DROSOPHILA INFORMATION SERVICE

Number 58

August 1982

Prepared at the
Division of Biological Sciences
University of Kansas
Lawrence, Kansas - USA

For information regarding submission of manuscripts or other contributions to Drosophila Information Service, contact P. W. Hedrick, Editor, Division of Biological Sciences, University of Kansas, Lawrence, Kansas 66045 - USA.

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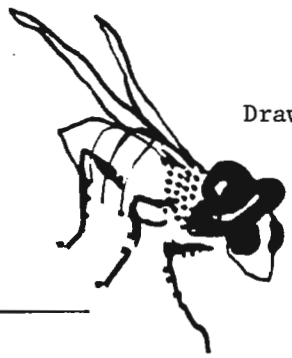
TECHNICAL NOTES



Drawings by C. Katz

ERRATA, DIS 56

Correction: Rahman, R. and D.L. Lindsley. "Ysu(f)$^{-}$, a spontaneous derivative of Ymal^{+}." DIS 56:108. Ysu(f)$^{-}$ should be designated: KL·bb^{+}? ot^{+}--1(1)Q-56^{+} bb? KS, and not as stated.

Correction: Traut, H. "An approximate χ^2 test as applied to mutation experiments with D. melanogaster." DIS 56:140-141. In formulae (1) to (4) the symbols | | have been misprinted as [ ].

Genetic Mapping Computer Program: Listings of a BASIC computer program that calculates maximum likelihood estimates of recombination frequencies in the presence of additive or multiplicative viability effects are available from Andrew G. Clark (new address: Institute of Ecology and Genetics, University of Aarhus, DK-8000 Aarhus-C, Denmark) or from Winifred W. Doane, Dept. of Zoology, Arizona State University, Tempe, AZ 85287. The program also computes linkage, using the classical model for comparison, as well as goodness-of-fit statistics. More information about the procedure appears in: Clark, A.G. (1981) The estimation of linkage in the presence of multiplicative viability effects. Genetics 98(3): in press.

Handbook of Drosophila Development: This handbook is due for publication in spring, 1982, and is intended to give a detailed introduction to various aspects of Drosophila development, suitable for both novice and research worker. It will be published by Elsevier/North Holland, and is edited by Robert Ransom (Open University). Contents: techniques (Ransom); gametogenesis (Bownes and Dale); embryonic development (Bownes); imaginal discs (Russell); eye and head development (Ransom); nervous system (Campos-Ortega); thoracic segments (Adler); abdominal development (Roseland); genitalia and sex determination (Laugé); internal organs (Ransom). It is hoped the cost will be kept below or around $20.

REQUESTS

Request for material for the new Red Book. We are currently in the process of revising the Red Book. We urgently request all Drosophila workers to contribute to this project by providing material. We are anxious to receive the following types of information:

1. Corrections of all errors found in the first edition.
2. Suggested deletions of material presented in the first edition.
3. Additions to entries in the original edition; these additions can be to any category of information itemized in the earlier version as well as to new categories "molecular biology" and "alleles". In many cases, alleles will henceforth be treated under the description of the nominate allele.
4. New entries describing genes or alleles not included in the earlier edition, or DNA sequences that have been characterized in some way. Please document all contributions with published or unpublished references.
5. References to work that contains descriptive information about genes or chromosomes of D. melanogaster. Send reprints if you've no time to abstract the information.
6. Mapping information--cytological, genetic, and molecular.
7. Illustrative material--diagrammatic, artistic, or photographic--to be considered for inclusion.

Please send all material to: Dr. Dan L. Lindsley, Dept. of Biology, University of California-San Diego, La Jolla, California 92093.

Drosophila breeding sites: In an effort to bring together as much information as possible for a general review, we would appreciate reprints, preprints, or unpublished records on the breeding sites of the world's drosophilidae. Information on substrate types, where and when collected, and numbers of flies reared from them would be most helpful. Write to: John Jaenike and David Grimaldi, Dept. of Biological Sciences, State University of New York, Binghamton, New York 13901, USA.

Deficiencies (2L) wanted: I am urgently looking for D. melanogaster stocks with deficiencies in chromosomal region 21B3 to 21B7. If you have such stocks or pertinent information please write to: Gerhard H. Müller, Institut für Biologie I (Zoologie) der Universität, Schanzlestrasse 1, D-7800 Freiburg, West Germany.

Request for stocks: bw^{59} and bw^{81} are no longer available from stock centers or other labs listed in the Computerized Stock List. If you have one or both of these stocks, please contact Dr. James L. Farmer, 591 WIDB, Brigham Young University, Provo, Utah 84602, USA.

The 23rd Annual Drosophila Conference was held March 25-28 at the University of Connecticut. Below is a list of the invited speakers and their topics.

DNA sequence variation at the alcohol dehydrogenase locus in D. melanogaster.
--Martin Kreitman

Factors influencing rDNA redundancy and function in Drosophila.
--H. Krider, F.L. Dutton, B. Yedvobnick, D. Durica and B.I. Levine

Cellular and molecular responses of imaginal disc cells to positional information.
--Paul Adler

Gene organization and expression at the adh locus.
--Cheeptip Benyajati

Decapentaplegic. A gene complex affecting morphogenesis.
--W.M. Gelbart, F.M. Hoffman, F.A. Spencer, V. Irish and C.T. Wu

Genetic analysis and molecular cloning of an RNA polymerase II locus.
--Arno Greenleaf

Sex and biorhythms in Drosophila: A genetic analysis.
--C.P. Kyriacou and J.C. Hall

Developmentally regulated gene expression in Drosophila larval fat bodies.
--Michael Levine

Evolutionary genetics of fitness and senescence in a melanogaster population.
--Michael Rose

Workshop on gene transfer methods.
--Allan C. Spradling, Gerald Rubin and Kevin O'Hare

At the end of the meeting, the following resolution was adopted by acclamation:

Whereas, female scientists comprise 31% of all junior and 15% of all senior faculty in the field of genetics, and

Whereas, women have been either un- or under-represented among the invited lecturers at the Annual Drosophila Conferences,

Therefore, be it resolved that:

(1) Drosophila Research Workers affirm their commitment to equal professional opportunities for all active scientists and that,

(2) Future conference organizers act on that commitment by ensuring representation for female scientists in all conference activities at a level commensurate with their availability.

Abstracts - 1980

Abstracts of some of the talks given at the 1980 Drosophila Research Conference at Snowbird, Utah, were included in DIS 56. This one was unfortunately omitted, and is therefore presented herein.

The Genetics of Male Fertility

Dan L. Lindsley
(Studies in collaboration with R. W. Hardy, J. Kennison, C. Pearson, R. Rahman, S. Rokop, D. Stern and K. Tokuyasu)

Normal spermatogenesis depends on the normal functioning of a number of genes; it has certain chromosomal requirements as well. Consider first the genic requirements. Based on the relative mutability of X-linked and autosomal genes to male sterilizing and lethal alleles and the estimated number of lethally mutable genes we estimate that there are some 600 loci that can mutate to male sterility; the observation that most such mutants are male specific leads us to propose that they are spermatocye specific in their activity. In addition several authors have observed that approximately 30% of temperature-sensitive lethals, when raised under permissive conditions, are male sterile, either immediately or after a suitable period at restrictive temperature; the observations that these mutants interfere with both normal development and spermatogenesis and that most sterilize females as well demonstrates that these genes are not spermatogenesis specific. Assuming the two populations of genes to be disjunct gives an estimate of 600 + 1800 genes or 36% of the genome that is crucial to male fertility.

A special set of six genes required for male fertility is carried by the Y chromosome--four on the long arm and two on the short. Ultrastructural studies of spermatogenesis in males deficient for specific Y-chromosome segments reveal that these genes control specific structural events in spermatid development in much the same way as do X-linked and autosomal genes. Among other things they demonstrate that the absence of nuclear structures and the formation of needle-shaped crystals, both of which are characteristic of spermatocytes of XO males, are attributable to the absence of different Y-chromosome segments. The two deficiencies that result in the absence of the tubuli and reticular aggregates from the primary spermatocyte nucleus also result in the absence of the outer dynein arms associated with the peripheral microtubule doublets of the sperm tail axoneme.

At least three different classes of rearranged sex-chromosome constitutions also lead to male infertility: (1) translocations between the X chromosome and either chromosome 2 or 3 [T(X;A)] and proximal heterochromatic X-chromosome deficiencies in combination with either (2) duplications for proximal X heterochromatin or (3) T(Y;A)'s.

Roughly speaking, T(X;A)'s that interchange fewer than ten numbered salivary-gland-chromosome divisions of X plus autosomal material are male fertile, whereas those interchanging more are male sterile; also fertile are T(X;A)'s whose X-chromosome breakpoint is at the proximal extremity of the proximal heterochromatin of the long arm or in the short right arm. All other reciprocal X-autosome translocations are male sterile. Insertional T(X;A)'s are sterile if more than ten numbered salivary-gland-chromosome divisions of either X or autosome are translocated and fertile if less than that quantity is inserted. These results are interpreted in terms of a model that postulates that the inactivation of X-chromosome genes in advance of autosomal genes in the primary spermatocytes is crucial to normal spermatogenesis. Translocations are viewed as removing segments of the X from a proximally located X-inactivating system and placing autosomal segments under its control.

Male genotypes in which the X chromosome carries a proximal deficiency and the Y chromosome carries a compensating duplication are frequently sterile or show drastically reduced fertility. Combinations of a number of different deficiencies with several different duplicated Y chromosomes suggest that the deleterious effects on fertility are correlated with the size of both the duplication and the deficiency. It is as though the proximal region of the deficient X chromosome, i.e., the heterochromatin, were competing with the duplication for some substance required for normal spermatogenesis, e.g., for cis inactivation of the X chromosome in the primary spermatocyte.

Deficiencies for segments of the proximal heterochromatin of the X chromosome that are male fertile in combination with a normal Y chromosome are male sterile in combination with Y-autosome translocations that are themselves male-fertile in combination with a normal X. In combinations tested to date, deficiencies that exclude bb^+ have been male fertile, whereas those that include it have been sterile. All T(Y;A)'s tested have sterilized deficiency-bearing males except those with absolutely terminal breaks in the autosome and those that insert an autosomal segment into the Y. This male sterility is dominant; i.e., it is not possible to rescue fertility by the addition to the genotype of either a normal Y or a duplication for the material missing from the X. T(Y;A)'s with subterminal breaks in both the Y and the autosome allow the recovery in sons of both products of adjacent I disjunction. These aneuploids demonstrate that only the autosome capped by Y derived material is responsible for male sterility.

Aizenzon, M.G. and E.S. Belyaeva. Institute of Cytology & Genetics, Novosibirsk, 630090, USSR. Genetic loci in the X-chromosome region 2A-B.

In the region 2A1-2 (3-4) - 2B17-18 restricted by the proximal margin of Df(1)At127 and covered by duplication Dp(1)y^2Y67g the series of mutations affecting viability has been located. These mutations seem to involve nine genetic loci (Belyaeva et al., this volume). Cytogenetic mapping of these loci with the help of a number of deletions and duplications (Table 1) allowed them to be arranged from the left to the right as follows: 1(1)BA11, 1(1)BA12, sta, o.c.c., (dor and swi are not separated cytogenetically), 1(1)HM38, and 1(1)HM40 (not separated), and 1(1)HM32. Fig. 1c details cytological intervals of the region 2A-B where these loci are located.

During cytogenetic mapping an interesting peculiarity of loci 1(1)BA12 and sta has been found: lethal alleles of these loci being together with some duplications at 25°C lose their

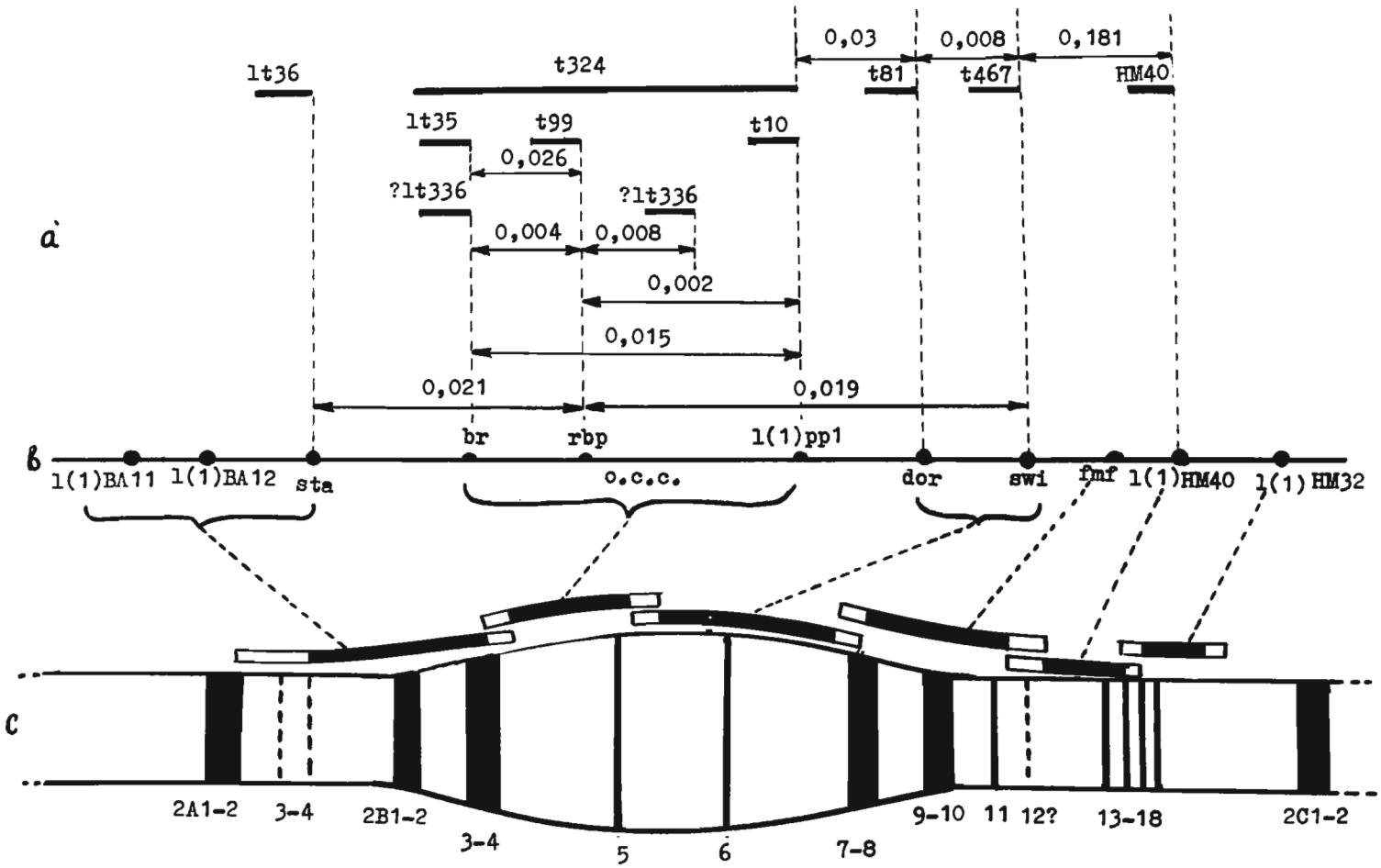


Fig. 1. Results of cytogenetic and genetic mapping of the loci in the 2A-B region. (a) Complementation map with indication of recombinational distances between representatives of complementation groups. (b) Loci. (c) Chromosome map of Bridges (Lindsley & Grell 1968) with indication of cytological intervals of the loci. Unshaded areas represent uncertainty in the exact position of the breakpoints.

Table 1. Interference between loci and rearrangements in the region 2AB (25°C): viable +; lethal -.

Rearrangements and localization of break points	Genetic loci										
			sta								
	1(1)BA11	1(1)BA12	sta^{lt36} sta^{lt3}	Df(1)sta	T(1;3)sta	o.c.c.	dor	swi	1(1)HM38	1(1)HM40	1(1)HM32
Df(1)RA19; 1E3-4 -2B11-12	-	-	-	-	+ **	-	-	-	+	+	+
Df(1)sta; 1E1-2 - 2B3-4	-	-	-	-	+ **	+	+	+	+	+	+
Dp(1;f)101 1A1-2 - 2B1-2±	+	+ *	-	-	+ **	-	-	-	-	-	-
$Dp(1)y^{59b}Y(2)$; 1A1-2 - 2B1-2±	+	+	+ *	+ *	+ **	-	-	-	-	-	-
Dp(1)Y Sz280; 1A1-2 - 2B3-4; 2B7-8 - 2C1-2	+	+	+	+	+	-	-	-	+	+	+
Df(1)S39; 1E1-2 - 2B5	-	-	-	-	+ **	-	+	+	+	+	+
Df(1)St472; 2B6-8 - 2B11-12	+	+	+	+	+	+	-	-	+	+	+
$Dp(1)y^{2}Y67g24.2$ Dp(1)y2Y53T; 1A1-2 - 2B6-8	+	+	+	+	+	+	+	+	-	-	-
Dp(1)Y2E; 1A1-2 - 2B13-15	+	+	+	+	+	+	+	+	+	+	-
$Dp(1)y^{2}Y67g$; 1A1-2 - 2B17-18	+	+	+	+	+	+	+	+	+	+	+
Df(1)At127; 1E3-4 - 2A1-4	+	+	+	+	+	+	+	+	+	+	+

* rough small eyes, scutellars reduced, notches on wings, low viability.
** phenotype sta (Lindsley & Grell 1968).

Table 2. Viability and fertility of males with deficiencies and duplications.

Genotype ♂♂	Ratio males to females (1/1 expected)			No. of males tested	Fertile males (%)	Males viable 8 days after hatching
	25°C	29°C	18°C			
Df(1)RA19/Dp(1)y^{2}Y67g24.2	218/473	10/102	5/51	62 (25°C)	2*(3.2)	0
Df(1)A94/Dp(1)y^{2}Y67g24.2	67/133	56/123	13/52	-	-	-
Df(1)St472/Dp(1)y^{2}Y67g24.2	37/316	19/104	58/320	20 (25°C) 19 (29°C)	0 0	0 0
Df(1)RA19/Dp(1)y^{2}Y53T	173/401	36/259	3/40	58 (25°C)	6 (10.3)	2
Df(1)A94/Dp(1)y^{2}Y53T	85/73	12/97	14/45	63 (25°C)	1 (1.6)	3
Df(1)St472/Dp(1)y^{2}Y53T	0/78	0/80	0/147	-	-	-
Df(1)RA19/Dp(1)y^{2}Y22T	180/156	117/136	34/33	121 (25°C)	115 (95)	120
Df(1)A94/Dp(1)y^{2}Y22T	123/85	76/61	45/39	30 (25°C)	29 (97)	26
Df(1)RA19/Dp(1)y^{2}Y2E	36/24	75/82	-	18 (25°C)	17 (95)	17
Df(1)A94/Dp(1)y^{2}Y2E	29/29	50/69	-	31 (25°C)	29 (97)	28

* in F_2 from these cultures males of Df(1)RA19/Dp(1)y^{2}Y67g24.2 were again obtained which had viability and fertility reduced.

Table 3. Genetic mapping within 2AB region.

No. of experiment	Female genotype	No. of female progeny (N)	No. (n) & genotype of male crossovers	Distance between mutations (m.u.)	Confidence interval $2n_1/N \cdot 100\% - 2n_2/N \cdot 100\%$
1	2	3	4	5	6
1	$\frac{y^+ \quad t467 \quad wpn}{y \quad HM40 \quad w^a}$	30,811	28 (y $t467^+$ $HM40^+$ wpn)	0.181	0.130 - 0.244
2	$\frac{y^+ \quad t467 \quad wpn}{y \quad lt81 \quad w^+}$	105,432	4 (y^+ $lt81^+$ $t467^+$ w^+)	0.008	0.003 - 0.016
3	$\frac{y^+ \quad lt81 \quad wpn}{y \quad t324 \quad w^+}$	40,367	6 (y^+ $t324^+$ $lt81^+$ w^+)	0.030	0.014 - 0.055
4	$\frac{y^+ \quad t99 \quad wpn}{y \quad t467 \quad w^+}$	32,337	3 (y $t99^+$ $t467^+$ wpn)	0.019	0.006 - 0.043
5	$\frac{y^+ \quad t99 \quad wpn}{y \quad lt36 \quad w^+}$	47,347	5 (y^+ $lt36^+$ $t99^+$ w^+)	0.021	0.009 - 0.041
6	$\frac{y^+ \quad t99 \quad wpn}{y \quad t10 \quad w^+}$	100,090	1 (y $t99^+$ $t10^+$ wpn)	0.002	0.0002 - 0.008
7	$\frac{y^+ \quad t10 \quad wpn}{y \quad lt336 \quad w^+}$	103,934	8 (y^+ w^+)	0.015	0.008 - 0.026
8	$\frac{y^+ \quad t99 \quad wpn}{y \quad lt336 \quad w^+}$	105,452	2 (y^+ w^+)	0.004	0.0008 - 0.011
9	$\frac{y^+ \quad t99 \quad wpn}{y \quad lt35 \quad w^+}$	57,850	7 (y^+ w^+)	0.026	0.012 - 0.043

lethal effect but cause numerous phenotypic changes. This phenomenon is influenced by temperature: at 29°C the effect is greater, at 18°C it is practically absent. In this case lethals are likely to be partly overlapped by duplications (Table 1).

In the region 2B7-8 - 2B12 we could not induce mutations affecting viability but in males lacking this genetic material both viability and fertility are greatly reduced (such males possess deletions removing the bands 2B7-8 - 2B11-12 and duplications covering essential loci of the region including swi and involving the bands 1A - 2B7-8; see Table 2).

For this reason we place in this region (2B7-8 - 2B12) a factor of male fertility, fmf (Fig. 1b).

Given the genetic map of the region 2A-2B we do not solve the problem of the exact number of genetic loci, since not all of them could be detected by the methods of inducing mutations affecting viability used in the experiment. Moreover, genetic organization of locus o.c.c., having a complex complementation map, remains unknown (Belyaeva et al. 1980).

Genetic mapping of mutations was carried out according to a standard scheme: females heterozygous for two lethals and flanking markers were mated to males v^{74k} or ct v f. All the females in the progeny were alive and those males that were crossovers without lethals, i.e., half of all crossovers since a reciprocal class with two lethals died. If N is the number of females and n is the number of crossover males, then the distance between the lethals is equal to $2n/N \cdot 100\%$. The limits of confidence interval (n_1, n_2) were calculated in such a way that mathematical expectations were within it with a probability of 90%. Arrangement of the lethals involved was ascertained according to combination of the flanking markers.

Results of the recombinant analysis are presented in Table 3 and Fig. 1a. They demonstrate that locus swi is situated to the right od for and confirm the cytogenetic localization of the other loci. Thus we have found that mutation br (contained in o.c.c.) is placed to the left of dor in contradistinction to previous mapping of these mutations with respect to yellow (Lindsley & Grell 1968).

The recombinant analysis indicates considerable size of the locus o.c.c.: the smallest distance between alleles of o.c.c. is equal to 0.002 m.u. and the largest is equal to 0.028 m.u. Taking into consideration that crossing over takes place in the distal X end as many as 5-10 times rarer than in the central one we suggest the maximal locus size to be equal to about 100kb. However, these data are to be considered as preliminary since in experiments 7-9 all the crossovers obtained were sterile and their genotype was not examined by the progeny.

As a whole the complementation map of the locus o.c.c. is co-linear with the genetic map, but in experiment 8 (Table 3) two types of crossovers have been found, so the arrangement of groups br and rbp within the o.c.c. locus is unclear. Mapping of the o.c.c. locus is to be continued using different crosses.

The authors are grateful to M.O. Protopopov for help in this work.

References: Belyaeva, E.S. et al. 1980, Chromosoma 81:281-306; Lindsley, D.L. and E.H. Grell 1968, Carnegie Inst. Wash. Publ. 627.

Alexandrov, I.D. Research Institute of Medical Radiology, Academy of Medical Sciences of USSR, Obninsk, 249020, USSR.

The w^{spA} - a new allele of site 7 within the white locus of D. melanogaster and its genetics.

The white-spotted (w^{sp}) phenotype is relatively rare among spontaneous or induced white mutations in D. melanogaster. Only four w^{sp} alleles were described since 1944. Among them w^{sp2} is phenotypically darker than w^{sp1} (Lindsley & Grell 1968), but w^{sp3} (Green 1959) and w^{sp4} (LeFever 1973) are identical with w^{sp1}. Thus, only two phenotypically different w^{sp} alleles are known so far. Occupying site 7 of the locus in question, w^{sp} alleles are known to reveal two unique features, namely, to affect the expression of zeste (z) and to complement with the w nondeficient (point) mutations only (Green 1959). In spring 1976, recovered among a number of visible eye-color mutations induced on the X-chromosome of st c(3)G ca males by gamma-rays (4000 r) was the w^{76d} with darker and more speckled phenotype than that of w^{sp2}. Females with the genotype w^{sp1}/w^{76d} or w^{sp2}/w^{76d} have lighter speckled eye color than w^{76d}/w^{76d} females, indicating that w^{76d} is possibly an allele of site 7 dominating over all other w^{sp} alleles. The new allele was named the white-spotted of Alexandrov (w^{spA}). Assuming the w^{spA} to be a change of site 7, each would be expected to suppress z and to complement with w point mutations. Further genetic tests confirmed these expectations. First, heterozygotes y sc z

w^{spA}/+ + z + were found to have wild-type eye color, as was to be expected if w^{spA} suppresses z. Second, w^{spA} proved to complement with w point mutations only (see below). It is important to know what the complementary features of w^{spA} are in comparison with those of other w^{sp} alleles. To answer this question, quantitative estimates, more sensitive than visual observation, were applied. For this purpose the red eye pigment contents in w^{sp1}, w^{sp2} and w^{spA} homozygotes, in heterozygotes from crosses inter se, and in heterozygotes for different w mutations were determined by employing the spectrophotometric method, the details of which have been described elsewhere (Alexandrov & Soluyanova 1974). The photometric measurements were performed with a Spektromom 201 (Hungary) at a wavelength of 480 m u. The data are expressed as the extinction (E) per 10 heads extracted per 1 ml of solvent (30% AEA).

Table 1. Relative amounts of eye red pigment in w^{sp1}, w^{sp2} or w^{spA} homozygotes, in heterozygotes from crosses inter se, and in compounds with different w mutations.

w	w^{sp1}		w^{sp2}		w^{spA}	
mutations	M*	Conf. limits**	M	Conf. limits	M	Conf. limits
w^{sp1}	0.045	0.050-0.040	0.046	0.048-0.044	0.099	0.105-0.093
w^{sp2}	0.046	0.048-0.044	0.066	0.073-0.059	0.088	0.100-0.076
w^{spA}	0.099	0.105-0.093	0.088	0.100-0.076	0.158	0.161-0.155
w^{258-45}	0.034	0.048-0.020	0.049	0.065-0.033	0.052	0.064-0.040
w^{67e}	0.040	0.041-0.039	0.047	0.054-0.040	0.088	0.108-0.068
w^{72b}	0.037	0.042-0.032	0.037	0.045-0.029	0.049	0.057-0.041
w^{76b55}	0.030	0.033-0.027	0.024	0.028-0.020	0.035	0.036-0.034
w^{66g}	0.170	0.186-0.154	0.194	0.216-0.172	0.358	0.374-0.342
w^{67g}	0.068	0.072-0.064	0.098	0.107-0.089	0.179	0.184-0.174
w^{74k}	0.180	0.181-0.179	0.289	0.311-0.267	0.418	0.438-0.398
w^{79b6}	0.131	0.151-0.111	0.274	0.285-0.263	0.336	0.354-0.318

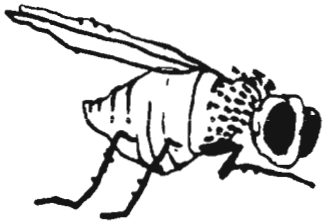
* means at least of 3-6 replicas
** confidence limits at $P_{0.05}$

The deletions for all (w^{258-45}) or a portion (w^{67e}, w^{72b}, w^{76b55}) of the white locus and w point mutations (w^{66g}, w^{67g}, w^{74k}, w^{79b6}) were used. All above-mentioned new w mutations excepting w^{79b6} as neutron-induced were obtained after gamma-ray irradiation of w^{+} alleles from D-32 stock. Besides, w^{76b55} and w^{66g} have proved to suppress z while all other w point mutations and microdeficiencies do not suppress z.

According to quantitative data (see Table 1) all wsp alleles tested show complementation with w point mutations only. It is important to note that, first, w^{sp} alleles under study differ from each other by extent of complementation and, second, in all cases the heterozygotes for w^{spA} have greater quantities of the red pigment than heterozygotes for w^{sp1} or w^{sp2}. It is essential, too, that w^{spA} distinctly complements (even by visual observation) with w point mutations such as w^{67g}, for which the complementation in compounds with other w^{sp} is questionable (particular under visual survey).

From these quantitative experiments it is apparent that w^{spA} is a new and less hypomorphic allele of site 7 within the white locus in D. melanogaster. Furthermore, it is obvious that the existence or lack of complementation is more clearly displayed in compounds with w^{spA} than with w^{sp1} or w^{sp2}. Therefore, w^{spA} seems to enable more precise visual discrimination of point alterations from minute rearrangements within the w locus.

References: Alexandrov, I.D. and Z.V. Soluyanova 1974, DIS 51:32; Green, M.M. 1959, Z. indukt. Abstamm. -u. Vererb. 90:375-384; LeFever H.M. 1973, DIS 50:109-110; Lindsley, D.L. and E.H. Grell 1968, Carn. Inst. Wash. Publ. 627.



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Comparative frequency and spectrum of w mutations induced by gamma-rays and neutrons in D. melanogaster.

0.3-0.4 MeV neutrons have both maximum LET (Kellerer and Rossi 1972) and RBE for such genetic end-points as chromosome damage in human lymphocytes (Sevankaev et al. 1979) or somatic mutations in Tradescantia (Underbrink & Sparrow 1974). Since visible mutations in plants are minute rearrangements rather than true gene mutations (Muller 1956; Mottinger 1970) the dependence of yield of gene mutations on the neutron energy remains obscure. In this context, it would seem worthwhile to report the RBE of 0.35 (dose-rate of 0.4 Gy/min) and 0.85 (dose-rate of 2.6 Gy/min) MeV fission neutrons (from reactor BR-10) for the gene and chromosome white mutations induced in mature sperm of D. melanogaster. The gamma-ray contamination of neutron flux was less 5%. As standard radiation a ^{60}Co source (Gammacell 220, Atomic Energy of Canada, Ltd.) was used with a dose rate of 6 Gy/min. In all experiments virgin males 3-4 days old from wild-type stock D-32 were irradiated and then individually mated to five virgin females of the genetic constitution y sc^{SI} dl49 sc^{8} w^{a}, b cn vg. The males were discarded after 24 h. The inseminated females were then permitted to lay eggs for two consecutive 3-day periods. Since different radiations under study may differently inactivate sperm including that with w mutations, the yields of the latter after treatment by neutrons or gamma-rays were investigated on the isoeffective doses yielding the same survival of zygotes, namely, the same averages of progeny from one male irradiated. According to preliminary data the isoeffective doses are about 10 and 40 Gy for neutrons under study and gamma-rays, respectively.

Among F_1 all w mutants were scored and first tested for fertility and viability in homo- or hemizygotes. Frequencies of sterile, lethal and viable w mutants induced by isoeffective doses of radiation studied and the RBE values established are presented in Table 1. It may be seen that at the same survival the RBE of 0.35 and 0.85 MeV neutrons for w mutations in toto are 7 and 3, respectively. Although the RBE values obtained for the three major classes of w mutations vary significantly, the effectiveness of 0.35 MeV neutrons is everywhere higher than that of 0.85 MeV neutrons. It is true for sterile and lethal w mutations, which are known to be determined predominantly by chromosome rearrangements, as well as for viable w mutations. The

Table 1. Frequencies of sterile, lethal and viable w mutations induced by gamma-rays and 0.35 or 0.85 MeV neutrons in Drosophila mature sperm and RBE values.

Radiation (dose) and RBE values	Total chromosomes studied	Classes of w mutations			
		Sterile	Lethal	Viable	Total
Gamma-rays (40 Gy)	32,325	1.16*(15)**	0.15 (2)	0.46 (6)	1.78 (23)
0.85 MeV n° (10 Gy)	21,349	2.34 (5)	1.41 (3)	2.34 (5)	6.09 (13)
RBE		2.0	9.4	5.1	3.4
0.35 MeV n° (10 Gy)	9,129	3.29 (3)	3.29 (3)	5.48 (5)	12.05 (11)
RBE		2.8	21.9	11.9	6.8

* All frequencies x 10^{-7} per locus per rad.
** In parentheses the number of mutations found is given.

latter are supposed to be a mixture of point (true gene) and chromosome mutations. To determine the share of gene mutations all viable w mutants were tested for fecundity, complementation with w^{spA} (a new allele of w^{sp} described elsewhere in this issue), suppression of z and normal recombination in the y —— spl region. According to these data (Table 2) five out of six of gamma-ray induced w mutants are true gene mutations since only one (w^{74b166}) appears to be a kind of rearrangement showing a marked decrease in recombination in the region studied and sterility in homozygotes as well. On the other hand, only one (w^{79b6}) out of 10 neutron-induced w mutants seems to be a true gene mutation, whereas all others reveal some properties of gross or minute chromosome rearrangements. The majority of those do not complement with w^{spA} and, therefore, may be deficiencies including all or a part of the white locus. Taking these

Table 2. Genetics of viable w mutants induced by gamma-rays and 0.35 or 0.85 MeV neutrons in Drosophila mature sperm.

Radiation	w mutants	Phenotype of ♂	Sterility of ♂ or ♀	Complementation with w^{spA}	Supression of z	Recombination in the y—spl region
Gamma-rays	w^{67a}	white	0*	+**	0	normal
	w^{67g}	white	0	+	0	normal
	w^{74b}	ecru	0	+	0	normal
	w^{74b166}	apricot	+	+	...	depressed
	w^{74d50}	white	0	+	0	normal
	w^{74d145}	white	0	+	0	normal
0.85 MeV n°	w^{79b6}	white	0	+	0	normal
	w^{79b8}	white	+	0	0	increased
	w^{79d4}	white	+	0	...	depressed
	w^{79d5}	white	0	0	0	normal
	w^{79d6}	white	+	0	...	depressed
0.35 MeV n°	w^{79b2}	white	+	0	...	depressed
	w^{79b3}	white	0	0	...	depressed
	w^{79b4}	white	0	0	...	Tr(X;2)
	w^{79b10}	white	+	0	...	depressed
	w^{79b11}	apricot	0	+	...	reduced

(* lack) and (** existence) of property under study, respectively

data into account, the intralocus mutation rate for gamma-rays and 0.85 MeV neutrons are 0.38 and 0.47 x 10^{-7} per locus per rad, respectively (i.e., RBE is 1.2), whereas 0.35 MeV neutrons appear not to be mutagenic (for induction of the true gene mutations) at all. 0.35 MeV neutrons are, however, more efficient than 0.85 MeV neutrons for inducing such genetic end-points as chromosome rearrangements. This observation on Drosophila is strictly comparable to the mammalian or plant cell results. Therefore, there is a different dependence of yield of gene mutations and chromosome rearrangements on neutron energy. In the light of the data described it is obvious that the intial alterations at the molecular level resulting in gene mutations and chromosome changes are essentially different from the radiation mutagenesis in the animal cells.

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Reversible analysis of genetic alterations at the molecular level in radiation induced white mutants of D. melanogaster.

According to one of the dogmas of molecular genetics the base-pair substitution mutations may just revert by means of new base-pair substitution and the frameshift mutations, by means of new frameshift (Auerbach 1976). Bearing this in mind, the analysis of molecular alterations in radiation-induced w point mutants of D. melanogaster was begun by means of induction of $w \rightarrow w^{+}$ reversions in somatic cells of eye discs, either with 1,4-bisdiasoacetyl butane (DAB) causing mainly GC——TA transversions (as had been shown on Actinomyces by Gumanov et al. 1966) or with a derivative of diazapiren (3,7-diamino-4,9-dioxy-5,10-dioxo-4,5,9,10-tetrahydrodiazapiren (DDDTDP) inducing clearly frameshift mutations (as had been identified in S. typhimurium by Abilev et al. 1979). Reversion as expected would result in a pigmented spot in the otherwise unpigmented eye. This approach has been successfully used for scoring both spontaneous intragenic recombinations (Stern 1969) and reverse mutations induced by alkylating agent (Mollet and Würgler 1974).

Table 1. The frequency of mosaic eyes in w mutants developed on media with DAB or DDDTDP.

Mutagene (conc.)	w^{66g}			w^{67a}			w^{67g}			w^{72b}			w^{79b6}		
	Females		Males	Females		Males	Females		Males	Females		Males	Females		Males
	n*/N**	%†	n/N	n/N	%	n/N	n/N	%	n/N	n/N	%	n/N	n/N	%	n/N
DAB (5 mg/vial)	13[a]		8[a]	1[a]		6[a]	11[a]		5[a]	0		0	6[a]		1[a]
	1[b]		1[b]	1[b]		0	4[b]		0	0		0	1[b]		0
	2[c]		2[c]	2[c]		0	2[c]		0	0		0	0		0
	1328	1.2	920	764	0.5	830	904	1.9	238	378	0.0	76	288	2.4	106
DDDTDP (0.5 mg/vial)	0		0	0		0	0		0	0		0	0		0
	1076	0.0	1092	770	0.0	774	792	0.0	842	444	0.0	434	492	0.0	574
Control	0		0	0		0	0		0	0		0	0		0
	1368	0.0	1460	1316	0.0	1276	1132	0.0	1068	438	0.0	442	440	0.0	438

	DAB			DDDTDP			Control		
	Females		Males	Females		Males	Females		Males
	%		%	%		%	%		%
	1.2		1.2	0.0		0.0	0.0		0.0
	0.5		0.7	0.0		0.0	0.0		0.0
	1.9		2.1	0.0		0.0	0.0		0.0
	2.4		0.9	0.0		0.0	0.0		0.0

* The number of eyes with the spots: a, 1-2 facets; b, 3-4 facets; c, more than 5 facets.
** The number of eyes examined.
† The frequency of mosaic eyes among all eyes examined.

Aqueous solutions of DAB (conc. 5 mg/vial) or DDDTDP dissolved in 2-3 drops of dimethylsulfoxide (conc. 0.5 mg/vial) were supplemented to media with mutant first-instar larvae. These concentrations of mutagenes used have been proved to increase significantly (by a factor of about 8) the frequency of sex-linked recessive lethals above that observed in untreated male larvae. The control cultures were similar to the treatment ones except that the mutagenes were omitted.

The w mutants used in the study represent an essentially unselected sample of four (w^{66g}, w^{67a}, w^{67g}, w^{72b}) gamma-ray induced and one (w^{79b6}) neutron-induced alterations at the locus in question. Genetics of w^{66g} (synonym w^{10gA}) have been described earlier (Alexandrov & Soluyanova 1974) and those of w^{67a}, w^{67g} and w^{79b6} are reported elsewhere in this issue (Alexandrov). All four of the named mutants have been proven to show themselves as point mutations. w^{72b} seems to be connected with a viable chromosome rearrangement including a deficiency since it does not complement with any of w^{sp} and reduces the recombination in the y——spl region markedly.

After eclosion, the eyes of imagoes were scored for the presence of colored spots under a dissecting microscope at a magnification of 25X. The coloration and sizes of spots in terms of number of eye facets involved were recorded as precisely as possible.

Three independent reversible experiments have been performed, the summarized data of which are presented in Table 1. It is seen that DAB but not DDDTDP produces eye mosaicism in w point mutants only. As in the DDDTDP series, no eye mosaics were recovered from three control experiments. The sizes of spots induced by treatment of DAB vary from 1-2 to 8-10 facets which had a maroon-like phenotypic appearance. However, the majority of spots (about 80%) consisted of 1-2 facets, which is remarkable since the primordial eye cells had been treated at the first instar. As a rule, each of the mosaic eyes had a single spot only. This allowed the comparative sensitivity of the different w mutants to induction of spots to be expressed as a share of mosaic eyes among all eyes examined. As will be seen from the table, the frequency of mosaic eyes in w point mutants ranges from 0.6% to 2.0% (means for the males and females in toto). It is of some importance that a doubling of the dose of w allele (in the genome of females) does not give a doubling in the frequency of spots. In

other words, the males are more sensitive to induction of eye spots than females. This higher susceptibility of males relative to females is in keeping with previous observations on the production of single spots by alkylating agent (Mollet & Würgler 1974). It is important to note that eye mosaic spots are lacking in w^{72b} mutants treated by an active inductor as DAB. That was to be expected if w^{72b} is a rearrangement connected with the deletion of all or part of the w locus. In such a case, the appearance of eye spots in the w point mutants tested may be regarded as manifestation of reverse events. If so, the w point mutations under study must bring about base-pair substitutions rather than frameshifts. However, the assumption that the eye spots induced by treatment of DAB may be morphosis of all kinds rather than phenotypic reflection of genetic events at the molecular level cannot be ruled out at once. Therefore, more direct and independent proofs of a complete or partial restoration of the wild-type gene function via histological studies of mosaic spots must undoubtedly be obtained. Such investigations are in progress now.

Grateful acknowledgments are made to Dr. A.M. Serebryany for DAB and to Dr. S.K. Abilev for DDDTDP they put at my disposal.

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Alexandrov, I.D., S.E. Alexandrova and M.A. Ankina. Research Institute of Medical Radiology, Academy of Medical Sciences of USSR, Obninsk, 249020, USSR. Formaldehyde-induced lethality and mutagenesis in Drosophila males defective in DNA metabolism.

The known phenomenology of formaldehyde-induced inactivation and mutagenesis (Auerbach et al. 1977) allows us to suppose that induction of both lethal and premutational lesions in DNA as well as repair and/or damage fixation are the most significant stages in the mechanisms of the action of formaldehyde (FA) on living cells. However, the true nature of initial lesions and repair systems is obscure so far. It is hardly possible to assume a priori that the mechanisms of lethal and mutagenic actions of FA are identical or that they would work in the same way in somatic cells as in germ cells. On the other hand, this possibility cannot be dismissed a priori, either. Comparative analysis of both lethal and mutagenic action of FA in repair-deficient mutants might be one of the means to make further progress in this field. Proceeding from this consideration, investigations on such Drosophila mutants are being carried out. Preliminary results of experiments with single mei-9 (defective in excision repair), mei-41 (defective in postreplication repair), and mus 102 (defective in nonidentified repair) mutants as well as with the mus 102/mei-41 double mutant are reported in the present communication. Besides their deficiency in DNA repair the mutants in question appear to be defective in other pathways of DNA processing (Baker et al. 1976).

Comparative FA-food inactivation and mutation induction in male larvae of the following genotypes were studied: (1) wild-type D-32, (2) wild-type Hikone R, (3) mei-9ATI, (4) g^2 mei-41^{AI} f, (5) mus 102^{AI} cv v f car, and (6) y mus 102^{AI} g^2 mei-41^{AI} f. Five pairs of 2-3 day old flies were mated (nos. 3-6 males were mated with y f:= females) in vials containing a standard semolina-sultana-golden syrup-yeast-agar media supplemented with the subtoxic (for wild-type stocks) concentration of FA (2.0×10^{-2} M). After 48 h, the parents were removed from the vials and every two days another 0.1 ml of FA of the same concentration was added to each of the vials with larvae. The cultures were maintained at 25°C. Three sets of experiments including altogether 8-10 vials for each of the genotypes were performed. The lethal action of FA was estimated by means of number of males emerging from one vial. Every two days after emergence FA-treated males were mass mated to y sc^{SI} d149 sc^8 w^a, b cn vg females which were allowed to oviposit for three days, after which the parents were discarded. The emerging F_1 flies were inbred pairwise in vials. After 14 days, the F_2 progeny were scored for the presence of patroclinus males. Vials with no such males were classified as lethals. In each case the presence of heterozygous females was verified in the F_3. Vials with no progeny at all (indicating sterility of the F_1 females) were only counted as steriles when both parents were still alive at the time of scoring.

Table 1. Egg to adult male viability, sterile F_1 females and frequency of sex-linked recessive lethals in male germ cells of genotypically different larvae developed on media with 2.0×10^{-2} M FA.

Genotype of male larvae	Means of males emerging from 1 vial and 95% conf. limits	Mean percent of sterile F_1 females and 95% conf. limits	Mean percent of lethals in the F_2 and F_3 and 95% conf. limits		
			T*	F_2	F_3
1. D-32	13.7 10.5 - 16.9	8.3 6.5 - 10.1	830	1.93 (16)** 0.98 - 2.86	1.32 (11) 0.54 - 2.10
2. Hikone R	24.7 18.5 - 30.9	6.4 4.8 - 8.0	854	1.76 (15) 0.86 - 2.66	0.82 (7) 0.20 - 1.44
3. mei-9ATI	27.6 20.6 - 34.6	4.7 3.3 - 6.1	858	1.98 (17) 1.04 - 2.92	0.93 (8) 0.29 - 1.57
4. g^2 mei-41AIf	0.0	-	-	-	-
5. mus 102^{AI}cv v f car	1.6 0.8 - 2.4	16.9 13.8 - 20.0	476	26.26 (125) 22.24 - 30.28	2.31 (11) 0.95 - 3.67
6. y mus 102^{AI} g^2 mei-41^{AI} f	1.3 0.5 - 2.1	12.7 9.7 - 15.7	426	9.39 (40) 6.57 - 12.21	1.88 (8) 0.58 - 3.18

* Total number of the X chromosomes tested.
** In parentheses the number of mutations found is given.

The results of cytotoxic tests (see Table 1) indicate that both mus 102AI single-mutant larvae and mus 102AI/mei-41AI double-mutant larvae are equally much more sensitive to killing by FA treatment than larvae of two nonmutant strains studied. The mei-9ATI and mei-41AI larvae display a directly opposite pattern of sensitivity, namely, the former show wild-type level of sensitivity, whereas the latter are dramatically eliminated at 2.0×10^{-2} M FA. For this reason the mutagenic response of mei-41AI to FA concentration employed was not subject to investigation. The mutagenic responses of other mutants, as seen in Table 1, appear to be the same and that which wild-type strains display if the frequencies of sex-linked recessive lethals observed in the F_3 are taken into account. Therefore, the sensitivity of mus 102AI to lethal action of FA does not correlate with that of mutagenic action of aldehyde in question. This implies that mus 102AI identifies the pathway for repair lethal rather than mutagenic lesions induced by FA. Further, it is important that the excision pathway identified by mei-9ATI seems not to be essential for repair of FA-induced premutational lesions as well. Thus, taking into account the lethals verified in the F_3 at least two (mei-9ATI and mus 102AI) in the three repair pathways under study appear not to be involved in FA-food mutagenesis. Further experiments are currently underway to study the mutagenic response of mei-41^{AI}, being deficient in a postreplication repair, at more low concentrations of FA.

Two findings require additional comments. First, the higher frequency of sterile F_1 females among the progeny of FA-treated mus 102AI males (see Table 1) implies that FA induces just one more kind of genetic lesion in larval spermatocytes, which appears to be abolished by the mus 102AI repair system. Second, when comparing the frequencies of lethals observed in the F_2 and F_3, it is obvious that some of the mutations are unstable, namely, they are found in the F_2 and not transmitted to F_3. The number of such lethals is much greater in the progeny of mus 102AI males than in those of other males tested.

Therefore, the genetic instability induced by FA seems to be abolished by the mus 102^{AI} repair system as well. In general, the results obtained show that repair mechanisms of fixations of FA-induced lesions leading to cytotoxic and mutational effects are clearly different.

Grateful acknowledgment is made to Prof. P.D. Smith, Emory University, for supplying the repair-deficient mutants under study.

References: Auerbach, C., M. Moutschen-Dahmen and J. Moutschen 1977, Mutation Res. 39: 317-362; Baker, B.S. et al. 1976, Proc. Natl. Acad. Sci. USA 73:4140-4144.

Alexandrov, I.D. and Yu.A. Siomin.
Research Institute of Medical Radiology, Academy of Medical Sciences of USSR, Obninsk, 249020, USSR. Cyto- and genotoxicity of formaldehyde and products of its interaction with lysin and ATP for different genotypes of D. melanogaster.

Lethal and mutagenic actions of formaldehyde (FA) added to the yeast media of Drosophila larvae have been known for a fairly long time (Rapoport 1947). Although the reaction of FA with adenylate in the food seems to be essential and sufficient in the mediation of the mutagenic activity of FA (Alderson 1961), the formed monomethylol derivative of adenosine is as such highly labile (Auerbach et al. 1977). However, the products of the reaction of FA with amino acids have proved to react with nitrogen bases two orders of magnitude as active as FA (Siomin et al. 1974). Among formed compounds, the product of the interaction of FA with lysin and ATP (named as AFAL) is exceptionally stable (Poverenny et al. 1978) and may bring about the biological effects of FA. Proceeding from this consideration, comparative analysis of lethal and mutagenic actions of FA and AFAL in the different genotypes of D. melanogaster was undertaken.

AFAL was selcted by means of paper chromatography ("Whatman 3", W&R Balston, Ltd., England) of a mixture containing 2.5 x 10^{-2} ATP, 0.4 M lysin and 0.1 M FA in 0.1 M sodium phosphate buffer (pH 6.8) which was dissolved in n-butanol/formic acid/water (7.1:1:1.3) solution. Before chromatography the mixture was incubated for 72 h at 50°C.

The effects of FA (conc. 2.0 - 4.0 x 10^{-2} M) and AFAL (conc. 0.9 - 1.5 x 10^{-3} M) added to a standard semolina-sultana-golden syrup-yeast-agar media on egg to adult viability were first studied for the following genotypes: (1) D-32 wild-type strain, (2) y sc^{SI} d149 sc^{8} w^{a} (M5), (3) hybrids of ♀ D-32 x ♂ M5, (4) y, (5) w, and (6) b cn vg. Five pairs of 2-3 day old flies were mated in the vials containing media supplemented with different concentrations of FA or AFAL. After 48 h, the parents were removed from the vials and every two days another 0.1 ml of FA or AFAL was added to each of the vials in the concentrations initially used. Control cultures were developed on a standard media unsupplemented by chemical agents.

The results of these cytotoxic tests are presented in Table 1 and merit the following conclusions. First, with increase in concentration of FA or AFAL there is increasing lethality of the larvae. Second, under the same survival the sensitivity of different genotypes to AFAL appears to be 13-20 times as high as that of genotypes to FA. Third, among genotypes studied the b cn vg was found to display the greatest sensitivity to both agents in question. It is important to note that both FA and AFAL cause a stop of ontogeny and lethality on the same developmental stage (mainly larval). However, the former was found to stretch the development of surviving offspring up to 20 days, whereas the latter does for 1-2 days only.

To test the mutagenic activity of FA and AFAL, the hybrid males from crosses of D-32 females to M5 males surviving on the media supplemented with FA (2.0 x 10^{-2} M) or AFAL (1.3 x 10^{-3} M) were individually mated to five y sc^{SI} d149 sc^{8} w^{a}, b cn vg females, so that tests for visible mutations at the y, w, b, cn and vg loci and sex-linked recessive lethals could be carried out simultaneously. Fresh females were provided for males treated every day to produce four successive broods of offspring. Lethals were scored in the F_2 with the aid of standard procedures and were verified in the F_3.

The pooled data for all broods studied (first to fourth day of mating) are presented in Table 2 and are essentially self-explanatory. As seen, no visible mutations at the selected loci appear to be induced by FA or AFAL in the concentrations tested. On the other hand, growth of the larvae on media supplemented with 2.0 x 10^{-2} M FA or 1.3 x 10^{-3} M AFAL, which produce comparable survival, has significant effects (compared to control) on inducing the amount of sex-linked recessive lethals if the latter according to tradition in the F_2. Moreover, mutagenic activity of AFAL is fivefold as small as that of FA. However, according to F_3 data AFAL does not reveal the mutagenic effect at all, whereas in experiments with FA the frequency of lethals is still one tenth as low as that of control. Therefore, the frequency of transmitted

Table 1. Egg to adult viability of different genotypes of D. melanogaster on the media supplemented by different concentrations of FA or AFAL.

Genotypes	Means* of adults emerging from one vial					
	Control	FA (final conc. in vial x 10^{-2} M			AFAL (final conc. in vial x 10^{-3} M	
		2.0	3.0	4.0	0.9	1.2	1.5
1. D-32	109.4 ± 9.2	36.0 ± 9.8	0.0	0.0	49.6 ± 3.8	23.6 ± 7.1	19.6 ± 3.2
2. M5	83.7 ± 6.3	20.1 ± 4.1	0.0	0.0	44.1 ± 2.1	19.4 ± 3.2	14.5 ± 3.5
3. Hybrids (D-32/M5)	168.2 ± 7.4	59.5 ± 13.7	6.5 ± 1.6	0.0	51.0 ± 5.7	21.0 ± 5.7	7.0 ± 2.8
4. y	154.0 ± 6.9	14.6 ± 5.3	0.0	0.0	34.0 ± 4.6	24.3 ± 4.0	13.6 ± 3.8
5. w	193.0 ± 14.2	8.4 ± 2.1	0.0	0.0	65.6 ± 4.5	27.0 ± 4.6	19.6 ± 4.7
6. b cn vg	135.5 ± 14.6	4.0 ± 1.4	0.0	0.0	15.3 ± 6.1	7.0 ± 3.0	0.0

* Means of 4-8 vials.

Table 2. Frequency of visible mutations and sex-linked recessive lethals in male germ cells of D-32/M5 hybrid larvae developed on media containing FA (2.0 x 10^{-2} M) or AFAL (1.3 x 10^{-3} M).

Genetic tests	Control		FA		AFAL	
	T*	% mutations and 95% conf. limits	T	% mutations and 95% conf. limits	T	% mutations and 95% conf. limits
y, w, b, cn and vg mutations	78,917	0.0 (0)**	28,467	0.0 (0	69,723	0.0 (0)
Lethals observed in the F_2	3,989	0.12 (5) 0.11 - 0.13	819	3.42 (28) 2.16 - 4.68	2,443	0.65 (16) 0.62 - 0.68
Lethals observed in the F_3	3,989	0.075 (3) 0.066 - 0.084	819	0.85 (7) 0.21 - 1.49	2,443	0.041 (1) 0.033 - 0.049

* Total number of the chromosomes tested.
** In parentheses the number of mutations found is given.

lethals induced by AFAL, if it is not zero, is one or probably more orders of magnitude lower than with doses of FA that produce comparable survival. As seen in Table 2, 15 out of 16 AFAL-induced lethals were not transmitted from F_2 to the next generation, i.e., all of those are actually unstabilized. It is essential to note that a definite amount of lethals (up to 75%) induced by FA are unstable as well. Since the levels of instability for the two compounds under study are quite different this implies that instability must be taken into account in comparative studies and the lethals observed in the F_2 must be verified in the F_3 at least.

The data described allow us to assume that lethal action of FA on Drosophila larvae may be brought about by AFAL since (1) AFAL is more effective in producing larval death than FA and (2) the lethal action of both FA and AFAL on the different genotypes is similar. However, the mutagenic action of FA seems to be hardly exerted by AFAL, the lethal action of which was found to be accompanied little if at all by induction of the transmitted mutations. Therefore, the mechanisms of lethal and mutagenic action of FA are assumed to be quite different. It is important that the lethal action of FA (via AFAL, probably) is clearly defined by genotypically predetermined cell metabolism. It is possible that the observed high sensitivity to killing of the b cn vg larvae to both FA and AFAL could be the consequence of some defect in repair of lethal damage that is intrinsic in the genotype in question. To test more precisely the role of repair systems in the formation of lethal and mutagenic effects of FA in Drosophila, experiments with some repair-deficient mutants were undertaken and the results are reported elsewhere in this issue.

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Band, H.T. and R.N. Band. Michigan State University, East Lansing, Michigan. Induced cold hardiness in D. melanogaster third instar larvae.

The susceptibility of D. melanogaster larvae to subzero treatment is well established in the literature. We have found this to be the case also in contrast to the cold hardiness of C. amoena larvae (Band & Band 1980a,b). Comparison of the recovery rates after 22 hrs at -2°C for Missouri C. amoena (woods) third instar larvae with that of Michigan C. amoena (apples), D. algonquin and D. melanogaster third instar larvae, all from E. Jordan, gave the expected result that Michigan C. amoena larvae recovered the fastest, the surprising result that some D. melanogaster larvae not only recovered, one pupated and emerged before D. algonquin larvae pupated. The experiment was repeated with younger D. melanogaster third instar larvae. Again the order of recovery after 22 hrs at -2°C was Michigan C. amoena, Missouri C. amoena, Michigan D. algonquin; D. melanogaster did not recover. All had come from cultures maintained at room temperature, 22°C, and had been grown on high protein chymomyzid medium (Band 1981) several months to over a year.

Timed emergence sequences in a controlled environment of 18 hr light, 22°C demonstrated that larvae had to be 6 to 7 days old to recover from -2°C; pupation begins on day 8 in this environment. More, however, appeared able to recover and pupate than emerge when grown under the long light, 22°C conditions. Adults emerging from larvae grown at room temperature and subjected to -2°C failed to be fertile, however. Cultures at room temperature were then subjected to overnight chilling in a refrigerator for three consecutive nights before larvae were transferred to -2°C for 22 hours. Supercooling points made on 5 larvae per culture for each of the 3 days indicated no difference between room temperature cultures and those at room/8°C. Accumulated data are given in Table 1, including the number of trials. Oregon-R controls grown on regular Drosophila medium are included. Table 2 summarizes the results for Michigan D. melanogaster larvae recovering from -2°C, pupating and emerging as adults after treatment at -2°C as late instars.

Glycerol/polyol determinations were made in 10 larvae each for E. Jordan and Lansing 1979 and 1980 stocks after 5 days at 4°C (temperatures in the refrigerator may vary considerably). Values ranged from 0.002 to 0.004 mg/mg larval weight, again in agreement with determinations made previously for this species (Band & Band 1980a,b).

Walker and Williamson (1980) and Walker et al. (1980) have found that the leucine amino-peptidase-D enzyme in D. melanogaster is activated in the presence of protein in the diet.

Table 1. Emergence of D. melanogaster adults after -2°C treatment at the late third instar stage for 18 to 22 hours. All grown on chymomzid medium at least several months except Oregon R, maintained on drosophila medium.

Source	No. trials	Environment	Supercooling point	No. larvae	No. emerge
Oregon-R	2	room (22°C)		54	0
Lansing	3	room		68	9
	2	18L/22°C	-12.8 ± 0.67	30	2
	1	room/8°C	-12.8 ± 1.51	15	0
Rose Lake	1	18L/22°C	-13.8 ± 1.04	15	0
	1	room/8°C	-13.7 ± 0.67	15	4, fertile
E. Jordan	2	18L/22°C	-13.0 ± 0.78	10	0
	2	room	-13.0 ± 0.86	20	1

Supercooling points represent the mean for 5 larvae prior to the -2°C treatment.

Table 2. Summary for Michigan D. melanogaster larvae subjected to -2°C.

	Room	18L/22°C	Room/8°C	Total
no. larvae	88	55	30	173
no. adults emerging	10	2	4	16
%	11	3.6	13	9.2

This probably accounts for the "acquired" ability for the late instar D. melanogaster larvae to recover from -2°C after 18-24 hours; 48 hours is lethal. However, the data offer no support that cold hardiness is a single step event. Age of larvae as well as diet appear to be important while a period of chilling prior to cold treatment seems necessary to insure subsequent adult fertility. In all environments tested, many more larvae appear able to recover, become active and pupate than can successfully complete development and emerge.

Throckmorton (1975) has postulated that diversity of feeding habitats characterized primitive drosophilids. The ability of Missouri C. amoena (woods) to use apples as a food source and the cold hardiness induced in D. melanogaster larvae on a high protein diet as might be found in nut breeders support Throckmorton's hypothesis. Results also indicate that diet can have unexpected physiological consequences.

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Band, H.T. and R.N. Band. Michigan State University, East Lansing, Michigan. C. amoena: freeze-tolerant, supercooling or both?

Insects which supercool in winter to avoid freezing typically have a supercooling point (SCP) lower than the freezing point (FP). The SCP is also the lower lethal temperature (LLT). Insects which are freeze-tolerant in winter typically have an elevated SCP (above -10°C). The SCP is also identical to the FP. The LLT may be considerably below the SCP. Freeze-tolerant insects may be freeze-susceptible in summer and insects which supercool in winter to avoid freezing may also vary the SCP seasonally. Insects which utilize proteins for cryoprotectants may be in either category; adults, larvae or both have been found to be overwintering stages (Zachariassen & Hammel 1976a,b; Duman 1979).

C. amoena overwinters as a larva, uses protein to achieve cold hardiness (Band 1981; Band & Band 1980a,b; Zachariassen, pers. comm.) and data obtained in the laboratory on larvae grown on chymomyzid medium indicated that it supercooled. Larvae obtained from apples in summer, 1981, both in Lansing and E. Jordan indicate this species may be either freeze-tolerant or supercool in contrast to D. algonquin larvae also in the same E. Jordan apples which have displayed only the freeze-tolerant characteristic of the FP = SCP. In particular C. amoena

larvae taken from near the apple seeds or from heavily parasitized apples have an SCP = FP; this is also the lower lethal temperature in summer.

Determinations were made in a freezing chamber cooling to -25°C. Individual larvae were attached by vaseline (Somme 1964) to the probe of a Model YS142SC telethermometer which was connected to a Sargent Recorder Model SF. Cooling was at the rate of 2 to 3°C per minute, comparable to Tucic's (1979) studies on egg, larval and pupal stages of D. melanogaster. Larvae were 4-5 mm in length, enabling D. melanogaster, D. algonquin and C. amoena larvae to be distinguished in the apples collected at E. Jordan.

In Lansing C. amoena adults were seen on small green fallen apples on a farm 7/5, apples collected 7/8, inspected for C. amoena eggs then held in the laboratory at 22°C, collected again on the farm 7/27 and the FP and SCP determined for some larvae the same day. At E. Jordan apples collected 7/18 had few C. amoena eggs, were collected again at two sites on 8/18 and 8/20, three sites on 8/29. Apples from one site were dissected 8/17 and appropriate sized larvae measured. Appropriate sized larvae were obtained from two sites in the 8/29 collection. Data for C. amoena larvae are given in Table 1, for D. algonquin larvae in Table 2.

Table 1. Freezing points and supercooling points obtained for "freeze-tolerant" and supercooling C. amoena larvae in Lansing and E. Jordan apple collections.

			Summer, 1981 "Freeze-tolerant"		Supercooling		
Site	Date	Measured	FP = SCP	n	FP	SCP	n
Lansing	7/08	7/28	- 7.1 ± 0.25	4			
	7/27	7/27	- 7.4 ± 1.7	5	-11.9 ± 1.4	-12.4 ± 1.3	4
	7/31	7/31	- 7.0 ± 0	2			
	9/14	9/25	-12.0 ± 2.8	2	- 9.5	-11	1
E.J., D	8/16	8/17	- 7	1	-10.8 ± 2.5	-12.2 ± 2.8	3
	8/20	8/21	-12.2 ± 3.9	5			
E.J., V	8/29	8/31	- 7	1	-10.9 ± 1.6	-12.0 ± 2.8	2

Table 2. Freezing/supercooling points obtained for D. algonquin larvae at two sites in apples shared with C. amoena larvae at E. Jordan, MI.

Site	Date	Measured	FP/SCP	n
D	8/16	8/17	- 7.0 ± 0	2
	8/20	8/21	-12.3 ± 4.6	3
V	8/29	8/31	- 6.0	1

At E. Jordan and Lansing sites, some C. amoena larvae give evidence of supercooling, more of relying on freeze-tolerance mechanisms to survive winter, as previously hypothesized (Band 1981). The difference appears to be due to a dietary component, either naturally available or added by larvae of pest species since C. amoena larvae taken from near the seed or from heavily parasitized apples have an FP = SCP. Baust et al. (1979) have shown that different species of insects within the same ball gall hibernaculum may manifest different overwintering strategies. Here a single species seems to have two different mechanisms.

In July in Lansing values range between -5 and -9 for larvae whose FP = SCP. This is also true for both C. amoena and D. algonquin larvae at E. Jordan in mid-August. In the last third of August, this dropped to -12 for both at D site, which is less well protected than V site. D. algonquin behaved similarly to the potentially freeze-tolerant C. amoena on all three collecting dates. At the Lansing site, the SCP for the potentially freeze-tolerant group had dropped to -12 also by mid-September.

Therefore if results from larval collections in these two areas 200 miles apart can be generalized for mid- to northern lower Michigan, C. amoena larvae begin fall with an average SCP of -12 whether by supercooling or by potentially freeze-tolerant mechanisms. Since average SCPs of -18.7 ± 3.68 and -19.5 ± 1.96 have been reached in the laboratory by E. Jordan September 1979 and Lansing March 1980 lines respectively after storage at 5 and 10°C for longer than two weeks, what the average maximum SCP or lower lethal temperature for populations in either locality may be remains unknown. Glycerol/polyol determinations for the E.J. set after four weeks at 5°C when the SCP averaged -18.7 ± 3.68 did not differ from previous results.

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s6; _____ and _____ 1980b, Experientia 36:1182-1183; Baust, J.G. et al. 1979, Physiol. Zool. 52:572-580; Duman, J.G., J. Insect. Physiol. 25:805-810; Somme, L. 1964, Can. J. Zool. 42: 87-101; Tucic, N. 1979, Evolution 33:350-358; Zachariassen, K.E. and H.T. Hammel 1976a, Nature 262:285-287; _____ and _____ 1976b, Norw. J. Zool. 24:350-352.

Banerjee, S. and P.K. Chinya. University of Calcutta, India. Effect of Mitomycin C on polytene chromosome replication of Drosophila.

Earlier reports on the Dipteran polytene chromosomes confirmed the replication process to be a temporally ordered sequence and replicating units as autonomous with respect to their termination (Häegele 1970; Mukherjee et al. 1973; Chatterjee & Mukherjee 1975; Mukherjee et al. 1975, 1976). Mitomycin C (MC), the antibiotic, inhibits DNA synthesis by introducing cross-links in the DNA chains at the G-C sites, at the initial stage of replication cycle. It may, therefore, play a significant role in interfering with the initial as well as terminal phase of the replication cycle. For this we took up the present series of investigations on larval salivary glands of D. melanogaster, by feeding them with the antibiotic.

Late third instar larvae of D. melanogaster were separated into two batches, one batch fed for 2 hours on MC-treated Drosophila culture medium (conc. 0.1 ml MC/5 gm of food and 0.4 ml MC/5 mg of food), and the other on normal medium. They were then sacrificed for autoradiography.

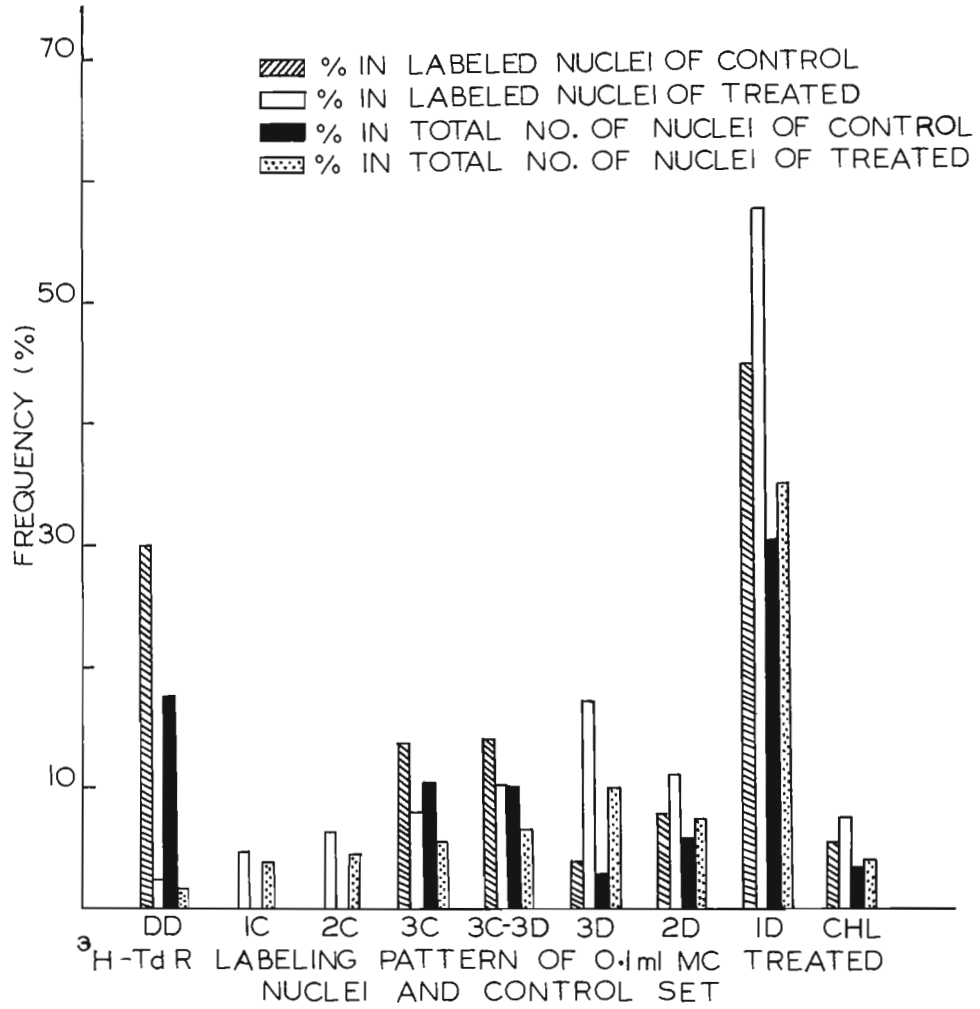

Fig. 1.

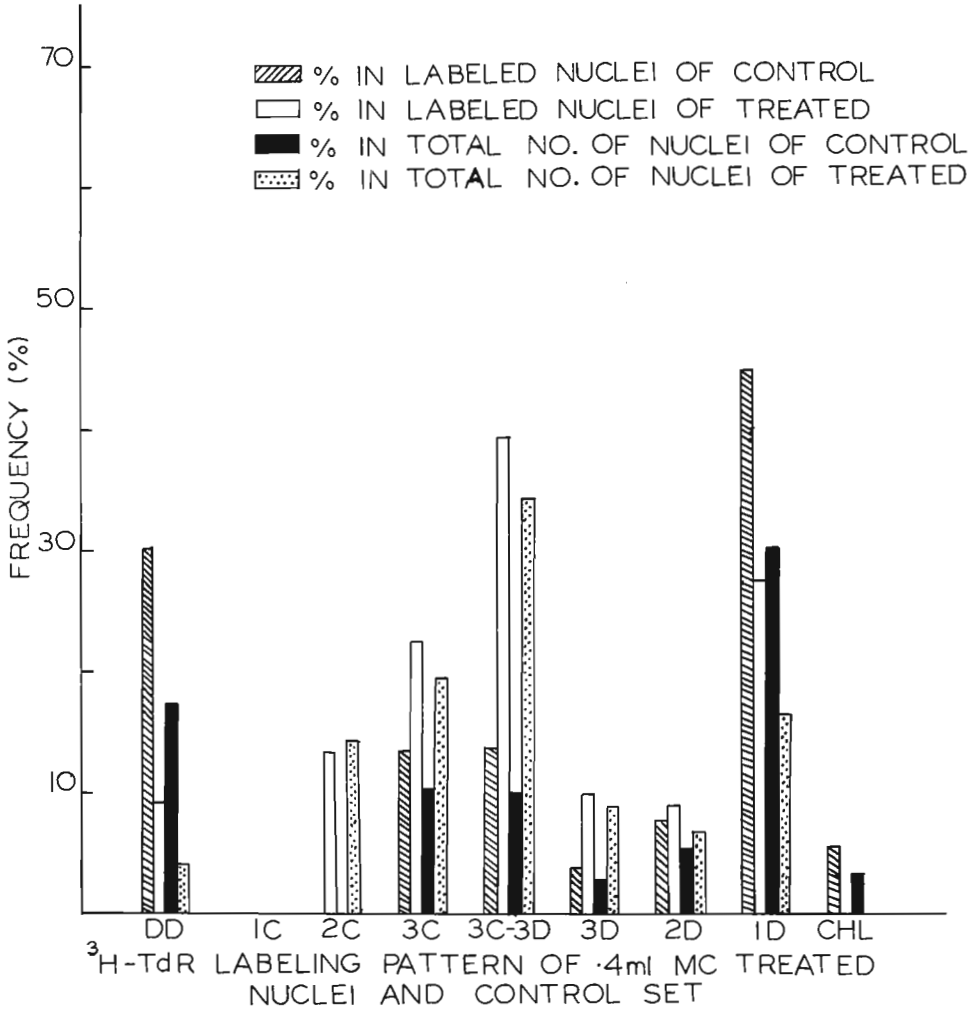

Fig. 2. Frequency (%) of ^{3}H-TdR labelling pattern of control as well as 0.4 ml treated nuclei of mitomycin C (MC).

In Drosophila, the ^{3}H-thymidine labelled polytene chromosomes, the following types of replication pattern is observed: "DD" (dispersed discontinuous), 1C, 2C, 3C (continuous, with increased labelling frequency and intensity), 3C-3D, 2D, 1D (discontinuous, with decreased labelling frequency and intensity), CHL (chromocenter labelling). It can be observed (Figs. 1 and 2) that relative frequencies of the "DD" types among the labelled nuclei, as well as within the total number of nuclei was much higher in the control than treated series of glands. It has also been observed that relative frequency of 1D pattern in 0.1 ml of MC-treated nuclei was very high, but remarkably decreased in the case of 0.4 ml of MC-treated nuclei. On the other hand, there was a net increase of 3C-3D type of nuclei in the 0.4 ml MC-treated series. The absence of CHL-labelling in 0.4 ml of MC-treated group may be attributed to comparatively less numbers of nuclei recorded. It is evident (Figs. 1 and 2) that "DD" is drastically reduced in treated nuclei. Now if "DD" type had been the late pattern of the cycle, then there is no reason for such decreased frequencies. At the same time "D" type of nuclei cannot be the initial phase, as we did not observe any reduction in this group; rather we observed a net increase in treated series for this type. So the results obtained in the present set of experiments provide evidence in favor of the DD-C-D sequence of replication in polytene chromosomes of Drosophila.

This work was supported by a U.G.C. Junior Research Fellowship to S. Banerjee.

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Barclay, H. Pacific Forest Research Center, Victoria, B.C., Canada. Yeast quality and the fertility of D. melanogaster.

It is common knowledge that yeast is of major importance in the diet of D. melanogaster (Chiang & Hodson 1950; Demerec 1950). Usually live yeast is added to the prepared food as a nutritional supplement (Demerec & Kaufmann 1973) but it is quite possible to maintain healthy cultures using only the pulverized yeast which is normally added to the food medium during the cooking process. The quality of the yeast in the latter case, however, must be adequate nutritionally. This was graphically illustrated recently in some evolutionary experiments of mine. In these experiments, described elsewhere (Barclay & Gregory, 1981), I took measurements on age-specific survivorship and fertility of the flies. Twenty 2-day-old flies (ten males and ten females) were put into each of 16 jars with medium and transferred to new jars with fresh medium every four days. The flies were counted at each transfer and the resulting offspring were counted about 15 days after each transfer until all of the original 320 flies had died.

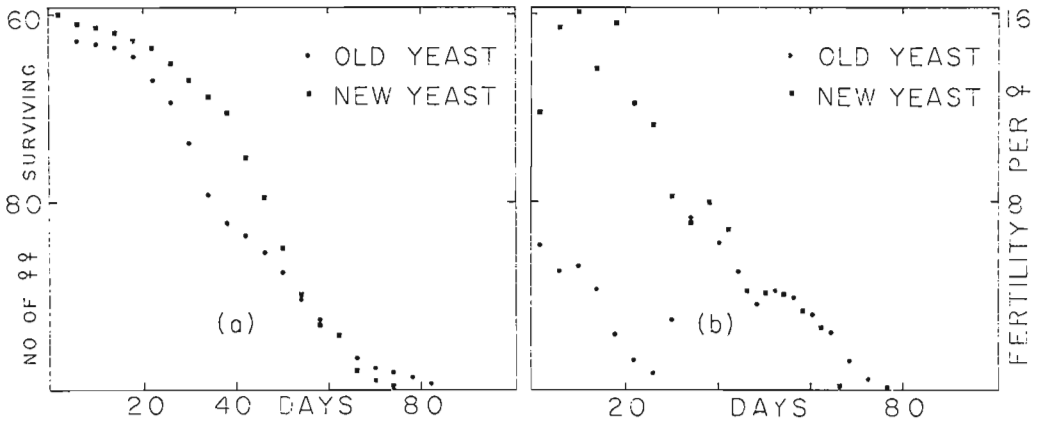

Fig. 1. Survivorship and fertility curves for D. melanogaster fed on old yeast and on new yeast. (a) Survivorship of the females. (b) Production through eclosion per female per day.

