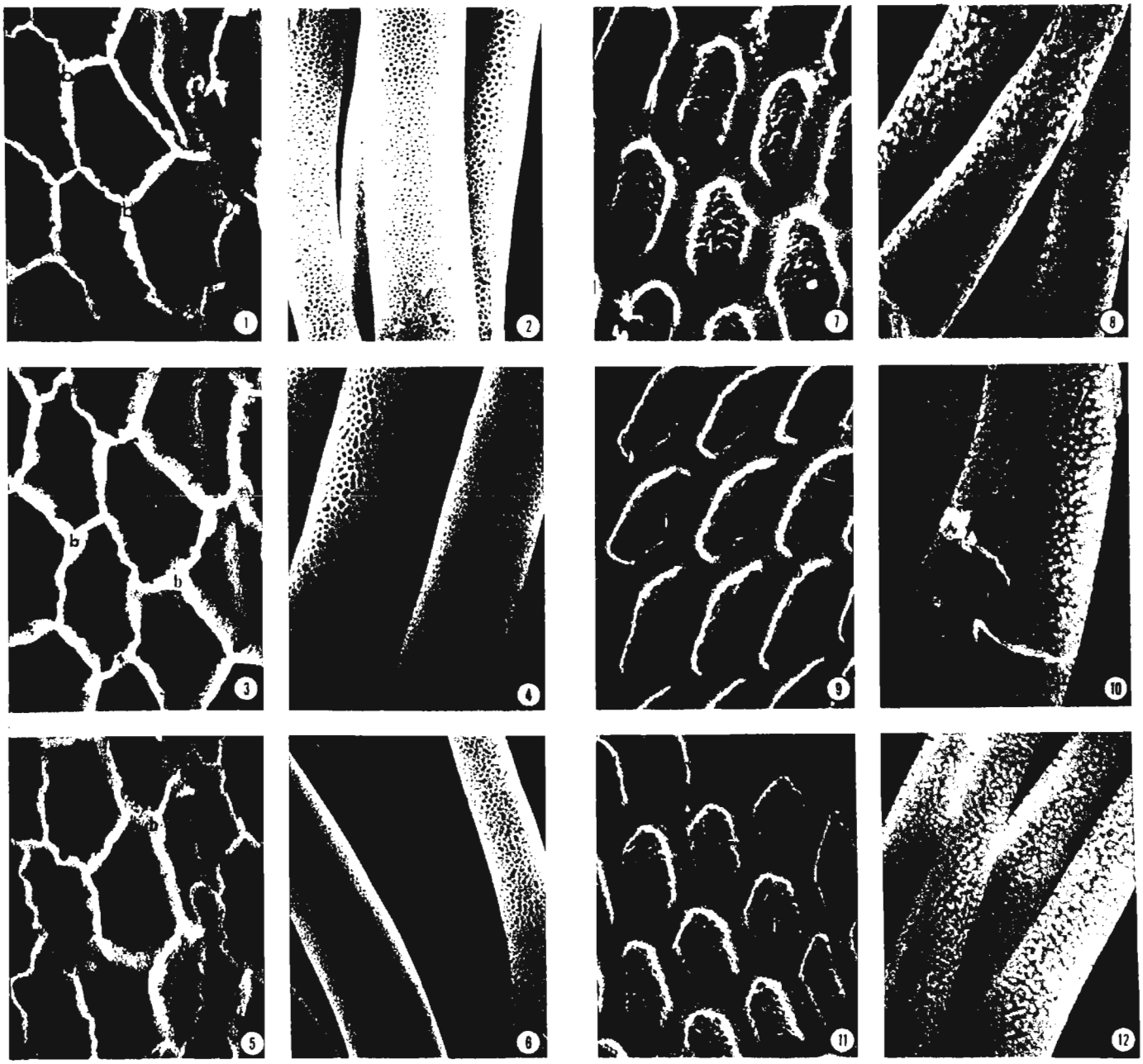


Figure Legend. Follicle imprints and respiratory filaments from eggs of *D. silvarentis* (Figures 1 - 6) and *D. mimica* (Figures 7 - 12) obtained from different sources.

D. silvarentis: Figures 1 and 2 represent eggs obtained from a museum specimen (K47B1) collected 1/13/67 by Dr. Ken Kaneshiro. Figures 3 and 4 were obtained from eggs of a female killed and kept in a closed tube for 30 days before egg processing, and Figures 5 and 6 were from freshly oviposited eggs.

D. mimica: Figures 7 and 8 show eggs from a museum specimen collected 5/10/72 by Dr. M. Delfinado. Figures 9 and 10 are from a female killed and kept in a vial for 30 days, and Figures 11 and 12 are from oviposited eggs.

The chorionic structures are very different between the two species. However, we do not find differences among the various preparations of eggs from the same species. Notice in particular the borders of the follicle imprints (b) and the porosity of the respiratory filaments (Figures 2, 4, 6, 8, 10, 12).

overnight at room temperature or for longer periods of time at 4°C.

The eggs are then dehydrated with increasing concentrations of ethanol (from 0 to 100%), critically point dried, and placed on an SEM stub. At this step, we always use a dissecting microscope to obtain the most desirable orientation of the egg. In order to observe a chorionic cross section and the floor of the chorion, we rupture one or two eggs with forceps and roll the egg over on the double-sided tape of the SEM stub. The eggs are then coated with carbon and an alloy of gold/palladium (60/40) and viewed under the SEM.

We have had good results in analyzing the chorion from rare Hawaiian *Drosophila* species as shown in Figures 1-12. The chorionic structures from specimens collected more than 25 years ago are well-preserved. The eggs from museum specimens (Figures 1, 2, 7, 8) are indistinguishable from specimens of freshly oviposited eggs (Figures 5, 6, 11, 12). In addition, we show that eggs from dead flies which had been stored in closed tubes for a month, by which time to soft tissues of the fly had decomposed, remained intact and the chorionic structures perfectly preserved (Figures 3, 4, 9, 10). This observation is useful for long term field collections. Instead of putting the flies in ethanol (at which point preparations of eggs for SEM is difficult if not impossible), we either put one or two samples in a microfuge tube for SEM analysis when we return to the lab, or if the specimen is rare, we will remove the abdomen and store it in a microfuge tube for chorionic analysis, placing the rest of the specimen in ethanol for the collection.

Although we have developed this technique for Hawaiian *Drosophila* species, we believe it can be adapted to be used for other *Drosophila* and other orders of insects as well.

Acknowledgments: Research supported by NSF grant BSR 89-18650 and NYU Research Challenge Fund Award.

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[1]Institute of Cytology and Genetics pr. akad. Lavrentyeva 10, 630090 Novosibirsk, Russia; [2]Institute of Biomedical Advanced Technology, C.N.R., via Ampere 56, 20131 Milan, Italy. A computing system for protein-coding regions prediction in Diptera nucleotide sequences.

Prediction of protein-coding genes in newly sequenced DNA becomes very important in large genome sequencing projects. This problem is very difficult due to exons/introns of the eukaryotic genes. Usually exons correspond to the coding fragments of genes, introns are non-coding regions which are spliced out at acceptor and donor splice sites during mRNA maturation.

To predict coding regions and splice sites a number of approaches were developed. The coding regions prediction methods miss most short exons and can not reliably define the exon/intron boundaries, while the splice site prediction discovers some of real splice sites with a great number of false sites. For these reasons, the most effective methods are based on the combined approach suggested by Shapiro and Senapathy (1987), Nakata, *et al.* (1985), Kolaskar and Reddy (1985), and Staden (1984). These techniques use the information about potential splice sites in combination with the coding potential. Based on this approach several methods were created for the coding regions prediction in mammalian sequences (Gelfand, 1990; Mural, *et al.*, 1992; Guigo, *et al.*, 1992; Hutchinson and Hayden, 1992; Solovyev and Lawrence, 1992; Milanesi, *et al.*, 1993; Snyder and Stormo, 1993). For *C. elegans* sequences the GM program was developed (Fields and Soderlund, 1990). However, the methods for exon/intron structure prediction for Diptera sequences are still to be developed.

We propose the first version of the system for exon/intron structure prediction specially adopted for Diptera sequences. The same approach for gene prediction had been used for human genome analysis (Milanesi, *et al.*, 1993). For the learning set we have selected Diptera protein-coding sequences from the EMBL Data Library (r38). The following procedures are involved in the algorithm for protein coding gene prediction: A. identification of the potential splice sites; B. construction of a Potential Coding Fragment (PCF); C. estimation of the potential of the revealed PCF; D. construction of potential genes having the maximal coding potential (program ORFJ); E. best PCFs selection (program BEX).

Now we consider the listed steps in more detail:

A. Identification of the potential splice sites. For the splice sites prediction we have used the set of consensuses,

Table 1. Consensus sequences of donor splice sites.

N	Number of sites corresponding to a certain consensus	Consensus
1	504	nnn/GURAGn
2	292	nnG/GUnnGU
3	289	nAG/GUnnnn
4	248	nMn/GUAnGn
5	213	nnG/GUAAnn

obtained by classification analysis (Milanesi, *et al.*, 1993). The Diptera donor splice sites were described by the 5 relatively similar consensuses (Table 1) and the sample of acceptor sites by those 20. The results of classification were tested on independent control samples which involve 84 Diptera acceptor sites and donor sites. The 5%-10% error of first kind (false negative) in prediction of splice sites was found. A sequence region matching any consensus from the set is considered as potential splice site. A second step performs a verification of the potential splice sites using an alternative technique based on weight matrix (Shapiro and Senapathy, 1987).

B. Construction of a Potential Coding Fragments (PCF). From the set of potential splice sites of the sequence under investigation, stop codons, and potential AUG codons, all possible PCF are constructed.

Table 2. Examples of exon/intron structure prediction in control genes

Entry in EMBL Data Library	Total length	Real exons from	Real exons to	PCFs predicted by ORFJ program from	PCFs predicted by ORFJ program to	PCFs predicted by BEX program from	PCFs predicted by BEX program to
DM04239	4395	?		196	306	?	
		703	870	829	870	?	
		933	994	871	994	?	
		1139	1483	?		1139	1483
		1538	2358	1538	2358	1710	2358
		2410	2671	2443	2671	2410	2671
		2725	3293	?		?	
		3350	4293	3426	4293	3350	3883
DMDSOR1	2777	412	521	?		?	
		1166	1796	1227	1796	1257	1409
		1991	2317	1991	2317	1991	2317
		2389	2499	2389	2499	?	

C. Estimation of the protein coding potential. For the estimations of the protein coding potential of revealed PCF we use a dicodon statistic (Claverie, *et al.*, 1990). Coding potential Pi of the i-th PCF is calculated as a sum of dicodon weights, characteristic of the given PCF. The PCFs with a score of coding potential Pi over 0 are selected for further analysis as potential fragments of gene coding regions.

D. Gene construction (program ORFJ). The set of revealed PCF is used for construction of potential genes with a maximal coding potential. For two PCF to be presented in one gene and separated by an intron, they should satisfy the two following rules of compatibility:

(i) Minimal intron length between two PCFs cannot be less than 50 bp.

(ii) Let A and B be two sequential PCFs (PCF A being positioned upstream relative to B). After eliminating the separating intron, these two PCFs can form a longer fragment only on condition that they retain the same translation frame.

The coding potential Pg of gene G, consisting from m PCF separated by m-1 introns, is calculated as a sum of all PCF coding potentials. The problem of searching for a set of potential genes with maximal coding potential is solved using dynamic programming techniques.

The algorithm was tested on a set of control data: DAADH2A (909 bp), DM04239 (4395 bp), DM790 (1342 bp), DMACT701 (2172 bp), DMARRA (2411 bp), DMBSHHB (2672 bp), DMDBP45A (1997 bp), DMDSOR1 (2777 bp), DMGLTFAC (4605 bp), DMNANOS (3422 bp), DMVERM (3043 bp). This set of sequences contains 45 exons with total length 15606 bp. The Table 2 shows the examples of gene structure prediction for DM04239 and DMDSOR1 sequences. In general, the comparison between real data and the computational results shows that 27% of real exons were not predicted and 13% non-real exons were predicted. For the correctly predicted exons 7% of total exon length was not found and 3% was overpredicted. The sequence DFRGSPACE (5134 bp) known to have no protein-coding genes (this sequence is coding rRNA genes) was also analyzed. No potential exons were found for direct and complementary strand.

E. Significant PCFs selection (program BEX). We select the most significant PCF among all generated at step C. Two levels of significance for best PCFs are used:

(i) Excellent PCF. The comparison between real data and the computational results for this level showed that 50% of true exons were lost (underprediction) and 2% false exons were found (overprediction). For correctly predicted exons 25% of the total length was lost and about 1% was overpredicted. The Table 2 shows the examples of excellent PCFs prediction for DM04239 and DMDSOR1 sequences.

(ii) Good PCF. The probability to lose real exons is nearly 35%, but the probability that the chosen exon is not real is about 5%. If no significant exons are found, the ORFJ program results are not statistically confirmed and can be considered as overprediction.

In comparison with the mode of best exons prediction the overprediction is greater, but the large portion of real

exons is correctly predicted.

Programs of the described system were implemented in FORTRAN 77 and C programming languages and run under UNIX (SUN SPARCstation 10).

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Thatcher, J.W., and W.J. Dickinson. University of Utah, Salt Lake City, UT. Rapid preparation of larval cuticles for mutant phenotype analysis.

The following are two quick and easy procedures ideal for use in screens of larval cuticle mutants. Cuticles are fixed and cleared in a single step, producing visibility comparable to Hoyer's medium, but with less effort and without use of choral hydrate (which, at least in the United States, requires a controlled substance license). The mounting procedure for medium 1 is somewhat simpler than for medium 2, but the latter provides permanent slides and somewhat greater transparency. The CMCP-10 used in medium 2 is a commercial mounting medium available from Polyscience, Inc.