Table 1. Demographic data on flies fed on old yeast and on new yeast. Each experiment resulted from 160 males and 160 females distributed evenly among 16 jars.

Age of yeast	Total adult production	Net reproductive rate	$e_o(\sigma)$*	$e_o(♀)$
old, then new	24,644	77.01	46.85	38.90
new only	80,062	250.19	53.98	44.37

*e_o is the life expectancy in days at eclosion.

Throughout the experiments I used only pulverized yeast obtained from our departmental supply store. Their supply was ordered in bulk and was used only by the genetics class and myself; prior to my measurements their most recent shipment of yeast (Schwartz-Mann #1762) had been received four years previously. During the course of my measurements the yeast supply became exhausted and new yeast had to be quickly obtained. I started using the new yeast (Fisher Y1) on the 20th day of the measurements and about one week later the fertility increased dramatically (Fig. 1). A subsequent repeat of the measurements using only the new yeast produced a fertility curve more in accord with commonly observed fertility curves (Fig. 1). I had no reason to believe that the physiological condition of the flies in the second set of measurements was any different from that of those in the first set other than as a result of nutrition due to the newer yeast; care was taken to duplicate all other conditions as closely as possible. The major demographic effect of yeast age observed was a reduction in fertility (Fig. 1; Table 1). Life expectancy was also reduced with older yeast (Table 1) but not nearly as drastically as was fertility.

I feel that the very different results obtained from the two yeast stocks can be attributed to their different ages rather than to the fact that they were different brands. Obviously in any laboratory devoted to Drosophila culture, this problem is unlikely to arise due to the volume of yeast used. For casual or intermittent users, however, the problem of yeast quality may well determine the outcome of experiments which measure life history features.

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Fruitless experiments with fruit flies: the "sex-ratio" chromosomes of D. pseudoobscura.

The "sex-ratio" (SR) trait is of interest to many population geneticists. SR is an X-linked condition with three non-overlapping inversions in D. pseudoobscura. Progeny of male carriers consist almost entirely of females (95-100%), but there is no apparent segregation effect in female carriers. There are many unresolved problems concerning the mechanism of SR action, its genetic basis, and its maintenance in natural populations at non-trivial frequencies. Several possible experiments spring immediately to mind: (1) Search for modifiers of SR meiotic drive. (2) Simulation of the behavior of SR in natural populations using laboratory population cages. (3) and (4) Genetic analysis of the SR chromosome by isolating recombinants between or within inverted regions, by introgression or other methods.

Each of us has independently and unsuccessfully attempted several of the above experiments. The purpose of this note is to provide a collective description of those fruitless and mostly unpublished experiments. We hope that the list will prevent duplication of unsuccessful experiments. It is not our intention to discourage efforts to analyze the problems posed, but to emphasize that significant improvements in the experimental method will be necessary for their successful solution.

(1) Attempts to identify modifiers of Sex-Ratio drive from natural populations: A Sex-Ratio (SR) chromosome collected at a citrus grove in Riverside, California, was made homozygous by recurrent inbreeding. SR/SR females were mated individually with about 50 wild males collected from the same locality. One F_1 male from each cross was mated with SR/ST females, and the resulting progeny were examined for evidence of dominant autosomal or Y-linked modifiers, as indicated by an excess of males over the usual few percent fathered by SR males. There was no evidence of modification of SR drive.

Similar tests for modifiers have been performed using an SR chromosome collected at Jasper Ridge, San Mateo County, California. Tested autosomes and Y chromosomes derived from Jasper Ridge; Tucson, Arizona; Mesa Verde, Colorado; Salt Lake City, Utah; Napa, California; and Dallas, Texas. A minimum of 35 F_1 males were tested for each locality. No evidence of modification of drive was observed.

Some evidence for modification of SR drive comes from crosses of SR to an or/or (orange) marker stock. While most of the SR/Y ; or/or give rise to the usual progeny sex ratio, a few males produced approximately 10% male progeny, all of which were sterile.

(2) Population cage experiments: Six population cages were established with an unknown mixture of 10-20 independently derived SR chromosomes and laboratory and wild ST X-chromosomes and autosomes. One cage became fixed for SR after approximately six months. Females from that cage were used to initiate three more cages, with males derived from three standard laboratory stocks. There is no evidence of increase of SR frequency in the three replicate cages as observed in the original. Possible presence of a modifier of SR drive is being tested.

(3) Introgression experiments: We have made two independent attempts to introgress the right arm of D. pseudoobscura ST X-chromosomes into a D. persimilis genetic background, motivated by the observation that D. pseudoobscura ST and D. persimilis SR are cytologically indistinguishable. In one experiment, females from a D. pseudoobscura yellow-singed-vermillion-compressed-short stock were crossed with ST D. persimilis males. Hybrid females were individually backcrossed to D. persimilis males for five generations, retaining only those cultures in which compressed and short (closely linked to SR) were segregating. No fertile males carrying compressed or short and the persimilis Y and autosomes were observed. In a comparable experiment of similar design, a characteristic allele of Esterase-5 was used to identify pieces of the X-chromosome introgressed into the D. persimilis background. After five generations of backcross, no fertile males carrying the D. persimilis Y-chromosome and the D. pseudoobscura Esterase-5 allele were observed, based on 260 males tested.

(4) Attempts to isolate recombinants between Est-5 and SR: Several workers have reported extremely tight linkage between SR and Est-5. An attempt was made to enhance recombination in that region using the inter-chromosomal effect. Crosses were designed to generate females heterozygous for SR, Est-5, and various third-chromosome inversions (obtained from Dr. W. Anderson). 278 male progeny were tested for SR drive and analyzed for Est-5 genotypes. No recombinants were observed.

Bentley, M.M. and J.H. Williamson. University of Calgary, Alberta, Canada. Conditional synthetic lethality of cin^3 v and dietary allopurinol.

The cin^3 allele was originally isolated as a female sterile following EMS-mutagenesis of a y cv v f marked X-chromosome (Mohler 1977) and later characterized as a cin allele (Bentley & Williamson 1979). Preliminary investigations of the cin^3 y cv v f chromosome indicated that it was viable in a stock with C(1)DX, y f but that the males produced were hypersensitive to allopurinol administered in the medium at doses from 0.125 - 0.5 mg/ml, yielding practically no survivors. Subsequently, cin^3 y cv^+ v^+ f^+ and cin^+ y^+ cv v f recombinants in stocks with C(1)DX, y f were tested for sensitivity to allopurinol and though each genotype displayed some sensitivity, as indicated by a deficiency of surviving males, neither approached the sensitivity of the original genotype. v and y genotypes have been tested and display some lethality and none, respectively. Allopurinol has been shown to inhibit tryptophan pyrolase (oxogenase), the product of v^+ (Becking & Johnson 1967) and xanthine dehydrogenase (XDH) to produce ry phenocopies (Glassman 1965; XDH is severely depleted in cin genotypes). The combination of a cin allele and a v allele appears hypersensitive to the dietary administration of allopurinol, hence the term conditional synthetic lethal.

This observation has several implications. Any subsequent screens for cin alleles should not employ an allopurinol screen for ry phenocopies and an X-chromosome marked with v. This combination may preclude the survival of newly induced cin alleles or at least severely restrict the variety of alleles isolated. This observation also indicates an efficient method of carrying out further fine structure analyses of the v locus. Allowing recombination in a female of the genotype cin^3 v^x / cin^3 v^y and subsequent progeny development on allopurinol containing medium may kill all flies which are not the result of intragenic recombination or conversion events at the v locus (only cin^3 v^{x+} v^{y+} will survive assuming this effect is not allele specific). We trust that this report will be useful for any investigators pursuing further work on either of these systems.

Acknowledgment: Thanks to D. Baillie for discussions on the v fine structure implications. Supported by the Natural Sciences and Engineering Research Council of Canada and by the University of Calgary Research Policy and Grants Committee.

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Biémont, C. Université Lyon I, Villeurbanne, France. Opposite effects of low and high temperatures on mortality rate during development of inbred D. melanogaster.

Natural populations of Drosophila carry deleterious variants which reduce viability of their carriers when homozygous as a result of inbreeding. Dying of inbred offspring ranges from early embryogenesis to larval and pupal stages. The extent of inbreeding depression during development depends on a maternal effect (Biémont 1978), therefore, on the cytoplasmic constitution of the oocyte, and varies according to the control of expressivity of the lethal factors involved (Ives 1945). The inbred embryos may then be sensitive to modifying environment (Lerner 1954) either during their development or during oocyte maturation in their mothers. We have used various (16, 18, 25, 28 and 30°C) temperatures to perturb nuclocytoplasmic interactions regulating morphogenesis, hoping thus a change in the extent of inbreeding depression in early or late development (Lints 1961; Lints & Lints 1965). This effect was analyzed in regard to the different sensitivity to inbreeding of the individuals (Biémont 1978).

Flies from a wild stock of D. melanogaster were reared in an axenic maize-dried yeast-agar medium (David 1959) at 25°C. 4-to-6 day old fertile brother-sister couples were isolated in separate plastic boxes and allowed to lay eggs during a 24 h period at 25°C. For each pair of sibs, the egg hatchability was evaluated as the proportion of fertilized eggs that hatched; the hatching-to-adult survival as the proportion of adults obtained from the hatched eggs. This test performed at 25°C permits classification of the brother-sister couples into three groups depending on the effect of inbreeding on egg hatchability and larvo-pupal development (Biémont 1978). We distinguish (a) couples sensitive-sensitive, with embryonic mortalities (the embryonic development seemed complete but the larva failed to hatch) and larvo-pupal

mortalities; (b) couples insensitive-sensitive, for which only larvo-pupal mortalities were observed; and (c) couples insensitive-insensitive, for which no mortality was observed either during embryonic or larvo-pupal developments.

To test the effect of developmental temperature, 50 of the sib couples previously isolated were permitted to lay eggs at 25°C during 4 h periods. The eggs were then placed at the new temperature. Hence, for each sib couple, the mortality rate during development of their eggs was estimated at 16, 18, 28 and 30°C successively. After each tested temperature, a control was performed at 25°C to take into account the effect of aging of the sib couples (15 days old at the end of the experiment). The results are summarized in Table 1. Two-way variance analyses performed within each group of inbreeding sensitivity show that 18 and 28°C do not lead to any significant effect on both embryonic and larvo-pupal developments. A striking effect is observed at 16 and 30°C. 16°C decreases egg hatchability for the three groups of sibs, while 30°C mainly decreases hatching-to-adult survival. 30°C, however, also decreases the hatchability of eggs laid by the sensitive-sensitive group of couples. To analyze this latter result, two sets of 6-day-old brother-sister couples were maintained at 16°C or 30°C during three days before mortality rate of their eggs developing at these temperatures was determined. As seen in Table 2, 16°C affects embryogenesis but not larvo-pupal development which is perturbed, however, at 30°C. Then, a shift in temperature from 25 to 30°C applied during development of eggs laid by sensitive-sensitive couples (Table 1) is more efficient in increasing embryonic mortality than a 30°C temperature applied on parental couple and during laying and development of their eggs (Table 2). Such inbred eggs have low homeostasis (Lerner 1954), poor power of regulation that makes them more sensitive to a shift in temperature (Boesiger 1969), as caused by unefficient factors regulating inbreeding depression during development (Biémont 1976, 1978). In the group of insensitive-insensitive couples (Table 1), the effect of 16°C is low and of 30°C even non-significant. This suggests interactions between inbreeding depression and temperature. A particular genetic structure of certain inbred embryos rather than an averaged unefficient homeostasis may then be involved in inbreeding effects.

Table 1. Influence of developmental temperature on egg hatchability and hatching-to-adult survival. The eggs were laid at 25°C. The homogeneity of controls performed at 25°C after each tested temperature, was tested by two-way variance analyses on arcsin$\sqrt{\ }$ transformed data. This lead to grouping the data into two sets of values corresponding to two periods of experiment (16°C-18°C and 28°C-30°C) between which an aging effect was observed. Within each period the 25°C controls were not significantly different; their values were then pooled. The probability (P) is associated to the F values of two-way variance analyses. The underlined values are significantly different from the non-underlined. NS: > 0.05. For the characteristics of the different kinds of couples, see text.

Characteristics	No. of couples	Developmental temperature 25	18	16	P	25	28	30	P
	Sensitive-sensitive couples								
Egg hatchability	12	0.868	0.917	<u>0.782</u>	<0.01	0.887	0.891	<u>0.761</u>	<0.01
Hatching-to-adult survival	12	0.841	0.860	0.860	NS	0.765	0.799	<u>0.711</u>	<0.01
	Insensitive-sensitive couples								
Egg hatchability	10	0.967	0.961	<u>0.880</u>	<0.01	0.951	0.924	0.920	NS
Hatching-to-adult survival	10	0.886	0.939	0.900	NS	0.834	0.839	<u>0.723</u>	<0.05 >0.01
	Insensitive-insensitive couples								
Egg hatchability	21	0.988	0.977	<u>0.941</u>	<0.05 >0.01	0.983	0.981	0.953	NS
Hatching-to-adult survival	21	0.964	0.950	0.940	NS	0.902	0.889	0.867	NS

Table 2. Influence of temperature of laying on egg hatchability and hatching-to-adult survival. The eggs developed at the temperature of laying. The probability (P) is associated to the F values of two-way variance analyses. The insensitive-insensitive couples are excluded from this table; their number was too small for valuable statistical analysis. NS: > 0.05. For the characteristics of the different kinds of couples, see text.

Characteristics	No. of couples	25	16	P	No. of couples	25	30	P
	Sensitive-sensitive couples							
Egg hatchability	12	0.797	0.725	<0.05	6	0.790	0.760	NS
Hatching-to-adult survival	12	0.771	0.802	NS	6	0.740	0.680	<0.05
	Insensitive-sensitive couples							
Egg hatchability	8	1.00	0.950	<0.05	16	0.980	0.980	NS
Hatching-to-adult survival	8	0.804	0.821	NS	16	0.654	0.566	<0.05

We conclude for our results that low (16°C) and high (30°C) temperatures applied during the development of oocytes or embryos promote opposite effects on early and late developments. Since all couples used in calculations were fertile and only their fertilized eggs were cnnsidered, we eliminate the explanation of temperature action by male sterility or bad success of fertilization (Laugé 1973; Cohet & David 1978). Our results may then be interpreted in terms of processes controlling development. Properties of proteins vital for sustaining development (Biémont 1978; Davidson 1968; Medvedev 1966) could be different or not altered in the same way at low and high temperature.

In natural populations of Drosophila, the population size is sensitive to seasonal thermal fluctuations. The constraints of natural selection varying according to temperature, therefore, to season, from embryonic to larvo-pupal stages, lead thus to the maintenance of a "phenotypic" polymorphism (Shapiro 1976) or "epigenetic load" (Cohet & David 1978). Since the offspring the more sensitive to inbreeding were also the more sensitive to temperature, this selective system would enhance the adaptability of the population to varying environmental conditions and would reduce genetic load when these conditions limit effective size leading thus to inbreeding.

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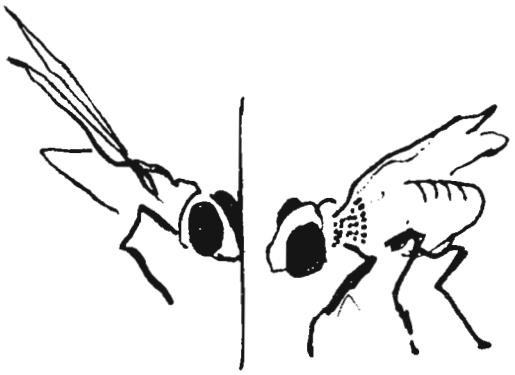


Borowsky, B. Yeshiva University, New York, New York. Altered secondary sex ratio with male age when sperm are not stored in D. melanogaster.

Older D. melanogaster males have a greater proportion of female offspring than do younger males (Yanders 1965; Gopalakrishnan & Rathnasabapathy 1973). Interestingly, a similar phenomenon occurs in humans (Novitski 1953). Both Yanders and Gopalakrishnan & Rathnasabapathy held virgin males for different lengths of time before permitting them to mate. Thus the change in sex ratio observed by these workers could have been explained by the earlier incapacitation of the Y, as opposed to the X, sperm during storage. But sperm storage on this scale is probably rare in humans. The present experiment was conducted to determine whether sex ratio would change with male age in D. melanogaster even when there was minimal sperm storage.

Flies were taken from a stock synthesized from several field-caught and several Oregon-R founders. Experimental animals were kept at 25°C on banana flakes-Karo syrup medium in single pair mating vials. Each of 60 six-hour-old virgin males was placed in a vial with a female. Each pair remained together for two days. Then each male was placed in a fresh vial with another six-hour-old virgin female. After two days, each male, now four days old, was placed in a vial with another virgin female. In this manner, each male was aged, but produced offspring continuously as he aged. Male parents were never discarded, but were permitted to die naturally; they were mated with a new female every two days until they died. Each female, on the other hand, after being in a vial with a male for two days, was transferred to a fresh vial. There she remained for two more days, continued to lay eggs, and then was transferred to a third vial for two more days. She was then discarded. Thus, each female laid eggs for only the first six days of her life, in three successive vials. This procedure allowed the female's age to be held constant while permitting males to get older. All transfers of parents were performed without anesthesia. Offspring were removed, anesthetized, sexed, and discarded, every two days. All vials were observed until the last F_1 had emerged and been classified.

Table 1. Changes in secondary sex ratio and number of offspring with increasing paternal age.

Paternal age (days after eclosion)	No. of broods	No. of male offspring	Total offspring	Percent male offspring	Mean no. offspring per brood
2-12	260	11,531	23,156	49.8	89.06
14-28	243	10,465	21,293	49.1	87.63
30-62	228	10,165	20.909	48.6	91.71

The data were divided into three consecutive age classes, each with approximately equal numbers of successful matings (Table 1). It should be noted that although the experiment was begun with 60 males, only five were still reproducing by day 62. Thus, although the number of broods is similar, fewer males are represented in each consecutive age class. The data show that although there was no significant change in fecundity as males aged ($F_{2,715} = 0.6001$, $p > 0.05$), the proportion of male offspring progressively decreased ($\chi^2 = 6.208$, d.f. = 2, $p < 0.05$). These results suggest that in Drosophila, as in humans, differential sperm death resulting from sperm storage cannot explain the change in sex ratio with paternal age. Rather, the change probably occurs during meiosis or after fertilization.

References: Gopalakrishnan, C.A. and V. Rathnasabapathy 1973, Cheiron 2:15-18; Novitski, E. 1953, Science 177:531-533; Yanders, A. 1965, Genetics 51:481-486.

Bos, M. and A. Boerema. University of Groningen, Haren (Gn.), The Netherlands. Drosophila and yeast conditions.

Laboratory studies using Drosophila have shown that winning in competition for food is largely determined by: a high rate of feeding, a short duration of the molting periods, a low food requirement, a high initial larval weight, resistance to possible disoperative effects, resulting from crowding (Bakker 1961). However, the environment can change the relative abilities of genotypes. Barker (in press) mentions experiments from which it becomes clear that the outcome of competition cannot be predicted simply on the basis of food exploitation. Many characters of the food medium can affect the outcome

e.g. biotic residues in the medium (Weisbrot 1966), the condition of the medium surface (Moore 1952), medium quality (Claringbold & Barker 1961), yeast genotype (Bos et al. 1977). All these conditions are realized in nature, but also in laboratory cultures. Therefore it is not surprising that the composition of the laboratory culture of Drosophila is complex enough to maintain genetic variation, since this variation is positively correlated with environmental heterogeneity.

Yeast is a main component of the Drosophila diet in nature and of many laboratory food media. Little is known about the effects of within-yeast variability. We are reporting here the survival of three Drosophila strains on three wild-type Saccharomyces cerevisiae cultures (1. a commercial dry yeast; 2. Baker's yeast, grown under aerobic conditions = O.P. = oxygen present; 3. Baker's yeast grown anaerobically = O.A. = oxygen absent) and two multiple mutant yeast strains (see Table 1). Mean survival to adult in five replicate vials with 50 larvae each, is presented.

Table 1. Survival of Drosophila on Saccharomyces cerevisiae yeast variants (mean number ± s.e.)

mg dry yeast/vial		D. melanogaster Adh-FF	D. melanogaster Adh-SS	D. simulans white
1. Wild-type yeast (dry commercial)	100 mg	40.6 ± 0.8	---	37.5 ± 5.4
	50	36.0 ± 0.9	35.8 ± 2.5	41.8 ± 1.1
	25	31.4 ± 1.7	---	39.4 ± 0.8
2. Wild-type O.P.	100 mg	31.0 ± 1.1	40.8 ± 1.5	23.8 ± 0.9
	50	36.2 ± 1.1	28.6 ± 1.7	24.0 ± 2.5
	25	31.2 ± 0.9	36.4 ± 2.1	28.2 ± 2.4
3. Wild-type O.A.	100 mg	16.6 ± 2.8	1	11.0 ± 2.1
	50	1	0	3
	25	0	0	0
4. Mutant 1*	100 mg	39.8 ± 1.3	29.0 ± 1.3	41.2 ± 1.7
	50	37.6 ± 1.3	38.4 ± 1.9	39.8 ± 3.0
	25	20.6 ± 2.3	24.4 ± 1.8	33.8 ± 1.2
5. Mutant 2*	100 mg	36.0 ± 1.6	38.0 ± 1.6	34.0 ± 1.7
	50	37.6 ± 0.7	32.8 ± 2.5	36.2 ± 1.7
	25	14.4 ± 3.6	10.6 ± 1.6	31.6 ± 1.9

*The genotype of the mutants was resp. α-ade,leu,his,ura,acr and α-ade,leu,his,ura,allNmr (Middelhoven 1977). Yeasts were grown on yeast complete medium (yeast extract 0.5 g, caseine hydrolysate 0.5 g, pepton 0.3 g, glucose 4.0 g, 100 ml water).

On all types of yeast (except wild-type O.A.) survival on 100 mg and on 50 mg dry-weight yeast/50 larvae is about the same and there is no difference between the Adh F and S strains (Adh = alcoholdehydrogenase). On wild-type O.A. yeast, however, survival of all strains is extremely low and survial of the Adh S strain on 100 mg is significantly lower than that of the Adh F strain.

In almost all experiments survival is decreased if only 0.5 mg dry yeast/larva (25 mg/50 larvae) is present. This is most evident with the D. melanogaster strains on the mutant 2 yeast.

In conclusion it can be said that condition as well as genotype of the yeast can influence survival of Drosophila drastically. The low viability on wild-type O.A. yeast is probably a question of low food quality. Yeast, grown anaerobically with a high glucose concentration contains low quantities of essential food components like sterols, phospholipids and vitamins, and -- depending on yeast strain and conditions -- differing amounts of ethanol. The presence of ethanol (a substrate of the Adh enzyme) can bring about reproducible changes in Adh allele frequencies in Drosophila populations (Gibson 1970; Bijlsma-Meeles & Van Delden 1974), but ethanol concentrations in our O.A. yeast are less than 2% and cannot effect the differential survival of the two Adh strains. The cause is, therefore, probably differential food requirement for the essential food components mentioned above.

Our experiments confirm that yeast variability can have a large effect on the fitness of Drosophila genotypes.

References: Bakker, K. 1961, Archs. neérl. Zool. 14:200-281; Barker, J.S.F., in prep., Biology and Genetics of Drosophila (eds. Wright and Ashburner); Bos, M. et al. 1977, Evolution 31:824-828; Bijlsma-Meeles, E. and Van Delden, W. 1974, Nature 247:369-371; Claringbold, P.J. and J.S.F. Barker 1961, J. Theor. Biol. 1:190-203; Gibson, J.B. 170, Nature 227:959-960; Middelkoop W.J. 1977, J. Gen. Microb. 100:257; Moore, J.A. 1952, Evolution 6:406-420; Weisbrot, D.R. 1966, Genetics 53:427-435.

Boulétreau-Merle, J. Université Lyon I, Villeurbanne, France. Variability of initial retention in natural populations of D. melanogaster.

Females of D. melanogaster become receptive for mating one day post-emergence. Inseminated females begin egg-laying rapidly and their daily egg production is high. If the females are not allowed to mate, they achieve a first batch of eggs but keep them in retention in the distal part of the ovarioles for several days. The duration of this period of initial retention differs in tropical and temperate populations (Bouletreau-Merle, in prep.). In laboratory conditions (25°C, total darkness) the initial retention of virgin females of the first laboratory generation proceeding from African flies trapped in the field is lower than the retention of offspring from French wild flies (France I) (Table 1). The means are statistically different ($t = 4.15$).

However, there was noticeable variability of the duration in the same population. A study of the genetic variability of this characteristic was performed in another French population (France II), with the offspring of 86 wild flies collected monthly, from May to November. Three virgin daughters were observed individually for 15 days, for each isofemale line. The variability between the lines is shown in Fig. 1. For each sample, the distribution of initial retention was almost normal but the means varied according to the time of year. However, the average duration was similar to France I.

Table 1. Initial retention in natural populations of different geographic origins.

Origin	Retention (days)	C.V.	n
Congo	5.36 ± 0.49	65.48	50
France I	8.52 ± 0.58	48.64	51
France II	8.26 ± 0.23	45.46	257

In several cases the three observed sisters presented the same extreme physiological characteristics, i.e., either short initial retention followed by relatively high egg deposition, or long initial retention (more than 15 days) followed by low egg deposition. The persistence of these genetic characteristics was tested after 12 or 14 generations without selection, in a few families chosen among the extreme isofemale lines reared in mass from the sisters of the three virgin females first tested. The results for two isofemale lines in each physiological tendency are presented in Fig. 2.

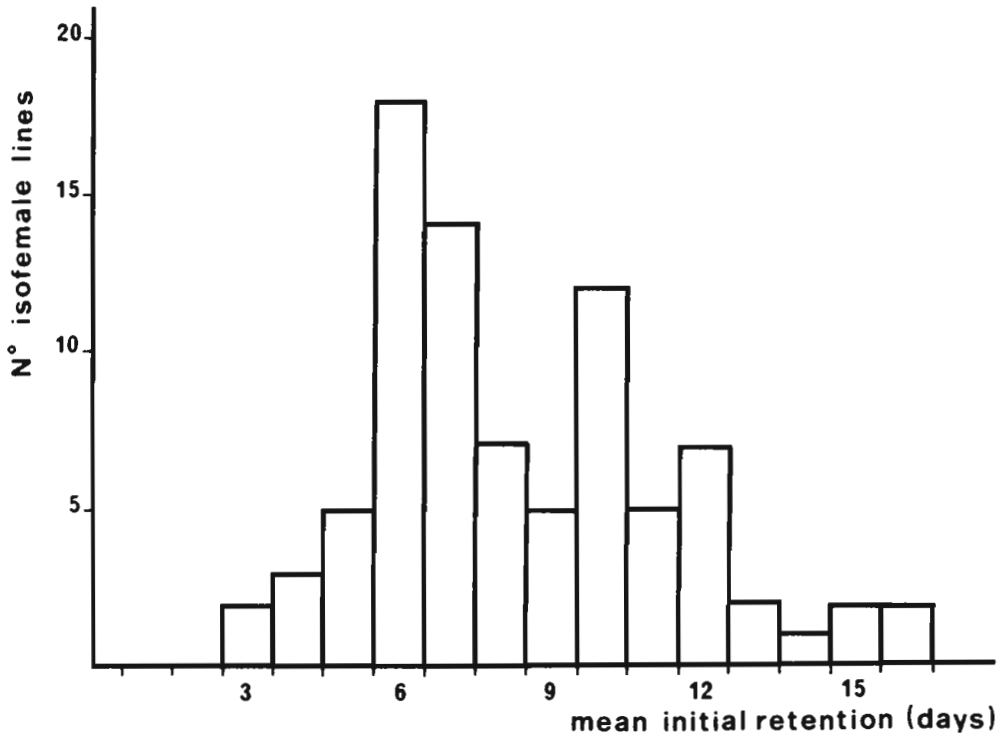


Fig. 1. Initial retention variability between virgin females of the first laboratory generation of isofemale lines founded by wild flies collected throughout the year in a French natural population.

The initial tendency was preserved through the generations in the majority of the lines picked out but a few of them, especially in the high retention group, had drifted near the mean value of the population (cf. female 48).

An 80% selective pressure was carried out for the following two generations in order to improve the characteristics in each tendency, then, the selection was relaxed for the next seven generations. Afterwards, the selected lines

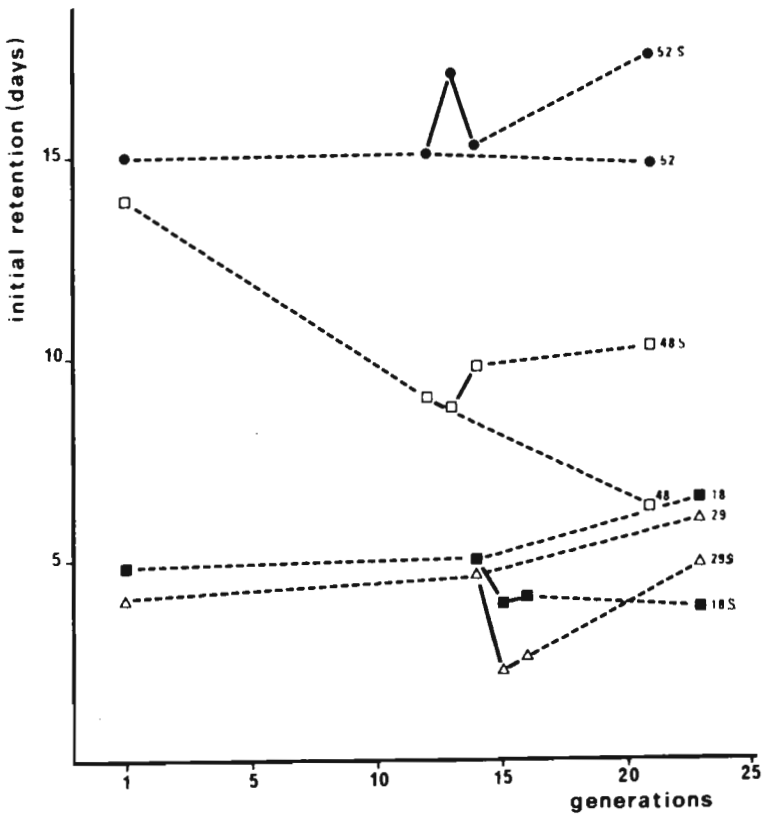


Fig. 2. Evolution of the initial retention in chosen isofemale lines with (——) or without (---) selection for long (52 and 48) or short (18 and 29) duration.

and the free lines were tested once more. Selection for only two generations did not really improve the mean durations which were already not far from their physiological limits either for long or for short retention but it stabilized the drifting lines.

The first choice which selected extreme families among the 86 isofemale lines was apparently sufficient in the majority of cases to isolate two physiological types which are genetically determined and some of the lines were homozygous enough to keep the characteristics unchanged from allicot females and without selection for a number of generations.

These physiological types, determined by the initial retention of virgin flies actually measured the capacity of the females to control oviposition in the virgin state. Recent observations showed seasonal variations of their relative frequencies in a French natural population (Boulétreau-Merle, in prep.). The existence of these different physiological capacities must have adaptive value for natural populations in temperate regions (Boulétreau-Merle, in prep.).

Boussy, I.A. University of California, Davis, California. Determining configuration of whole-arm translocations.

I recently was faced with the problem of determining whether a number of homozygous-lethal whole-arm 2-3 translocations in D. melanogaster were 2L·3R, 3L·2R or 2L·3L, 2R·3R. They had been detected by pseudolinkage tests and by the lack of apparent breakpoints in salivary gland chromosome preparations. I first attempted to confirm the 2L·3R, 3L·2R configuration of Jaakko Puro's homozygous-viable whole-arm translocation FM-46 (Puro 1973). To do this, I recombined the markers dumpy (dp^{ov}; 2L,13.0) and ebony (e^{11}; 3R,70.7) to FM-46. I then crossed heterozygous FM-46(dp,e)/FM-46(+,+) males to dp,e stock females and looked for assortment products, confirming the physical linkage of 2L to 3R, and the 2L·3R, 3L·2R configuration of FM-46.

I then crossed FM-46 to each of my whole-arm translocations, and crossed F_1 males to virgins of Compound 2 or Compound 3 stocks [C(2L)P4 dp, C(2R)P4 px and C(3L)P3 ri, C(3R)P3 sr were used]. If the translocation being tested had the same configuration as FM-46, then two bivalents would form at metaphase I, and only euploid gametes would form (Fig. 1a); thus no offspring would develop in a cross to a compound chromosome, which yields only gametes aneuploid for whole arms (Holm 1975). If the translocation were of different configuration from FM-46, then alternate disjunction from the metaphase I ring would produce euploid gametes, but both adjacent disjunctions would produce gametes aneuploid for whole arms (Fig. 1b); such gametes should complement the gametes produced by compound chromosome stocks. Noting which crosses produced progeny and which were sterile scored each translocation as 2L·3R, 3L·2R or 2L·3L, 2R·3R. These assignments corroborated completely previous groupings of the translocations into two sets, based on making F_1's between them, crossing the F_1's to compounds, and scoring sterility or fertility.

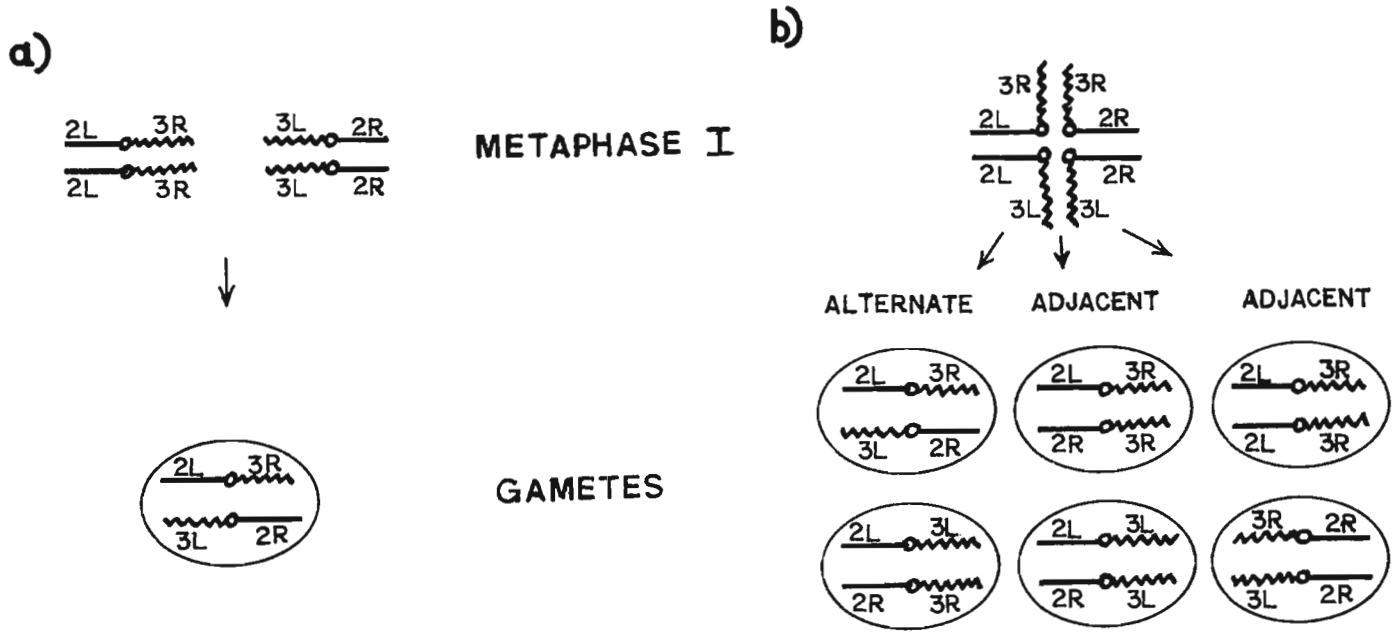


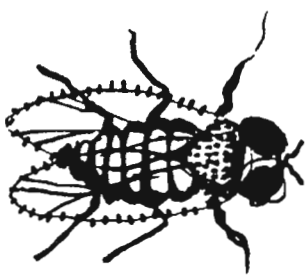
Fig. 1. Metaphase I and gametic products of heterozygotes for a whole-arm translocation of known configuration (2L·3R, 3L·2R) and whole-arm translocations of (a) the same configuration, and (b) of different configuration (2L·3L, 2R·3R).

The technique can be applied easily to any whole-arm translocation, whether homozygous viable or lethal. It consists simply of crossing the unknown to FM-46 (or any other whole-arm translocation whose configuration is known), then crossing F_1's to compounds and scoring whether or not offspring are produced. If so, the translocation is of different type from FM-46 (or the test translocation used); if not, it is of the same type. Since compound females preferentially produce segregational gametes (as opposed to males which do not; Holm 1975), the latter cross is best done with male F_1's and compound females.

References: Holm, D.G. 1975, Ch. 13 in: Holm, D.G. and E. Novitski, eds., 1975, Genetics and biology of Drosophila, Vol. 1b, Academic Press, NY; Puro, J. 1973, Hereditas 75:140-143.

Appendix: A supplementary note by J. Puro.

The method described above by Boussy is perhaps the simplest genetic way to determine the configuration of whole-arm translocations. It may be supplemented by cytological study of F_1 larvae carrying the whole-arm translocation being tested and a translocation whose configuration is known. Viz., the two alternative configurations diagrammed in Figs. 1a and 1b can be distinguished, by virtue of an intimate homologous synapsis, in mitotic prophase of neuroblasts (see Puro 1973). The fertile crosses between F_1's and flies bearing autosomal compounds result in the production of offspring which I have previously called tricomplex heterozygotes by virtue of the fact that each offspring has three partially homologous autosomes plus a single compound. For another genetic method to determine the configuration of a whole-arm translocation, see the note by Puro and Kiiskilä, this issue of DIS.



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Visual orientation of Drosophila mutants in a multiple Y-maze.

Table 1. Y-maze orientation.

Group	Mutant	Choice Coeff.
Wild type	WT	0.75
Receptor	so	0.55
	w	0.49
	ey^2	0.70
	sev^{LY3}	0.74
	ora^{JK84}	0.50
	$rdgB^{KS222}$	0.58
ERG	X-37*	0.65
	X-61*	0.66
	X-72*	0.53
	$nonA^{H2}$	0.54
	$nonA^{H17}$	0.47
	$nofC^{B1}$	0.48
	$nofC^{B2}$	0.48
	$nofC^{B5}$	0.53
	$nofC^{B8}$	0.49
	$nofC^{B9}$	0.52
	$nofC^{B10}$	0.51
	t	0.45
no object fixation	$nofA^{S100}$	0.81
	$nofD^{B11}$	0.59
	$nofE^{B12}$	0.42
	$nofF^{S71}$	0.48
	$nofG^{S131}$	0.73
	apo^{S129}	0.51
Optomotor-blind	nbA^{H18}	0.65
	nbA^{H47}	0.53
	elf^{H37}	0.75
	omb^{H31}	0.26
	opm53*	0.54
	e	0.64
Motor	Hk^1	0.63
Brain	sol^{KS58}	0.41

* laboratory name

The orientation of flies towards a 5x5° dark spot in a multiple Y-maze was tested in mutants known for partial or complete blindness or other defects in the processing of visual information. The maze resembles "Galton's Board" and consists of 14 consecutive decision points at which the running fly has to decide to go either to the left or to the right (Bülthoff, Götz & Herre 1982). A probability of choice $0.5 < p < 1$ indicates preferred orientation towards the dark spot whereas a probability $0 < p < 0.5$ is associated with orientation away from the pattern. A probability around 0.5 suggests inability to orientate visually towards the pattern. The choice coefficients in Table 1 are calculated from the end distributions of Y-maze runs with approximately 1300 flies for most mutant strains, and for none with less than 500 flies.

A few of the mutant strains show normal orientation effects as compared to the behavior of the wild type. These strains include the eyeless mutant (ey^2) with rudimentary compound eyes and the mutant sevenless (sev^{LY3}) in which the inner rhabdomere 7 is missing, the mutant with an extra lamina fiber (elf^{H37}) (Heisenberg 1979) and, surprisingly, also two of the S-mutants ($nofA^{S100}$, $nofG^{S131}$) which have been selected because of a defect in their attitude towards visual objects (Heisenberg & Wolf 1979). The orientation effect is almost absent in the blind mutant sine oculis (so), in the mutant white (w) lacking screening pigment, in the receptor mutants ora^{JK84} and $rdgB^{KS222}$, and mutants disturbed in the primary process of phototransduction (X), no on-response (non), night blind (nb^{H47}) and tan (t) (Pak 1975). The same holds for the ERG mutants of the nofC group (Bülthoff 1980), for the no-object-fixation mutants ($nofF^{S71}$, apo^{S129}, $nofD^{B11}$, $nofE^{B12}$ (Heisenberg 1979; Bülthoff 1980) and for the hyperkinetic (Hk^1) mutant (Kaplan & Trout 1969) and the recently described small optic lobe (sol^{KS58}) mutant (Fischbach & Heisenberg 1981). Most surprising are the scores obtained for the optomotor blind mutant omb^{H31} (Heisenberg, Wonneberger & Wolf 1978), a mutant with severely diminished movement induced course control response which is structurally impaired in the lobula plate giant neurones of the visual system. The flies of this strain avoid the visual object in the Y-maze. The avoidance reaction, or antifixation is remarkably strong ($p = 0.26$) and seems to be in contradiction to the almost normal fixation response obtained in other experimental paradigms (Bülthoff, Götz & Herre 1982).

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Isolation of sex-linked mutants disturbed in visual orientation.

A multiple Y-maze paradigm was used to select D. melanogaster mutants disturbed in visual orientation. Sex-linked recessive mutations were obtained by EMS mutagenesis (Lewis & Bacher 1968). Screening of a total of 15,000 flies resulted in 11 mutants which belong to six complementation groups. The probability of visual attraction towards the pattern is shown in the last column of the following table. A probability around 0.5 suggests absence of preferred orientation towards the pattern, which is 0.75 in the wild type. The probability of 0.26 in the optomotor blind mutant omb^{H31} (Heisenberg, Wonneberger & Wolf 1978) indicates an equally strong avoidance response in this particular strain.

Group	Mutants	ERG	Pig.	DPP	Sens.	Y-Maze
ERG						
1	$nofC^{B}$ (1,2,5,8,9,10)	-	+	+	0.001	0.48-0.53
OPTIC						
2	$nofI^{B3}$	+	-	-	1	0.55
3	$nofK^{B6}$	+	-	-	1	0.56
4	$nofL^{B7}$	+	-	-	1	0.56
BEHAVIOR						
5	$nofD^{B11}$	+	+	+	1	0.59
6	$nofE^{B12}$	+	+	+	1	0.42

abbreviations: ERG - Electroretinogram; DPP - Deep Pseudopupil
Pig. - Screening Pigment; Sens. - Sensitivity

The physiological and structural integrity of the peripheral stages of information processing in the visual system of the mutants were checked by measuring the elctroretinogram (ERG) and observing the deep pseudopupil (DPP). The mutants in the first group belong to one complementation group with abnormal transient responses (off-transient of lamina potential is absent) but can be separated further into a group of normal ($nofC^{B1}$, $nofC^{B2}$, $nofC^{B5}$) and abnormal ($nofC^{B8}$, $nofC^{B9}$, $nofC^{B10}$) time course of the receptor potential. In some individuals of the second group the return to baseline after light-off is delayed by 2-5 s. In other flies the receptor potential is not sustained during light-on. The decay to base line during the light stimulus is similar to that of the transient receptor potential group (trp). The absolute sensitivity (Sens.) as shown by the ERG is reduced by a factor > 1000 for all mutants of Group I tested so far. The ERG defects in the mutants of the present complementation group partially overlap with known ERG defects in mutants of different genotype. The deep pseudopupil (DPP) and the screening pigment (Pig.) was normal in all mutants of Group I.

The three optic mutants (different complementation groups), however, show normal ERG responses, but abnormal eye color and DPP's. The eye color in these three mutants is darker and more brownish than in wild type. This is probably due to a disrupted screening pigment which can be observed in semi-thin sections. These mutants also show irregularities in the pattern of the rhabdomere endings, which can be investigated by the optical neutralization technique (Franceschini & Kirschfeld 1971). In some flies the endings are packed more closely than in the wild type and are arranged in a more circular pattern which deviates from the normal trapezoidal arrangement. This, however, was not due to a missing receptor as in the mutant sevenless (sev^{LY3}). The number of receptors in a rhabdome was normal in all of the mutants. In none of the mutants, however, the DPP's were visible except for a few individuals in which the DPP was blurred. The defect is most probably due to a displacement of the distal rhabdomere endings from the focal plane of the dipotric apparatus of the ommatidia. This is seen by comparison of real and virtual images of the antidromically illuminated receptor endings in individual intact ommatidia through adjustment of the focal plane of the microscope. For the mutants $nofI^{B3}$, $nofK^{B6}$ and $nofL^{B7}$ the receptor endings are located between the lenslet and its focal plane. This would suggest that the flies are unable to respond in the Y-maze, because the tiny test pattern is blurred so much that it falls below the detection threshold.

Of the 11 selected mutants only $nofD^{B11}$ and $nofE^{B12}$ do not seem to be disturbed in early visual processing. While optics and ERG appear to be normal, these mutants differ significantly from wild type in object-induced orientation. The disturbed orientation behavior of $nofE^{B12}$ can be seen already in the culture vial or in free flight: Mutant flies seem to turn more often than wild type. It seems that $nofE^{E12}$ is not able to maintain a straight course towards an object over extended periods of time.

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Cadieu, N. Université Paul Sabatier, Toulouse, France. A possible case of behavioral heterosis depending on an environmental condition: differential responsiveness of inbred and outbred D. melanogaster to sugar stimuli.

For behavioral patterns, as for other kinds of phenotypes, intraspecific hybridization can give rise either to an intermediate or to a heterotic transmission. According to Bruell (1967) and others, behvaior patterns contributing to the individual fitness would be heterotically transmitted: the F_1 hybrids performing better, on an average, than both inbred parental strains. However, this opinion does not necessarily imply that heterosis for a given phenotype is an absolute criterion of its contribution to individual fitness.

On the other hand this point of view ignores the occurrence of statistical interactions between genetic and environmental sources of variation; i.e., an effect of heterosis may well be obvious under a particular environmental condition and fail under a different one. We describe in this note an effect of behavioral heterosis relative to a controlled environmental condition: the fasting duration preceding every determination of the tarsal reflex (T.R.) threshold.

The T.R. is an extrusion of the proboscis, released by the stimulation of contact chemoreceptors located on the foretarsi of the fly; in this experiment, the stimulus was a solution of sucrose.

The threshold of T.R. was measured through the method of Vaysse and Médioni (1973). Flies of three inbred lines, all derived from the wild strain Basel-06 (at least 57 generations of brother x sister matings) were used, as well as all possible F_1 between these lines taken in pairs, and their reciprocals.

For every group (15 males, 15 females) the level of responsiveness to sucrose was measured after either 12, 16 or 24 hours of hydric starvation. To avoid eventual seasonal drift effects, the parental lines and the hybrids were tested in strict synchrony. The experimentation was performed with the same climatic parameters as breeding (t°: 25 ± 0.5°C; R.H.: 85 ± 10%) on 7 ± 1 day old animals. A total of 810 flies were individually handled in this experiment.

As the thresholds were consistently higher in females than in males for a given starvation degree, the data were processed for each sex separately by a three-way analysis of variance. The main results can be summarized as follows:

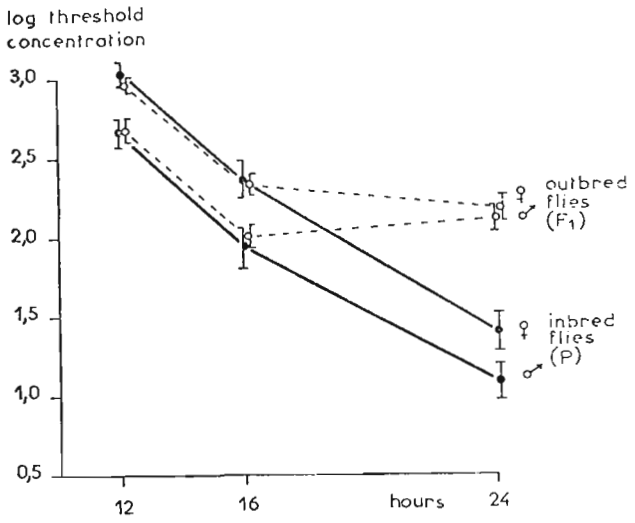

Fig. 1. Variations of tarsal responsivity (log. threshold concentration of sucrose, ordinates) as a function of previous fasting duration (hours, abscissae).

(1) On the whole T.R. thresholds decrease with increasing fasting duration ($P = 0.001$).

(2) The other two principal effects (father vs. mother ancestry) reveal threshold values lower in the descendants for one of the inbred lines than of the other two ($P = 0.01$ for the males; $P = 0.05$ for the females).

(3) Among the first-order interactions, only one is significant: paternal x maternal ancestry ($P = 0.001$ in males and in females). This interaction discloses a possible heterosis effect, threshold values being on the whole higher in outbred than in inbred lines.

(4) The second-order interaction (fasting duration x father ancestry x mother ancestry) is highly significant ($P = 0.001$ in each sex). As a matter of fact, the F_1 hybrids do not differ from their inbred parents, but after the longest fasting duration: in inbred flies the T.R. threshold keeps on decreasing between 16 and 24 hours starvation, whereas this value keeps steady in F_1 hybrids during the same interval (Fig. 1).

On that basis a contribution to the fitness of the phenotype "level of responsiveness to sugary substrates" could be considered, as the discovery of a food-source, as well as its sustained exploitation, is favored by the T.R. This response could be only differentially aroused in inbred and outbred flies under a very severe food deprivation. This condition could lead the hybrids to select the carbohydrate sources that are profitable enough; such a mechanism was already pointed out for the honeybee (von Frisch 1964). On the other hand, inbred flies could start responding to minimal sucrose concentrations, by no means sufficient to ensure an adequate caloric supply.

So speculative an interpretation should nevertheless be rated with caution: first of all, one should search for an inbreeding depression effect, complementary of the assumed heterotic mechanism (Cadieu, in prep.). Furthermore, an experiment still in progress will show whether the previous speculations fit in with biological aspects of heterosis (fertility, survival to fasting) which are more classical.

However that may be, the present study is proving of an obvious methodological interest for students in behavioral genetics: for it makes clear that genetic effects of great potential significance from an evolutionary viewpoint, like heterosis, may become patent only under propitious environmental circumstances.

Work supported by the Centre National de la Recherche Scientifique.

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Carson, H.L.[1], C.B. Krimbas[2] and M. Loukas[2]. [1]University of Hawaii at Manoa, USA; [2]Agricultural College of Athens, Greece. Slime fluxes a larval niche of D. subobscura Col.

D. subobscura Collins, a paleoarctic member of the obscura species group and the most common in many European countries, has been intensively investigated in studies of population genetics, ecology and evolution. In this respect it is the counterpart of the North American D. pseudoobscura and D. persimilis.

Until now very little has been known of its breeding sites in its main wild biotope, fir forests. Larvae of this species are found in decaying fruits, oranges and even lemons (Krimbas, unpubl. data) not only in Greece but apparently also in Israel, Tunesia and other Mediterranean countries, in decaying fruits of Zizyphus jujuba in Greece (Loukas 1981), in Cornus berries in Northern Italy (Buzzati-Traverso 1948), in rowan berries in England (Begon 1975), blackberries and snowberries in England (Kearney 1978), in apples in England (Hummel 1978), in several soft and juicy fruits in Switzerland: those of Atropa belladona, Fragaria vesca, Juglans regia, Prunus avium, Prunus spinoza, Rubus fructicosus, Rubus idaeus, Sambucus edulus, Sambucus nigra (Schatzman 1977), in fruits of Magnolia grandiflora in France (Lachaise 1978). Also in diseased Iris roots in England (Smart 1945), oak galls of Biorrhiza pallida in England (Basden 1952) and in mushrooms especially in Amanita phalloides in England (Shorrocks, pers. comm.), in Amanita rubescens in Spain (Galicia) (Fontdevila 1978). All this rather long catalog of larval niches would indicate that the fly is polyphagus in the sense that larvae feed on yeasts cultured in several fermenting substrates. Since some of these substrates are cultivated fruits the fly is partially commensal to man.

However, in spite of all this, practically nothing was known of the breeding site of D. subobscura in fir forests, its most usual wild biotype, since decaying fruits or mushrooms, at least in Greece, could not justify the extremely high densities of flies observed, amounting in August to 2 flies per m^2 in Mt. Parnes, giving thus a panmictic size of 160,000 flies. It has been suggested that in coniferous forests in the USA D. pseudoobscura and D. persimilis breed in slime fluxes, and in Spain Prevosti (1959) found larvae of D. ambigua (another obscura group species) in slime fluxes.

At the end of May this year (May 28, 1981) several slime fluxes were prospected in Mt. Parnes forest: they originate generally out of old scars on tree trunks but most of the fermenting exudate is concealed below the bark. We saw females attracted to it and eggs apparently deposited in the upper region of the slime flux.

One such slime flux on Abies cephallonica collected on May 28, 1981 produced D. subobscura which started hatching on June 20, 1981; in total 39 males and 39 females. One male and one female were produced from a small piece of another slime flux of Abies cephallonica taken to the laboratory and put in a bottle of Drosophila medium as in the case of the first one. Iden-

tification of D. subobscura was made by crossing individually the wild males to virgin females of the ch cu strain (homozygous for the recessive mutants cherry eyes and curly wings). All F_1 were of wild type. Females produced were collected still virgin and crossed individually to ch cu males; the wild type F_1 produced were crossed in order to check whether mutant F_2 would also be produced (another check that wild females were virgins indeed).

From a third slime flux on Populus tree in the same region 9 males and 3 females were produced. The 3 females crossed individually to ch cu strain and thus proved to be D. subobscura. Of the 9 males, one fertilized a ch cu virgin female, one died and seven did not take with ch cu females. They were electrophorized for esterases and proved to be a different species from D. subobscura, at the present moment unidentified.

A slime flux collected near Karpenissi in a fir forest in June, 1981 did not produce any flies.

Although these data are preliminary we believe that they indicate that slime fluxes could be considered as a major (if not the) larval site of D. subobscura in fir forests. If this is so an important blank in the biology of wild D. subobscura would be clarified and many difficulties in studies of population genetics of wild populations of this animal will be overcome. Indeed this finding opens new experimental possibilities.

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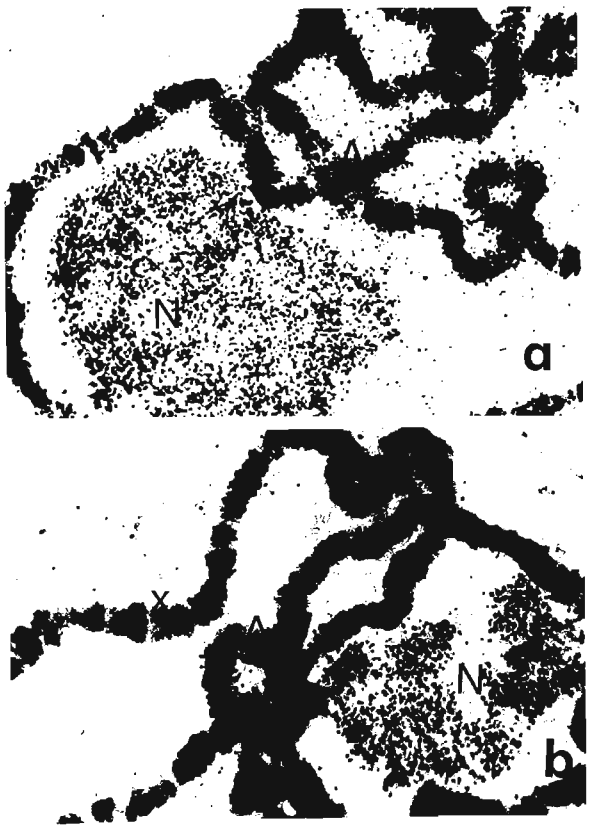
Chatterjee, R.N. and A.S. Mukherjee. University of Calcutta, India. Effect of α-amanitin on the ^{3}H-uridine incorporation of the D. melanogaster salivary gland chromosomes.



α-amanitin, a mushroom toxin, inhibits in vivo or in vitro RNA synthesis (mainly chromosomal RNA synthesis) in higher organisms primarily through its effect on RNA polymerase II (Jacob et al. 1970; Chambon 1975). It seems that the response of a specific subcellular organism to this inhibitor, amatoxin, which also selectively affects chromosomal RNA synthesis in dipteran polytene chromosome, may help to elucidate the regulation of transcription of chromosomal and nucleolar RNA synthesis.

For the present experiments, salivary glands from third instar larvae of D. melanogaster were dissected out by hand in Drosophila Ringer (pH 7.2), incubated either in Ringer or in α-amanitin containing Ringer solution for 10 minutes, and then incubated for 10 minutes in ^{3}H-uridine (300 μCi/ml; specific activity 7500 mCi/mM, Bhaba Atomic Research Centre, Bombay). The concentrations of α-amanitin were 1, 5, 10, 20, 30 and 50 μg/ml. Cytological preparations of chromosomes were then made and processed for autoradiography.

Results show that, while in the control sets, the nucleolus is densely labelled and chromosomes show either localized labelling on various active sites or dense labelling all through (Fig. 1a); in the treated series at the concentrations used the nucleolar labelling is not much affected but

Fig. 1. Photomicrographs showing the nucleolus and chromosomal labelling with ^{3}H-uridine in control (a) and after treatment of the salivary gland with 20 μg/ml α-amanitin (b) for 10 minutes followed by pulse labelling in Ringer. N = nucleolus; X = X-chromosome; A = autosome.

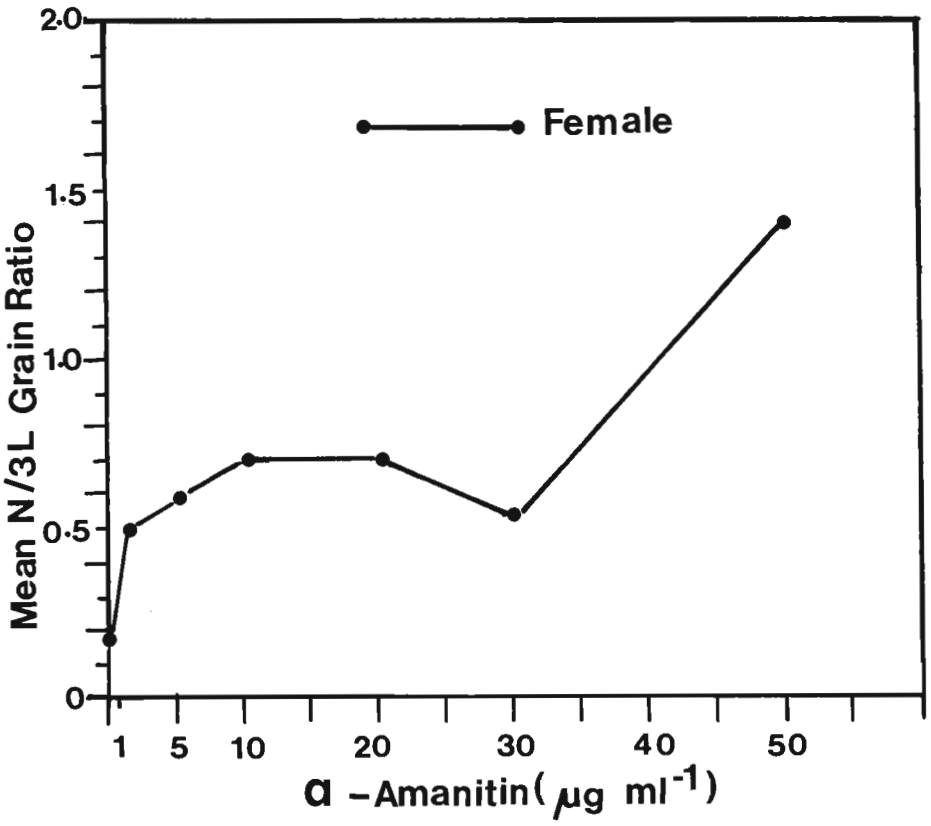


Fig. 2. Chart showing the effect of α-amanatin on the relative transcriptive activity pattern of the nucleolus in female as measured by mean grain ratio of a unit area of nucleolus and 3rd chromosome 68B-74F (N/3L).

Table 1. Data on the ^{3}H-uridine labelling pattern of the autosome (3L) and a unit area of the nucleolus of D. melanogaster in normal and after in vitro treatment of salivary glands in different concentrations of α-amanitin.

Treatment	Sex	Mean grain number with S. E.		Mean grain ratio (N/A)	Correlation coefficient (r_i)
		3rd Chromosome (68B-74F) Y	Nucleolus (unit area) X		
Ringer/Control	♀	268.20 ± 22.39 (20)*	48.80 ± 3.59 (20)	0.18	0.59
1 μg/ml α-amanatin	♀	79.75 ± 14.37 (20)	31.15 ± 3.96 (20)	0.39	0.90
5 μg/ml α-amanatin	♀	108.79 ± 20.80 (20)	36.36 ± 4.70 (20)	0.33	0.75
10 μg/ml α-amanatin	♀	86.75 ± 15.28 (16)	36.63 ± 3.68 (16)	0.42	0.56
20 μg/ml α-amanatin	♀	47.44 ± 6.21 (25)	25.44 ± 3.25 (25)	0.54	0.67
30 μg/ml α-amanatin	♀	48.40 ± 6.84 (22)	21.36 ± 3.16 (22)	0.44	0.94
50 μg/ml α-amanatin	♀	5.57 ± 1.27 (19)	7.00 ± 1.30 (19)	1.26	0.95

*The number in parentheses denotes the number of nuclei examined.

chromosomal labelling is drastically reduced with a disperse distribution of silver grains (Fig. 1b).

In order to obtain precise evaluation the effect of α-amanitin on chromosomal and nucleolar RNA synthesis, grains were counted over a unit area of the nucleolus (100 μ^2) and on 3rd chromosome (68B-74F) of the female from two experimental autoradiograms (control and treated series of experiments). The results of these investigations are presented in Table 1. It is evident from the data that α-amanitin drastically inhibits chromosomal labelling than that of nucleolar labelling. Yet, there is a positive correlation between chromosomal and nucleolar RNA synthesis for all experimental sets. The analysis of interclass correlation indicates that the degree of correlation is not homogenous in nature (data not included here). This may imply that the normal coordination of synthesis of nucleolar and chromosomal RNA has been altered due to the effect of α-amanitin. Furthermore, when the grain ratios of the nucleolus to 3L (N/3L) segment are plotted against the concentration of α-amanitin, a biphasic curve is obtained (Fig. 2). The biphasic nature of the curve may imply that two levels of sensitivity of α-amanitin

might be existing in the cellular RNA polymerase system.

This work is financially supported by U.G.C. Research Grants.

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Choo, J.K. Chungang University, Seoul, Korea. Effect of gene frequency on heavy metal compounds in D. melanogaster.

Caged populations of flies expressing normal viability on the second chromosome, composed of D. melanogaster collected from Banweol area, a suburb of Seoul, were exposed to lead acetate and cadmium chloride. Pb and Cd compound chemicals were mixed in the medium at 50 mg/l, and flies collected from the cages at generations 5, 10, 15 and 20 to investigate the effect of gene frequency on the second chromosome.

Frequency of recessive lethal mutant concelaed in second chromosomes after generation 20 was 5.8% in the control cage; in the chemical treatment cages, frequencies were 8.1% and 7.3% for Pb and Cd, respectively.

Effect of low viability mutation induced by heavy metal compounds occurred in generation 15, as shown in Fig. 1. After generation 20, the frequency of semilethal chromosomes was 13.9% in the control cage; those in the Pb and Cd compound cages increased directly and the populations have maintained 38.2% and 30% in semilethal contents.

All second homozygote viability consisting of lethal genes after generation 20 was estimated to be 22.17 in the control cage. On the other hand, viability decreased in the Pb and Cd compound cages to 17.15 and 18.55, respectively.

From the results of this experiment, both lead and cadmium compounds have low mutageneic effects in complete lethal second chromosomes. However, the viability polygene mutation induced by Pb and Cd chemicals seemed somewhat higher than those in control populations.

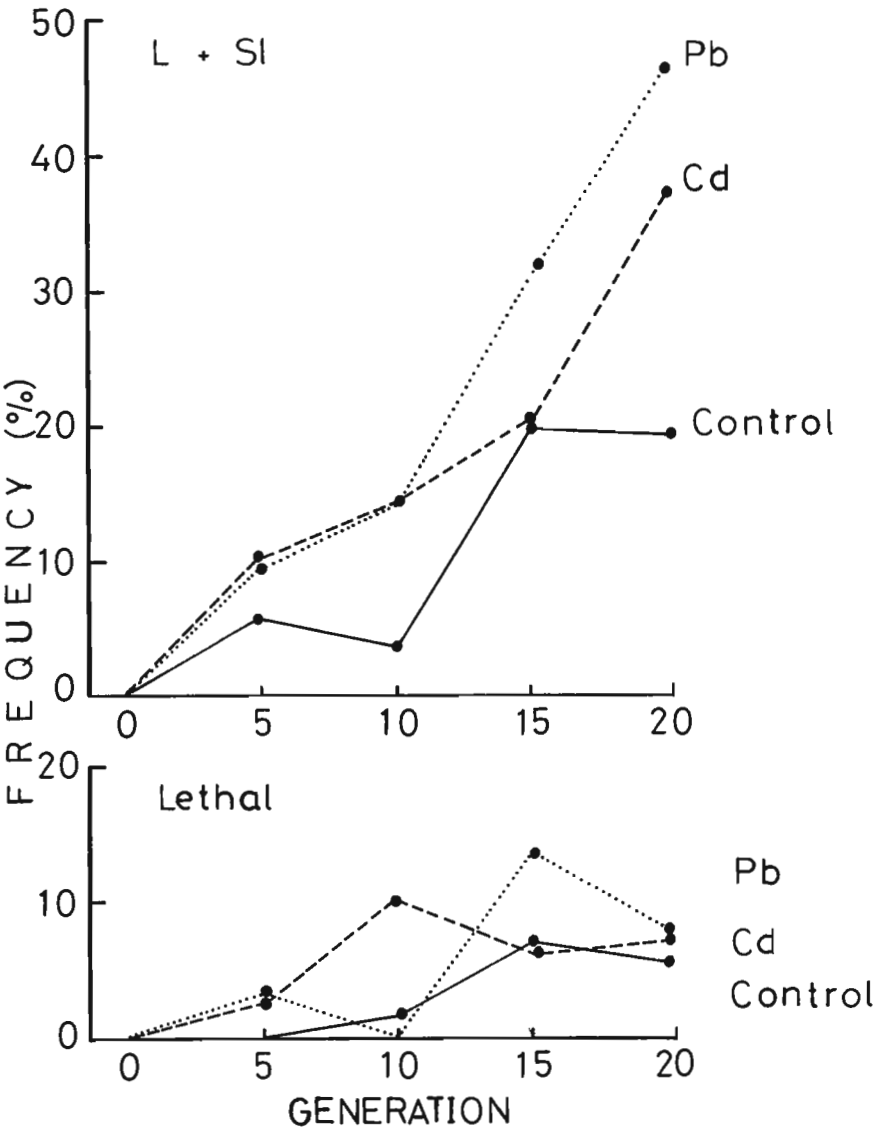

Fig. 1. Changes in frequency of lethal and L + SL chromosomes isolated from different chemical populations.

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Seasonal studies on Drosophila fauna of Khallikote Ghats, Orissa, India.

Seasonal variation of climate, and the consequential impact on the environmental milieu, have been known to affect population size. However, related studies on the tropical species of Drosophila are very meager (Pipkin 1953; Gupta 1974; Reddy & Krishnamurthy 1977, 1978; Prakash & Reddy 1979) and this prompted us to undertake the analysis of the seasonal changes vis à vis the population size of Drosophila at different localities of Khallikote Ghats, Orissa, situated between 19°15' and 19°5' N latitude and 84°20' and 85°15' E longitude.

Collections were made in the first week of every month from January through December, 1980, by the usual trapping method. Forty traps with fermented banana bait were tied to the branches of trees. The traps were fixed exactly at the same positions to rule out the possible effects of microhabitat variation, if any, with respect to food supply. The vegetation here consisted largely of fruit and timber-yielding trees as well as thick bushes. The collection data during the year of collection is depicted in Table 1.

Table 1. Collection data on Drosophila from Khallikote Ghats (1980).

Months		malerkotliana	kikkawai	takahashii	rajasekari	bipectinata	suzukii	nigra	melanogaster	Total
Jan.	M	154	78	8	2	2	-	-	-	421
	F	73	50	23	17	11	-	3	-	
Feb.	M	79	-	12	3	5	1	-	-	259
	F	98	-	29	8	11	1	5	7	
Mar.	M	12	-	3	3	1	-	3	4	65
	F	18	-	3	6	5	-	-	7	
Apr.	M	22	-	1	3	-	-	-	3	117
	F	50	-	12	12	8	-	-	6	
May	M	14	-	2	2	2	-	-	-	60
	F	24	-	6	4	4	-	-	2	
June	M	16	-	1	3	2	-	1	-	55
	F	14	-	7	4	6	-	1	-	
July	M	141	54	12	16	25	-	2	-	390
	F	77	27	10	16	10	-	-	-	
Aug.	M	154	23	64	30	8	-	-	-	457
	F	89	18	43	22	6	-	-	-	
Sep.	M	291	50	68	40	38	-	2	-	766
	F	145	22	53	25	32	-	-	-	
Oct.	M	205	55	59	43	21	-	4	-	625
	F	132	49	28	18	11	-	-	-	
Nov.	M	109	65	27	18	2	-	2	-	408
	F	85	44	34	15	3	-	4	-	
Dec.	M	167	124	69	43	12	-	3	-	697
	F	115	103	38	12	9	-	2	-	

A total of 4,320 flies belonging to eight species were collected during the period of study. Of these, four species (D. malerkotliana, D. takahashii, D. rajasekari and D. bipectinata) appear to be seasonally versatile, occurring year-round, while others remained restricted only to some months of the year. Barring the population of D. melanogaster, the dry pre-monsoon period (March-June) appears to be most inhospitable as the population size of all the species has been reduced to low levels. Further, it is observed that D. kikkiawai is completely absent during this period. However, during both monsoon and post-monsoon periods there was a noticeable increase in the population size of the majority of the species. D. suzukii, however, was available only in the month of February. Plausibly, the onset of monsoon modifies the habitat which stimulates the growth of almost all Drosophila species. The marked seasonal fluctuation in the population size of most of the Drosophila species in our studies are apparently due to rainfall which, as far as we can see, is the sole macroclimatic variable.

The award of fellowship to D.P.D.M. by the C.S.I.R., New Delhi, is thankfully acknowledged.

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Drosophila fauna from three localities of Orissa State, India.

Drosophila fauna of several areas of this country have, no doubt, been explored but many more parts still remain to be surveyed. The State of Orissa is one such virgin field. The present note is a preliminary report on Drosophila fauna from three localities of Orissa: Nandankanan, Bhanja Bihar and Golabandha. The collections were made in the months of October-November, 1980, on yeasted banana mash. The bottles containing the bait were tied to branches of trees. The collection data from these localities are shown in Table 1.

Table 1. Drosophila fauna of Nandankanan, Bhanja Bihar and Golabandha, Orissa.

Species	Nandankanan M	Nandankanan F	Bhanja Bihar M	Bhanja Bihar F	Golabandha M	Golabandha F	Total
Melanogaster species group:							
D. malerkotliana	30	60	30	60	55	60	295
D. kikkawai	5	12	12	28	20	40	117
D. bipectinata	10	15	8	20	11	15	79
D. rajasekari	3	5	6	12	10	22	58
D. takahashii	15	12	20	28	12	25	112
Immigrans species group:							
D. nasuta	25	20	--	--	--	--	45
Total:							706

The flies, so collected, consist of six species. Five of these (D. malerkotliana, D. kikkawai, D. bipectinata, D. rajsekari and D. takeshii), which belong to the melanogaster species group, were available in all the localities under study. This clearly indicates a great versatility of these species. However, D. nasuta of the immigrans species group is restricted to Nandankanan area. It has also been observed that D. malerkotliana numerically dominates over all other species in all these localities. As we see from the table, the females outnumber the males in five of the six species. The imbalance in the sex ratio in the above wild populations needs to be explained through further investigations.

The award of a Senior Fellowship to D.P.D.M. by the C.S.I.R., New Delhi, is thankfully acknowledged.

Dekker, W. University of Groningen, Haren, The Netherlands. Two Drosophila species utilizing different feeding substrates in one habitat.

In the field populations of Drosophila species often occur at low densities (Burla et al. 1950; Crumpacker et al. 1973; Begon et al. 1975; Begon 1978) and are therefore difficult to study population dynamically. In 1976 Hummel et al. (1979), however, accidentally discovered that natural populations of D. limbata occurred in very high densities in cucumber greenhouses in Hoogezand-Sappemeer in the north of the Netherlands: top population densities amounted to an estimated 2500 to 5000 flies/100 m^2. These observations were confirmed in later years: during summertime comparable high densities were reached in the greenhouses studied. In 1979 we found in a particular cucumber greenhouse (area 1000 m^2) that, in addition to D. limbata, D. phalerata, another species also belonging to the quinaria species group, was present at high density, though still at a lower density than D. limbata, while other Drosophila species were nearly absent. A representative sample of flies, obtained by net sweeping in July, contained 6391 D. limbata, 797 D. phalerata, 3 D. busckii and 1 D. funebris. On a refuse heap 10 m apart from the greenhouse, however, in addition to the species occurring in the greenhouse, the following species were also found: D. hydei, D. subobscura, D. melanogaster and D. fenestrarum. Further research was directed to the problem why both closely related species of the quinaria group are so abundant in this greenhouse. This particular greenhouse was exceptional as it contained a large population of Agaricus campestris, probably because the soil was derived from a mushroom nursery.

In order to trace the breeding sites of the two species, material of all potential oviposition and larval feeding substrates was sampled. For this purpose rotten cucumbers (Cucumis sativus), mushrooms (Agaricus c.f. campestris and Lepista nuda) and berries of the black night-

shade (Solanum nigrum), were collected and stored separately in buckets closed with cheesecloth, at room temperature (± 20°C) for at least 30 days. Each bucket was examined for the presence of adult flies at irregular intervals, but at least once a week (both living and dead adults were counted). The results are given in Table 1. Because the numbers of D. busckii and D. funebris obtained in the greenhouse were extremely low, these species were neglected in further calculations.

Table 1. A: Number of Drosophila adults emerged from diverse substrates collected in the greenhouse. B: Number of flies in a sample collected by net sweeping in the greenhouse.

		Drosophila species			
A	Substrate	limbata	phalerata	busckii	funebris
	Cucumis sativus	48	1	11	0
	Agaricus campestris	111	180	0	0
	Lepista nuda	0	0	0	0
	Solanum nigrum	0	0	0	0
	Totals from substrate:	159	181	11	0
B	Greenhouse	6391	797	3	1

Table 2. For both substrates, and for the greenhouse, proportion of flies pro species given in angles ± standard deviation.

	Drosophila species	
Substrate	limbata	phalerata
Cucumis	84.74 ± 7.44	5.26 ± 7.44
Agaricus	38.73 ± 3.79	51.27 ± 3.79
Greenhouse	68.16 ± 6.07	21.85 ± 6.07

Table 3. For both Drosophila species, proportion of flies pro substrate, given in angles ± standard deviation.