Materials:

medium 1 - 9:1 glacial acetic acid : 85% lactic acid
medium 2 - 4:2:1 glacial acetic acid : CMCP-10 : 85% lactic acid
NaCl-Triton (0.7% NaCl, 0.2% triton X-100)
small petri dishes or 24 well trays
microscope slides and coverslips
clear fingernail polish

Methods:

1. Eggs are collected from apple juice plates (Wieschaus and Nusslein-Volhard, 1986), washed and incubated in NaCl-Triton until larvae hatch.
2. Remove as much NaCl-Triton from larvae as possible.
3. Add 1 to 1.5 ml of either medium 1 or medium 2 to larvae.
4. For larvae treated with medium 1:
   At room temperature, leave vessel containing the larvae in fixative solution uncovered overnight under a fume hood. Prop container at an angle. This will cause the larvae to concentrate in the angle between the wall and bottom of the dish, facilitating their removal when mounting. Within 24 hours, most of the acetic acid will evaporate, leaving behind a viscous lactic acid solution which serves nicely as a mounting medium and clearing agent. For superior clearing, leave larvae under the fume hood for an additional 24 hours (48 hours total).
   For larvae treated with medium 2:
   Leave vessel containing the larvae covered at room temperature for 24 hours. Then, under a fume hood, prop container at an angle as described above and remove the cover. Allow medium to sit for approximately one hour, or until it becomes highly viscous. Be careful. If the medium sits too long, it will become too viscous to mount.
5. To mount, recover larvae with a micropipettor set to the indicated volume (following table) and place on a microscope slide.

Coverslip size	Volume (ul) medium 1	Volume (ul) medium 2
18 mm x 18 mm	30-35	45-50
22 mm x 22 mm	40-45	60-65
22 mm x 40 mm	75-80	100-110

6. Carefully place coverslip over the medium.
7. (For larvae mounted in medium 2 only.) Allow to harden overnight.
8. (Optional for larvae mounted in medium 1.) Seal coverslip by applying a thin layer of clear fingernail polish along each side of the coverslip.

Reference: Wieschaus, E., and C. Nusslein-Volhard 1986, In: *Drosophila: A Practical Approach* (Roberts, D.B., ed.), pp. 199-227, IRI Press, Oxford.

Thatcher, J.W., and W.J. Dickinson. University of Utah, Salt Lake City, UT. Rhodamine phalloidin staining without hand peeling.

Phalloidin staining provides a convenient method for visualizing actin cytoskeleton during embryogenesis. The standard procedures (Wieschaus and Nusslein-Volhard, 1986; Ashburner, 1989) recommend that the vitelline membrane be removed by hand because binding of phalloidin to f-actin is blocked by the methanol used to remove vitelline membranes in connection with other staining methods (Mitchison and Sedat, 1983; Tautz and Pfeifle, 1989). As hand peeling requires much time and effort to prepare the large samples needed to study details at all embryonic stages, we sought an alternate method of vitelline membrane removal. We found that 95% ethanol can be substituted for methanol and that the resulting phalloidin stain is not significantly different in pattern or intensity from that obtained with hand-peeled embryos.

Our procedure follows Wieschaus and Nusslein- Volhard (1986) except as noted:

1) Dechorionate and fix embryos as described (either contained in a basket or free in the two phase fixation system).
2) Embryos in a basket: Transfer embryos into a 50 ml screw cap centrifuge tube and remove as much fluid as possible. Add 10 ml each of 95% ethanol and heptane to the tube.
   Embryos free: Withdraw as much of the aqueous (lower) layer as possible without losing embryos at the interface. Add a volume of 95% ethanol equal to the remaining heptane.
3) Shake vigorously for 30 to 60 seconds. This treatment will rupture and subsequently remove the vitelline membrane from most embryos. Those embryos which have been devitellinized will drop to the bottom of the ethanol layer, while those which have retained the vitelline membrane will remain at the interface between the ethanol and heptane layers.
4) Transfer the embryos in ethanol to a different container, allow to settle, remove the ethanol and rinse in PBS with 0.1% triton X-100 (PBS-TX).
5) Refix the embryos in 4% formaldehyde in PBS-TX for 20 to 60 minutes.
6) Remove fixative and rinse embryos with PBS-TX for at least 10 minutes.
7) Stain embryos with phalloidin as described.

References: Ashburner, M. 1989, *Drosophila: A Laboratory Manual*, pp. 204-204, 211, Cold Spring Harbor Laboratory Press; Mitchison, T.J., and J. Sedat 1983, Dev. Biol. 99:261-264; Tautz, D. and C. Pfeifle 1989, Chromosoma 98:81-85; Wieschaus, E., and C. Nusslein-Volhard 1986, In: *Drosophila: A Practical Approach*, (Roberts, D.B., ed.), pp. 199-227, IRI Press, Oxford.

Dickinson, W.J, K. Rodriquez and J.W. Thatcher. University of Utah, Department of Biology, University of Utah, Salt Lake City, UT 84112. Replica plating *Drosophila* lines and parallel processing of larval cuticles from multiple lines.

Some genetic screens require "destructive analysis" to score the phenotypes of interest (*e.g.*, biochemical traits or anatomical features only visible in dissected, sectioned or cleared material). In such cases, mutagenized chromosomes must be established in separately maintained lines so that mutations can be recovered, for example, in sibs or descendants of the individuals actually examined. The methods described here facilitate these procedures by allowing sets of 24 lines to be maintained and processed in parallel. We also described the specific application of this approach to the analysis of

cuticle phenotypes in first instar larvae.

The system employs three types of modules based on standard 24 well tissue culture plates (*e.g.*, Falcon 3047). 1) Unmodified culture plates serve as "base units" holding standard fly food. 2) "Adult modules" use 15 ml tubes in matching arrays to provide living space for flies when joined to base units. 3) "Egg collection modules" also can be joined to adult modules and have mesh bottoms that allow eggs or larvae to be washed and processed through various solutions.

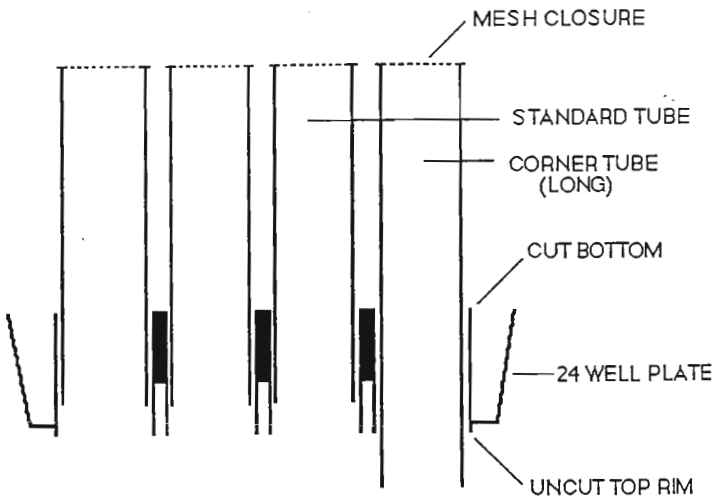

Figure 1. Section through end row of an "adult module."

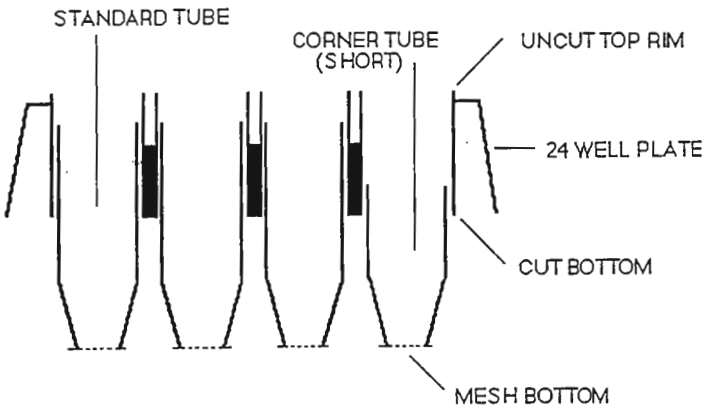

Figure 2. Section through end row of an "egg collection module."

Fabrication of Modules:

A schematic of a section through one row of an adult module is shown in Figure 1. Each unit is fabricated from standard laboratory plasticware in four steps.

1) Remove well bottoms from 24 well plates. This is conveniently done by slicing the whole plate just above the bottom with a band saw.
2) Cut 15 ml conical polypropylene centrifuge tubes (*e.g.*, Corning 25319-15 or Fisher 05-539-5) into two pieces. The upper sections are used in adult modules; the bottoms are saved for egg collection modules (below). Use the calibration marks on the tubes as cutting guides. In our hands, tubes cut at 4.5 ml mark are satisfactory. The criterion is that the cut end of the upper section can be wedged securely through the open bottom of a well (sliced as above) without protruding past the top (uncut) rim. Check this fit with a sample of your material and adjust the position of the cut as needed. Tubes then can be cut in masse with a band saw by leaving them in the foam racks in which they are supplied. (Save the bottoms in the foam.) Cut a smaller number of tubes (about 10% of the total) to yield top pieces 1-2 cm longer than the others (so that they will protrude above the top rim of a well).

3) Remove caps and close tube tops with nylon or stainless steel mesh. Pre-cut squares can be glued or heat fused into place. For the latter, use a hot plate and press the tube top down onto the mesh until it melts slightly around the mesh. If using nylon, determine in advance a temperature setting that softens the tube without destroying the mesh. Permanent mesh tops (instead of cotton plugs) allow modules to be washed intact for reuse.
4) Force tubes from the bottom securely into the openings of the 24 well plate. It may be necessary to clean cut edges with sandpaper or a utility knife to get a snug fit between tube and tray. Glue should not be necessary. Use the longer tubes on two diagonal corners and force them through to protrude about 1 cm above the top rim of the well. These ends hold the module in proper register when joined with other modules. However, if additional tubes protrude, it is difficult to fit modules together quickly.

A section through one row of an egg collection module is shown in Figure 2. They are fabricated as follows.

1) Remove the bottoms from 24 well plates as described above.
2) Cut the tips off of the 15 ml tube bottoms saved from above (about 1 cm from the bottom). Again, these can be cut *en masse* in the foam racks (cutting right through the foam).
3) Glue or fuse (as above) stainless steel or nylon mesh over the lower (tapered) ends. Choose mesh with openings small enough to retain eggs.
4) Force these tubes into the cut 24 well plate from the top. Push them down until the top edge of each tube is below the top (uncut) rim of the well. On the diagonal corners, use shorter tubes that can be forced about 1 cm below the rim (allowing the corner wells to accept the longer tubes protruding from the adult modules described above).
5) It is important to align the bottom (mesh covered) ends of the tubes. Tap or press against a flat surface to bring them into a plane.

Establishing and maintaining cultures:

In this application, adult modules are fitted to base units containing standard fly food. Dispense food into wells in advance (about 3/4 full).

1) Invert an adult module on a bench top (mesh ends down) and brush anesthetized flies into the tubes.
2) Invert a base unit (with food) over the adult module, matching the corner wells to the protruding tubes. Secure the plates together with rubber bands. Note that for modules constructed as described, the two 24 well plates are face-to-face with the uncut upper rims of matching wells in contact. In our experience, this gives a tight enough fit to prevent flies or larvae from escaping.
3) Once flies awaken, the joined units can be incubated upright or on their sides (to minimize loss of adults trapped in food).
4) To replicate the block of 24 cultures, invert the combined modules, tap gently to knock adults down into the adult module, and remove and replace the base unit.
5) The initial base unit (with eggs/larvae) can be combined with a clean adult module to retain adults that subsequently emerge.
6) We routinely place one set of replicate cultures at 15-18°C to slow development until we analyze some of the progeny from another.
7) We label an entire module with a date and number on a piece of tape. Individual lines can subsequently be identified by row and column.

Collecting and processing eggs/embryos/larvae:

In these applications, the egg collection module is substituted for the simple base unit. Covers supplied with the 24 well plates are used as trays to hold medium in contact with the mesh-covered openings. This is adequate to promote oviposition as described below. These trays also can hold various solutions through which samples are processed.

1) Fill trays with apple juice medium (Wieschaus and Nusslein-Volhard, 1986).
2) Rest egg collection modules on top of apple juice medium, mesh ends down. Add a drop of thick yeast suspension to each tube and let sit (covered with cheesecloth) until the yeast forms a firm surface (such that adults walking on it will not be trapped). The yeast is in contact with the apple juice medium through the mesh.
3) Use these combined units in place of a standard base unit to join adult modules as described above

(face-to-face). Females oviposit into yeast, and adults feed on the yeast and/or through the mesh. The medium also keeps the yeast moist during the collection period and provides stimuli for oviposition.

4) At the end of the desired collection period, remove the egg collection module and wash away the yeast by directing a stream of water in turn through each well. Eggs and larvae are retained. It may help to first soak in a tray of water to soften yeast.
5) Transfer samples through any series of solutions held in trays or base units.

Parallel processing for cuticle preparations:

1) Collect and wash eggs as described. We normally collect for 24 hours.
2) Return egg collection module to a tray or base unit with enough water to cover mesh by 1-2 mm.
3) Incubate for another 24 hours to hatch eggs. Larvae accumulate in the water.
4) Transfer egg collection module to a base unit with about 2 ml/well of the fixation/clearing/mounting medium 1 described in an accompanying note (Thatcher and Dickinson).
5) We find it most convenient to recover larvae from the mesh bottom wells at this stage. Transfer in about 1 ml of fixing/clearing/mounting medium to a clean 24 well plate.
6) Complete processing and mounting (as described in Thatcher and Dickinson).

Reference: Wieschaus, E., and C. Nusslein-Volhard 1986, In: *Drosophila: A Practical Approach*, (Roberts, D.B., ed.), pp. 199-227, IRI Press, Oxford.

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A new autonomous P element vector with a multiple cloning site for insertion of a variety of DNA sequences.