	Drosophila species	
Substrate	limbata	phalerata
Cucumis	71.56 ± 6.28	13.86 ± 19.60
Agaricus	18.44 ± 1.80	76.14 ± 5.62
Total	90.00 ± 8.01	90.00 ± 24.99

Table 2 gives for the data in Table 1 the proportion of total flies per substrate converted to angles ($y = \arcsin \sqrt{x}$), giving a total of 90° in each row. It is evident from the data of Tables 1 and 2 that most of the larvae of D. phalerata feed in Agaricus. The data for D. limbata, however, are not conclusive. Unfortunately it is not well possible to estimate the relative amounts of suitable breeding substrates (rotten cucumbers can, fresh cucumbers cannot be used as breeding substrate). Therefore, at this stage, there is no clear evidence in what substrate D. limbata breeds preferentially. In order to derive quantitative information concerning the breeding substrates of D. limbata and D. phalerata, we proceeded along the following line of reasoning. The flies emerging from Cucumis have a species ratio (D. limbata/D. phalerata) of 48/1; for Agaricus this is 111/180. Assuming that: (1) migration is neglectable, (2) Cucumis and Agaricus are the only breeding substrates, and (3) the proportion of adult life stage on total life span is the same for both species, the species ratio of the flies caught by net sweeping must be an average of these two substrate species ratios, weighted by the amount of flies emerging from each of both substrates. From Table 2 we can solve the proportion of all flies emerging from Cucumis by multiplying the Cucumis row with 0.6395 and the Agaricus row with 0.3605, giving the desired ratio of species. Now we can derive that 0.6395 x 84.74° = 54.19° of all flies are D. limbata and breeding in Cucumis, and that 0.3605 x 38.73° = 13.96° of all flies are D. limbata, breeding in Agaricus. Therefore 54.19 x 90°/(54.19 + 13.96) = 71.56° of all D. limbata breed in Cucumis, and 13.96 x 90°/(54.19 + 13.96) = 18.44° of all D. limbata breed in Agaricus. In this way Table 3 is obtained (90° in each column).

These results should, at this moment, not be overemphasized, because first, the experimental data are limited, and secondly, we have multiplied two times with factors treated as deterministic values, which are actually statistical ones, possibly giving rise to bigger variances.

Nevertheless, the conclusion is that D. limbata in the larval stage mostly feeds on Cucumis (71.56° = 90%), while D. phalerata feeds in the larval stage mostly on Agaricus (76.14° = 94%). For the greater part, therefore, these two closely related species have different feeding sites, which provide them with different niches in the same habitat. The cause of this difference in breeding substrate is investigated at the moment. There are strong indications that differen-

tial needs for sterols are responsible for this phenomenon.

I am very grateful to Mr. P. Boerland, owner of the greenhouse, who kindly permitted me to do my experiments in his greenhouse, and to Dr. W. van Delden for his very useful comments on the manuscript.

References: Begon, M. 1978, Ecol. Entom. 3:1-12; Begon, M., O. Milburn and D. Turner 1975, J. Nat. Hist. 9:315-320; Burla, H., A.B. da Cunha, A.G.L. Cavalcanti, T. Dobzhansky and C. Pavan 1950, Ecology 31:393-404; Crumpacker, D.W. and J.S. Williams 1973, Am. Midl. Nat. 91:118-129; Hummel, H.K., W. van Delden and R.H. Drenth 1979, Oecologia 41:135-143.

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Biochemical loci of the "fruit fly" (Drosophila melanogaster).†

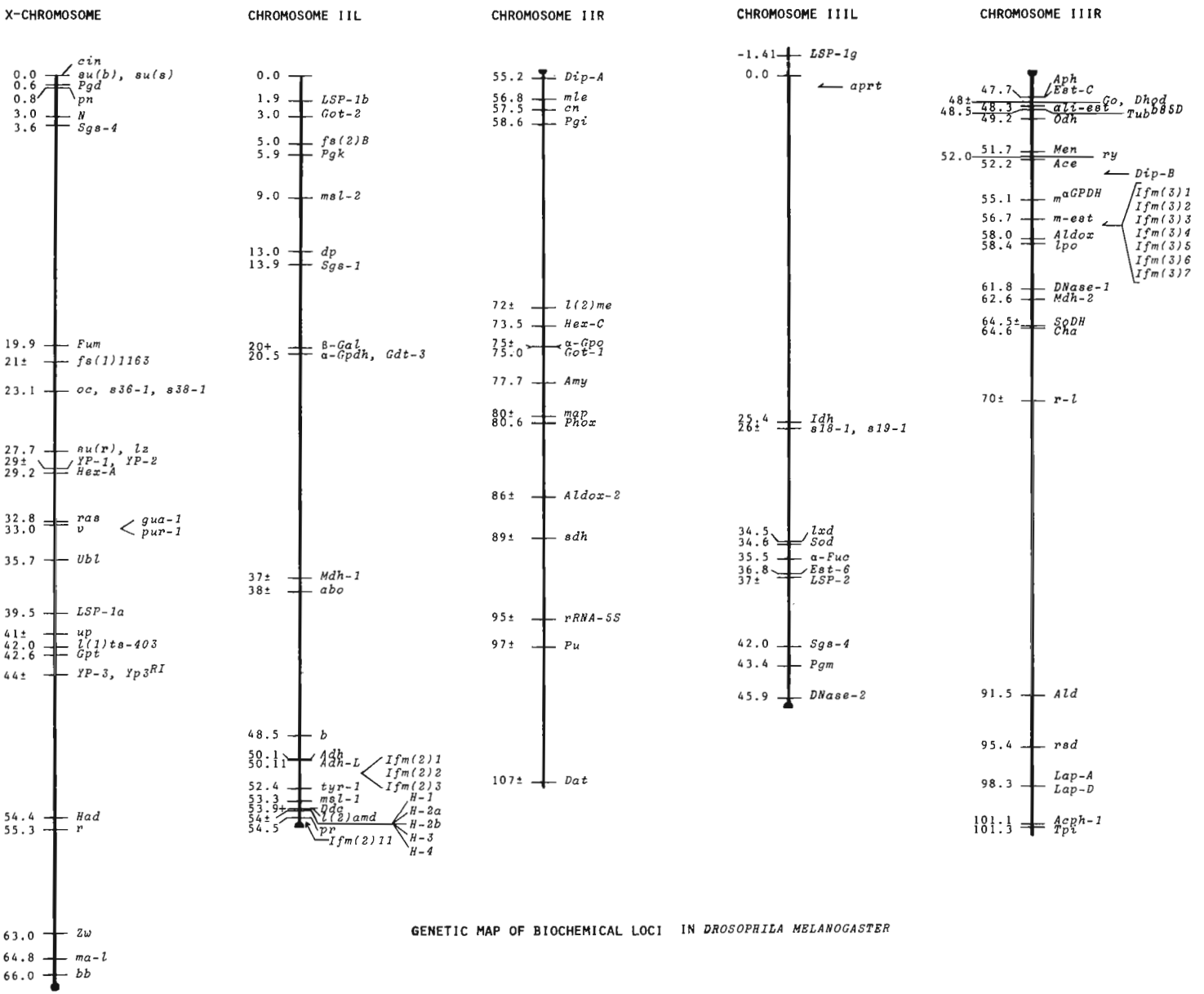
2N = 8

The genetic map of biochemical loci in D. melanogaster is an updated version of earlier revisions (97, 400, 490). Information from general references (92, 253, 268, 301), and new material compiled by February 1, 1982 are included. An effort was made to screen all published articles, research notes, books and personal communications that have withheld the test of time. Some papers cited do not contain mapping data but were considered useful in the interpretation of mapping data. Certain loci have been so extensively studied that it is not feasible to give a full list of references for them; review articles are thus cited. We have attempted to resolve cases of synonymous symbols and use of the same symbol for unrelated loci. Bracketed symbols are for unnamed loci for which we suggest the symbol indicated. Genes located cytologically by segmental aneuploidy and/or by in situ hybridization studies, but not by recombination analysis, are listed with the chromosome or chromosome arm in which each occurs shown parenthetically.

Loci for which there are physical mapping data from restriction enzyme and/or nucleotide sequencing studies are referenced by a superscript (P). Structural genes that code for the primary amino acid sequence of a given protein or polypeptide are indicated by an asterisk (*) where the data are convincing and by two asterisks (**) where the data are suggestive but less critical. No attempt has been made to include mapping information on dispersed repeated gene families such as copia, 412, 297 or other transposable elements; this has been reviewed (374). We thank E. Kubli for editing the tRNA genes. A cytogenetic map of tRNA loci, prepared by Kubli, accompanies our map†.

Gene Symbol	Genetic Map Locus	Cytological Map Limits	Enzyme, Protein, or Nucleic Acid Affected	Reference
abo abnormal oocyte	2-38		rDNA redundancy	233 234 274 329 353 445 469
**Ace* (= *l(3)26*)	3-52.2	87E1-5	acetylcholine esterase	156 173
**Acph-1*	3-101.1	99D-99E	acid phosphatase-1	24-5 123 208 266 290-1
**actin genes:				
{*A1*}	(3R)	88F	actins I, II, III	130^{P} 131^{P} 189 396^{P} 455^{P}
{*A2*}	(1)	5C		
{*A3*}	(2R)	42A		
{*A4*}	(2R)	57A		
{*A5*}	(3R)	87E		
{*A6*}	(3L)	79B		
**Adh*	2-50.1	35B2-3	alcohol dehydrogenase	31-2^{P} 142 149^{P} 162 275 279 303 354 407 410 434-5
Adh-L	2-50.11	35B2-3	ADH control	184 391
***{*Ak*}	(3L)	66B-F	arginine kinase	128
**Ald*	3-91.5	97A-B	aldolase	308 414 416
**Aldox* (= *Aldox-1*)	3-58.0	88F9-89B5	aldehyde oxidase	73 77 84 90-1 116 282 368 414 431

†In: Genetic Maps, S.J. O'Brien, ed., Vol. 2, March, 1982. Lab. Viral Carcinogenesis, N.C.I., N.I.H., Bethesda, Maryland 20205.



GENETIC MAP OF BIOCHEMICAL LOCI IN *DROSOPHILA MELANOGASTER*

February 1, 1982

Aldox-2	2-86		aldehyde oxidase-2	29
**ali-est*	3-48.3		ali-esterase	306 307
**Amy*	2-77.7	54B-55	α-amylase	12-14 93 94 97 228
**Aph*	3-47.7		alkaline phosphatase	22 421
aprt	3-(right of *ru*)		adenine phosphoribosyl transferase	209 210 253
b black	2-48.5	34E5-35D1	β-ureidopropionase (?) β-alanine deficient	16 253 471
bb bobbed	1-66.0	20C1-2	rRNA: 2S, 5.8S, 18S, 28S	61 87^{p} 140 146 147^{p} 148^{p} 195^{p} 212-13^{p} 224-5^{p} 230^{p} 259-61^{p} 273^{p} 319^{p} 331^{p} 340 341 346-8^{p} 429^{p} 443^{p} 452^{p}
B2t (see *Tub*^{b85D})				
***Cat*	(3L)	75D-76A	catalase	265
**Cha* (= *Cat*)	3-64.6	91B-D	choline acetyltransferase	154 155 172 174
chorion genes: see shell proteins (*s*)				
cin cinnamon	1-0.0		modifier of: xanthine dehydrogenase, aldehyde oxidase, pyridoxal oxidase, sulfite oxidase	17 30 49 116 314 459 493
***cn* cinnabar	2-57.5	43E3-14	kynurenine hydroxylase	141 318 385 453
cr compensatory response	(1)	20C1-2	rDNA redundancy	329
**Dat*	2-107	60B1-10	dopamine acetyl transferase	194 277
**Ddc*	2-53.9+	37C1-2	dopa decarboxylase	186 276 438-41
***Dhod*	3-48	85A-C	dihydroorotate dehydrogenase (mitochondrial)	333 337
**Dip-A*	2-55.2	41A-42A3	dipeptidase-A	310 411 414
***Dip-B*	3-(near *red*)	87F12-88C3	dipeptidase-B	310
***Dip-C*	(3R)	87B5-87B10	dipeptidase-C	310
**DNase-1*	3-61.8	90C2-E	deoxyribonuclease-1	89 160
DNase-2	3-45.9		deoxyribonuclease-2	160
dnc dunce	1-(between *y* and *cho*)	3D4	cAMP-phosphodiesterase, form II	50 85 101 226 227
dp dumpy	2-13.0	24E2-25A2	orotate phosphoribosyl transferase	43 151 253
ecdysteroid-inducible polypeptide (EIP) genes:				
**{EIP28/EIP29}*	(3L)	71C3-D2	28K EIP (28-I,-II,-III), 28.5K EIP, 29K EIP (29-I,-II,-III,-IV)	57 58 461
**{EIP40}*	(2R)	55B-D	40K EIP (40-I,-II,-III)	
----	(3L)	63F2-4	EIP(?) RNA	
**Est-C*	3-47.7	84B2 or 84D3-12	esterase-C	23 308 309 401
**Est-6*	3-36.8	69A1-5	esterase-6	2 23 70 83 123 344 345 463
Est-9	2-		esterase-9	264
fs(1)1163	1-21		yolk protein-1 control, *cis*-acting	45 46 132 481
fs(2)B female sterile(2)Bridges	2-5.0		thymidylate synthetase	53

*α-*Fuc*	3-35.5		α-fucosidase	267
**Fum* (= *Fuh*)	1-19.9	5C-6C11	fumarase (fumarate hydratase)	271 323
***Gapdh*	(2R)	50D-51A2	glyceraldehyde-3-phosphate dehydrogenase	128
*β-*Gal*	2-20+	26A7-9	β-galactosidase	267
***Gart*	(2L)	27C	glycinamide ribotide transformylase(?)	180[p]
Gdt-3	2-20.5		*cis*-temporal control for α-*Gpdh*	34
β-Glu	(3R)		β-glucuronidase, I & II	241
**Go* (= *Hex-1*)	3-48.5	84CD	glucose oxidase	54
Got-1	2-75.0		glutamate oxaloacetate transaminase-1	161
**Got-2*	2-3.0 (or 4.8?)	22B1-4	glutamate oxaloacetate transaminase-2	159 161 460
*α-*Gpdh*	2-20.5 (or 17.8?)	25F-26B	α-glycerophosphate dehydrogenase	36-8 71 157 298-300 460
α-Gpo	2-75	52B-52F	α-glycerophosphate oxidase (mitochondrial)	298 300
**Gpt*	1-42.6	11F1-12A2	glutamate pyruvate transaminase	247 414
gua-1	1-left of *v*	9E1-3	guanosine 1	211 480
histone genes:				
*{*H-1*}	2-54	39D3-E2	histone 1	40 59 218 252 289 316 351[p] 458 462 488
*{*H-2a*}			histone 2a	
*{*H-2b*}			histone 2b	
*{*H-3*}			histone 3	
*{*H-4*}			histone 4	
**Had*	1-54.4		hydroxy acid dehydrogenase	44 397
**Hex-A* (= *Hex-A,B*)	1-29.2	8D4-E	hexokinase-A	293 412 414
**Hex-C* (= *Hex-3 = Fk*)	2-73.5		hexokinase-C (hexokinase-3)	207 272 293 294
heat shock cognate (HSC) genes:				
hsc70 (= *hsc1*)	(3L)	70C	HSC 70 RNA (= HSC RNA 1)	79 474[p]
hsc2	(3R)	87D-88B, 88E	HSC RNA 2	79
hsc3	(3R)	95C-E	HSC RNA 3	
heat shock genes and related elements:				
**hsp22*	(3L)	67B	22K heat shock protein	75[p] 80[p] 188[p] 197[p] 219[p] 255[p] 322 376 418[p] 419[p]
**hsp23*			23K heat shock protein	
**hsp26*			26K heat shock protein	
**hsp28* (= *hsp27*)			28K heat shock protein	
**hsp68*	(3R)	95D	68K heat shock protein	10 79 187-8[p] 355 376
**hsp70*	3-	87A7 & 87C1	70K heat shock protein	9[p] 10 51 81[p] 133 150[p] 171[p] 179 187[p] 188[p] 196[p] 198[p] 199 200 201-2[p] 216[p] 251 254-6[p] 258[p] 281 283[p] 322 357 375-6 395 399[p] 464[p] 468

*_hsp83_ (= _hsp82_)	(3L)	63BC1	83K heat shock protein	10 187[P] 188[P] 255[P] 376
α gene, β gene, γ gene	(3R)	87C1 & chromocenter	heat shock RNAs: αβ RNA; αγ RNA	171[P] 254[P] 255[P] 256[P] 257
_63B-T2_	(3L)	63B	heat shock RNA 63B-T2	302[P]
93D hs-DNA	(3R)	93D	heat shock polyA⁻ RNA (mostly)	239 248
10F hs-DNA, 54E hs-DNA, 63F hs-DNA, 73D hs-DNA, 88B hs-DNA	(1), (2R), (3L), (3R)	10F1, 54E1, 63F1, 73D1, 88B1	minor heat shock RNAs	255[P]
*_Idh_ (= _Idh-NADP_)	3-25.4	66B-D	NADP-isocitrate dehydrodgenase	122 308 335 381
indirect flight muscle (IFM) myofibrillar protein genes:				
(see _up_)				
Ifm(2)1, _Ifm(2)2_, _Ifm(2)3_, _Ifm(2)11_, _Ifm(3)1_, _Ifm(3)2_, _Ifm(3)3_, _Ifm(3)4_, _Ifm(3)5_, _Ifm(3)6_, _Ifm(3)7_	2-between _b_ and _pr_; 2-near _pr_; 3-between _st_ and _e_ (_Ifm(3)4_ between _red_ and _ss_)		IFM myofibrillar proteins	479
**_Kf-I_	(3R)	91B-93F	kynurenine formamidase-I	288
**_Kf-II_	(2L)	25A-27E	kynurenine formamidase-II	288
_l(1)ts-403_ lethal(1)temperature sensitive-403	1-42.0		control of heat shock proteins	110
l(2)amd lethal(2)α-methyl dopa	2-53.9+	37B10-C4	(? dopa decarboxylase system)	276 367 437-40
_l(2)me_ lethal(2)meander	2-72		protease activity	42 50 99 456
*_Lap-A_	3-98.3		leucine aminopeptidase-A	23 113
*_Lap-D_	3-98.3		leucine aminopeptidase-D	23 113 350
larval cuticle protein (LCP) genes:				
{_L3CP1_}, {_L3CP2_}, {_L3CP3_}	(2L)	44D	17.5K LCP 1 (3rd instar); 17.5K LCP 2 (" "); 9K LCP 3 (" ")	127 366[P] 463
{_L3CP5_}	3-		LCP 5 (" ")	127 366[P] 463
**_lpo_	3-58.4		pyridoxal oxidase (low)	72 116 423
larval serum proteins (LSP) genes:				
*{_LSP-1α_}	1-39.5	11A7-B9	larval serum protein-1α	47 342 365[P] 477[P]
*{_LSP-1b_}	2-1.9	21D2-22A1	larval serum protein-1β	47 249[P] 342 365[P] 477[P]
*{_LSP-1g_}	3-(-1.41)	61A1-6	larval serum protein-1γ	47 249[P] 342 365[P] 477[P]
*{_LSP-2_}	3-37	68E3-4	larval serum protein-2	2 3 249[P]
lxd low xanthine dehydrogenase	3-34.5		modifier of: xanthine dehydrogenase, aldehyde oxidase, pyridoxal oxidase, sulfite oxidase	73 103 143 313 423 459 493
_lz_ lozenge	1-27.7	8D4-8E2	monophenoloxidase; diphenoloxidase	20 285 320 321 422
mα-GPDH	3-55.1		modifier of α-_Gpdh_	229

ma-l maroon-like (= *mal*)	1-64.8	19D1-3	modifier of: xanthine dehydrogenase aldehyde oxidase pyridoxal oxidase uricase octanol dehydrogenase phenoloxidase	63 77 100 115 117-21 126 143-4 423 492-3 459
map midgut activity pattern	2-80		α-amylase *trans* control	1 95
**Mdh-1* (= *cMdh*)	2-37	31B-E	NAD-malate dehydrogenase (cytoplasmic)	4 158 297 298 414 415
**Mdh-2* (= *mMdh*)	3-62.6	90C-91A3	NAD-malate dehydrogenase (mitochondrial)	414 415
**Men* (= *Mdh-NADP*)	3-51.7	87D1-2	NADP-malate dehydrogenase (malic enzyme)	125 135 308 414 417
m-est	3-56.7		esterase-6 and leucine aminopeptidase modifier	68 69
mle maleless	2-56.8		control of X-linked enzymes (G6PD, 6PGD, FUM)	26 129 386
msl-1 male-specific lethal-1	2-53.3		control of X-linked enzymes (G6PD, 6PGD, FUM)	26 27
msl-2 male-specific lethal-2	2-9.0		control of X-linked enzymes (G6PD, 6PGD, FUM)	26 27
muscle protein genes: (see actin, *Ifm*, *TM*, *up*)				
**{*MHC*}	(3R)	87E & 88F	myosin heavy chain (?)	33 483
** ----	(3R)	87B	22.5K myofibrillar contractile protein	33 483
N Notch	1-3.0	3C7	mitochondrial respiratory enzymes: NADH oxidase NADH dehydrogenase succinate dehydrogenase α-glycerophosphate dehydrogenase	222 223 253 392 393 394 425 426
oc ocelliless (associated with *In(1)7F1,2-8A1,2*)	1-23.1	7F1-8A2	stable position effect on shell protein-36-1 & shell protein-38-1	246 372^{p} 373^{p} 377
**Odh*	3-49.2	86C1-D8	octanol dehydrogenase	76 78 309
proteins, miscellaneous genes: (see EIP genes)				
**P1*	(3L)	70CD	110K larval fat body protein P1	249^{p}
**P6*	(3L)	70CD	29K larval fat body protein P6	249^{p}
* ----	(3L)	80C	26K embryonic cytoplasmic protein	39^{p}
**6-Pgd*	1-0.6	2D3-4	6-phosphogluconate dehydrogenase	35 138 166-8 170 450
**Pgi*	2-58.6		phosphoglucose isomerase	414 417
**Pgk*	2-5.9	22D-23E3	3-phosphoglycerate kinase	60 416
**Pgm*	3-43.4	72D1-5	phosphoglucomutase	185 402 403 416
**Phox*	2-80.6		phenol oxidase	19
pn prune	1-0.8	2D5-6	guanine triphosphate-cyclohydrolase control	109 253
***pr* purple	2-54.5	37B2-40B2	sepiapterin synthase-enzyme A (Mg^{2+}-requiring)	98 235 253 398 449

Pu Punch	2-97	57BC(?)	GTP cyclohydrolase	253 305
pur-1	1-left of *v*	9E1-3	purine 1	211 480
**r* rudimentary	1-55.3	15A1	carbamyl phosphate synthetase aspartyl transcarbamylase dihydroorotase	48 52 111-2 114 205-6 245 253 296 334 336 404
ras raspberry	1-32.8	9E1-3	guanine triphosphate-cyclohydrolase control	109 253 454 480
**r-l* rudimentary-like (= *ral*)	3-70	93B4-13	orotate phosphoribosyltransferase	74 242 332 333 484
**rp*	(3R)	99D	ribosomal protein 49	408 494[P]
rRNA genes: (see *bb*)				8 28 214[P] 263[P]
{*rRNA-5S*}	2-95	56EF	5S-rRNA	324[P] 328 330 349 388-9 405[P] 432-3 458 495
rsd raised	3-95.4		actin III metabolic regulation	240 253
**ry* rosy	3-52.0	87D12-13	xanthine dehydrogenase (with *cis* control)	63 64-5 136-7 144 183 278 304 448
shell protein genes (chorion genes):				
**s15-1* (= *c15* = *A1*)	(3L)	66D11-15	shell protein 15-1	163 369[P] 378
**s16-1* (= *c16* = *A2*)	(2R)	54CD	shell protein 16-1	163
**s18-1* (= *c18* = *B1*)	3-26 (right of *se*)	66D11-15	shell protein 18-1	369[P] 370 378 496
**s19-1* (= *c19* = *B2*)	3-26 (right of *se*)	66D15	shell protein 19-1	370 496
**s36-1* (= *c36* = *C1*) **s38-1* (= *c38* = *C2*)	1-23.1	7F1-2	shell protein 36-1 shell protein 38-1	369[P] 371 372[P] 377 378 444
sdh	2-89		mitochondrial succinate dehydrogenase	243
salivary gland structural (SGS) proteins genes:				
***Sgs-1*	2-13.9	25A3-D2	SGS-1	409
**Sgs-3*	(3L)	68C	SGS-3	2 231 478[P]
**Sgs-4*	1-3.6	3C10-D1	SGS-4	21 231-2 280[P] 295[P]
***Sgs-6*	3-42.0	71C1-F5	SGS-6	491 475
**Sod* (= *To*)	3-34.6 (or 32.5?)		superoxide dismutase (tetrazolium oxidase)	124 207 301 313 460
**Sodh* (= *SoDH*)	3-64.5	91B-93F	NAD-sorbitol dehyrogenase (cytoplasmic)	41 42
su(b) suppressor of black	1-0.0		β-alanine	487
su(r) suppressor of rudimentary	1-27.7		dihydrouracil dehydrogenase	15 111 383 384
su(s) suppressor of sable	1-0.0	1B11 to C2-3	suppresses *pr*, *s*, *sp* & *v* 30-100K protein tRNA (asn, asp, his, tyr) modifiers	178 203 204 269 406 427
***Sucr*	(2L)	31CD-EF	sucrase	311
TM	(3R)	88F2-5	tropomyosin	382
**Tpi*	3-101.3	99B-E	triosephosphate isomerase	414 416

**_Treh_	(2R)		55B-E	trehalase	312
transfer (4S) RNA genes:					
{_tRNA^{Ala}_}	(3L)		63A & 90C	alanyl tRNA	359
{_tRNA^{Arg-2}_}	(2R)		42A	arginyl tRNA-2	458 486^{p} 102^{p} 107 175^{p} 191^{p} 358 364^{p} 446-7^{p}
	(3L)		84F		
{_tRNA^{Asn-5}_}	(2R)		42A, 59F & 60C	asparaginyl tRNA-5	102^{p} 191^{p} 358-9 446^{p} 447^{p}
	(3L)		84F		
{_tRNA^{Asp-2}_}	(2L)		25D, 29D, 29E	aspartyl tRNA-2	102^{p} 175^{p} 360
	(3L)		70A		
{_tRNA^{Glu-4}_}	(2R)		52F & 56EF	glutamyl tRNA-4	6^{p} 190^{p} 237
	(3L)		62A		
{_tRNA^{Gly}_}	(2R)	(?)	58A	glycyl tRNA (anticodon GAA)	470
	(3L)		84C & 90E		
{_tRNA^{Gly-3}_}	(2L)		22BC & 35BC	glycyl tRNA-3	175^{p} 177^{p} 181^{p} 458
	(2R)		56EF		
{_tRNA^{His}_}	(2R)		48F & 56E	histidyl tRNA	7^{p} 102^{p} 359
{_tRNA^{Ile}_}	(2R)		42A & 50AB	isolucyl tRNA	191^{p} 343^{p} 446^{p} 447^{p}
{_tRNA^{Leu-2}_}	(2R)		44E	leucyl tRNA-2	176 470
	(3L)		66B5-8 & 79F		
{_tRNA^{Leu}_}	(2R)		50AB	leucyl tRNA-3(?)	343^{p}
{_tRNA^{Lys-2}_}	(2R)		42A, 42E, 50B & 56EF	lysyl tRNA-2	88^{p} 139^{p} 165 175^{p} 177^{p} 191^{p} 358 363 446^{p} 447^{p} 466^{p} 476
	(3L)		63B		
{_tRNA^{Lys-5}_}	(1)	(?)	12E	lysyl tRNA-5	164 165 175^{p} 470 489^{p}
	(2L)		29A & (?)29DE		
	(2R)		48F-49A		
	(3R)		84A & 87B		
{_tRNA^{Met-2}_}	(2R)		48B5-7	methionyl tRNA-2	107 175^{p}
	(3L)		72F1-2		
	(3R)		83F-84A		
{_tRNA^{Met-3}_}	(1)	(?)	19-20	methionyl tRNA-3	107 165 175^{p} 177^{p} 362^{p} 428 485^{p}
	(2R)		46A1-2 & 56EF		
	(3L)		61D1-2 & 70F1-2		
{_tRNA^{Phe-2}_}	(2R)		56EF	phenylalanyl tRNA-2	5^{p} 165
{_tRNA^{Ser-2b}_}	(3R)		86A, 88A, 94A6-8	seryl tRNA-2b	176 470
{_tRNA^{Ser-4}_}	(1)		12DE	seryl tRNA-4	106^{p} 175^{p}
	(2L)		23E		
	(2R)	(?)	56D		
	(3L)	(?)	64D		
{_tRNA^{Ser-7}_}	(1)		12DE	seryl tRNA-7	106^{p} 175^{p}
	(2L)		23E		
	(2R)	(?)	56D		
	(3L)	(?)	64D		
{_tRNA^{Thr-3}_}	(2R)		47F	threonyl tRNA-3	165 176 470
	(3R)	(?)	87B		
{_tRNA^{Thr-4}_}	(3R)		93A1-2	threonyl tRNA-4	176 470

{*tRNA^{Tyr}*}	(1)	19F	tyrosyl tRNA	176 236 467^{p}470
	(2L)	22F & 28C		
	(2R)	41AB, 42A, 42E, 50C & 56D		
	(3R)	85AB		
{*tRNA^{Val-3a}*}	(3L)	64D1-2	valyl tRNA-3a	175^{p} 470
{*tRNA^{Val-3b}*}	(3R)	84D, 90BC, 92B	valyl tRNA-3b	105^{p}106^{p}165 175^{p}
{*tRNA^{Val-4}*}	(2R)	56D3-7	valyl tRNA-4	104 106^{p}165 175^{p}
	(3L)	70BC		457
	(3R)	89B & 90C		
----	(2L)	22A-C	4S-tRNA, unidentified	356
----	(1)	3D, 3F, 5F-6A & 11A		
----	(2L)	22DE, 24DE, 28D & 28F-29A		108
----	(2R)	41CD, 48CD, 49F-50A, 54A, 54D, 55F, 58AB & 60E		
----	(3L)	67B & 70DE		
----	(3R)	87F-88A, 90DE, 95F-96A, 97CD & 99EF		
tubulin genes:				
**Tuba67C*	(3L)	67C4-6	α-tubulin 67C	215^{p} 315 352
**Tub^{a84B}*	(3R)	84B3-6	α-tubulin 84B	
**Tub^{a84D}*	(3R)	84D4-8	α-tubulin 84D	215^{p} 284 315 352
**Tub^{a85E}*	(3R)	85E6-10	α-tubulin 85E	
*{*Tub^{b85D}*}	3-48.5	85D4-7	β-tubulin, testis specific	220 221 315
(= *B2t* = *ms(3)KK^{D}*)				
tyr-1 tyrosinase-1	2-52.4		monophenoloxidase (tyrosinase) diphenoloxidase (dopa oxidase)	250 285 361 422
***Ubl* (= *RPII* = *PolIII* = *AmaC4* = *l(1)L5*)	1-35.7	10C2-D4	RNA polymerase II subunit (α-amanitin resistant)	152 153 292 413
***up* upheld (*up^{2}* = *wupB*)	1-41		IFM myofibrillar protein, Z-band	286 287 465 472
***v* vermilion	1-33.0	10A1	tryptophan oxidase (tryptophan pyrolase)	11 203 217 244 387 454
wupB wings-up B (see *up*)				
Xh^{abo}	1-	20C1-2	rDNA redundancy	317 353
yolk proteins genes:				
(see *fs(1)1163*)				
**Yp-1*	1-29	9A1-B1	yolk protein-1	193^{p}473^{p} 18^{p} 45
**Yp-2*	1-29	9A1-B1	yolk protein-2	325-8
**Yp-3*	1-44	12BC	yolk protein-3	339^{p}424

$Yp3^{RI}$	1-near *Yp-3*		yolk protein-3 control, *cis*-acting	481
*$\overline{Zw}$ Zwischenferment	1-63.0	18D1-E2	glucose-6-phosphate dehydrogenase	134 169 379-81 430 450 451

*structural gene
**possible structural gene
^{p}references with physical mapping data

Authors' Note

Some of the genetic symbols listed should be considered as tentative. We have attempted to apply the rules governing nomenclature for genetic loci in *D. melanogaster* (see 253), but are not entirely satisfied with the results. Nevertheless, we made some changes where rules were broken. All subscripts for tRNA genes were moved to superscripts, e.g., $tRNA_2^{Arg}$ is designated $tRNA^{Arg-2}$. Since Greek letters are not to be used in symbols, we have replaced α, β and γ with *a*, *b* and *g*, where feasible; but this could not be done where it would change the alphabetical listing of the symbol. We are still not satisfied with symbols of excessive length. This revised map of "biochemical loci" supercedes all earlier versions.

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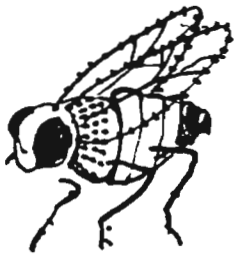
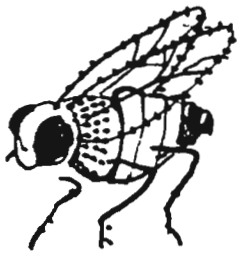
Acknowledgements

We thank those investigators who provided us with unpublished data, reprints, and corrections or clarification of information presented in earlier maps. We apologize to those whose work may have been inadvertently overlooked or misrepresented and hope that we will be informed of any such oversights or errors. This compilation was supported by NIH grant GM-25255.





Ehrman, L. and T. Miller. State University of New York, Purchase, New York, and University of Miami, Coral Cables, Florida. Florida Drosophila.

We wish to update the field records of Carson and Heed (1964) and Wheeler (1943) by itemizing the following species collected during late January, February and March, 1981, by baiting, netting and aspirating. (Note especially the D. willistoni group entries, and the melanogaster nongarbage collections).

Site in Florida	Remarks	Species
Coral Gables	Campus, near greenhouse and environs	D. acutilabella, D. cardini, D. latifasciaeformis, D. melanogaster, D. peninsularis, D. simulans, D. willistoni, willistoni group
Hollywood	Perimeter of golf course, near pool	D. acutilabella, D. cardini, D. melanogaster, melanogaster group, D. peninsularis, D. simulans, D. willistoni, willistoni group
Big Cyprus National Preserve	Pinecrest (not on garbage)	D. acutilabella, D. cardini, D. melanogaster, melanogaster group, D. simulans
Pigeon Key	3.5 acres, not open to public	D. simulans
Near Homestead Redlands agricultural area	Avocado, citrus orchards	D. cardini, cardini group, D. melanogaster, melanogaster group, D. peninsularis, D. simulans, D. willistoni, willistoni group

We thank Drs. S. Green, R. Hofstetter and P. Luykx as well as Mrs. E. Russell and Dan Cary for sabbatical leave hospitality and for assistance with collections; all are affiliated with the Dept. of Biology, University of Miami at Coral Gables. Professors M. Wasserman, Queens College, and J.R. Powell, Yale University, assisted with the species identification.

References: Carson and Heed 1964, P.N.A.S. 52:457; Wheeler 1943, U. Texas Publ. No. 4313.

El-Zawahri, M.M. and A.F. Khishin. Assiut University, Assiut, Egypt. On the mutagenic effects of Cannabis.

In a recent laboratory study, marihuana was reported to cause damage to human chromosomes. But a study of long-term heavy consumers of marihuana does not uphold this finding (Bear 1977). Marihuana is a derivative of Cannabis, a drug commonly abused in the Middle and Far East (Chopra 1958). The question whether Cannabis causes detectable genetic damage is yet to be settled. And the study of the probable mutagenic effects of the drug becomes all important for a number of reasons. Smoking of Cannabis, and its prolonged misuse in other different ways, is common among many peoples (Paton 1974). Recent reports suggest that the drug persists in the body and accumulates with repeated dosage (Carlini et al. 1976). Last and not least is its possible therapeutic use in medicine. With these points in view, a research plan has been set up in our laboratory to study the mutagenic effects of Cannabis. The present paper reports the results of experiments on D. melanogaster.

Table 1. Percentages of dominant lethal mutations (DL) induced by crude Cannabis in D. melanogaster.

Concent. of Cannabis %	Successive days 1 T	1 LD%	2 T	2 LD%	3 T	3 LD%	4 T	4 LD%	Average T	Average LD%
Control	1210	4.9	988	4.9	1017	4.8	1310	5.2	1131	4.9
0.5	653	10.5	559	9.7	562	12.6	591	14.5	591	11.8
1.0	512	19.6	531	16.1	503	18.5	495	16.9	510	17.8
2.0	320	24.5	294	29.8	347	21.5	397	23.7	339	24.9

T = total number of eggs counted.

Table 2. Recessive sex-linked lethal mutations (r.s.l.l.) induced by various concentrations of crude Cannabis in D. melanogaster.

Brood (3 days)	Concent. of Cannabis %	No. of males tested	No. of chromosomes tested	No. of lethals	Lethals %
1	0.0	50	1250	5	0.40
	0.5	50	1029	8	0.78
	1.0	30	620	7	1.13
	2.0	22	445	15	3.37
2	0.0	50	970	3	0.31
	0.5	45	866	7	0.81
	1.0	31	510	8	1.57
	2.0	20	380	11	2.89
3	0.0	50	927	4	0.43
	0.5	30	672	5	0.74
	1.0	30	411	11	2.43
	2.0	20	354	13	3.67

Crude Cannabis was added to the media of Drosophila. The concentrations tested were 0.5, 1, 2, 5, and 10%. Adult flies emerging from larvae thus treated were tested for genetic damage observed as induced dominant, and recessive sex-linked lethal mutations.

Flies fed and reared on the control media completed their life cycle in 9 days. Those reared on media containing 0.5, 1 and 2% Cannabis completed their life cycle in 10, 11, and 13 days, respectively. In groups fed on media containing 5 and 10% of the drug, hatched eggs produced undersized larvae which died rapidly and never reached the adult stage. It was also observed that the number of eggs laid was less in all treated groups than in the control, and the higher concentrations of Cannabis were more drastic in their effects than lower ones.

Table 1 shows that the percentages of unhatched eggs were greater in all treated groups than in the control. The average figures for D.L. percentages were 11.8, 17.8 and 24.9 for groups fed on 0.5, 1 and 2% Cannabis media compared to 4.9% in the control. These differences were found to be highly significant by the χ^2 test ($P < 0.01$). It is also seen that dominant lethals induced by Cannabis increased with increased concentration.

The frequency of the recessive sex-linked lethal mutations was slightly higher in males fed on 0.5% Cannabis than control (Table 2). However, no significant change in mutation frequency was detected in the three broods, but the consistency of the results suggests that though no one of the individual χ^2s was large, a persistent bias might lead to a significant value for the merged data. In males fed on 1% and 2% Cannabis, the frequency of mutations was higher than in the control. Statistical analysis showed that the differences are significant at the 95% level. The results again showed an increase in the recessive sex-linked lethals percent by increasing the concentration of Cannabis.

References: Bear, A.S. 1977, "The Genetic Perspective", W. Saunders, London; Carlini, E.A., C. Lindsey, R.E. Musty and J.M. Monti 1976, in: "Pharmacology and Cannabis" (M.C. Brude and S. Szara, eds.), Raven Press, New York; Chopra, I.C. 1958, Proc. of Symp. held at the Postgraduate Institute of Medical Education and Research, Chandigarh; Paton, W.D.M. 1974, U.S. Govt. Printing Office, Washington, DC, USA.

Faccio Dolfini, S. and A. Bonifazio Razzini. University of Milan, Italy.

Cytochemical localization of glucose-6-P dehydrogenase in D. melanogaster.

Direct visualization of the activity of an enzyme in single cells may have wide applications in somatic cell genetics.

For this purpose, optimal reaction conditions for the cytochemical localization of the X-linked enzyme glucose-6-P dehydrogenase (Young et al. 1964) in different cells of Drosophila melanogaster were determined. The usual procedure for the detection of this enzyme is based on two coupled reactions: (1) in presence of the substrate (G-6-P) and of the enzyme (G-6-PD), the coenzyme NADP is reduced to NADPH; (2) in presence of nitroblue tetrazolium chloride (NBT), the coenzyme is reoxidized and the reduced NBT precipitates as insoluble colored granules of formazan in correspondence of the sites of enzyme activity. The method devised for animal (rat) and plant (corn) tissues by Negi and Stephens (1977), which takes advantage of the presence in the medium of a viscous stabilizer preventing the diffusion of the enzyme, was followed. The reaction mixture is prepared dissolving NBT (10 mg), NADP (10 mg) and G-6-P (60 mg) in 20 ml of 22% polyvinyl alcohol (PVA) solution in Tris-maleate buffer (0.2 M, pH 7.2).

Different cell systems of D. melanogaster were tested for the presence of the enzyme: cells of two established lines, 1.XII and Ca3 (Mosna and Barigozzi 1976; Halfer 1978) and cells obtained from wild-type embryos. Cells of the lines were harvested on coverslips in plastic dishes (35 mm diameter) in D225 medium supplemented with 18% fetal calf serum (Echalier and Ohanessian 1970). After 16-20 hours, the coverslips with adherent cells were removed, rinsed with 0.7% NaCl and let air dry at room temperature. The dried coverslips were then placed in plastic dishes, covered with reaction mixture prewarmed at 37°C and incubated for 20-30 min. After that time, the coverslips were removed, rinsed with distilled water, air dried and finally mounted. Control coverslips were incubated in the same medium lacking substrate (G-6-P).

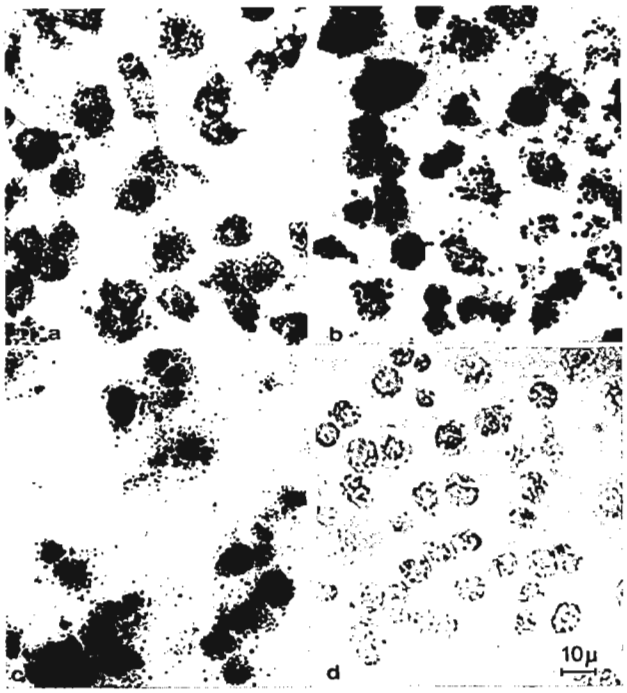


Fig. 1. (a) and (b) Cells of 1.XII and Ca3 lines covered by formazan granules revealing the presence of G-6-PD. (c) Embryonic cells from the wild stock Varese showing the same pattern. (d) Control cells of Ca3 line exposed to the reaction mixture without the substrate (G-6-P).

Embryonic cells were dissociated from eggs laid by females of the Varese wild strain over a period of six hours, according to the procedure of Horikawa and Fox (1964) and harvested on coverslips following the same steps above described for cell lines. The results obtained clearly reveal the activity of the enzyme G-6-PD both in cell lines (Fig. 1 a and b) and in embryonic cells (Fig. 1 c). The control slides on the contrary showed a complete absence of formazan granules (Fig. 1 d).

This method, devised by Negi and Stephens (1977), therefore turned out to be valid also for D. melanogaster cells, disclosing various potential applications.

References: Echalier, G. and A. Ohanessian 1970, In Vitro 6:162-172; Halfer, C. 1978, Chromosoma 68:149-163; Horikawa, M. and A.S. Fox 1964, Science 145:1437-1439; Mosna, G. and C. Barigozzi 1976, Experientia 32:855; Negi, D.S. and R.J. Stephens 1977, J. Histochem. Cytochem. 25:149-154; Young, W.J., J.E. Porter and B. Childs 1964, Science 143:140-141.

Fujikawa, K., K. Asanoma and E. Inagaki.
Hiroshima University, Hiroshima, Japan.

Visible mutations induced in spermatozoa of Drosophila by mitomycin C.

A potent antibiotic, mitomycin C (MC) can act either as a monofunctional or as a polyfunctional alkylating agent on DNA after chemical or enzymatic conversion (Szybalski & Iyer 1964). The present experiments with a specific-locus method were conducted to examine mutagenic property of MC in spermatozoa of D. melanogaster.

In all the experiments, Canton-S, wild-type, 3-day-old virgin males were fed MC (50, 100 and 200 µg/ml in 1% sucrose for the treated series) and 1% sucrose (for the control) for a 24-h period according to the method of Lewis and Bacher (1968). To test for dumpy mutations induced in spermatozoa, these males were individually mated for a 24-h period with 4 virgin females of the genetic constitution, echinoid (ed, II-11), dumpy (dp, II-13.0), clot (cl, II-16.5). With the same MC solutions and same treatment conditions, tests for sex-linked recessive lethals were also made by the use of Basc method.

The recovered dumpy mutants, either complete or mosaic, were classified according to their phenotypes (olv, ov, ol, lv, o, v and cm; see Carlson & Oster 1962), and mated with fresh ed dp cl males or females to test for their transmissibility.

Table 1. Frequencies of F_1 dumpy mutations detected after exposure of spermatozoa to MC.

Exposure (µg/ml)	No. of flies scored (T)	No. of mutants: Mosaic (M)	No. of mutants: Complete (C)	Mutation frequency $(\frac{M+C}{T}\times 100)$
50	39,531	40	20	0.152[a]
100	68,324	46	23	0.101[a]
200	7,582	11	2	0.171[a]
control	21,847	5	0	0.023

[a]Significant at 1% level from the control.

Table 2. Transmissibility of F_1 dumpy mutants induced by MC.

	Mosaic	Complete	Total
No. tested	97	45	142
No. fertile (F)	75	34	109
No. transmitted (T)	24 (8)	19 (15)	43 (23)
Transmissibility $(\frac{T}{F}\times 100)$	32	56	39

( ) No. of mutants whose gonads were completely mutated.

The statistical significance of the difference between the results of the MC-treated series and those of the control was tested by using Kastenbaum and Bowman's tables (1970). Frequencies of F_1 dumpy mutations observed in the MC-treated series and control series are given in Table 1. All concentrations of MC produced a significantly higher frequency of dumpy mutations than that in the control (Table 1), indicating that MC is an effective mutagen when analyzed with a specific visible test at the dumpy locus. However, no link could be observed between the increase in the concentration and the shift in the mutation frequency (Table 1). This was also true for sex-linked recessive lethal mutations observed under the present experimental conditions, i.e., 1.26% (23/1828) at 50 µg/ml, 1.57% (47/2999) at 100 µg/ml and 0.83% (4/482) at 200 µg/ml.

As shown in Table 2, of the 109 fertile F_1 dumpy mutants, 39% were transmitted to F_2, and not all phenotypic complete mutants were transmitted as gonadal completes. When the phenotypic completes that were gonadal mosaic or non-mutant were reclassified as mosaics, the frequency of mosaicism was calculated to be 86%. These values of percent transmissibility and percent mosaicism do not differ much from those (25-35% and 94-98%, respectively) obtained in the previous studies of Carlson and his co-workers for dumpy mutations with monofunctional alkylating agents ICR-170, EMS and NMU (Carlson & Oster 1962; Jenkins 1967; Corwin 1968).

Another finding in the present study relates to the nature of MC-induced dumpy mutations. As in the studies of Carlson and his co-workers with the monofunctional alkylating agents, genetic analysis of F_1 and transmitted dumpy mutants indicated that the majority were produced without apparent breakage. Thus no mutants of the ov type showed variegated position effects. Although a locus whose deficiency results in a Minute phenotype lies close to the dumpy locus (Carlson & Southin 1962), no Minute bristles in the olv types could be observed. Furthermore, it was noted in the present study that the fertility of the (olv, ov) types of F_1 dumpy mutants did not differ significantly ($\chi^2 = 2.12$, d.f. = 1) from that of the (ol, lv, o, v, cm) types, a class of dumpy mutants which are usually free from aberration phenomena (Carlson & Southin 1962; Fujikawa & Inagaki 1979), i.e., 75% (52/63) vs. 78% (57/79). Since mutants involving

chromosomal abnormalities are generally assumed to have lower fertility than those due to point mutational events, such a similarity in fertility may reinforce the paucity of evidence for MC-induced breakage in the dumpy region of spermatozoa. These data correlate well with the results of Schewe et al. (1971), which have shown that feeding of MC (500 µg/ml in 1% sucrose) to adult males does not cause chromosomal interchanges in post-meiotic germ cells. It has been reported that the interchanges occur predominantly in spermatogonia (Schewe et al. 1971).

References: Carlson, E.A. and I.I. Oster 1962, Genetics 47:561-576; Carlson, E.A. and J.L. Southin 1962, Genetics 47:321-336; Corwin, H.O. 1968, Mutation Res. 5:259-270; Fujikawa, K. and E. Inagaki 1979, Mutation Res. 63:139-146; Jenkins, J.B. 1967, Genetics 57:783-793; Kastenbaum, M.A. and K.O. Bowman 1970, Mutation Res. 9:527-549; Lewis, E.B. and F. Bacher 1968, DIS 43:193; Schewe, N.J., D.T. Suzuki and V. Erasmus 1971, Mutation Res. 12:255-267; Szybalski, W. and V.N. Iyer 1964, Science 145:55-58.

Gallego, A., A. García-Dorado and C. López-Fanjul. Universidad Complutense de Madrid, Spain. A new allele (H^{St}) at the Hairless locus with an effect on sternopleural bristle number.

In a line from the Dahomey population of D. melanogaster selected for low sternopleural bristle number, a delayed response to selection of 1.6 bristles (0.7 standard deviations of the base population) was observed from generation 13 to 17, accompanied by a threefold increase of the variance of the line with respect to its previous value. This late response can be attributed to the increase in frequency of an allele at the Hairless locus for which the H^{St} symbol is proposed because of its effect on sternopleural bristle number.

A lethal analysis showed that the H^{St} allele is a recessive lethal located at chromosome 3. Heterozygotes can be easily distinguised from wild type homozygotes as vein L5 does not reach the wing margin; this same effect can often be observed for vein L4. In addition, all heterozygotes show suppressions of postorbital bristles, and suppressions of ocellar, dorsocentral and scutellar bristles were very frequently observed. An allelism test indicated that H^{St} is a new allele of the Hairless series (location 3-69.5).

The phenotypic distributions of sternopleural bristle number of wild type homozygotes and H^{St} heterozygotes do not overlap, and their means differ by 2.2 bristles, the heterozygote class being that with the lower mean. From generation 13 onwards all selected individuals (12 out of 120) happened to be heterozygous for the H^{St} allele.

As far as we know this is the first time in which an allele of the Hairless series has been shown to have an effect on sternopleural bristle score (Nash 1965; Lindsley & Grell 1968).

References: Lindsley, D.L. and E.H. Grell 1968, Genetic Variation in D. melanogaster, Carnegie Inst. of Wash. Publ. No. 627; Nash, D. 1965, Genet. Res. Camb. 16:175-189.

Gasaryan, K.G., A.K. Shahbazyan, N.Yu. Sakharova and S.G. Smirnova. Institute of Molecular Genetics, USSR Academy of Sciences and Moscow State University, Moscow, USSR. Mutations obtained in Drosophila after microinjections of Rous sarcoma viruses into early embryos.

After early Drosophila embryos were microinjected with the RNA-containing avian Rous sarcoma viruses two mutations affecting the eye-antennal disk morphogenesis were obtained. No changes occurred in the control experiment.

The first mutation which manifested itself in the second generation of flies obtained from the injected eggs led to a wide spectrum of deviations from the normal eye morphology: the destruction of the facet pattern, the emergence of protrusions of different size and shape covered by a facet layer or by chitin. All changes occurred in the forepart of the eye. Genetic analysis showed this mutation, which we termed eye deformed (ed), to be recessive, with vary expressivity and decreased penetrancy (60-80%). The mutation is localized in the second chromosome in the vicinity of point 74 and is temperature-sensitive. If the mutant stock was kept at a lowered temperature (16°C) all through the period of development the majority of flies had the normal phenotype (the mutation's penetrancy was reduced to 0%). Histological analysis of the developing eye-antennal imaginal disks of this mutant stock suggests that it contains foci of abnormally high proliferation activity.

The second mutation was obtained as a derivative of the first mutant stock and manifested itself phenotypically in a partial or complete reduction of the eyes and in a diminished and altered shape of the head. It proved to be dominant, with 100% penetrancy and vary expressivity. This mutation, which we termed eye reduced (er), is also localized in the second chromosome in the vicinity of point 72. Lowered temperature did not change the penetrancy of this mutation but the phenotype showed a tendency towards normalization. Histological analysis of the development of the er mutant stock revealed a decreased proliferation ability and a higher cell death rate in the eye disks.

[The Rous sarcoma virus was kindly donated by Dr. F.L. Kiselyov, All-Union Oncologic Research Centre, Moscow.]

Gerasimova, T.I. Institute of Molecular Genetics, USSR Academy of Sciences, Moscow, USSR. Mutator effects of an unstable cut allele in D. melanogaster.

The second MR-h12 autosome carrying the MR-factor which causes male recombination was used for inducing unstable insertion mutations in the cut locus. To this end we crossed male MR-h12/Cy X female Maxy/FM4 and screened the progeny for visible sex-linked recessive mutations in 16 genes of the Maxy chromosome. The following mutations were found in a total of 50,000 chromosomes: w-2, rb-5, ct-4, sn-18, and ras-3. The mutations were unstable and reverted to the wild type at a rate of 10^{-3} to 10^{-4}. The results are consistent with those of M. Green (1977). The present paper, however, deals with unstable mutations in the cut locus, which other authors had failed to obtain. Our study was concentrated on the ct^{MR2} allele. This mutation reverts to the wild type at a rate of 1×10^{-3}. The ct^{MR2} mutants show the characteristic ct phenotype: sharply cut wings. The instability of ct^{MR2} shows it to be an insertion mutation caused by the integration of a putative transposon (hereinafter referred to as the MR-transposon) into the cut locus by dint of the MR-h12 factor. The ct^{MR2} allele proved to be a strong mutator. A number of its derivatives were obtained, including ct^{+} wild-type revertants. Both stable (not a single new mutation in 10,000 chromosomes) and unstable revertants were found (Table 1). The unstable revertants mutate to the initial state or to new ct alleles.

Table 1. Characterization of derivative mutations of the unstable ct^{MR2} allele.

Mutations	Stability	Mutability
ct^{MR2}	unstable	sex-linked lethals, y, w, sn, rb, m, ct^{+} and other ct alleles
ct^{PN10}	unstable	ct^{+}, ct alleles
ct^{+1}	unstable	ct, sn
ct^{+2}	stable	---
ct^{+3}	stable	---
ct^{e8}	stable	---

Mutations in other genes (rb, sn) were also found in these strains. Presumably the stable revertants emerge as a result of an accurate excision of the MR-transposon while the unstable revertants keep the MR-transposon though it is in some way blocked. The excision of the MR-transposon may be inaccurate and then new stable ct alleles appear. These are probably deletion mutations. The ct^{18} lethal also seems to be a deletion mutation. It seems to result from the excision of the right-hand lethal part of the cut locus (Johnson & Judd 1979). Apart from the stable derivatives of the ct^{MR2} allele, new unstable ct mutations were found; ct^{PN10} leads to multiple notches at the edge of the wing and reverts to the wild type at a rate of 2×10^{-3} or to other ct alleles, which means that it contains the MR-transposon. The mutator effects of the ct^{MR2} allele extend to other X-chromosome genes as well as the cut locus. Various crosses exhibited unstable mutations in the y, w, rb, m and sn loci. The effects of the ct^{MR2} on mutagenesis were also tested by the standard M-5 procedure. In 5920 chromosomes 54 sex-linked recessive lethals were obtained. Some of them proved unstable and reverted to the wild type.

Thus the ct^{MR2} allele obtained by means of the MR-h12 chromosome proved to be a strong mutator with respect to the cut locus and other genes in the X chromosome. The mutator action of the ct^{MR2} allele was shown to be of two types. In the first case new mutations occurred in other loci (y, w, sn, rb, m, lethals) as a result of a $ct^{MR2} \rightarrow ct^{+}$ reversion, i.e., the MR-transposon was excised from the cut locus and integrated into other genes, blocking their transcription. In the second case the ct^{MR2} allele was preserved while new unstable mutations

appeared in other genes, i.e., the MR-transposon was not excised from the cut locus and the emergence of new mutations was probably due to new copies of the MR-transposon (its amplification).

References: Green, M.M. 1977, Proc. Natl. Acad. Sci. USA 74:3490-3493; Johnson, T.K. and B.H. Ludd 1979, Genetics 92:485-502.

Ghosh, M. and A.S. Mukherjee. University of Calcutta, India. Nucleolar chromatin structures in D. hydei.

The nucleolus in the giant cells of larval salivary glands of Drosophila reveals typical thread-like structures which have been shown to be DNA in nature (Barr & Plaut 1966; Rodman 1969). Very likely these DNA threads are looped out from the main chromatin and contain the rDNA sequences in the manner the DNA loops out in the puffs (Chowdhry & Godward 1979). However, an interesting feature observed about these threads in Drosophila is that the pattern of the thread-like connection in the matrix of the nucleolus is not constant. The pattern varies not only within species (Barr & Plaut 1966), but within the same species there is a considerable degree of variation in the morphological configuration of the threads.

A wide variety of morphological conformations of nucleolar chromatin thread (NCT) in the salivary gland nuclei can be grouped into four types on the basis of condensation and complexity of the structures as follows:

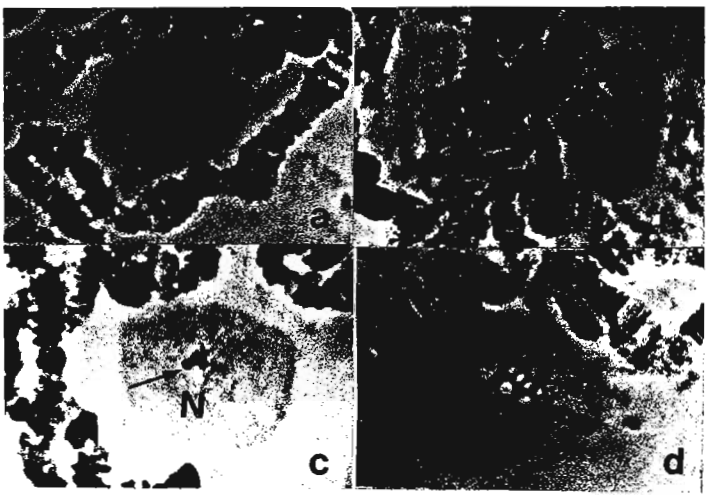


Fig. 1. Photomicrographs showing four types of nucleolar chromatin threads (NCT) in D. hydei. Type I = a, Type II = b, Type III = c and Type IV = d. "N" represents the nucleolus and the arrow indicates nucleolar chromatin thread (NCT).

Type I: The thread is very small, branched into thin fibrils with beads and granules (Fig. 1a).

Type II: The thread is ramified and granules are more densely stained but the connection between the main thread and the granules is not visible (Fig. 1b).

Type III: The thread is condensed and positively stained and fewer granules or beads restricted to the central zone (Fig. 1c).

Type IV: More than one thread, ramified and with less decondensed granules scattered over the whole nucleolar mass (Fig. 1d).

We then became interested in getting more information about the NCT and for this we used a number of chemicals --(1) deoxyadenosine, (2) 5'-bromodeoxyuridine (BudR), (3) α-amanatin, and (4) 2:4, Dinitrophenol (Dnp)-- which may reveal more detail of the structures.

Observation shows that there was a good deal of unmasking of the nucleolar matrix without any apparent conformational changes of the nucleolar chromatin structure or pattern.

Data on the frequency of these four types of nucleolar chromatin structures show that while nuclei treated with deoxyadenosine, BudR and α-amanatin exhibit a much higher frequency of Type IV NCT over the control, that of Type I NCT shows a greater reduction in its frequency. In contrast, the 2:4, Dinitrophenol (Dnp) was not very effective in changing the conformational pattern of the different NCT types (see Fig. 2 on next page).

We may conclude, therefore, that the variability in the pattern of the nucleolar chromatin thread may not really be a manifestation of the genetic endowment but is rather an expression of gene function.

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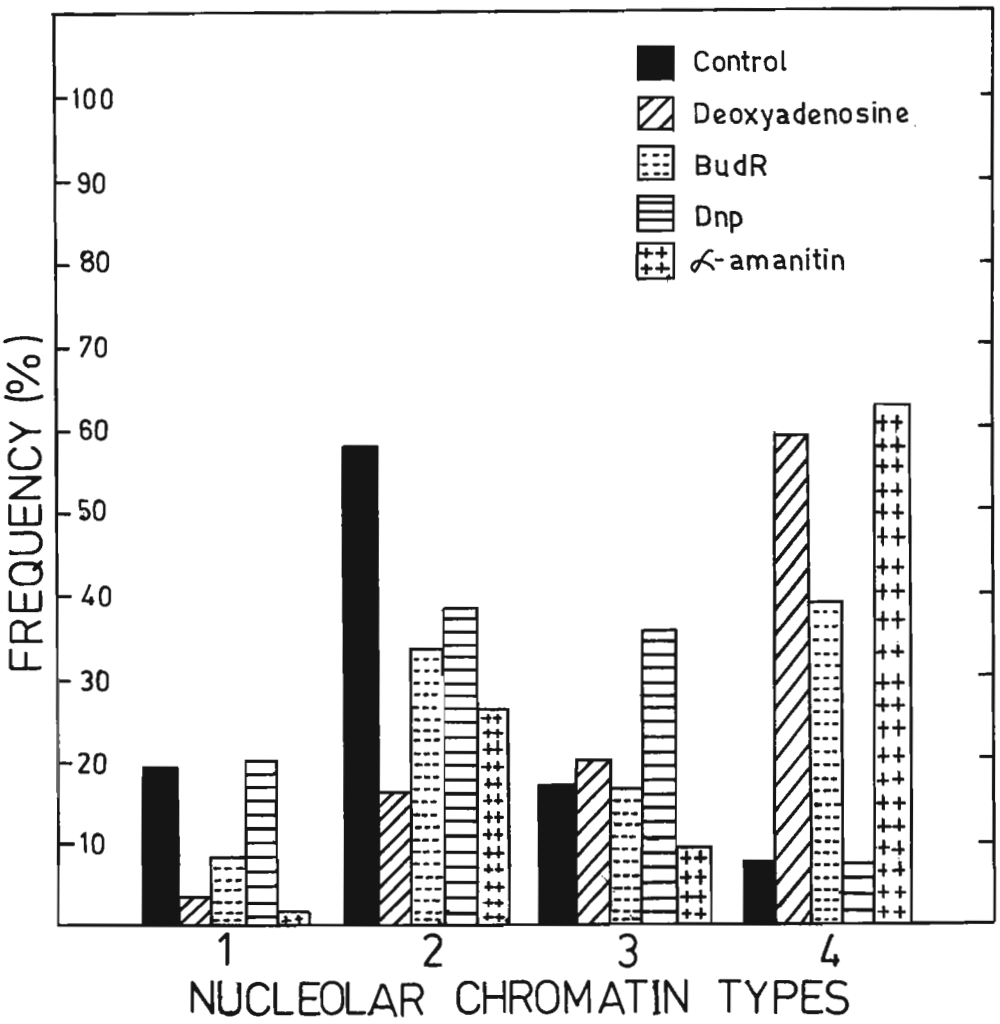


Fig. 2. Histogram displaying the frequency of different types of NCT in D. hydei under normal physiological condition as well as following different chemical treatment procedures.

Gould, A.B. and A.M. Clark. University of Delaware, Newark. Stability of adult life span in laboratory stocks of D. melanogaster.

Adult life span in D. melanogaster is a genetic trait which is characteristic for any given strain or mutant. This was demonstrated many years ago by the group working with Raymond Pearl (Pearl & Parker 1922; Gonzales 1923). In the late 1960's we were working with two wild type (Ore R and Swe-c) and two mutant (vg and vg^{np}) strains to demonstrate the genetic control of adult life span (Clark & Gould 1970). At that time our method involved crossing the mutant strains repeatedly into the wild type strains, each of which had different mean adult life spans. The mutant strains thus acquired the genetic background of either Ore R or Swe-c. The determinations of adult life span made on the backcrossed mutant strains showed that their adult life spans had been modified in the direction of those of the wild type strains. For example, Ore R was the longer-lived strain; vg bred into Ore R had its adult life span increased by 28% (Table 1) whereas the vg^{np} strain, whose adult life span was similar to Ore R, did not change greatly (9% longer). Swe-c was shorter-lived. vg in a Swe-c background had a slightly shortened life span (8%) and the adult life span of vg^{np} bred into Swe-c was shortened by 26%.

During the years since 1969 these stocks have been carried by mass transfers every six weeks, and kept at 18°C. Recently we became curious about what might have happened to their adult life spans during those intervening years. Hence such determinations were made in the spring of 1981, using the method of previous years (Clark & Gould 1970), with the difference

that instead of single fly isolation they were now housed ten flies to an 8-dram vial. Temperature was the same as before, 25°C, but only males were tested.

Table 1. Mean adult life spans for males of strains of D. melanogaster.

Strain	N	Life span in days 1967-69	N	Life span in days 1981
Ore R	104	67.3 ± 1.4	41	74.2 ± 1.3
"	107	71.1 ± 1.7		
Swe-c	218	37.1 ± 0.9	40	45.0 ± 1.4
"	108	41.0 ± 1.6		
vg	103	36.8 ± 1.8	60	44.8 ± 2.4
vg^{np}	103	62.2 ± 2.3	58	58.0 ± 3.2
vg (Ore R)	108	51.0 ± 1.8	62	66.4 ± 2.4
vg^{np} (Ore R)	108	71.2 ± 1.7	59	63.3 ± 1.8
vg (Swe-c)	108	29.8 ± 1.7	46	41.4 ± 2.9
vg^{np} (Swe-c)	106	45.8 ± 2.4	61	64.3 ± 2.1

The results likewise are in Table 1. Ore R and Swe-c have maintained their characteristic adult life spans, with our recent determinations being higher than but close to the range of those made in 1967-69. vg^{np} has also remained at approximately its 1967 life span, whereas vg has increased its adult life span by about 18%. The mutants which were bred into the wild type stocks behaved differently. vg with the Ore R background showed an increase in adult life span nearly into the range of Ore R itself, and vg bred into Swe-c likewise showed an increased adult life span, being now similar to the original Swe-c stock. vg^{np} with Ore R background showed a shorter adult life span than in the previous experiments, whereas vg^{np} with Swe-c background had a lengthened adult life span.

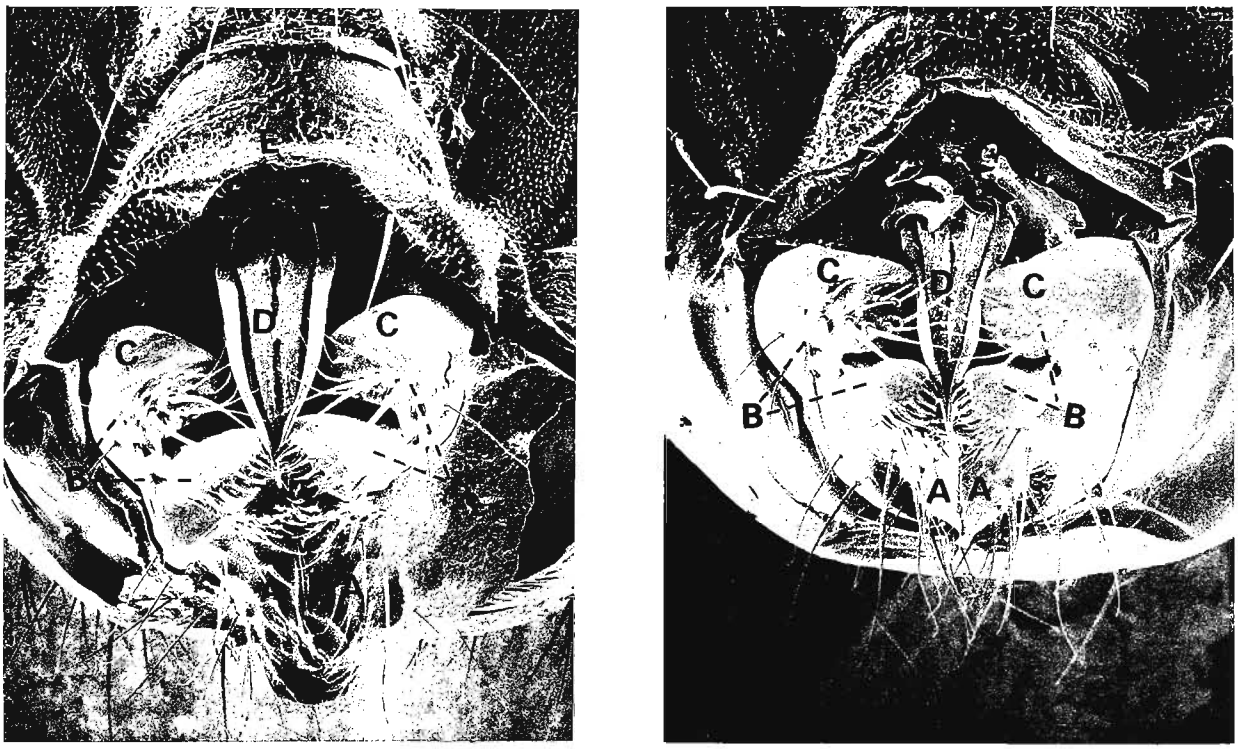
Adult life span is a polygenic trait which can be modified by mutation or recombination of genes in the pool of a population. It is also sensitive to environmental influences. But it is still a remarkably stable trait. These recent life span determinations show this stability with respect to the wild type strains. They also show that there has been some selection for longer adult life span in the vg lines. The behavior of the vg^{np} lines can be explained by the greater amount of genetic variability evident in these strains, as reflected in the larger size of their standard errors compared to either vg or wild type.

References: Clark, A.M. and A.B. Gould 1970, Exp. Geront. 5:157-162; Gonzales, B.M. 1923, Amer. Nat. 57:289-325; Pearl, R. and S.L. Parker 1922, Amer. Nat. 56:174-187.

Gupta, A.P. Harvard University, Cambridge, Massachusetts (present address: Cidade Universitaria, Rio de Janeiro, Brazil).
Scanning electron microscope study of phenotypic differentiation between D. pseudoobscura and D. persimilis.

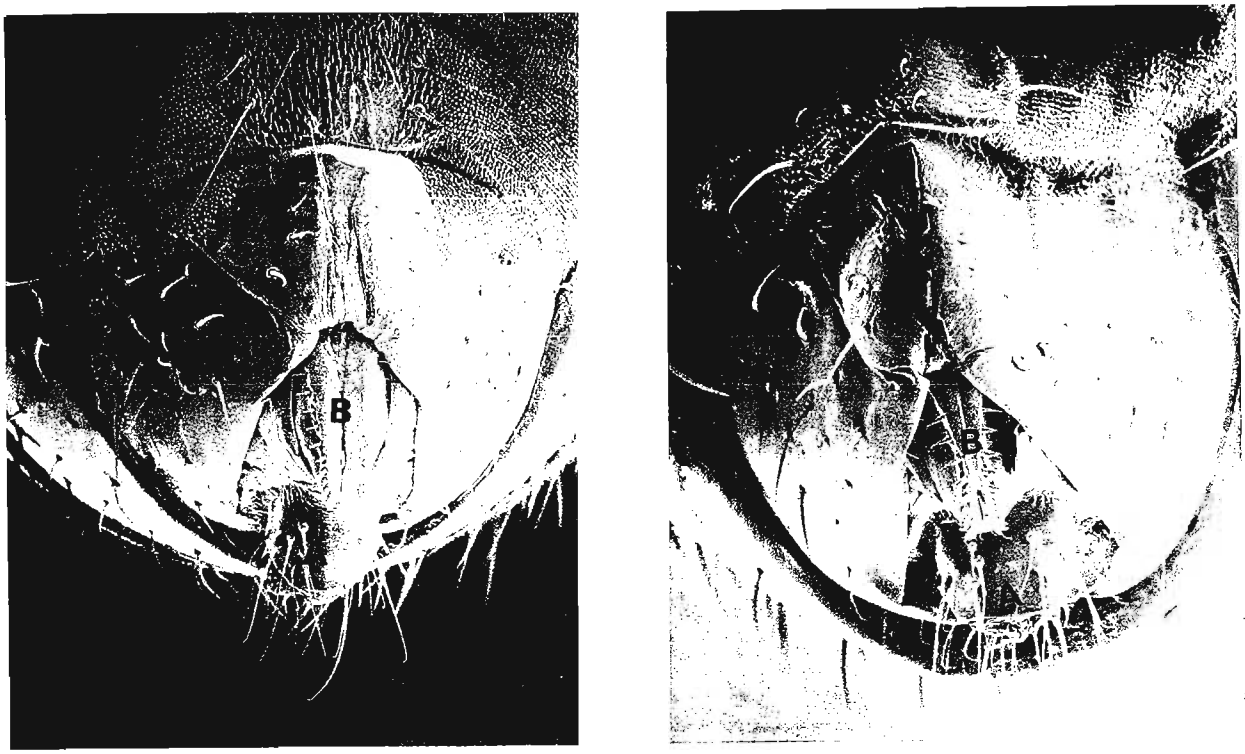
D. pseudoobscura and D. persimilis are morphologically alike and considered sibling, but they exhibit significant genetic differences. Crosses between them produce sterile F_1 males and fertile F_1 females. Backcrosses of these females to the males of either species produce progeny with poor viability and most are sterile. These species are, however, distinguishable by the shape, size and index of penis (Rizki 1951) although there is some overlap. However, a consideration of all morphological characteristics does not allow us to differentiate between individual flies of each species.

The present work was designed to differentiate an individual of either sex of D. pseudoobscura from an individual of the same sex of D. persimilis using scanning electron microscope. Six isofemale strains of D. pseudoobscura from Strawberry Canyon, California and four isofemale strains of D. persimilis from Fish Creek, California were used. A total of 40 flies (34 males and 6 females) from D. pseudoobscura and 36 flies (29 males and 7 females) from D. persimilis were examined with the scanning electron microscope. Males and females from both species were placed separately in vials containing 10% alcohol overnight to remove any contaminating particles. The flies were fixed and dried on metal stubs using standard procedures for the preparation of material for the scanning electron microscope studies. Scanning at different magnifications and tilts were performed for genital organs only for each sex from both the species. A comparison of several characteristics of males--size and shape of penis, size and shape of anal plate, size and shape of forceps, and size and shape of claspers and the distance between the two bristles above the base of the penis--and a comparison of size and shape of the anal plate the the ovipositor in females showed some variation in these characters in different individuals of each species. However, no species-specific differences in either sex were observed (Figs. 1 and 2). Therefore, it is not possible to distinguish individuals of D. pseudoobscura and D. persimilis on the basis of genitalia.



D. pseudoobscura ♂ D. persimilis ♂

Fig. 1. A comparison of genitalia between males of D. pseudoobscura and D. persimilis by scanning electron microscope: (A) anal plate, (B) forceps, (C) claspers of the forceps, (D) penis, and (E) two bristles above the base of the penis.



D. pseudoobscura ♀ D. persimilis ♀

Fig. 2. A comparison of genitalia between females of D. pseudoobscura and D. persimilis by scanning electron microscope: (A) anal plate and (B) ovipositor.

This work was supported by NIH Grant GM 21179 to R.C. Lewontin. I thank him for the encouragement during this work. I would also like to thank Professors A.R. Cordeiro and L.A.S. Monjelo.

Reference: Rizki, T.M. 1951, Proc. Nat. Acad. Sci. USA 37:156-158.

Hägele, K. and H.A. Ranganath. Ruhr-Universität Bochum, Germany. A centric fusion in D. nasuta albomicana.

D. nasuta nasuta (2n = 8) and D. n. albomicana (2n = 6) are cross fertile chromosomal races. They belong to the nasuta subgroup of the immigrans species group of Drosophila (Wilson et al. 1969; Ranganath & Krishnamurty 1981).

Recently, we have discussed that during the karyotypic evolution of D. n. albomicana there were three centric fusions and one pericentric inversion. Because of the repeated occurrence of centric fusions, it was concluded that D. n. albomicana is a product of "karyotypic orthoselection" (Ranganath & Hägele 1981). This is defined as the tendency for similar structural changes to establish themselves in one chromosome member of the karyotype after another (White 1975).