In the course of a series of experiments to examine the population dynamics of marked P elements in *Drosophila melanogaster*, we have created an autonomous P element vector containing a number of unique restriction sites. Our goal was to facilitate the cloning of genes and other DNA sequences within an autonomous P element while still maintaining the functionality of the transposase gene. This construct could be widely used to introduce a variety of genes into the genome of *D. melanogaster* by microinjection of embryos.

This note reports the construction of an autonomous P element with a multiple cloning site (MCS) located between the third open reading frame (ORF3) of the transposase gene and the 3' terminal inverted repeat. We further report the results of transformation experiments using both this vector, named pπ2A.2, and also a derivative construct in which a fragment of bacteriophage lambda DNA was inserted into the MCS.

The structure of the vector pπ2A.2 is given in Figure 1. The strategy used in its design is similar to that employed by Karess and Rubin (1984) for the construction of Pc[*ry*]B. The first 2885 bp are identical to those of the 2907 bp P element present in pπ25.1 (O'Hare and Rubin, 1983). Adjacent to the truncated 3' inverted repeat there is a MCS polylinker region derived from pBluescript II KS (Strategene, La Jolla, CA) where it does not disrupt cis or trans functions for transposition. This MCS polylinker is then followed by a 496 bp fragment containing a duplicated 3' end of the P element with a functional 3' inverted repeat. A derivative of pπ2A.2 containing lambda DNA as a molecular marker was constructed by subcloning a *Bam*HI-*Eco*RV fragment of lambda (positions 5505 to 6681). This vector is designated as pπ2A.2 lambda1.

Table 1. Results of microinjection experiments with pπ2A.2 and pπ2A.2λ1 constructs.

Construct	No. injected embryos	No. larvae surviving	No. eclosed flies	No. fertile flies G_o	No. PCR^+ flies, G_o	No. independent transformants
pπ2A.2	409	108 (26%)	46 (42.6%)	37 (80%)	5	3
pπ2A.2λ1	680	180 (26.5%)	106 (58.9%)	75 (70%)	38	17

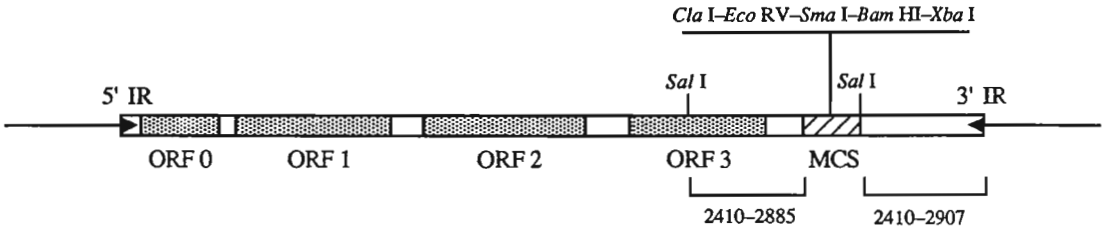

Figure 1. Structure of the P element vector pπ2A.2. The location of the transposase open reading frames (ORFs), the unique restriction sites in the multiple cloning site (MCS), the terminal inverted repeats (IRs) are shown. Numbers refer to positions in the nucleotide sequence the canonical P element from pπ25.1 (O'Hare and Rubin, 1983).

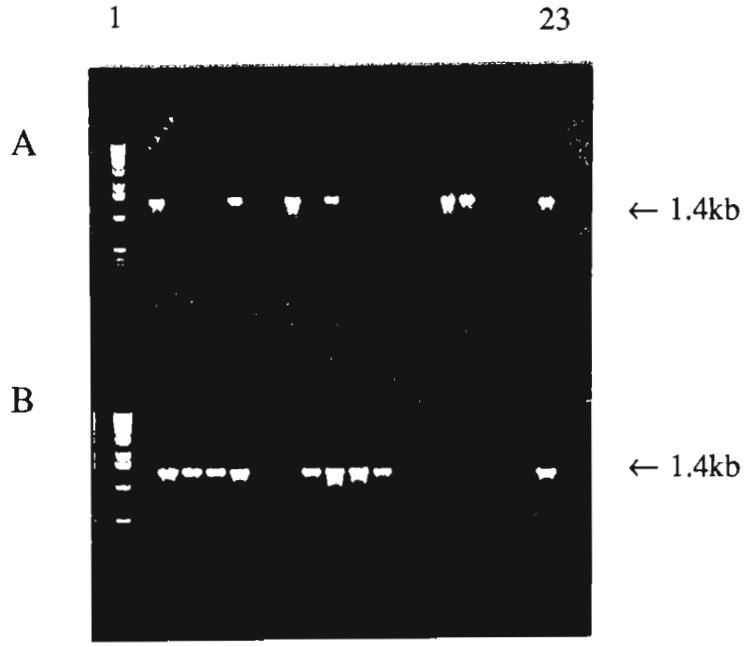

Figure 2. Results of the PCR analysis of single-fly DNA for 32 G_0 individuals microinjected with p 2A.2 lambda1, lanes 3-21 (panel A) and 3-17 (panel B). Panels A and B, lane 1, 1 kb ladder DNA size markers; lanes 22 (A) and 18 (B), PCR reaction with single-fly DNA of the original CS-brown line (negative control), respectively; lanes 23 (A) and 19 (B), PCR reaction with p 2A.2 lambda1 DNA (positive control), respectively. The size of the expected PCR product containing the lambda DNA insert (1.4 kb) is indicated.

Both constructs were transformed into Canton-S brown (M strain) embryos using standard microinjection methods (Rubin and Spradling, 1982) with some modifications as described elsewhere (Prokchorova *et al.*, 1989). A summary of transformation results for both constructs are presented in Table 1.

Putative transformants were screened in the absence of any visible phenotypic or selectable markers using the single fly PCR method described by Engels *et al.* (1990) and Gloor and Engels (1992). Individual micro- injected flies were tested for the presence of P element constructs using a P element primer (5' - CCCCACGGACATGCTAAGGG, beginning at nucleotide 2749) and a MCS-specific primer (5' - ACGGTATCGATAAGCTTGATATCG). Figure 2 illustrates the results of the PCR analysis obtained from single injected flies (G_0). Progeny from individuals which gave PCR product were used to establish transformant lines. The G_1 progeny from these lines were again tested by PCR in pools of 10 flies to verify the presence of the P element constructs. Approximately 90% of the flies showing a strong amplification signal in the G_0 analysis were confirmed as transformants. We estimate that screening 20 G_1 progeny from each G_0 fly having an initial positive PCR signal is sufficient to assure the establishment of several independent transformed lines.

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Balajee, A.S., and A. Szakmary. Institute of Radiation Genetics and Chemical Mutagenesis, University Leiden, The Netherlands, present address for A.S.B., Lab. of Mol. Genetics, NIA, NIH, Baltimore, MD 21224 and for A.S., Lab. of Mol. Genetics, NIEHS, NIH, P.O.Box 12233, Res. Triangle Park, NC 27709. Fluorescent *in situ* hybridization (FISH) on polytene chromosomes with multiple, differently labeled DNA-probes.

When labeled bands are very close so that the signals merge or when signals can not be easily attributed to a certain DNA probe by their location alone, different colours can help. In this protocol we describe how to label polytene chromosomes with biotin (blue or green), digoxigenin (red) and fluorescein (green) simultaneously.

PREPARATION OF SLIDES

Dissect well-fed larvae in 45% acetic acid, remove fat body cells as well as possible and anterior part of the gland. Use preferentially female larvae from non-crowded bottles. Transfer the salivary glands into a small drop of lactic acid:water:acetic acid (1:2:3) on a siliconized coverslip (we use 24 x 60 mm). After fixation (3 - 5 min) the coverslip can be picked up with a clean slide (we use ones treated with Deinhardt solution) and the cells squashed. Check your squashes for spreading of the chromosomes and continue only with the best ones. Keep the slides at 4°C for some hours and freeze them in liquid nitrogen or deep freeze (-110°C). Flip off the coverslip and transfer immediately into chilled ethanol (70%, chill with dry ice). Dehydrate with 90% and 100% ethanol (chilled) and air dry.

PROBE LABELING

Labeling by Nick-translation: Biotin-11-dUTP labeling

Add the following to a sterile eppendorf tube: (for digoxigenin or fluorescein replace biotin-11-dUTP by 25 uM digoxigenin-11-dUTP or fluorescein-12-dUTP plus 25 uM of dTTP.

22 ul filtered distilled water
5 ul Nick translation buffer (10x NTB)
5 ul Dithiothreitol (0.1 M DTT)
5 ul Deoxynucleotides triphosphate solution (0.5 mM dATP, dGTP, dCTP, 0.1 mM dTTP)
6 ul Bio-11-dUTP (0.4 mM)
1 ul DNA probe (1 ug/ml)
5 ul DNase I, diluted 1:1000 from 1 mg/ml stock solution.
1 ul DNA polymerase I (10 units/ul) to get a final volume of 50 ul

Place the eppendorf tube for 2 hours in a 15°C waterbath placed in a cold room (4°C). Stop the reaction by adding 5 ul EDTA (0.5 M, pH 7.4).

Labeling by Random priming (A Random priming kit is available from Promega Biotech, USA).

Mix the following in an eppendorf tube placed on ice:

10 ul 5x labeling buffer; 250 mM Tris.Cl, pH 8.0; 25 mM $MgCl_2$; 10 mM DTT; 1 mM Hepes, pH 6.6; 20 ng random hexanucleotide primers).
dATP, dCTP, dGTP (2ul of a 500 uM stock, to give a final concentration of 20 uM)
25-100 ng denatured DNA template
10 mg/ml acetylated BSA (2 ul of a 400 ug/ml stock)
2.5 ul of 1 mM Biotin-16-dUTP
5U of Klenow enzyme (100 U/ml)
Add sterile water to make up the volume to 50 ul. Mix gently and incubate at RT for 60 min.
Add EDTA to a final concentration of 20 mM and purify as described below.

PROBE PURIFICATION

Prepare a Sephadex G-50 chromatography column by placing a bit of sterilized glass wool in a sterile Pasteur pipette (150 mm) and fill evenly with Sephadex.
Equilibrate and wash with TE-buffer (10 mM Tris.Cl, pH 7.5, 1 mM EDTA, 2-3 times)

Add 50 ul Salmon sperm DNA (1 mg/ml) and elute with 600 ul of TE (0.1 M)
Add the probe (55 ul) and elute with 545 ul TE (0.1 M). Repeat with 600 ul of TE and start collecting the last fraction in a sterile tube when the solution level reaches the upper edge of the resin (this fraction contains the labeled probe).

PROBE PRECIPITATION
Split the probe in two Eppendorf tubes (300 ul/tube) and add to each tube:
25 ul Total human DNA (2.0 ug/ul)
2.5 ul Salmon sperm DNA (stock conc. 10 mg/ml)
32.8 ul Sodium acetate (3 M, pH 5.6)
720 ul ice-cold ethanol (100%)
Store the tubes for at least an hour at -20°C
Spin down the pellet at 1200xg for 30min at 4°C
Decant the supernatant, dry the pellet, dissolve both pellets in hybridization buffer at a concentration of 25 ng/ul overnight at 4°C. Labeled probes can be kept at -20°C.

IN SITU HYBRIDIZATION
a) RNase treatment (optional):
Wash the slides for 5 min with PBS (without Mg^{++} or Ca^{++}) at RT
Dehydrate the slides in an ethanol series (70, 90, and 100%, 5 min intervals)
Incubate the slides with RNase (100 ug/ml in 2xSSC) for 1 h in a moist chamber at 37°C, (add 100 ul and cover with a 24 x 60 mm coverslip)
Wash 3x for 5 min with 2xSSC, remove coverslip after first wash and wash once with PBS.

b) Pepsin treatment (to remove proteins and decrease non-specific binding)
Incubate the slides with a pepsin solution 0.005% in 10 mM HCl, for 10 min in a waterbath at 37°C.
Wash for 5 min with PBS at RT.
Wash for 5 min with PBS/$MgCl_2$ (50 mM) at RT (helps to protect chromosome morphology).
Post-fixation for 10 min with 1% formaldehyde (3 ml in 100 ml) in PBS/$MgCl_2$ (50 mM), at RT (helps maintaining chromosome integrity).
Wash for 5 min with PBS at RT.
Dehydrate the slides in a ethanol series (70, 90, and 100%, 5 min intervals). Keep slides in slide box at RT.

c) Probe competition (required when your probe contains repetitive sequences, not required if it is a single copy gene or cDNA sequence).
Transfer the amount of labeled probe you need in a sterile tube, add total genomic DNA (50-100 fold excess) if you did not add as carrier DNA in precipitation step and incubate for 5 min at 70°C in water bath without shaking. Chill on ice, leave there for 5 min and incubate the tube for 2 h at 37°C in water bath.

d) Prehybridization
Prehybridization can be started about 30 min before the end of probe competition.
Incubate slides with 100 ul of 70% formamide/2xSSC under a 24 x 60 mm coverslip for 4 1/2 min at 80°C. Immediately remove coverslip and transfer slides in ice-cold 70% ethanol for 5 min.
Dehydrate the slides in an ethanol series (70, 90, and 100%, 5 min intervals) at RT.
In case of no competition mix probe with the hybridisation mix described above on the slide and denature at 80°C for 4 1/2 min.

e) Hybridization
Incubate the slides with 12 ul probe (conc. of probe is 300 ng/slide) under a coverslip, seal corners of the slides with glue, and place them in an inverted position in a moist chamber with 60% formamide/2xSSC pH 7.0.
Next day remove glue, wash 4x for 5 min each with 50% formamide/2xSSC pH7.0 in a waterbath at 42°C. Stringency depends on the formamide concentration. For less stringency reduce formamide to 30%. Remove coverslip after first wash.
Wash 3x for 5 min each with 0.1 SSC in a waterbath at 60°C.
Wash for 5 min with 4xSSC/0.05% Tween-20 at RT.

IMMUNOFLUORESCENCE DETECTION

Double hybridizations with biotin and digoxigenin probes

1) Preincubate slides in immunological buffer (100 ul), 4xSSC/5% non-fat dry milk under a coverslip (24 x 60 mm) for 15 min at RT to block non-specific binding of the antibodies.
2) Wash 2x with 4xSSC/5% non-fat dry milk
3) Incubate with 100 ul Avidin-FITC (5 ug/ml, final conc.) under a coverslip 24 x 60 mm for 30 min at RT in the dark.
4) Wash twice with 4x SSC/0.05% Tween-20 followed by a 5 min wash in TNT buffer.
5) Incubate with Goat-anti-Avidin-biotin + (0.5 mg/ml) and Mouse-anti-digoxigenin (2.5 ug/ml) diluted in TNB buffer (0.1 M Tris.Cl, 0.15 M NaCl, 5% Boehringer Mannheim dried milk powder) for 30' at RT or 37°C in the dark.
6) Wash 3x with TNT buffer
7) Incubate with Avidin-FITC + Sheep-anti-Mouse-digoxigenin (2 mg/ml) diluted in TNB buffer for 30' at RT or 37° in the dark.
8) Wash 3x with TNT buffer as above
9) Incubate with Sheep-anti-digoxigenin-TRITC (2 mg/ml) diluted in TNB buffer for 30' at RT or 37°C in the dark.
10) Wash 3x with TNT buffer.
11) Dehydrate the slides in an ethanol series (70, 90, and 100%, 5 min intervals) at RT.
12) Embed the air-dried slides in Vectashield mounting medium under a coverslip (24 x 60 mm, 25 ul per slide).

For triple hybridizations with biotin, digoxigenin and fluorescein

1) Preincubate slides in immunological buffer (100 ul), 4xSSC/5% non-fat dry milk under a coverslip (24 x 60 mm) for 15 min at RT.
2) Wash 2x with 4x SSC/5% non-fat dry milk
3) Incubate with Avidin-AMCA (5 ug/ml) under a 24 x 60 mm coverslip for 30 min at RT in the dark.
4) Wash 3x with 4xSSC/0.05% Tween-20 followed by a 5 min wash in TNT buffer.
5) Incubate with Goat-anti-Avidin-biotin + (0.5 mg/ml), Mouse-anti-digoxigenin (2.5 ug/ml) + Rabbit-anti-FITC (5 ug/ml) diluted in TNB buffer under coverslip for 30 min at RT or 37°C.
6) Wash 3x with TNT buffer
7) Incubate with Avidin-AMCA (5 ug/ml)+ Sheep-anti-Mouse-digoxigenin (2.5 ug/ml) + Goat-anti-rabbit-FITC (2.5 ug/ml) under coverslip for 30 min at RT or 37° in the dark.
8) Wash 3x as above in TNT buffer.
9) Incubate with Sheep-anti-digoxigenin-TRITC (2 mg/ml) for 30 min at RT or 37°C in the dark.
10) Wash 3x in TNT buffer.
11) Dehydrate the slides through an ethanol series (70, 90, and 100%, 5 min intervals) at RT.
12) Embed the air-dried slides in Vectashield antifade solution under a coverslip (24 x 60 mm, 25 ul per slide).

Additional comments and recommendations

Try a range of 4-5 min for chromosome denaturation, we had our best results with 4 1/2 min.

In both examples (double and triple hybridization) the immunostaining steps 5-8 can be repeated to increase sensitivity. Keep in mind that the background increases as well.

When the end label is FITC embed in Vectashield/PI.

When the end label is TRITC or TRITC and FITC embed in in Vectashield/DAPI.

When the end label is AMCA with FITC or TRITC use only Vectashield.

Equipment

We use an Olympus BH2-RFC (reflected light fluorescent attachment) microscope, equipped with BP490 and BP545 excitation filters for blue and green excitation. The EY455 filter is particularly useful while observing FITC stained specimens in blue irradiation.

References (related protocols): Lim, J.K., 1993, Dros. Inf. Serv. 72:73; Kim, W., and M.G. Kidwell 1994, Dros. Inf. Serv. 75:44.

Reagents required

Formamide	Baker cat.no. 7042, store at RT
Rubber cement	Simson

Dextran sulphate	Pharmacia, cat.no.17-0340-01.
DNA polymerase	Promega, cat.no. M205A, store at -20°C
DNase I	Boehringer, cat.no.104159, store at -20°C
Sephadex G-50 fine	Pharmacia
DNA from Salmon testes	10 mg/ml TE-buffer, Sigma
Tween-20	Sigma, cat.no.P1379
Pepsin	Serva, cat.no.31855
RNase A	Boehringer, cat.no.109142
Blocking reagent	Boehringer, cat.no. 1093657
acid-free formaldehyde (37%)	Merck, cat.no. 3999
Deoxynucleotide triphosphates (100 mmol/l; 250 ul)	
-dATP	Boehringer cat.no. 1051440
-dCTP	Boehringer cat.no. 1051458
-dGTP	Boehringer cat.no. 1051466
-dTTP	Boehringer cat.no. 1051482
Biotin-11-dUTP (dissolve in 50 mM Tris pH 7.5)	Sigma, cat.no. B7654
Digoxigenin-11-dUTP	Boehringer, cat.no. 1093088
Fluorescein-12-dUTP	Boehringer, cat.no. 1373242
Avidin-FITC (5mg/ml)	Vector Laboratories, cat.no. A-2001
Goat-anti-Avidin-biotin (0,5mg/ml)	Vector Laboratories, cat.no. BA-0300
Avidin-TRITC (2.5 mg/ml)	Vector Laboratories, cat.no. A-2002
Avidin-AMCA (5 mg/ml)	Vector Laboratories, cat.no. A-2008
Sheep-anti-digoxigenin (2 mg/ml)	Boehringer, cat.no. 1330713
Mouse-anti-digoxigenin (0.1 mg/ml)	Sigma, cat.no. D8156
Sheep-anti-Mouse-digoxigenin (200 ug/ml)	Boehringer, cat.no. 1214624
Sheep-anti-digoxigenin-FITC (200 ug/ml)	Boehringer, cat.no. 1207741
Sheep-anti-digoxigenin-TRITC (200 ug/ml)	Boehringer, cat.no. 1207750
Rabbit-anti-Mouse-FITC (solution)	Sigma, cat.no. F7506
Rabbit-anti-Mouse-TRITC (solution)	Sigma, cat.no. T2402
Goat-anti-Rabbit-FITC (solution)	Sigma, cat.no. F9262
Goat-anti-Rabbit-TRITC (solution)	Sigma, cat.no. T2527
Goat-anti-Mouse-FITC (solution)	Sigma, cat.no. F9006
Goat-anti-Mouse-TRITC (solution)	Sigma, cat.no. T5393
Rabbit-anti-Goat-TRITC (solution)	Sigma, cat.no. T6028
Rabbit-anti-FITC (solution)	Dako(patts), cat.no. V403
DAPI (4,6-diamidino-2-phenylindol.2HCl)	Serva, cat.no. 18860
PI (propidium iodide)	Sigma cat.no. P5264
Vectashield mounting medium	Vector Laboratories, cat.no. H-1000

Solutions required

Pepsin prepare a 10% pepsin solution in water, dissolve at 37°C, store aliquots at -20°C
add 50 ul 10% pepsin to 100 ml 0.01 N HCl when needed.

1% acid free formaldehyde/1xPBS/50 mM $MgCl_2$ (150 ml)
3 ml acid free formaldehyde (37%)
5 ml 1M $MgCl_2$
92 ml 1x PBS

10x PBS 8.0 g NaCl + 0.2 g KCl + 1.15 g Na_2HPO_4 + 0.2 g KH_2PO_4 per liter dist. water

RNase A dissolve 50 mg RNase A in 5 ml 2xSSC
place solution for 10 min in boiling water bath (to remove DNase activity)
cool down, aliquot and store at -20°

20x SSC 175.3 g NaCl + 88.2 g Na citrate, dissolve, adjust to pH 7.0 and add till 1 liter

70% resp. deionized formamide/2x SSC/50 mM sodium phosphate (1 ml)
700 ul 100% deionized formamide
100 ul 0.5M sodium phosphate buffer pH 7.0

100 ul filtered 20x SSC
100 ul filtered bidist. water
prepare 60% formamide/SSC and 50% formamide/SSC for moistening hybridization chamber and washing after hybridization the same way
10x Nick translation buffer (10 ml)
0.5 M Tris.HCl pH 7.8, 50 mM $MgCl_2$, 0.5 mg BSA (nuclease free)/ml; store at -20°C
Dithiothreitol (DTT) (100 mM) store at -20°C
Nucleotides mix 0.5 mM dATP, 0.5 mM dCTP, 0.5 mM dGTP and 0.1 mM dTTP; store at -20°C
TE-buffer, pH 7.8, 10 mM Tris.HCl, 1 mM EDTA
DNase I dissolved in 20 mM Tris.HCl, 50 mM NaCl, 1 mM DTT, 0.1 mg BSA/ml, 50% glycerol (v/v), store at -20oC
Low molecular DNA (Salmon testes and Human placenta DNA (= competitor)
dissolve 100 mg DNA in 100 ml 0.3 M NaOH in TE-buffer and boil for 20 min
neutralize with 5 ml 2 M Tris.HCl pH 7.5/7.5 ml 4 M HCl/12 ml 2 M NaAc
precipitate, centrifuge and dissolve in TE-buffer
20% dextran sulphate (10 ml)
dissolve 2 g dextran sulphate in 50% deionized formamide/2x SSC/50 mM sodium phosphate pH 7.0 for 3 h at 70°C, aliquot and store at -20°C.
10% dextran sulphate/50% formamide/2x SSC/50 mM sodium phosphate pH 7.0
0.1 M Tris.HCl/0.15 M NaCl/0.05% Tween-20 (TNT, prepare fresh)
0.1 M Tris.HCl/0.15 M NaCl/0.5% Boehringer blocking reagent (TNB, prepare fresh)
4x SSC/0.05% Tween-20 (4T, prepare fresh)
4x SSC/5% non-fat dry milk (4M, prepare fresh)
Vectashield-PI/Vectashield-DAPI dissolve 1 ug/ml of PI or DAPI in Vectashield antifade solution.

Park, Sangbin, and J.K. Lim. Department of Biology, University of Wisconsin-Eau Claire, Eau Claire, Wisconsin 54702-4004. Limjk@UWEC.EDU FAX: (715)836-5089. A microinjection technique for ethanol-treated eggs and a mating scheme for detection of germ line transformants.

We found the following microinjection technique to be a rather simple and efficient method for P-mediated germ line transformation. A broad outline of the technique is to immerse the eggs in 95% ethanol for a few minutes, arrange the eggs on a slide coated with agar, and inject the DNA solution into the eggs without dechorionating. The plasmids are injected into eggs produced by flies homozygous for *w*; delta2-3(99B), the flies from injected eggs are mated with either *C(1)DX, y w f* females or *FM6, w* males, and their progeny screened for germ line transformants. Our data accumulated during the last few years indicate that we can expect about one germ line transformant in every 20 injected eggs.

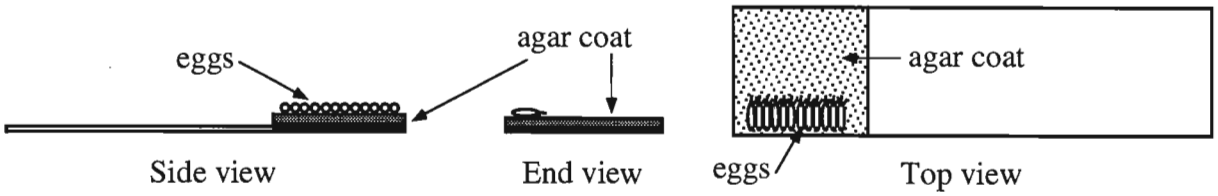
I. Microinjection technique and rationale:

1. Prepare egg collection plates by autoclaving 6 gm of agar in 150 ml orange juice (We used both Minute maid and Tropicana brands and both brands worked fine for us) at 250°F for 10 minutes. Leave at room temperature for about 10 minutes, then add 1.5 ml of ethyl acetate (emits a pungent banana-like odor) and mix well. Pour the agar solution onto a watch glass and allow to gel.

2. Prepare agar-coated slides by autoclaving 6 gm of agar in 100 ml of orange juice for 10 minutes at 250°F. Leave at room temperature for several minutes, and add an antibiotic solution (we add 600 ml of ampicillin solution at 25 mg/ml concentration). Dip the frosted end of the microscope slides into the agar solution (the agar solution sticks better on the frosted surface of the slide) and scrape the agar off the smooth side of the slides. Let the agar solution on the slides gel. Prepare 40 to 50 slides at a time, place in Wheaton slide staining dishes with glass cover, and store in refrigerator. The coated slides keep well for several days in the refrigerator.

3. Collect rapidly-laid eggs. Our practice is to feed yeast paste to one-day-old homozygous *w*; delta2-3(99B) flies for three to four days and collect eggs on the egg collection plates from these flies for one hour and discard these eggs to eliminate eggs that were withheld. Our practice is to place about 300 pairs of flies in an empty 1/2 pint milk bottle, fasten an egg collection plate on the opening of the bottle to avoid the flies from escaping, and place the bottle mouth-side down (egg collection plate-side down) in a dark box (We use an empty Dispo pipets container from Scientific Products). Then collect eggs on a new egg collection plate for about 25-30 minutes, flood the eggs on the plate

with 95% ethanol, keep the eggs completely immersed in ethanol for three to four minutes, collect the eggs with a feather or brush to the center of the plate, and transfer them to a drop of sterile water on the agar-coated slide to wash the eggs and arrange them for injection (see diagram below). From this time on, the flies were provided with a new egg collection plate every 25-30 minutes to collect rapidly-laid eggs. The strain of *w*; delta2-3(99B) originating in Bill Engels laboratory is an excellent egg producer and more than 90% of the eggs collected in this manner hatched. The delightful feature of the strain is its ability to produce eggs all day long without obvious signs of sagging in egg production (three cheers)!