During hybridization experiments between D. n. nasuta and D. n. albomicana, we found a centric fusion between two long dot chromosomes of D. n. albomicana. This was recorded in one individual of F_1 hybrids. Fig. 1a presents a C-banded F_1 metaphase plate (2n = 7) having the normal long dot chromosome of D. n. albomicana. An F_1 metaphase plate with the fusion product of two long dot chromosomes of D. n. albomicana is shown in Fig. 1b. This fusion product appears as a small metacentric which reveals the same C-banding differentiation in both the chromosome arms (Fig. 1c).

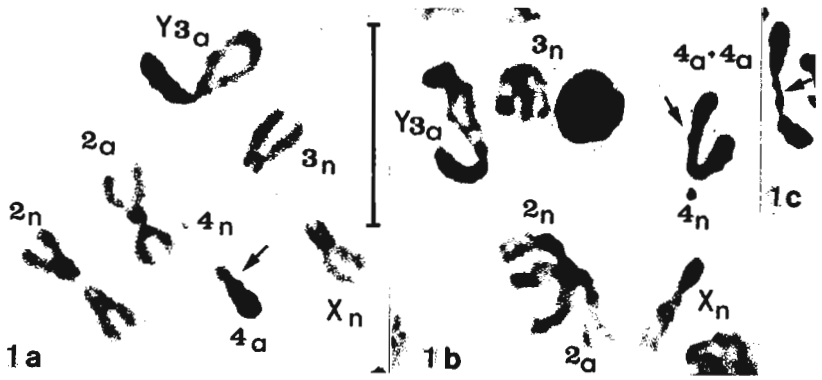
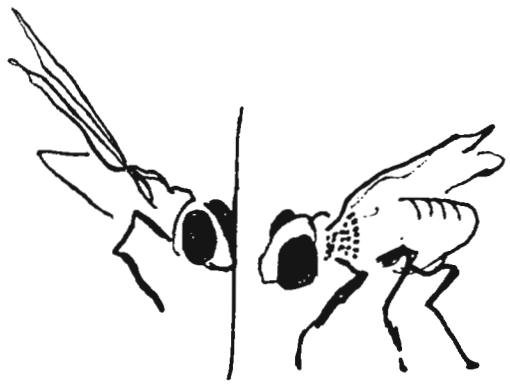


Fig. 1. C-banded metaphase chromosomes of F_1 hybrids from D. n. nasuta and D. n. albomicana (2n = 7). The chromosomes are designated to indicate the race to which they belong: n = D. n. nasuta, a = D. n. albomicana. The bar in the figure represents 10 µm.
(a) Metaphase with a normal long dot chromosome from D. n. albomicana (arrow).
(b) Metaphase with the centric fusion of two long dot chromosomes of D. n. albomicana (arrow).
(c) Demonstration of the same C-banding pattern in both arms of the fusion product of the long dot chromosomes. Arrow points to the centromere position.

The present finding of an isolated, floating case of a centric fusion product in one individual of D. n. albomicana does not show any evolutionary implications. However, it may indicate the possible persistance of a fusion tendency in the karyotypic organization of D. n. albomicana.

References: Ranganath, H.A. and K. Hägele 1981, Naturwissenschaften 68:527; Ranganath, H.A. and N.B. Krishnamurty 1981, The J. Hered. (USA) 72:19; White, M.J.D. 1975, Genetics 79:63; Wilson, D.W., M.R. Wheeler, M. Harget and M. Kambysellis 1969, V. Univ. Texas Publ. 6918:207.



Hanna, P.J. and O. Hess*. Deakin University, Victoria, Australia; *Universität Düsseldorf, Germany. Growth of testes in relation to fertility of repleta group Drosophila.

During recent work with several species of the repleta group of Drosophila it was important for us to know about timing of spermatogenesis, and in particular, when males became fertile after eclosion. It was thought that if some species exhibited long delays in attaining fertility then these delays would add to the already known difficulties in culturing the species. The study and its findings are presented here to assist others who are planning to work with Drosophila of the repleta group.

The experiment was performed by taking a sample of newly emerged males (0-3 hours) and regularly removing a few flies from the storage flasks (held at 21±1°), for dissection in Drosophila Ringer's solution on microscope slides. Testes were then carefully uncoiled and their lengths measured against a graduated scale on a white card placed below the slides.

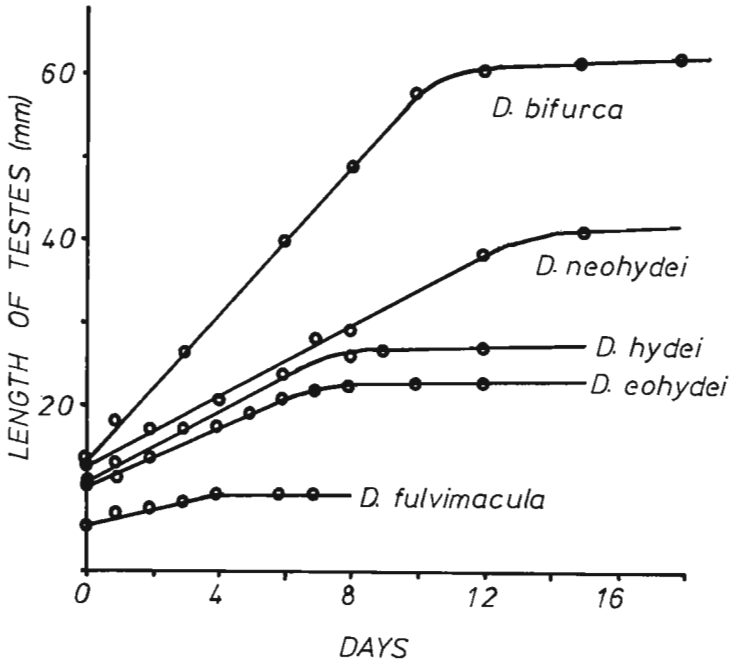


Fig. 1. Growth of testes in adult repleta group Drosophila. Day 0 represents eclosed adults, but wings still folded.

In each of the five species studied newly eclosed males were sterile as testes had not fully grown (Fig. 1). At eclosion, D. fulvimacula had the shortest testes (approximately 5.5 mm), whereas D. bifurca had the longest testes (approximately 13 mm). The growth of testes immediately following eclosion appeared linear in all species and during that time the males remained sterile yet some were observed to mate with virgin females. When growth of testes neared a maximum, which was found to be species-specific, motile sperm were found in the seminal vesicles and soon after in the ejaculatory bulbs. A little variation in time for motile sperm to be produced was observed between flies of the same species, as well as the testes of individual flies. It is clear that, when fertility was attained, D. bifurca had the longest testes of the five species (approximately 62 mm) and also took the longest time to attain fertility (more than two weeks).

Obviously males of newly emerged flies of the repleta group cannot produce fertile matings and storage of the males is essential. We have found it desirable to transfer males in storage into new flasks every few days. This provides good nutritional conditions and prevents the flies from adhering to old media which usually shrinks away from the sides of the flask. Single-pair matings have best been obtained by aging males until testes would have reached their maximum length (see Fig. 1) and then placing them with newly eclosed virgin females in new flasks.

If the observed increase in length of testes is a "passive" phenomenon, due entirely to elongation of spermatid bundles (Stern 1941), then the data presented here raise questions in regard to the relationship between testis length and sperm length. One might expect the longest, fully developed testes, namely those of D. bifurca with testes of about 62 mm, to produce the longest sperm. However, Hess and Meyer (1963) found that the sperm lengths of a few isolated sperm from the species of D. repleta, D. nigrohydei, D. eohydei, D. bifurca, D. neohydei and D. hydei to be 2.5, 1.2, 2.6, 5.5, 6.4 and 6.6 mm, respectively. The conflicting data might simply be explained by increased numbers of elongating spermatid bundles in species such as D. bifurca and D. neohydei, but our observations suggest that this is not a likely explanation. Further studies of sperm lengths in the different species are required.

References: Hess, O. and G.F. Meyer 1963, J. Cell Biol. 16:527-539; Stern, C. 1941, J. Expl. Zool. 87:113-158.

Hanna, P.J., W. Liebrich* and O. Hess*. Deakin University, Victoria, Australia; *Universitat Dusseldorf, Germany. Spermatocytes in Drosophila not appearing to be produced by synchronous divisions of definitive spermatogonia.

During independent studies of D. hydei spermatogenesis we observed that the numbers of primary spermatocytes in pre-meiotic cysts, as well as the numbers of spermatids in post-meiotic bundles, exhibited a variation. The data obtained for D. hydei (Hanna, Liebrich & Hess 1982) indicated that the well documented view of primary spermatocyte production by a 2, 4, 8, 16 ... $(2)^n$ progression of definitive spermatogonia is an oversimplification of the real situation. The findings were best explained by partial asynchrony of mitotic divisions in definitive spermatogonia.

Six other Drosophila species were subsequently examined for germ cell variation within primary spermatocyte cysts and spermatid bundles. The results (Liebrich, Hanna & Hess 1982) exhibited variations within each species and from these variations species-specific means were calculated.

A brief summary of the total data is presented here so that others who are working on Drosophila, and in particular Drosophila spermatogenesis, may similarly examine additional species which are held in different laboratories throughout the world.

Testes of pupae of approximately 2 days (i.e., free of the fat bodies) were dissected as for culture of isolated cysts (Liebrich 1981) and all undamaged primary spermatocyte cysts were then examined for the number of germ cells within. In addition, cross sections of male testes were prepared as described elsewhere (Hanna 1981). Photographs were taken of the sections and these were used to count the number of spermatids within each bundle.

Typical cross-sections of adult testes of D. neohydei and D. eohydei, together with cysts of the most common primary spermatocyte types, are shown in Figures 1 and 2. It is clear that within a single testis different bundles can contain different numbers of spermatids. The distribution of numbers of spermatids within bundles correlated with direct counts of primary spermatocytes (assuming that normal meiosis produces a fourfold increase in germ cells from primary spermatocytes through to spermatids). Table 1 summarizes the data for some Drosophila species, namely D. hydei, D. bifurca, D. neohydei, D. eohydei, D. virilis, D. immigrans, D. simulans, D. melanogaster and D. fulvimacula.

Drosophila species	number of cysts counted	most common number of spermatocytes per cyst	number of bundles*	most common number of spermatids per bundle	estimate of spermatocytes bundles were derived from
hydei	2311 (pupae=p)	8	106 (116) (p)	32	8
	574 (adult=a)	7	812 (860) (a)	28	7
bifurca	274 (p)	6	105 (106) (a)	20	5
neohydei	487 (p)	6	206 (212) (a)	20	5
eohydei	830 (p)	7	155 (161) (a)	28	7
virilis	593 (p)	8	125 (120) (a)	32 28 (28%)	8 7
immigrans	291 (p)	10-14	50 (57) (a)	64 [60 (10%)	16 15]
simulans	266 (p)	13,14,15	711 (103) (a)	64	16
melanogaster	370 (p)	16	60 (86) (a)	64 [32 (20%)	16 8!]
fulvimacula	419 (p)	13	205 (221) (a)	52 [48 (25%)	13 12]

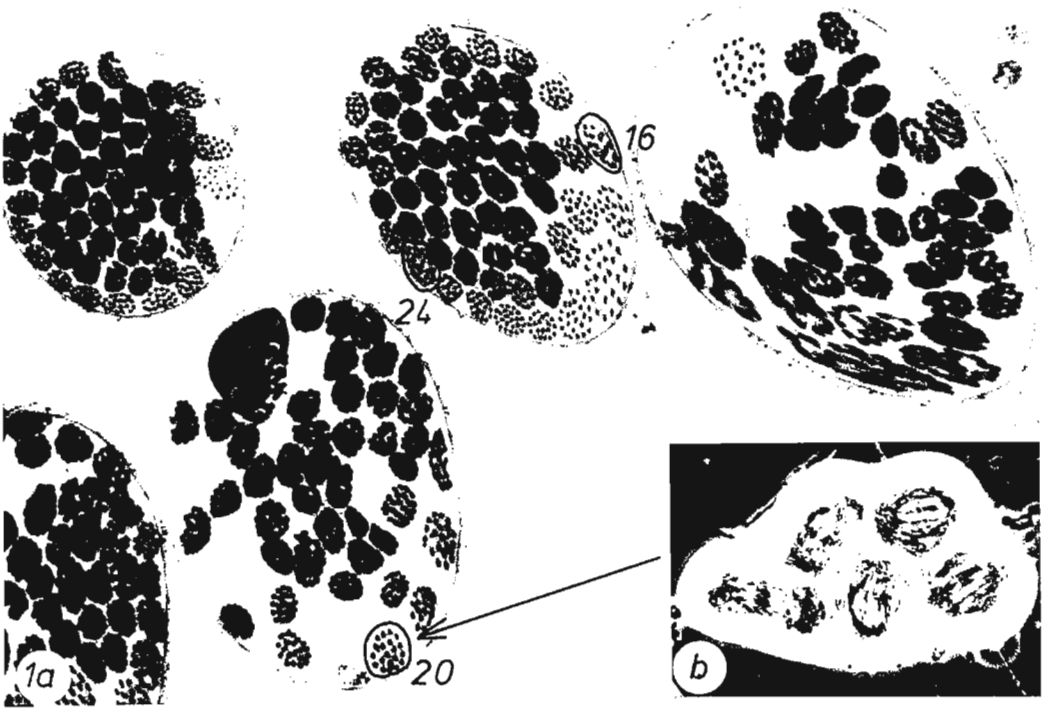
*The first number indicates the quantity of spermatid-bundles whose number of spermatids is dividable by four; in brackets the total number of bundles counted.

We have found variation of germ cell number within spermatocyte cysts and spermatid bundles to occur (1) between individuals, (2) between species, and (3) between pupae and adult stages of development (Hanna 1981; Hanna et al. 1982). It will be interesting to see whether other Droso-

phila species show similar results. These data raise important questions regarding the control and nature of mitotic divisions in definitive spermatogonia.

The authors wish to thank U. Glos for technical assistance. One of the authors (W.L.) was supported by the Deutsche Forschungsgemeinschaft.

References: Hanna, P.J. 1981, Experientia 47:951-952; Hanna, P.J., W. Liebrich & O. Hess 1982, Gamete Res. 6 (in press); Liebrich, W. 1981, Cell & Tissue Res. 220:251-262; Liebrich, W., P. J. Hanna and O. Hess 1982, submitted to Int. J. Invert. Reprod.



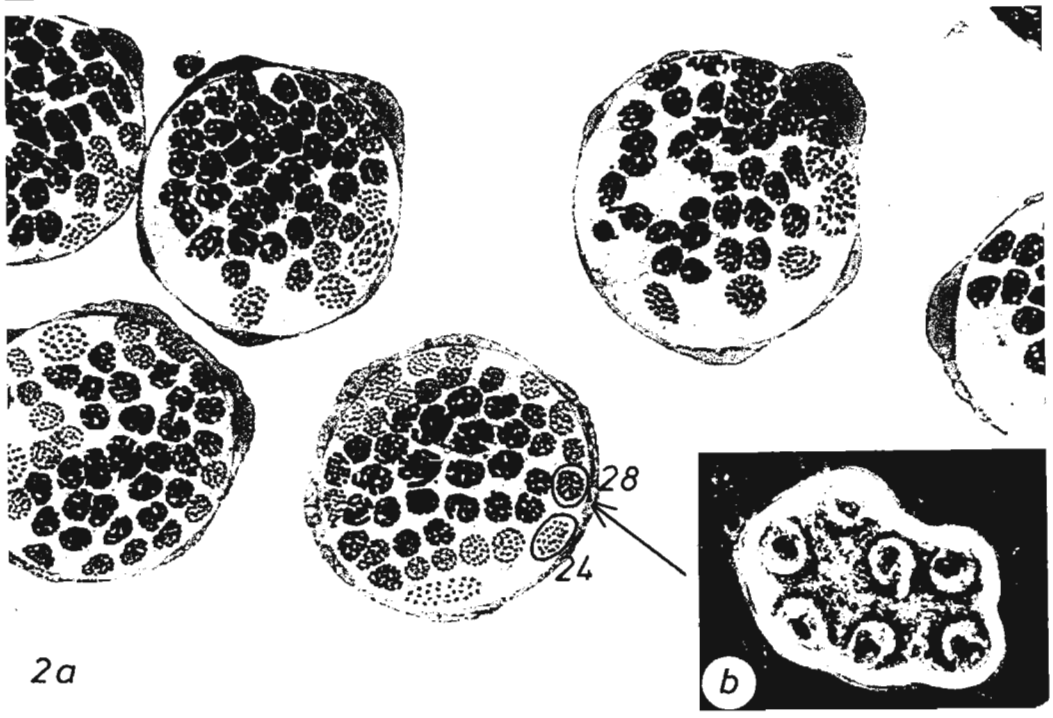


Fig. 1. (a) Section through a coiled testis of D. neohydei. The numbers 16, 20 and 24 indicate the numbers of spermatids within the encircled spermatid bundles. The most common type is 20. (b) Pre-meiotic cyst of D. neohydei showing 5 primary spermatocytes. Such a cyst would be expected to give a post-meiotic bundle of 20 spermatids (arrow).

Fig. 2 (a & b) As per Fig. 1 but showing a section (a) and cyst (b) of a testis of D. eohydei.

Hardy, R.W. University of California, San Diego. Needle-like crystals in spermatocytes and spermatids of XY males of D. melanogaster.

Recently we examined testes from males having a normal Y chromosome. In one stock a small thin needle-like crystal was seen in the nucleus of most primary spermatocytes. Other laboratory stocks failed to exhibit either nuclear or cytoplasmic crystals in the primary spermatocytes; however, thin needle-like crystals are seen in spermatids.

Testes are dissected into a drop of either Drosophila Ringer's (Meyer) or a balanced salt solution on a siliconized slide. The latter is necessary to allow an extremely flat preparation to be made before it dries out. The testes are cut at about 1/3 their length from the closed end and a coverslip is slowly lowered onto the preparation. Excess fluid is withdrawn from beneath the coverslip with a piece of filter paper and the testicular contents become expressed. Spermatids are viewed with an oil phase objective.

The crystals are most easily seen in the cytoplasm of spermatids that are in the "onion" stage or have just begun elongation. The numbers and amounts of crystal material is variable between cysts in a testis. In D. melanogaster neither the occurrence of crystals nor their shape seems to depend on age of the males or other genotypic differences examined including the alleles of Stellate which change needle-like crystals in XO spermatocytes to star-shaped (Hardy 1980; Hardy et al. 1981). Crystals were not found in the one stock of D. simulans examined.

At present work is underway to further characterize the occurrence of the crystals using light and electron microscopy.

References: Hardy, R. W. 1980, DIS 55:54; Hardy, R. W., K. T. Tokuyasu and D. L. Lindsley 1981, Chromosoma (Berl.) 83:593-617.

Hickey, D.A. and R.S. Singh*. Brock University, St. Catharines, Ontario, Canada; *McMaster University, Hamilton, Ontario, Canada. A spontaneous X-linked mutation affecting aldehyde oxidase and xanthine dehydrogenase activities in D. melanogaster.

The genetic control of the enzymes xanthine dehydrogenase (XDH) and aldehyde oxidase (AO) in D. melanogaster has been extensively studied (see Courtright 1976 for a review). The structural genes for these two enzymes are both on the third chromosome (Chovnick et al. 1977; Dickinson 1970). However, the expression of both genes is affected by a number of other gene loci. Mutations at two loci on the X-chromosome, cinnamon (1-0.0) and maroon-like (1-64.8), cause a reduction in the activities of both enzymes and, also, affect the eye color of the mutant flies (Baker 1973; Collins & Glassman 1969). A third-chromosome mutation, low xanthine dehydrogenase (lxd, 3-33), affects the activity of both enzymes but does not affect the eye color of mutant flies (Keller & Glassman 1964). Finally, a second-chromosome mutation, Aldox-2 (2-86), has been recently discovered, which affects aldehyde oxidase activity but not xanthine dehydrogenase activity (Bentley & Williamson 1979a). The modes of action and interaction of these four "controlling" loci are not yet fully understood, though some models have been proposed (Courtright 1976; Meidinger & Williamson 1978). Since two of these loci, cinnamon and maroon-like, also affect the eye color of mutant flies, it is possible that other eye-color mutants of D. melanogaster might have reduced (or absent) XDH or AO activities. This study involved a search for any such mutants. One strain was found which had appreciable, but significantly reduced, levels of XDH and AO.

Thirty-three stocks of D. melanogaster which contained different eye-color mutations were obtained from the Mid-American Drosophila Stock Center. Enzyme activity was visualized after electrophoresis of crude fly homogenates on 5% polyacrylamide gels. Electrophoresis and gel staining for xanthine dehydrogenase activity was as described by Singh et al., 1977.

Most of the Drosophila strains examined had close to wild-type levels of both xanthine dehydrogenase and aldehyde oxidase. As has been previously described, the rosy stock lacks XDH activity only (see Chovnick et al. 1977 for a review) and the maroon-like stock lacks both enzyme activities (see Courtright 1976). However, from Fig. 1 we see that the maroon stock also is deficient in both XDH and AO. This suggests that the mutation which causes the maroon eye phenotype also causes a reduction in enzyme activity as first suggested by Forrest et al. (1956). Nevertheless, a number of further observations and experiments demonstrated that the effects on eye color and enzyme activity in this strain are genetically separate.

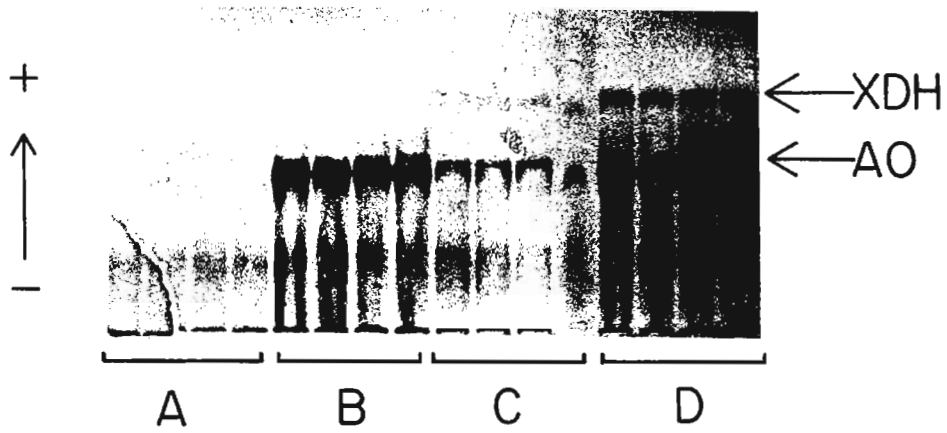


Fig. 1. Polyacrylamide gel stained for xanthine dehydrogenase and aldehyde oxidase activity. There are four replicate samples of each genotype. (A) maroon-like; (B) rosy; (C) maroon; (D) maroon-like X maroon F_1 females. Each sample consisted of two female flies, one week old.

When wild-type males were crossed with maroon females, the F_1 females had normal levels of enzyme activity but the F_1 males had the reduced activity levels characteristic of their maroon mothers. This indicated that the mutation affecting enzyme activity was on the X-chromosome rather than on the third chromosome (which carries the maroon locus). Moreover, the F_1 males, which had reduced enzyme activities, had wild-type eye color.

The next phase of the work was to map this X-linked mutation. Maroon males were crossed with virgin yellow, Bar females and the F_1 flies interbred. All of the yellow and yellow, Bar F_2 males had wild-type levels of enzyme activity while Bar and wild-type (for body, color and eye shape) F_2 males had reduced levels of activity. This meant that the mutation affecting enzyme activity was closely linked to the yellow locus. The results of a parallel experiment using the closely linked markers yellow and white confirm this result. Fifteen recombinants were recovered in the yellow white region, but none resulted in the separation of yellow from the wild-type levels of enzyme activity. The recombination data are supported by the observation that crosses of maroon virgin females with sc^8 Y males yield F_1 males with wild-type levels of enzyme activity, indicating that the wild-type allele is carried on the sc^8 translocation.

This mutant was tested for complementation with the three other mutations which are known to reduce both aldehyde oxidase and xanthine dehydrogenase activity. Two of these mutations are on the X-chromosome, maroon-like (mal) and cinnamon (cin), while low-xanthine dehydrogenase (lxd) is on the third chromosome. F_1 heteroallelic females from all three crosses had wild-type levels of enzyme activity (see Fig. 1 for results of maroon X maroon-like cross). This suggests that this new mutation is at a separate genetic locus, although it is recombinationally inseparable from cinnamon. Those females which were heteroallelic with cinnamon had wild-type eyes as well as normal levels of enzyme activity and were also fully viable.

Table 1. Aldehyde oxidase activity of cin^2 flies expressed as a percentage of wild-type (Oregon-R) activity (± 95% confidence intervals).

Developmental stage	Males	Females
3rd instar larvae	31 ± 12	29 ± 11
1 hour post-eclosion	6.5 ± 4	14.6 ± 5
8 days post-eclosion	2.5 ± 5	32 ± 8

A summary of the relative enzyme activities (for aldehyde oxidase) of the mutant strain is shown in Table 1. Larvae of both sexes have approximately 1/3 of the wild-type levels of activity; young adult females have about half of this relative amount (approximately 15% of wild-type) but this increases with age to reach approximately 1/3 of wild-type levels after one week. Adult males, in contrast, have very much reduced enzyme activities (<10% of wild-type levels when newly eclosed) and this amount decreases with age (Table 1).

We have not found any evidence for temperature effects on enzyme expression. However, temperature does affect the viability of the strain. Viability is comparable to that of the Oregon-R strain at 19°C, but is reduced to zero at 29°C. Both adult and larval stages are temperature-sensitive.

The results demonstrate that the maroon stock of D. melanogaster, besides having an altered eye-color phenotype, is deficient in xanthine dehydrogenase and aldehyde oxidase activities. The effect on enzyme activity is not genetically linked to the maroon locus, but is controlled

by a gene on the X-chromosome. This gene locus is closely linked to the yellow locus, and thus, to the cinnamon locus (Bentley & Williamson 1979b). In many respects this mutation is similar to those alleles placed in the cinnamon Complementation Group C by Bentley and Williamson (1979b). However, it has more than 15% of wild type enzyme activity in adult females. Moreover, female flies which were made heterozygous with the cin^1 allele showed wild-type levels of enzyme activity, had red eyes and were completely viable. Because it shares some, but not all, of the characteristics of the cinnamon mutation which was described by Baker (1973), and because it maps close to the cinnamon locus, we suggest the label $cinnamon^{leaky}$ (cin^{ℓ}) for this mutation. However, it could be argued that the complementation with cin^1 in heteroallelic females is a basis for a separate nomenclature. It remains to be seen whether this allele will complement all of the cin alleles which have recently been isolated by Bentley and Williamson (1979b).

We found that the viability of the maroon stock is temperature-sensitive. Experiments are now in progress to test if this temperature sensitivity is genetically linked to the locus controlling the enzyme expression.

Acknowledgments: David Townsend and Roger Paterson provided technical assistance. Drosophila stocks were provided by the Mid-America Drosophila Stock Center.

References: Baker, B.S. 1973, Dev. Biol. 33:429-440; Bentley, M.M. and J.H. Williamson 1979a, Z. Naturforsch. 34C:304-305; _____ and _____ 1979b, Can. J. Genet. Cytol. 21:457-471; Browder, L.W. and J.H. Williamson 1976, Dev. Biol. 53:241-249; Chovnick, A., W. Gelbart and M. McCarron 1977, Cell 11:1-10; Collins, J.F. and E. Glassman 1969, Genetics 61:833-839; Courtright, J.B. 1976, Adv. Genet. 18:249-314; Dickinson, W.J. 1970, Genetics 66:487-496; Forrest, H.W., E. Glassman and H.K. Mitchell 1956, Science 124:725-726; Keller, E.C. and E. Glassman 1964, Genetics 49:663-668; Meidinger, E.M. and J.H. Williamson 1978, Can. J. Genet. Cytol. 20: 489-497; Singh, R.S., R.C. Lewontin and A.A. Felton 1976, Genetics 84:609-629.

Honisch, S. and J.A. Campos-Ortega. Institut fur Biologie III, Freiburg, West Germany. giant, a segment-specific defect during embryonic development.

Fully developed embryos hemizygous for the large Df(1)JC19 (2F3;3C5) were found to show characteristic morphological defects at both cuticle and internal organs (Campos-Ortega & Jiménez 1980). The larval cuticle of those embryos lacks partially or completely ventral setae (denticle belts) at abdominal segments A5-A7 whereas the remaining cuticular structures do not show any apparent abnormality (Fig. 1a). Whole-mounts of 20h old mutant embryos stained with acidic fuchsin (Zalokar & Erk 1977) show that the corresponding neuromeres are absent, the abdominal 8th neuromere and some remnants of the defective segments being clearly separated from the rest of the CNS (Fig. 1b). Finally, the head does not complete involution, pharynx and pharyngeal chitinized sclerites (H-piece) are shorter and brain lobes somewhat smaller than in wild-type embryos of the same age. Histological analysis of increasingly aged embryos uncovers extensive cell death within epidermal and neural components of the af-

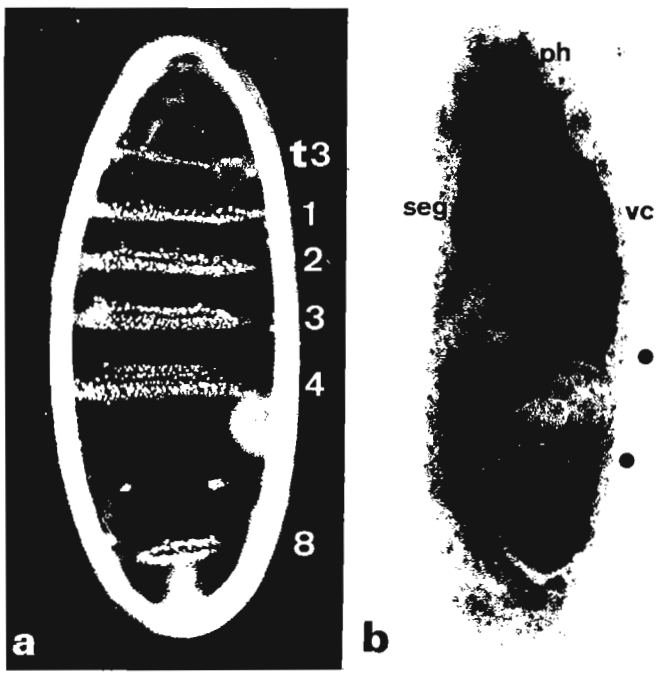


Fig. 1. a is a preparation of the cuticle of a hemizygous Df(1)62g18 embryo photographed in dark field. Setae in metathorax (t3) and abdominal segments 1-4 and 8 are clearly visible. Seate at A5-A7 are essentially absent. b is a micrograph of a fuchsin stained, whole-mounted $l(1)gt^{13z}$ embryo showing the interruption of the ventral cord (vc), marked with dots, referred to in the text. ph: pharynx; seg: supraesophageal ganglion.

fected segments. This process becomes clearly evident at about 7h and is completed at about 13h. The use of smaller deletions mapping within the territory of the Df(1)JC19 permitted restriction of the phenotype described above to the region uncovered by the Df(1)62g18. This region comprises four genes, gt, tko, z and zw1 (Judd et al. 1972). Analysis of lethal point mutations at three of those four loci (Fig. 2) demonstrates that the mutant phenotype found in Df(1)JC19 embryos is exclusively due to the deletion of the gt locus. Since the phenotype of $1(1)gt^{x11}$ and $1(1)gt^{13z}$ hemizygous embryos is identical to that of hemizygous Df(1)JC19 embryos it can be further concluded that, besides at 3A1, the locus of gt (Judd et al. 1972), there is no other gene involved in embryonic morphogenesis between 2F3 and 3C5.

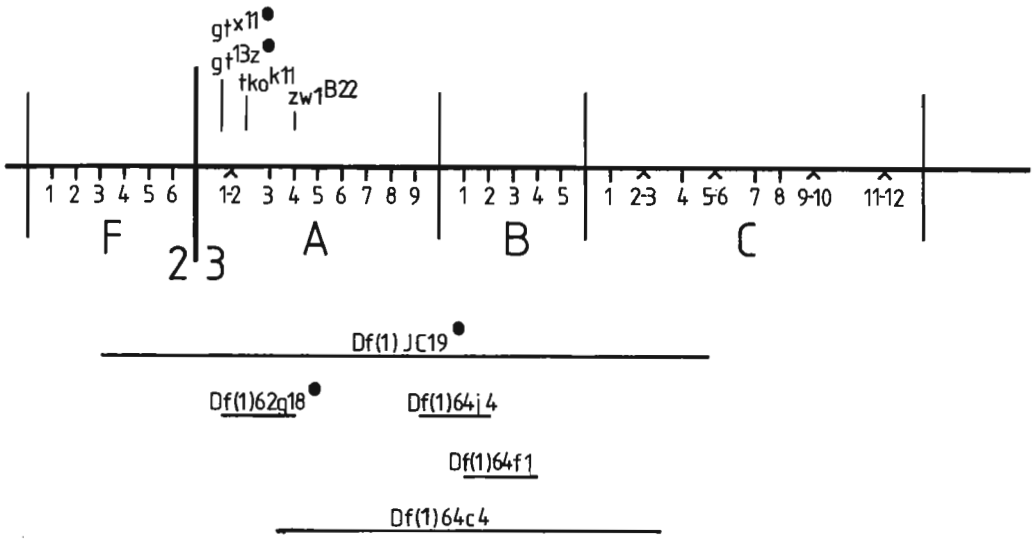


Fig. 2. Localization of deletions and point lethals used in this study. Black dots signify stocks in which the described mutant phenotype is visible.

We gratefully acknowledge Drs. L. Craymer and B.H. Judd for mutant stocks and the Deutsche Forschungsgemeinschaft (SFB 46) for financial support.

References: Campos-Ortega, J.A. and F. Jiménez 1980, in: Behavior and Development of Drosophila (eds. O. Siddiqi, L.M. Hall and P. Babu), Plenum Press; Judd, B.H., M.W. Shen and T.C. Kaufman 1972, Genetics 71:139-156; Zalokar, M. and I. Erk 1977, Stain Technol. 52:89-95.

Hougouto, N. and A. Elens. Facultés Universitaires Notre Dame de la Paix, Namur, Belgium. Phototactical differences brought about by a selection for reproductive characteristics.

The present paper is concerned with the phototactical behavior of some highly inbred D. melanogaster lines. At the outset of our experiments, these lines had been maintained by brother-sister crossing for more than 330 generations; moreover, a bidirectional selection for male sexual activity gave a high activity (HA) and a low activity (LA) line. These two lines present many adaptive differences: the LA line has a lower fecundity, a higher sex ratio, a higher genetical load. However, it is considered as genetically heterogeneous, even more than the HA line in spite of a presumably higher consanguinity (Kaidanov and others 1969; Kaidanov 1980).

Starting from the 264th generation, a one-way selection for the abdominal bristle number was begun in both HA and LA lines. For the first one it has been easy to obtain a so-called HA^+ line having a higher bristle number but remaining for the other characteristics very similar to the HA line. In the LA line, however, the selection for abdominal bristle has succeeded

only when it has been lead together with a selection for a higher female fecundity. By the way of such a "double selection" it has been possible to obtain a so-called LA^+ line characterized by a higher abdominal bristle number (as high as in the HA^+ line), a higher fecundity, a normal sex ratio, a lower genetical load, and even a higher male sexual activity (Hougouto and others 1980).

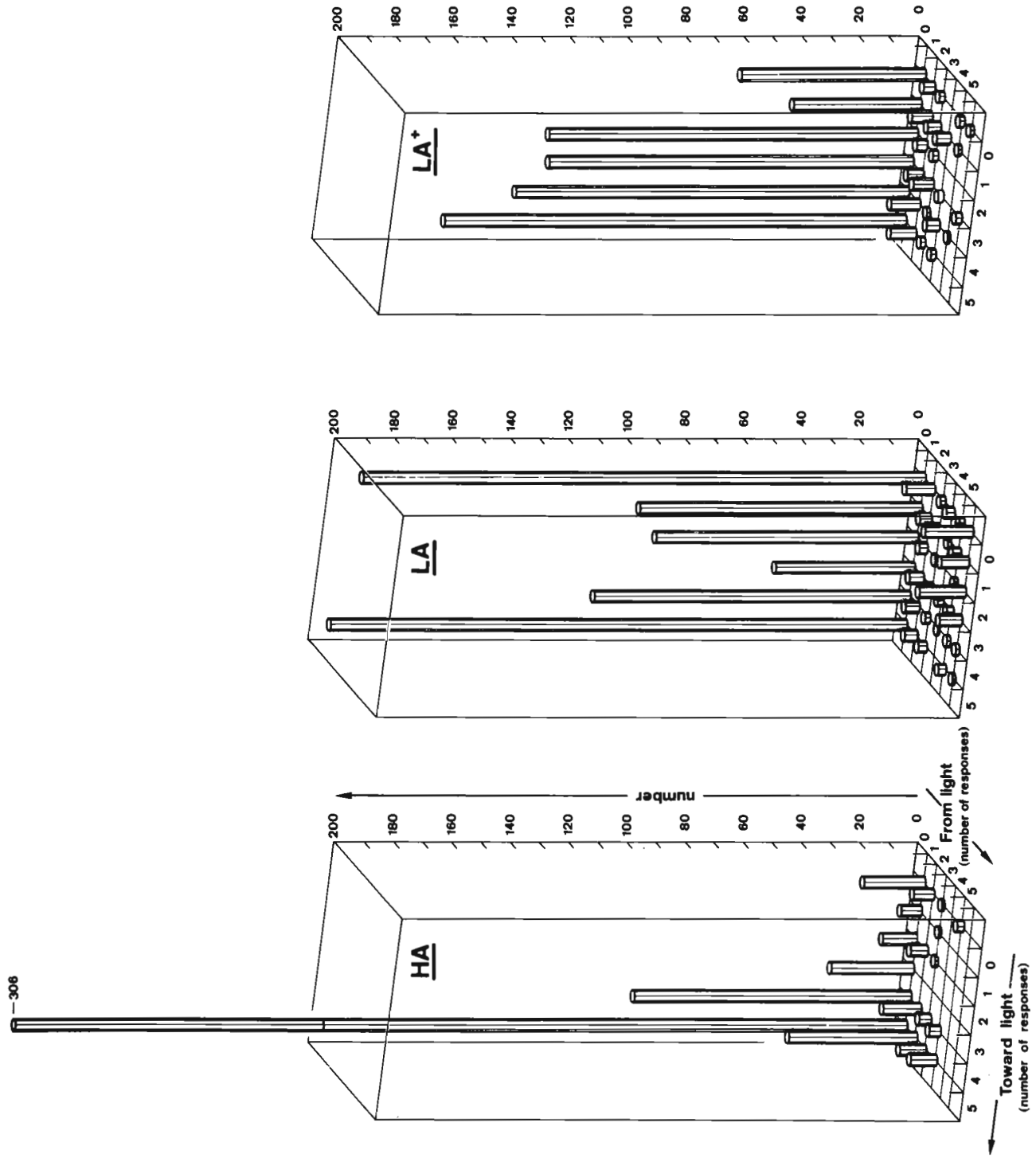


Fig. 1. Fractionation for positive and for negative phototactism.

In order to investigate if such a selection had brought as well some behavioral differences between HA, LA, AND LA^+ lines, the flies have been submitted to the "countercurrent distribution test for phototactical behavior", according to Benzer (1967, 1973). To have enough flies for one test, we used the descendants of crosses between 5 males and 5 females from the 330th generation. Six repetitions were done. The flies were 5 days old. Males and females were tested separately, but the data have been combined (the differences between sexes are not significant).

In Fig. 1, the results are presented in a three-dimensional representation. The height of the columns give the number of flies in each tube, after five choices "to light" and five choices "from light".

The differences between the flies of the three lines are evident, the HA line being the most positively phototactic and the most locomotrically active one. The most interesting fact is that the LA line appears to be the most heterogeneous one: some flies are even "negatively phototactic", contrary to the flies of the other lines. Such an observation is in good agreement with the previously reported ones: the LA line is characterized by a strong genetical load but also by a high mutability. Such a high mutability seems to be a consequence of the negative selection for male sexual activity; it is considered as a reaction against "unadaptive selection" (Kaidanov 1980; Hougouto and others 1980). Generally speaking, the behavioral differences between the three lines result probably from the preceding generations of selection combined with the high consanguinity.

Again, the present study shows that highly inbred lines are not necessarily homozygous. They can remain strongly heterogeneous, this heterogeneity being independent above all from the environmental conditions as well as from the direction of selection.

References: Benzer, S. 1967, Proc. Nat. Acad. Sci. USA 58:1112; _____ 1973, Scient. Amer. 229:24; Hougouto, N., N.V. Glotov and L.Z. Kaidanov 1980, Genetika (Russ.) 16,7,1228; Kaidanov, L.Z., I.S. Kuksinskaja and N.S. Meksina 1969, Genetika (Russ.) 8,8,84; Kaidanov, L.Z. 1980, Genetica 52/53:165.

Hougouto, N. and A. Elens. Facultes Universitaires Notre Dame de la Paix, Namur, Belgium. Phototactism as measured by use of the Kekič maze and of the Benzer method.

It is well known that the phototactical behavior of Drosophila is always a function of the experimental procedure used. The data here presented (Fig. 1, Table 1) are a further illustration of it. Four strains of D. melanogaster, characterized by different alcohol dehydrogenase (ADH) activity levels and by different oviposition site preferences regarding environmental alcohol (Hougouto et al. 1982), have been assayed for phototactism according to Kekič (1981) and to Benzer (1967). In both methods, the negative as well as the positive responses to light are determined. But in the Benzer "countercurrent" method the flies are submitted, besides, to repeated mechanical stimuli. In the Kekič maze the most "sluggish" flies tend to remain in the central chamber ("start"), the positively phototactic flies go to the right and the negatively phototactic flies go to the left. In the Benzer "countercurrent" method the most sluggish flies remain in the "0,0" tube, the most positively phototactic ones concentrate in the "5,0" tube and the most negatively phototactic flies concentrate in the "0,5" tube (for the "LA" strain only, some flies are really negatively phototactic in the present experiment).

A three-dimensional representation shown in Fig. 1 seems to give a better description of these behavioral characteristics. But one is not allowed to conclude that a disruptive selection based on the use of the Benzer method would necessarily give better results than those obtained by Kekič and Marinkovič (1974).

Table 1. Distribution of the flies after a one hour test in the Kekič maze.

Strains	Chambers / Light, in lux	1 500	2 800	3 2000	4 4500	5 6500	Total number
HA		95	490	538	385	233	1741
LA		44	81	642	211	139	1117
AO-null		200	293	362	268	223	1345
ADH-null		82	154	1037	120	119	1512

5 repetitions have been done. The data relative to both sexes have been combined.

References: Benzer, S. 1967, Proc. Nat. Acad. Sci. 58:1112; Hougouto, N., M.C. Liétaert, M. Libion-Mannaert, E. Feytmans and A. Elens 1982, Genetica (in press); Kekič, V. and D. Marinkovič 1974, Behav. Genet. 4:285-300; Kekič, V. 1981, DIS 56:178.

[Fig. 1 on following page.]

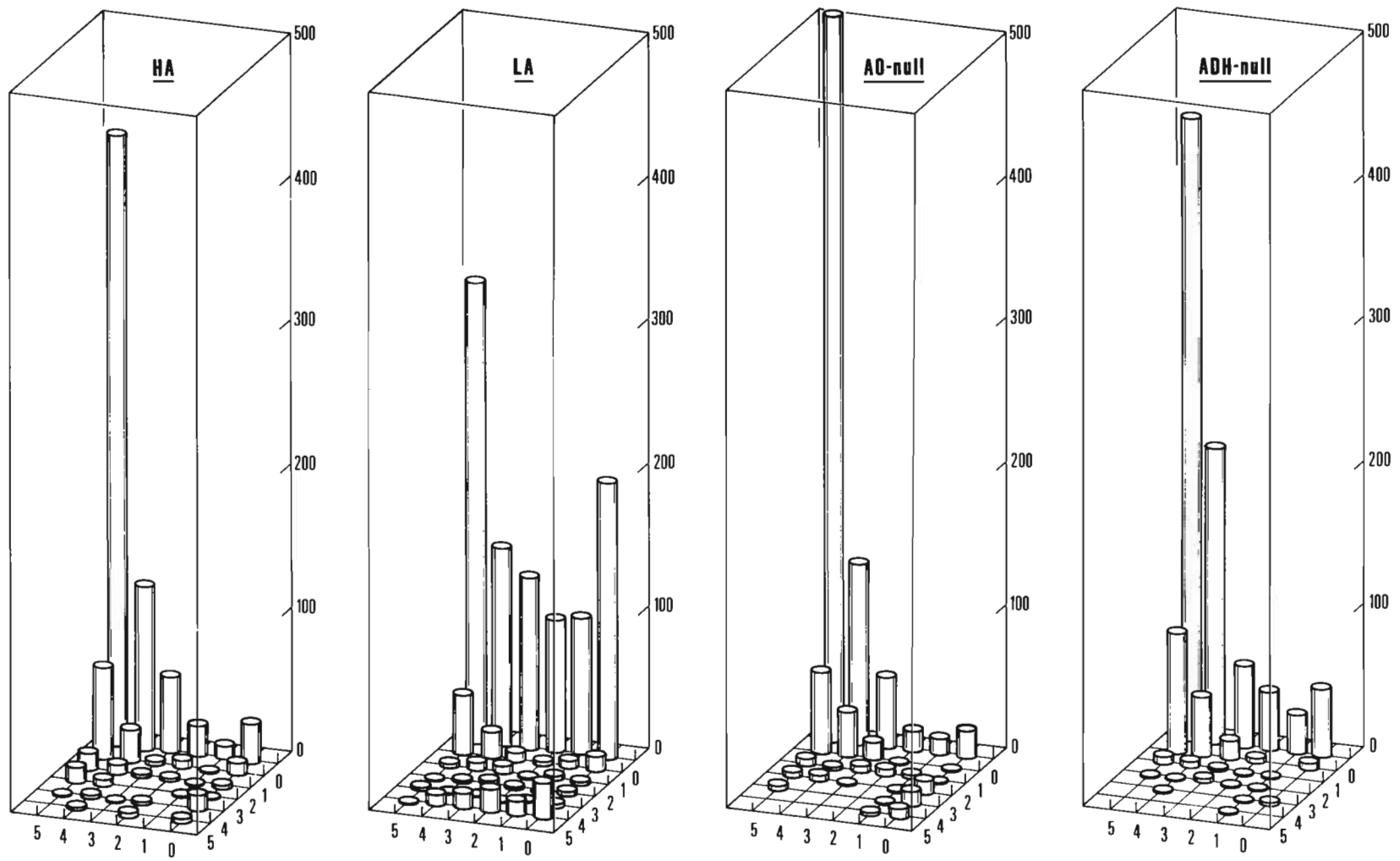


Fig. 1. Distribution of the flies after a test for positive and for negative phototactism, according to Benzer (one minute runs).
ordinate: number of flies in the tube
front view abscissa: number of positive responses (toward light)
side view abscissa: number of negative responses (from light)
Ten repetitions have been done. The data relative to both sexes have been combined.

Ikeda[1], H., O. Kitagawa[2] and A. Fukutami[3]. [1]Ehime University, Matsuyama, Ehime, Japan. [2]Tokyo Metropolitan University, Tokyo, Japan. [3]Josai Dental University, Saitama, Japan. Colonization of D. mercatorum in Africa.

Wasserman (1962) noted that D. mercatorum probably arose originally in the Brazilian and Bolivian lowlands and spread into the Nearctic and the Neotropical regions. In addition, this species has been found in the Hawaiian Islands, Australia (Patterson & Wheeler 1949), Spain (Prevosti 1950) and Samoa (Wheeler & Kambysellis 1966). On September 4, 1979, we collected three females of D. mercatorum in a city market of Nairobi, Kenya and were able to establish two isofemale laboratory stocks, L75 and L169. The eye color mutant (bright red eye color, autosomal recessive) was isolated from L75. The metaphase karyotype of the stock consisted of one pair of large metacentrics, one pair of small metacentrics, two pairs of rods, a rod-shaped X and a short rod-shaped Y.

The collection trip was supported by the Ministry of Education of Japan (No. 404149).

References: Patterson, J.T. and M.R. Wheeler 1949, Univ. Texas Publ. 4920:207-233; Prevosti, A. 1950, DIS 27:110; Wasserman, M. 1962, Univ. Texas Publ. 6205:63-71; Wheeler, M.R. and M.P. Kambysellis 1966, Univ. Texas Publ. 6615:533-565.

Ives, P.T. Amherst College, Amherst, Massachusetts. Increasing the length of the Bridges crossover maps.

In many routine linkage tests with standard wild-type strains I have never got as much crossing over in the middle of the second chromosome as the Bridges map indicates. (See Ives et al. 1953 for one example.) In some 1976 tests with a new wild-type strain, M3 (Kidwell et al. 1977), I finally observed even more crossing over there than the Bridges map calls for. This was true only in A type tests, not in B type. (A starts with wild males x marker females; B is the reverse.) The results are given in the accompanying table, together with comparable data from tests of standard Oregon-R (Ore-R) and the net b cn bw (nbcb) stock I synthesized in 1938 using mutants out of males collected at the Porch site of the South Amherst natural population.

Table 1. Percent of crossing over in mid-chromosome 2.

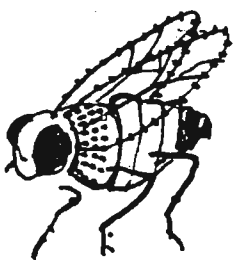
Strain	Flies	nub-lt	lt-stw	stw-sca	Total units
Standard Map	...	8.0	0.1	11.6	19.7
M3 A	646	15.8	0.8	13.9	30.5
M3 B	667	3.7	0.0	6.4	10.1
Ore-R A	603	3.3	0.0	10.4	13.7
Ore-R B	896	4.1	0.1	11.8	16.0
nbcb A	740	4.9	0.1	10.5	15.5
nbcb B	675	4.6	0.1	8.4	13.1

(These data are extracted from the results of tests with the al nub lt stw^3 sca^2 sp^2 marker stock.)

The M3 A cross increased the map distance between nub and sca by 10.8 map units, mostly between nub and stw. The other series showed only about half the map distance recombination between nub and lt. Male recombination rates in sibs of these females were 40 times as high over the whole chromosome in M3 A as in Ore-R and nbcb A and B tests, and M3 B was 12 times as high. (The male data, from 262,183 flies, are being published separately.

Bridges, who reportedly used the highest spontaneous crossover values he could obtain for his maps, would have delighted in such a strain as M3 for his nearly always A-type female crossing over tests, because it would have made much more room on the maps for new mutant loci in the "tight" mid-regions of the large autosomes.

References: Ives, Fenton, Yost and Levine 1953, PNAS 39:1134-1141; Kidwell, Kidwell and Ives 1977, Mutation Res. 42:89-98.



Ives, P. T. Amherst College, Amherst, Massachusetts. The effects of radiation on male crossing-over.

Males from stocks of Oregon-R (Ore-R) and net^{38j} b^{38j}cn^{38j}bw^{38j} (nbcb), and males from a wild-type strain out of the South Amherst natural population, M3 (Kidwell et al. 1977) were crossed to al nub lt stw^3 sca^2 sp^2 (anlsss) females. This is the A cross of Kidwell and Kidwell (1975). Virgin F_1 males from these crosses were mated singly, some as controls and some after receiving 1 kr of hard X-rays, to successive harems so as to test five sperm broods, days 1-3, 4-5, 6, 7-8, and 9-10, for male crossing-over. Also tested similarly were heterozygous males from the B mating, M3 females x anlsss males.

Table 1. Minimum frequencies of spontaneous male crossing-over with anlsss.

			Region					
			al-nub	nub-lt	lt-stw	stw-sca	sca-sp	
Strain	Type	Flies	1	2	3	4	5	Total
Ore-R	A	51,489	2	3	0	2	4	11
		%	.004	.006	.000	.004	.007	.021
nbcb	A	60,277	2	5	0	2	2	11
		%	.003	.009	.000	.003	.003	.018
M3	B	80,829	44	78	0	43	34	199
		%	.054	.097	.000	.053	.042	.246
M3	A	69,588	125	179	6	144	131	585
		%	.180	.257	.009	.207	.188	.841

Table 2. Minimum percentage of spontaneous crossing-over in successive sperm broods.

M3, Type A

		Region					
Brood	Flies	1	2	3	4	5	Total
1	20,890	.20	.27	.01	.19	.19	.86
2	22,678	.19	.26	.01	.22	.21	.89
3	14,435	.17	.27	.01	.24	.17	.86
4	6,502	.17	.28	.00	.17	.18	.80
5	5,083	.12	.14	.00	.18	.14	.57

M3, Type B

		Region					
Brood	Flies	1	2	3	4	5	Total
1	24,210	.05	.10	.00	.07	.03	.25
2	26,546	.05	.10	.00	.06	.05	.26
3	18,255	.05	.12	.00	.04.	.02	.23
4	7,055	.09	.09	.00	.01	.06	.24
5	4,763	.06	.08	.00	.00	.08	.23

The sums of crossover data from all control broods together are given in Table 1, and for the separate control broods of M3 A and B tests in Table 2. These data show the minimum frequencies of crossing-over. The occasional and generally small clusters of more than one crossover fly in a given region were counted as only one crossing-over event in any one sperm brood of M3 A and B tests.

Table 3. Minimum frequencies of male crossing-over with anlsss in brood 4 sperm after exposure to 1 kr of X-rays.

Strain	Flies	Region 1	2	3	4	5	Total
Ore-R	4,469	5	12	5	7	5	34
	%	.11	.27	.11	.16	.11	.76
nbcb	5,104	9	12	7	7	4	39
	%	.18	.24	.13	.13	.08	.76
M3 B	6,717	13	28	12	13	15	81
	%	.19	.42	.18	.19	.22	1.21
M3 A	4,207	22	19	4	14	25	84
	%	.52	.45	.10	.33	.59	2.00

The M3 A and B rates (Table 2) remain constant in successive sperm broods. The effects of irradiation on the rate of crossing-over appeared first in brood 4 (days 7-8) sperm, the data from which are given in Table 3. In these tests, too, clusters of crossovers within a region were small and infrequent, and they were counted as only one crossover event each.

In Table 1, both Ore-R and nbcb heterozygote males gave spontaneous crossovers at a total rate of about one crossing-over event per 5000 test flies. The M3 B frequency is roughly 12 times higher and the M3 A frequency 40 times higher than the Ore-R and nbcb frequencies. These are clearly 3 different frequencies of male crossing-over.

The 1 kr of X-rays added approximately the same amount of crossing-over to each of the 4 kinds of male heterozygotes as is shown in Table 3. The effects of the radiation are clearly additive and not synergistic in M3 A and B heterozygous males.

Sobels and Eeken (1981), testing a combination similar in principle to M3/anlsss, reported an additive effect of X-rays on the production of translocations, which are also the result of exchanges between chromosomes. They found a synergistic effect in the production of sex-linked mutations, which are chiefly "point" effects on one chromosome.

The lack of synergistic effects of X-rays in producing chromosomal exchanges in heterozygous males that show those exchanges in appreciable frequencies spontaneously suggests that the several spontaneous phenomena of hybrid dysgenesis may not be due simply to a deficient DNA repair mechanism as was proposed by Yamaguchi (1976).

Spontaneous crossing-over in all of the 4 kinds of heterozygous males reported here occurred rarely if at all in heterochromatin. (Region 3, lt-stw, consists almost entirely of heterochromatin.) In this respect the male crossing-over resembles female crossing-over. It differs notably in euchromatic distributions: 56% of all male crossing-over was observed in regions 2 and 4 euchromatin which is proximal to the large central segment of heterochromatin. Only 17% of spontaneous female crossing-over occurs in these regions, according to the standard Bridges map. The X-ray treatment induced enough male crossing-over in the heterochromatin that 12% of the observed crossovers were in region 3. The other 88% were distributed in euchromatic regions in roughly the same proportions as the spontaneous crossovers. Thus it appears that there are at least 3 distinct maps of chromosomal distributions of crossing-over in D. melanogaster: standard female, spontaneous male, and X-rayed male, rather than just female and male maps as proposed in 1974 by Becker.

The proportionate distribution of spontaneous crossing-over in the 5 regions set off by anlsss does not differ in M3/anlsss males from that in Ore-R and nbcb heterozygotes in Table 1. Transposable elements (Green 1980) may be operating in M3/anlsss but probably not in the Ore-R and nbcb heterozygous males. If so, they appear to have many operational euchromatic sites along the whole chromosome in M3/anlsss sperm cells during late mitosis or early meiosis.

I am indebted to Dr. M. G. Kidwell for several helpful suggestions and criticisms in preparing this report.

References: Becker 1974, Naturwissenschaften 61:441-448; Green 1980, Ann. Rev. Genet. 14: 109-120; Kidwell and Kidwell 1975, Nature 253:755-756; Kidwell, Kidwell and Ives 1977, Mutation Res. 42:89-98; Sobels and Eeken 1981, Mutation Res. 83:201-206; Yamaguchi 1976, Mutation Res. 34:389-406.

Jackson, F.R., D.A. Gailey and R.W. Siegel. University of California, Los Angeles. The conditioned modification of male courtship behavior in D. melanogaster is affected by biological rhythm mutations.

The capacity of fruit flies to exhibit experience-dependent behavioral changes has been amply demonstrated (Thorpe 1939; Quinn et al. 1974; Menne & Spatz 1977; Dudai 1978; Siegel & Hall 1979; Booker & Quinn 1981). Recently it was shown that the courtship activity of individual males is affected by previous experience. Furthermore, the integration of information bringing about this behavioral modification can be disrupted by mutations that affect associative behavior in Drosophila (Siegel & Hall 1979; Gailey et al. in prep).

In classical conditioning, animals make associations between temporally delivered stimuli. The time interval between these stimuli is a demonstrably important determinant of conditioning efficiency; therefore, an organism with an abnormal sense of time might respond inefficiently to stimuli delivered in a standard temporal sequence. With this in mind, we have examined the conditioning behavior of mutants previously isolated on the basis of having abnormal biological timers.

Mutations that affect both daily (circadian) rhythms (Konopka & Benzer 1971; R. Konopka & R. Smith, pers. comm.) and the much shorter-term courtship song oscillation of the male (Kyriacou & Hall 1980) have been reported. The period alleles, per^{ℓ}, per^{s} and per^{o}, specify longer period, shorter period and arrhythmic daily behavior, respectively. These mutations affect the male song rhythm in an analogous fashion; per^{s} shortens and per^{ℓ} lengthens the period, while per^{o} abolishes song rhythmicity. But for song periodicity, the courtship behavior of the per mutants is indistinguishable from wild-type; this has made it possible to assess the role of endogenous rhythms in the conditioning of courtship. Studies of the per strains and two newly isolated clock mutants have led to the discovery of a relationship between a specific type of clock defect and the ability of males to be conditioned.

Fruit flies to be used in conditioning experiments were raised on a medium of cornmeal, sucrose, dextrose, wheatgerm, yeast and agar. Males and C(1)DX, y f (attached-X) females were collected under very light ether anesthesia and then maintained in vials of the standard medium in a light-dark (LD 12:12) cycle for five days prior to experiments (males were individually housed; females were kept 10/vial). For all experiments, conditioning was performed by pairing flies in cylindrical chambers (0.2 cm^3) of a mating wheel (Hotta & Benzer 1976). The conditioning paradigms used in the present study have been described (Siegel & Hall 1979; Gailey et al., in prep). In brief, five-day-old Canton-S males, which have been paired for 30 minutes with fertilized females, afterwards exhibit a marked reduction in their courtship of test virgin females. Control males, paired with virgin females or with mature males, or simply rested for 30 minutes, thereafter avidly court virgin females. The second conditioning task is an analog of the fertilized female conditioning, but makes use of the observation that immature (< 1 day old) males stimulate courtship. In this paradigm, C-S males display a subsequent decrement in courtship of immature males after being conditioned by an immature male.

Table 1. Biological-rhythm parameters and courtship-conditioning indices for wild-type (C-S) and clock-defective strains.

Strain	Circadian period (hr)	Courtship song-rhythm period (sec)	Average conditioning index (± SEM)	
			Fertilized female	Immature male
$per^{\ell 1}$	28[a]	82.1 ± 3.3[b]	58 ± 11[c]	57 ± 12[d]
$per^{\ell 2}$	28[e]	nt[f]	51 ± 14[c]	73 ± 11[c]
per^{o}	arr[a]	arr[b]	76 ± 13	84 ± 14
per^{s}	18[a]	41.5 ± 1.0[b]	93 ± 7	97 ± 2
Andante	25.5[g]	nt	21 ± 25[d]	46 ± 16[d]
psi-2	25.5	~70	35 ± 12[d]	24 ± 17[d]
C-S	23.8	54.0 ± 0.8[b]	91 ± 3	98 ± 1

[a]Konopka & Benzer 1971.
[b]Kyriacou & Hall 1980.
[c]statistically different from wild-type, $p < 0.05$ (Student's t-test, 2-tailed).
[d]statistically different from wild-type, $p < 0.01$.
[e]R. Konopka, pers. comm.
[f]not tested.
[g]R. Smith, pers. comm.

The percentage of time a male devotes to courtship activity during a test observation period (ca. 10 min.) is defined as the Courtship Index (CI). CIe and CIc represent courtship indices for experimental and control situations, respectively. A C-S male, following an exposure to a noncopulating (= immature), courtship-stimulating, virgin female will readily court a subsequent virgin female ($\overline{CIc}$ = 38). After experiencing a fertilized female, however, there is a dramatic depression in courtship of a virgin ($\overline{CIe}$ = 4). This effect can be quantified by a Conditioning Index (Δ) which is defined as follows:

$$\Delta = 1 - \frac{CIe}{CIc} \times 100$$

In contrast to wild-type, males from four different long-period mutant strains are not effectively conditioned. Table 1 summarizes the average conditioning indices and biological clock characteristics for C-S males and for males from a collection of clock-defective mutant strains. Clearly, only those mutations which bring about slower rhythms have an effect on conditioning performance. per^{l1}, per^{l2}, Andante and psi-2 lengthen circadian periods (i.e., slow down internal timing processes) and males carrying these mutations are not conditioned in a normal manner. In contrast per^{s} and per^{o}, which presumably affect the same gene product as per^{l} but speed up timing mechanisms, do not significantly alter conditioning performance.

It appears that mutations which lengthen period intervals also interfere with conditioning. We have, in fact, ruled out several alternative explanations for the present results. For example, the conditioning defect displayed by per^{l} males is neither a result of variation in genetic background nor a consequence of a mutation outside the clock locus. In addition, the mutant site responsible for the aberrant conditioning behavior of per^{l} males maps genetically to an interval (y-cv) in which the per locus itself resides.

There are several different mechanisms by which mutations that lengthen periods could affect conditioning. The defective behavior of per^{l} males cannot be interpreted as an alteration of a possible diurnal pattern of conditioning, for their average conditioning index is the same at all times of day. Furthermore, per^{l} males elicit the conditioning cue(s) from fertilized females in a normal manner (data not shown). Thus, we have adopted the working hypothesis that there exists a physiological relationship between internal clocks and conditioning. If this is so, then internal timers may indirectly affect or be integral components of the conditioning apparatus.

Acknowledgments: We are grateful to Ronald Konopka and Randy Smith for providing stocks of per^{l2} and Andante before they had published data on the mutants. F.R.J. was supported by NIH-PHS Genetics Training Grant GM 07104 (05). His current address is Dept. of Genetics, Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.

References: Booker, R. and W.G. Quinn 1981, Proc. Nat. Acad. Sci. USA 78:3940-3944; Dudai, Y. and G. Bicker 1978, Naturwissenschaften 65:495-496; Hotta, Y. and S. Benzer 1976, Proc. Natl. Acad. Sci. USA 73:4154-4158; Konopka, R.J. and S. Benzer 1971, Proc. Natl. Acad. Sci. USA 68:2112-2116; Kyriacou, C.P. and J.C. Hall 1980, Proc. Natl. Acad. Sci. USA 77:6729-6733; Menne, D. and H.C. Spatz 1977, J. Comp. Physiol. 114:301-312; Quinn, W.G., W. Harris and S. Benzer 1974, Proc. Natl. Acad. Sci. USA 71:708-712; Siegel, R.W. and J.C. Hall 1979, Proc. Natl. Acad. Sci. USA 76:3430-3434; Thorpe, W.H. 1939, Proc. Roy. Soc., B, 127:424.

Kalisch, W.-E. Ruhr-Universität Bochum, Germany. Chromosome spreading of salivary glands and Malpighian tubules in D. hydei.

Recently we introduced a new surface spreading technique for salivary gland chromosomes in D. melanogaster, Chironomus th. thummi and Ch. tepperi, which effects longitudinal and lateral chromosome spreading up to 6-8 times in comparison with the corresponding values of polytene chromosomes in squash preparations. This technique is now available for detailed cytological analyses of banding patterns and for photo maps of polytene chromosomes (Kalisch & Hägele 1981). In connection with the autoradiographic method spread chromosomes may even be used for in situ hybridization of cloned nucleic acids (in prep.). However, modifications of the technique are sometimes necessary even in salivary gland chromosomes of different species as well as always in polytene chromosomes of different tissues in the same species. The reason for this different spreading behavior may probably result from the degrees of polyteny as well as quantitative and/or qualitative differences of the chromosomal proteins.

We now have preliminary results in D. hydei, a species from which comparable studies of banding patterns in different tissues already exist (Berendes 1963, 1966).

Different from the technique already described (Kalisch & Hägele 1981), we used 33% instead of 66% propionic and citric acid (1:1, ten min.) as pretreatment solution for the Malpighian tubules. Pretreatment of salivary glands in D. hydei took two min. longer (ten min.)

than in D. melanogaster. A microscope mirror (WILD) was used instead of a spreading trough. On its concave surface a drop of spreading medium was placed (2 cm in diameter; 4 mol urea, 0.1 mol HCl).

Fig. 1 shows distal tips of the salivary gland X-chromosome after spreading (b) and squashing (c). Obviously, the spreading technique promotes a well-focused picturing even of faint

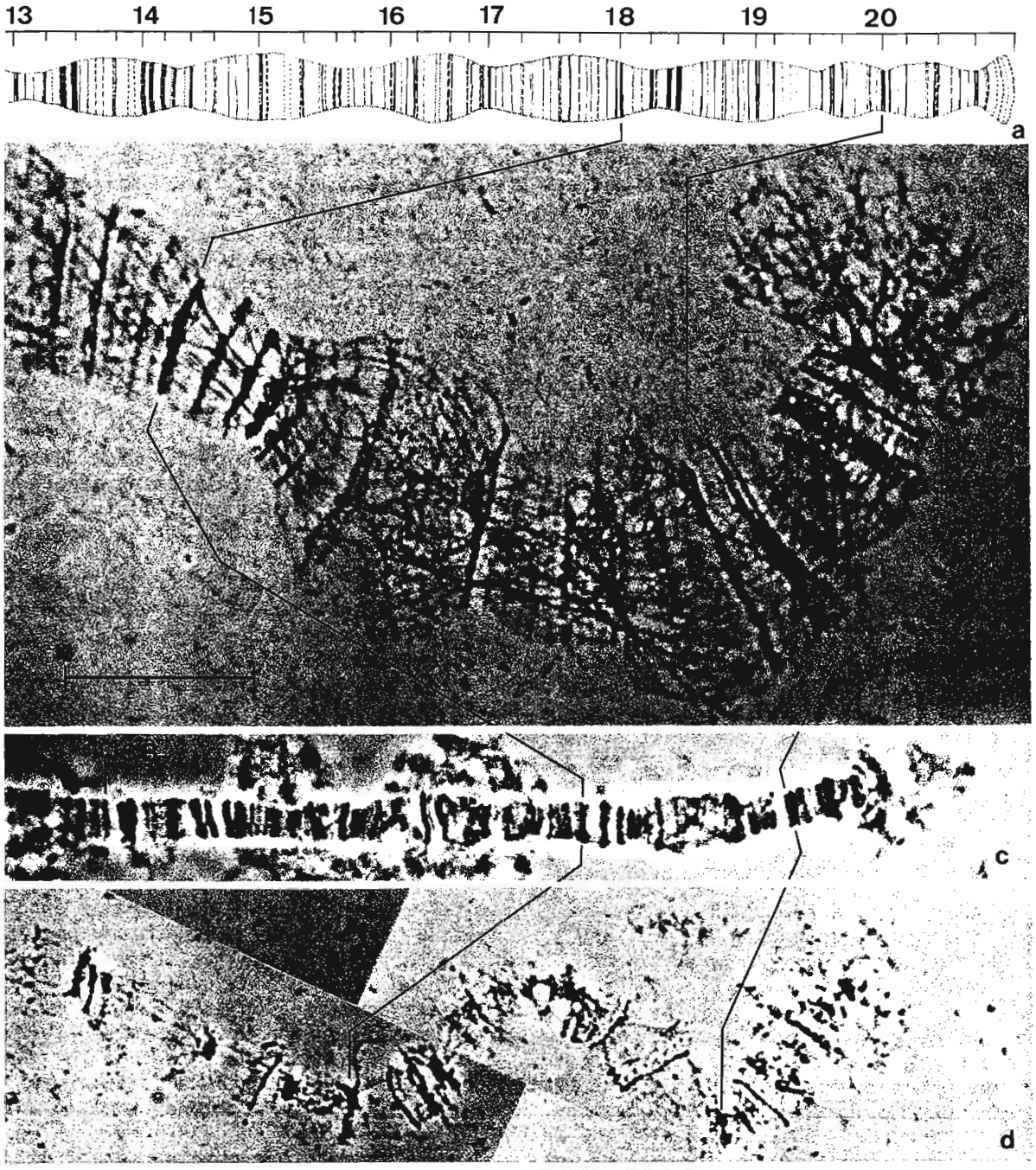


Fig. 1. Distal end of the X-chromosome in D. hydei, Orcein-staining, Neofluar 63/1. 25 Ph, same magnification in (b) - (d), Bar = 20 µm. (a) Chromosome map (on the basis of 40-50 different salivary gland cells) and reference numbering according to Berendes, 1963. (b) Surface spread salivary gland X-chromosome (total length: 617 µm; diameter: 18-30 µm). (c) Squash preparation (total length: 287 µm; diameter: 5-6 µm). (d) Surface spread X-chromosome of Malpighian tubules (total length: 543 µm; diameter: 2.5-8.5 µm).

bands and small interbands. Whether this technique is suitable to judge between single and double polytene structures as can be done by the EM-technique (Berendes 1970; Lossinsky & Lefever 1978) has not yet been studied. Salivary gland chromosomes with lower spreading degrees nearly show proportional spreading, laterally and longitudinally. Exceptions are the excessively spread nucleolus, some prominent chromosome bands (which are constricted in diameter compared with their neighboring structures) and the tip of the X-chromosome, which often is already flared in the squash preparation and always so after spreading (20 D in Fig. 1b). Even in spreading preparations the distal ends of the four long autosome arms are frequently synapsed, either all four ends together or two by two.

Actual considerations about correlations between genes and polytene structures insist the relevancy of detailed analyses in banding patterns of different tissues in the same species. However, analyses of squashed chromosomes in Malpighian tubules are complicated by the low degree of polyteny (diameter of squashed chromosomes in D. hydei: Malpighian tubules ca. 1-2 µm, salivary glands ca. 5-8 µm). Preliminary studies show (Fig. 1d) that our spreading technique is even available for the chromosomes of Malpighian tubules, whereby diameters similar to those of squashed salivary gland chromosomes can be reached.

This work is financially supported by the Deutsche Forschungsgemeinschaft.

References: Berendes, H.D. 1963, Chromosoma (Berl.) 14:195-206; _____ 1966, J. Exp. Zool. 162:209-218; _____ 1970, Chromosoma (Berl.) 29:118-130; Kalisch, W.-E. and K. Hägele 1981, J. of Cell Biol. 31:91-138; Lossinsky, A.A. and H.M. Lefever 1978, DIS 53:126-131.

Karakin, E.I. Institute of Cytology & Genetics, Siberian Branch, USSR Academy of Sciences, Novosibirsk, 630090, USSR.

Antigenic characteristics of malignant invasive neuroblastoma in larval brain transplants of lethal 1(2)gl^4 D. melanogaster mutants.

It was demonstrated that the 1(2)gl^4 D. melanogaster mutation transforms the imaginal optic primordia in the larval brain into an invasive and lethal neuroblastoma similar to vertebrate neoplasms in basic characteristics (Gateff & Schneiderman 1974). One may expect in transplants some changes of water-soluble antigen patterns (Fel & Shvemberger 1968). Comparisons of antigen patterns of the primary cerebral hemispheres from homo- and heterozygous larvae for 1(2)gl^4 demonstrate their almost total similarity (Fig. 1a and 1b). Successful transplants of the hemispheres of 1(2)gl^4 homozygous larval brain into the abdomens of D. melanogaster female hosts, where they grew rapidly, not only retained some of the primary antigens (Fig. 1c, points over the precipitation peaks) but also contained several "new" antigen fractions (Fig. 1c, +); some antigens are absent (Fig. 1a, -). The presence of the new antigen fractions cannot be attributed to the contamination by hemolymph antigens of female hosts (see control, Fig. 1d). It seems that the new antigens are neuroblastoma markers and specifically characterize the given invasive malignant neoplasm.

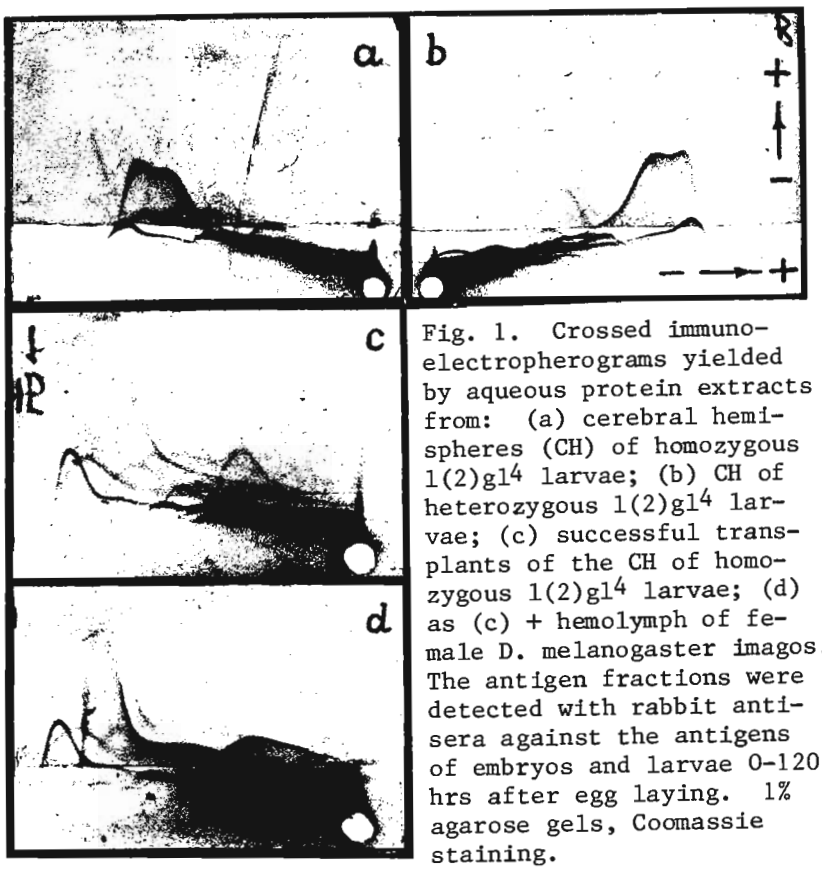


Fig. 1. Crossed immunoelectropherograms yielded by aqueous protein extracts from: (a) cerebral hemispheres (CH) of homozygous 1(2)gl^4 larvae; (b) CH of heterozygous 1(2)gl^4 larvae; (c) successful transplants of the CH of homozygous 1(2)gl^4 larvae; (d) as (c) + hemolymph of female D. melanogaster imagos. The antigen fractions were detected with rabbit antisera against the antigens of embryos and larvae 0-120 hrs after egg laying. 1% agarose gels, Coomassie staining.

References: Fel, V.Ya. & I. N. Shvemberger 1968, Morphological & Immunological Study of Cytodifferentiation in Experimental Tumors, Leningrad, Nauka (in Russian); Gateff, E. & H.A. Schneiderman 1974, Wilhelm Roux Arch. EntwMech. Org. 176:23.