4. Inject the eggs within 60 minutes from collection. The injection apparatus we used is similar to the one described by Simmons and Drier (1993). The Needles are pulled from omega dot capillary tubes (Capillary tubing with omega dot fiber for rapid fill, Cat. No., 30-30-0-075, Frederick Haer and Company, Brunswick, ME 04011, Tel: (800) 326-2905). The vertical pipette puller we used was from David Kopf (Vertical pipette puller, model 720, David Kopf Instruments, Box 636, Tujunga, CA 91042, Tel: (818) 352-3274), and the composition of plasmid DNA solution that we injected into eggs was: 5 mM KCl, 0.1 mM NaH_2PO_4 (pH 6.8), 5% by volume of food color that has been autoclaved (Schilling Green food color) and ampicillin (100 mg/ml of the buffer). Any food color should work but we used green. For those of you who are learning to inject, we suggest injecting the above solution without plasmid DNA first, and try the same injection buffer with plasmid DNA. You will note that you can inject more than 100 eggs without DNA molecules, but if you try to inject plasmid DNA in the same injection buffer, you will have a plugged needle tip after injecting only several eggs! Our interpretation is that the plasmid DNA molecules in the injection buffer form a complex with DNA-binding proteins from the cytoplasm of the eggs. As long as you do not have DNA molecules, as in the injection buffer alone, you will not have a problem with the DNA-binding proteins that get into the needle. For this reason, the concentration of the DNA solution we inject is about 1.0 - 0.5 mg/ml. With practice, one can learn to break away a very small part of the needle tip thereby unplugging the tip. The "excellent" needle has a needle tip with a large enough hole to avoid plugging but small enough to readily penetrate the egg shell without causing too much damage to the eggs. We usually inject about 200 eggs with one ml of the DNA solution which suggests we are injecting about 5 nanoliter of DNA solution per egg. You can inject a DNA solution of concentration higher than 1.0 mg/ml, but the higher the DNA concentration, the more likely you will have problems with the plugged needle tips.

5. Collection of larvae from injected eggs: Our practice is to inject about 30 to 40 eggs at a time on the same slide. After injection, the eggs on the slide are placed on a square Petri plate filled with fly food about 1/6 inch deep. The larvae from the injected eggs usually take more than 24 hours at room temperature to hatch. We collect the larvae between 24 to 50 hours after injection. A dark-colored fly food in the Petri plate helps spotting the newly-hatched larvae.

II. Mating scheme for detection of germ line transformants

The female flies recovered are mated with *FM6, w*/Y males individually, and the males from the injected eggs are individually mated with *C(1)DX, y w f* virgin females. We usually brood three-day intervals to establish two or three broods and examine all progeny for colored eyes. As shown in the following table, one can hope to expect more than

Table 1. A summary of P-mediated germ line transformation experiments.

Plasmids used*	*pSB 5*	*pSB 6*	*pSB 11*	*pSB 12*	*pSB 13*	*pSB 14*	*pPDC6*	*pW8X*	*placZwh*	Total
Plasmid size in kb	10	10	11	11	11	11	28	13.5	14	N/A
No. eggs injected	no data	4,515	846	508	no data	1,301	1,723	1,012	1,267	11,172+
No. larvae recovered	110	404	59	52	244	290	544	413	660	2,756
No. pupa collected	71	219	27	17	167	130	329	281	388	1,629
No. flies eclosed	56	186	21	7	138	106	248	210	334	1,306
No. fertile flies tested	38	86	9	2	91	70	136	148	185	765
No. transformants**	15	35	3	1	37	12	12	64	43	222

* all plasmids, except the last two, are *CaSpeR-hsp70* with an insert.

** The number of fertile flies in the preceeding box that produced at least one transformant.

one germ line transformant out of every 20 injected eggs in some cases. One of us (SBP) injected all the eggs shown in the table. Therefore, the complications resulting from the injection are constant and minimized. The first six columns in the table were results from experiments performed during June and July 1994, and the last three columns were the results of the injections carried out during February, 1995. The results of injection experiments performed during 1991 - 1994 by other injectors in our laboratory are consistent with the ones shown in the table below. In agreement with observations by others, the larger plasmids showed a lower efficiency of transformation (*i.e.*, pPDC6 which was 28 kb). In establishing the transformants, one must get rid of flies with variegated eyes and preferably use a single fly with solid-colored eyes to set up a line.

We wish to acknowledge that we were unaware of the article by Bartoszewski and Gibson (1994) which appeared in DIS Vol. 75 (July 1994). We did not receive our Vol. 75 until April 1995 by an unwitting error in shipping of the standing order. We also wish to acknowledge that one of us (JKL) has known since 1966 that immersing the fly eggs in 95% ethanol will not kill the eggs.

References: Simmons, M.J., and E.A. Drier, 1993, Dros. Inf. Serv. 72: 80-81; Bartoszewski, S., and J. Gibson 1994, Dros. Inf. Serv. 75: 205-206.

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, Department of Zoology, University of Oklahoma, Norman, OK 73019.

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. The editor reserves the right to make minor grammatical and stylistic changes if necessary.

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.

Format: Manuscripts are now being entered into computer files so that a diskette version can eventually be distributed with the printed copy. In order to make the text as simple as possible for different printer formats and search commands, we have tried to minimize the use of special symbols (e.g., ul is used for μl, "female" and "male" are written out in the text in place of the short-hand symbols, Greek letters are written out, and so forth). The meaning should be clear from the context in all cases.

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.

Stock Lists, Specialized Bibliographies, and Long Technical Articles: Long or complex material can generally not be accepted unless it is submitted on diskette, with a printed copy for editorial guidance. Special justification is needed for material, such as bibliographic lists, that are often readily available by other means. A page charge of $30.00 per page will be requested to help defray publication costs of accepted material. Inquiries about format and content are welcomed.

Figures: Both line drawings and half-tone illustrations will be accepted, but there will be a special charge of $10.00 per half-tone illustration to help cover the cost of their preparation for printing.

Equipment and Supplies Available

Frank Butterworth. Oakland University.

The following pieces of equipment, supplies, and books are immediately available FREE.

Tissue transplantation equipment: Three units that are used to make glass needles for transplanting organs and tissues into *Drosophila* larvae or adults or other small animals following the procedure of Dietrich Bodenstein in *Biology of Drosophila* by Demerec. This equipment is custom made according to Dietrich's directions, modeled after Dietrich's own equipment with some improvements.

1. Constriction-producing device and micro manipulator needed to put constrictions in the glass tubing that will determine the size of the payload chamber and regulate the entry/exit speed of the transplant.
2. Diamond grinding wheel (low speed, power driven) needed to sharpen the needle point and most important, to smooth off jagged edges.
3. Stereo microscope system to view both the above operations.

There is also some required, sundry equipment and supplies such as transplantation needle holders, plastic tubing and couplings, glass tubing for the transplant needles, and very fine embroidery needles and holders for dissection. I cannot over-estimate the value of this material since some of it is very difficult to find. Total approximate weight - 50 lbs.

Two population cages made of Lucite plastic and stainless steel mesh according to the directions of Allan Spradling approximately 18" on a side that can be used to generate massive amounts of fly embryos. Approximate weight each - 15 lbs.

Slides of sectioned adult male and female abdomens. These are 2 micrometer, serial sections (sagittal, transverse, and cross) of various experimental and control animals. Most of these experiments have been published (experimental details available) and many of the sections are stained with a variety of cytochemical procedures. More details on request. Approximately 100 boxes at 2 lb. per box.

Books and journals: a variety of books associated with *Drosophila* biology, insect biology, the complete set of bibliographic books of Herskowitz, and sundry reprints.

Classical, half pint, wide-mouth, glass milk bottles, complete with paper caps and heavy steel holding/carrying cases. There are approximately 1000 bottles some of which are still in their original boxes and about 10 stainless steel cases.

This material is immediately available on a 'first-come, first-served' basis **subject to approval by Oakland University**. It is free, but arrangements will have to be made for shipping and packing. I advise to act as soon as possible. Please contact me by phone: 810-370-3555; phone/fax: 810-652-2116; email: butterwo@vela.acs.oakland.edu; or write: Frank Butterworth, Biological Sciences, Oakland University, Rochester, MI 48309.

Seeking Genetic Materials in 14C, 96D1-2, 97D1-2

Daria Siekhaus. Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305-5307, (415)-723-6925, FAX (415)-725-6044, e-mail daria@leland.stanford.edu

I am looking for P-element insertions, deficiencies or chromosomal abnormalities in the regions listed. For 96D1-2 I already have Bloomington Df2366, and P-element insertions rJ244, rJ880, and rJ851. For 97D1-2 I already have Df T19QRX, DF ro60b, and Df roXB3, EMS l(3)117 from K. Anderson, P-element RK344 and A6-3-24 from r. Bodmer, as well as F336 ry+ lacZ from K. Anderson. Many many thanks to anyone who responds.

Position Available

Peter Lawrence. MRC Laboratory of Molecular Biology, Cambridge, England.

An unusual job is open for applicants. Paul Johnston, Peter Lawrence's assistant and collaborator of 20 years died in March. Peter is now looking for someone to fill this post which is funded by the Medical Research Council to be held in Cambridge, England at the MRC Laboratory of Molecular Biology. The person should be well experienced with flies (practical *Drosophila* genetics, antibodies and RNA in situs, preparing eggs for injection, dissecting and mounting adults, etc.). The work would be to assist Peter in his own experiments and when he collaborates with others. The style of the lab is old fashioned: Peter works at the bench and the assistant would be, in the main, a coauthor on the papers. The most important quality sought is meticulousness and high standards. Salary would depend on paper qualifications, such as whether the person has a PhD. A BA degree or equivalent would be necessary. A work permit might be obtainable for an ideally qualified person.

Interested? If so please email C.V. and relevant information to Peter Lawrence at pal@mrc-lmb.cam.ac.uk or write to him at the MRC Laboratory of Molecular Biology, Hills Rd., Cambridge, CB2 2QH, England. Tel: 44 1223 402282,402226,402274; Fax: 44 1223 412142

Standing Orders for DIS

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 for forthcoming regular and special issues and a Standing Order Invoice to facilitate prepayment. If you would like to be added to the Drosophila Information Mailing List, please write to the editor, Jim Thompson, at the address at the front of this issue.

Back Issues

The following annual back issues of DIS are still available:

DIS 70 (1991)	$ 8.00 plus shipping
DIS 71 (1992)	$12.00 plus shipping
DIS 72 (1993)	$12.00 plus shipping
DIS 75 (1994)	$12.00 plus shipping

Shipping costs:

U.S. (UPS)	$ 3.00 per copy
Foreign surface	$ 6.00
Foreign air:	
Canada	$ 7.00
Mexico and Central America	$13.00
South America	$21.00
Europe	$23.00
Other foreign destinations	$27.00

All orders must be prepaid by a check in U.S. currency drawn on a U.S. bank. Please make checks payable to "*Drosophila* Information Service" and mail to James Thompson, Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019.

Special DIS Issues

DIS 73: *Drosophila* Genes/Chromosomes
DIS 74: Bibliography of *Drosophila*, 1982-1993

published by the FlyBase Consortium

DIS 73 is a compendium of four tables. Three, compiled by the FlyBase Consortium, are major revisions of their DIS 69 counterparts describing *D. melanogaster* genes. The fourth is a detailed description of the polytene maps of *D. melanogaster*.

GENETIC LOCI lists genes sorted alphabetically by abbreviation and listing gene name, map position, gene product information and literature citations.

GENE FUNCTION lists loci assorted by the function of the gene's product.

GENE and ALLELE SYNONYMS lists synonyms alphabetically together with their corresponding valid genetic symbols.

SALIVARY GLAND CHROMOSOME MAPS by T.I. Heino, A.O. Saura and V. Sorsa consist of light and electron micrographs of the polytene chromosomes, and histograms of chromatin and DNA per band.

DIS 74, also compiled by the FlyBase Consortium, is an exhaustive reference list of *Drosophila* from 1982 through 1993 inclusive, presented in standard bibliographic order.

Each volume is about 675 pages. Each costs $29.00 plus shipping. Domestic UPS (U.S.A. Only) is $4.50 per copy; Canada and Mexico is $7.00 per copy; other foreign surface shipping is $6.00 per copy. Foreign air parcel post costs per copy are: Canada and Mexico $8.00; Central and South America $13.00; Europe $22.00; other foreign destinations $30.00.

Copies of DIS 73 and 74 must be prepaid by check in U.S. currency drawn on a U.S. bank. Make checks payable to DIS-73/74. Purchase orders or standing orders cannot be accepted. Address your purchase requests or questions to: DIS 73/74, c/o Dawn Rowe, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge MA 02138-2097 U.S.A. Phone: (617) 495-2906, FAX: (617) 495-9300, E-mail: dis73-74@morgan.harvard.edu.

The Encyclopaedia of Drosophila CD-ROM

Macintosh Compatible only
Release 1.0

The Berkeley Drosophila Genome Project (BDGP) and FlyBase announce Release 1.0 of the Macintosh CD-ROM version of the Encyclopaedia of Drosophila (EofD). This is the same version of the EofD CD-ROM that was distributed to registrants at the April 1995 Drosophila Research Conference in Atlanta.

The EofD is a graphical interface based on the ACeDB software first developed for the *C. elegans* database and now in use as a powerful browsing and querying tool for many genome databases. Multiple data sets are displayed on chromosomal maps in which text descriptions of data items are displayed on command by mouse-driven "pointing and clicking".