Karakin, E.I. and T. Ya. Lerner. Institute of Cytology & Genetics, Siberian Branch, USSR Academy of Sciences, Novosibirsk, 630090, USSR. Changes in the antigenic composition of the salivary glands and brain-ventral ganglion complex in developing D. melanogaster third instar larvae.

The tissues of D. melanogaster third instar larvae differ in their antigenic composition (Boavida & Roberts 1975; Karakin et al.[1] 1977). Using our micromodification of Laurell's method (Laurell 1965) we analyzed the antigenic composition of the salivary glands (SG) and the brain-ventral ganglion complex (BVG) which were extirpated from synchronized larvae of D. melanogaster Canton S (wild type) 72, 84, 96, 108 and 120 hrs after egg laying (Figs. 1 and 2). To detect tissue proteins, we used rabbit antisera against the antigens of D. melanogaster embryos and larvae, i.e., during 0-120 hrs of development (Karakin et al.[1] 1977; Karakin et al.[2] 1977).

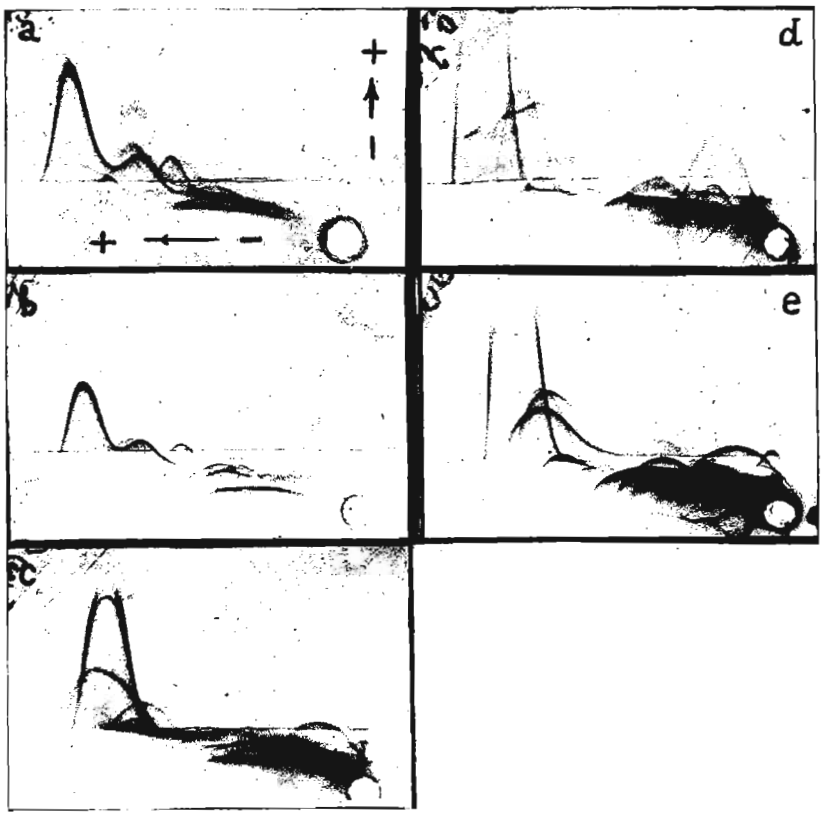


Fig. 1. Crossed immunoelectropherograms yielded by the protein extracts from D. melanogaster larval salivary glands: (a) 72; (b) 84; (c) 96; (d) 108; (e) 120 hrs after egg laying; 1% agarose gels, Coomassie staining. (a) and (b) - aqueous solutions; (c), (d) and (e) - extracts in Tris-EDTA-borate buffer, pH 8.6, containing 0.1 M ditiothreitol.

The antigenic composition of the BVG was found to be less variable during development than that of the SG (Figs. 1 and 2). The addition of ditiothreitol to the extracting solution for the SG was efficient in restoring disulphide bonds (more complete antigenic patterns) only at 96, 108, and 120 hrs of development (Beckendorf & Kafatos 1976); an aqueous solution was used for extraction of proteins from the BVG.

The higher stability of the antigen patterns of the BVG during development seems to comply with the constancy of its physiological functions during the period studied; their antigen pattern is modified in the prematamorphosic state (120 hrs).

If the synthesis of the secretion is, indeed, the terminal step of the developmental process in the larval salivary glands, then the maximum changes in the antigenic patterns of these glands occurring at 96, 108 and 120 hrs of larval development may be useful in further studies of the organ-specific function of the salivary glands (Karakin et al.[2] 1977).

References: Beckendorf, S.K. and F.C. Kafatos 1976, Cell 9:365; Boavida, M. and D. Roberts 1975, J. Insect. Physiol. 21:1587; [1]Karakin, E.I., T.Ya. Lerner, I.I. Kiknadze, L.I. Korochkin and S.M. Sviridov 1977, Cytology 19:111 (in Russian); [2]Karakin, E.I., T.Ya. Lerner, V.A. Kokoza and S.M. Sviridov 1977, Dokl. AN SSSR 233:698; Laurell, C.-B. 1965, Analyt. Biochem. 1:358.

[Fig. 2 on following page]

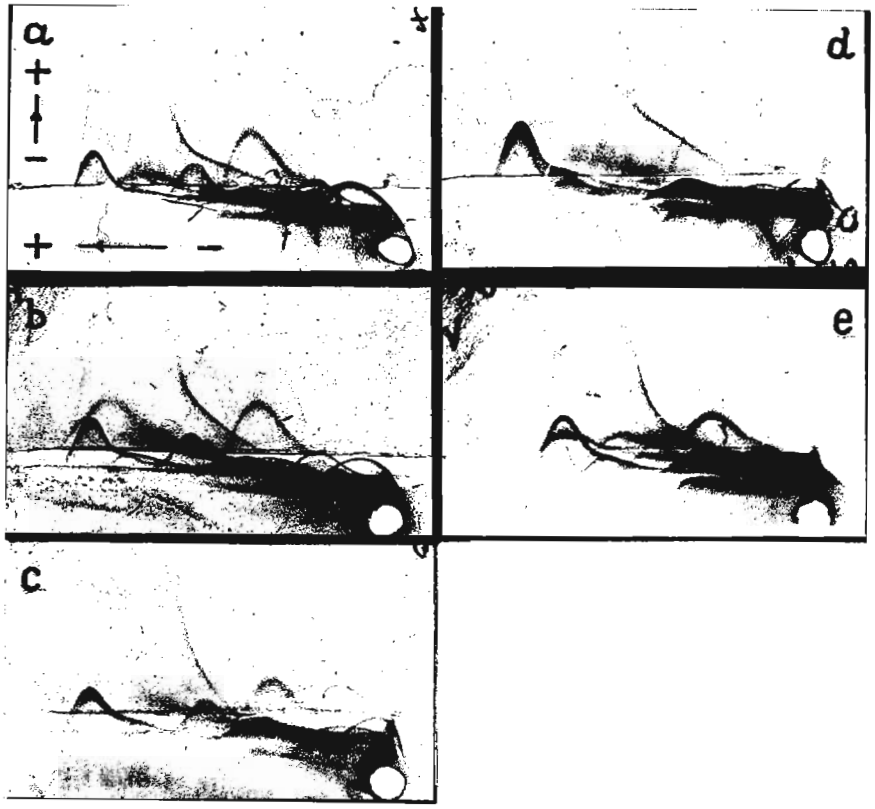


Fig. 2. Crossed immunoelectropherograms of aqueous protein extracts from brain-ventral ganglion complex of D. melanogaster larvae: (a) 72; (b) 84; (c) 96; (d) 108; (e) 120 hrs after egg laying; 1% agarose gels, Coomassie staining.

[Karakin & Lerner]

Kearney, J.N. University of Leeds, England. Intraspecific competition amongst the larvae of D. subobscura feeding on Sorbus fruits.

D. subobscura is the dominant woodland Drosophila around Leeds, in the latter half of the year. Its population density, however, increases markedly in November - the "autumn flush" (Basden 1953) - which coincides with a peak of emergence from one particular fruit, rowan (Sorbus aucuparia L.). A laboratory experiment was carried out to determine what levels of larval crowding within individual berries would result in intraspecific competition; and to assess whether this degree of crowding is likely to occur in the field.

A sample of rowanberries, all of a similar size, were removed directly from the tree. They were allowed to rot/ferment in a jar for seven days, during which time they were periodically stirred. Each berry was then removed with sterile forceps, a small cut was made in the pericarp with a sterile scalpel, and a number of freshly hatched sterile D. subobscura larvae (Sang 1956) were placed into the slit. The berry was placed onto moist peat in a cotton-plugged 3" x 1" tube, the whole of which had been previously autoclaved. The plugged end was covered with aluminum foil to prevent evaporation. All tubes were maintained in an incubator at 18°C ± 0.5° and with constant illumination. The wing length of emerging adults and the percentage of the larvae reaching the adult stage were determined in each case and used as indices of competition (Fig. 1).

Despite the large confidence limits, general trends could be observed. In both sexes, there was little or no change in wing length until a density of five larvae per berry was reached. With respect to survivorship there was probably no real major change until a density of eight larvae per berry was used.

A sample of 200 berries was collected from beneath Sorbus trees in late October. They were individually dissected and the number of larvae were counted. Although most berries contained no larvae, none of them contained more than three larvae. On the basis of these

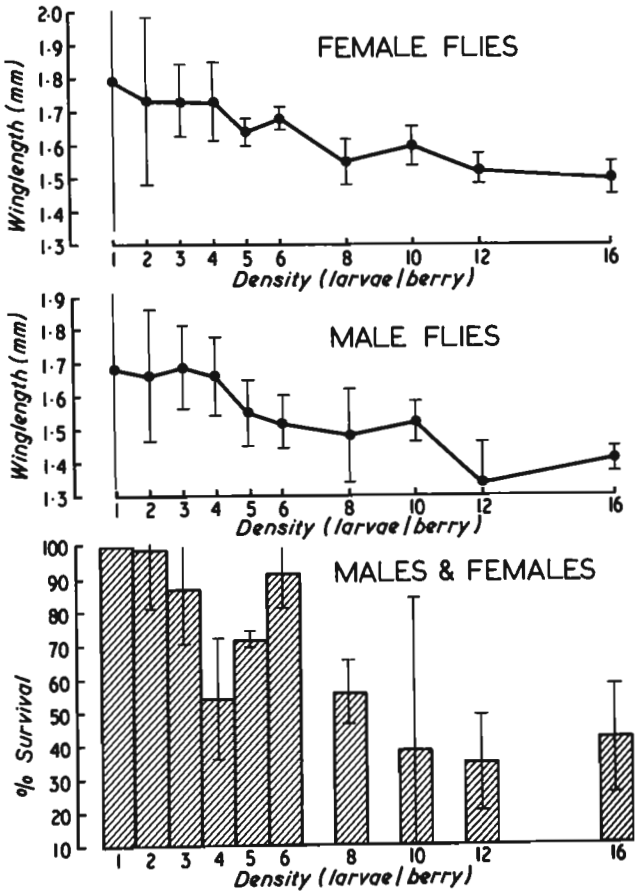


Fig. 1.

preliminary findings it is suggested that intraspecific competition amongst the larvae of D. subobscura feeding on Sorbus fruits is not likely to be important in this particular instance.

References: Basden, E.B. 1953, Nature 172:1153; Sang, J.H. 1956, J. Exp. Biol. 33:45-72.

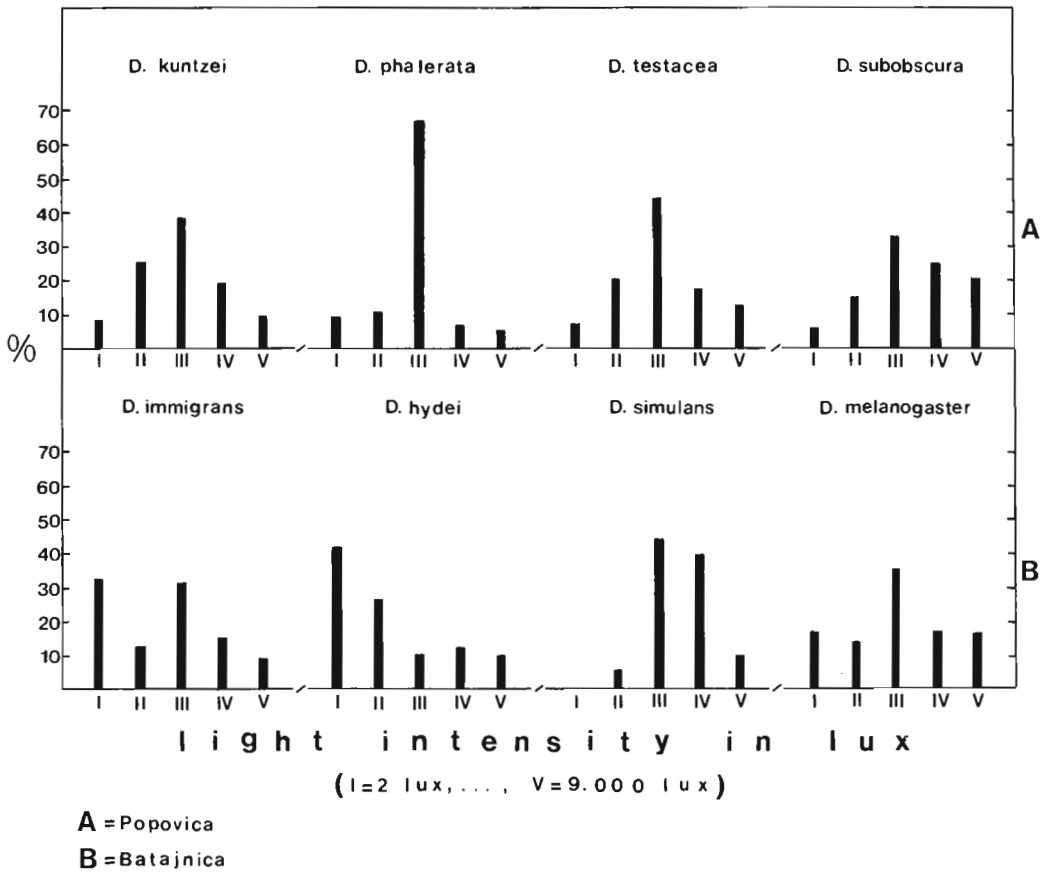
Kekić, V. Institute of Zoology, Faculty of Science, University of Belgrade, Bel-Grade, Yugoslavia. Phototactic behavior of Drosophila spp. under laboratory conditions.

Using a maze (Kekić 1981) which enables the subject fly to choose one out of five different light intensities (in this experiment: 2, 200, 900, 4000 or 9000 lux), we registered the distribution of individuals from eight Drosophila species, collected in the neighborhood of Belgrade at two localities: Popovica and Batajnica (60 km away from one another). All the flies were tested under virtually identical conditions: F_1 flies of the same age (6 days) were used, raised on a corn medium at 19°C and RH 60% in mass culture, but without competition. Before testing, the flies spend 24 hours in the dark. All manipulations with the flies prior to testing were done using an aspirator, i.e., avoiding anesthetizing of flies. Males and females of the same species were tested together, but different species were tested separately. The tests were made in a climate-controlled room at 20°C and RH 60%. All other important factors of the procedure were the same as those described in our previous paper (Kekić 1981).

This tedious description of experimental procedure, as all students of behavior know well, is unfortunately necessary. In this case small changes of the above mentioned "determinants" of phototactic behavior under laboratory conditions also may considerably influence the "free choice" of flies.

Concerning the results (shown in Fig. 1, following page) it is important to notice that the differences between populations within one species may, sometimes, be greater than the difference between species captured at the same time in the same geographic locality (see Kekić and Marinković 1979).

Fig. 1.



References: Kekić, V. 1981, DIS 56:178-179; Kekić, V. and D. Marinković 1979, Aquilo Ser. Zool. 20:118-128.

King, J. and J. McDonald. University of Georgia, Athens, Georgia. Genetic localization of a trans-acting modifier gene in D. melanogaster+.

There have been several studies reporting the existance of regulatory genes not physically linked to the structural gene they control (e.g., Rawls & Luches 1974; Scandaldos et al. 1980; Luis & Paigen 1975). It has also been shown that such modifier genes in some instances exist on different chromosomes than the structural gene they control (e.g., McDonald & Ayala 1978a). In D. melanogaster the gene coding for α-glycerophosphate dehydrogenase (E.C.1.1.1.8.) is located on chromosome 2 (2-17.8, Grell 1967): It has been shown that α-GPDH activity is modified by elements that associate with chromosome 3 (Laurie-Alberg et al. 1980). Here we report the results of a mapping study which localizes the modifier effect to map position 3-55.1 in D. melanogaster .

Two strains of flies were used in this study. A wild-type strain (F2, $\frac{+..+..+}{+..+..+}$) made completely homozygous for the first, second and third chromosomes was derived from a single wild caught male collected in Napa County, California in September 1974 (McDonald & Ayala 1978a). A second substituted strain (F2, $\frac{ru..cu..ca}{ru..cu..ra}$) was constructed to have the same second (and thus the same α-gpdh genotype) and X-chromosome constitution as the wild-type strain but homozygous for the multiply marked third chromosome carrying roughoid (ru, 3-0.00), hairy (h, 3-26.5), thread (th, 3-43.2), curled (cu, 3-50.0), stripe (sr, 3-62.0), ebony-sooty (e^s, 3-70.7) and claret (ca, 3-100.7). Our method of substituted strain construction utilizes the balancer stock $Cy/B1L^2$ Sb Ser/e^{11} and has been fully described earlier (McDonald & Ayala 1978b). α-GPDH activity was measured on an ACTA II spectrophotometer according to McDonald & Avise (1976).

Activity is expressed as ΔOD/fly. Eacy assay homogenate consisted of 10 flies (♂♂) per 0.5 ml/Tris-HCl buffer, pH 8.6.

The relative α-GPDH activities of the strains used in our study were as follows: F2, $\frac{+...+...+}{+...+...+}$ = 1.36; F2, $\frac{ru...cu...ca}{ru...cu...ca}$ = 1.00; and F2, $\frac{ru...cu...ca}{+...+...+}$ = 1.33. In every case within-strain error (S.D./mean activity) was 5.0%. The above results demonstrate a highly significant ($p < 0.001$, based on a Students' t-test) difference in α-GPDH activity between the strain homozygous for ru...cu...ca third chromosome and the strain homozygous and heterozygous for the wild third chromosome. These data are consistent with earlier reports of an α-GPDH third chromosome regulatory effect (Laurie-Ahberg et al. 1980).

Table 1. Relative α-GPDH activities of recombinant and parental phenotypes. See text for details.

Visible phenotype	Relative α-GPDH activity
ru...cu...ca	1.00
+...+...+	1.33**
ru...h	1.30*
h...e^s	1.10
th...e^s	1.11
sr...e^s	1.19**
cu...sr	1.03
cu...+	1.21**
+...sr	1.26*

*P < 0.01;
**P < 0.001 based on a Students' t-test comparison with the ru...cu...ca control.

In order to map the location of the third chromosome gene or genes responsible for the α-GPDH regulatory effect, a backcross was made between the heterozygous (F2, $\frac{ru...cu...ca}{+....+....+}$) and the homozygous marker strain (F2, $\frac{ru...cu...ca}{ru...cu...ca}$). The resulting recombinant progeny were scored for visible phenotype and α-GPDH activity. Due to the fact that a multiple fly homogenate was needed for measuring α-GPDH activity, it was necessary to pool recombinant classes. The mapping experiment proceeded, therefore, in a stepwise fashion such that the modifier effect was first associated with a broad chromosomal region and then more precisely located to a specific position within that region.

The results of our study are presented in Table 1. By comparing the α-GPDH activity of the recombinant classes we localized the modifier effect between two markers cu and sr at 55.08 ± 0.99 (Fig. 1) (cu and sr). Details of the mapping procedure have been submitted to "Genetics" for publication.

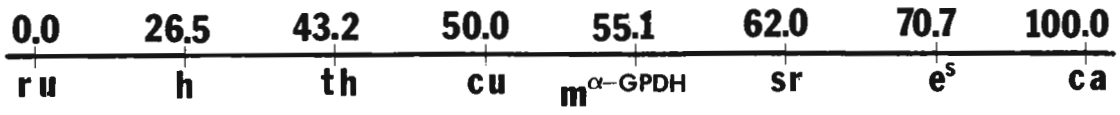


Fig. 1. The map location of an α-GPDH modifier gene on chromosome 3 in D. melanogaster.

References: Grell, E.H. 1967, Science 158:1319-1320; Laurie-Ahlberg, C.C., G. Maroni, G.C. Bewley, J.C. Luechesi and B.S. Weir 1980, Proc. Natl. Acad. Sci. USA 77:1073-1077; Luis, A.J. and K. Paigen 1975, Cell 6:371-378; McDonald, J.F. and J.C. Avise 1976, Biochemical Genetics 14:347-355; McDonald, J.F. and F.J. Ayala 1978a, Genetics 89:371-388; _____ and _____ 1978b, Can. J. Genet. Cytol. 20:159-175; Rawls, J. and J. Luchesi 1974, Genet. Res., Camb. 24:59-72; Scandalios, J.G., D.-Y. Chang, D.E. McMillin, A. Tsaftaris and R.H. Moll 1980, Proc. Natl. Acad.Sci. USA 77:5360-5364.

+This work was supported by NSF Grant #DEB 7815466 to John McDonald.

Kokoza, V.A. and E.I. Karakin. Institute of Cytology & Genetics, Siberian Branch, USSR Academy of Sciences, Novosibirsk, 630090, USSR. The investigation of some biochemical characteristics of the salivary gland secretion proteins of D. melanogaster.

Two different electrophoretic buffer and gel systems are known for the separation of the salivary gland secretion proteins of D. melanogaster (Korge 1975, 1977; Beckendorf & Kafatos 1976); we describe here the method of two-dimensional electrophoresis combining both of these two systems.

The secretion samples of D. melanogaster Oregon were prepared as described by Korge (1977); for the first electrophoretic separation of the saliva proteins we used 7% PAAG with 8M of urea in acid buffer system (Korge 1977). After the electrophoresis a strip with the separated saliva proteins was cut out from the first gel, equilibrated with SDS-containing buffer (Beckendorf & Kafatos 1976) and transferred to the top of the second 10-20% gradient SDS-PAAG. Standard protein molecular weight markers were used as references.

Fig. 1. The salivary gland secretion proteins of D. melanogaster: (a) Coomassie staining; 1, 3, 4, 5, and 6 - the number of separated proteins; the position of the spots on the two-dimensional gel are marked with arrows. (b) PAS staining of the secretion proteins. (c) Immunodiffusion test of the SP-3 and SP-4 bands (wells 3 and 4, respectively) with the antiserum against the whole salivary gland antigens (the central well).

In the first dimension (Fig. 1a) a well-known pattern of the secretory proteins (Korge 1975) was obtained. During the second separation the secretory protein 3 (SP-3) was divided, however, into the four protein spots (with the molecular weights of about 65 kD, 80 kD, 140 kD and more than 140 kD), while each of the other secretion proteins, SP-4 (m.w. 80 kD), SP-5 (m.w. 20 kD) and SP-6 (m.w. 10 kD), are represented as a single discrete spot. All of these secretory proteins except SP-5 demonstrate the presence of the carbohydrate moieties (Fig. 1b) when the secretion samples were separated in the first dimension. As to the second one, the spots with m.w. of 80 kD and 65 kD derived from the SP-3 do not demonstrate any PAS positive reaction (Fig. 1b).

To characterize the immunochemical properties of the separated saliva proteins the bands were cut out from the first gel and tested with two different antisera: I - against the salivary gland antigens, and II - against the major organ-specific antigen fraction of the secretion (Karakin et al. 1977). As a result SP-3 and SP-4 were shown to be organ-specific proteins of the salivary glands because both these fractions reacted with antiserum of type II. It was an unexpected result that SP-3 and SP-4 demonstrated immunochemical similarity (Fig. 1c) because these proteins are controlled by two nonallelic genes located on the third chromosome (for SP-3) and on the X-chromosome (for SP-4) (Korge 1975, 1977; Akam et al. 1978; Kokoza et al. this issue).

References: Akam, M.E., D.B. Roberts, G.P. Richards and M. Ashburner 1978, Cell 13:215-225; Beckendorf, S.K. and F. Kafatos 1976, Cell 9:365-373; Karakin, E.I., T.Ya. Lerner, V.A. Kokoza and S.M. Sviridov 1977, Dokl. Biol. Sci. 233(1-6):102-105; Korge, G. 1975, Proc. Natl. Acad. Sci. USA 72:4550-4554; _____ 1977, Develop. Biol. 58:339-355.

Kokoza, V.A., S.G. Kazakova and E.I. Karakin. Institute of Cytology & Genetics, Siberian Branch, USSR Academy of Sciences, Novosibirsk, 630090, USSR. The genetic localization of genes responsible for two salivary gland secretion proteins of D. melanogaster.

Some electrophoretic variants of D. melanogaster saliva proteins were mapped (Korge 1975; Akam et al. 1978; Velissariou & Ashburner 1980). We used PAAG electrophoresis (Korge 1975) with some modifications for the detection of the new variants of the secretory protein 4 and by means of recombination analysis we localized the genes responsible for the secretory proteins 4 and 3.

The secretion samples were prepared according to Korge's procedure (1977); 15% PAAG was used for the resolution of low molecular weight secretory proteins. Under these conditions the "standard" secretory proteins 2, 3, 4 and 5 were resolved as well as some additiol proteins 6a, b; 7a, b when the secretions of D. melanogaster Oregon were adjusted to the electrophoresis (Fig. 1a).

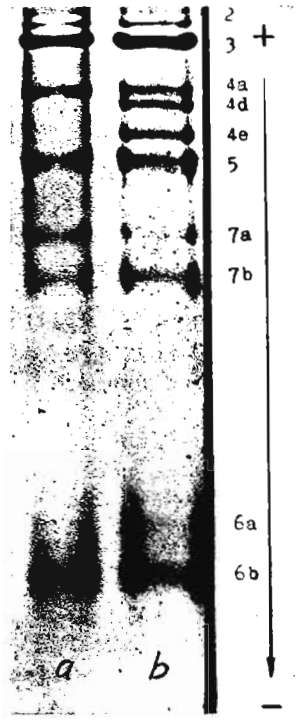


Fig. 1. Saliva proteins of some Drosophila stocks: (a) Oregon; (b) Odessa.

In natural populations of Odessa, Kishinev and Evpatoria we identified the genetic polymorphism of saliva protein 4 (Fig. 1b). All of these variants demonstrated sex-linked inheritance and one variant (protein 4e) was used for precise localization of Sgs-4 locus on the X-chromosome genetic map. Sgs-4 gene was shown to be located between 3 and 5 genetic map units (Korge 1975), so for recombination analysis we used recessive mutations w^a and rb located at 1.5 and 7.5 map units, respectively (Lindsley & Grell 1968). A stock homozygous for protein 4e was obtained from Odessa population and the following crosses were done:

$$(1)\quad ♀♀ \frac{w^a\ \text{Sgs-4a rb}}{w^a\ \text{Sgs-4a rb}} \times ♂♂ \frac{+\ \text{Sgs-4e}\ +}{Y}$$

$$(2)\quad ♀♀ \frac{w^a\ \text{Sgs-4a rb}}{+\ \text{Sgs-4e}\ +} \times ♂♂ \frac{w^a\ \text{Sgs-4a rb}}{Y}$$

From the F_2 generation about 2,500 males were screened and 97 recombinant males were selected. All recombinant males were crossed to $\overline{XX}/Y$ females, and salivary gland secretions of F_3 male larvae were tested by electrophoresis.

Table 1.

Crossover I type (recombination between w^a and Sgs-4)	+ Sgs-4a rb	10	24
	w^a Sgs-4e +	14	
Crossover II type (recombination between Sgs-4 and rb)	w^a Sgs-4a +	39	73
	+ Sgs-4e rb	34	
Total number of recombinants			97

The results of this analysis (Table 1) show that Sgs-4 gene is located at a position 24/97ths of the genetic distance between w^a and rb; taking the map distance as 6 units, the position of Sgs-4 is 3.0 ±.

In a previous paper (Korge 1975) it was reported that the gene Sgs-3 responsible for the major secretory protein 3 is located between hairy and thread on the genetic map of the third chromosome. We also investigated this locus to determine the position of the Sgs-3 gene on the genetic map more precisely. Formosa stock with fast migrating variant 3d (Fig. 2b) and a mutant stock, se ri with 3a variant (Fig. 2a), were used for this aim. Formosa wild-type females were crossed to se ri males and then heterozygous se ri/+ females were crossed to se ri e males. From their progeny 100 recombinant males were selected and were individually crossed to homozygous se ri e females. Two classes of F_3 larvae can be readily distinguished: those that are se ri e homozygotes and have darkened color of spiracle sheaths due to homozygosity for e (Lindsley & Grell 1968), and those that are heterozygous for e; these individuals carry one "recombinant" and one se ri e chromosome. The secretory proteins of this last class of larvae were analyzed. These results are summarized in Table 2, and show the Sgs-3

a b

Fig. 2. Saliva proteins of two Drosophila stocks: (a) mutant se ri; (b) Formosa.

gene to be located at a position 43/100th of the genetic distance between se and ri; taking the distance as 21 map units (Lindsley & Grell 1968), the Sgs-3 gene is located at 35.0 ± position of the genetic map of the third chromosome.

Table 2.

Crossover I type (recombination between se and Sgs-3)	se Sgs-3d +	17	43
	+ Sgs-3a ri	26	
Crossover II type (recombination between Sgs-3 and ri)	se Sgs-3a +	34	57
	+ Sgs-3d ri	23	
Total number of recombinants			100

References: Akam, M.E., D.B. Roberts, G.P. Richards and M. Ashburner 1978, Cell 13:215-225; Korge, G. 1975, Proc. Natl. Acad. Sci. USA 72:4550-4554; _____ 1977, Develop. Biol. 58:339-355; Lindsley, D. and E. Grell 1968, Carn. Inst. Wash. Publ.; Velissariou, V. and M. Ashburner 1980, Chromosoma (Berl.) 77: 13-27.

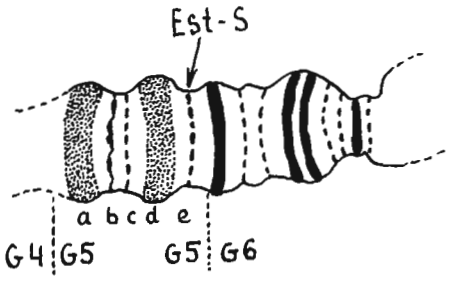
Korochkin, L.I. and M.B. Evgeniev. Institute of Developmental Biology, Institute of Molecular Biology, Moscow, USSR.

Analysis of a gene coding for organo-specific esterase in D. virilis.

1. It was observed that the structural genes coding for isozymes of esterase in Drosophila of the virilis group were located on the second chromosome.

2. It was shown by the analysis of hybrids D. virilis x D. lummei (with the inversion on the second chromosome, which was marked by the dominant mutation Shorty) that esterase genes were located proximally concerning the inversion 2F8 Griffin cytogenetic map.

3. We used the insertion of segments of the second chromosome of D. lummei into the second chromosome of D. virilis to find the position of esterase genes on the cytogenetic map. The existence of two clusters of esterase genes--α-esterases 2G1-2G2, β-esterases proximally concerning 2G4--can be seen as a result of these experiments.



4. The heterochromatic insertion into the zone G4-G5 decreases the activity of organospecific esterase in the ejaculatory bulb of D. virilis.

5. On the basis of the different genetic and cytogenetic data we conclude that the gene Est-S coding for the organospecific esterase is located on the region G5e (see illustration).

6. Recently G. Enikolopov et al. (in press) isolated DNA sequences containing the structural genes of S-esterase. It was shown by the method of DNA-DNA hybridization in situ that this DNA was hybridized on the region G5e.

References: Korochkin, L. 1980, Genetic regulation of isozyme patterns in Drosophila during development, in Isozymes, vol. 4, Alan Liss., Inc., New York, pp. 159-202.

Krimbas, C. and M. Loukas. Agricultural College of Athens, Greece. Additions to the Drosophila species in Greece.

Drosophila helvetica has been recorded for the first time in Greece in a sample from Karpenissi. It should also be noted that D. cameraria (alias D. pallida) was known in Greece from Krimbas (1963) and thus in this respect is not a new species for Greece as in Triantaphyllidis and Tsacas (1981). Thus until now 22 species are recorded from Greece (Krimbas 1963, 1964; Tsacas 1963; Triantaphyllidis & Tsacas 1981).

References: Krimbas, C. 1963, DIS 37:95; ____ 1964, DIS 38:76; Triantaphyllidis, C.D. and L. Tsacas 1981 (in press); Tsacas, L. 1963, DIS 37:135-136.

Krimbas, C. and M. Loukas. Agricultural College of Athens, Greece. Obscura group Drosophilas from USSR.

Two strains of obscura group species of Drosophila were sent to us by Dr. Golubovsky who collected them in USSR. Identification of species was performed by crosses. One strain from Vitebsk proved to be D. obscura; the other from Alma Ata, D. ambigua. They differ slightly morphologically from other European strains of the same species. Thus measurements of wing indices were found to be as shown in the table.

	D. obscura (Vitebsk)	values reported by Burla
costal index	2.5 - 3.2	2.7 - 3.1
4 v index	1.8 - 2.3	1.9 - 2.1
4 c index	0.8 - 1.1	0.9 - 1.0
5 c index	1.5 - 2.0	1.3 - 1.7
	D. ambigua (Alma Ata)	**values reported by Burla**
costal index	2.7 - 3.2	2.8 - 3.0
4 v index	1.8 - 2.0	1.8 - 1.9
4 c index	0.8 - 0.9	0.9
5 c index	1.5 - 2.2	1.3 - 1.5

References: Burla, H. 1951, Revue Suisse de Zoologie 58(2):23-175.

Kuhn, D.T. and D.F. Woods. University of Central Florida, Orlando, and Thimann Laboratories, University of California, Santa Cruz. Location of tuh-3 in D. melanogaster.

Table 1 details the results of two experiments designed to map the tuh-3 gene. In 1a, 129 crossovers within a 12 map unit region between cu (3-50) and sr (3-62) were recovered from cu tuh-3^+ sr/ cu^+ tuh-3 sr^+ females. Ninety-four crossovers occurred left of tuh-3 (cu tuh-3 sr^+) and 35 to the right (cu tuh-3^+ sr^+). Progeny from the cultures found to possess tuh-3 (94 cultures) showed an average of 61.0% penetrance of the tumorous-head trait, while progeny from the other 35 cultures were all normal and lacked the tumorous-head trait. Of the 94 cultures judged to carry tuh-3 by examination for the tumorous-head phenotype, 88 were tested for the sac-testis trait which is a second abnormality attributed to tuh-3. Males from each strain showed sac-testes (28.8% penetrance), thereby substantiating the presence of tuh-3. Thirty-three of the 35 cultures lacking tuh-3 were tested for the sac-testis trait. None of the 33 showed the sac-testis trait (0.3% penetrance); therefore, the tuh-3^+ gene must have been present, placing tuh-3 at 50+(94/129x12) = 58.7+.

Table 1b presents data demonstrating that the tuh-3 gene is not located between sbd (3-58.2) and bx (3-58.8). From a stock containing sbd^2 bx^3 pbx/tuh-3 females, 20 males were isolated that were sbd^2 but lacked bx^3. Therefore, some of these males could possibly carry sbd^2 tuh-3 and others sbd^2 tuh-3^+ if tuh-3 was between sbd and bx. Results from testing the 20 crossover types demonstrated that they were sbd^2 tuh-3. All possessed tuh-3 as indicated by 88.5% penetrance of head abnormalities and 46.7% penetrance of sac-testes. These results are not in agreement with Bournias-Varidiabasis and Bownes (1978) who mapped by the identical technique tuh-3 to 3-58.5. Kuhn et al. (unpublished) found tuh-3 located to the right of pbx and left of fl.

An attempt to map tuh-3 in aneuploids using the tumorous-head effect as a criterion is presented in Table 2 (following page). Analysis of the experimental results shows that tuh-3 cannot be localized by this technique. Knowing that tuh-3 must be to the right of pbx and

Table 1. Results of genetic mapping tuh-3 by (a) crossovers between curled (3-50.0) and stripe (3-62.0), (b) crossovers between stubbloid (3-58.2) and bithorax (3-58.8).

	Tumorous-head trait			Sac Testis trait		
	# c/o tested	N	% with trait	# c/o tested	N	% with trait
(a) c/o between cu-sr						
cu tuh-3	94	9,649	61.0	88	6,529	28.8
cu tuh-3^+	35	4,085	0.0	33	3,692	0.3
total	129	12,877		121	10,221	
	maps to 3-58.7+					
(b) c/o between sbd^2-bx^3						
sbd^2 tuh-3	20	1,486	88.5	20	772	46.7
sbd^2 tuh-3^+	0	0	0.0	0	0	0.0
total	20			20		

Table 2. Comparative frequencies of the tumorous-head trait in flies deficient for various regions of chromosome 3R and their non-deficient sibs.

	Matings		tuh-3/tuh-3^+		tuh-3/Df		
Males	(x)	Females	Number eclosing	% with trait	Number eclosing	% with trait	Sex
Df-sbd^{105}/rucuca		tuh (UCF)	646	32.9	727	50.2	♂ + ♀
Df-sbd^{105}/rucues		tuh (UCF)	420	20.0	399	27.1	♂ + ♀
Df-bxd^{100}/TM1		v, tuh-1/Y; 2; 3	240	71.2	215	56.3	♀
Df-bxd^{100}/Canton-S		v, tuh-1/Y; 2; 3	258	64.0	213	54.5	♀
Df-P2/T(2;3)ap^{Xa}		tuh (UCF)	699	33.3	750	21.7	♂ + ♀
Df-P10/TM1		$tuh\text{-}1^h$; sbd^2 tuh-3	319	27.6	279	17.6	♂ + ♀
Df-Ubx^{109}/Xa		tuh (UCF)	644	26.7	679	37.4	♂ + ♀
Df-P9/Dp-P5		tuh (UCF)	754	16.5	518	9.1	♂ + ♀
Dp-bxd^{100}-Df-P115/TM1		v, tuh-1/Y; 2; 3	737	84.8	126	61.9	♀
Df-P115/TM1		v, tuh-1/Y; 2; 3	657	77.5	106	42.5	♀
Df-P115/Canton-S		v, tuh-1/Y; 2; 3	718	68.7	49	65.3	♀
Df-P115/TM1		tuh (UCF)	603	28.3	210	23.8	♂
Df-C4/Dp-P5		tuh (UCF)	1048	24.0	115	9.6	♂ + ♀
Df-bxd^{111}/TM1		tuh (UCF)	708	26.6	59	28.8	♂
			tuh-3/tuh-3				
tuh (UCF)		tuh (UCF)	564	75.8			♂ + ♀
tuh (UCF)		v, tuh-1/Y; 2; 3	1446	86.2			♂ + ♀

left of fl (Kuhn et al. unpublished) the ten deletion stocks were grouped into those definitely not uncovering tuh-3 (Df-sbd^{105}, Df-bxd^{100}), those that may uncover tuh-3 (Df-P2, Df-P10, Df-Ubx^{109}, Df-P9, Dp-bxd^{100}-Df-P115, Df-P115, Df-C4), and one that definitely must cover tuh-3 (Df-bxd^{111}). Df-bxd^{111} uncovers both pbx and fl which flank tuh-3. All deletions were maintained in the heterozygous condition, so when crossed with $tuh\text{-}1^h$; tuh-3 ($tuh\text{-}1^h$ with tuh-3 produces the tumorous-head trait) females the offspring were of two types: those carrying tuh-3 and the deletion chromosome (which may or may not carry a tuh-3^+ locus) and those that were heterozygous tuh-3/tuh-3^+.

We originally hoped to see a significantly higher penetrance of the tumorous-head phenotype when tuh-3 was uncovered in aneuploids (tuh-3/Df) than in their heterozygous sibs (tuh-3/tuh-3^+). This expectation was met only when Df-sbd^{105}, Df-Ubx^{109}, and Df-bxd^{111} were tested, in spite of the fact that Df-sbd^{105} cannot possibly uncover tuh-3. Df-bxd^{111} undoubtedly uncovered tuh-3 yet penetrance was insignificantly higher in the Df-bxd^{111}/tuh-3 flies (28.8%)

than for their heterozygous sibs (26.6%). The fact that Df-Ubx^{109}/tuh-3 flies have a higher penetrance of the tumorous-head trait (37.4%) than their sibs (26.7%) could suggest tuh-3 was uncovered. However, if Df-Ubx^{109} uncovered tuh-3 so must have Df-P9, Df-P115, and Df-C4 which were all more extensive deletions. The data show penetrance of the tumorous-head trait lower in Df-P9/tuh-3 flies (9.1%) than in their Dp-P5/tuh-3 sibs (16.5%). It was again lower in all three crosses involving Df-P115 (42.5%, 65.3%, 23.8%) as compared to their respective sibs (77.5%, 68.7%, 28.34%), and penetrance of Df-C4/tuh-3 (9.6%) is lower than in their sibs Dp-P5/tuh-3 (24.0%).

As a matter of fact all remaining deletions tested showed the same trend; Df/tuh-3 flies consistently had lower penetrance for the tumorous-head trait than did their tuh-3^{+}/tuh-3 sibs. Df-bxd^{100} does not uncover tuh-3, yet in both tests the Df-bxd^{100}/tuh-3 flies had fewer head abnormalities (56.3%, 54.5% respectively) than did their sibs TM1/tuh-3 and Canton-S/tuh-3 (71.2%, 64.0% respectively). Limits of influence of Df-P2 and Df-P10 probably do not include the tuh-3 locus, yet the characteristic suppression of the tumorous-head phenotype remained present. Df-P2/tuh-3 flies showed 21.7% penetrance while their tuh-3^{+}/tuh-3 sibs showed 33.3% penetrance. In Df-P10/tuh-3 flies, penetrance was 17.6% with penetrance in their tuh-3^{+}/tuh-3 sibs 27.6%. The effects of Dp-bxd^{100}-Df-P115 may or may not extend into the region expected to contain the tuh-3 locus. Here Dp-bxd^{100}-Df-P115/tuh-3 flies possessed lower penetrance (61.9%) than their tuh-3^{+}/tuh-3 sibs (84.8%).

An indication of the complexity of the tumorous-head system can be seen when one compares penetrance between the crosses. When deletion-bearing males were mated with tuh(UCF) females, penetrance was always lower than if the females were v tuh-1^{h};Y 2; 3. Both the tuh(UCF) and v tuh-1^{h} X-chromosomes carry the tumorous-head maternal effect gene. However, v tuh-1^{h} is more efficient at promoting greater maternal effect activity as additionally confirmed when tuh(UCF) males were mated to tuh(UCF) females (75.8% penetrance) compared to those mated to v tuh-1^{h}; Y; 2; 3 females (86.2% penetrance). Using the sac-testis maternal effect as criterion for tuh-3, the gene deletion mapped to 89E4,5 (Kuhn et al., Genetics 99:99-107, 1981).

Research supported by NSF Grant PCM77-13966 and NIH Grant AG01846.

References: Bournias-Vardiabasis, N. and M. Bownes 1978, DIS 53:190.

Kurokawa, H., Y. Oguma and N. Tachibana. University of Tsukuba Sakura-mura, Ibaraki, Japan. Sexual isolation among four species of D. auraria complex.

Four sibling species of D. auraria complex can be experimentally crossed and produce offspring. Among these, three species of D. auraria, D. biauraria and D. triauraria are widely distributed in Japan and Korea, while D. quadraria is recorded only from Taiwan. The sexual isolation experiments between these members were carried out by using male multiple choice technique. As shown in Table 1, the isolation coefficients represented between different species were highly significant. Intense sexual isolation was one-sidedly demonstrated in most cases, particularly in the crosses between D. auraria and D. quadraria, and between D. triauraria and D. quadraria. The isolating conditions among the three species of D. auraria, D. biauraria and D. triauraria were similar to those reported previously by Kurokawa (1960), excepting the cross between D. auraria and D. triauraria, which represented a higher value than in the previous report. A relationship among the four siblings with respect to sexual isolation is provisionally re-

Table 1. Sexual isolation among four sibling species of D. auraria complex.

Cross			Homogamic		Heterogamic		Homogamic	Heterogamic	Coefficients
Type	Female	Male	(+)	(-)	(+)	(-)	%(+)	%(+)	of isolation
A x B	A541 + B16	A541	119	50	27	143	70.4	15.9	0.751
		B 16	50	50	0	100	50.0	0.0	1.000
	A542 + B3	A542	81	18	11	88	81.8	11.1	0.871
		B 3	72	16	2	85	81.8	2.3	0.973
A x T	A541 + T544	A541	74	26	22	78	74.0	22.0	0.689
		T544	55	45	3	97	55.0	3.0	0.927
	A542 + T540	A542	81	18	5	94	81.8	5.1	0.940
		T540	99	25	6	123	79.8	4.7	0.942
A x Q	A541 + Q	A541	124	33	110	42	79.0	72.4	0.096
		Q	137	21	7	153	86.7	4.4	0.956
	A542 + Q	A542	102	8	65	43	92.7	60.2	0.479
		Q	119	11	4	124	91.5	3.1	0.975
B x T	B16 + T544	B 16	84	33	2	118	71.8	1.7	0.973
		T544	59	47	8	101	55.7	7.3	0.830
	B3 + T540	B 3	56	13	0	69	81.2	0.0	1.000
		T540	83	36	10	109	69.7	8.4	0.863
B x Q	B16 + Q	B 16	92	58	16	133	61.3	10.7	0.787
		Q	141	6	27	120	95.9	18.4	0.880
	B3 + Q	B 3	58	18	22	58	76.3	27.5	0.635
		Q	107	10	25	94	91.5	21.0	0.825
T x Q	T544 + Q	T544	86	73	126	33	54.1	79.2	-0.337
		Q	135	13	74	76	91.2	49.3	0.567
	T540 + Q	T540	80	37	101	13	68.4	88.6	-0.307
		Q	143	25	75	94	85.1	44.4	0.529

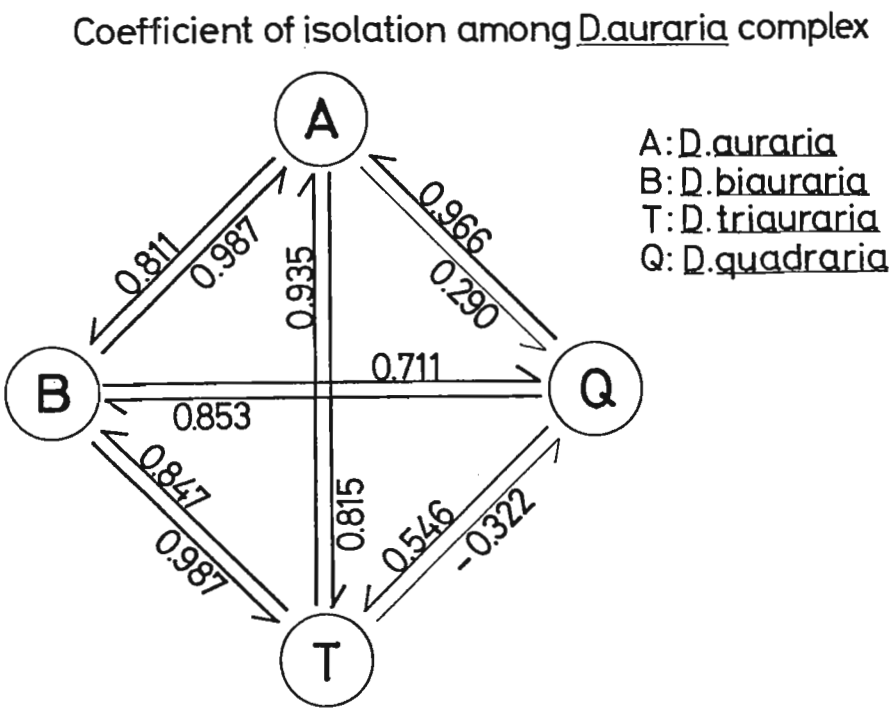


Fig. 1. Relationship in respect to isolation coefficients among four sibling species of D. auraria complex.

presented in Figure 1, where each arrow being directed from male to female flies mated means an intensity of the isolation between the two. The figures put along the arrows refer to the average coefficients of the joint isolation. This interspecific relationship is in accordance with that represented by a morphological study on structural asynapses found in polytene chromosomes of the hybrid F_1. It is explicable in this complex that the sexual isolation proper has enough of a role in order to maintain discrete gene pools as species.

Reference: Kurokawa, H. 1960, Japan. J. Genet. 35:161-166.

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The right breakpoint of In(1)sc^{V2} subdivides the ribosomal DNA.

In(1)sc^{V2} was described by Valencia and Muller (DIS 21:70) as having breakpoints identical to those of In(1)sc^{8} (with an Xh breakpoint proximal to the bobbed locus and near the centromere). Recent observations, however, do not accord with that determination. Both In(1)sc^{4L}sc^{V2R} and In(1)sc^{V2L}sc^{8R} (recovered as reciprocal recombinants between In(1)sc^{V2} and In(1)sc^{4L}sc^{8R}) were shown to survive normally in combination with we bb^{1}; in addition In(1)sc^{4L}sc^{V2R} was observed to survive normally in XO males; In(1)sc^{V2L}sc^{8R}/O was not tested. These results indicate that In(1)sc^{V2} carries ribosomal cistrons at both ends and suggest that the right breakpoint is within the region containing the ribosomal DNA. Accordingly the distribution of ribosomal DNA sequences in In(1)sc^{V2} was assessed by means of in situ hybridization. ^{3}HcRNA was prepared from pDm238, as plasmid containing 18S and 28S ribosomal DNA but not intron sequences (obtained from D. Glover). This was hybridized to mitotic chromosome preparations of In(1)sc^{V2}/+ females (+ = Canberra wild type). The numbers of silver grains over the two ends of In(1)sc^{V2} and over the + chromosome were determined in 12 nuclei. The mean numbers of grains over In(1)sc^{V2} and + were 7.1 and 7.3, respectively. The grains on In(1)sc^{V2} fall into two groups of approximately equal size (Fig. 1, following page); there is a tendency for one group to be slightly larger than the other and in several mitotic anaphase preparations the larger block of grains was associated with the non-centromeric end of the chromosome (i.e., the end nearer the former mitotic plate). [If the In(1)sc^{V2} grain counts are partitioned into a small and a larger group, the mean grain counts in each are 2.8 and 4.3, respectively.] Thus there is not duplication of ribosomal cistrons within In(1)sc^{V2}, and the right inversion breakpoint is almost in the middle of the region containing the ribosomal DNA sequences. Survival of In(1)sc^{4L}sc^{V2R}/O also shows that the proximal portion of the ribosomal DNA sequences is sufficient to support normal viability. Cytological examination of somatic chromosomes indicates that the proximal breakpoint of In(1)sc^{V2} dimidiates Xh.

Fig. 1. Mitotic metaphase chromosome preparation from $In(1)sc^{V2}/+$ females after in situ hybridization with ^{3}H-cRNA synthesized from the plasmid pDm238. The arrow indicates the $In(1)sc^{V2}$ chromosome.

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Altitudinal variations of Afro-tropical D. melanogaster populations.

Adaptation of natural populations to local environmental conditions is a most important problem of ecological genetics. In D. melanogaster, it was previously shown (David et al. 1977) that latitudinal genetic clines between Europe and tropical Africa can be evidenced by biometrical analysis of variou morphological traits measured on laboratory reared flies. However, this previous study included only populations living at low altitude.

In Europe, D. melanogaster can be found in mountains during summer but it is likely that altitudinal habitats are recolonized each year by adults migrating from the valleys (Kimura et al. 1978). In tropical countries, permanent populations can be found in mountains at altitudes over 2000 m.

Comparing Drosophila living at different altitudes offers a good opportunity to look for a genetic differentiation and adaptation among these populations. In this study we compare 12 strains collected in Cameroons at different altitudes and studied in the laboratory a few months after their capture. These strains were structured in three groups: low altitude (180 m., 2 strains); middle altitude (average 1000 m., 5 strains); high altitude (2000 m., 5 strains). These Cameronian strains were compared to a fourth group of three strains available from East Africa, collected at altitudes varying from 1000 to 1500 m. For each strain, samples of 30 males and females, reared at 25°C, were studied; measured traits were: fresh weight, wing length and breadth, thorax length, number of abdominal and sternopleural chaetae. Data were also obtained upon more numerous individuals, for the duration of development and percent of preadult mortality. Statistical samples are constituted by population average data.

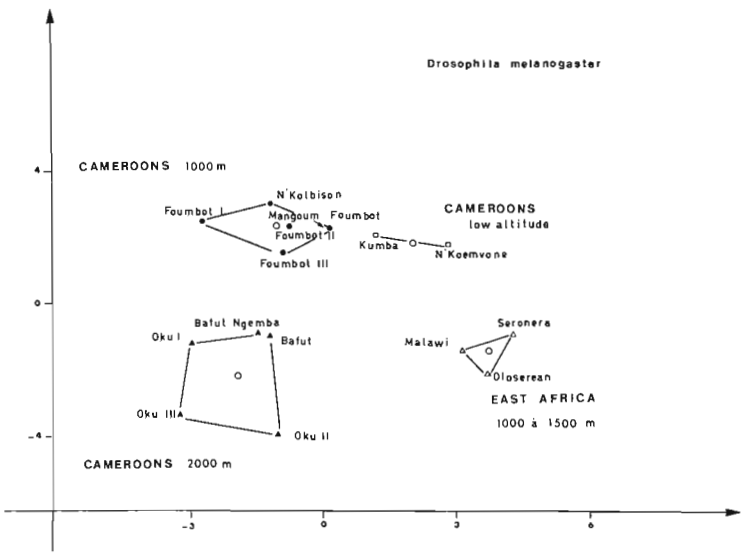


Fig. 1. Cameronian D. m. populations (males) projected on the first two canonical axes (z_1 horizontal) origined at the diagram center, compared to the East African strains.

In this study, we tried to maximize the discrimination between the

the four groups using Canonical Discriminant Analysis (BMDP7M Program, Dixon 1977). Calculations (Louis & Lefevre 1971) made separately upon males and females provided similar discrimination: this could be expected since within a strain, the values of the two sexes are correlated (David 1979). We therefore present only the analysis concerning the male measurements (except for preadult mortality which concerns both sexes).

The results are shown in Figure 1. Values of each strain are plotted on the first two factorial canonical axes whose eigenvalues express respectively 48% and 40% of total dispersion. The third axis (12%), now shown, would mainly help to discriminate between low and middle altitude groups from Cameroons.

Table 1. Central point original coordinates of the four groups of the figure (hollow circles).

	Cameroons			East Africa
Characters	low altitude (N=2)	1000 m (N=5)	2000 m (N=5)	1500 m (N=3)
Weight (mgx 10^{-2})	76.7	78.5	85.3	88.2
Abdominal chaetae	30.1	33.2	33.5	34.1
Sternopleural chaetae	16.3	13.5	14.7	17.4
Thorax length (mm x 10^{-2})	88.2	88.9	91.4	91.9
Wing length (id.)	174.8	182.5	193.4	181.5
Wing breadth (id.)	82.8	87.6	92.2	87.9
Development time (hours)	213.4	209.1	207.4	211.4
Pre-imaginal mortality (%)	34.1	31.5	24.8	18.8

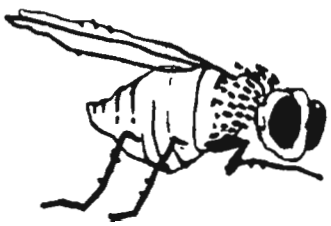
The average values of the eight measured characters in the four groups are given in the Table. The most discriminative variables provided by the stepwise discriminant analysis are wing length, wing breadth and mortality. We could suspect that some populations were misclassified because of the previous grouping of the strains according to their origin. This objection was ruled out by a further calculation of MAHALANOBIS distance (D^2) which shows that all wintin-group distances were lower than the between-group ones. The figure shows that a linear combination of eight characters allows a good discrimination of the populations here studied. The laboratory techniques allow the conclusion that the observed separation reflects a genetic divergence between local populations (David 1979).

The separation of the three Cameronian groups suggests, but does not demonstrate, that environmental variations correlated with altitude play a direct role in the genetic variations here observed. Other genetic differences related to altitude were also observed in Mexico by Pipkin et al. (1973). That altitude alone is not the only environmental selective factor is demonstrated, in this study, by the position of the East African group: although collected at middle altitude, it is highly separated from corresponding Cameronian group; some kind of longitudinal genetic divergence is also likely to occur in tropical African populations.

Concerning the method used, the results suggest that in order to compare neighboring populations, it would be of interest to include within-population variability in Discriminant Analysis treated data.

It is now well established that natural populations of D. melanogaster show a lot of genetic divergences when populations living in different continents are compared. This study shows that a significant differentiation can also be observed over much shorter distances and also that altitude must be considered as an environmental selective parameter.

References: David, J. 1979, Aquilo, Ser. Zool. 20:49-61; David, J., Ch. Bocquet, and M. De Sheemaeker-Louis 1977, Genet. Res. 30:247-255; Dixon, W.J. 1977, BMDP Programs, Univ. Calif. Press, Berkeley, Los Angeles, London, 880 pp.; Kimura, M.T. 1978, Kontyu Tokyo 46:585-595; Louis, J. and J. Lefevre 1971, Biom. Prax. 12:1-40; Pipkin, S.B., C. Rhodes and N. Williams 1973, Journ. of Heredity 64:181-185.



Lumme, J. and P. Lankinen. University of Oulu, Finland. Free male crossing over in D. littoralis: a new tool for studies on meiosis.

Free male crossing over in D. littoralis Meigen was discovered recently (Lumme & Lankinen 1981). Some additional information has been gathered since the first brief report. The free male crossing over has now been ascertained in several crosses involving many stocks from various geographical areas. Our first report on this topic described results obtained with material which contained one visible mutant and four enzyme markers. In the following list, we describe some additional visible mutants and enzyme markers now available in this species. We want to emphasize that any resemblance of names of the visible mutants to the names of melanogaster mutants is purely coincidental. On the other hand, the enzyme designations are thought to represent homology. The identification of chromosomes is based on enzyme genes (Lumme 1981).

First chromosome: cos: costata. Spontaneous, phenotype resembles Chymomyza costata in that the fly keeps the wings upwards and shakes them. This has nothing to do with courtship behavior. v: vermilion. Two spontaneous alleles from different stocks have been isolated. Homologous with v of D. virilis (non-complementary in species cross). sw: short wings. Spontaneous, wings hardly cover the abdomen. dia: diapause. A gene locus which is variable in wild populations, has an influence on the expression of the photoperiodic adult diapause (Lumme 1981).

In addition to these loci, it is known that the enzyme loci Fu (Fumarase), Hk (Hexokinase) and 6-Pgdh (6-Phosphogluconate deh.) are located in the X chromosome. No electrophoretic variants have been isolated, but they were localized in species crosses with D. virilis and/or D. kanekoi.

Second chromosome: Acph-1: Acid phosphatase-1. Two allozymes have been isolated. Lap: Leucine aminopeptidase. At least two electrophoretic forms have been isolated. Me: malic enzyme (NADP-dependent malate dehydrogenase). Two variants isolated in laboratory stocks. Est-4: α-Esterase-4. Different forms exist in our laboratory stocks. cu: curled wings. Spontaneous, similar to cu in melanogaster. Recessive suppressor of diapause (Lumme 1981). dt: detached. Spontaneous, longitudinal veins fail to reach the wing margin. Penetrance incomplete, especially with cu.

Fused chromosome 3-4: Mdh: Malate dehydrogenase (soluble). Stocks with different allozymes available. Pgm: Phosphoglucomutase. As above. dh: dahlia. Spontaneous, dilutes the eye color of v, cn and st to yellowish, but difficult or impossible to see alone. re: red. Spontaneous, eye color red instead of the dark brown of the wild type. Has no influence on v, cn or st, i.e., impossible to see with them. cn: cinnabar. Spontaneous, vermilion-like eye color; two alleles have been isolated. ev: extra veins. Spontaneous, posterior crossvein branched, occasionally blistered. ar: arrow. Spontaneous, wing shape resembles that of the imaginary creature (the male) drawn in Fig. 1, p. 129 in Ehrman (1978). Cdl: Critical daylength for photoperiodic diapause. The locus responsible for quantitative genetic variation, which forms a latitudinal cline (Lumme 1981).

Autosomal markers, the chromosomal location of which is not yet known: st: scarlet. Spontaneous, vermilion-like eye color. May be in 5, because it is not linked with any of the markers above. cst: coastal. Spontaneous. Wings and especially legs short, preadapted to windy coasts. rl: rolled. X-ray induced (5 krad), wings curved slightly downward. ro: rough. Spontaneous, eye rough and slightly smaller than wild type, often changes in wing veins.

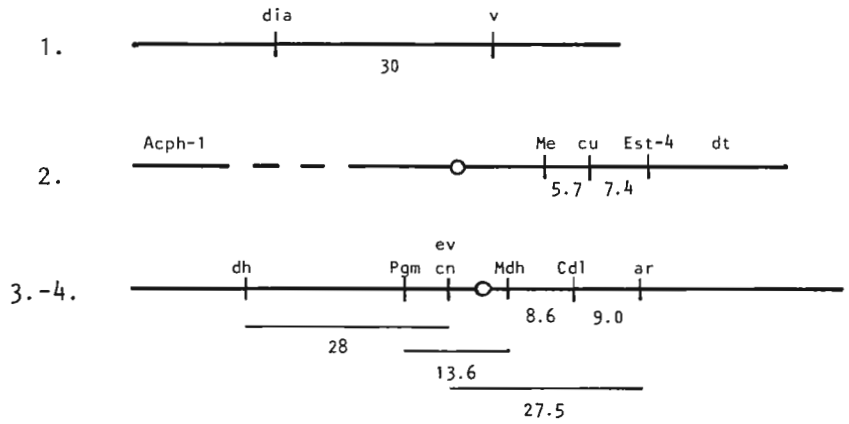


Fig. 1. Relative locations of genetic markers of D. littoralis.

The approximate linkage relations of the markers (if known) are given in Fig. 1. The data are very preliminary.

When a species is available which has male crossing over, it can be helpful in finding the answer to an important question: why is crossing over lacking in males of most Drosophila species? We now have stocks available with multiple marker systems in the second and the 3-4 fusion chromosome. A stock Me^{97} cu $Est\text{-}4^{95}$ dt represents the second chromo-

some. For the fused chromosome 3-4 a stock dh Pgm^{100} cn Mdh^{100} ar has been recently constructed. Suitable stocks for use in crosses, having different alleles at enzyme loci, are also available from a wide geographical area. We offer these stocks to anybody willing to use them. As a return service we would like to have possible new mutants. We are mainly interested in new mutants mapped between cn and ar, as well as in chromosomal rearrangements in this region.

It is worth mentioning that the lack of male crossing over has been confirmed in still another species of the D. virilis group. The close relative of D. littoralis, D. ezoana, was studied with respect to the fourth chromosome markers Mdh and α-Gpdh. The recombination frequency was 37.4% (n = 198) in females and zero (n = 90) in males.

References: Ehrman, L. 1978, in: The Genetics and Biology of Drosophila (Ashburner, M. and T.R.F. Wright, eds., Academic Press) Vol. 2b:127-180; Lumme, J. and P. Lankinen 1981, Hereditas 94:285-286; Lumme, J. 1981, Hereditas 94:241-244.

Lyttle, T. W. University of Hawaii, Honolulu. Generation of marked Fs(2)D chromosomes in D. melanogaster by induced gonial recombination.

The desire for an SD (Segregation distorter) chromosome in D. melanogaster which carries a dominant female-sterile [Fs(2)] allele recently led us to produce such a second chromosome by irradiation induced gonial recombination in SD/Fs(2) males. Here SD = SD(ROMA), while Fs(2) = Fs(2)D, maintained as Cy0S/Fs(2)D/Ms(2) bw^D. A second variant, Fs(2)D-M, maintained as T(1;2) Bld/T(1;2)64/Y;Fs(2)D-M, is generally accepted to be identical to Fs(2)D, and the two are apparently listed synonymously as Fs(2)1 in the computerized stock list of Lindsley and Zimm (1979). However, in our hands Fs(2)D exhibits both a wing effect (similar to Serrated) and shortened, thickened bristles, neither of which appear with Fs(2)D-M. Furthermore, the former exhibits complete insensitivity, and the latter full sensitivity, to segreation distortion. The most reasonable conclusion is that there are in fact not one but two independent Fs(2) derivations, which may or may not be allelic.

Each of the two Fs(2) chromosomes looks completely normal in preparations of polytene cells.

We chose to use Fs(2)D because of its insensitivity to distortion, and 5-7 day old males of the genotype constitution (1) Sp SD bw^D/Fs(2) or (2) al dp SD sp^2/Fs(2) were subjected to 4000 R of γ-rays from a Cobalt-60 source at the University of Hawaii. These were then mated to cn bw or In(2L+2R)Cy, dp^{lvI} Bl L^4sp^2/ab^2 ix bw sp^2 females, in a ratio of 1 male:2 females. The irradiated males were transferred to new containers and provided with a similar number of new females on days 4, 8, 12, 18 and 22 post-irradiation, while mated females were also brooded into new bottles on the same schedules. The distribution of recovered mitotic recombinants in the progeny (those carrying only one of the two SD tip markers) is given below:

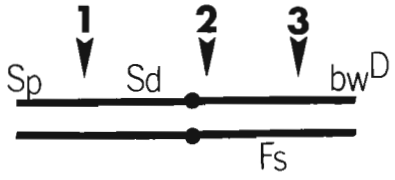
Day	0-3	4-7	8-11	12-17	18-21	22-	Total
	1	1	3	72	39	20	136

Each recombinant was backcrossed to cn bw females, and the segregation ratio in the progeny was used to ascertain the presence of SD (except that an extra generation of backcrossing was required for the al dp SD sp^2 recombinants). Female sterility was determined by a mass mating of recombinant/cn bw females by sib males.

Only five recombinants were recovered from cross (2), three of genotype al dp Fs(2) and two Fs(2)sp. The balance of the recombinants (131) were obtained from cross (1). The 116 which survived to be analyzed are presented by genotype in Table 1. Several interesting conclusions can be drawn from this table. First, the number of bw^D recombinants (59 + 8 lost = 67) is approximately equal to the number of Sp lines (57 + 7 lost = 64). Thus, there is apparently no overall bias in recovery for the two mutants. Individual classes of reciprocal recombinants are somewhat skewed, although this may be partially attributable to the improper inclusion of lines showing mild segregation distortion (i.e., recombinant:cnbw ratio of about 60:40) in the SD^+ class when they in fact carry SD. This might inflate the number of Sp SD^+ Fs types, for example, at the expense of the Sp SD Fs class.

The conspicuous paucity of Sp and SD Fs bw^D recombinants seems to argue that these are the putative triple recombinant classes which would be obtained from the map order Sp SD Fs^+ bw^D. Since SD is located cytologically in 37DE (Ganetzsky 1977), while the table suggests recombination in the Sp-SD interval is as frequent as in the Fs-bw^D interval, this would argue that Fs(2)D is located in the proximal euchromatin of 2R. The wing and bristle abnormalities described above always segregate with Fs(2) and thus seem to be a direct effect of that lesion rather than simply a spurious secondary association. It must be emphasized that no quantita-

Table 1.



Crossover event in:			TOTAL
1	Sp Fs 18	SD bw^D 7	25
2	Sp SD Fs 21	bw^D 44	65
3	Sp SD 16	Fs bw^D 8	24
1+2+3	Sp 2	SD Fs bw^D 0	2
TOTAL	57	59	116

tive mapping is attempted here owing to the possibility that some of the recovered lines may represent clusters of mitotically derived descendents of single recombination events; thus different cluster sizes may serve to change the relative proportions of the recombinant classes.

This research was supported by United States Public Health Service Grant #GM24083.

References: Ganetzky, B. 1977, Genetics 86:321-355; Lindsley, D. and G. G. Zimm 1979, DIS 54:1-266.

Markow, T. A. and M. Manning. Arizona State University, Tempe, Arizona. Female olfaction, vision, and reproductive isolation between D. melanogaster females and D. simulans males.

The factors by which D. melanogaster females discriminate males of their own species from males of the sibling species D. simulans have been studied by Manning (1959a, b), Von Schilcher and Manning (1975), and Barker (1962). Manning (1959b) suggested that D. melanogaster females are not particularly stimulated by visual cues but that their receptivity to cross specific males could be enhanced by mechanically altering their antennae and hence their chemosensory system. Courtship songs of D. melanogaster males differ significantly from D. simulans (Von Schilcher & Manning 1975) and may also be important. Most investigators feel that one of the sensory modes will be found to be of primary importance for female discrimination, but neither visual, olfactory, or auditory cues have been shown to be without any role.

Table 1. Mating success of wild type melanogaster and simulans males.

	Number mating	
Female genotype	melanogaster males	simulans males
D. melanogaster W.T.	41	0
D. simulans W.T.	0	46
D. melanogaster norp A^{P24}	56	0
D. melanogaster smb B	40	3

Table 2.

Female	Average time until mating
D. melanogaster W.T.	6.44 minutes
D. simulans W.T.	12.20 minutes
D. melanogaster norp A^{P24}	2.33 minutes
D. melanogaster smb B	10.317 minutes

We wished to use sensory deficient mutants to examine the role of vision and olfaction in mate selection by D. melanogaster females presented with a choice between D. melanogaster and D. simulans males. Wild type D. melanogaster and D. simulans were collected at a deserted citrus orchard in Tempe, Arizona in April 1979. Two mutant strains of D. melanogaster, norp A^{P24} ("no receptor potential"), which is blind, and smb B ("smell blind") were obtained from Dr. William Pak and Dr. Jeff Hall, respectively. Both of these genetic lesions are presumed to be highly specific, affecting only the sensory organs for vision or olfaction, and therefore avoid the complications of mechanically modifying flies. Flies were reared under uncrowded conditions in half-pint milk bottles containing standard cornmeal-molasses-agar medium.

Virgins were separated under light ether anesthesia and stored 10 flies/vial until used in experiments at 4 days of age.

Female choice experiments were conducted by placing a single female in an 8-dram vial containing one simulans and one melanogaster males. Vials were observed for one hour and the successful male was recorded. Four replications of about 10-15 vials each were carried out on separate mornings. From Table 1 it is clear that wild type females of each species do not make "mistakes". D. melanogaster females from the norp A strain, though unable to perceive visual cues, did not ever mate with simulans males. Three of the melanogaster females having olfactory deficiencies mated with simulans males. The average time until mating for each group is shown in Table 2. While eliminating vision did not increase the probability of mating with a simulans male, mating speed was certainly shorter among norp AP^{24} females. Smell-blind females took longer to mate.

The results support the idea that in D. melanogaster females, the visual system plays a minor role at most in mate selection, but that females rely in part on olfactory cues to detect males of their own species.

References: Barker, S.F. 1962, Amer. Natur. 96:105-115; Manning, A. 1959a, Behavior 15: 123-145; _____ 1959b, Anim. Behav. 7:60-65; Von Schilcher, F. and A. Manning 1975, Behavior Genetics 5:395-404.

Marks, R. W.* and R. D. Seager†. University of California, Davis, California. [Current addresses: *Harvard University, Cambridge, Massachusetts; †University of Georgia, Athens, Georgia.] An estimate of map distance between Adh and α-Gpdh in a natural population of D. melanogaster.

In the course of an experiment to measure the degree of multiple mating in a natural population of D. melanogaster, we have collected data that allow us to estimate the recombination fraction between the second chromosome electrophoretic loci Adh and α-Gpdh. Females were collected at baited traps, and immediately placed individually into 6-dram shell vials. These females were allowed to oviposit for 24 hours, then were transferred to fresh vials. After 8 days of this regime, the genotypes of the females were assayed on starch gels. As the progeny emerged they were collected and frozen until they could be assayed. An average of more than 57 progeny per female were scored. In those cases in which the females were heterozygous at both linked loci and showed no evidence of multiple mating, the distribution of genotypes among the progeny allows calculation of map distance.

Of 11 females heterozygous at both loci, 7 showed no evidence of using sperm from more than one male. From these 7 we calculate a mean distance (recombination fraction) of 33.5 ± 6 centimorgans (cM). Approximate 95% confidence limits are given. This datum is in fairly good agreement with the 32.3 cM reported in Lindsley and Grell (1968), and with the 29.6 cM reported by Cavener (1977). It contrasts rather sharply with the value of 21.7 cM reported by Milkman and Zeitler (1977) for a laboratory population.

Milkman and Zeitler attribute the low value they obtain to technical problems of mapping (presence or absence of interior markers) and to properties of the second chromosome (2L). Our datum suggests that this may not be the explanation; that Milkman and Zeitler's value is, in fact, abnormally low. We believe that their data are of more interest than originally supposed.

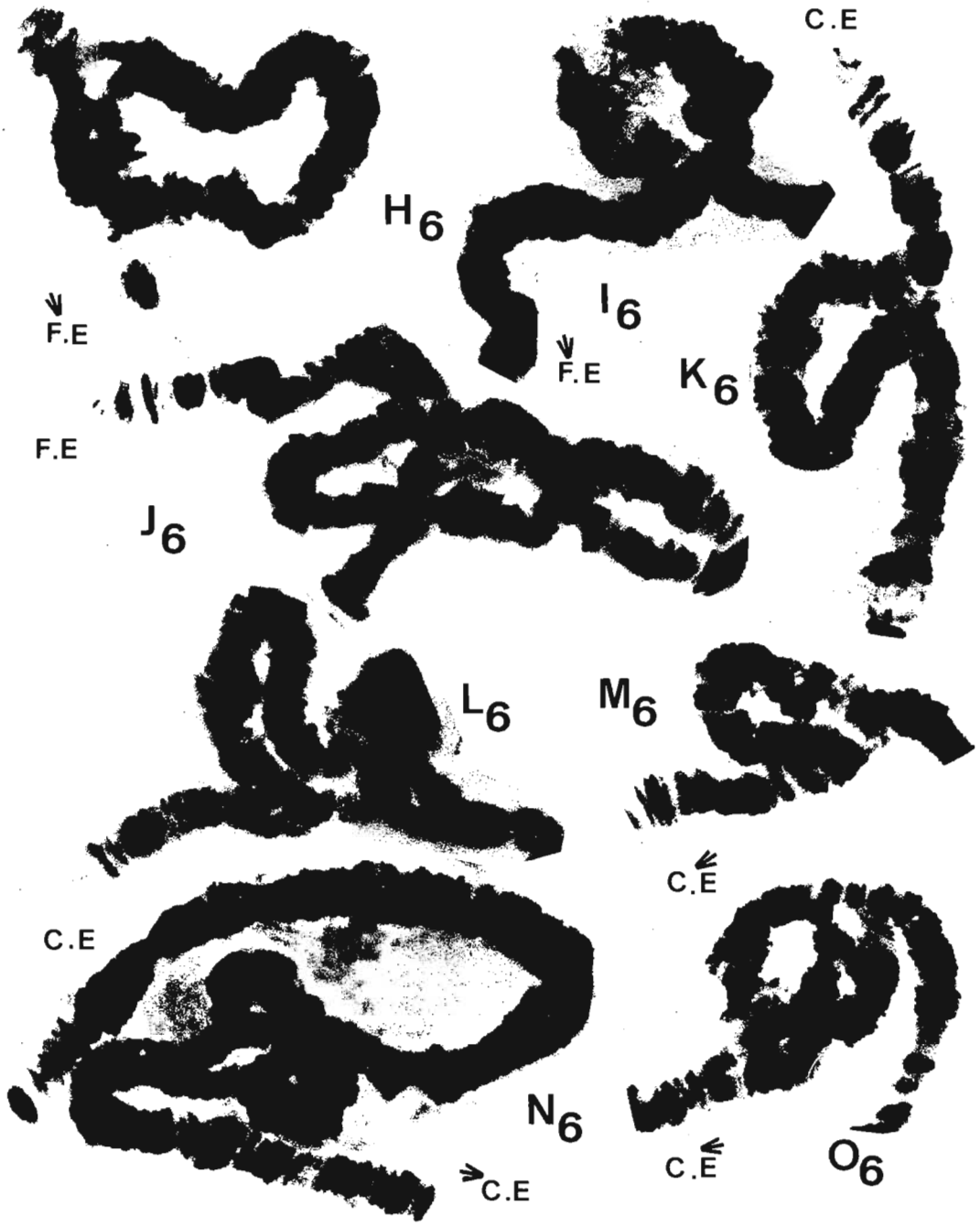
References: Cavener, D.R. 1977, DIS 52:120; Lindsley and Grell 1968, Gen. var. in D. mel.; Milkman, R. and R. Zeitler 1977, DIS 52:61.