Release 1.0 combines data from the BDGP and from FlyBase in an integrated view. In Release 1.0, all BDGP data (except DNA sequences) and much (but not all) FlyBase data are contained. In contrast, the FlyBase World Wide Web home page (URL = http://morgan.harvard.edu/) contains more FlyBase data but only a subset of BDGP data. Our long term goal is that both the EofD and the FlyBase home page will provide different views of the same comprehensive data set. However, we are probably about a year away from achieving this goal.

Before purchasing the EofD, you should make sure you have access to a Macintosh computer with the following attributes:

The EofD CD-ROM can only be run on higher end Macintosh computers, those equipped with 68040 or PowerPC processors and a CD-ROM drive. The application runs in native mode on the Power Macintosh and we recommend the Power Macintosh for optimal performance.

The EofD requires System 7.1 or higher. System 7.5 is required to use the EofD Guide help facilities. In addition, QuickTime is required to play movies supplied on the CD-ROM.

The EofD requires a minimum of 16 Megabytes (MB) of RAM. We recommend turning on Virtual Memory and setting it to at least 32 MB.

The costs of the EofD, including shipping and handling, are, per CD-ROM and user manual:

$10 for U.S. purchases and shipment to a U.S. address
$15 for international purchases and shipment

Order forms must be accompanied by checks or money orders in US dollars made out to "EofD". Only prepaid orders will be accepted.

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Drosophila Information Newsletter

The Drosophila Information Newsletter (DIN) is going away. The network newsgroup bionet.drosophila is functioning well and can carry out the functions of DIN with greater timeliness and less effort. Volume 20 will be the final edition of DIN. Thank you to all who contributed to DIN over the past five years.

We encourage readers of DIN to make use of the bionet.drosophila newsgroup. You can participate in bionet.drosophila via newsreader software on your local system, by an e-mail subscription to bionet.drosophila, or through the archive in FlyBase/News/bionet.drosophila. Message traffic on bionet.drosophila averaged 2 messages per day from 1/1/95 through 6/28/95. Contact your local computer whiz if you want to access bionet.drosophila with newsreader software. If you want an e-mail subscription to bionet.drosophila, send a message to biosci-server@net.bio.net with the following line in the body of the message:

subscribe drosophila

The subject line can be blank. Send this message from the account at which you wish to receive postings to bionet.drosophila.

To post a message to the group, use your newsreader software, or send your post as an e-mail message to dros@net.bio.net. Unlike subscription messages, messages to be posted must include a subject in the subject line of the message.

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TABLES OF CONTENTS

DIN Volume 16 (October 1994)

DIN Volume 17 (January 1995)

DIN Volume 18 (April 1995)

DIN Volume 19 (July 1995)

ANNOUNCEMENTS, VOL. 16-19

NEW STOCK CENTER AT INDORE, INDIA

A new Drosophila Stock Center has been established at Devi Ahilya Vishwavidyalaya, Indore, India; it emphasizes the collection of P insertion, enhancer trap, and other mutant lines. The center is funded by the Indian government's Department of Biotechnology, and is run by Pradip Sinha. Dr. Sinha invites all Drosophila workers to contribute P insertion and other mutant stocks to the collection. The center can be reached by mail at School of Life Sciences, Devi Ahilya Vishwavidyalaya, Vigyan Bhavan, Khandwa Road, Indore-452001, India, or by FAX at 91-731-473063 (e-mail is not yet available). The Indian Stock Center stock list will be added to FlyBase as soon as a computerized version is available.

BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 16

* We will be away for a FlyBase meeting (plus some vacation) October 6 through October 17. Requests received by 11AM on Wednesday, October 5, will be shipped October 10. Requests received between 11AM Oct. 5 and 11AM Oct. 20 will be shipped October 24.

* Funding for the stock center has been renewed for the next 5 years, effective September 15, 1994. The collection is now jointly supported by NSF (Biological Instrumentation and Resources) and NIH (the National Center for Research Resources, the National Institute of General Medical Sciences, and the National Eye Institute). The program officers with responsibility for the stock center award are Dr. Machi Dilworth at NSF (mdilwort@nsf.gov) and Dr. Elaine Young at NIH (elainey@ep.ncrr.nih.gov). Thanks to everyone who helped bring this about.

Our current funding agreement requires that we institute a cost-recovery program. We will finalize the plans for this program and contact each user group with complete details latter this fall.

* The stocks search function on FlyBase has been changed to search all three melanogaster center lists at once. A stock center code precedes each stock number (B for Bloomington, M for Mid-America, and U for Umea) and the center's name and an e-mail address for ordering stocks is included as the last line of each stock record. Please order stocks from the correct center.

* The Bloomington stock list on FlyBase is updated whenever new stocks become available. If a stock does not appear on our list we do not have it.

BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 17

* Beginning January 1, 1995, the weekly stock request deadline will be 5:00 PM on Wednesdays. All requests that include our stock numbers and your Bloomington User Number and are in by the deadline will be shipped to you the following Monday.

* The Bloomington Stock Center now has a cost-recovery program. Minimal users of the stock center are not required to contribute to the center, but all users must be registered with the center and provide their Bloomington User Number (BUN) when requesting stocks. Send e-mail to matthewk@indiana.edu for more information on this program.

BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 18

* We will be closed the week of April 3. Requests received between 5:00 PM March 29 and 5:00 PM April 12 will be shipped April 17.

* The linking of Bloomington stocks to alleles and aberrations in FlyBase has begun. Use the FlyBase WWW homepage (http://morgan.harvard.edu/) to make use of this new feature. Options to follow links to stocks are available through the CytoSearch and SymbolSearch tools. Approximately 700 alleles and aberrations in Bloomington stock genotypes are not yet linked to objects in FlyBase.

* A new version of WAIS has caused some odd problems with wild-card searching of the Stock Center stock lists on FlyBase. We hope to solve this problem in the next few weeks, but in the meantime, to be sure you see all available stocks with alleles of a given gene, search for both gene-symbol* and gene-symbol[*. This can be done in one step by including both in the same query, or separately. For example, if you want to see all stocks with wg alleles, enter your query as: wg* wg[* This will produce a complete list.

BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 19

* We will be closed the week of September 17 (road trip!). Requests received by 5:00 PM Wednesday, September 13 will be shipped on Monday, September 18. Requests received between 5:00 PM on the 13th and 5:00 PM on Wednesday, September 27th will be shipped on Monday, October 2. Mark your calendar.

* We have begun a collection of stocks carrying generally useful GAL4 and UAS constructs. Stocks that are ready for release are listed below with the names, pattern information, and references provided by the donors. These short names will be replaced by full genotypes when FlyBase curators have assigned valid construct symbols and insertion identifiers. You may order these now by stock number. We hope to have additional lines by the end of the summer. If you have GAL4/UAS stocks of general interest that you would like to contribute to the collection please contact Kathy Matthews (matthewk@indiana.edu) or Thom Kaufman (kaufman@bio.indiana.edu).

GAL4 constructs
1734 1J3 (hairy) [1,4,5]
1747 71B (imaginal discs) [1]
1767 24B/TM3 (embryonic mesoderm) [1]
1774 69B/TM3 (embryonic epiderm, imaginal discs) [1,4]
1782 32B/TM3 (imaginal discs) [1]
1795 30A/CyO (imaginal discs) [1,6]
1799 hs-Gal4[89-2-1]/TM3 (heat shock Gal4)
1803 55B (follicle cells) [3]
1822 31-1 (CNS/PNS)
1824 pGawB (basal expression)
1854 1-76-D (epidermal stripes)
1874 389 (embryonic CNS)
1878 T80 (ubiquitous in 3rd instar imaginal discs) [4]
1947 RG1 (paired) [1,5]
1967 34B [1,6]
1973 e22c (ubiquitous)
2017 ptc-Gal4 [8,4]
2023 hs-sev-Gal4 [9]

UAS constructs
1776 UAS-lacZ[4-1-2] (cytoplasmic beta-gal, insert on 2) [1]
1777 UAS-lacZ[4-2-4B]/TM3 (cytoplasmic beta-gal)
2025 D-Ras2[Val14] (constitutively activated Drosophila Ras2) [3]
2033 deltaD-Raf[F179] (constitutively activated Drosophila raf) [3]
2074 deltaC-Raf1[ra2] (constitutively activated form of human Raf1) [3]
2075 wg[tsM7-2-1] [4]
2076 en [5]

References
[1] Brand and Perrimon (1993) Development 118: 401-415
[2] Brand, Manoukian and Perrimon (1994) Methods in Cell Biology, Vol 44: 635-654
[3] Brand and Perrimon (1994) Genes and Dev. 8: 629-639
[4] Wilder and Perrimon (1995) Development 121: 477-488
[5] Yoffee et al. (1995) Development, in press
[6] Ingham and Fietz (1995) Current Biology 5: 432-440
[7] Staehling-Hampton et al. (1994) Cell Growth and Differentiation, in press
[8] Speicher et al. (1994) Development 120: 535-544
[9] Ruberte et al. (1995) Cell 80: 889-897
[10] Greig and Akam (1993) Nature 362: 630-632
[11] Hinz et al. (1994) Cell 76: 77-87

DROSOPHILA CONFERENCE

The 1996 meeting will be held in San Diego, California, April 27-May 1. Jim Posakony will be the organizer.

POSITIONS AVAILABLE AT INSTITUTE OF ECOLOGICAL GENETICS

This is to inform you of the existence of a brand new Institute of Ecological Genetics to study Amazonian biodiversity. For further information write to:

Dr. Hugo Hoenigsberg
Instituto de Genetica-Ecologica y Biodiversidad Amazonica
Cra.4 No.71-69
Bogota D.C.COLOMBIA.
FAX: 612 7369

You can send your C.V. to our personal address above. We are considering applications to fill posts as research scientific staff members. Evolutionary biologists, geneticists, ecologists, systematist, botanists, zoologists, mathematicians and other Ph.D. individuals interested in neo-tropical biological research with at least 10 years of research experience preferably, but not exclusively, in the tropics, and about 10 published scientific papers will be considered. This new Institute will study Amazonian biodiversity. Although its main purpose is research it will by inclination help, not only to preserve the Amazonian biodiversity, but also to disseminate the gospel of international management of the most wonderful world natural reserve for which it is worth to dedicate one's life. There will also be graduate degrees to be dealt with. For academic life within the Institute, please contact the Rector of the Amazonian University as follows:

Dr. Ernesto Fajardo
Universidad de la Amazonia
Florencia, Caqueta
Colombia.
FAX: (988 35) 8231

Florencia is the capital of the State of Caqueta, and Caqueta is one of the three Amazonian States of Colombia. We will be working closely with Peruvian and Brazilian scientists interested in Amazonian biodiversity. Our Central offices and research labs will be in Florencia's surroundings.

DEMEREC'S BIOLOGY OF DROSOPHILA

Cold Spring Harbor Laboratory Press has published a facsimile edition of Demerec's classic on BIOLOGY OF DROSOPHILA. It was first published by John Wiley and Sons in 1950. Until its appearance, no central, synthesized source of biological data on *Drosophila melanogaster* was available, despite the fly's importance to science for three decades. Ten years in the making, it was an immediate success and remained in print for two decades. However, original copies are now very hard to find.

Contents

*Normal Spermatogenesis in *Drosophila* (K.W. Cooper)
*The Early Embryology of *D. melanogaster* (B.P. Sonnenblick)
*Histogenesis, Organogenesis, and Differentiation in the Embryo of *D. melanogaster* Meigen (D.F. Poulson)
*The Postembryonic Development of *Drosophila* (D. Bodenstein)
*External Morphology of the Adult (G.F. Ferris)
*The Internal Anatomy and Histology of the Imago of *D. melanogaster* (A. Miller)
*Collection and Laboratory Culture (W.P. Spencer)

632 pp., illus., indexes, ISBN 09-87969-441-6; Cloth $39. To place an order, contact Cold Spring Harbor Laboratory Press at cshpress@cshl.org, or phone 1-800-843-4388, or fax them at 516-349-1946.

DROSOPHILA BOARD MEETS IN ATLANTA

The North American Drosophila Board will meet at 2:00 PM, Wednesday, April 5, 1995, in Tower Room 1, Westin Peachtree Plaza Hotel, Atlanta, GA. If you have concerns you would like brought before the Board, contact your regional representative.

Mariana Wolfner, 1993 President of the Board, posted the following explanation of the Board to bionet.drosophila on December 7, 1994. It is reprinted here for those who missed it and aren't familiar with the Drosophila Board.

The Drosophila Board meets annually during the Flymeeting to discuss issues of relevance to the fly community. The Board is composed of:

- regional representatives who represent *you*.
- the present, immediate past and future meetings organizers.
- ex officio members representing stock centers, DIS, DIN, FlyBase.

Between meetings, the Board is polled by its president on any other issue that may need attention. Board reps serve three-year terms; the terms are staggered.

With this posting I am listing the main issues that were discussed at the last Board meeting. Please contact your regional rep if you would like further details, or if you have comments or issues that you want brought up at future Board meetings.

The current regional reps are:

Northeast: Welcome Bender
Mid-Atlantic: Margarete Heck
Great Lakes: Helen Salz
Midwest: Bill Engels
Heartland: Juan Botas
California: John Tower
Northwest: Susan Parkhurst
Canada: Tom Grigliatti

The current Board President is Claire Cronmiller. Addresses of all these individuals are in FlyBase.

Agenda of last Board meeting:

1. Administrative matters related to financial and organizational issues.
2. Annual flymeeting format, in general, and related issues
3. Statistics for the current meeting (V. Finnerty, organizer)
4. Future meetings (meeting locations rotate: east coast - west coast - central)
   The dates, sites and organizers for the next several meetings are:
   1995, Atlanta, April 5-9, A. Spradling
   1996, San Diego, April 27-May 1, Jim Posakony
   1997, Chicago or New Orleans, dates TBA [about April 16-20], Organizer TBA
   1998, East Coast site TBA, dates TBA, Organizer TBA
   1999, Seattle, dates TBA, Barbara Wakimoto and Susan Parkhurst
   If you wish to volunteer to organize one of the meetings, please contact Claire Cronmiller.
5. Reports on operations, statistics and administration from:
   Larry Sandler Lectureship Committee
   Stock Centers
   DIN
   DIS
   FlyBase

CRETE 10th-ANNIVERSARY MEETING

Spyros Artavanis-Tsakonas, Crete Workshop Organizing Committee, Boyer Center for Molecular Medicine, Room 236, 295 Congress Avenue, Yale University School of Medicine, New Haven, CT 06510 USA.