Mather, W. B. and G. Balwin. University of Queensland, Brisbane, Australia. Inversions from the River Kwai, Thailand: fifth report.

In December 1979, 21 isolines of D. s. albostrigata, 5 isolines of D. albomicans and 7 of D. kohkoa were established from the River Kwai region of Thailand.

The inversions from the region were last reported on from a collection made in June 1979 (Mather & Balwin 1981).

(a) D. s. albostrigata. Five simple and one complex inversions were detected. All inversions except one had previously been detected at the River Kwai. A photograph of the new inversion (H_6) is presented and breakpoints assigned (in relation to the standard photographic map - Thongmeearkom 1977). The heterozygosity frequency of all inversions detected is given in Table 1.



C.E. = centromere; F.E. = free end

Table 1. D. s. albostrigata.

Inversion	Chromosome	Simple	Complex	Breakpoints	Het. %
E	II L	X			28.6
A_5	II L	X			90.5
B_5	III	X			9.5
C_5	II R	X			19.0
D_5	II L		X		23.8
H_6	II R	X		17.7 - 26.6	4.9

Table 2. D. albomicans.

Inversion	Chromosome	Simple	Complex	Breakpoints
C	III	X		
S_5	II L	X		
U_5	II L		X	
W_5	III	X		
X_5	III		X	
E_6	III		X	
J_6	II L		X	6.6 - 21.3
I_6	III	X		36.2 - 42.4

Table 3. D. kohkoa

Inversion	Chromosome	Simple	Complex	Breakpoints
E^1	II L	X		
K_6	III	X		38.0 - 45.4
L_6	III		X	34.3 - 45.4
M_6	III	X		35.0 - 39.2
N_6	III	X		19.7 - 24.6
F_6	III	X		
O_6	III		X	32.9 - 39.4
G_6	II L		X	

Mather, W.B. and G. Balwin. University of Queensland, Brisbane, Australia.

Inversions from the River Kwai, Thailand. Sixth report.

In July 1981, 63 isolines of D. s. albostrigata, 13 isolines of D. albomicans and 2 of D. kohkoa were established from the River Kwai region of Thailand. The inversions from the region were last reported on from a collection made in December 1979 (Mather & Balwin, this issue).

(a) D. s. albostrigata. Seven simple and one complex inversions were detected. All inversions have been previously found at the collection site. The heterozygosity frequency of the inversions detected is given in Table 1.

(b) D. albomicans. Four simple and four complex inversions were detected. Six of the eight inversions had previously been detected at the River Kwai. Photographs of the two new inversions (J_6 and I_6) are presented and breakpoints assigned (in relation to the standard photographic map - Mather & Thongmeearkom 1980). See Table 2.

(c) D. kohkoa. Five simple and three complex inversions were detected and of these eight, five (K_6, L_6, M_6, N_6 and O_6) are new; photographs are presented and breakpoints assigned (in relation to the standard photographic map (Mather & Thongmeearkom 1978). See Table 3.

The material was collected and the isolines established by W.B.M. The laboratory work was carried out by G.B.

References: Mather, W.B. and G. Balwin 1981, DIS 56:91; Mather, W.B. and P. Thongmeearkom 1978, DIS 53: 150; _____ and _____ 1980, DIS 55: 101; Thongmeearkom, P. 1977, DIS 52: 154.

Table 1. D. s. albostrigata.

Inversion	Chromosome	Simple	Complex	Het. %
E	II L	X		38.1
A_5	II L	X		71.4
D_5	II L		X	41.3
Q_5	II L	X		1.6
C_5	II R	X		41.3
C	III	X		4.8
W_2	III	X		3.2
B_5	III	X		1.6

(b) D. albomicans. Nine simple and three complex inversions were detected. All of the inversions had previously been found at the River Kwai. [See Table 2 on following page.]

(c) D. kohkoa. One isoline was inversion free and the other had inversion I and P, both on chromosome III. This is the first time they have been recorded at the River Kwai. Previous records had recorded them only in Cebu and Luzon

Table 2. D. albomicans.

Inversion	Chromosome	Simple	Complex
R_5	I	X	
S_5	II L	X	
U_5	II L		X
C_6	II L	X	
C	III	X	
L_3	III	X	
W_5	III	X	
B_6	III	X	
D_6	III	X	
E_6	III		X
I_6	III	X	
X_5	III		X

(Mather & Thongmeearkom 1972, Cebu; 1973, Luzon).

The material was collected and the isolines established by W.B.M. The laboratory work was carried out by G.B.

References: Mather, W.B. and P. Thongmeearkom 1972, DIS 48:40; _____ and _____ 1973, DIS 50:60.

Mather, W.B. and G. Balwin. University of Queensland, Brisbane, Australia. Inversions from Kenting, Taiwan: second report.

In June 1980, 85 isolines of D. albomicans were established from Kenting in Southern Taiwan.

A collection had been previously made in August 1971, when 131 isolines were analyzed (Mather & Thongmeearkom 1972).

Table 1.

Inversion	Chromosome	Het. % 1971	Het. % 1980
E^1	II L	39.1	85.9
B^1	III	47.7	3.5

The two simple inversions detected had previously been detected but in very different frequencies (Table 1). Eleven of the 85 isolines were inversion free.

The breakpoints of these inversions in relation to a standard map are recorded in Mather and Thongmeearkom (1980).

The material was collected and the isolines established by W.B.M. The laboratory work was carried out by G.B.

References: Mather, W.B. and P. Thongmeearkom 1972, DIS 49:110; _____ and _____ 1980, DIS 55:101.

Mather, W.B., M. Clyde and G. Balwin. University of Queensland, Brisbane, Australia. Inversions in D. sulfurigaster albostrigata from Hidden Valley Springs, Luzon, Philippines: second report.

In January 1974, D. sulfurigaster albostrigata was examined for inversions at Hidden Valley Springs, Luzon, Philippines (Mather & Clyde).

As part of a long-term project the species was again sampled in this locality in January 1975, January 1977, July 1977, and July 1979 (see Table).

It will be noted that both inversions C and E remain in substantial frequencies from 1974-1979 but that G was not detected in 1977 or 1979.

	Percent heterozygosity frequency				
Inversion	Jan. 1974 (79 isolines)	Jan. 1975 (132 isolines)	Jan. 1977 (22 isolines)	July 1977 (31 isolines)	July 1979 (44 isolines)
C	51	43	18	36	41
E	84	81	91	65	73
G	8	10	--	--	--

The material was collected and the isolines established by W.B.M. The laboratory work for the 1975 collection was carried out by M.C., and for the 1977 and 1979 collections by G.B.

References: Mather and Clyde, DIS 52:147.

Maveety, N. and M. B. Seiger. Arizona State University, Tempe, Arizona, and Wright State University, Dayton, Ohio.

Variation in photoresponse in a population of D. immigrans.

Five isofemale strains of D. immigrans, started from flies collected in the Wright State Biology Preserve, Dayton, Ohio, were compared for their photopreference in a Hirsh-Hadler photo-choice maze under GE cool-white lights providing 180 foot-candles of light at the surface of the maze. All stocks were raised on cornmeal-molasses-agar medium with tegosept and tested when 2-8 days old at 70°F. Flies entering the maze make 15 consecutive choices between light and dark pathways and emerge in 16 collecting tubes at the end of the maze. Flies making all dark choices emerge in tube #1 (highly photonegative); flies making all light choices emerge in tube #16 (highly photopositive). A population of flies which makes an approximately equal number of light/dark choices will have a mean phototactic score of 8.5 (photoneutral).

Table 1. Weighted means of light preference in five isofemale lines of D. immigrans females.

Strain	X ± SE	(n)
F-1	8.63 ± 0.16	304
F-3	9.77 ± 0.17	199
F-7	8.60 ± 0.23	137
F-10	9.88 ± 0.21	145
F-18	9.91 ± 0.15	291

Flies from each strain were tested in three trials and the results pooled for final analysis (Table 1). Because the males were found to be especially ether sensitive and died before entering the maze, only the photoscores of the females are presented. However, when males could be scored, their responses were similar to the female scores.

A one-way analysis of variance using the three weighted means in each of the five strains showed significance at the 5% level (Table 2). Since all the tests were stringently controlled, there could have been very little effect of environmental disparities. Therefore, the variability must be attributed to genetic differences among the strains. This corroborates the results of Markow and Scarvarda (1977), who found that intrapopulation variation had some genetic basis. Since the strains were initiated from sympatric females, this study would indicate that there is genetic variability in photoresponse in the population of D. immigrans sampled, even though the average for each female line is near a neutral light preference. Since the strains used here were samples of a population collected at one time, the variation might be the result of segregation in which certain types are favored. Alternatively, the variation might be the consequence of selection favoring different genotypes in a variety of subniches of the habitat of a population. Different photoresponses could serve to direct individuals of diverse genotypes to those subniches in a heterogeneous habitat where they will be most fit.

Table 2. One-way anova of photopreferences in five isofemale lines of D. immigrans females.

Source	D.F.	SS	MS	F
strains	4	6.48	1.62	4.66*
error	10	3.48	0.35	
residual	14	9.95		

*significant at the 5% level.

References: Markow, T.A. and N. Scarvarda 1977, Behavior Genetics 7:139-146.

Mglinetz, V. A. Institute of Medical Genetics, USSR Academy of Medical Sciences, Moscow, USSR.

Formation of compartment boundaries due to differences in cell adhesiveness.

Compartmentalization in Drosophila ontogeny plays an important role in imaginal pattern formation. There is rather strong evidence that each step of compartmentalization is controlled by respective homeotic selector genes (Garcia-Bellido 1977). The role of selector genes is to activate subordinate realizator genes. The latter group seems to include the genes controlling the synthesis of adhesor molecules (Steinberg 1964). Then, the action of a selector gene must lead to formation of two compartments differing in adhesor molecules (AM) composition. However, the Mittenthal's model (1979) fails to explain the affinity of cells within a compartment being greater than that to the cells of a neighboring compartment: according to the model most of their AM are similar. According to Steinberg, the differences in cell adhesiveness may be due not only to differences in types of adhesor sites on the cell surfaces, but also to differences in their spatial distribution and

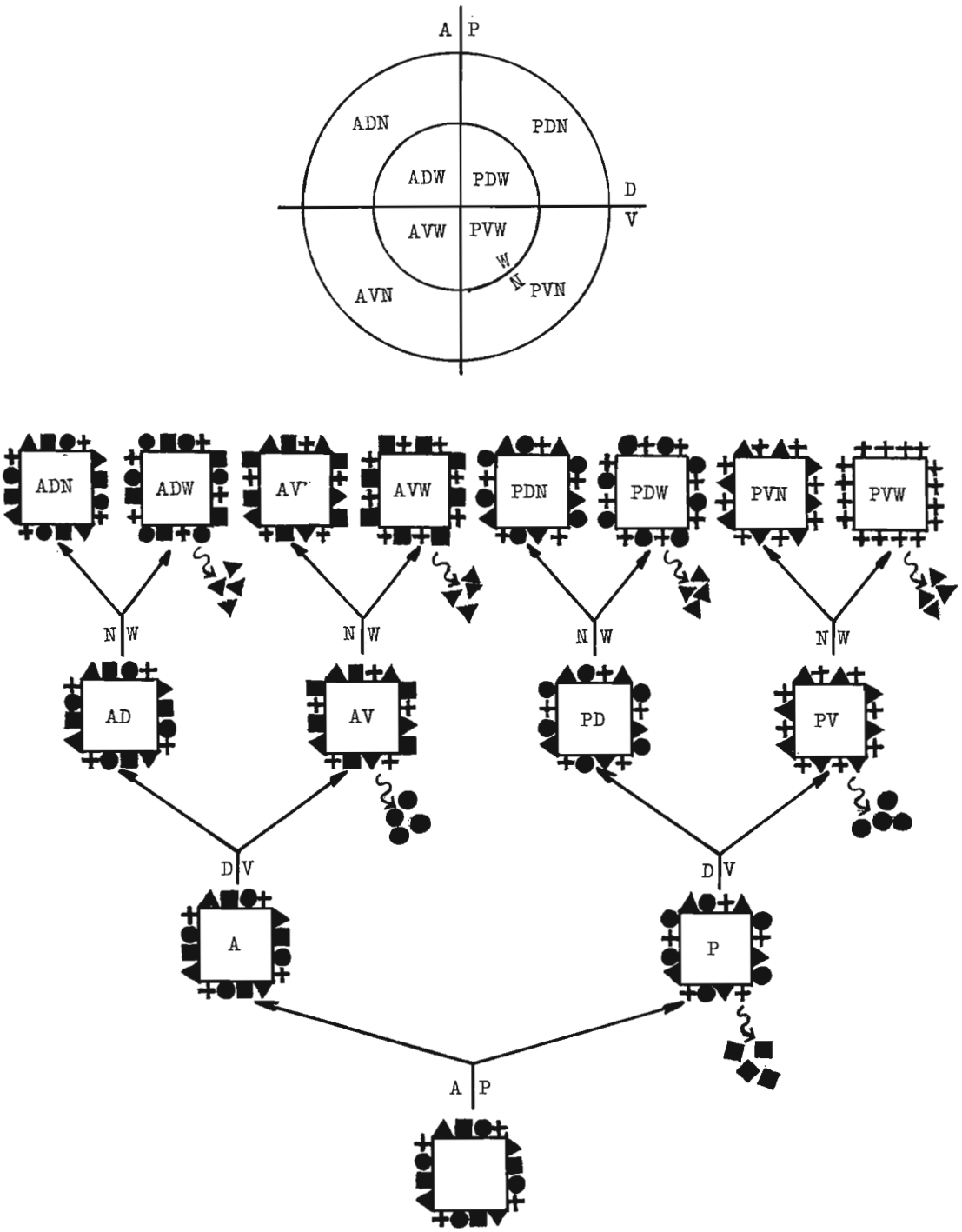


Fig. 1. Scheme illustrating the subdivision of the wing imaginal disc into anterior and posterior (A/P), dorsal and ventral (D/V), and notum and wing (N/W) compartments (upper part). Squares represent the cells; symbols ▲ ■ ● ✚ correspond to four types of AM.

density. A model may be suggested accounting for all the three causes of differences in cell adhesiveness in the neighboring compartments. The line of reasoning may be exemplified by compartment formation in the wing imaginal disc of Drosophila (see Fig. 1). The cell surfaces in initial imaginal discs must bear at least four types of AM (▲ ■ ● +). In every one of the three steps of compartmentalization a selector gene suppresses, as shown in the figure, (or activates) in one of the compartments a specific realizator gene controlling a certain type of AM, e.g., ■ in anteroposterior compartmentalization or ● in dorsoventral. Then the cells of every final compartment will differ in the AM set. This leads to a basic suggestion: on suppression of a specific realizator gene in one of the alternative compartments the sites of respective AM's in that compartment do not remain vacant, but are filled by available AM's, as shown in the figure. Then, the density of preserved AM's is increased and the pattern of their distribution is changed. As a result, the cells within any compartment exert great affinity to one another due to similar distribution of AM's on their surfaces. On the other hand, in spite of differences in only one type of AM, the affinity of cells of different compartments is essentially lower, as they differ also in the distribution of common AM's. It may also be suggested that, depending on completeness of initial sets of AM's, the cells of different compartments may also differ in absolute values of adhesiveness, for instance, 4AM → 3AM → 2AM → 1AM. This suggestion seems to be open to experimental testing. A similar mechanism may be involved in segmentation process.

References: Garcia-Bellido, A. 1977, Am. Zool. 17:613; Mittenthal, J.E. 1979, J. Theor. Biol. 81:129; Steinberg, M.S. 1964, in: Cellular Membranes in Development (M. Locke, ed.), Acad. Press, New York, p. 321.

Miglani, G. S. and A. Thapar. Punjab Agricultural University, Ludhiana, Punjab, India. Temperature induces crossing-over in Drosophila males.

The effect of temperature on the induction of crossing over in D. melanogaster males was studied. Three (second chromosome) genetic markers--dumpy (dp), black (b) and cinnabar (cn)--were used. Virgin dp b cn/dp b cn females were pair-mated with Oregon-K wild-type males. Thirty pair-matings were initiated. The pairs were allowed to lay eggs at 25°C for two days after which the adults were removed. Ten of these vials were shifted to 19°C, another ten to 28°C, and the remaining were allowed to proceed at 25°C. From here onwards the experiments were completed at these three different temperatures. The F_1 (+ + +/dp b cn) males were crossed with dp b cn/dp b cn females. The frequencies of crossing-over in the test cross progeny were calculated in region 1 (dp-b) and region 2 (b-cn). For testing the significance of difference in the frequencies of crossing-over, z-test was used.

25°C is the most favorable temperature for culturing D. melanogaster. No crossing-over was observed when the experiment was performed at this temperature. This experiment served as a control for the other two experiments conducted at 19°C and 28°C. At 19°C, the frequencies of crossing-over in regions 1 and 2 were 4.9% and 4.1%, respectively. These values were highly significant ($p < 0.001$) as compared to the control. At 28°C, the frequencies of crossing-over in regions 1 and 2 were 14.9% and 20.6%, respectively. These values were again highly significant ($p < 0.001$) as compared to the control. The results suggested that a continuous treatment of flies with a low (19°C) or a high (28°C) temperature during their entire lives led to induction of crossing-over in D. melanogaster males.

Morgan, D. G. and R. S. Singh. McMaster University, Hamilton, Ontario, Canada. Effect of temperature shift on developmental rate of D. pseudoobscura.

Due to its effect on fitness parameters, altered developmental rate of an organism is considered to be of primary evolutionary importance (Lewontin 1965). In many species of Drosophila, at a constant temperature, females develop faster than males. The effect of temperature on developmental rates has been widely investigated, but in all these experiments a constant or fluctuating temperature was used throughout the development of the organisms (e.g., see David & Clavel 1966, 1976). To understand the nature of the difference between developmental rates of the sexes and also to see if there are any temperature sensitive developmental stages in D. pseudoobscura, a temperature shift experiment was performed in which flies were allowed to com-

plete part of their development at one temperature and were then shifted to another temperature. The eggs used in the experiment were collected within an hour of noon. Fifteen to 20 eggs were placed in a 25 mm x 95 mm glass vial containing 10 ml of Carpenter's medium. A set of three replicate vials were used for each day. The emerging flies were sexed and counted once a day at noon. Developmental rate is defined as the weighted mean number of days taken to complete development from the egg to adult stage.

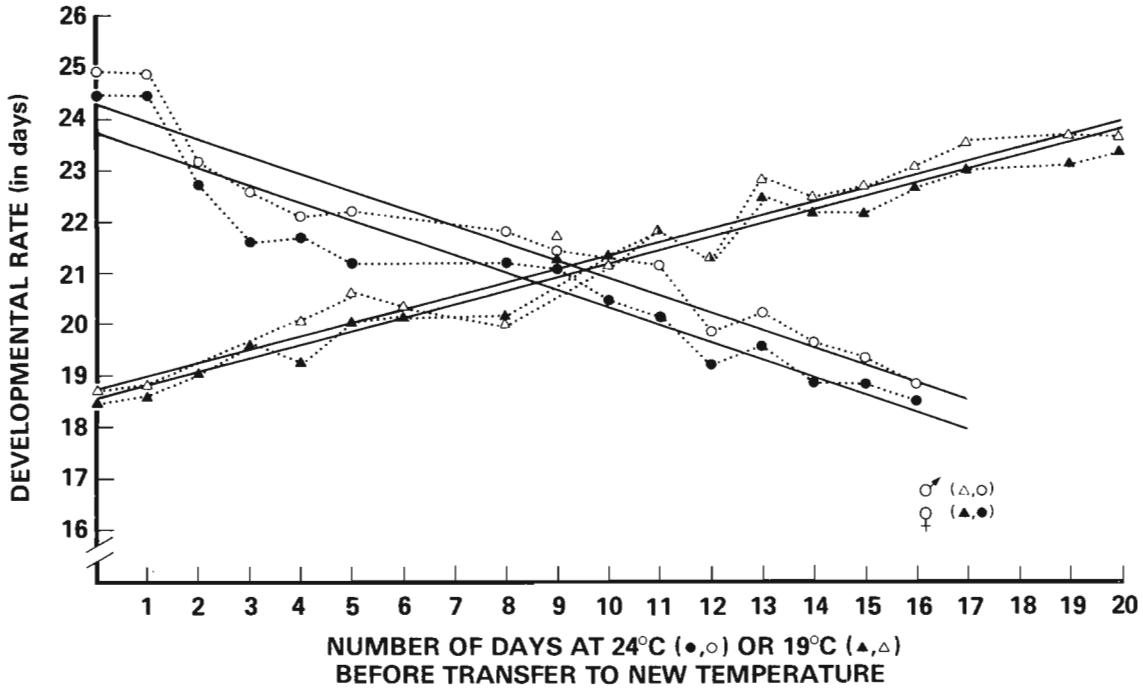


Fig. 1. Effect of temperature shift on developmental rate of males and females of D. pseudoobscura.

Table 1. Effect of temperature shift (from high to low and vice versa) on developmental rate (in days) of males and females of three different strains of D. pseudoobscura.

		From high to low		From low to high	
Strain	Sex	Slope	Y-intercept	Slope	Y-intercept
		24° - 19°C		19° - 24°C	
SC-E8	M	-0.37	22.60	0.28	17.00
	F	-0.38	22.14	0.27	16.76
		(r=0.99)**		(r=0.99)**	
BO-12	M	-0.34	24.29	0.26	18.76
	F	-0.34	23.28	0.25	18.57
		(r=0.99)**		(r=0.99)**	
		24° - 14°C		14° - 24°C	
SC-E8	M	-1.31	36.49	0.50	18.88
	F	-1.28	35.24	0.50	18.62
		(r=0.99)**		(r=0.99)**	

Significance level = * $p < 0.05$, ** $p < 0.01$

The result of such a reciprocal temperature shift experiment is shown in Fig. 1. The obvious point to note is that the difference in the developmental rate of males and females is reduced when the temperature shift is from low to high but increased when the temperature shift is from high to low. For both curves, the points appear to fall on a straight line except during the embryonic or eclosion stages. In the high to low temperature shift experiment, development is rapid during the embryonic stage and in the low to high temperature shift experiments, there appears to be a plateau during the eclosion period.

The regression analysis of data from two sets of temperature shift experiments (24-14°C and 24-19°C) for three isofemale lines of D. pseudoobscura is given in Table 1. The main points to note are as follows:

1. The developmental rate of males and females are highly correlated.
2. The difference in the developmental rate of males and females is larger when the temperature shift is from high to low than when from low to high (compare y-intercepts).
3. Embryonic stages appear to be sensitive but only in the high to low temperature shift experiments.
4. The difference in the developmental rate between the sexes is larger when the temperature shift involves a larger temperature range (compare data for SC-E8 at 24-14°C vs. 24-19°C).
5. Bogota (BO_{12} is slower in its development than Strawberry Canyon (SC-E8). This is true for all Bogota strains (Singh, unpublished).

The results of this study show that the sex difference in developmental rate is very much dependent upon the pattern of temperature variation provided during the development of Drosophila.

References: David, J. and M.F. Clavel 1967, Naturaliste Canada 94:209-219; ____ and ____ 1966, C. R. Acad. Sci. Paris 262D:2159-2162; Lewontin, R.C. 1965, in: The Genetics of Colonizing Species, Acad. Press, New York.

Niesel, D. and G. C. Bewley. North Carolina State University, Raleigh, North Carolina. Isoelectric focusing of glycerol-3-phosphate dehydrogenase isozymes in Drosophila.

The isoelectric point of glycerol-3-phosphate dehydrogenase isozymes (GPDH, NAD^+ oxidoreductase, E.C. 1.1.1.8) was determined in both the larval and adult stages of development by analytical isoelectrofocusing according to Vesterberg and Svensson (1966). Each separation was achieved in an LKB isoelectric focusing column using 2% LKB carrier ampholytes which generated a linear pH gradient from 4 to 6.

In crude adult homogenates bearing the fast electrophoretic allele (Fig. 1A), two distinct peaks of GPDH activity are observed at equilibrium. Peak I, which focuses at a pH of 5.2, has been identified as $GPDH^F$-1 on the basis of its thermal stability at 50°C (Bewley et al. 1974). Peak III, which focuses at a pH of 5.6, exhibits a loss of activity at 50°C characteristic of $GPDH^F$-3. Fractions 74-77 exhibit a thermal stability intermediate to peaks I and II and probably reflect the decay of a population of $GPDH^F$-2 molecules. The shoulder, Peak II, of GPDH activity at these fractions is suggestive of this assignment. A previous report of the isoelectric focusing of purified preparations of $GPDH^F$-1 and $GPDH^F$-3 (Niesel et al. 1980) gives

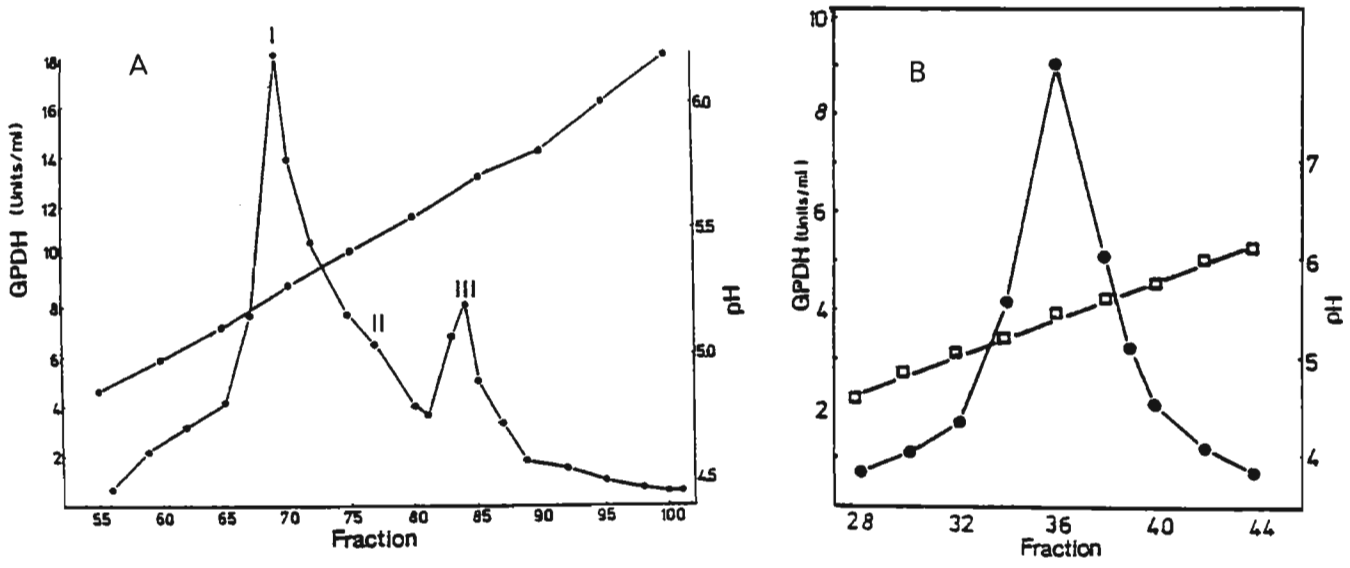


Fig. 1. (A) The isoelectric focusing profile of adult homogenates in a 2% ampholine gradient, pH 4-6. Activities were determined in duplicate from 1.0 ml fractions of a 110 ml LKB analytical isoelectric focusing column. Peak I ($GPDH^F$-1) = pI 5.2; Peak II = pI 5.4; Peak III ($GPDH^F$-3) = pI 5.6. (B) The isolelectric focusing profile of larval homogenates under identical conditions with (A). Peak I ($GPDH^F$-3) = pH 5.6.

identical pI estimates and therefore indicates that no alteration of charged properties and thus conformation occurs during the process of purification of these isozymes.

In crude adult homogenates, $GPDH^F$-1 and $GPDH^F$-2 (Peaks I and II, Fig. 1A) comprise 75-80% of the total GPDH activity, while GPDH-3 (Peak III) contributes the remaining 20-25%. These estimates are in agreement with determinations from starch gel staining intensity and thermal stability studies (Bewley et al. 1974)

In crude larval preparations (Fig. 1B), a single sharp peak of GPDH activity is recovered during focusing under the same conditions. The peak focuses at pH 5.6 and exhibits a thermal stability characteristic of $GPDH^F$-3. No evidence is observed for multiplicity in larval extracts which is at variance with a recent report of six electrophoretic forms observed by microelectrophoresis of various larval tissues (Bienz & Deak 1978).

Supported by NIH Grants GM-23617 and AG-01739.

References: Bewley, G.C., J.M. Rawls, Jr., and J.C. Lucchesi 1974, J. Insect Physiol. 20: 153-165; Bienz, M. and I.I. Deak 1978, Insect Biochem. 8:449-455; Niesel, D.W., G.C. Bewley, S.G. Miller, F.B. Armstrong and C.Y. Lee 1980, J. Biol. Chem. 255:4073-4080; Vesterberg, O. and H. Svensson 1966, Acta Chem. Scand. 20:820-834.

Nigro, L. and B. Shorrocks. The University, Leeds, Great Britain. Habitat choice in a natural population of Drosophila.

By using mark, release, recapture experiments, Taylor and Powell (1978) have shown that D. persimilis and D. pseudoobscura tend to return to their area of origin, or to an ecologically similar area, when transplanted away. If this phenomenon of habitat choice, by individual flies, was of wide occurrence it would have important implications for the maintenance of genetic variation in natural populations.

The experiments reported below were carried out on D. subobscura, in Adel Dam, a mixed woodland of 50 ha, 8 km north of Leeds, England (Shorrocks 1975). The traps used in the experiments were baited with cotton-wool soaked in "Guinness". Flies were collected using a net and pooter.

Two experimental areas, 100 m apart, were chosen. Area I was rather dark and the soil dry and consisted mainly of mature beech trees. Area II was light and the ground wet, with a few alder trees. Flies were collected from both areas, marked with fluorescent dust of different colors and released midway between both areas. On the following two days, flies were recaptured from both areas I and II and from a third area, III, which was also dark and dry, although the trees were mature sycamore. These latter collections were carried out because Taylor and Powell (1978) observed that flies also returned to ecologically "similar" areas. The experiment was repeated on three different occasions during July and August 1980. A heterogeneity chi-square test was not significant ($\chi^2_6 = 9.0$) and in the following table the data from the three experiments have been pooled.

Table 1. Contingency tables showing recaptured flies according to area of original capture and recapture. Figures in parentheses indicate numbers expected if random movement occurred.

(a)

		Site of recapture I	Site of recapture II	
Site of original capture	I	78 (66)	56 (68)	134
	II	33 (45)	58 (46)	91
		111	114	225

(b)

		Site of recapture III	Site of recapture II	
Site of original capture	I	30 (24)	56 (62)	86
	II	14 (24)	58 (26)	72
		44	114	158

The first comparison between site I and II is shown in Table 1a. There is a significant tendency ($\chi^2_1 = 9.58$, $p < 0.01$) for flies to return to their area of first capture. Table 1b shows that flies also show a tendency to return to an ecologically similar area as that in which first captured. Proportionately more flies originally caught in area I were recaptured in area III than in area II ($\chi^2_1 = 3.51$, $p = 0.05$).

References: Shorrocks, B. 1975, J. Anim. Ecol. 44:851-964; Taylor, C.E. and J.R. Powell 1978, Oecologia (Berl.) 37:69-75.

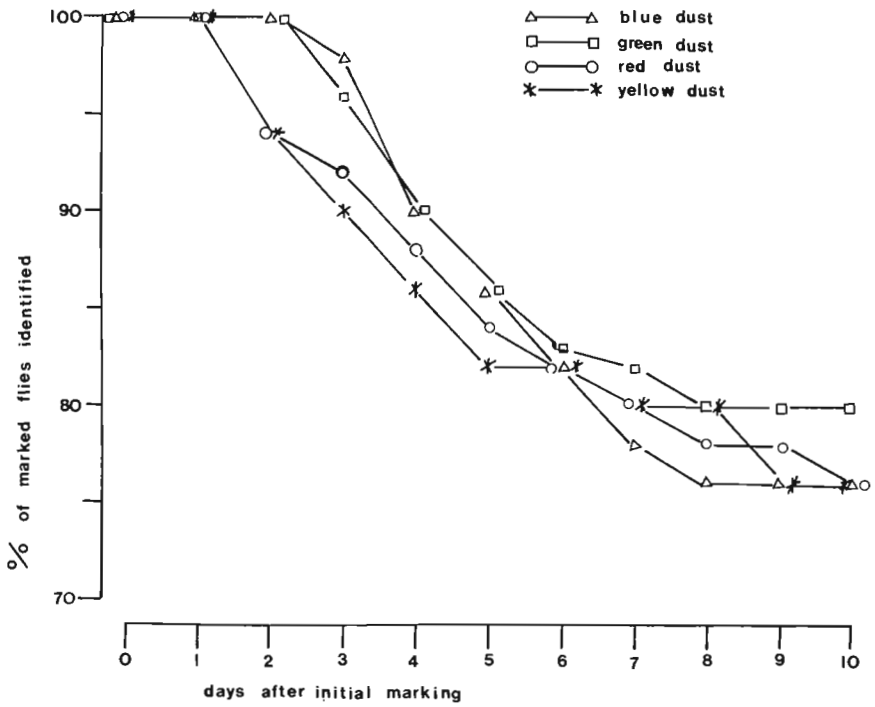
Nigro, L. and B. Shorrocks. The University, Leeds, Great Britain. Effectiveness of fluorescent dust for marking Drosophila.

In recent years, micronized fluorescent dust has been used by a number of workers to mark wild flies for dispersal and density studies (Crumpacker & Williams 1973; Begon 1978; Dobzhansky et al. 1980). The effectiveness of such dusts in marking the flies over several days has been tested by Crumpacker (1974) on D. pseudoobscura and Moth and Beker (1975) on D. simulans. We report below a similar experiment on D. subobscura, in which, however, the process of identifymarks is similar to that in a field experiment.

Four colors of dust were tested (Heffner Co. Ltd., Redhill, Surrey, U.K.) on both males and females. The experimental flies, F_1 progeny of wild caught females, were marked by rolling in a 75 mm x 25 mm glass vial thinly coated with dust. The marked flies (25 male and 25 female for each color of dust) were placed into perspex cages in an outdoor insectary and examined each day for visibility of marks. For examination the marked flies were placed individually into glass vials and examined under a binocular microscope with U.V. illumination in a dark room. An equal number of unmarked flies, also in glass vials, were mixed with the experimental animals. Thus the person scoring marks had to separate marked and unmarked flies as in a field experiment.

The results are shown in Figure 1. There was no significant difference between male and female data and these have been combined for each color of dust. For three days after marking almost 100% of the marked flies could be recognized. Even by day ten, 80% of the marks were still visible. There was no difference in marking ability between dusts.

It would appear that micronized fluorescent dusts are a suitable marking agent for short term field experiments on dispersal and density.



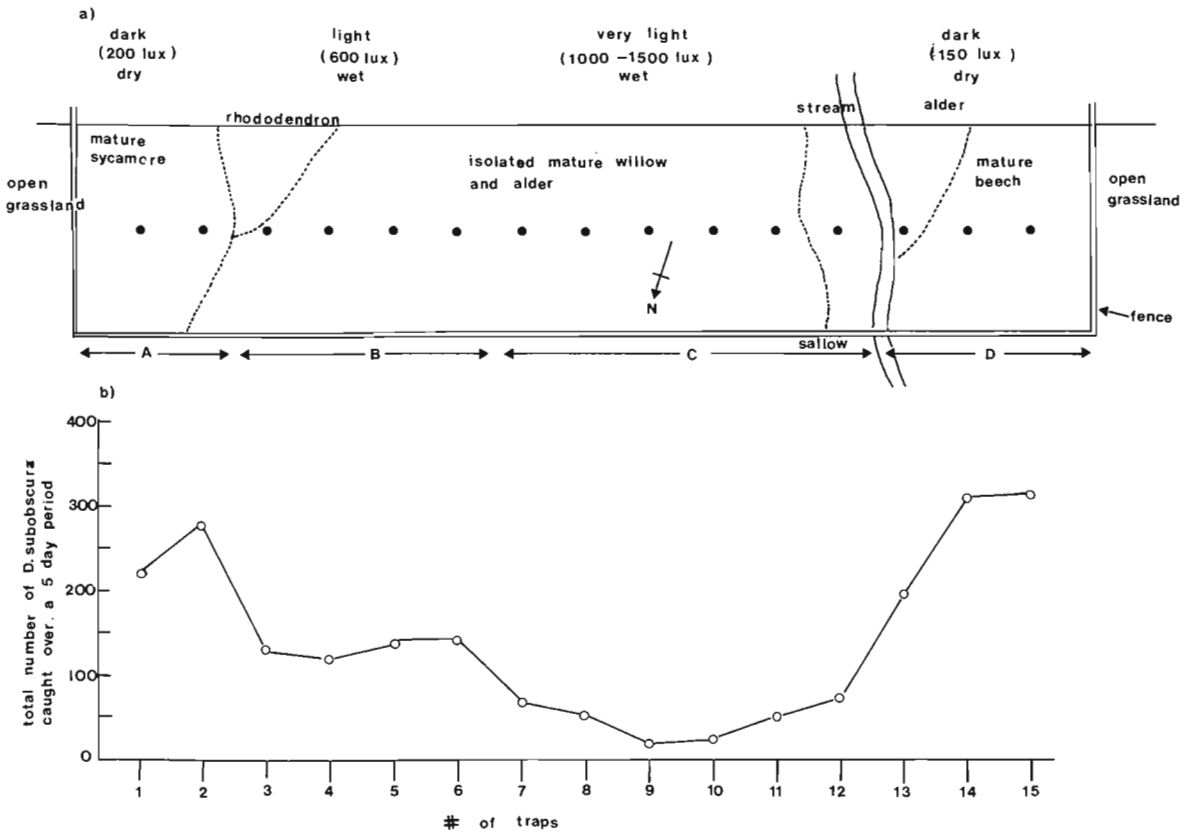
References: Begon, M. 1978, Ecol. Entomol. 3:1-12; Crumpacker, D.W. 1974, Amer. Mid. Nat. 91:118-129; Crumpacker, D.W. and J.S. Williams 1973, Ecol. Monogr. 43:499-538; Dobzhansky, Th., J.R. Powell, C.E. Taylor and M. Andregg 1979, Am. Nat. 114:325-334; Moth, J.J. and J.S.F. Beker 1975, J. Nat. Hist. 9:393-396.

Nigro, L. and B. Shorrocks. The University, Leeds, Great Britain. Microdistribution of D. subobscura in an English woodland.

An examination of microdistribution is an important preliminary to many kinds of field investigation, including habitat selection, dispersal studies and density estimates. The collections described below were carried out during July 1980 in Adel Dam, a deciduous woodland, 8 km north of Leeds, England (Shorrocks 1975). It provided the background for a later study of habitat selection, also outlined in this issue.

Like many English woodlands, the quantity and quality of vegetation in Adel Dam varies over quite short distances. Figure 1a shows a simplified map of part of the woodland chosen for the present study. The area represents the northern edge of a small nature reserve, bounded by a fence and open ground on three sides. Fifteen traps, each 10 m apart and baited with cotton-wool soaked in "Guinness", were put out along a transect. Traps were opened in the morning and collections made all day using a net and pooter, for five consecutive days. The results are shown in Figure 1b.

There are quite marked differences in the numbers of D. subobscura coming to traps, even a short distance apart. Four areas or microhabitats have been delimited and are labeled in Figure 1a. In general the flies appear to prefer "dark and dry" habitats (areas A and D) and to avoid those that are "light and wet" (areas B and C). This finding confirms previous work by Taylor and Powell (1978) on D. pseudoobscura and Atkinson and Miller (1980) on D. subobscura. Area C is so unfavorable that it is unlikely to support a resident population of any kind. There was also an interesting difference in sex ratio between the two microhabitat types. In the "dark and dry" areas there were 50% females while in the "light and wet" areas it was 70%.



References: Atkinson, W.D. and J.A. Miller 1980, Heredity 44:193-199; Shorrocks, B. 1975, J. Anim. Ecol. 44:851-864; Taylor, C.E. and J.R. Powell 1978, Oecologia (Berl.) 37:69-75.

Nowak, J. and M. J. Piechowska. Polish Academy of Sciences, Institute of Biochemistry and Biophysics, Warsaw, Poland.

Glutamate dehydrogenase activity during development of D. melanogaster.

The role of glutamate dehydrogenase (GDH), a mitochondrial enzyme, in insect metabolism still presents an open problem. Some authors suggest that the enzyme is involved in respiratory metabolism of the cell. Bond et al. (1968) stress the fact that the high level of free amino acids, especially glutamate, glutamine and proline, in insects' haemolymph may point to their role as respiratory substrates for appropriate enzymes, among them GDH. Those authors have proved experimentally that the activity of GDH is regulated by "energy charge" (according to the terminology of Atkinson, 1966). On the other hand, Bursell (1975) and Hansford et al. (1975) have found that this enzyme plays an important role in oxidation of proline in the tse-tse fly (Glossina morsitans) and Japanese beetle (Popilla japonica).

In the present work, GDH activity was determined in enzyme preparation from mitochondria of D. melanogaster, strain Oregon-R, during nine days of development--from egg to imago--under the following culture conditions: 25°C, medium supplemented with yeast and sterilized to prevent contamination with the yeast mitochondrial enzymes, 12 hr light:12 hr dark photoperiod. The age of insects was synchronized from 4 hr egg-laying periods. The mitochondria were isolated from 2 g of insects, from 10% homogenate prepared in the following buffer: 10 mM Tris, 60 mM sucrose, 240 mM mannitol, 0.2 mM EGTA, pH 7.4 (Storey et al. 1978) with β-mercaptoethanol (1%). The nuclei and cell debris were removed at 2,200g for 6 minutes, and the supernatant was centrifuged at 7,000g for 10 minutes. The sedimented mitochondria were washed once with isolation buffer and then with 0.1 M potassium-phosphate buffer pH 7.6. The mitochondria were treated with 1% Lubrol PX to release the matrix enzymes. The membranes were removed by centifugation at 100,000g for 30 minutes, and the supernatant obtained was used for the determination. The activity of GDH was assayed at 340 nm in a VARIAN-CARY spectrophotometer with recorder, in 10 mM L-glutamate, 6 mM NAD, and 0.1 M potassium-phosphate buffer pH 7.6. Protein was determined by a method of Lowry et al. (1951). The results are expressed as micromoles of NAD utilized during one minute of the reaction by 1 mg of the extract protein.

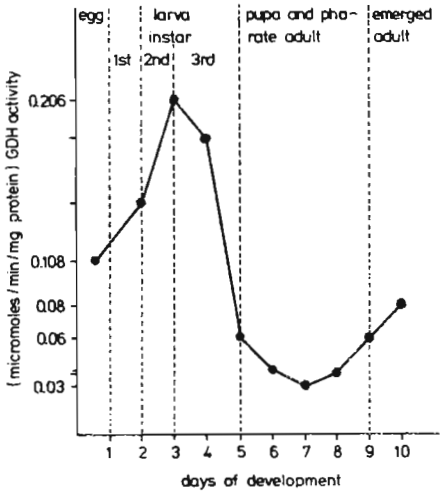


Fig. 1. The activity of glutamate dehydrogenase from D. melanogaster mitochondria during the insect development. Each point on the curve represents a mean from 5 experiments.

GDH from D. melanogaster mitochondria shows high activity (0.108 μm/min/mg protein) as early as in 19-hour-old embryos. The activity increases with growth of the larva, reaching the highest value of 0.206 μm/min/mg protein on the third day of development. On the other hand, by the end of the late third instar (5th day), the activity decreases rapidly (to 0.06 μm/min/mg protein) and continues to decrease until the 7th day, when it reaches the lowest value (0.03 μm/min/mg protein). Subsequently, the activity gradually increases but it does not reach again the maximum value, only a much lower level of 0.08 μm/min/mg protein at the imago stage.

The developmental profile of the D. melanogaster GDH activity was compared with an idealized representation of the behavior of several Drosophila dehydrogenases: alcohol-, β-hydroxybutyrate-, malate-, and NADP-dependent isocitrate- presented by Rechsteiner (1970). The course of these two curves differs greatly. First of all, the dehydrogenases considered by Rechsteiner showed no activity in embryos and the maximum of their activity was observed on the 5th day. Although in both cases the activity was lowest on the 7th day, the curves for the pupal stage differed in character: the GDH activity followed a U-shaped curve at variance with the above-mentioned dehydrogenases.

On the other hand, changes in the activity of GDH during D. melanogaster development were closely similar to those observed for cytochrome oxidase (Chen 1966) as well as for oxygen consumption (Chen 1966; Fourche 1969; Wigglesworth 1966). Chen (1966) detected cytochrome oxidase activity as early as by the end of embryonal development of D. melanogaster: the activity increased during the larval stage and decreased in pupae. On the other hand, Fourche (1969) demonstrated that oxygen consumption was the highest on the third day of development (at the

larval stage) and the lowest on the seventh day (at the pupal stage); at the pupal stage the utilization of oxygen followed a U-shaped curve. An identical profile of oxygen consumption was obtained by Guillet (1980) in pupal mitochondria. These profiles showed close similarity with the curve representing changes in GDH activity at the pupal stage.

Convergence of these results seems to suggest that GDH plays a role in the respiratory metabolism of D. melanogaster.

References: Atkinson, D.E. 1966, A. Rev. Biochem. 35:85; Bond, P.A. and J.H. Sang 1968, J. Insect Physiol. 14:341; Bursell, E. 1975, Insect Biochem. 5:289; Chen, P.S. 1966, Adv. Insect Physiol. 3:67,93; Fourche, J. 1969, Ann. Biol. 8:334; Guillet, C. 1980, DIS 55:53; Hansford, R.G. and R.H. Johnson 1975, Biochem. J. 148:389; Lowry, O.H., N.J. Rosenborough, A.L. Farr and R.J. Randall 1951, J. Biol. Chem. 60:310; Rechsteiner, M.C. 1970, J. Insect Physiol. 16:1179; Storey, K.B. and E. Bailey 1978, Insect Biochem. 8:73; Wigglesworth, V.B. 1966, in: Insect Physiology, ed. Methuen and Co., London, Wiley and Sons, New York.

Oguma, Y., M. Tomomatsu, M. Nishino and H. Kurokawa. University of Tsukuba, Ibaraki, Japan. Photomap of salivary gland chromosome of D. auraria.

D. auraria belongs to the auraria complex with the other siblings of D. biauraria, D. triauraria and D. quadraria. Metaphase karyotype consists of two Vs, one rod and one dot. Several studies on the external morphology and the sexual isolation using these species have been published (Kurokawa 1960, 1962, 1963; Lee 1974), while information of the chromosomes is still poor. This paper presents exclusively a standard photomap of salivary gland chromosomes of D. auraria.

Female flies collected in 1978 at Tsukuba, Ibaraki-pref. were singly kept in vials and allowed to lay eggs. Among these, inversion-free isofemale line was selected and used for making the photomap. Salivary glands of the third instar larvae were dissected in a 1:1 mixture of Drosophila Ringer's solution and 45% acetic acid, then transverred to an aceto-lactic orcein mixture and kept for one hour. The photographs were taken under phase contrast microscope. The salivary gland nuclei show five long euchromatic arms and one very short strand radiating from the common chromocenter (Fig. 1). The whole chromosome is divided into 102 sections on the basis of certain major bands (Fig. 2).

X-chromosome (Sections 1-20): This chromosome is characterized by five distinct bands at 1D/E and a remarkable puff at 2A/D. The other landmarks are two puffs at 9A and 9C/D, and the contiguous but distinct bands at 19C/D and 20B/D.

2L-chromosome (Sections 21-40): The remarkable features of this chromosome are three thick bands at 21D, two distinct bands at 22A, thick bands at 28A/29B, 39A/C and four thick bands at 40A/C. The landmark puff arises in 37A.

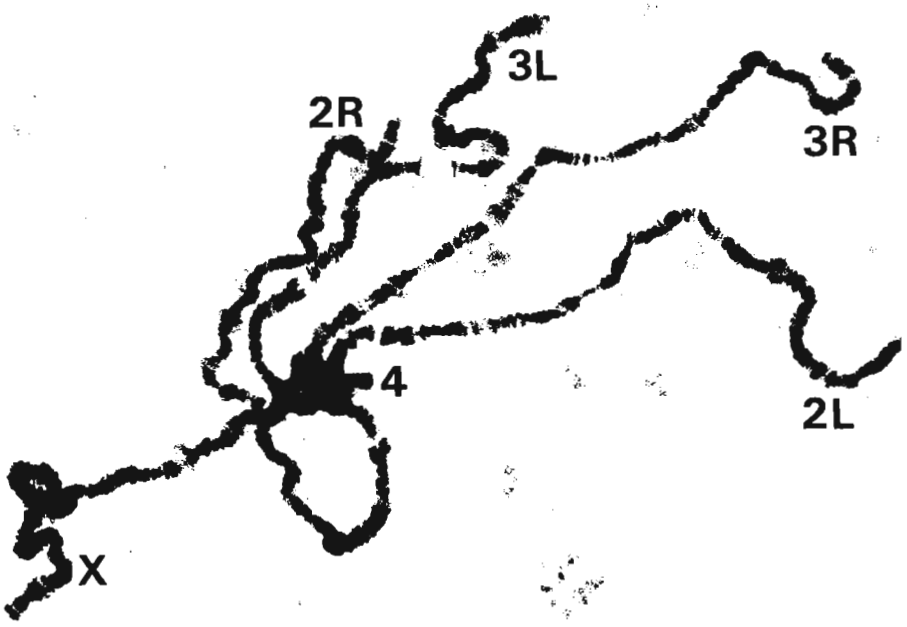


Fig. 1. Salivary gland chromosomes of D. auraria.

2R-chromosome (Sections 40-60): This chromosome is characterized by thick bands at 42D/E and faint band region between thick bands at 56A/E and 57A/D. The major puffs arise at 52B/D and 53C.

3L chromosome (Sections 61-80): This chromosome is easily recognized by the presence of the prominent puff at 64D. The short asynaptic regions at 77B and 79A/B can be seen in the proximal part of the chromosome.

3R-chromosome (Sections 81-100): This chromosome is marked by the presence of two puffs at 88A and 89C/D, five distinct bands

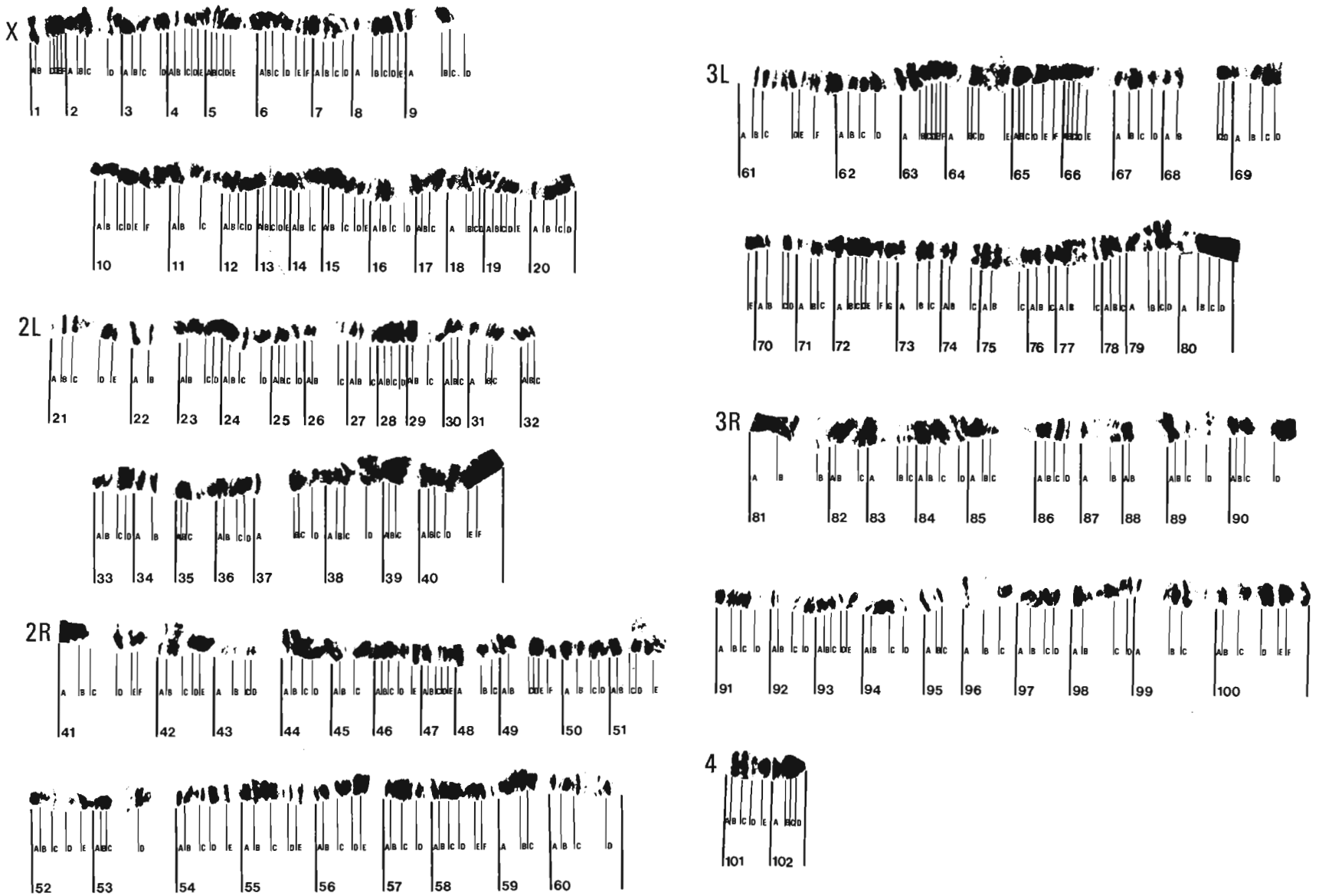


Fig. 2. Photomap of salivary gland chromosomes of D. auraria. [Oguma et al.]

at 91B/C and three thick bands at 98A. Also two short asynapses at 83A/C and 87A/B appear at the proximal region.

We could not observe Balbiani rings which have been reported in this species by Kastritsis and Grossfield (1971).

References: Kastritsis, C.D. and J. Grossfield 1971, DIS 30:120; Kurokawa, H. 1960, Japan. J. Genet. 35:161-166; ____ 1962, Japan. J. Genet. 37:510-517; ____ 1963, Japan. J. Genet. 38: 1-5; Lee, T.J. 1974, Japan. J. Genet. 49:305.

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New data on genetic and cytogenetic localizations of esterase-C and octanol dehydrogenase in D. melanogaster.

Esterase-C (Est-C) has been mapped at 51.7 (which should lie to the right of p, 48.0) on the third chromosome in D. melanogaster (Triantaphyllidis & Christodoulou 1973). In this case, Est-C should have been included in the large inversion which distinguishes D. melanogaster from D. simulans (84B-85A; 93F) and should lie closely to the left of pe (= p) in D. simulans. However, that it was not included in that inversion is indicated by the relative D. simulans map positions: Est-C, 59.6 (Triantaphyllidis & Christodoulou 1973) and pe, 101. Therefore, in order to examine the possibility that the locus lies to the left of p, the following crosses were carried out in D. melanogaster: A wild type stock with an Est-C variant allele (Est-$C^{4 \text{ or } 8}$) was crossed to two multiple marker stocks [ri pP (Cross I) and st pPcu (Cross II)] which carry Est-C^6. The mutant abbreviations are as follows (Lindsley & Grell 1968): ri-radius incompletus, 3-47.0; p^P-pink peach, 3-48.0; st-scarlet, 3-44.0; cu-curled, 3-50.0. F_1 females obtained from the crosses were backcrossed to males of the respective multiple marker stocks. The offspring were electrophoretically analyzed after scoring the visible marker phenotypes. As seen in Table 1, Est-C was mapped at 47.7 (Cross I) or 47.8 (Cross II) on 3R, confirming that the position is to the left of p^P (48.0). Since ri and pP represent the two closest flanking markers, we arbitrarily chose 47.7 as the locus of Est-C.

Table 1. Genetic mapping of Est-C in D. melanogaster.

	Phenotype of visible markers	Est-C^6/Est-C^6	Est-C^6/Est-$C^{4 \text{ or } 8}$	Genetic map position
Cross I	+ p^P	9	1	47.7 (47.5-47.9)*
	ri +	6	6	
Cross II	+ p^P cu	52	2	47.8 (47.6-48.0)*
	st + +	4	58	

*The 95% binomial confidence interval.

In order to examine cytogenetically the results, deficiency stocks (see Table 2) in that region of 3R were crossed to an Est-C^8 stock. The appearance of Est-C^8 hemizygous progeny would indicate the inclusion of Est-C within the deficiency. As shown in Table 2, only when Df(3R)$Antp^{NS+R17}$ was heterozygous with Est-C^8 could we detect the hemizygous Est-C^8 phenotype. In all other cases, we observed the Est-C^6/Est-C^8 phenotype in the F_1. Therefore, we can conclude that Est-C is cytogenetically located between 84B2 and 84D9.

Table 2. Results of cytogenetic mapping of Est-C in D. melanogaster.

Stock	Deficiency	Phenotype when heterozygous with Est-C^6	Est-C^8
Df(3R)Scr*	84A1; A6-B1	6	6-8
Df(3R)$Antp^{NS+R17}$*	84B1; 84D11-12	6	8
Df(3R)dsx^{D+R2}**	84D9; 84F16	6	6-8

*Kaufman, Lewis and Wakimoto (1980)
**Duncan and Kaufman (1975)

In addition, we found that Df(3R)cu^{40} (86C1-2; 86D8, kindly provided by Dr. M. Ashburner) included octanol dehydrogenase (Odh) in D. melanogaster. The cytogenetic localization is consistent with the genetic mapping of Odh (Courtright et al. 1966, 49.2±).

References: Courtright, J.B. et al. 1966, Genetics 54:1251-1260; Duncan, I.W. and T.C. Kaufman 1975, Genetics 80-733-752; Lindsley, D.L. and E.H. Grell 1968, Carn. Inst. Wash. Publ. 627; Triantaphyllidis, C.D. and C. Christodoulou 1973, Biochem. Genet. 8:383-390; Kaufman, T.C., R. Lewis and B. Wakimoto 1980, Genetics 94:115-133.

Ohnishi, S. and R. A. Voelker*. National Institute of Genetics, Mishima, Japan; *National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. New data on the genetic mapping of isocitrate dehydrogenase in D. melanogaster.

Fox (1971) reported that NADP-dependent isocitrate dehydrogenase (Idh) has been located at 3-27.1, which lies 0.6 map unit to the right of h (hairy, 3-26.5), i.e., to the right of se (sepia, 3-26.0) in D. melanogaster. However, Ohnishi and Voelker's data (1979), in which Idh was mapped to the left of se in D. simulans, suggested that Idh might be localized to the left of se in D. melanogaster, as there are no reported inversions in that region of the arm (Horton 1939). In order to examine this possibility, a wild type stock with a variant allele (Idh^6) was crossed to a multiple marker stock [jv (javelin, 19.2), se, by (blistery, 48.7)] which carries the common allele (Idh^4). F_1 females obtained from the cross were backcrossed to males of the marker stock. The offspring were electrophoretically analyzed after scoring for the visible markers. The results are shown in Table 1.

Table 1. Genetic mapping of Idh in D. melanogaster: Frequencies of Idh genotypes among recombinants obtained from the following cross:

$$\frac{jv\ se\ by\ (Idh^4)}{+\ +\ +\ (Idh^6)}\ ♀♀ \times \frac{jv\ se\ by\ (Idh^4)}{jv\ se\ by\ (Idh^4)}\ ♂♂$$

Phenotype of visible markers	Idh^4/Idh^4	Idh^4/Idh^6
jv se +	125	0
+ + by	0	110
jv + +	3	17
+ se by	27	0
jv + by	1	3
+ se +	6	1

As shown in the first lines of Table 1, Idh was located to the left of se at 25.4 [= 19.2 + (5/58 x 6.8)], rather than to the right of se as was previously reported. The 95% binomial confidence interval extends from 24.9 to 25.9. This result is consistent with the sequence of genes expected from the data of D. simulans.

Rawls and Lucchesi (1974) have cytologically localized Idh to 66B-67D. Hn (23.0) and h (26.5) have been localized, respectively, to 66A-B and 66D (Lindsley and Grell 1968). Thus, Idh must lie somewhere in the 66A-D interval.

References: Fox, D.J. 1971, Biochem. Genet. 5:69-81; Horton, I.H. 1939, Genetics 24:234-243; Lindsley, D.L. and E.H. Grell 1968, Carnegie Inst. Wash. Publ. No. 627; Ohnishi, S. and R.A. Voelker 1979, Japan. J. Genet. 54:203-209; Rawls, J.M. and J.C. Lucchesi 1974, Genet. Res. 24:59-72.

Oishi, K. and T. Ota. Kobe University, Kobe, Japan. Rudimentary ovary of tra-2^{OTF}-transformed "males": a transplantation experiment.

The action of a sex-transformation mutant, tra-2^{OTF}, is apparently leaky such that the transformed "males", X/X; tra-2^{OTF}/tra-2^{OTF}, have various structures with female characteristics (Fujihara et al. 1978). One of such structures is the gonad, which is ovary-like, though usually quite rudimentary. At some low frequency the gonad develops and becomes a nearly mature-looking ovary with eggs. Whether the larval gonads from these "males" can become functional if placed in normal surroundings was examined by transplanting them into normal third-instar female larvae. Host females were allowed to develop, and eclosed flies were test-crossed to determine if they produced any donor ovary-derived progeny. Table 1 shows the results.

Table 1. Results of transplantation of mid-third instar larval gonads of X/X; tra-2^{OTF}/tra-2^{OTF} into mid-third instar normal female larvae, and of progeny test-crosses.

	Host	Donor	No. hosts implanted	No. hosts eclosed	No. fertile	No. donor-derived progeny produced*
Experimental	A	B	75	56	54	0
Control	A	C	68	47	42	22

A: y w f/y w f; +/+
B: X,+/X,+; tra-2^{OTF} a px or/tra-2^{OTF} a px or
C: X,+/X,+; tra-2^{OTF} a px or/SM1(Cy)
*Results of test-crosses with y w f/B^sY; tra-2^{OTF}/tra-2^{OTF} males.

Donor larvae were first selected on the basis of gonad size (transformed flies have female-size gonads; Fujihara et al. 1978) and then separated on the basis of or color of Malpighian tubules into B and C.

In the experimental group none of the eclosed and fertile host flies (54) produced any donor ovary-derived progeny upon test-crossing. In the control group, however, 22 out of 42 host flies eclosed and were fertile, produced some donor ovary-derived progeny. Forty-eight out of 56 host flies eclosed in the experimental group were dissected. None had three mature-sized ovaries (two host-derived and one donor-derived). In many cases even rudimentary (donor-derived) gonads were not found, but in 15 flies there was at least one mature-sized egg floating freely inside the abdomen, suggesting that the transplantation itself was successful. In the control group, 44 out of 47 eclosed were dissected and 36 were found to have three mature-sized ovaries. Another set of experiments showed that the larval gonads from normal females (same as C in Table 1) transplanted into X/X; tra-2^{OTF}/tra-2^{OTF} larvae (same as B in Table 1) can develop into mature-looking ovaries. In each and all of the hosts eclosed and dissected (7/20), one mature-sized ovary plus rudimentary ovaries were detected. The mature-sized ovary was always donor-derived (large ovaries sometimes seen in tra-2^{OTF}-transformed flies can be easily distinguished from normal ones by morphology).

In conclusion, the larval gonads of X/X; tra-2^{OTF}/tra-2^{OTF}, which in situ develop into rudimentary ovaries, are at least by the mid-third instar so affected that they cannot develop into functional ovaries even when transplanted into normal female larvae.

Reference: Fujihara, Kawabe and Oishi 1978, J. Hered. 69:229.

Pilares, G. L. and M. P. Suyo. National University of San Marcos, Lima, Peru.

Distribution of different species of Drosophila from Peru (South America).

The fruit flies of the genus Drosophila are very well defined in the different biogeographical zones of the world. In South America, Peru is an excellent area due to its accidental geography and its inter-andean valleys separated from the tropical zone by a great geographical barrier: the Andes Cordillera. Usually, the species tend to expand from dispersion centers so that theoretically in a period of time they can occupy all those places where they find appropriate ambient conditions.

In some cases when a certain species arrives at a new place, it undergoes modifications to enable its adaptation and this produces a transformation into new species (Jordan & Kellog 1915). This is why there are species that although living in different zones are morphologically very similar, but with notable differences especially in sexual behavior and in chromosome number. From the point of view of geographical isolation this is of great value and can lead to an understanding of the mechanisms of speciation.

Based on collections done in some regions of Peru since 1973 (Pilares and Vásquez 1977) and due to the versatility of the zones and season of collection (Pilares et al. 1981), we have been able to increase until 1980 a more representative number of species from nine departaments and some provinces. The zonification also considers altitude above sea level from the back sides of the Andes, from 300 m above sea level on the western slope. The collection was done at pre-established points (Fig. 1), such as domestic and wild habitats on fruits, flowers, green leaves, mushrooms and traps of fermented bananas.

For identification we have used the keys by Brncic (1957), Kikkawa (1938), Pavan (1949), Wheeler (1959, 1970), Patterson (1948), and Spencer (1950b). We have identified a total of 69 fruit fly species and two sub-species (Ayala 1973) and have registered six sub-genera (Table 1):

Dorsilopha	(1)	Scaptodrosophila	(2)	Sophophora	(15)
Hirtodrosophila	(1)	Drosophila	(39)	Phloridosa	(2)

From the total number of species, nine are not located in any of the sub-genera. The more important collection sites are:

- Northern Coast	= La Libertad	: Trujillo
- Central Coast	= Ancash	: Huaráz, Bolognesi, Huaylas
	= Lima	: Lima, Huaral, Cieneguilla, Lomas de Lachay
	= Ica	: Pisco
- Southern Coast	= Tacna	: Tacna
- Central Sierra	= Junin	: Huancayo
- Southeast Sierra	= Cusco	: Cusco

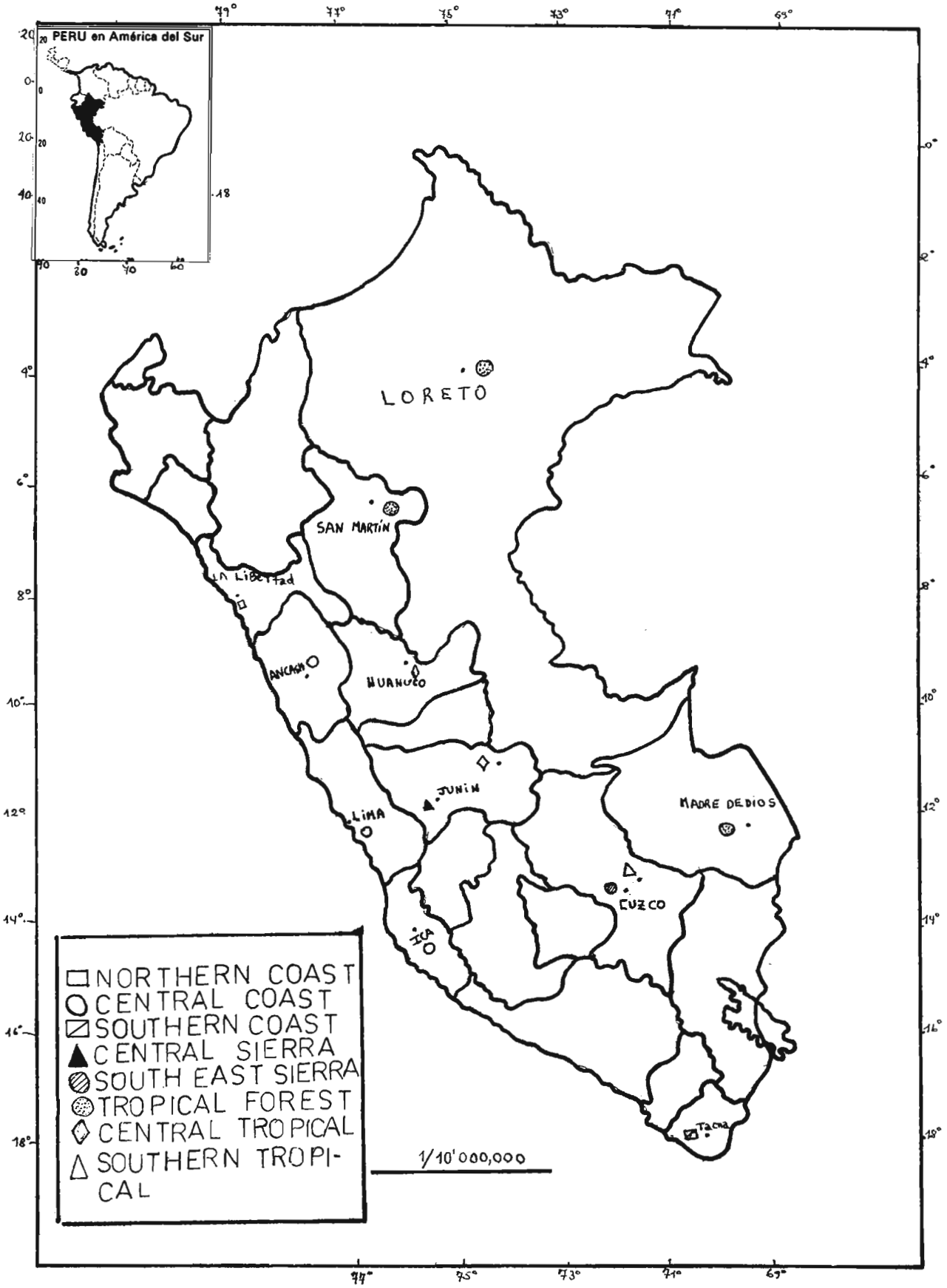


Fig. 1. Distribution of Drosophila species in 11 departaments of Peru.

- Tropical Forest sea level	= Loreto	: Iquitos, Maynas, Sungarococha, Cantón
	= Madre de Dios	: Tambopata, Callanga
	= San Martin	: Tarapoto
- Central Tropical Forest high altitude	= Junin	: Chanchamayo, La Merced
	= Huanuco	: Tingo María
- Southern Tropical Forest high altitude	= Cusco	: La Convención, Machu Picchu, Quillabamba, Paso de Lares, Pilcopata

Table 1. Distribution of different species of Drosophila in 11 departaments of Peru.

Sub-genera	Species		
1. Dorsilopha	1. busckii		
2. Drosophila	2. similis	15. mesophragmatica	28. mediostriata
	3. annularis	16. cardini	29. annulimana
	4. flavopilosa	17. procardinoides	30. guaranunu
	5. lindae	18. prosimilis	31. paranaensis
	6. pallidipennis	19. peruviana	32. fulvimacula
	7. quadrum	20. viracocha	33. cardinoides
	8. andina	21. orkui	34. limensis
	9. calloptera	22. triangulina	35. mercatorum
	10. inca	23. lugubripennis	36. repleta
	11. novemaristata	24. polymorpha	37. nigricuria
	12. virilis	25. mediosignata	38. pachua
	13. hyalipennis	26. hydei	39. immigrans
	14. dentata	27. neoguaranunu	40. divisa
3. Hirtodrosophila	41. glavifrons		
4. Phloridosa	42. florícola	43. alei	
5. Scaptodrosophila	44. mirim	45. latifasciaeformis	
6. Sophophora	46. emarginata	51. willistoni	56. kikkawai
	47. melanogaster	52. willistoni willistoni	57. prosaltans
	48. simulans	53. willistoni quechua	58. fummipennis
	49. ananassae	54. nebulosa	59. capricorni
	50. succinea	55. tropicalis	60. bocainensis
Species not registered in sub-genera	61. cuzcoica	64. latibuccata	67. peruensis
	62. canescens	65. maculifrons	68. pulvera
	63. impudica	66. obscuricolor	69. onicophora

Financial assistance from PNUD/UNESCO RLA/78-024 and "Antonio Raimondi" Institute of San Marcos University is gratefully acknowledged.

References: Ayala, F. 1973, J. Hered. 64(3); Brncic, D. 1957, Monografía Universidad de Chile; Jordan, D.S. and Kellogg, V.L. 1915, Evolution and Animal Life, Appleton, New York; Kikkawa, H. and R.T. Peug 1938, Jap. Jour. Zool. 7:507; Pavan, C. 1949, Especies Brasileñas de Drosophila; Patterson, J.T. 1943, Univ. Texas Publ. 4313:7; Pilares, G.L. and E.J. Vásquez 1977, Rev. Per. Entom. 20(1):106; Pilares, G.L., V. Rafael, M.P. Suyo and J. Vásquez 1981, Vol. 23(1); Spencer, W.P. 1950b, John Willey & Sons, Inc.; Wheeler, M. 1959, Univ. Texas Publ. 5914:181-205; Wheeler, M. 1970, Museu de Zoologia Universidade de Sao Paulo.

Polanco, M. M. E. and H. F. Hoenigsberg. Universidad de Los Andes, Bogotá, D. E., Colombia. D. pseudoobscura from the Colombian Altiplano.

After Hunter and Dobzhansky collected Drosophila pseudoobscura in Bogotá in 1963, there have been several collections made in the Altiplano which reported variable findings (1963, 1964, 1965, 1966, 1969, 1970, 1972-1980). While collections done by Hoenigsberg in 1956, 1957, 1958, 1959 and 1961 did not report D. pseudoobscura in the Altiplano, those after 1963 have found them. The so-called Bogotá collection (Dobzhansky et al. 1963) did come from the city of Bogotá. However, Hoenigsberg's collections (unpublished results) came from various natural woods distant from Bogotá and throughout the Cordillera Oriental of the Colombian Andes. The collections which report D. pseudoobscura from Colombian Andes are:

Collector(s)	Year	Locality	Altitude (mts)
Hoenigsberg, H.F.	1963	Cátedra	2600
	1964	Mosquera	2600
	1965	Sopó	2640
	1966	Sopó	2640
		Tabio	2660
		Chia	2660
		Zipaquirá	
Hoenigsberg, H.F.	1969	Torobarroso	2660
& Dobzhansky, Th.	1970	Torobarroso	2660
	1972	Aguas Calientes	2680
	1973	Tenjo	2680
	1974	El Recreo	2690
Hoenigsberg, H.F.	1975-1979	Torobarroso	2660
		Tunja	2650
		Paipa	2550
		Lanceros	2550
Palomino, J.J.	1977	Pamplona	2500
Hoenigsberg, H.F.,	1978-1980	Sochagota	2550
Polanco, M.M.E.,		Duitama	2500
Barrera, I., &		Sogamoso	2500
Herrera, O.L.		Santillana	2660
		Torobarroso	2660
		Aguas Calientes	2680

References: Dobzhansky, Th., A.S. Hunter, O. Pavlovsky, B. Spassky and B. Wallace 1963, Genetics 48:91-103.

Puro, J. and E.-L. Kiiskilä. University of Turku, Finland. Cytogenetics of T(2;3)rn.

T(2;3)rn is a whole-arm transfer between two major autosomes of D. melanogaster. Carlson (1956) assumed that in both chromosomes the breaks are slightly to the right of the centromere. If so the left arm of 2 would be joined to the right arm of 3 and vice-versa. Oksala (1958), on the other hand, considered it probable that one break lies in the heterochromatin of 2L and the other in the proximal euchromatin of 3R. Duncan and Kaufman (1975) reported that the translocation breakpoints are in 40-41 and 80-81, i.e., in the chromocenter and, in addition, that there is an inversion, In(3R)81F; 84D. The locus of rn was mapped in or just proximal to 84B1,2.

Since the linkage configuration still is obscure we report here results showing that the configuration of T(2;3)rn is 2L·3L; 2R·3R. The test involved crossing T(2;3)rn to two previously analyzed whole-arm transfers T(2;3)FM29 = 2L·3L; 2R·3R and T(2;3)FM46 = 2L·3R; 2R·3L (Puro 1973). The arms of chromosome 3 were marked, in FM29, by ru and h (3L) and ca and bv (3R) and, in FM46, by in and ri (3L) and pP (3R). Both types of doubly heterozygous males were then tested for the type of distribution of 3L and 3R by mating to females homozygous

for the respective markers. Random distribution of the markers is evidence for similarity and (pseudo)linkage for dissimilarity between the translocations in the males tested. Following numbers of offspring were counted:

Males tested	ru h ca bv or in ri p^p	wild	ru h or in ri	ca bv or p^p
T(2;3)rn/T(2;3)FM29, ru h ca bv	61	57	76	65
T(2;3)rn/T(2;3)FM46, in ri p^p	79	101	0	0

The configuration of T(2;3)rn having been determined it was then of interest to ask if the rn gene is transmitted by 2L·3L or 2R·3R. Since, in the first cross, the recombinant classes ru h and ca bv were derived respectively from the types of sperm where 2R·3R and 2L·3L of T(2;3)rn had been segregated, one of them should carry the gene for rotund wings. Therefore, ru h and ca bv male offspring were mated to females heterozygous for T(2;3)rn and also to females heterozygous for rn^3, which is an X-ray induced point mutation allele of rn (Hanna-Alava, unpublished). Corroborating the localization of rn at 83B1,2 by Duncan and Kaufman, our results showed that rn is transmitted by 2R·3R.

References: Carlson, E.A. 1956, DIS 30:109; Duncan, I.W. and T.C. Kaufman 1975, Genetics 80:733-752; Oksala, T. 1958, Cold Spring Harbor Symp. 23:197-210; Puro, J. 1973, Hereditas 75: 140-143.

Puro, J. and E. Novitski. University of Turku, Finland, and University of Oregon, Eugene, Oregon. In(3L)F removed from In(3LR)DcxF.

In(3LR)DcxF is a complex rearrangement with In (3L)62; 67 [designated here as In(3L)F according to its discoverer Federova] superimposed on In (3LR)CxD (Lindsley & Grell 1968). Because of being a good crossover suppressor and carrying the dominant marker, Dichaete, In(3LR)DcxF is widely used as a balancer for chromosome 3 mutants. Dichaete was lost, however, in a stock where In(3LR)DcxF was used to balance Dp(1;3)B^S, a duplication carrying Bar^S and some heterochromatin on the tip of 3L. [Dp(1;3)B^S was obtained by B. Leigh of the University of Leiden and was later used as material to generate autosomal compounds consisting of two entire chromosome 3's; see Novitski, Grace & Stommen, Genetics 98, 1981.] Nevertheless, the derivative chromosome still balanced Dp(1;3)B^S. Salivary gland preparations made of Dp(1;3)B^S/the derivative balancer (see figure) reveal in 3L a heterozygous terminal knob and a loop of an inversion with the breakpoints at 62B and 67D. Apparently In(3L)F was separated from In(3LR)CxD, the other component of In(3LR)DcxF, by rare crossing over and was subsequently selected because of a better viability as compared to the Dichaete bearing DcxF chromosome. Such a change is expected to take place in stocks carrying In(3LR)DcxF to balance lethals or other genetic material in distal segments of 3L. However, without a dominant marker, like Bar^S in the present case, a loss of Dichaete in such a stock would probably be misinterpreted as a contamination.

In(3L)F is homozygous lethal and has no phenotypic effect in heterozygotes. Since B^S is easily reverted to wild type in Dp(1;3)B^S/In(3L)F, In(3L)F is currently being kept in combination with TM1, Me and is available in the Laboratory of Genetics, University of Turku and in the European Drosophila Stock Center at the University of Umeå, Sweden.

Ringo, J. M. and D. F. Wood. University of Maine, Orono, Maine. Gregarious pupation site selection in D. melanogaster and D. simulans.

We studied the distribution of pupae of D. melanogaster and D. simulans in 35 ml culture vials. An outcrossed wild-type stock of each species was used. Culture vials containing cornmeal-molasses-yeast medium were stoppered with cotton plugs so that the food-to-stopper distance was 66 mm. Fifty eggs, 0-6 h old, were placed on a moist square (approximately 5 mm square) of aseptic tissue and this tissue was placed on the surface of the culture medium in a vial. Fifteen replicate vials were prepared for each species. After incubating 9-10 d at 22 ± 0.5°C, 12:12 LD cycle, each vial was marked off in six 11 mm wide bands with a felt tip pen. The bands were subdivided into 8 equal sections by 8 vertical lines also marked with a felt tip pen.

On the vial walls, neither species exhibited a light bias (D. simulans: $\chi^2 = 11.23$, df = 7, $0.10 < p < 0.25$; D. melanogaster: $\chi^2 = 8.26$, df = 7, $0.25 < p < 0.50$), and on the medium D. melanogaster was unbiased ($\chi^2 = 1.63$, df = 3, $0.50 < p < 0.75$) while D. simulans tended to pupate on the side farthest from the light ($\chi^2 = 26.52$, df = 3, $p < 0.005$). Furthermore, when rings were lumped within vials, there was no tendency to clump. This was determined by comparing the number of coefficients of dispersion greater than 1.0 out of the 15 replicates with the expected number (7.5); for D. melanogaster, $\chi^2 = 1.67$, $0.10 < p < 0.25$, and for D. simulans, $\chi^2 = 3.27$, $0.05 < p < 0.10$.

In D. melanogaster, pairs, triplets, or other n-tuples of contiguous pupae were observed on each vial wall. The mean number of pupae (± SE) per vial arranged in this way was 9.3 ± 0.1, or about 20% of the flies. In D. simulans, contiguous pupation was observed only on the medium, where it could not be scored accurately. We have seen similar behavior--contiguous, parallel pupation--in casual observations of other species of Drosophila, for example, D. busckii and D. virilis. Gregariousness of adult Drosophila, particularly ovipositing females (del Solar & Palomino 1966; Spieth 1968), is a well-documented tendency that may affect fitness by helping to produce optimal larval densities (Sang 1949; Lewontin 1955; Lewontin & Matsuo 1963). We can only speculate that pupal gregariousness may be a fitness character.

References: del Solar, E. and H. Palomino 1966, Amer. Natur. 100:127-133; Lewontin, R.C. 19755, Evolution 9:27-41; Lewontin, R.C. and Y. Matsuo 1963, Proc. Nat. Acad. Sci. (USA) 49: 270-278; Markow, T.A. 1979, Behav. Genet. 9:209-217; Sang, J.H. 1949, Physiol. Zool. 22:202-210; Spieth, H.T. 1968, Evol. Biol. 2:157-193.

Robertson, H. M. University of the Witwatersrand, Johannesburg, South Africa. Female preference for normal or wingless males in D. melanogaster.

Eastwood and Burnet (1979) found that when presented with the choice of a normal and a wingless male, single Novosibirsk females mated randomly. Averhoff and Richardson (1974), without presenting experimental techniques or data, report similar results. In the light of the clearcut disadvantage of wingless males in single-pair matings (e.g., Manning 1967) and mass-matings (e.g., Ewing 1964) this result is surprising. I therefore repeated this experiment with Canton-S males which I had previously established are extremely disadvantaged when wingless in single-pair matings (Robertson, submitted to Anim. Behav.).

Single 3-day-old females were placed in a small observation chamber (25 mm diameter, 8 mm deep) with a normal and a wingless male (both 3 days old; wings surgically removed on day 2) and observed till copulation or for 30 min. The normal male copulated in 36 of the 46 trials, the wingless in 4, and 6 did not lead to copulation. Unlike those of Averhoff and Richardson (1974) these wingless males were highly competent in all aspects other than final successful copulation, i.e., they usually started courting first and usually ousted the normal male from the position behind the female. The males' jostling for position behind the female appeared to agitate and inhibit her so that many trials were prolonged before copulation. The winged male usually copulated when briefly courting the female alone. It seemed that a single, undisturbed, normal courtship bout was sufficient and important for copulation.

Clearly the wingles males' disadvantage is retained in these results, which strongly support the contention that, at least in this strain, the wing vibration provides important, even if not essential, stimuli. Eastwood and Burnet's (1979) suggestion that the importance of these stimuli differs in various strains best explains these, their and Averhoff and Richardson's (1974) results.

References: Averhoff, W.W. and R.H. Richardson 1974, Behav. Genet. 4:207-225; Eastwood, L. and B. Burnet 1979, Experientia 35:1159-1160; Ewing, A.W. 1964, Anim. Behav. 12:316-320; Manning, A. 1967, Anim. Behav. 15:239-250.

Robertson, H. M. University of the Witwatersrand, Johannesburg, South Africa. Courtship duration decreases with age in young D. melanogaster females.

Table 1. Change in number of females mating and courtship duration with female age.

Female age (hours)	N	Courtship duration (secs) Mean	S.D.
12-15	0	--	--
15-18	0	--	--
18-21	2	914	795
21-24	3	430	285
24-27	9	550	445
27-30	--	--	--
30-33	8	385	297
33-36	--	--	--
36-39	10	288	187
39-42	8	395	239
42-45	10	297	183
45-48	8	314	277
48-51	10	186	92
51-54	--	--	--
54-57	10	207	155
78-81	10	194	200

Manning (1967) found that young D. simulans females, if receptive, exhibited uniformly short courtship durations before mating, suggesting that switch-on of female receptivity is independent of the "courtship summation process" which ostensibly controls courtship duration. Yacher and Spiess (1973) and Cook (1973) independently found that mating speed and courtship duration in D. persimilis and D. melanogaster increased and decreased respectively, suggesting that switch-on and courtship duration are not independent and both are controlled by the courtship summation threshold.

I collected Canton-S females every hour and tested them in single-pair matings with 3-day-old males which had been kept singly for one day. The tests were done in a perspex chamber (25 mm diameter, 8 mm deep) between 0800 and 1100 hours, so that females could be categorized in three-hour brackets, for 30 min each with ten females tested per three-hour bracket. Most females were receptive after 24 hours, but courtship duration continued to decrease until 48 hours, after which it was constant (Table 1).

These results agree with those of Yacher and Spiess (1973) and Cook (1973). This effect may, however, only be the result of a decrease in the length of the "female latency" resulting from the females' agitation (Robertson, submitted to Anim. Behav.). Eastwood and Burnet (1977) found such an effect for the male latency to courtship. Switch-on of female receptivity may yet be shown to be independent.

References: Cook, R.M. 1973, J. Insect Physiol. 19:397-406; Eastwood, L. and B. Burnet 1977, Behav. Genet. 7:359-372; Manning, A. 1967, Anim. Behav. 15:239-250; Yacher, T.H. and E.B. Spiess 1973, Anim. Behav. 21:359-370.

Robertson, H. M. University of the Witwatersrand, Johannesburg, South Africa. Effect of age and sexual deprivation on courtship of D. mauritiana females by D. melanogaster males.

Few studies have been made on the effect of age and sexual deprivation on the specificity of courtship responses in Drosophila. Pruzan et al. (1979) have shown that virgin females of some D. paulistorum "semispecies" and the sibling species D. melanogaster and D. simulans aged 9 and 11 days respectively are usually as unlikely to mate interspecifically as are 3-day-old virgins. What about the propensity of males to court interspecifically?

I compared the propensity of 2- and 12-day-old D. melanogaster males to court 3-day-old D. mauritiana females. For each age class 20 single pairs were observed in a small observation chamber (25 mm diameter, 8 mm deep) for 10 min each. Of the 2-day-old males, six only tapped the female and then left her. Another three started courting by following and directing wing vibration at the female, but eventually tapped her again and stopped courting. The remaining 11 continued courting until they licked the female, and then stopped after tapping her vigorously. Thus within 10 min all 20 2-day-old males stopped courting the D. mauritiana females.

Of the 12-day-old males, one stopped courting after licking the female and another three after making full attempts at copulation. The remaining 16 males courted throughout the 10 min observation period and six attempted copulation (they had some difficulty because the females avoided them by running). Clearly age and/or sexual deprivation reduced the specificity of the males' responses. Further experiments with old but non-deprived males are needed.

References: Pruzan, A., L. Ehrman, I. Perelle and J. Probber 1979, Experientia 35:1023-1025.

Robertson, H. M. University of the Witwatersrand, Johannesburg, South Africa. Male activity during Drosophila copulation.

Despite numerous reports of measurements and comparisons of copulation durations, Drosophila flies are always described as being inactive or quiescent during copulation. During a series of experiments on the Canton-S strain of D. melanogaster, a locally caught strain of D. simulans, and a D. mauritiana strain, I noticed that during copulation in all three species the male repeatedly extended and vigorously rubbed or vibrated his meso- and metathoracic legs against the sides of the female's thorax and legs. The mesotarsi in particular were rubbed against her thorax and reached onto her eyes. This is not the same as the "massaging" behavior noted by Bennet-Clark et al. (1980) in Zaprionus copulation. In D. melanogaster these bouts of rubbing were initially brief (± 0.5 s) and frequent (up to 5 per min) and later became longer (± 1 s) and less frequent (about 1 per min). Each copulation contained 25.4 ± 9.9 (S.D.; N = 10) bouts of rubbing (copulation duration = 17.2 ± 2.2 min).

To investigate whether this rubbing somehow enhanced fertility, perhaps by promoting sperm transferal, single D. melanogaster females were placed in food vials, each with two males with various degrees of amputation of the meso- and metathoracic legs. The vials were examined for progeny after 20 days. Removal of meso- and metatarsi (N = 15), meso- and metatarsi and mesotibiae (N = 30), or meso- and metatarsi and tibiae (N = 20) did not have any obvious qualitative effect since most vials contained large numbers of progeny. In each test a few vials contained no progeny, presumably because the males were unable to copulate.

Observations of a few copulations by these amputated males showed that they still attempted to rub the female's thorax, but, especially in the last test, their legs did not reach further than her abdomen. These copulations were also of normal length. Dissection of females inseminated within the last 24 hours by these latter males showed no obvious difference in the sperm content or placement in the seminal receptacle or spermathecae when compared to females similarly inseminated by normal males.

The initially high frequency of rubbing while the female is making what appear to be rejection movements, e.g., kicking and pushing with the hind legs, might suggest that the rubbing induces female quiescence, but later when the pair was quiet, the males' vigorous rubbing instead induced female activity. Also, females were as quiet with amputated males. Therefore, as yet, no function can be ascribed to this, in fact, prominent male activity during copulation.

Reference: Bennet-Clark, H.C., Y. Leroy and L. Tsacas 1980, Anim. Behav. 28:230-255.

Robertson, H. M. University of the Witwatersrand, Johannesburg, South Africa. Polygenic autosomal control of male genitalic shape in D. simulans and D. mauritiana.

The study of the genetics of species differences is usually limited by the prevalence of hybrid sterility. Thus species pairs which produce fertile hybrids, and exhibit structural or behavioral differences, are particularly useful for such studies. D. simulans and D. mauritiana produce fertile female hybrids, and differ dramatically in the shape of the posterior lobe of the genital arch of the male genitalia. This structure is large and flat with a large ventral hook in D. simulans, and narrow and straight in D. mauritiana (Tsacas & David 1974).

Table 1. Shape of the posterior lobes of the genital arches in male backcross progeny of hybrids of D. simulans and D. mauritiana.

F_1 female / Male	sim♀-mau♂ mau	mau♀-sim♂ mau	sim♀-mau♂ sim	mau♀-sim♂ sim	Total
F_1	39	44	34	44	161
Intermediate	108	136	117	110	471
Parental	52	51	43	73	219

In hybrids from both reciprocal crosses (240 males examined) this structure was uniformly intermediate, i.e., midway in breadth with a small ventral hook. This suggests autosomal control with no dominance. The backcross progeny showed continuous variation from this F_1 shape to the shape of the appropriate male parent. They were therefore broadly classed as being F_1, intermediate, or male parental (Table 1). At least two independently assorting loci must be involved since the ratios approximate 1:2:1

proportions. The continuous variation which is artifically compartmentalized in this classification suggests that more loci are involved. Again there is no evidence of dominance or sex linkage.

Reference: Tsacas, L. and J. David 1974, Bull. Soc. Entomol. Fr. 79:42-46.

Roca, A., F. Sanchez Refusta, C. Graña and M. A. Comendador. University of Oviedo, Spain. Chromosomal polymorphism in a population of D. melanogaster.

Natural and laboratory populations of D. melanogaster are frequently polymorphic for chromosomic inversions (Ashburner & Lemeunier 1976). Considering their geographic location and their frequency, the inversions have been categorized by Inoue and Watanabe (1979) as follows: Common cosmopolitan inversions are the most frequent world-wide inversions on every major autosomal arm; Rare cosmopolitan inversions are less frequent than the above but are also world-wide in distribution; Recurrent endemic inversions are fairly frequent and widespread inversions found only in related populations; Unique endemic inversions are usually observed in a single individual or its brood from a single population, and never found in a different population.

In this note we are going to present some preliminary results found in a natural population of D. melanogaster. The studied population comes from a small chestnut grove near Oviedo (Spain). In September of 1980, 320 inseminated females were caught. From the offspring of these females 80 second chromosomes and 75 third chromosomes were isolated by crossings with the strain Cy L/Pm; TM_3/Pr Dr. This chromosome sample can be considered as representative of the population in the time of the capture (SE-80). Beside it, a couple from the offspring of each caught female was introduced in a cage to start a population (CPSE-80) which has been afterwards maintained at 21°C. This cage population was analyzed in June of 1981 (approximately 18 generations after its initiation). In February of 1981, 100 couples from the CPSE-80 were taken out, introduced in 10 bottles (10 couples in each bottle) and maintained by rotatory crossings at 24°C (CASE-80). Simultaneously to the analysis of this sample (May 1981) a new sample of the same population was captured in the wild (SE-81A). Another sample was taken 15 days later (SE-81B). Both collections were analyzed immediately after capture.

The chromosomic analysis of each individual was done through its offspring in crosses with individuals homozygotic for the standard arrangement published by Bridges. The lactic-acetic orcein staining procedure was used to visualize the polytene chromosomes of the salivary glands of the females in the third instar larvae.

The presence of a new reciprocal translocation [T(1;2) 7E;55A], which involves the first and second chromosomes, in an individual of the CPSE-80 sample, is remarkable, since this type of translocation is extremely rare in D. melanogaster. Among the 197 reciprocal translocations between these two chromosomes, described by Lindsley and Grell (1968), only two of them were of spontaneous origin and 190 induced.

The inversion frequencies found in each of the samples and their breakpoints are given in Table 1.

Table 1. Frequencies (%) of inversions per major autosome arms.

Inversion	Sample SE-80 (*)	CASE-80 (128)	CPSE-80 (260)	SE-81A (180)	SE-81B (238)	Category
In(2L)t 22D; 34A	20.00	47.66	40.77	15.00	21.01	Common cosmopolitan
In(2R)NS 52A; 56F	12.50	24.22	18.46	14.44	12.60	Common cosmopolitan
In(3L)P 63C; 72E	13.33	24.22	10.00	7.22	13.02	Common cosmopolitan
In(3R)P 89C; 96A	22.67	15.62	18.85	3.33	12.60	Common cosmopolitan
In(3R)C 92D; 100F	10.67	25.00	7.69	10.00	11.76	Common cosmopolitan
In(3R) 93E; 97D	---	0.78	1.15	---	---	Rare cosmopolitan
In(3L)Y 68F; 75C	---	---	---	---	1.26	Rare cosmopolitan
In(3LR) 69A; 83A	---	---	---	---	1.26	Unique endemic
In(2L) 33A; 34C	---	---	0.38	---	---	Unique endemic
In(3L) 67B; 70C	---	---	---	0.56	0.42	Unique endemic

( ) Number of chromosomes tested.
* 80 second chromosomes and 75 third chromosomes.

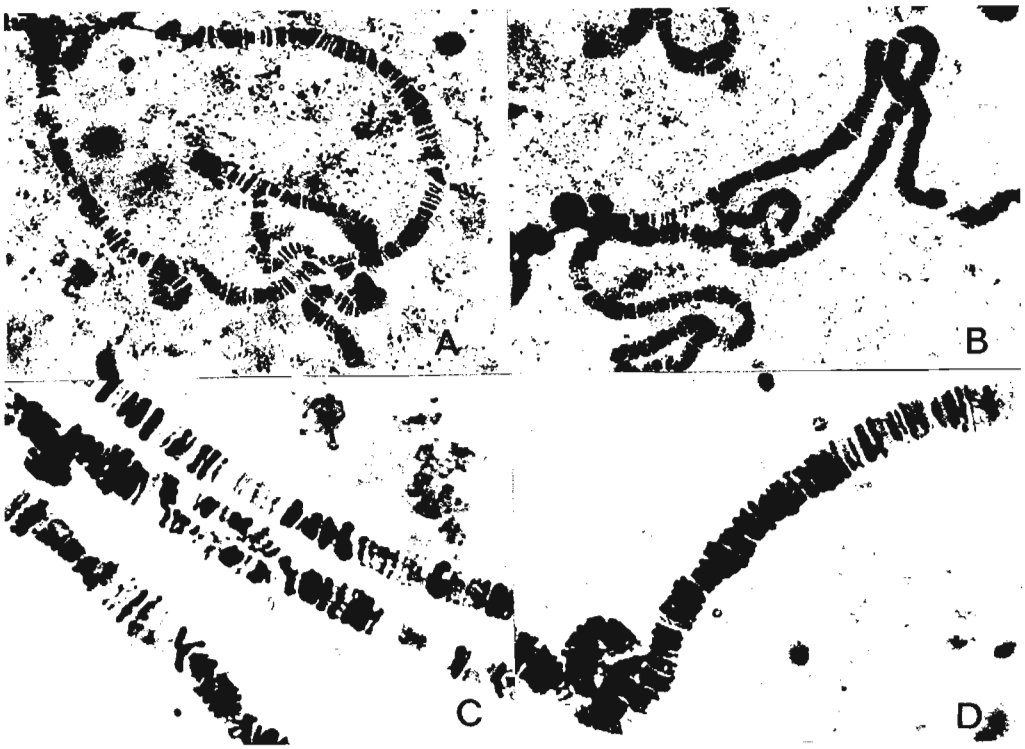


Fig. 1. (A) T(1;2)7E;55A. (B) In(3LR)69A;83A and In(3L)Y 68F;75C. (C) In(2L)33A;34C. (D) In(3L)67B;70C.

The photographs of the new inversions described here are shown in Fig. 1 (B-D).

The frequencies of common cosmopolitan inversions in samples of our natural population (SE-80, SE-81A and SE-81B) fall into the range of the values for other populations, and the only data to emphasize are the strong variation shown by 3RP. This inversion has been considered as the commonest in D. melanogaster (Ashburner & Lemeunier 1976), and shows great variations among different populations and along the years (Inoue & Watanabe 1979). At the present time, because of the lack of data from new captures, it is not possible to explain the reason for this variation.

On the other hand, it should be noted that strong variations of the common cosmpolitan inversions occurred when the samples were maintained in laboratory conditions (CASE-80 and CPSE-80) when they are compared to the original sample (SE-80). Alahiotis et al. (1977) have suggested that the frequency of 2Lt is increased in high density conditions. Our results are in agreement with this suggestion, because the samples maintained in the laboratory, where they have available a rich medium and consequently can reach higher densitites than in the restrictive conditions of the field, show a level of 2Lt of more than 40%.

The inversions 3RY and 3R 93E; 97D have been quoted as recurrent endemic from Japan and unique endemic from Japan respectively (Inoue & Watanabe 1979). According to our findings, both inversions should be considered as rare cosmopolitan. Moreover it is probable that 3LR 69A; 83A was produced on a preexisting 3RY since this one must be older because it is cosmopolitan.

With regard to the disputed origin of the unique endemic inversions as a result of the hybrid dysgenesis (Mettler et al. 1977) or the action of a "mutator" (Inoue 1979), we believe that our results support the last hypothesis for the following reasons: The frequency of these inversions per autosomal major arm is 0.31%, similar to the one detected in other populations in which the presence of the "mutator" seems indubitable (Yamaguchi 1976; Yamaguchi et al. 1976; Inoue 1979). In addition, the notable recurrence of unique endemic inversions in our population makes improbable the hypothesis of hybrid dysgenesis and then the presence of a "mutator" is postulated.

References: Alahiotis et al. 1977, DIS 52:106-108; Ashburner, M. and F. Lemeunier 1976, Proc. R. Soc. Lond. B. 193:137-157; Inoue, Y. 1979, Japan. J. Genet. 54:83-96; Inoue, Y. and T.K. Watanabe 1979, Japan. J. Genet. 54:69-82; Lindsley, D.L. and E.H. Grell 1968, Carn. Inst. Wash. Publ. 627; Mettler, L.E. et al. 1977, Genetics 87:169-176; Yamaguchi, O. 1976, Mutation Res. 34:389-406; Yamaguchi, O. et al. 1976, Genetics 83:409-422.

Sapunov, V. B. Department of Biology, Leningrad Medical Institute for Sanitation, Leningrad, 195067, USSR. Inter-line differences of juvenile hormone activity in D. melanogaster.

Reproductive function in insects depends on the gland corpus allatum (Engelmann 1970); the effect of this gland on male mating activity is different in different insect groups. Some data are contradictory. As far as we know, this question has not been studied in Drosophila. The purpose of this work was to study the activity of juvenile hormone (JH) in D. melanogaster lines differentiated by male mating activity.

We used the following lines: K-1, Canton-S (wild stock), LA and HA. The two latter lines are inbred, selected by differences in male mating activity (Kaidanov et al. 1978). JH activity was checked by the method of deWilde et al. (1968), with some modification. The flies (aged 5-7 days) were etherized and the quantity of flies of weight M was considered.

M was about 0.4-0.6 gram. It was homogenized and put into ester-alcoholic solution (6:1) with a weight of 5M. In 5 hours the hormone liquid was pipetted out from the homogenized flies. 5M of ester was poured into the rest of the fly homogenate. In two hours the second solution was added to the first and was evaporated. After evaporation the substances settled in the bottom of the glass were the lipid solution of JH.

The assignment of our hormone was designed to test JH in relation to its morphogenic activity in flies of Canton-S stock. Flies of this line appeared not to have any abnormal abdomen type (Sapunov & Kaidanov 1978). The appearance of this trait is proportional to morphogenic activity of the solution of JH that is applicable to insects of prepupal stage (Postlethwait 1974). Drops of juvenile hormone were applied in doses of 0.07 mkl over the surface of prepupae of Canton-S. Both male and female flies having the trait (abnormal abdomen) were checked after metamorphosis. The results are shown in Table 1.

Table 1. Morphogenic effectivity of JH from different lines of D. melanogaster.

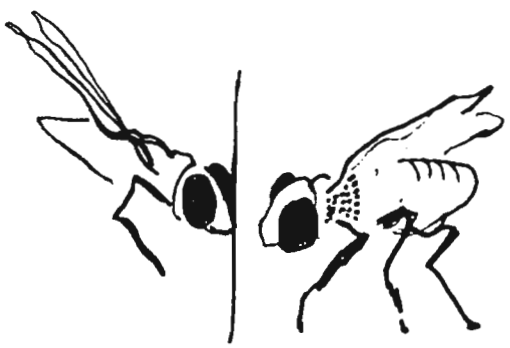
Sex of tested flies (Canton-S)	K-1 wild stock		LA		HA	
	n	%	n	%	n	%
Females	947	7.1 ± 0.83	1072	3.5 ± 0.56	914	9.0 ± 0.95
Males	1053	7.0 ± 0.84	1145	5.2 ± 0.66	1059	14.0 ± 1.07

The morphogenic activity of hormone in LA stock is lower than that in wild strain (K-1) by 1.5-2 times. The JH activity in flies of HA stock is maximal. The rate of abnormality is higher in females than in males.

The data suggest that the differences in male reproductive function are accorded to JH activity. Perhaps the selection in increasing or decreasing the male mating activity is related to the endocrine system, principally to the gland corpus allatum, which synthesizes JH. These data correspond to that of Foster (1967), who studied dung flies. According to his experiment, allatoctomized males showed no mating activity.

We suggest that the reproductive function of Drosophila may be determined by hormonal factors.

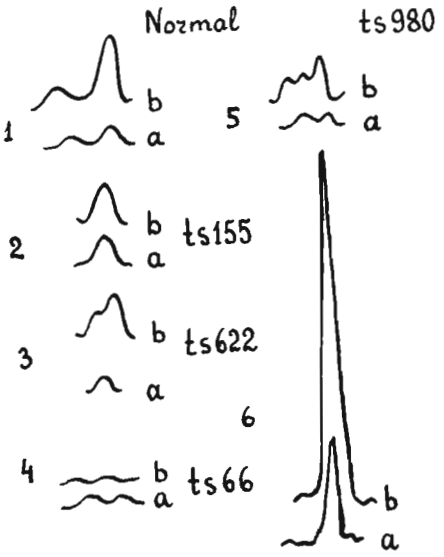
References: Engelmann, F. 1970, Physiology of Insect Reproduction, New York; Foster, W. 1967, Science 158:1569-1597; Kaidanov, L.Z., I.R. Pole and V.B. Sapunov 1978, XIV Intern. Cong. Gen., Contrib. Paper Sessions 1:553; Postlethwait, J. 1974, Biol. Bull. 147:119-135; Sapunov, V.B., L.Z. Kaidanov 1978, Biol. Sci. (USSR) 9:103-107 (Russ.); Wilde, J. de et al. 1968, Proc. Konikl. Nederl. Ac., Ser. C, 71:321-326.



Savvateeva, E., I. Labazova and L. Korochkin. Institute of Developmental Biology, Moscow; Institute of Physiology, Leningrad, USSR. Isozymes of cyclic nucleotide phosphodiesterase in Drosophila temperature-sensitive mutants with impaired metabolism of cAMP.

Temperature-sensitive mutations affecting metabolism of cAMP in the X-chromosome of D. melanogaster were obtained following treatment with EMS and screening for temperature-dependent hypersensitivity to eheophylline and propranolol in postembryonic development. Biochemical analysis of cAMP content and activity of phosphodiesterase (PDE) revealed two mutants with low activity of PDE (ts66, theophylline-sensitive, maps to the right from forked; and ts980, propranolol-sensitive, location yet unknown), one mutant with increased activity of the enzyme (ts398, ts-lethal, unspecific to inhibitors used, maps 1.39), and two mutants with increased content of cAMP (ts155, propranolol-sensitive, maps between cv and ct; and ts622, insensitive to propranolol, location yet unknown). All mutants have an altered behavioral performance at 29°C: their locomotor activity correlates with cAMP content; besides which ts398 at 22°C is more bright than normal flies in learning to avoid shock-associated odor and is completely dull at 29°C; ts622 is bright in learning at 29°C.

Five-day-old imagos were homogenized in 0.5% Triton X-100 and subjected to microelectrophoresis in polyacrylamide gel (Korochkin et al. 1977). The gels were stained according to Hrapchack and Rasmussen (1972) using equimolar amounts of cAMP and cGMP and snake venom. Areas of PDE activity could be identified by a white precipitate of calcium phosphate. The gels were scanned using microddensitomer IFO-451 (USSR). Microelectrophoregrams are shown in the figure.



Two fractions with moderate PDE activity, the slow one prevailing and becoming more prominent following temperature treatment, could be seen on electrophoregrams of normal flies (1a,b) as well as ts155 (2a,b) and ts622 (3a,b). The latter is indicative of the data that the increased content of cAMP in the mutants does not result from altered PDE activity.

In the case of ts66 having low PDE activity, two small peaks can bee seen in the origin area. Only traces of activity are visible after heating a fly at 29°C (4a, b). It is hardly possible to reveal PDE in gels of the second low-activity mutant, ts980; however, after heat treatment 2-3 areas of slight activity can be registered (5a,b). ts398 shows a prominent peak of PDE activity near the origin, and temperature pre-treatment leads to significant increase of the activity of this slow isozyme, while the minor fraction remains unchanged (6a,b). When cGMP was omitted from incubation media, the minor fraction could not be revealed. So one might assume that in Drosophila tissues two isozymes of cyclic nucleotide PDE are present: the more active slow-moving one splitting cAMP, and the fast-moving minor one splitting cGMP.

References: Korochkin, L. et al. 1977, Isozyme Genetics, Moscow, Nauka.

Seager, R. University of Northern Iowa, Cedar Falls. Generation length of D. melanogaster in population cages.

A variety of cages are used to study selection in Drosophila populations. In order to estimate fitness, the generation length must be known. However, generation length estimates for D. melanogaster in cages vary widely [e.g., 15 days (Frydenberg 1962), 20.5 days (Crow & Chung 1967), and 23 days (Barker 1962)]. I have measured generation length in commonly used cages (Tracey & Ayala 1974; Seager et al. 1982) similar to those described by Ayala (1968). The cages are made from one-quart refrigerator containers 14 cm high which taper from a 10 cm square at the lid or front end to an 8.5 cm square at the back end. On the top is a 3 cm diameter hole covered with wire mesh. Ten 1-oz. food cups containing standard cornmeal-molasses medium with a rolled up tissue and 2 ml of heavy yeast solution are screwed into holes in the cage--five on the bottom, two each on the two sides and one on the back end. Each food cup is changed on a rotating basis every 21 days.

Two cages with the second chromosome constitution SM5 Cy/Bl L^2 and two cages with the third chromosome constitution TM3 Sb Ser e^s/e^{11} were initiated and kept at 22°C. After about four weeks, Cy Bl L^2 eggs were introduced into the Sb Ser cages, and vice-versa. For 24 days, starting 10 days after the egg introduction, about 250 eggs were sampled from each cage, reared under uncrowded conditions, and scored. Generation time was estimated, first, as the time between introduction of the foreign eggs into a cage and the peak of egg laying by adults which developed from those eggs, and second, as the time between the first eggs laid by the introduced genotype and the first eggs laid by the next generation.

Average generation time estimates are 22.6 days for the Cy Bl L^2 cages and 25.5 days for the Sb Ser cages (overall average 24.0 days) by method one, and 19 and 19.5 days, respectively, (overall average 19.3 days) by method two. Considering that generation length appears to be genotype-dependent, a reasonable estimate of generation length in these cages is 21 days.

This work was supported by NSF Grant DEB-7918493 to W. Anderson.

References: Ayala 1968, DIS 43:185; Barker 1962, Gen.Res. 3:388-404; Crow and Chung 1967, Genetics 57:951-955; Frydenberg 1962, Hereditas 48:83-104; Seager, Ayala and Marks 1982, Genetics (in press); Tracey and Ayala 1974, Genetics 77:569-589.

Serra, L. and M. Monclús. University of Barcelona, Spain. Ecological relationship between D. andalusiaca and some species of Scaptomyza.

In a series of monthly samples, obtained during a two-year period in a Populus sp. forest at Bordils (Girona, Spain), a relatively important frequency of the species D. andalusiaca was observed. This species had previously been found, although only very few individuals, in the Iberian Peninsula, and very rarely in the northeast of Spain.

The flies were caught by means of a series of traps put on the tree branches, approximately 1.5 m high (group A) and on the ground among the weeds (group B). The first year, out of 4564 individuals caught in group A, 25 belonged to the genus Scaptomyza and none were found of the species D. andalusiaca. The frequency of Scaptomyza in this group is 0.0055 ± 0.0021 (0.5%). In group B, out of 14,186 individuals caught, 719 belonged to the genus Scaptomyza and 104 to the species D. andalusiaca. The frequencies obtained are 0.0506 ± 0.0036 and 0.0073 ± 0.0014, respectively.

As expected, the frequency of individuals of the genus Scaptomyza is significantly greater in group B than in group A, but it is the same (and even more) for the individuals of the species D. andalusiaca. This result let us carry out, in the second year, a sweeping of the weeds with a net, while keeping both groups A and B in the same places. The total number of flies caught on the weeds was 5949. The frequency of individuals of the genus Scaptomyza was 96.3% and of D. andalusiaca, 3.16%. The total number of flies caught in group B was 5020. The percentage of Scaptomyza was 3.76, and of D. andalusiaca, 0.27; the corresponding values for group A are 1827, 0.58% and 0%, respectively.

We believe these data suggest that the species D. andalusiaca has a natural habitat much more similar to that of the Scaptomyza than the other species of Drosophila.

Singh, R. S. McMaster University, Hamilton, Ontario, Canada. A new esterase of D. pseudoobscura and D. persimilis.

Carboxylesterases, in general, are highly polymorphic and show multiple (isozyme) loci in most organisms which have been studied electrophoretically (Zouros 1975; Singh 1976). In D. pseudoobscura four esterase loci have been shown to be highly polymorphic. They are esterase-1 (Singh 1979), esterase-4 (Morton & Singh 1981), esterase-5 (Lewontin & Hubby 1966), and esterase-6 (Prakash 1971). Of these, esterase-1 and -5 are β-esterase, and esterase-4 and -6, α-esterase. Here I report another α-esterase which is highly polymorphic in both D. pseudoobscura and D. persimilis. I have called it Esterase-3, as on electrophoresis on 5% polyacrylamide gel with ·1M Tris-Borate EDTA (pH 8.9) buffer it migrates between esterase-1 and esterase-4 (Figure 1). It was first observed on acrylamide gel with ·1M Tris-Borate EDTA (pH 7.2) buffer. Later it was found out that it can also be visualized at acrylamide gel run at pH 8.9 provided that the gel is presoaked in ·1M Tris-Borate (pH 7.2) buffer for an hour before staining. Presoaking in .5M boric acid also brought out the esterase-3 bands but they were not as good. Thus, esterase-3 seems to

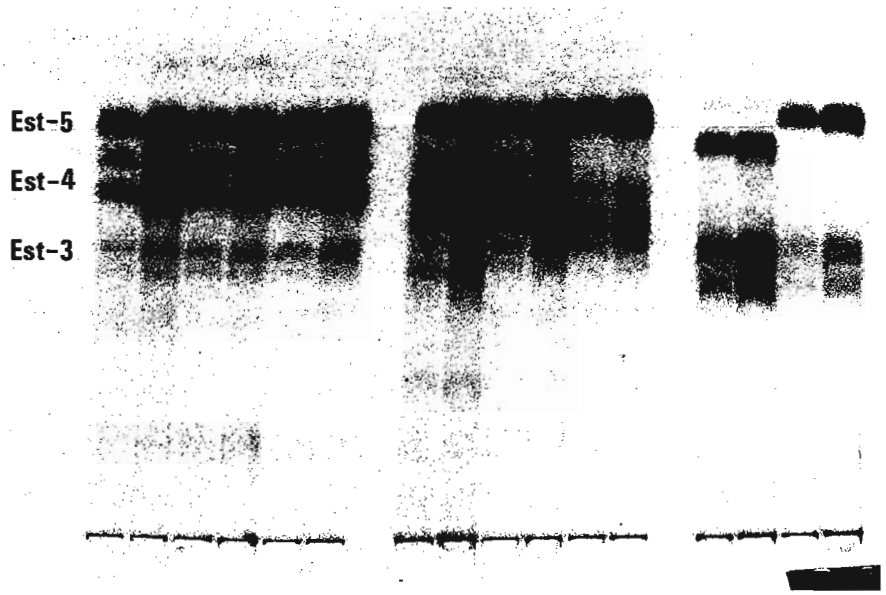


Fig. 1. A gel showing esterases of D. pseudoobscura.

require low pH for staining. This enzyme appears as a single major band and a minor trailing band which may or may not always appear. Heterozygous individuals show two major bands, suggesting that the enzyme is a monomer.

We have examined a total of 99 strains of D. pseudoobscura and 22 strains of D. persimilis and have found a total of 11 alleles of which 6 are shared between the two species (Table 1). Bogota has no unique alleles. We also observed a null allele which is quite common in some populations. Most populations of D. pseudoobscura are highly heterozygous. An equal level of heterozygosity is shown by D. persimilis.

Table 1. Frequency of electrophoretic alleles of Esterase-3 locus in various populations of D. pseudoobscura and D. persimilis. (Electrophoretic conditions: .1M Tris-Borate EDTA, pH 7.2; 5% gel.)

	D. pseudoobscura[1]							
Alleles	SC	CH	CE	MV	AU	BO	Total	D. persimilis[2]
Null	4	4	4	14	-	4	30	2
.85	2	10	4	-	-	-	16	-
.92	-	2	-	2	-	-	4	2
.95	2	10	1	-	-	20	33	-
.98	-	-	2	-	-	-	2	-
1.00	22	30	19	10	18	4	103	11
1.04	-	-	-	-	-	-	-	2
1.08	-	2	2	-	-	-	2	3
1.12	-	-	-	2	-	-	2	22
1.17	-	-	-	2	-	-	2	-
1.20	-	-	-	2	-	-	2	2
No. genes examined	30	58	32	32	18	28	198	44
Total alleles	4	6	6	6	1	3	10	7
Unique alleles	0	0	1	3	0	0	4	1
Heterozygosity	.436	.666	.608	.696	.000	.449	.671	.675

[1]D. pseudoobscura populations are: SC = Strawberry Canyon, Berkeley, California; CH = Mount Charleston, Nevada; CE = Cerbat Mountains, Nevada; MV = Mesa Verde, Colorado; AU = Austin, Texas, BO = Bogota, Colombia.

[2]D. persimilis = a mixture of isofemale lines from three different localities in California.

References: Lewontin, R.C. and J.L. Hubby 1966, Genetics 54:595-609; Morton, R.A. and R.S. Singh 1981 (in preparation); Prakash, S. 1977, Genetics 85:713-719; Singh, R.S. 1976, Genetics 82:507-527; ____ 1979, Genetics 93:997-1018; Zouros, E. 1975, Nature 254:446-448.

Skripsky, T. and J. C. Lucchesi. University of North Carolina, Chapel Hill, North Carolina. A genetic interaction affecting viability and sexual differentiation in D. melanogaster.

$Dp(1;3)sn^{13al}$ significantly lowers the viability of males carrying the multiply inverted Basc or Binsinscy chromosomes (Table 1, B Dp males). These males often exhibit abnormalities in their sexual phenotype. In contrast, males with the duplication and a noninverted X chromosome as well as females with the duplication are of a normal phenotype and survive equally well. Seven of the total 39 B Dp males had completely missing external analia and genitalia, both derivatives of the genital imaginal disc. The mean number of sex comb teeth in Basc males with or without the $Dp(1;3)$ sn^{13al} was identical (9.8); however, in Dp-carrying males, 5 out of 45 sex combs examined under a compound microscope had abnormally arranged (displaced) sex comb teeth. All 44 sex combs in the control Basc; Ki males were normal.

Table 1. Progeny from females (maternal genotype) which were crossed individually to 2-3 males of the genotype $Df(1)ct^{J6}/Y;Dp(1;3)sn^{13al}/Ki$.

Maternal genotype	y;Dp males	y;Ki males	B;Dp males	B;Ki males	B/Df;Dp females	B/Df;Ki females	+/Df;Dp females	+/Df;Ki females
Basc/ 1(1)f1-1251	136	121	24	150	191	108	164	98
Basc/y cm $Sxl^{f\#1}$ ct	38	32	5	34	42	23	0	36
Basc/Basc	-	-	10	89	139	158	-	-
Binsinscy/y 1(1)f1-1251	52	37	0	47	39	56	53	52

$Df(1)ct^{J6}$ includes the bands 6E1-7B7 (Lefevre & Johnson 1973).
Binsinscy, $In(1)sc^{s1L}$ sc^{8R} +d1-49, y sc^{s1} sc^{8} w sn^{x2} B·y 1(1)f1-1251 is a derivative of 1(1)ts-1251 of Arking (1978), which is female-specific lethal at all temperatures.

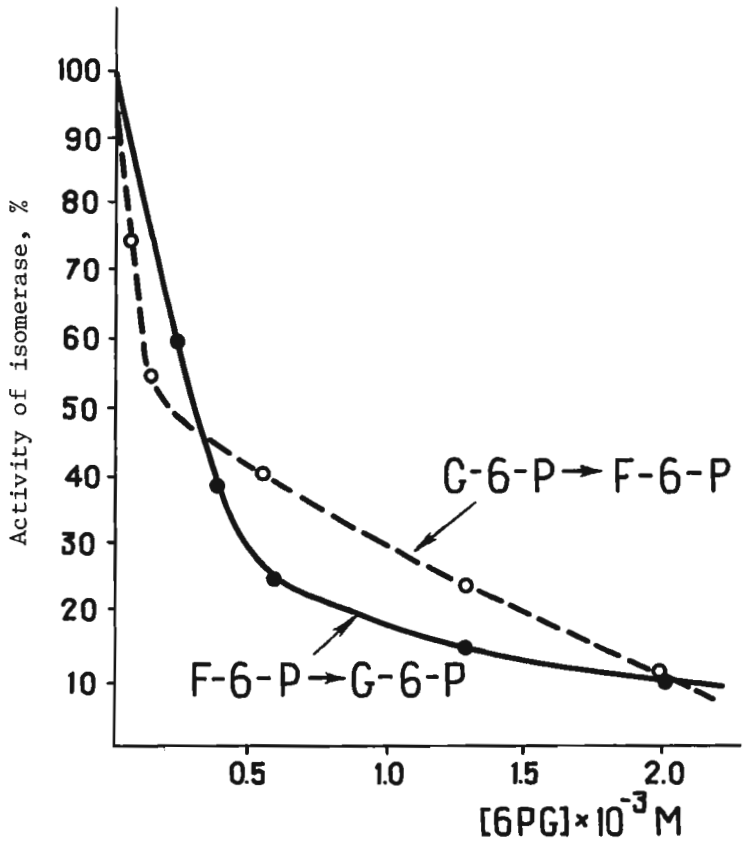
In an additional experiment, 10 C(1)RM, y v f females carrying the duplication $Dp(1;3)sn^{13al}$ and a Ki chromosome were crossed to Basc males. Only five of these females were fertile and their progeny were as follows: 53 Dp females, 20 Dp males; 158 Ki females, 82 Ki males. The apparent paucity of duplication females may reflect the preferential segregation of the duplication-bearing third chromosome from the attached-X. It may also indicate that the effect on viability is not sex-specific. Consistent with the results of the previous crosses, four out of the 20 duplication males were missing all or some parts of the genital disc derivatives. In one of these males a rudimentary ovary with several clearly identifiable oocytes was present.

$Dp(1;3)sn^{13al}$ includes the locus of Sxl and the involvement of this gene in the observed phenomenon seems to be probable because of its role in sex determination and dosage compensation. Whether the interactions are the results of position effects in the rearranged chromosomes (a breakpoint of the S inversion in the Basc chromosome is at 6A1-3, not far from 6F, the locus of the Sxl gene), alterations in the amounts of r-DNA or other proximal heterochromatic regions, or some other effects has yet to be determined.

References: Arking, R. 1978, Genetics 88:s4; Lefevre, G. and T.K. Johnson 1973, Genetics 74:633.

Smirnova, S. G., G. L. Kogan and V. A. Gvozdev. Institute of Molecular Genetics, USSR Academy of Sciences, Moscow, USSR. On the mode of action of lethal mutations affecting 6-phosphogluconate dehydrogenase in D. melanogaster.

We have found earlier that mutations in the Zw locus which encodes for glucose-6-phosphate dehydrogenase (G6PD) suppress lethal mutations in the X-linked Pgd locus (1-0.9) coding for 6-phosphogluconate dehydrogenase (Gvozdev et al. 1977). Other authors too have discovered the possibility of suppression of mutations in the Pgd locus by inactivating G6PD (Hughes & Lucchesi 1977). It was hypothesized that the death of individuals with a normal G6PD activity but with impaired or null 6PGD activity might be due to an accumulation of 6-phosphogluconate (6PG), which, at least in mammals, can inhibit the glycolytic enzyme glucose-6-phosphate isomerase. Indeed, in fly extracts this enzyme was shown to be considerably inhibited by 6-phosphogluconate in vitro. The figure shows that 6PG inhibits glucosophosphate isomerase both in respect to the conversion of glucose-6-phosphate into fructose-6-phosphate and in the opposite direction. The data of Hughes and Lucchesi (1978) suggest that 6-phosphogluconate



Inhibition of glucose-6-phosphate isomerase by 6-phosphogluconate (6-PG)

can exercise its inhibiting effect in vivo: these authors have shown the lethality of null-mutations in the Pgd gene to be at least partly overcome by a "diabetic" diet, i.e., a substitution of fructose for all glucose in the feed (Hughes & Luchesi 1978). We have observed a fivefold increase in the viability of males with a Pgd lethal from the cross

♀ 1(1)39pn/FM4, $y^{31d}sc^{8}dmB$

x ♂ FM^{4}, $y^{31}sc^{8}dm$ B

on a glucose-free fructose-containing diet.

If the effect of the Pgd mutations is indeed based on 6-phosphogluconate intoxication, then the Pgd mutations could behave like cell lethals, at least in some tissues. To study this possibility the y ac se w X-chromosome carrying a 1(1)93 or 1(1)94 lethal [both fully inactivating 6PGD (Grozdev et al. 1977)] was introduced into a stock with an unstable circular X chromosome R(1)2 w^{vc}. In the case of the two lethals adult progeny from the cross

R(1)2 w^{vc}/y 1w1z

x y ac scl(1) 93 wZw^{A}/w^{+}Y

contained gynandromorphs carrying tissues with both the XX and XO genotypes. The XX tissues looked normal with the exception of variegated eyes (w/w^{vc} heterozygotes). The XO tissues, resulting from the loss of the circular X chromosome, could be detected by the yellow color of the cuticle and bristles and the white color of the eyes. These tissues carry a Pgd lethal in the hemizygous state. The number of gynandromorphs came to 20-50% of the number of R(1)2w^{vc}/y ac sc 1(1) Pgd w females. Analysis of the distribution of body parts and organs with the yellow white phenotype made it possible to determine the proportion of imaginal discs and histoblasts giving rise to the XO tissues (Bryant & Zornetzer 1973). In some of the gynandromorphs the proportion of presumptive XO tissue germs came to 80%. However, the viability of adult flies carrying over 60% of XO tissues with Pgd lethal in the hemizygous state was considerably decreased. No anomalies were observed on the parts of body with the XO cells. Some of the gynandromorphs showed a drop in 6PGD activity to 20% of the control value, though no clear-cut correlation could be established between the proportion of XO tissue and the total level of 6PGD activity.

Genetic mosaics carrying the 139 and 193 lethals in the Pgd locus (described in Gvozdev et al. 1977) were also found in the stocks 1(1)39 pn Zw^{A}/Dp(1;f)R and y ac sc w 1(1)93 Zw^{A}/Dp(1;f)R where these lethals were covered by the normal Pgd allele within the free duplication Dp(1;f)R. Hence the duplication Dp(1;f)R may be lost with a low frequency during the cleavage of the fertilized egg, leading to the development of an individual in which part of the tissues are deprived of 6PGD activity.

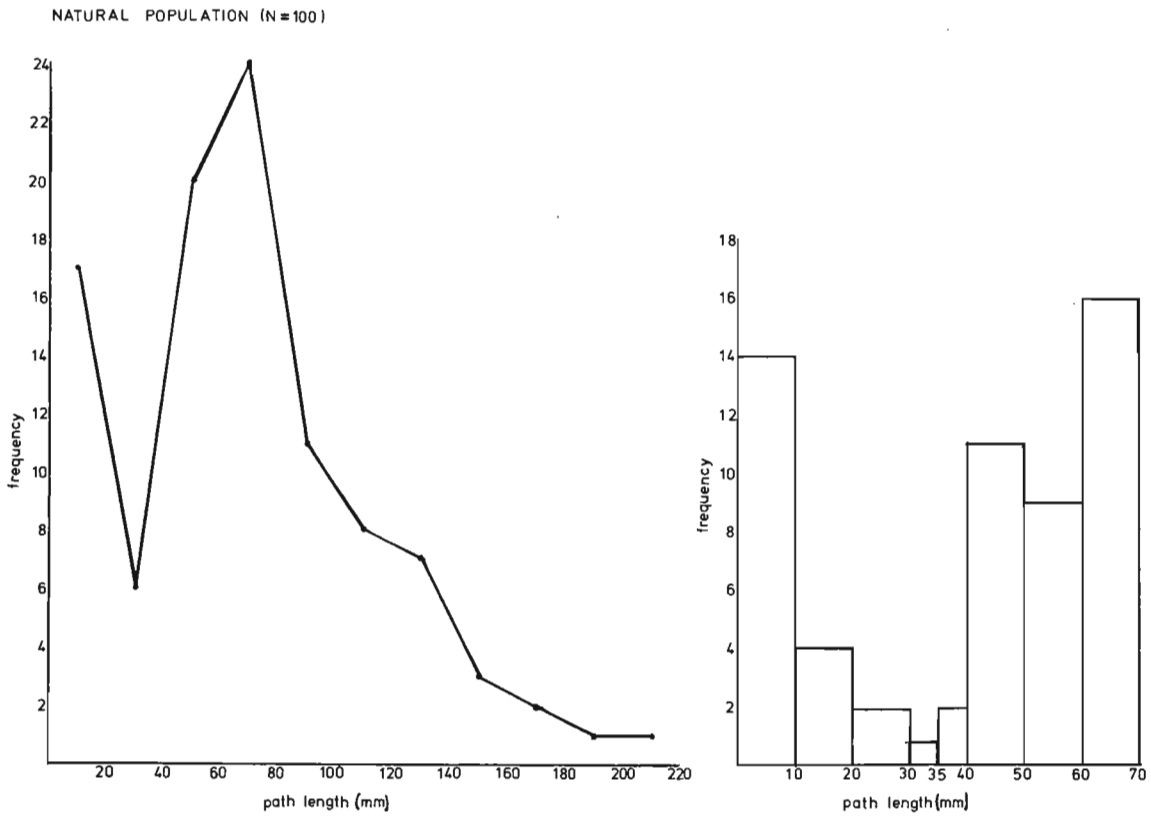
Our results show that the null Pgd mutations are not cell lethals. The viability of flies carrying a substantial proportion (50% and more) of Pgd-mutant tissue is probably maintained owing to the normal tissues. In our opinion the above observations scarcely conform to the concept that the effect of the Pgd lethals is mainly due to 6-phosphogluconate intoxication. The data for mosaics are more likely to indicate that the mutant tissues lack or are deficient in some vital metabolites, e.g., pentoses, coming from other, normal, parts of the body. On the other hand we cannot exclude the possibility that the normal tissues, even if their proportion is small enough, may utilize 6-phosphogluconate, preventing it from reaching critical concentrations.

References: Bryant, P.J. and M. Zornetzer 1973, Genetics 75:623-637; Gvozdev, V.A., T.I. Gerasimova, G.L. Kogan and O.Yu. Braslavskaya 1976, FEBS Letters 64:85-88; Gvozdev, V.A., T.I. Gerasimova, G.L. Kogan and Ya.M. Rozovsky 1977, Molec. Gen. Genet. 153:191-198; Hughes, M.B. and J.C. Lucchesi 1977, Science 196:1114-1115; ____ and ____ 1978, Biochem. Genetics 16:469-475; Parr, C.W. 1956, Nature 1781-1801.

Sokolowski, M. B. York University, Downsview, Ontario, Canada. Rover and sitter larval foraging patterns in a natural population of D. melanogaster.

Sokolowski (1980) identified a behavioral polymorphism in D. melanogaster larval foraging strategies which was attributed to differences in a single pair of chromosomes. Rover larval foragers traverse a large area whereas sitters cover a relatively smaller one while foraging on a yeast-covered petri dish. One criterion which quantitatively distinguished these morphs was the path length of the foraging trail. A larva which had a path length of greater than 35 mm was classified as a rover, whereas sitter larvae traversed 35 mm or less while feeding. In 1977, a population of D.melanogaster larvae from a pear tree was surveyed to determine if both rover and sitter larval foragers could be found in nature (Sokolowski 1980). Rover and sitter larval foragers were found to exist at relatively constant frequencies (72% rovers: 28% sitters) during the nine-week sampling period.

In the present study, by measuring larval path length distributions as well as the frequency



Figs. 1 and 2. The frequency distributions of path lengths in third instar larvae of Drosophila melanogaster sampled from a single pear is bimodal. (Fig. 1 is to the left, Fig. 2 is to the right.) Both of the forager types exist in this population, long path lengths characterizing the rover phenotype and short path lengths the sitter one. A magnification of the lower end of the path length distribution curve (utilizing path length categories of 10 mm rather than 20 mm in length) shows good separation of the rover and sitter phenotypes (see Fig. 2).

of rover as compared to sitter foragers, further support for the hypothesis that rover and sitter foragers exist in nature is acquired.

Nine fallen rotting pears were collected from a 1 m^2 area from a yard in downtown Toronto and brought into the laboratory. One pear was sampled at random. It contained more than 100 third-instar larvae. Each of these larvae was tested using the standard yeast-covered petri dish apparatus (Sokolowski 1980). A copy of the foraging trail was superimposed onto a grid so that the path length could be measured. All larvae were tested within 24 hours of bringing the pears into the laboratory.

Employing the criteria used to distinguish a rover from a sitter, 20 of the 100 larvae tested were classified as sitters and the remaining 80 were rovers. Figure 1 shows the distribution of path lengths in the natural population sample of 1979. Path length was divided into categories 20 mm in width. The distributions of path lengths are clearly bimodal. The first peak, found below 35 mm, is the sitter peak: 20% of the larvae score in the sitter range of path lengths. The second peak found about 35 mm is the rover peak. In Figure 2, path length categories range from 0 to 70 mm and have smaller intervals of 10 mm in width. The change in interval size magnifies the intersection of the two curves in Figure 1. By analyzing the foraging trails of the lab stocks, Sokolowski (1980) found that the dividing line between rovers and sitters was a path length of 35 mm. The same dividing line also separated the two path length distributions of larvae collected from nature. Only two of 100 larvae had a score for path length between 35 and 40 mm in length, and only one of 100 larvae had a score between 30 and 35 mm in length. Even if the discriminating criterion is not exactly 35 mm long, very few larvae would be scored in error. The distribution of rovers and sitters are clearly distinct in this sample.

References: Sokolowski, M.B. 1980, Behav. Genet. 10:291-302.

Sokolowski, M. B. York University, Downsview, Ontario, Canada. Temporal patterning of foraging behavior in D. melanogaster larvae.

Larval foraging behavior can be defined as the relative amount of feeding and locomotor behavior performed on the feeding substrate during a test period. The number of probes with the mouth hooks (shovels) and the number of muscular contractions passing along the body of the larva (crawls) are discrete measures of feeding and locomotor behavior (Sokolowski 1980). Burnet et al. (1977) and Sewell et al. (1975) studied larval feeding rate in an aqueous yeast suspension. Drosophila larvae also tend to crawl along the feeding substrate while shoveling. Larval feeding and locomotor behavior can be examined simultaneously when a larva is placed in a petri dish covered with a yeast paste so that a moving larva leaves a visible trail in the yeast. In Sokolowski (1980) I identified a behavioral polymorphism in D. melanogaster larval foraging patterns using this technique. Rover larvae traverse a large area whereas sitter larvae cover a small area while foraging on a yeasted petri dish. Genetic analyses using chromosome substitutions revealed that differences in these forager types could be attributed to the second pair of chromosomes, whereas differences in larval feeding rate were affected by both the second and third chromosomes.

It is of interest to determine whether the mean behavioral score per test is a

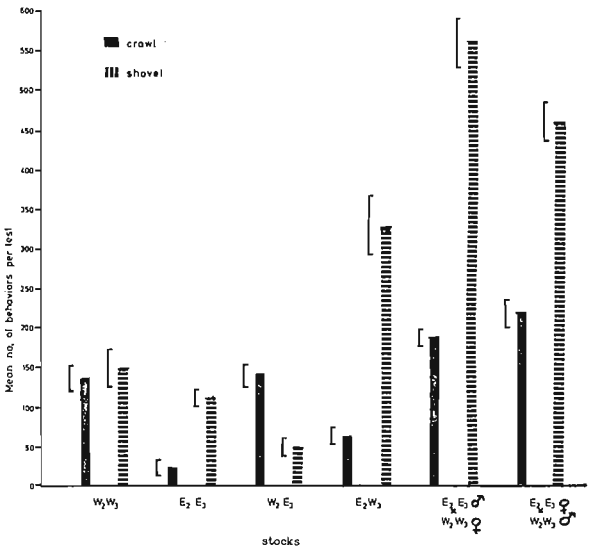
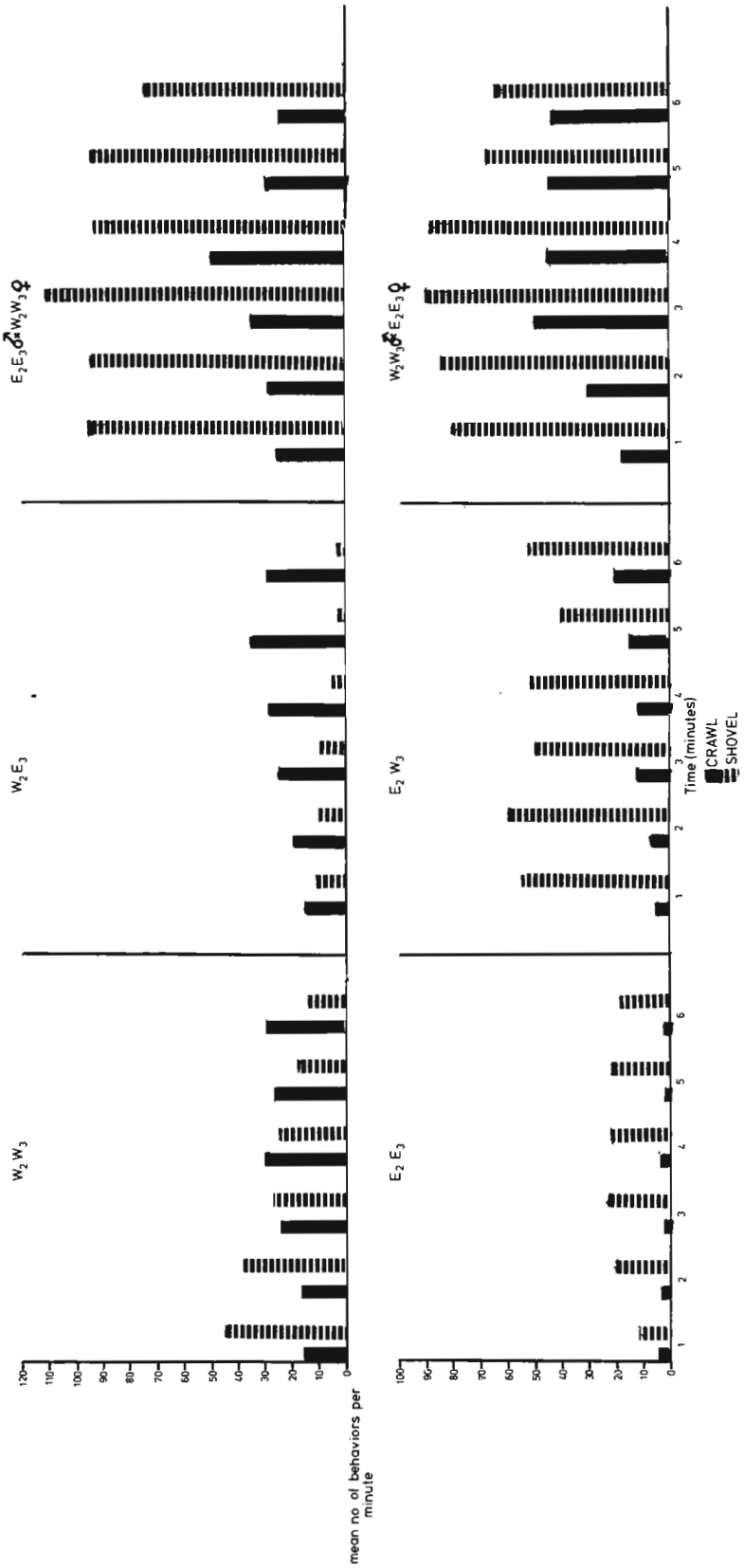


Fig. 1. This figure shows the mean ± S.E. crawling and shoveling scores performed by the original stocks (W2W3 and E2E3), the reconstructed stocks (W2E3 and E2W3) and the reciprocal crosses of the original stocks. 25 D. melanogaster larvae of each stock and 12 larvae of each of the reciprocal crosses were tested.



good representation of the patterns of behavior observed during the six minute test period.

Four stocks with isogenic 2nd and 3rd chromosome pairs were used: two original stocks, W2W3 and E2E3, and two reconstructed ones, W2E3 and E2W3. W2E3 had the same 2nd chromosome pair as W2W3 but differed in having the 3rd pair from E2E3. For further explanation of the stocks and methods used in this study, see Sokolowski (1980).

Figure 1 illustrates the mean crawling and shoveling scores ± S.E. of the original stocks, the reconstructed stocks and the reciprocal crosses of the original stocks. Stocks with the W2 homologs showed significantly higher ($p < 0.0001$, Mann-Whitney U test) crawling scores than did those with the E2 homologs. The heterozygote larvae of both reciprocal crosses showed very high crawling and shoveling scores compared to the scores of the original and reconstructed stocks. When high crawling score larvae were mated with low crawling score larvae, both male and female resultant hybrid progeny exhibited high levels of crawling behavior, i.e., high levels of crawling behavior is dominant and the X-chromosome does not significantly affect crawling behavior. The differences in shoveling behavior could not be attributed to a single autosome.

Figure 2 illustrates that the stocks' mean crawling and shoveling scores well represented the temporal differences in foraging behavior during the test period. W2W3 and W2E3 stocks performed consistently more crawling per minute than did the E2E3 and E2W3 stocks. In general, in the former stocks the amount of crawling performed per minute increased during the first 4 minutes. This may reflect behavioral adjustments to a

Fig. 2. This figure shows the temporal patterning of crawling and shoveling behavior in early third instar D. melanogaster larvae of the original stocks (W2W3 and E2E3), the reconstructed stocks (W2E3 and E2W3) and the reciprocal crosses of the original stocks.

new environment, a fairly common occurrence. The relative amounts of shoveling performed per minute tended to decrease with time. Larvae which have a high genetic potential for crawling behavior, rovers, reduced the amount of stationary feeding (stationary feeding was reflected by high shoveling and low crawling scores) as the time spent in the new environment (in this case, a yeast-covered petri dish) increased. The patterns exhibited by each of the reciprocal crosses were similar to each other. Larvae of the reciprocal crosses tended to increase the amount of crawling behavior performed per minute during the course of the experiment as did the larvae of W2W3 and W2E3 stocks. It was concluded that the temporal patterning of these behaviors does not contradict any of the results presented in Figure 1.

References: Burnet, B., D. Sewell and M. Bos 1977, Genet. Res. Camb. 30:149-161; Sewell, D., B. Burnet and K. Connolly 1975, Genet. Res. Camb. 24:163-173; Sokolowski, M.B. 1980, Behav. Genet. 10:291-302.

Spofford, J. B. University of Chicago, Illinois. Allopurinol enhances variegation.

Allopurinol (AP) has recently been used in a screen for ry (=XDH) variegation (Rushlow & Chovnick 1981) inasmuch as low XDH levels are fully inhibited by lower AP concentrations than are needed to yield ry phenocopies in wild type. AP actually probably also improves the chance of recovering a variegating rearrangement by affecting the variegation process itself. Fowle (1980) found AP reduced bristle pigment in several y-variegating rearrangements.

To determine whether AP affects the variegation process per se or a later step in the path to bristle pigment, I screened the effect of AP on five other phenes in six different variegation-causing rearrangements. Powdered AP (gift of J. Fowle) was added to a single batch of cornmeal medium before the latter solidified, giving concentrations (w/v): 0, 0.02%, 0.04%, and 0.08%. One or two fertile vial cultures were reared at 25°C for each rearrangement at each AP level, with a pair of parents taken from the same stock culture. Usually no males and few females survived at 0.08% AP, with the surviving females very strongly mosaic when they had the variegating rearrangement.

In the accompanying table (1) w mosaicism was measured as (a) percent of eye area pigmented, visually estimated, ± sample σ for the large-spot mosaic $Y^{S}w$ y.$Y^{L}y^{+}$/Y; $Su(var)^{S}$ Dp(1;3) $w^{m264-58}$/$Su(var)^{S}$ and as (b) drosopterin content per head in O.D. units after paper chromatography in 4:4:1 n-butanol:H_2O:acetic acid, ± sample σ for the pepper-and-salt mosaic In(1) w^{m51b} males. Values for Oregon-R males are given in (c) as control for XDH inhibition. (2) m mosaicism was scored as [number with m patches on either wing/total number] in each sex for $In(1)m^{K}$. (3) Lethal variegation was scored as [number ♂/number ♀] in In(1)lv59/sc^{8}Y/C(1)DX, y w. (4) ac mosaicism was scored as the number of dorsocentrals per fly ± S.E. for Dp(1;1) sc^{V1}, y ac/Y. (5) y mosaicism was confirmed also in these last males, reported as mean number of yellow thoracic bristles per fly, with no correction for the reduced number of bristles at 0.04% AP. (6) B expression is crudely expressed as [number antero-posterior facet rows X number dorso-ventral facet rows] typically seen in $B^{S}Y$ males.

Gene	Genotype	0	0.02% AP	0.04% AP	0.08% AP
w	a) $DP(1;3)w^{m264-58}$	99.9 ± 0.2	85.5 ± 0.3	79.7 ± 0.6	--
	b) $In(1)w^{m51b}$	32.3 ± 2.9	28.2 ± 2.6	17.8 ± 6.0	--
	c) Oregon-R	32.8 ± 3.9	28.2 ± 4.8	22.0 ± 4.3	--
m	$In(1)m^{K}$ ♂♂	1/29	4/38	6/30	4/5
	$In(1)m^{K}$ ♀♀	0/30	1/27	6/22	4/6
1^{V59}	In(1)lv59/sc^{8}Y	122/127	70/59	41/45	6/20
ac	$Dp(1;1)sc^{V1}$, y ac	2.73 ± 0.25	2.55 ± 0.39	1.25 ± 0.24	--
y	$Dp(1;1)sc^{V1}$, y ac	0.18	0.64	1.12	--
B	$B^{S}Y$	10 x 22	9 x 21	8 x 20	4.5 x 17.5

Thus 0.04% AP appears to intensify variegation in most of the tested systems. Roughly 1/4 of the facets of the w^{m51b} eyes appeared white when viewed under mineral oil versus none in the control Oregon-R eyes at that level. Whether there was an effect on lv59 variegation is un-

clear. The effect on B was counter to the others, since it would be expected that intensified variegation would reduce B expression. Nevertheless, given the number of different rearrangements and phenes assayed and the fact that no other variegation modifier is universal in its effect, either, AP is shown to be an enhancer of the variegation process itself.

References: Fowle, J.R. III 1980, Ph.D. Dissert., The George Washington Univ.; Rushlow, C.A. and A. Chovnick 1981, Genetics 97:s92.

Takada, H. and M. J. Toda. Sapporo University and the Institute of Low Temperature Science, Hokkaido University, Sapporo, Hokkaido, Japan. Notes on Arctic Canadian Diastatidae and Drosophilidae (Diptera).

During July 13 to August 11, 1980, the junior author had an opportunity to make drosophilid fly collections at Inuvik (68°22' N, 133°45' W) and Tuktoyaktuk (69°26' N, 133°03' W), Northwest Territories of Canada, in the Permafrost Expedition of Hokkaido University. We report here the list of 16 species collected, including a new species. The description of new species has been reported in the Journal of the Faculty of General Education, Sapporo University, No. 18, 1981.

Arctic Canadian species of Diastatidae:

1. Diastata tenuipes (Walker). Inuvik, 1 female, 1 male, August 2, 1980.
2. D. eluta Loew, 1863. Inuvik, 1 female, August 7, 1980.
3. Campichoeta griseola (Zetterstedt), 1855. Inuvik, 3 males, 1 female, August 2, 1980.

Arctic Canadian species of Drosophilidae:

4. Amiota quadrata Takada & Toda n. sp. Inuvik, 22 males, 7 females, August 8, 1980.
5. Chymomyza aldrichii Sturtevant, 1916. Inuvik, 2 females, 1 male, July 31 to Aug. 8, 1980.
6. Ch. tetonensis Wheeler, 1949. Inuvik, 1 female, 2 males, July 31 to Aug. 8, 1980.
7. Ch. costata (Zetterstedt), 1838. Inuvik, 54 females, 153 males, July 31 to Aug. 8, 1980.
8. Ch. caudatula Oldenberg, 1914. Inuvik, 1 female, July 31 to Aug. 8, 1980.
9. Scaptomyza montana Wheeler, 1949. Inuvik, 5 females, 2 males, August 7, 1980.
10. S. trochanterata Collin, 1953. Inuvik, 2 females, 1 male, August 7, 1980.
11. Drosophila athabasca athabasca Sturtevant & Dobzhansky, 1936. Inuvik, 420 females, 254 males, July 31 to Aug. 11, 1980; Tuktoyaktuk, 5 females, 1 males, July 13-26, 1980.
12. D. montana Stone, Griffen & Patterson, 1941. Inuvik, 18 females, 25 males, July 31 to Aug. 8, 1980; Tuktoyaktuk, 2 females, July 13-26, 1980.
13. D. borealis Patterson, 1952. Inuvik, 1 female, July 31 to Aug. 8, 1980.
14. D. putrida Sturtevant, 1916. Inuvik, 5 females, 6 males, July 31 to Aug. 11, 1980; Tuktoyaktuk, 1 female, July 13-26, 1980.
15. D. subquinaria Spencer, 142. Inuvik, 27 females, 37 males, July 31 to Aug. 11, 1980; Tuktoyaktuk, 1 female, 1 male, July 13-26, 1980.
16. D. rellima Wheeler, 1960. Inuvik, 87 females, 86 males, July 31 to Aug. 11, 1980; Tuktoyaktuk, 10 females, 13 males, July 13-26, 1980.

Triantaphyllidis, C., J. Panourgias and Z. Scouras. University of Thessaloniki, Greece. Genic variation in a Greek wild population of D. melanogaster.

The present communication constitutes a further report of a much wider investigation in isozyme variation in Greek D. melanogaster and D. simulans populations. The project was started ten years ago and is still in progress.

Polymorphisms of seven enzyme systems, namely, of esterase-6 (Est-6), esterase-C (Est-C), acid phosphatase (Acph), alcohol dehydrogenase (Adh), α-glycerophosphate dehydrogenase (αGpdh), phosphoglucomutase (PGM) and malate dehydrogenase (Mdh-1) were studied in wild sympatric D. melanogaster and D. simulans populations. The sample was collected in early June from University Farm, a locality at a distance of about 15 km from the town of Thessaloniki. Our results for D. melanogaster are shown in Table 1. The results of the D. simulans are not given because the sample was too small. Hardy-Weinberg tests indicated that the studied sample was in equilibrium for all loci, except Adh, where $P < 0.05$. The mean observed heterozygosity (H) was 0.179, while the expected was 0.190.

Table 1.

Locus	Alleles	Frequencies
Est-6	n=213	
	73	0.0024
	79	0.0070
	S	0.5587
	F	0.4319
	H = 0.531/0.501	
Est-C	n=202	
	S	0.0743
	F	0.9208
	107	0.0049
	H = 0.129/0.147	
Acph	n=246	
	S	0.0020
	F	0.9980
	H = 0.005/0.004	
ADH	n=296	
	S	0.1503
	F	0.8497
	H = 0.213/0.255	
α-Gpdh	n=261	
	S	0.2586
	F	0.7395
	110	0.0019
	H = 0.352/0.386	
PGM	n=147	
	F	1.00
MDH	n=289	
	S	0.9810
	F	0.0156
	S_1	0.0034
	H = 0.02/0.037	

For Est-C^F allele a contingency χ^2 test for heterogeneity indicated that two samples taken from the same region (Triantaphyllidis et al. 1973, and this investigation) were significantly different at the 0.05 level. This is not the case for Est-6^S alleles. The same test applied to two samples (September, Triantaphyllidis et al., in prep.; June, this report) of the same locality indicated that the September collection differed significantly from the June collection only in the Mdh^S allele.

Our results and the results reported by Eisses et al. (1979) indicated that the genetic distance between D. melanogaster and D. simulans is about 0.450. It is interesting that Nei (1975) proposed a formula for estimating the phylogenetic divergence time (t years) from the genetic distance (D); that is, $t = 5 \times 10^6 D$. The present authors used this formula and the genetic distance between the two sibling species for estimating the divergence time between them; thus the divergence time is estimated as 2.25 million years. Although we should keep in mind that the estimation of divergence time from electrophoretical data is subject to a large amount of error, our estimate is clearly in the range of species divergence within a genus (Nei 1975; Carson 1976).

Supported by the National Hellenic Research Foundation.

References: Carson, H. L. 1975, Nature 259:395; Eisses, K. et al. 1979, Evolution 33:1063; Nei, M. 1975, Molecular Population Genetics and Evolution, North Holland, Oxford; Triantaphyllidis, C. et al. 1973, Hereditas 74:25.