The Crete meeting on the Molecular and Developmental Biology of Drosophila will be held from July 14th through July 20, 1996. All subsequent meetings are also scheduled for the middle of July. Although a formal announcement will be made in the Fall, applications to attend the meeting are currently being accepted. This will be the tenth anniversary of the Crete Workshop.

6TH EUROPEAN SYMPOSIUM ON DROSOPHILA NEUROBIOLOGY

Stephan Schneuwly

The next European Symposium on Drosophila Neurobiology will be held on September 15-19, 1996 in Regensburg, Germany. Regensburg is a beautiful historic bavarian city, which can be easily reached by car, train and airplane (International Airports of Munich and Frankfurt). To keep the meeting costs as low as possible we are planning to communicate mainly through E-mail and FlyBase. If you would like to be posted on the mailing list, send a short message to the following mailbox: sekretariat.schneuwly@biologie.uni-regensburg.de

MATERIALS AVAILABLE, VOL. 16-19

Arthur Chovnick, Dept. of Molecular & Cell Biology, U. of Connecticut, Storrs, CT 06269-2131, USA. chounick@uconnvm.uconn.edu.

The Chovnick laboratory will not be able to continue to maintain and provide cultures of rosy region stocks beyond January 1, 1995. We will honor requests for these stocks until then.

This collection includes rosy wildtype isoalleles with known sequence differences as well as mutations induced on the known isoalleles. Mutant sites cover the entire gene from 5' promoter to the 3' poly T site, and include Ambers, Opals, Frameshifts, electromorphs, transitions, transversions, deletions, duplications and transposable element insertions. They include complimenters, non-complimenters, leaky mutants, nulls and a tissue specific overproducer site located in the large 5' intron, as well as 5' and 3' splice site mutants. The available rosy stocks include the mutants summarized in Lindsley and Zimm, pages 606 through 614.

Also, we will be discarding stocks carrying mutations in genes surrounding rosy, and located in 87C, 87D, 87E as well as a set of overlapping deficiencies in this area. See Lindsley and Zimm, pages 399 through 402 and page 873.

STOCKS AND CLONES

Burke Judd, NIEHS, P.O.Box 12233, Research Triangle Park, NC 27709-2233, USA. Phone: 919-541-4690, FAX 919-541-7593, judd_b@niehs.nih.gov.

Some of the following are unique combinations of mutants that will be discarded soon after I leave the NIEHS, March 31, 1995. If you can use any of them please let me know. After March 31, contact Jim Mason (mason-j@vaxe.niehs.nih.gov), who will keep the stocks for another few weeks.

z[v77h] w[+] is from Oregon-R
z[v77h] w[67c23]
w[zm]
sc z[1] w[zm]
z[v77h] w[zm]
y z[a] w[zm]
y w[zl]
z[1] w[zl]
y z[a] w[zl]
z[77h] w[zl]
sc z[1] w[zvl]

In(1)w[m4], y
In(1)w[m4], y sn[3]
In(1)w[m4], spl sn[3]
In(1)w[m4], y z[1]
In(1)w[m4], z[v77h] sn[3]
In(1)w[m4h], z[v77h]
In(1)rst[3]
In(1)rst[3], y z[1]
In(1)rst[3], z[v77h]

Df(1)rst[2], y
Df(1)rst[2], z[1]

Df(1)Su(z)J93, y z[1]/FM7: The distal breakpoint of this deficiency is 35 to 60 kb proximal to the w locus and extends through rst and vt but does not include N. Deficiency acts as a dominant suppressor of z[1] apparently by acting on the w locus in cis. It also exhibits rst, vt, reduced viability and female sterility. From deletion mapping against various rst and vt deficiencies, the suppressor of z[1] element is proximal to rst-vt. Deficiency occurs at very low frequency as an ectopic exchange product from females heterozygous for y[2] w[sp-2] and z[1] w[zm] or z[1] w[zl]. Several strains were recovered from both types of heterozygotes. Original recombinant chromosomes contained the w[zm] or w[zl] alleles. These have been replaced by crossingover with w[+] from Oregon-R or with w[65a25]. All versions of these derivatives and the parental chromosomes are available. CaSpeR plasmid clones of part of the region from +100 to +163 (white locus map) are available. A transformed line carrying approximately 17.5kb extending from +122.5 to + 142 is available. Johng Lim, who did the transformation, also has a copy of this line.

z[J91]: This allele occurred spontaneously in z[1] w[65a25] spl sn[3]. It causes lemon-yellow eye-color in z[J91] w[+] males and z[J91] w[+]/z[+] w[+] females. It acts only in cis, however, thus most likely is acting on the w locus.

z-w lethals: One allele of each of 13 lethals located in the region between the z and w loci will be deposited in the Mid-America Stock Center at Bowling Green. As many as three alleles for each locus will be kept here until sometime in April.

The deficiencies generated by ectopic recombination in the region flanking the w locus that are described in Montgomery et al., (1991) Genetics 129: 1085-1098, will be available for a few more weeks.

echinus locus: We have cloned and sequenced genomic and cDNA that we believe to be the ec locus. However, a transformed line carrying the genomic sequences fails to rescue ec mutations, thus we have not yet published this. Fly strains and clones are available to anyone who is interested in a collaboration to complete this analysis. Contact Bibba Goode, Laboratory of Reproductive Toxicology, NIEHS, P.O. Box 12233, RTP, NC 27709.

NEW MUTATION AFFECTING EYE-ANTENNAL DISC DERIVATIVES

Byeong-ryool Jeong, Department of Biology, Box B79, Indiana Univ., Bloomington, IN 47405, USA. Phone: 812-855-8175, bjeong@ucs.indiana.edu.

I found a dominant mutation that resides on the third chromosome in a maintained stock of labial[vd1]. Genetic mapping indicates that this mutation is between curled and ebony. The adult phenotype of this mutation includes: irregular facets and hairs in the eyes (frequent), duplicated or irregular postorbital bristles (frequent), tufted or mirror-image-duplicated vibrissae (frequent), very small eyes (rare), duplication of the antennae (rare), and crooked bristles on top of the head. All of these defects seem to be derived from the eye-antennal disc, and when I looked at the eye-antennal disc of the third instar larvae, I could find morphological defects in some of the discs, such as humps on the eye or the antennal disc. Expression pattern of labial seemed to be unaffected. I named this mutation "Dead[BJ1]" for "Defective eye-antennal disc."

In addition to the above phenotypes, this mutation seemed to be temperature-sensitive dominant lethal at 25oC, and to have very low fecundity. I failed to separate the mutation from lab[vd1], therefore, the genotype of the stock is lab[vd1] Dead[BJ1]/TM6B, Tb Hu.

This stock will be discarded about the middle of April. If anyone wants this stock, please contact me at the e-mail address above.

TOLIAS OVARIAN gt22A cDNA LIBRARY

Peter P. Tolias, Public Health Research Institute, 455 First Ave., New York, NY 10016, USA. Phone: (212) 578-0815 office; (212) 578-0816 lab; Fax: (212) 578-0804; E-mail: tolias@phri.nyu.edu.

The following information updates that published in DIN Vol.9 and DIS 72.

The available amplified aliquots of the Tolias ovarian gt22A directional cDNA library were titred at 3 x 108 pfu/ml (99% inserts) before freezing. The original complexity of this sample was 500,000 independent clones (99.7% inserts).

The 5' end has unique sites for EcoRI and SalI (GAATTCGTCGACCCACGCGTCCG), the 3' end has a unique NotI site. Use a fresh Y1090 O/N grown in LB amp (50 ug/ml), 0.2% maltose and 10 mM MgSO4 as recommended by most protocols. If you want to screen this library by PCR, I suggest that you use a small fraction of this aliquot to reamplify and use only reamplified samples for PCR.

This library has been widely distributed in the USA, Canada and Europe. To conserve the remaining aliquots, please check oogenesis labs in your area first before requesting it. If it is not available, please send us a Federal Express number to facilitate shipment. When you receive the library, divide it into 50 ul aliquots in siliconized microfuge tubes, add one drop of chloroform, store one of the aliquots at 4 deg C and freeze the rest at -70 deg C. When a frozen aliquot is required, thaw, use and store at 4 deg C but do not freeze again.

This library should be referenced in publications as: "Stroumbakis, N.D., Li, Z. and Tolias, P.P. (1994). RNA- and single-stranded DNA-binding (SSB) proteins expressed during Drosophila melanogaster oogenesis: a homolog of bacterial and eukaryotic mitochondrial SSBs. Gene 143, 171-177."

REQUESTS FOR MATERIALS, VOL. 16-19

MATERIALS in 99C1-E1

David Bilder, Dept. of Developmental Biology, Stanford Univ. School of Medicine, Stanford, CA 94305-5427, USA. (415)497-2057, bilder@cmgm.stanford.edu.

I would appreciate hearing about genetic and molecular information for working in the 99C1-E1 region: P insertions, lethal complementation groups, chromosome aberrations, chromosome walks etc. Thank you.

DEFICIENCIES AND P{w[+]} LINES

Joan E. Wilson, Dept. of Biological Sciences, Gilbert Building, Stanford University, Stanford CA 94305-5020. 415-725-8778, fax/9688, wilsonje@leland.stanford.edu.

Looking for deficiencies in 29C-D, 30D-31B, 44C-46C, 48, 91, 92D-93C. Also any P{w[+]} inserts in or around 29-31 or 91-93.

P{w[+]} SIMULANS STRAINS

Dominique Joly, CNRS, Lab. Populations, Genetique et Evolution 91198 Gif sur Yvette Cedex, France. joly@sunbge.bge.cnrs-gif.fr, Fax: 33 1 69 82 37 34.

I am studying the genetic basis of sperm length in Drosophila. For that purpose, I would be very interested in any D. simulans strains carrying a P-white[+] element. I am to your disposal for any further indications on the experiments that I would like to realize with those strains. Thanks in advance for your help.

GENETIC MATERIALS IN 51B-C

Michele Crozatier, Centre de Biologie du Developpement, Batiment 4R3, 118, route de Narbonne, 31062 Toulouse, France. Tel: (33) 61.55.82.87, Fax: (33) 61.55.65.07, Email: seroude@cict.fr.

I am looking for deficiencies, P insertions or chromosome aberrations in 51B6-C3. Thanks in advance for your help.

CALL FOR STRUCTURAL DATA ON NERVOUS SYSTEM DEVELOPMENT

Karl-Friedrich Fischbach, Institut fur Biologie III, Schanzlestr. 1, 79104 Freiburg i. Brsg., Germany. kff@sun1.ruf.uni-freiburg.de; 0761-203-2730, Fax: 0761-203-2745.

Many Drosophila labs have started or are planning to provide useful information in www. Without well organized linking knots, this information is somewhat chaotically distributed and not so easy to access. Therefore, we plan to build such a knot with regard to nervous system development, first of all for our own use, but - due to the nature of www - this should be usable by the whole Drosophila community.

We shall enter in 1995 as much of our own data as possible (some copyright problems have to be solved and

conflict with conventional publishing to be avoided). Our emphasis will be on the optic lobe and its larval and pupal development. These data will be crosslinked to the structural data given free by your lab. Thus a decentralised international dataweb about the development of the fly's nervous system will evolve. This undertaking differs from, but is not in conflict with, the Drosophila Brain Database just launched by Nick Strausfeld and Kim Kaiser; it will rather heavily rely on many crosslinks to this database. Entries in the Tucson-Glasgow Drosophila Brain Database will be carefully checked by an oversight group, while we shall simply list www-links under appropriate headings. In order to minimise electronic traffic on our local computer net, and in order to maximise speed for your own data retrieval, we would welcome, if you download those links to your own system.

If you want to participate, E-mail your www-homepage and/or any comment to kff@sun1.ruf.uni-freiburg.de. Our www-homepage is: http://deep-thought.biologie.uni-freiburg.de/data/kff.html

ATLAS OF AN INSECT BRAIN

Katsuo Furukubo-Tokunaga, Zoological Institute, Univ. Basel, Rheinsprung 9, CH-4051 Basel, Switzerland. E-mail: tokunagak@ubaclu.unibas.ch.

Looking for one or two copies of the brain anatomy book ATLAS OF AN INSECT BRAIN (N.J. Strausfeld, Springer-Verlag) for educational purpose. If you have this book only to fill your book shelf, please contact me before you start to think about cleaning up the space. Thank you.

REPRINTS/PREPRINTS ON DEVELOPMENT, GENETICS & EVOLUTION

Richard Gordon, Department of Radiology, University of Manitoba, ON104, Health Sciences Centre, 820 Sherbrook Street, Winnipeg, Manitoba, Canada R3A 1R9. E-mail: gordonr@cc.umanitoba.ca, Fax: (204) 783-8565.

I am finishing a book about the intersection of three major fields of biology: Gordon, R. (1995). The Hierarchical Genome and Differentiation Waves: Novel Unification of Development, Genetics, and Evolution (Singapore: World Scientific), in prep.

If your work might be relevant to this review, I would appreciate reprints or preprints (mailed to the address above) as soon as possible, or an update, if I've been in contact with you previously. Thanks for your help. Please be sure to send your e-mail address, in case I have any questions.

If you are curious, condensed accounts are given in: Gordon, R. & G.W. Brodland (1987). Cell Biophysics 11, 177-238. Gordon, R. (1993). In: Beysens, D., N. Boccara & G. Forgacs, eds. Dynamical Phenomena at Interfaces, Surfaces and Membranes. Commack, N.Y.: NOVA Science Publishers, 99-111. Gordon, R., N. K. Bjorklund & P. D. Nieuwkoop (1994). Int. Rev. Cytol. 150, 373-420. Bjorklund, N. K. & R. Gordon (1994). Computers & Chemistry 18(3), 333-345.

MUTATIONS IN 15

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I am searching for mutations within division 15 of the X chromosome. I have inflated, rudimentary, bazooka, M(1)15D and forked alleles. I would like any others, can anybody help?

GENETIC MATERIALS IN 58D1-2

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I am looking for deficiencies, P insertions or chromosome aberrations in 58D1-2 or near that region. Thanks in advance for your help.

GENETIC MATERIALS IN 8A-C

Brenda Lilly, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA. phone (713) 798-3569, FAX (713), 798-5386, lilly@bcm.tmc.edu.

I am looking for deficiencies, P-elements and chromosome aberrations in the 8A-C region of the X-chromosome. Any materials or information would be greatly appreciated. Thanks.

TECHNICAL NOTES, VOL. 16-19

AN IMPROVED DEVITELLINIZATION TECHNIQUE WITH A HIGH YIELD OF X-GAL STAINED EMBRYOS

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X-gal staining (5-Bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) is frequently employed to study the spatio-temporal expression of Drosophila genes. These include enhancer trap studies that involve the insertion of P-lacZ transposons in the vicinity of desired genes (1) and studies involving fusion of the lacZ gene to promoters of developmentally expressed genes (2). X-gal staining is the most popular and convenient method for studying the developmental expression of a lacZ reporter gene (2,3). Devitellinization of embryos, however, is not practiced during X-gal staining since hand peeling of the membrane is time consuming and cumbersome while chemical devitellinization (6) results in diffusion of the stain. Lack of a technique for fast and effective devitellinization of X-gal stained embryos limits the scope of this technique in the study of lacZ expression in embryos.

We present here an adaptation of the chemical devitellinization technique (6) which meets these requirements. The protocol involves standard X-gal staining that includes dechorionation of the embryos in bleach (5% Na hypochlorite) and subsequent treatments with 0.7% NaCl and 1% Triton X-100 for 5 min each. Embryos are then fixed in equal volumes of 3.7% formaldehyde in citric phosphate buffer, pH 7.6 (4), and heptane for 15 min. The solution is then drained and the embryos are gently dried to allow traces of heptane to evaporate. This is followed by a rinse in citric phosphate buffer (4) and overnight incubation at 30[o]C in incubation buffer (4) with an increased concentration of Triton X-100 (0.5%) as compared to the usual (0.02%) and a saturating amount (4) of X-gal. Stained embryos are then fixed again with 3.7% formaldehyde, 50 mM EGTA and an equal volume of heptane for 15 min. Fixed embryos are flushed with heptane and after adding 1 ml of 5% TCA (Tricarboxylic acid) stained embryos were shaken for 2 min and then allowed to stand for 5 min. The solution is then removed and the embryos are flushed with methanol and shaken vigorously; the devitellinized embryos will sink down. The duration of this exercise must not last longer than 3-4 min. The solution is then replaced with a mixture of fresh incubation buffer and glycerol (1:1). The embryos will first shrink and then recover their normal shape in 1-2 hrs. The devitellinized X-gal stained embryos are then mounted in a glycero gelatin mountant (4). The time of exposure to methanol should be optimum as prolonged exposure of the embryos to methanol results in flaky appearance of the stain possibly due to precipitation of the proteins. In contrast, shorter periods of exposure give poor yields of devitellinized embryos. Use of a higher concentration of Triton X-100 during staining appeared to be important for retaining the X-gal staining of the devitellinized embryos. This technique overcomes the limitations of the handpeeling technique and also prevents the loss of resolution that results from chemical devitellinization methods due to precipitation of the proteins. This technique provides improved resolution of the X-gal stained embryonic cell types.

References:

1. E. Bier et al. (1989) Genes and Dev. 3: 1273- 1287.
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3. O'Kane, C.J. and Gehring, W.J. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 9123- 9127.
4. Ghysen, A. and O'Kane, C. (1989) Development 105: 35-52.
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NEED PRINS PROTOCOL FOR DROSOPHILA

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PRINS is a successful technique in human cytology but we did not succeed to apply it to Drosophila melanogaster mitotic chromosomes and also not satisfactorily to polytene chromosomes. Does anybody have a PCR protocol which works on polytene chromosome? We would be grateful to get some information.

AN INEXPENSIVE ACTIVITY MONITOR SUITABLE FOR DROSOPHILA

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Introduction

A commonly measured activity in Drosophila is locomotory activity. It is reduced in a large number of mutants. In addition one of us has used this measurement in investigating changes with age(Driver et alia 1986). The genetic dissection of this activity offers an readily accessible window into the link between activity and neurological function, particularly for an activity which changes with age. We have been unable to find a simple and inexpensive device to measure this and to simplify further measurements we have built an electronic activity meter. This meter responds to breaking an IR light beam and records each break as a separate digit. A novel feature of our design is the use of four calculators as four separate parallel recorders, which enabled a substantial reduction in cost. The total cost of components was under A$70. The device is portable and can be placed in a controlled temperature cabinet so that activities can be measured under controlled temperature and lighting conditions. The use of batteries as power sources isolates the device from power surges which might give false readings.

Construction

Circuit components were supplied by National Semiconductor.

IR beam. Four Photo-Interrupter pair (electronic catalog listing ZD1901 or SY-508) were supplied with the emitter and detector on the one block: these were cut into two at the base to separate the emitter and detector. These were separately mounted on either side of four holes on a particle board, which was sufficiently large to fit four vial (25mm x 75 mm) snugly.

Recording devices: Citizen LC-510N electronic calculators.

Power pack: a battery pack which carries four AA batteries. The use of rechargeable batteries has been found most convenient because the drain on the batteries necessitates frequent changes.

Conditioning of the signal from the photo transistor was by a Schmidt trigger flip flop (CD 4093) which converted interruptions of currents to pulses. The input and out leads of each of the four circuits on this chip were connected to the M+ key on the calculators. Inputs from the photo-interrupt were connected to the terminals labelled CONT A,B,C, and D respectively. The control voltage input was connected to the + side of the power pack.

The - side of the battery pack was connected to a push button switch and then via a 100 K ohm resistor to each photo-interrupt.An indicator LED was also wired in, connecting the side of the switch away from the battery to the + side of the battery pack via a 470 ohm resistor.

The circuitry was then fixed to the same board that the phot-interrupts were mounted on. Total dimensions: 450mm x80 mm

Operation

Vials containing 20 flies were used. The foam top was pushed down into the tube to give a gap between the foam and the medium of 150 mm. The tubes were inserted into the holes in the board. The power from the batteries was switched on, the calculators switched on and the number 1 entered into each calculator, in that order. At the end of 5 minutes the <memory return> button was pressed on each calculator to get an accumulated number. The vials were changed to another hole and the readings repeated. This procedure was repeated twice more so that each vial was measured once in each hole. This alternation permits systematic differences between recording sites to be evened out. In addition the flies were restimulated to move. Between readings the calculators were switched off, and the main power switched off, in that order.

If there is no event for a long period the calculators will turn off. This period is about five minutes, so if a calculator was found to be turned off, the reading was taken as zero.

Problems in Operation.

The device will measure activity in vials which have medium in them, provided that the surfaces are clean. If the light beam is blocked, difficulty will be experienced in operating the calculators: the keys do not respond. In most cases this problem could be overcome by repositioning the vials so that the IR beam is not blocked.

It was also found that after prolonged operation the calculator failed to respond to the keys, particularly during the switching off procedure.This appears to be due to the drain on the batteries. In addition, if the memory is not cleared after the last use, then extra-ordinarily high readings may be found after the next measurement. If the switching sequence above is used so that the device has periods of being off between readings, these problems can be minimised.

However frequent battery replacement (or recharging) is necessary.

Results and Discussion.

An individual tube filled with Canton-S flies up to 7 days old will give a reading of 50-200 counts at 20-25 degrees celsius, although very inactive mutants such as dunce, and older flies are considerably less active.

The activity measured is activity after alarm, i.e. an alarm response. If flies are left undisturbed on a vibration free surface for an hour or more, then counts measured over the next 20 minutes, without moving the vials, are in the order of 0-2. We are using this to genetically dissect alarm response and the way this changes with age. The results of this investigation will be reported elsewhere.

Reference

Driver, C.J.I.; Wallis, R.; Cosopodiotis, G.; Ettershank, G. Is a Fat Metabolite the Major Diet Dependent Accelerator of Ageing? Exp. Gerontol. 14:497-507; 1986.

GENETIC NOTES

A PROBLEM FRT STOCK

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We recently discovered that the Sco FRT40A chromosome which some of you may have obtained from our lab to build recombinants between FRT40A and a specific mutation (m) contains an additional lethal between the FRT and Sco. When this chromosome is used to build recombinants between m and the FRT element, some recombinants will be FRT l m or FRT l rather than the desired FRT m. If you have used this chromosome you should test the putative FRT m recombinant lines for the presence of either m or l. Since Sco is homozygous lethal the presence of the second lethal was not readily detectable. As far as we know the other FRT lines which have been recombined with dominant markers are not associated with a similar problem. We are sorry for any inconvenience this may cause you.

D. SIMULANS 3R INVERSIONS

Jerry Coyne, Dept. Ecology and Evolution, The Univ. of Chicago, 1101 E. 57th St., Chicago, IL 60637, and P. Sniegowski, The Center for Microbial Ecology, PSSB, Michigan State University, East Lansing, MI 48824. jcoyne@pondside.uchicago.edu.

We report here a third-chromosome balancer stock of D. simulans that contains previously undescribed paracentric inversions. A full description of the isolation of this chromosome will appear in DIS 75. The normal 3R of D. simulans differs from that of D. melanogaster by a paracentric inversion of 9 numbered divisions. Using the D. melanogaster system, the chromosome order of the normal D. simulans 3R is 61-84F/93F-84F/93F-100. Relative to D. melanogaster, the D. simulans balancer chromosome has the sequence: 61-81F1/[89E1-93F/84F-84E1/84B1-84E1/84B1-81F1]/89E1-84F/93F-100 (The region in brackets is the region inverted relative to the normal D. simulans 3R).

This aberration is associated with the dominant mutation Ubx[m] and Dl lies within the inverted region. Although the region balanced by this chromosome is rather small, the stock is useful because it allows one to keep two dominant alleles in trans condition without selection.

Conference Programs

In an effort to provide as diverse a source of information on *Drosophila* genetic research as possible, Drosophila Information Service will print programs for research conferences whenever space allows. The editor invites conference organizers or participants to submit copies of meeting programs and a brief description of the theme, location, and time of the conference. DIS will endeavor to publish authors, affiliation of the senior or corresponding presenter, and title of the talks or posters. This can then be used by readers of DIS to locate individuals pursuing problems of common interest, locate possible postdoctoral researchers, and find sources for materials or information. Even if space does not permit publishing all proceedings, we hope that a listing of regional and national conferences will be useful.

36th Annual *Drosophila* Research Conference 5 to 9 April 1995, Atlanta, Georgia, U.S.A.

The 36th Annual *Drosophila* Research Conference for the U.S.A. was held at the Westin Peachtree Plaza Hotel, and the 1995 Program Chair was Allan C. Spradling, Howard Hughes Medical Institute Research Laboratory, Carnegie Institution of Washington. The conference was sponsored by the Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814-3998. Approximately 670 slide and poster presentations were given. The plenary session lectures are listed here, grouped by topic as in the conference program.

Palazzolo, Michael. (Human Genome Center, Lawrence Berkeley Laboratory, Berkeley, CA). High throughput DNA sequencing of the *Drosophila* genome.

Cronmiller, Claire. (Department of Biology, University of Virginia, Charlottesville, VA). The genetic regulation of ovarian follicle morphogenesis.

Tully, Timothy. (Neuroscience, Cold Spring Harbor Laboratory, NY). Molecular genetics of memory.

Hays, Thomas. (Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN). The function of cytoplasmic dynein: a minus end-directed microtubule motor.

Rorth, Pernille. (Department of Embryology, Carnegie Institution of Washington, Baltimore, MD). Functional dissection of the *Drosophila* C/EBP transcription factor.

Savakis, Babis. (Institute for Molecular Biology, Heraklian, Greece). Transposition of the *Minos* element in *D. melanogaster*.

Abmayr, Susan. (Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA). Genes controlling embryonic development of the larval body wall muscles.

Dickinson, Michael. (Department of Organismal Biology and Anatomy, University of Chicago, IL). Grasping for air: the physiology and aerodynamics of *Drosophila* flight behavior.

Lin, Haifan. (Department of Cell Biology, Duke University Medical School, Durham, NC). Making a difference: the role of asymmetric germline cell divisions in oogenesis.

Carroll, Sean. (Laboratory of Molecular Biology - HHMI, University of Wisconsin, Madison, WI). Regulation and evolution of insect wing number, formation and pattern.

Hooper, Joan. (CSB, University of Colorado Health Sciences Center, Denver, CO). *Cubitus interruptus* drives transcriptional activation and is regulated by *hedgehog* signaling.

WORKSHOPS:

Bender, Michael. (Department of Genetics, University of Georgia, Athens, GA). Molecular biology of ecdysone response.

McKearin, Dennis. (Department of Biochemistry, University of Texas S.W. Medical Center, Dallas, TX). Oogenesis.

Rubin, Gerry. (Department of Molecular and Cell Biology - HHMI, University of California, Berkeley, CA). Genome analysis and databases.

Abmayr, Susan. (Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA). Muscle system.

Kimbrell, Deborah. (Department of Biology, University of Houston, TX). Immunity.

Salz, Helen. (Department of Genetics, Case Western Reserve University, Cleveland, OH). RNA processing.