Tsacas, L., C. Krimbas and C. D. Triantaphyllidis. Laboratoire de Biologie et Génétique Evolutives Gif-sur-Yvette, France; Agricultural College of Athens, Greece; Aristotelian University of Thessaloniki, Greece. The genus Drosophila in Greece.

In a recent catalog of the genus Drosophila in Europe, 16 species were reported from Greece (Rocha Pité & Tsacas 1979; see also Krimbas 1963, 1964; Tsacas 1963). After some new collections in different parts of this country, five species new for the Greek fauna were found (Triantaphyllidis & Tsacas, in press). More recently, D. helvetica Burla was collected in the mountains of Central Greece (Krimbas & Loukas, in press). Thus the total number of Drosophila species collected up to now in Greece reaches 22, 47% of the total number of species in Europe.

Five subgenera are represented. The subgenus Drosophila s. str. is the best represented (12 species, 55%) followed by the subgenera Sophophora (6 species, 27%), Scaptodrosophila (2 species, 9%), Lordiphosa and Dorsilopha (1 species each, 4.5%). Of these species eight are cosmopolitan (36%); the other 14 (64%) are species with a large European and sometimes extra-European distribution.

Among the five Mediterranean European countries in which more than ten Drosophila species are known, Greece occupies the last but one place with 22 species, before Italy (16 species). The other three countries, France, Spain, and Yugoslavia, have respectively 30, 29 and 24 species.

From these observations, other species may be expected to live in Greece. Biogeographical comparisons between Mediterranean countries will be possible only when Drosophilid fauna are better prospected.

List of Drosophila species collected in Greece:

Subgenus	Species group	Species	Distribution
Dorsilopha		busckii Coquillett	Cosmopolitan
Drosophila s. str.	funebris	funebris Fallén	Cosmopolitan
	immigrans	immigrans Sturtevant	Cosmopolitan
	quinaria	kuntzei Duda	Europe
		limbata von Roser	Europe
		phalerata Meigen	Europe
		transversa Fallén	
	repleta	buzzatii Patterson & Wheeler	Cosmopolitan
		hydei Sturtevant	Cosmopolitan
		repleta Wollaston	Cosmopolitan
	testacea	testacea von Roser	Holarctic
	not classified	picta Zetterstedt	Europe, localized
		cameraria Haliday	Europe, Madeira, Cyprus, Iran
Lordiphosa		andalusiaca Strobl	Europe, Morocco, Madeira, Cyprus, Israel, Syria
Scaptodrosophila	victoria	lebanonensis Wheeler	Europe, Israel, Lebanon, USA
		rufifrons Loew	Europe
Sophophora	melanogaster	melanogaster Meigen	Cosmopolitan
		simulans Sturtevant	Cosmopolitan
	obscura	ambigua Pomini	Europe
		helvetica Burla	Europe, India, Japan
		obscura Fallén	Europe
		subobscura Collin	Europe, Circummediterranean, Chile

References: Krimbas, C. 1963, DIS 37:95; _____ 1964, DIS 38:76; Krimbas, C. and M. Loukas 1982, DIS 58 (in press); Rocha Pité, M. T. and L. Tsacas, Bolm. Soc. port. Ciên. nat., 1979, 19:37; Triantaphyllidis, C. D. and L. Tsacas, Scient. Annal. Fac. Phys. and Math. Univ. Thessaloniki; Tsacas, L. 1963, DIS 37:135.

Valadé del Rio, E. Universidad de Santiago, Santiago de Compostela, Spain.

Simultaneous mutation and back-mutation in two sex-linked loci in D. melanogaster.

In a mutator-bearing strain of D. melanogaster, some yellow-body mutant males appeared. The new mutant behaves as sex-linked, and was symbolized y^{681}. It was found a strain with $\hat{X}\hat{X}$ females and these yellow males.

In order to study its genetic stability, a series of 19 crosses was performed using single $\hat{X}\hat{X}$ females and single y^{681} males as progenitors. The results obtained are presented in Table 1. Only a cluster of y^+ males was recovered, but surprisingly, the y^+ revertant flies simultaneously showed white eye color. It seems that the two mutational events (y^{681} to y^+ and w^+ to w^{781}) are related because of simultaneous occurrence in the same individuals and no appearance as single events. The new mutant behaved as sex-linked, and was symbolized w^{781}.

According to the hypothesis of insertion sequences (IS) that stem from somewhere in the genome and transpose to and integrate at the site of mutation (Green 1980), we can explain this result in the light that, in the beginning, the IS was located in the yellow locus and was transposed to the white one. As a consequence, the yellow mutants have back-reverted to

Table 1. Offspring of crosses made in order to study the stability of alleles.

Cross made				Revertants	
Female	Male	Replicates	Descendents	Phenotype	No. of clusters
$\hat{X}\hat{X}$	y^{681}	19	1,044	y^+ w^{781}	1 (4 individuals)
$\hat{X}\hat{X}$	w^{781}	17	1,735	0	0

wild color body and, simultaneously, wild color eyes have mutated to white color.

We established a strain with w^{781} males and $\hat{x}\hat{x}$ females, and performed a series of 17 crosses in order to study the stability of the new w^{781} mutant. Males carrying the w^{781} allele were mated with $\hat{x}\hat{x}$ females. The results obtained are presented in Table 1, where we can see that they have behaved as stable.

It seems that there is not any kind of chromosomal aberration associated with either mutant.

References: Green, M. M. 1980, Ann. Rev. Genet. 14:109-120.

Valadé del Rio, E. Universidad de Santiago, Santiago de Compostela, Spain.

Investigation during several generations of mutation and back-mutation of an unstable mutant of D. melanogaster.

In a mutator-bearing strain with an eye-color sex-linked mutant called "cast" (chestnut color eye, DIS 58), I have performed a series of crosses in order to investigate both mutation and back-mutation in successive generations. Males tested were individually mated with $\hat{X}\hat{X}$/Y females. The progenitor males in the first generation were sons of a single male, i.e., all of them carry a copy of the same cast allele. In every generation were found: (1) males carrying the mutant (or the revertant) that appeared in the previous generation, and (2) males carrying the alleles that behaved as stable in the same one. The scheme of crosses is presented in Fig. 1 and the results are presented in Table 1.

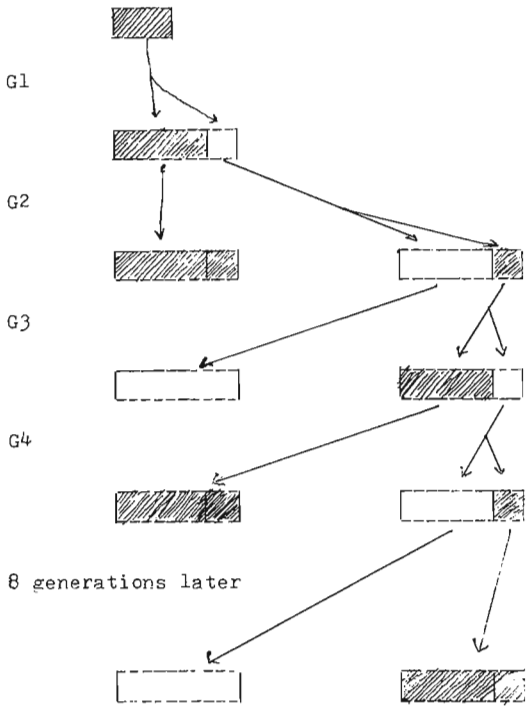


Fig. 1. Mating scheme. White square = wild type; black square = flies showing cast phenotype.

I recovered no mosaics, and that led me to believe that the cast alleles and their derivatives are stable in somatic lines. Other important data, not reflected in Table 1, is that in all cases of appearance of both mutations and reversions, the males appearing in the offspring were all mutant (or revertant) males, and in these single progeny no males like their father appeared. This is an unexpected result which raises questions yet unsolved, such as the nature of those mutations, and when they take place.

In Table 1 [following page], we can see that frequency of mutation and back-mutation are similar in the G1, G2 and G3 generations, but lower in G4. In G8 the instability is seen to be lost, and the tested alleles behave as stable. This is a normal behavior of other unstable mutants that we found in this unstable system: they are unstable during a few generations; after that, the alleles behave as stable and later, again, the instability appears.

I have not observed any chromosomal aberration associated with the several cast alleles.

Table 1. The phenotype of the offspring in successive generations. (Males were mated with XX/Y females.)

Generation	Genotype of male	Pairs	Clusters in progeny: Number	Clusters in progeny: Size	Progeny	Ratio	Mutational event
G 1	cast	27	1	39	1,765	0.0216	cast to cast$^+$
G 2	cast$^+$	26	1	28	1,477	0.0186	cast$^+$ to cast
	cast	18	0	0	1,693	---	---
G 3	cast	20	2	17, 23	1,543	0.0252	cast to cast$^+$
	cast$^+$	26	0	0	1,742	---	---
G 4	cast$^+$	23	1	14	1,621	0.0085	cast$^+$ to cast
	cast	19	0	0	1,547	---	---
G 8	cast	39	0	0	2,235	---	---
	cast$^+$	35	0	0	2,223	---	---

Valadé del Rio, E. and E. Costas. Universidad de Santiago, Santiago de Compostela, Spain. Interaction between genes for eye color in D. melanogaster.

Genotypes	Phenotypes
$\frac{cn^{rbr}\ bw^{979}}{cn^{rbr}\ bw^{979}}$	White eye
$\frac{cn^{rbr}}{cn^{rbr}} ; \frac{cast}{cast}$	Tangerine eye
$\frac{bw^{979}}{bw^{979}} ; \frac{cast}{cast}$	Brownish yellow eye
$\frac{cn^{rbr}\ bw^{979}}{cn^{rbr}\ bw^{979}} ; \frac{cast}{cast}$	White eye

In an unstable strain of D. melanogaster there have spontaneously appeared three different mutants whose phenotypical effect is on the eye color: cn^{rbr} rojo brillante, color bright red; bw^{979}, an allele of brown with color brownish yellow; and cast, color chestnut. The first and second mutants are located in the second chromosome; the third is sex-linked.

Between these three mutants there are interactions and epistatic effects, and so we can obtain the indicated genotypes that give their own phenotypes.

These results let us suppose that between the cn^{rbr} mutant and both bw^{979} and cast there are interactions, giving all double homozygote flies different and distinguishable phenotypes. But, between the bw^{979} mutant and the cast one, there is an epistatic effect, being the allele bw^{979} which behaves as epistatic.

Valente, V. L. S. and N. B. Morales. Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. Photomap and reference map of the salivary gland chromosomes of D. cubana Townsend.

Drosophila cubana Townsend belongs to the D. willistoni group as well as the species D. willistoni Sturtevant, D. paulistorum Dobzhansky and Pavan, D. equinoxialis Dobzhansky, D. tropicalis Burla and Da Cunha, D. insularis Dobzhansky, and D. pavlovskiana Kastritsis and Dobzhansky. It was raised to the category of species by Winge (1965) after an exhaustive series of interspecific hybridization experiments within the willistoni group. Before the publication of these results it had been considered as a subspecies of D. tropicalis and called D. tropicalis cubana (Townsend 1954).

The salivary gland chromosome complement of D. cubana consists of two pairs of submetacentric chromosomes, the X-pair and the pair II, and of one acrocentric pair, the chromosome III. The Y-chromosome is heterochromatic, not being distinguished from the chromocenter mass.

In the whole, five arms bound to a conspicuously dimensioned chromocenter can be observed.

The shortest arm is the X-chromosome's left arm (XL), followed in length crescent order by the X-chromosome's right arm (XR) and the second chromosome's left arm (IIL), which are both equivalent. Then follows the second chromosome's right arm (IIR) and chromosome III.

Figure 1 presents a photomap of the salivary chromosome's five arms from a third-stage larva. The D. cubana lineage used here came from Trinidad, Antilles, and was sent to our department by Dr. J. J. Townsend.

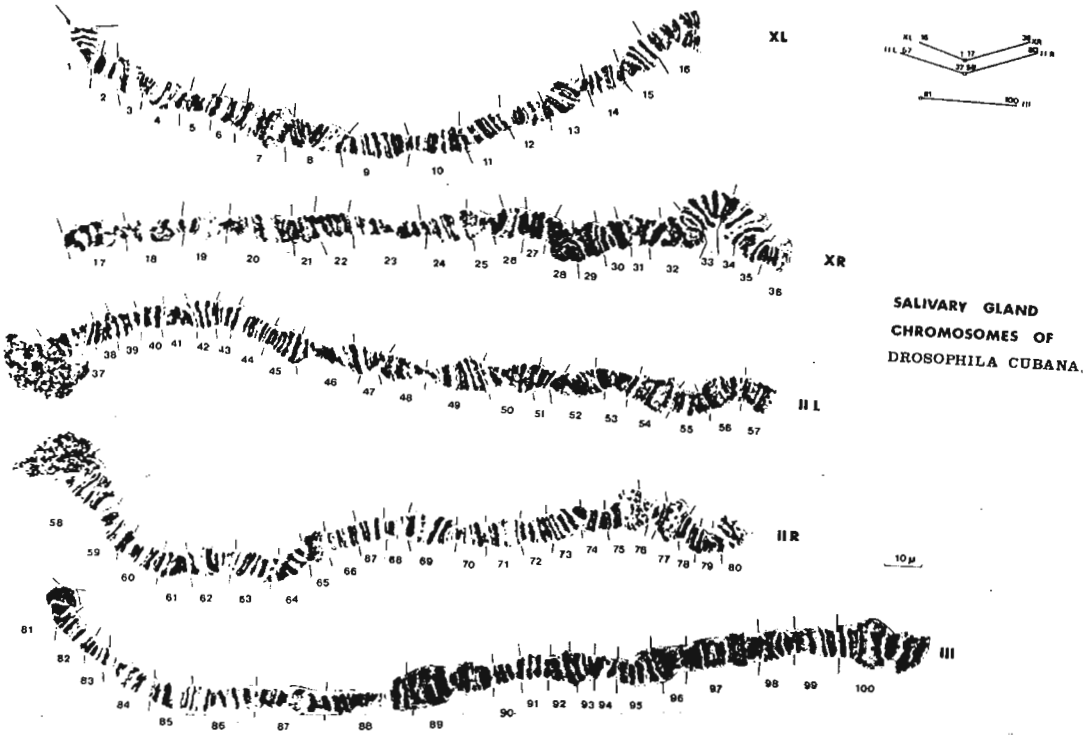


Fig. 1. Photomap of salivary gland chromosomes of D. cubana.

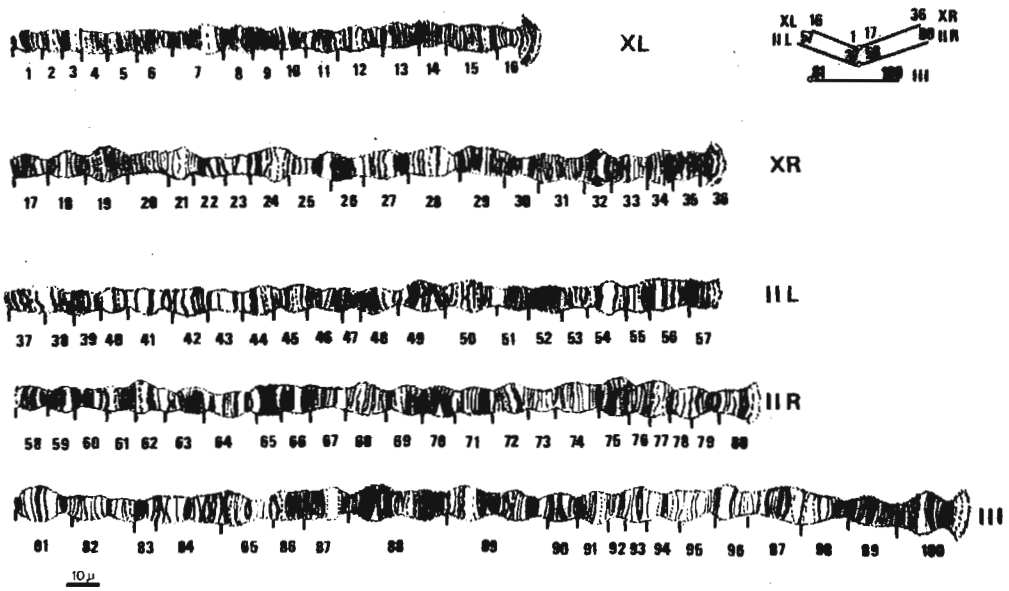


Fig. 2. Reference map of D. cubana.

Fig. 2 shows the reference map elaborated from camera lucida drawings after the observation of 25 individuals' cells and microphotography analyzing. The marker regions that are indicated in this map are described farther on.

D. cubana chromosome arms were subdivided into 100 sections of approximately the same size and according to the presence of diagnostic puffs and bands. Numbering is continuous from the XL base to the distal end of chromosome III.

XL was divided into 16 sections (1 to 16). It is easily distinguishable from the other arms by its short length, a fan-shaped tip and the presence of a characteristic puff in section 7.

XR was divided into 20 sections numbered from 17 to 36. Remarkable features in this chromosome are the tip section which is also spread like a fan as in XL but preceded by puffed sections, mainly the 34th where a "dotted line" band can be observed at the median region. Besides that, this arm is far longer than the left one; it presents a "repeat" in section 32 and a puff in region 19, in addition.

Chromosome IIL is similar in size to XR, being relatively free of puffs during the third stage and bearing a closed tip region shaped like a spatula. It was subdivided into 21 sections numerated from 37 to 57.

The right arm of chromosome II is quite long (23 sections numbered from 58 to 80). Near the tip it exhibits marker puffs in regions 75 and 77 which make it different from the terminal end of IIL; as reference features there are also a heterochromatic region in section 65 and a characteristic bulging base.

Finally, the third chromosome, which is the longest, has 20 sections numbered from 81 to 100 and presents typical base and tip. The base is paddle-shaped and the tip is opened after a constriction and a puffed region in section 100. Section 89 presents such a puff and wide bands pattern in the constriction of its medial region that make it dissimilar to any of the other sections found in the remaining four arms.

References: Townsend, J. I. 1954, Amer. Nat. 88:339-351; Winge, H. 1965, Heredity 20: 9-19.

Valentin, J. University of Göteborg, Sweden. Recombination pattern in mei-218 when target is inverted.

Many meiotic mutants, among them mei-218, cause a non-uniform reduction of recombination: distal ends are more affected than centromere regions. Several other alterations of recombination, notably Schultz-Redfield effects of heterologous aberrations, follow a similar pattern. Unfortunately nothing is known about the mechanisms creating this pattern. However, it is known that in Schultz-Redfield effects pattern is specific for the loci involved, not as one might think for distality or proximality (Ramel 1962). Thus if the target chromosome in which recombination is scored is reshuffled by homozygous aberrations, regions will respond to the Schultz-Redfield effect in the same manner as they did before, even if distal regions are moved to proximal and vice versa. This is also true for another meiotic mutant, mei-1 (Valentin 1973). Schultz-Redfield effects are proportional, not additive, to the effects of mei-218 which might indicate that these effects occur at different stages of meiosis (Valentin & Ståhl 1975). Therefore, mei-218 need not be locus-specific in its effects.

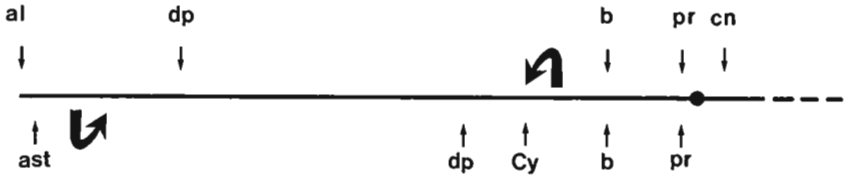


Fig. 1. Map positions of inversion breakpoints (big arrows) and of loci used in standard (above) and inverted (below) sequence, from Lindsley & Grell (1968) and this study.

Unfortunately, it is not so simple to devise good experiments to test this. Many nice homozygous viable inversions are X-linked, but so is mei-218. Thus for a model experiment, one would have to introduce not only suitable markers but also mei-218 itself into inversions by means of double crossovers. Since mei-218 is not repair deficient, progeny testing is always needed to verify its presence, so looking for the correct double crossover might prove tedious.

On the other hand, In(2L)Cy exists with several marker combinations, some of which are homozygous viable. Being paracentric it is not ideal since it does not make distal loci entirely proximal or vice versa. However, as a first approximation to the problem, recombination was scored in In(2L)Cy, al^2 Cy/In(2L)Cy, al^2 ast^3 dp b pr flies, which were also mei-218 homozygous or heterozygous (control). The results were compared with data of Carpenter and Sandler (1974) for corresponding loci in standard sequence.

Somewhat unexpectedly, the results of Table 1 [following page] rather suggest that the pattern specificity of mei-218 is also to target loci rather than to mere position along the chromosome. Obviously a more stringent test is needed before definite conclusions could be drawn. Nevertheless, it is titillating to speculate on mechanisms by which certain target loci might respond more pronouncedly than others to agents affecting recombination.

References: Carpenter, A.T.C. and L. Sandler 1974, Genetics 74:453-475; Lindsley, D.L. and E.H. Grell 1968, Carn. Inst. Wash. Publ. 627; Ramel, C. 1962, Hereditas 48:1-58; Valentin, J. 1973, Hereditas 75:5-22; Valentin, J. and G. Ståhl 1975, Genetics 81:325-331.

Table 1. Recombination frequencies in inverted and normal chromosomes 2 in mei-218 homo- and heterozygous flies (3 most significant digits given).

In(2L)Cy/In(2L)Cy, region:	ast - dp	dp - Cy	Cy - b	b - pr	N
y mei-218/y mei-218	5.23 %	0.169 %	0.338 %	1.01 %	1 185
y mei-218/+	31.9 %	5.14 %	3.49 %	5.79 %	5 237
Ratio $\frac{218/218}{218/+}$	0.164	0.033	0.097	0.174	-

Noninverted, region:	al - dp	dp ---------- b	b - pr	pr - cn
Ratio $\frac{218/218}{218/+}$	0.060	0.055	0.078	0.347

(Computed from Table 6 of Carpenter & Sandler 1974)

Vargo, M. and J. Hirsch. University of Illinois, Urbana-Champaign, Illinois.

Components of the proboscis extension reflex.

A food-deprived, water-satiated fly will show an increased probability of extending its proboscis to a water stimulus when that stimulus is preceded by a sucrose stimulus. This induced responsiveness has been termed the Central Excitatory State (CES) and has been characterized in the blow fly (Phormia regina) by Dethier, Solomon and Turner (1965). Through selection and single pair mating, Tully (1981) has fixed lines of P. regina for high and low expression of CES. Behavioral analysis has identified four components of the proboscis response: water responsiveness, water-induced CES (w-CES), sucrose-induced CES (s-CES), and conditioning. Tully's breeding analysis has shown that one major gene correlate is segregating in his pure breeding CES lines. Recently, the CES-induced proboscis response has been identified in D. melanogaster.

The test procedure we use to present evidence for CES (Fig. 1, following page) consists of a water pretest, a .25-M sucrose stimulation, a 45-sec interstimulus interval in which no stimuli are presented, and then a water posttest. All stimuli are administered to the prothoracic tarsi for 5 sec. The test has 12 trials with a 6-min intertrial interval (time from onset of pretest on one to onset of pretest on the next trial). Proboscis extension is scored as either 1 (extension) or 0 (no extension) resulting in a range of scores from 0 to 12 for pretest, sucrose, or posttest responses. Flies in the experiment were from the Berlin wild type strain (free-mating) obtained from the Mid-America Drosophila Stock Center at Bowling Green University. When tested all flies were approximately 48 hr old, water-satiated (Nelson 1971) and food-deprived for 36 hr.

Table 1. Mean response for pretest (PRE), sucrose (SUC) and posttest (POST) scores for each of six groups.

Group	PRE	SUC	POST	N
1	3.452	12.00	7.226	31
2	3.333	---	3.367	30
3	---	12.00	8.100	30
4	8.097	12.00	---	31
5	4.226	---	---	31
6	---	12.00	---	31

Our experiment tested 6 groups together, each receiving a different combination of the pretest-sucrose-posttest stimuli, in order to measure the effect of each stimulus condition on proboscis extension behavior. Referring to Table 1, the pretest and posttest scores of Group 1 differ significantly ($P < 0.001$, paired t-test) indicating that the posttest response is attributable to an excitation induced in the fly. Comparison of the pretest and posttest scores in Groups 1 and 2 indicated that only sucrose stimulation induces CES in Drosophila, whereas both water and sucrose have been shown to induce CES in the high-CES Phormia lines (Tully 1981).

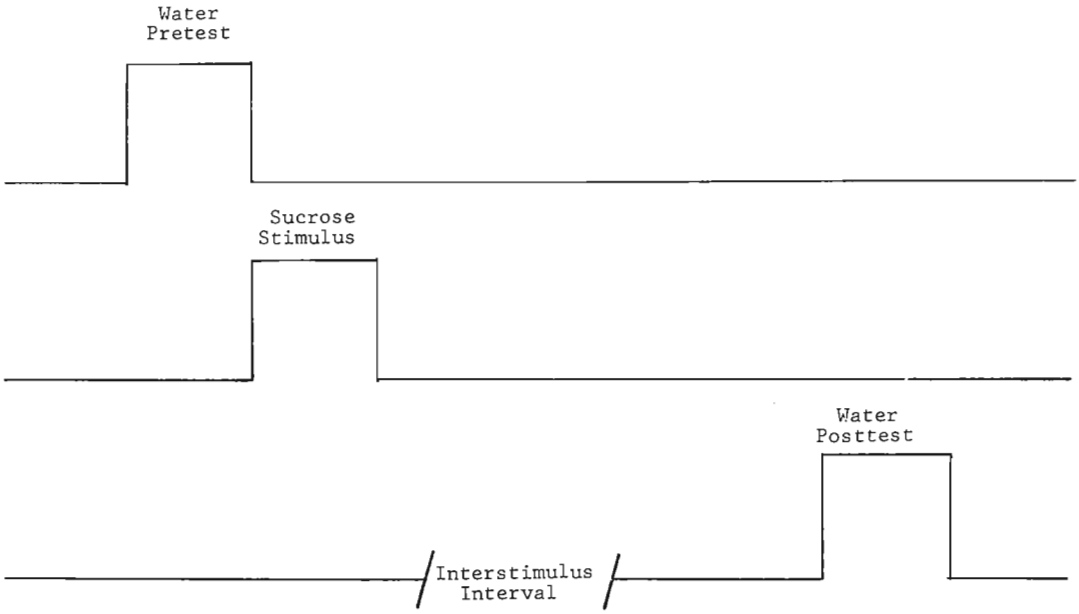


Fig. 1. Outline of the CES paradigm presented to an individual fly in one trial of the test. The length of the stimulus presentation is 5 sec, interstimulus interval is 45 sec, and intertrial interval is 6 min.

Comparing the pretest score of Group 4 with the posttest scores of Groups 1 and 3 reveals no significant difference, indicating (1) that pretest does not increase or decrease the number of posttest responses (comparison of Groups 1 and 3), and (2) that the excitation persists for at least 6 min in the fly (comparison of Groups 1 and 4). The pretest scores for Groups 1, 2 and 5 do not differ significantly, indicating that posttest stimulation does not increase or decrease pretest responsiveness.

In conclusion, each of the three stimuli in the test have a specific function. Sucrose (which produces the maximal number of responses throughout the experiment) induces an effect similar to the Central Excitatory State previously reported in Phormia. This excitation is measured by responsiveness to a water posttest and is completely dissipated by presentation of the posttest (comparison of pretest scores of Groups 1 and 5). The pretest acts as a measure of water responsiveness and does not itself induce any measurable excitatory state in the animal (comparison of pretest and posttest scores of Group 2).

The results of this experiment demonstrate the existence of a Central Excitatory State in D. melanogaster. Furthermore, this interpretation has been corroborated by the results of stimulating one foreleg only with water and the other one only with sucrose.

References: Dethier, V. G., R. L. Solomon and L. H. Turner 1965, J. of Comp. & Physiol. Psychol. 60:303-313; Nelson, M. C. 1971, J. Comp. & Physiol. Psychol. 77:353-368; Tully, T. P. 1981, Unpub. Ph.D. Dissert., Univ. of Illinois, Urbana-Champaign.

Voelker, R. A. and G. B. Wisely. NIEHS, Research Triangle Park, North Carolina.

Corrected and new information on $\ell(1)E12^{ts}$.

In our search for extant conditional lethal mutants which might be alleles of C4 (an allele coding for α-amanitin resistance of RNA polymerase II which maps at 1-35.66), we examined $\ell(1)E12^{ts}$ because of its reported location at 35.4 and its continuous temperature-sensitive phenotype. The initial tests for allelism showed that $\ell(1)L5$ (a nonconditional lethal allele of C4) and $Df(1)m^{259-4}$ (a deficiency known to include C4) were not allelic to $E12^{ts}$. Subsequent tests with other deficiencies which together spanned the cytological region from 10A to 11A failed to localize $E12^{ts}$. A genetic mapping experiment using y ct^6 ras f showed that $E12^{ts}$ mapped at 15.1 ± 1.0. We then attempted to cytologically map the locus, using deficiencies and duplications in the 5-7 cytological

region. The following deficiencies in that region were tested: Df(1)C149 (5A8-9; 5C5-6); Df(1)N73 (5C2; 5D5-6); Df(1)ct^{J6} (6E1; 7C1); and Df(1)ct^{J4} (7A2; 7C1). Only Df(1)N73 failed to complement E12^{ts}; thus, E12^{ts} maps in the 5C5-6 to 5D5-6 interval. This location was corroborated by the observation that T(1;2)rb^{+71g} (3F3-5E8 inserted in section 23) covered E12^{ts} and permitted survival at the restrictive temperature.

We suspect that the error in the reported map position can be accounted for by the following: Tarasoff and Suzuki do not report the data documenting the map positions of the four lethals described in their paper; they merely state that they map closely to the right of either cv or v and then give the respective distances for each mutant. If through some clerical error cv was replaced by v, their localization 2.4 to the right of cv = 13.7 (which is close to our 15.1) would become 2.4 to the right of v = 33.0, yielding their reported location of 35.4.

In the crosses involving Df(1)ct^{J6} and Df(1)ct^{J4} a peculiar phenotype was observed. Females heterozygous for either of these deficiencies and any of several wild-type or balancer X chromosomes exhibited a protuberance extending from the thorax in the region of the presutural and notopleural bristles. The penetrance of this trait was nearly 100%, with the expressivity varying from a slight bulge to a structure the length of a haltere. Since the effect was observed only in association with the two ct deficiencies, it is presumably attributable to some factor in the region common to both (7A2-7C1), perhaps the ct locus itself.

References: Greenleaf, A.L., J.R. Weeks, R.A. Voelker, S. Ohnishi and B. Dickson 1980, Cell 21:785-792; Tarasoff, M. and D.T. Suzuki 1970, Dev. Biol. 23:492-509; Craymer, L. and E. Ray 1980, DIS 55:200.

Walsh, B. and B. Dunlap. University of Washington, Seattle, Washington. Estimation of maximal reversion rates for the claret locus.

Homozygous ca females have claret eyes and aberrant meiotic chromosome behavior. Baker and Hall (1976) present evidence that cand is a double mutant, defective both at a locus controlling eye pigmentation and at one controlling meiosis. Two mutants, independently derived from wild type, ca (claret, normal disjunction) and ncd (wild type eyes, nondisjunctional) are both allelic to cand (O'Tousa and Szauter 1980), further supporting the hypothesis.

In the following experiment, we unsuccessfully attempted to determine whether cand is a double mutant by inducing reversions to ca^{+}. We mutagenized ca/cand males, mated them to ca/ca virgins, selected offspring with wild type eyes, and determined whether the ca or cand allele reverted by progeny testing for other linked markers. If cand had reverted, we would have determined its disjunctional phenotype, with the expectation that if cand were a double point mutant, the phenotypes should be separable by reversion, while if it were a deletion, it should not revert.

Approximately 10,000 progeny from males exposed to 4000R of gamma rays from a ^{60}Co source and 126,000 progeny from males which were treated with ethylmethane sulfonate (EMS) did not include any revertants for ca or cand.

It is possible to compute the maximum reversion rate consistent with these data. If v equals the probability of a reversion, (1-v) is the probability of no reversion, and $(1-v)^n$ is the probability of no reversions in n chromosomes. For n large and/or v small, we can approximate $(1-v)^n$ by e^{-nv}. To compute a 100α% upper confidence limit for v, we solve for v in the expression $e^{-nv} = 1 - \alpha$; which is the expression: Probability (no reversions in n trials given reversion rate v) = $1 - \alpha$. Thus, the 100α% upper confidence limit for the reversion rate is $v = -\ln(1-\alpha)/n$.

For our experiments, 126,000 progeny from EMS-treated males were counted. Half of these males had the mutagenized ca chromosome, while the other half had the cand chromosome, so that for both ca and cand, n = 63,000. Likewise for irradiated males, n = 5,000 for both ca and cand. Therefore, for EMS-induced reversion, the 95% upper confidence limit for the rate of reversion to ca is 4.75×10^{-5} and for gamma rays this maximum reversion rate is 5.99×10^{-4}.

References: Baker, B. S. and J. C. Hall 1976, in: Genetics and Biology of Drosophila, Vol. 1 (ed. E. Novitski and M. Ashburner), Academic Press, NY; O'Tousa, J. and P. Szauter 1980, DIS 55:119.

Zhimulev, I. F. and M. S. Feldman. Institute of Cytology and Genetics, Novosibirsk 630090, USSR. Genetic loci in the Lyra-deficiency region of the D. melanogaster 3L chromosome.

The salivary gland chromosome region removed by Df(3L)Ly (70A2-3 - 70A5-6 bands according to Lindsley & Grell 1968) was saturated with EMS-induced lethals and semi-lethals. The mating scheme is shown in Fig. 1. Eleven mutations were found (Table 1). Complementation mapping revealed three complementation groups (Fig. 2).

No one mutation showed phenotype Ly.

Ly^2 mutation (Waddle 1977) was found to be related to the same deficiency as Ly.

References: Waddle, F. 1977, DIS 52:3.

Table 1. Frequencies of mutations induced.

Experiment number	Mothers	No. of cultures	No. of fertile cultures	Mutations found
1	Sb/+	2,623	1,953	A1, A2, G1, G2
	D/+	1,564	1,016	F15, F16
2	Sb/+	3,187	2,123	F83, F85, F86
	D/+	1,053	1,053	F22, F68*

*Lost before analysis could be completed.

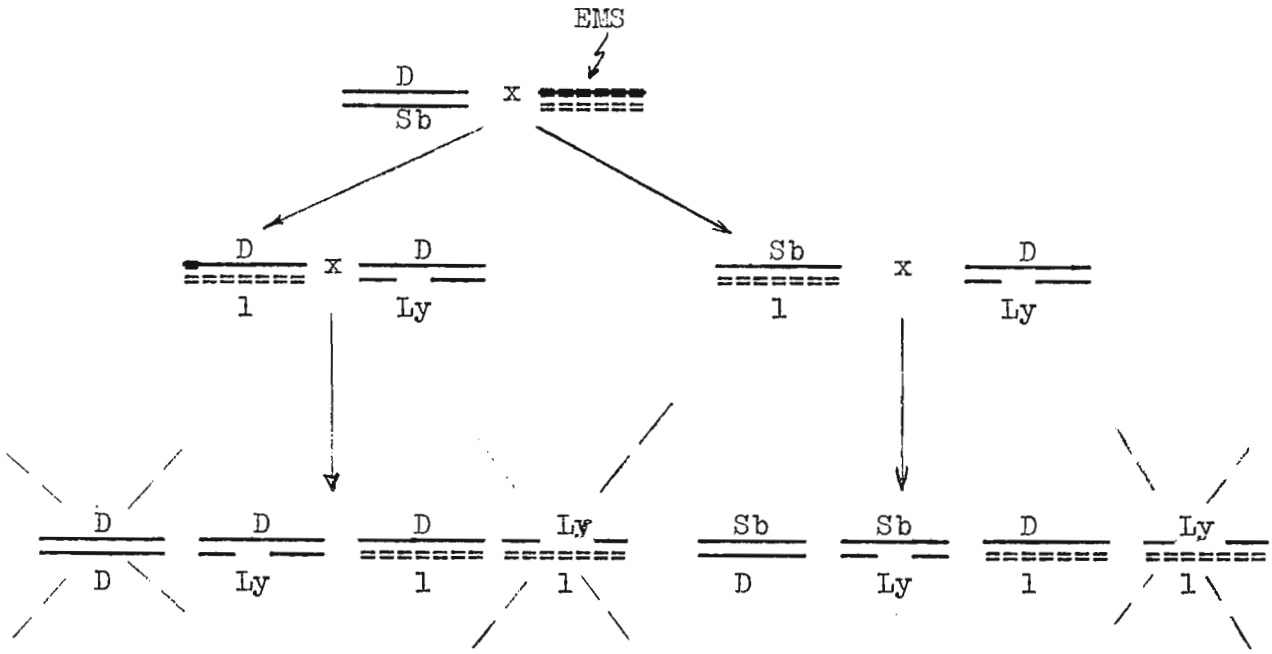


Fig. 1. Mating scheme for screening lethals in the Ly deficiency.

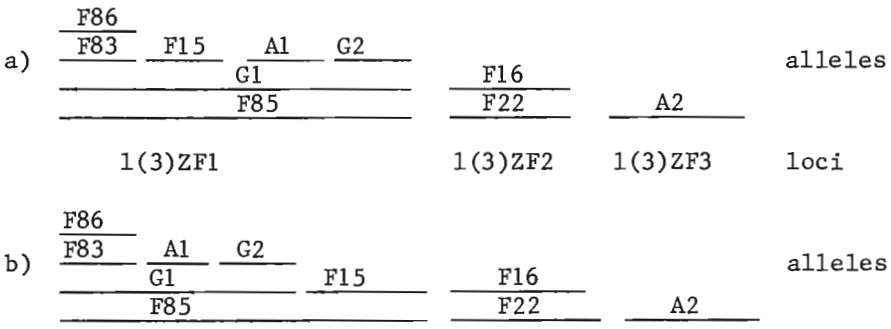


Fig. 2. Complementation map at 25°C (a) and 30°C (b).

Bird, S. R. and R. Semeonoff. University of Leicester, Great Britain. Electrophoretic analysis of many enzyme loci from a single fly homogenate.

We should like to report a convenient technique for electrophoretic analysis of Drosophila enzymes on cellulose acetate. The "12 Sample Super Z application system" together with "Titan III H cellulose acetate plates", both supplied by Helena Laboratories, 1530 Lindberg Drive, Beaumont, Texas, USA, can be used to analyze up to 12 samples from a single fly homogenate in a very short time in a buffer system originally designed for Hemoglobin electrophoresis.

Flies are homogenized using a rounded-off glass rod in 7 µl of distilled water in eppendorf centrifuge tubes and the tubes are spun for 30 seconds in an eppendorf micro-centrifuge. Enough supernatant is obtained to load each of the wells of the applicator with 5 µl as recommended. Electrophoresis is carried out at 200 to 300 volts for between 20 and 35 minutes. Staining is complete in a further 30 minutes. Thus in case of failure the samples are still available for a repeat run. Two plates can be run at the same time and since more than one locus can be analyzed on the same plate (e.g., ADH and αGPDH) a large number of systems can be scored in a little over two hours.

The following staining recipes have been developed. For ADH αGPDH and PGM electrophoresis is performed using the "Supre Heme" buffer of Helena Laboratories. For Est-6 we used a 0.015M sodium phosphate buffer, pH 7.0, and for ODH a TRIS-EDTA-Borate buffer, pH 9.0. All staining reagents are as supplied by Sigma Chemical Company. Staining recipes:

Est^6

Presoak plate in 0.015M sodium phosphate buffer, pH 7.0.
Run at 300 volts for 20 minutes using the same buffer.
Stain:
4 mg β-Napthyl acetate
50 mg Fast Blue B
15 ml 0.15M Na_2HPO_4
35 ml 0.15M NaH_2PO_4

PGM

Presoak plate in "Supre Heme" buffer.
Run at 300 volts for 20 minutes using the same buffer.
Stain:
2 mg MTT
0.7 mg PMS
10 µl G-6-PDH
7 mg G-1-P (with 1% G-1.6 Di PO_4)
1 mg NADP
10 ml 0.2M Tris buffer pH 7.4
10 ml 2% agar for overlay
(A stronger stain produced shadow bands ahead which made scoring difficult.)

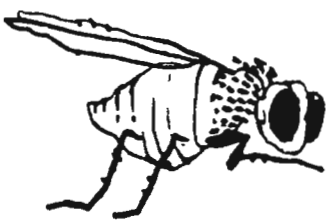
ADH AND αGPDH

Presoak plate in "Supre Heme" buffer.
Run at 200 volts for 30 minutes using the same buffer.
Stain:
2 ml Ethanol
8 mg MTT
3 mg PMS
8 mg NAD
10 mg αGP
8 ml 0.2M Tris buffer pH 8.0

ODH

Presoak plate in buffer 87 mM Tris, 8.7 mM Boric acid, 1 mM EDTA, pH 9.0.
Run at 200 volts for 30 minutes using the same buffer.
Stain:
0.7 ml of Octanol in 2 ml Ethanol
50 mg βNAD
40 mg MTT
5 mg PMS
100 ml 0.05M Tris buffer pH 8.5
With this recipe ADH also stains due to the presence of the Ethanol. This does not interfere with the ODH bands as ODH migrates far more rapidly than ADH.

All staining was done in darkness at 26°C. It is important that the cellulose acetate plates are placed on a horizontal surface.



Blount, J. L. Mount Union College, Alliance, Ohio. Bascy, an improved Basc stock.

A new Basc-derived stock, Bascy, containing a yellow mutant of spontaneous origin is now available. The yellow allele produces bright yellow body, brown bristles and hairs with yellow tips, and yellow wings and veins. The usual Basc markers are, of course, still present in the stock, but the scute phenotype is of increased penetrance. Crossover suppression and salivary cytology appear identical to those of Basc. Use of the Bascy stock in scoring recessive sex-linked lethals may increase the ease and speed of the process, since half of the classes possible in the F_2 can be identified by body color. Bascy may also be of use in additional screening processes, particularly those making use of a y^+ marked Y-chromosome. Bascy has been formed containing autosomes derived from Canton-S wild, and is available from the Bowling Green, Ohio, stock center.

Curtsinger, J. W. University of Minnesota, St. Paul, Minnesota. A control experiment for the Hirsch-Hadler classification maze.

The Hirsch-Hadler classification maze has been a fundamental tool of Drosophila behavior genetics research for several decades. Depending on the orientation and illumination of the maze, it can be used to study phototaxis or geotaxis in groups of flies (e.g., Dobzhansky & Spassky 1968). This note reports the results of control experiments in which flies moved through a horizontal and evenly illuminated maze. The performance of flies in the absence of light or gravitational cues reported here suggests a new component in the maze behavior of D. pseudoobscura.

A standard 10-level Hirsch-Hadler maze was obtained from Dr. S. Kessler, Stanford University. Experimental stocks of D. pseudoobscura originated from a large population cage founded with 40 isofemale lines collected in San Mateo County, California. Stocks were maintained at approximately 22°C on Carolina Instant Medium. The maze was used in horizontal position in a noise- and temperature-insulated environmental chamber at 22°C. The maze was mounted in a topless black box with cool fluorescent lighting in the plane of the maze, at the collection tube end. Foodless collection tubes were plugged with moist cotton during experiments. All experiments were initiated at 4-6 p.m. and continued for 24 hours without disturbance. Tested flies were males and females 5-7 days post-emergence at the commencement of experiments. Six experiments were initiated with 200 flies placed at the maze origin, seven experiments with 100 flies, and one experiment with 350 flies. Overall 74% of the flies completed the 10 levels of the maze within 24 hours. The maze was disassembled and cleaned after every experiment. All testing was completed in a four-week period.

Table 1.

Experiment	N	$\bar{L}$	SD	σ_L
1	172	5.02	2.83	1.58
2	164	4.44	2.91	1.57
3	132	3.71	2.78	1.54
4	137	7.18	2.48	1.45
5	174	6.63	2.86	1.49
6	133	5.54	2.72	1.57
7	280	3.58	2.57	1.52
8	73	7.17	2.62	1.45
9	46	5.41	2.52	1.58
10	21	5.38	2.67	1.58
11	79	6.85	2.15	1.49
12	84	4.23	2.55	1.57
13	70	2.94	2.57	1.45
14	65	3.77	2.01	1.54

N = number of flies completing the maze in 24 hours.
$\bar{L}$ = mean number of left turns in a horizontal and evenly illuminated 10-level Hirsch-Hadler maze.
SD = observed standard deviation in L.
σ_L = expected standard deviation based on a binomial distribution.

In moving through the maze from the origin to one of the 11 collection tubes, each fly must make a series of 10 turns. The collection tubes are numbered 0, 1, ... 10, such that a fly that makes L left turns and (10 - L) right turns in any sequence ends up in collection tube L.

The means and standard deviations of L in 14 control experiments with a horizontal and evenly illuminated Hirsch-Hadler maze are shown in Table 1. A total of 1,630 flies were tested. The grand mean of L is 5.07, based on pooling all individual scores. The grand mean is not significantly different from 5.00, indicating no overall left-right bias in the maze. The expected standard deviations based on a binomial distribution (with the mean estimated from $\bar{L}$ for each experiment) are shown in the fifth column of Table 1. Note that in every experiment the observed variance exceeds that expected on the basis of binomial sampling.

To interpret the overdispersion of maze scores, it is useful to consider the conditions for a binomial distribution (Yule & Kendall 1950): (1) For

each individual the probability of a left turn must be the same at each level of the maze. (2) The probability that an individual turns left at one level of the maze must be independent of turns at other levels. (3) The probability of a left turn must be the same for all individuals. If (1) is violated then the observed variance will be less than that expected for a binomial distribution. If (2) and/or (3) are violated, i.e., if an individual's turns are correlated and/or individuals differ in probabilities of making left turns in an undifferentiated maze, then the observed variance will be larger than that expected from binomial sampling. All three assumptions might be violated here, but the net effect is that violation of (2) and/or (3) overwhelms possible violation of (1), leading to inflated variance in each of the 14 control experiments. It can be concluded that D. pseudoobscura exhibits a correlation of turns and/or heterogeneity between individuals with respect to left-right turns in the absence of light or gravitational cues. This is evidence for a previously unacknowledged component of behavior in the standard Hirsch-Hadler maze.

I thank Dr. M. Seiger and Dr. T. Tully for critical comments.

References: Dobzhansky, Th. and B. Spassky 1969, Proc. Nat. Acad. Sci. USA 62:75-80; Yule, G. U. and M. G. Kendall 1950, An Introduction to the Theory of Statistics, 14th ed., Hafner, New York.

Fleming, R. J. Brandeis University, Waltham, Massachusetts. A quick method for visualizing the internal structures of Drosophila larvae.

This rapid staining technique allows one to see the clear three-dimensional arrangement of larval internal structures (see Fig. 1). The larval preparation is easy and takes only 15 minutes.

(1) Heat-kill a larva in 55-60°C water (2-3 second immersion). (2) transfer the larva to a depression slide and cut the epidermis and outer cuticle longitudinally with microscissors in saline solution. (3) Replace saline with Kahles fixative (6:1:46 solution of formalin:acetic acid:35% ethanol). Let stand 2-3 minutes. (4) Replace fixative with 0.1% Eosin Y in 200:1 solution of 70% ethanol:acetic acid. Let stand 1-2 minutes. (5) Wash out excess Eosin with fixative. Leave specimen in fixative on the depression slide and add 3 drops of 0.05% Fast Green in 95% ethanol. Let stand 1-2 minutes. (6) Wash out excess Fast Green with fixative. Leave specimen in fixative and cover the depression slide with a coverslip to prevent evaporation.

These preparations are not permanent and tend to gradually lose clarity after about an hour. The procedure can also be extended to adult internal structures.

Fig. 1. Late third instar larva cut mid-ventrally. B, brain lobe; E, eye disc; G, gut; L, leg discs; M, muscles; MP, malpighian tubules; PV, proventriculus; SG, salivary gland; VG ventral ganglion. X37.

Gasaryan, K. G. and A. K. Shahbazyan. Institute of Molecular Genetics, USSR Academy of Sciences and Moscow State University, Moscow, USSR. Multiple microinjections into Drosophila embryos.

Intracellular injections are increasingly used for various studies of early embryos. We propose a technique of multiple microinjections into Drosophila eggs. We have used the principle of a manipulating table and a fixed microcapillary (Fig. 1A). A mechanical manipulator was constructed on the basis of a microscope with an object guide and a temperature-controlled table attached to it. As a result it is possible to move the table in all three dimensions with the required accuracy and to perform the injections at a constant temperature. The injector consists of a microsyringe (10 µl), a piston operating microscrew and a needle-holder. The microsyringe is tightly connected to a glass microcapillary by means of a flexible tube; the diameter of the needle tip is 5-7 µm. The syringe and the tube are filled with water while the microcapillary is filled with the solution to be injected and vaseline oil. Drosophila eggs are deposited on a slit cover glass (6x20) (Fig. 1B) and lubricated by vaseline oil to prevent drying up. After the injection the eggs on the cover glass may be immediately transferred to food medium or incubated in a humid chamber.

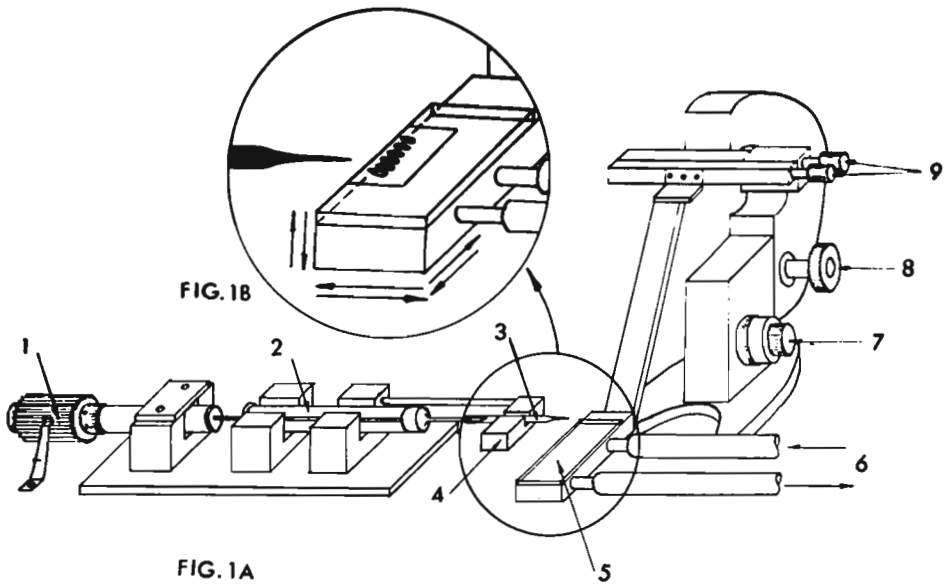


Fig. 1. (A) A mechanical manipulator and an injector. 1 - piston operating microscrew; 2 - microsyringe; 3 - microcapillary; 4 - microcapillary holder; 5 - temperature-controlled table; 6 - to refrigerator (thermostat); 7 - microscrew; 8 - macroscrew; 9 - microscrews of object guide. (B) A manipulating table.

Grimaldi, D. and J. Jaenike. State University of New York, Binghampton, New York. Laboratory culturing of mycophagous drosophilids.

Here we describe two techniques for raising mycophagous species in vials, and a method to collect eggs from these species. Those we have raised are D. falleni, D. recens, D. putrida, D. testacea, D. tripunctata, and Mycodrosophila dimidiata. Laboratory rearing of Leucophenga varia was not successful. A medium has been developed for studies requiring homogeneous vial lots and for larval substrate modifications. The following is the procedure for preparing the medium:

Ingredients:
500 g Agaricus bisporus (commercial) mushrooms
800 ml distilled water
30 g cellulose powder (Alphacel non-nutritive bulk, ICN Pharmaceuticals, Inc.)
50 ml Karo light corn syrup
12 g agar
7 g brewer's yeast
6 ml Tegosept (20%, in 95% ethanol)
1.4 g ascorbic acid
0.14 g Nystatin (dissolved in 2 ml 95% ethanol)

Chop mushrooms and mix with water in a blender until smooth. Add cellulose powder, yeast, corn syrup, and agar. Stir and then boil gently for about 5 minutes. Allow mixture to cool to 70°C, then add Tegosept, Nystatin, and ascorbic acid. Stir well and pour into vials while hot. Nystatin inhibits yeast growth and so prevents asphyxiation of larvae by a layer of yeast that can grow over the surface of the medium. Each vial with medium need be seeded with only

one grain of live baker's yeast. D. tripunctata and M. dimidiata were not raised on this medium, but the other Drosophila breed well on it. D. putrida and D. testacea thrive best on conditioned medium; as such, they require more larvae per vial than the other species for successful culturing.

To simply maintain lines and for generally easier culturing, empty vials are packed with about 1 g sterile cotton and a piece of Agaricus is placed on top. Agaricus appears to be more suitable for larval growth if it is first soaked for 2-3 hours in a suspension of live baker's yeast in warm water. Each piece of mushroom should include a portion of cap and stipe (stem), since larvae develop in both portions, while females generally prefer to oviposit on the stipe. Pupation occurs in the cotton. Because the rotted mushroom and the puparia adhere to the cotton, inverting the vial and tapping removes adult flies without having debris fall out.

In order to collect eggs, large numbers of flies were bred in population cages. The cages were (l x w x h) 30 cm x 25 cm x 20 cm; the sides and bottom were made of plywood, while the top was glass. At one end was an opening for a cloth sleeve. About 3 cm of sterile sand was placed on the bottom of the cages, and about 15 Agaricus mushrooms were placed on the sand. Several thousand flies will emerge in one generation. To collect eggs, flies are starved for about 24 hr, and then plugs of mushroom medium (topped with yeast) are placed in the cages overnight. The eggs laid on the medium can be removed easily by cutting off slices.

Hungate, F. P. Battelle, Pacific Northwest Laboratory, Richland, Washington.

A CO_2 anesthetizer.

The problem of making large numbers of pair matings or otherwise handling large numbers of flies, using ether as the anesthetic, is well recognized as is the alternative of using CO_2. We have examined several porous materials as potential surfaces for a continuous flow CO_2 anesthetizer for use under the binocular microscope. The material we selected is a porous semirigid plastic having a white smooth surface (Porex™ Glasrock Products, Inc., 7380 Bohannon Road, Fairburn, Georgia 30213). The unit we developed uses the 1/8" thick high density polyethylene (Porex™), having 35 micron pores, as the diffuser plate. A 10 micron pore material is also available but was not tested. The body of our unit is lucite (Fig. 1). The screws can be removed to wash or replace the porous sheet when it becomes dirty or contaminated. The front edge over which flies are removed into tubes can be taped or glued to the lucite base or fitted to a retaining groove in the lucite to assure full flow of CO_2 through the porous plastic.

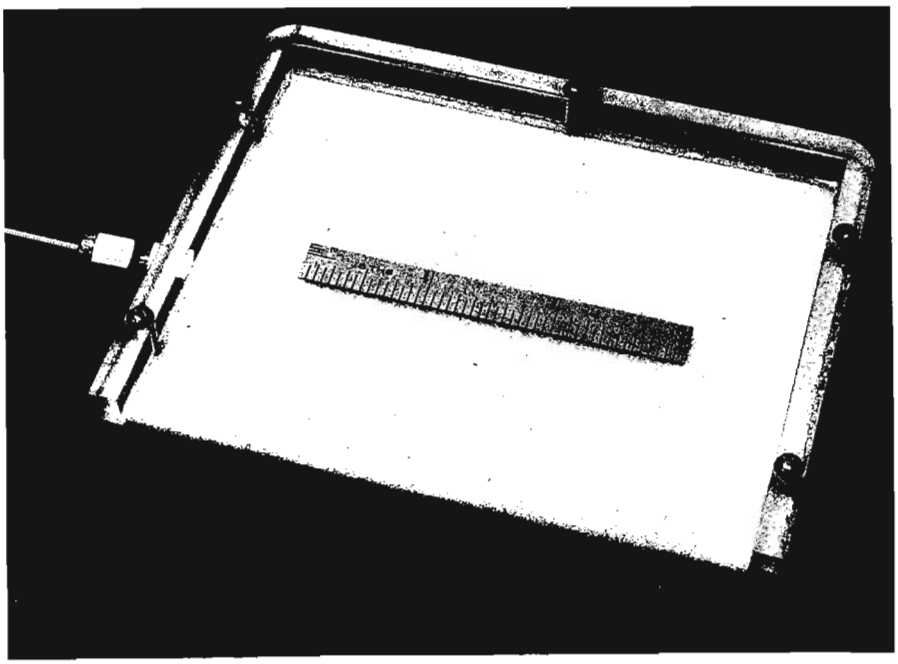


Fig. 1. Top view of anesthetizer showing diffuser plate held in place by a three-sided curb bolted to the chamber base.

This anesthetizer has been valuable in handling flies that have been treated with carcinogens since there is virtually no chance for the flies to escape. We have adapted our standard ether type anesthetizer with a rubber tube to supply CO_2 and, with a Y in the line from the CO_2 tank, the CO_2 can be valved or clamped with a hemostat to go to either the anesthetizer funnel or to the diffuser unit. A two-stage pressure reducing valve, preferably with a needle valve adjustment on the exit line, allows one to adjust CO_2 flow to the minimum required to immobilize the flies.

Anesthetization with CO_2 is near instantaneous and is much less toxic to the flies and to humans than is ether, and CO_2 presents no fire hazard. It should not be used to anesthetize flies carrying genes for CO_2 sensitivity.

Work supported by the U.S. Department of Energy under contract DE-AC06-76RL0 1830.

Koana, T.* and T. Miyake. Mitsubishi-Kasei Institute of Life Sciences, Tokyo, Japan. [*Present address: International Christian University, Tokyo.] Histochemical determination of the genotype of primary cultures from single Drosophila embryos.

In the analysis in vitro of developmental lethals of Drosophila, an abnormality which appeared in the expected frequency (1/4) in the cultures (from single embryos) has been presumed to be the mutant symptom since the lethal embryos could not be distinguished from heterozygous or normal ones (Donady & Seecof 1972; Cross & Sang 1978). To eliminate this ambiguity, we have developed an enzyme marker system to identify the genotype of cultures. It is a histochemical method to stain for glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49) activity (see Pearse 1972) in a primary culture. By linking Zw^n (G6PD$^-$, 1-63) to a lethal gene, the cultures from embryos hemizygous for the lethal gene could be identified by the absence of G6PD activity.

Our culture method was the same as the column drop culture of Shields et al. (1975), except that the floor of the culture chamber was made of a coverslip instead of a glass plate. For staining, cultures were first frozen by putting the coverslip with cells on a flat dry-ice surface. Then a drop of the reaction mixture A was layered on each culture. The reaction mixture A consisted of:

glucose-6-phosphate	15 mg
NADP, sodium salt	1.5 mg
polyvinyl pyrollidone	75 mg
0.5M Tris (pH 7.5) 0.005M $MgCl_2$ (aqueous)	1 ml

After 25 min at room temperature, a drop of the reaction mixture B was layered on each culture. The reaction mixture B consisted of:

nitroblue tetrazolium	4 mg
phenazine methosulphate	0.5 mg
0.5M Tris (pH 7.5) 0.005M $MgCl_2$ (aqueous)	0.5 ml
Methanol	0.5 ml

After 15 min in a light-tight box at room temperature, reaction was terminated by immersing the coverslips in tap water at 45-50°C for 30 sec. Positive reaction was indicated by the presence of deep purple diformazan precipitate.

When a wild-type culture was stained, cell aggregates were stained deep purple (Fig. 1a, 1b). On the other hand, Zw^n cultures were never stained positively (Fig. 1c, 1d). Although Geer et al. (1974) reported that Zw^n flies had approximately 10% of the normal level of G6PD activity, we found no detectable activity in any Zw^n cultures. The reaction mixture without substrates and/or dyes never stained wild-type cultures.

In the cross between heterozygous Zw^n females and normal males, 1/4 of the embryos were expected to be hemizygous for Zw^n, and therefore not to show G6PD activity. The other 3/4 were expected to be normal or heterozygous and to show G6PD activity. When homozygous Zw^n females were crossed with normal males, embryos with or without G6PD activity should be half and half. The results summarized in Table 1 agreed well with the expectation. No difference

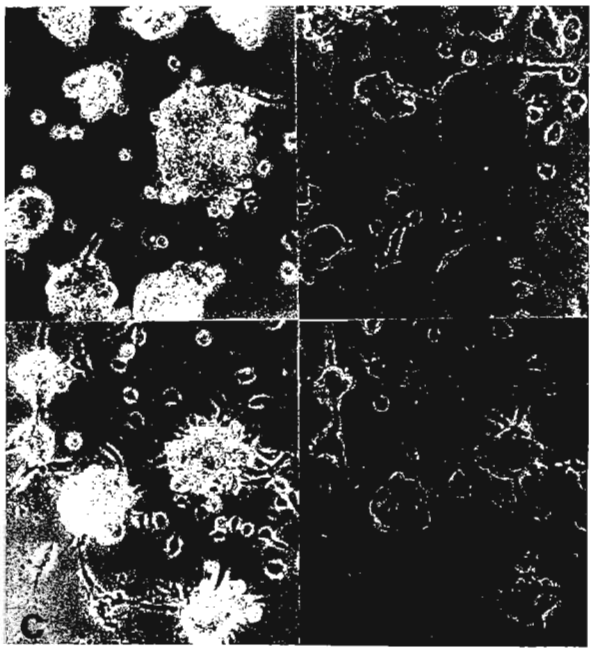


Fig. 1. G6PD staining of primary cultures from normal and Zw^n single embryos. (a) Wild-type culture before staining. (b) The same culture as in (a), after staining for G6PD activity. Cell aggregates were stained deep purple by diformazan precipitate. (c) Zw^n culture before staining. (d) The same culture as in (c), after staining. No precipitate formed. Phase contrast.

Table 1. G6PD staining of cultures from single embryos obtained from various crosses.

Parents' genotype ♀	♂	Expected frequency of Zw^n embryos	Number of cultures stained	unstained
+/+	+/Y	0.0	35	0
Zw^n/Zw^n	Zw^n/Y	1.0	0	27
$Zw^n/+$	+/Y	0.25	27	8
Zw^n/Zw^n	+/Y	0.5	12	11
y w $Zw^n/FM7^c$	+/Y	0.25	34	11

between $Zw^n/+$ cultures and +/+ cultures, or between Zw^n/Zw^n cultures and Zw^n/Y cultures could be found. The results in Table 1 also indicated that there was no sign of a maternal effect, and each culture was stained or unstained according to its own genotype.

As G6PD is water soluble and extremely sensitive to fixation (Kankel & Hall 1976), it is not very easy to tell whether each cell was stained or not, but there is no difficulty nor ambiguity in determining whether each culture was stained or not. It was made possible to use Zw^n as a marker gene for the identification of lethal embryos. When the embryos are taken from the cross 1-Zw^n/FM7 x +/Y, only the cultures from hemizygous lethal embryos are unstained and those from heterozygous or normal ones are stained. Thus, an abnormality found in a culture can be directly correlated to its genotype. We are now doing such an experiment and preliminary data show a good correlation between the G6PD activity and the mutant symptom in vitro.

References: Cross, D.P. and J.H. Sang 1978, J. Embryol. exp. Morph. 45:173-187; Donady, J.J. and R.L. Seecof 1972, In Vitro 8:7-12; Geer, B.W., J.T. Bowman and J.R. Simmons 1974, J. Exp. Zool. 187:77-86; Kankel, D.R. and J.C. Hall 1976, Dev. Biol. 48:1-24; Pearse, A.G. Everson 1972, Histochemistry, Theoretical and Applied, Vol. 2, Churchill Livingstone; Shields, G., A Dübendorfer and J.H. Sang 1975, J. Embryol. exp. Morph. 33:159-175.

Kotarski, M., R. J. MacIntyre and S. Pickert. Cornell University, Ithaca, New York. A rapid method for enzymatic spot testing.

The need for a large number of low and null activity mutants of α-glycerophosphate dehydrogenase (αGPDH) in D. melanogaster required the development of an efficient and reliable technique for assaying large numbers of single flies. Using the method presented below, 48 single flies can be assayed at one time for αGPDH activity.

Single flies are placed in the wells of a flat-bottomed, plastic microtiter plate (Falcon Plastics) and 20 µl of a homogenization buffer[1] is placed into each well. The flies are homogenized using a custom machined, 48 pronged aluminum homogenizer[2] (Fig. 1). The use of only 20 µl of buffer makes the homogenates viscous enough to stick to the ends of the homogenizing prongs. After homogenization is complete, the assemblage is inverted and the microtiter plate is lifted from the homogenizer leaving a drop of homogenate atop each of the 48 prongs. The

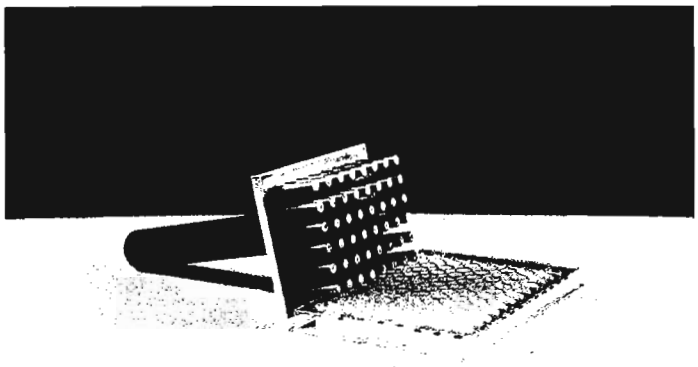


Fig. 1. The microtiter plate and homogenizer.

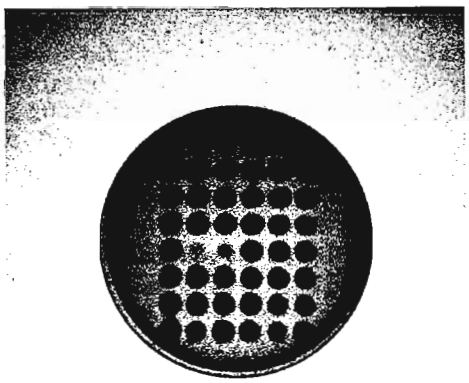


Fig. 2. The results of a spot test. Dark spots are $\alpha GPDH^+/Df$ homogenates; light spots are $\alpha GPDH^{null}/Df$ homogenates.

homogenizer is then inverted and pressed gently onto a petri dish containing substrates in an agar base[3]. The homogenizer is removed, transferring the drops of homogenate onto the substrate surface. After an incubation of 15-20 minutes (25°) the αGPDH positive homogenates have produced a very dark blue spot from the enzyme's reaction while the αGPDH negative homogenates produce very little or no color (Fig. 2).

This assay can distinguish $\alpha GPDH^{+}/Df$ from $\alpha GPDH^{null}/Df$ flies as long as the α-glycerophosphate concentration is kept limiting in the reaction. This test cannot discriminate between $\alpha GPDH^{+}/\alpha GPDH^{+}$ and $\alpha GPDH^{+}/Df$ flies.

This method has been used successfully to assay 23,910 single F_2 flies from an ethylmethane sulfonate mutagenesis (Lewis & Bacher 1968). This experiment produced eight null mutants and 21 low activity mutants (2-70% αGPDH activity). Among the low activity alleles are 15 electrophoretic variants.

This spot testing method should be adaptable to many other enzyme systems where large numbers of mutants are desirable.

[1]Homogenization buffer: One part tris barbitol-sodium barbitol buffer (Gelman, HR Buffer, Gelman Instruments), ionic strength 0.05, pH 8.8, and one part 0.1M tris HCl, pH 8.3.

[2]Homogenizer: 8.0 x 6.0 x 0.5 cm aluminum block base with 48, 0.5 cm diameter cylinders as prongs. Prongs are spaced 0.9 cm center to center. Produced by the Cornell University Physics Machine Shop, Ithaca, New York.

[3]Agar substrate: One part 1.25% agarose (SeaKem ME) which is allowed to cool slightly before mixing with one part 2.26mM β-NAD (from yeast 98%), 1.20 mM MTT tetrazoleum, 1.0 mM phenazine methosulfate and 1.40 mM DL-α-glycerophosphate hexahydrate (disodium salt). The agar and substrate phases are made in 0.05M tris HCl buffer, pH 8.3.

References: Lewis, E.B. and F. Bacher 1968, DIS 43:193.

Krasnov, P., A. Karavanov and L. Korochkin. Koltzov Institute of Developmental Biology, USSR Academy of Sciences, Vavilov Str. 26, Moscow 117334, USSR. New technique of isolation and purification in preparing quantities of bulbus ejaculatorius of Drosophila.

The past few years are characterized by the increased interest of many investigators in the problem of regulation of differential gene expression in eukaryotes. Successful solution of this problem is in many aspects dependent on the choice of experimental model. Such a model has to meet a number of criteria, the most important of which are: the presence of pronounced specialized function; the synthesis of well identifiable final gene product; the possibility of both qualitative and quantitative evaluation of changes of this synthesis in the process of development, differentiation and/or under the action of exogenous stimulus. In addition, a thorough genetical knowledge of such a model is desirable.

The traditional model of this laboratory--bulbus ejaculatorius of Drosophila--satisfied all these criteria (Korochkin 1980). This organ is characterized by highly specialized function, well identifiable synthesis of the organ-specific enzyme S esterase, with well defined periodical changes of its synthesis in the processes of development and differentiation; and thorough genetical knowledge of Drosophila requires no commentary. However, the use of this model for biochemical studies was considerably limited up to now by difficulties in obtaining preparative amounts of material: all the isolation and purification processes were performed by hand under microscopic control. In the present paper we report the development of a new rapid and simple procedure permitting isolation and purification of practically unlimited amounts of material by mechanical means.

The protocol described below was employed to isolate and purify bulbus ejaculatorius from 1000 D. virilis flies without preliminary separation of females. All the operations were carried out at 4°C.

Flies were placed into a 100.0 ml centrifuge tube and vaseline oil in a volume necessary to completely cover the flies was added. 60 polystirole beads 3 mm in diameter were thoroughly mixed with the vaseline oil and flies, followed by the addition of 40.0 ml of buffer A (0.2 M sucrose, 3 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.4). This mixture was homogenized for 1.5 min. at 2500 rpm with a motor-driven blade equipped homogenizer. In Figure 1 the appearance of such a homogenate is presented. One can see two layers: the upper one consists of the vaseline oil emulsion in buffer A and contains all the hitin capsules of the flies and parts of internal organs; the lower one contains the majority of internal organs including bulbuses.

such as eggs and salivary glands. Moreover, the application of vaseline oil for the separation of hitin capsules of flies may be useful for a number of other operations with Drosophila.

References: Korochkin, L. 1980, Isozymes 4:159 (M. Rattazzi, J. Scandalios and G. Whitt, eds.), Alan Liss, Inc., New York.

Lankinen, P. and J. Lumme. University of Oulu, Finland. An improved apparatus for recording the eclosion rhythm in Drosophila.

We have earlier described an apparatus for monitoring the pupal emergence rhythm of Drosophila (Lumme & Lankinen 1976). The apparatus was based on the "falling ball principle" originally presented by Truman (1972). Now we describe an improved version of our apparatus, which has proven to be reliable and to function faultlessly during several years of use. Furthermore, the new model is easier to construct and essentially less work is needed to keep it running in comparison to our older model.

We are well aware that even simpler devices than ours are available. Some of the most elegant are based on the "falling fly principle" (e.g., Maier 1973), and they have been used successfully for D. pseudoobscura and D. melanogaster. In that type of a machine, the flies slipper soon after eclosion on the inner walls of a teflon-coated funnel, and fall down through a registering photo-gate sensor. Unfortunately, our main subject of study, D. littoralis, is too clever for these devices. Emerged D. littoralis adults spend a variable long time in the funnel before dropping down into the soapy water.

The structure and function of the "eclosionometer":

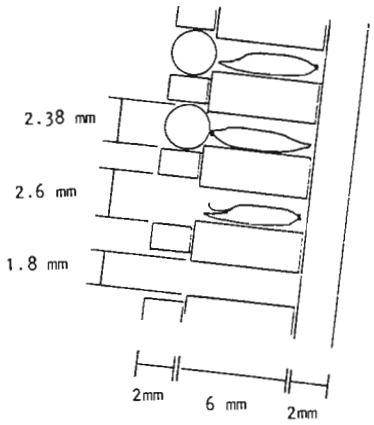


Fig. 1. Transection of the pupal plate.

Pupal plate. The heart of the device is the pupal plate (Fig. 1). It is made of three 80 x 100 mm pieces of clear acryl plastic. The thickness of the middle plate (for pupae) must fit the pupal length of the species under study. For the D. obscura group species 3-4 mm and for the D. virilis group 6 mm has proven to be suitable. In front of the middle plate there is the ball-plate, where the holes are wider than the pupal holes and concentric with them. The balls are stainless steel bearing balls, in our apparatus SKF RS 2.381 mm. The bottom plate has holes only for the four bolts keeping the three plates together. Each pupal plate has holes for 396 pupae.

Emergence box. Emergence boxes are made of commercially available clear plastic boxes (depth 80 mm, width 130 mm, height 150 mm). One to three pupal plates are placed into the box into a position tilted 5-10° from vertical. When a fly emerges from its pupal case, it pushes down the ball in front (or back) of it immediately. The falling ball rolls on the tilted plane to the microswitch and falls through it to the bottom of the box (Fig. 3, following page).

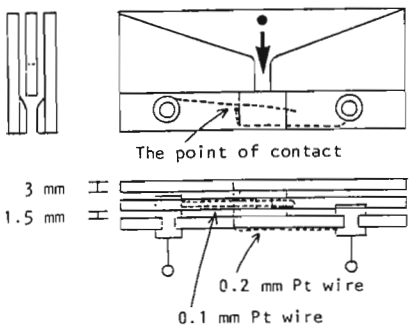


Fig. 2. The microswitch.

The microswitch. The structure of the switch is described in Fig. 2. The "open" structure has proved to be a necessity to prevent the switch from getting blocked by loose flies and pupal cases drifting in the box. The 1.5 mm wide slots in the switch allow the detritus to pass it freely. Only the balls are guided to the funnel, where they bend the upper platinum wire loop to make contact to the lower platinum wire placed crosswise. The weight of a fly is maximally one tenth of that of the ball (50 mg) and thus the flies are not able to bend the wire loop.

Amplifier. The thin platinum wires of the microswitch would burn without an amplifier due to the relatively high current (250 mA) needed to activate a pen of the event-recorder. A simple two-transistor amplifier works as a relay and reduces the current in the microswitch to 0.05

Fig. 1. Homogenate after the homogenization in the presence of vaseline oil.

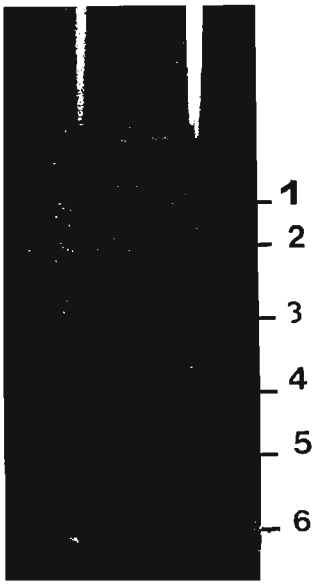


Fig. 2. Distribution of bulbuses among the layers of sucrose of different density.

The lower layer was collected with a glass pipette and another 40.0 ml of buffer A was added to the upper layer, followed by homogenization for 5 sec at 2000 rpm. This procedure was repeated until the combined volume of the lower layers reached 200-250.0 ml. After settling for 15 min it was divided into three layers: the upper layer containing the vaseline oil emulsion and oil-soluble products was removed with sterilized cotton, and the middle layer consisting of suspension of the internal organs' small particles was decanted. To the lower layer containing eggs, testes and bulbuses 100.0 ml of buffer A was added and the procedure of settling was repeated for another 15 min. Combined lower layers were transferred to a 25.0 ml centrifuge tube and brought to 20.0 ml with buffer A deprived of $MgCl_2$ and centrifuged for 10 min at 2500 rpm in the bucket rotor of a K-23 Janetcki (DDR) centrifuge. Prior to centrifugation the solution in the tube was underlaid with 20.0 ml of 1.3 M sucrose prepared on the same buffer A. The interphase after centrifugation was collected and washed once more with 10m0 ml of buffer A for 10 min at 2500 rpm. The supernatant was discarded. The pellet was resuspended in 0.5 ml of 1.5 M sucrose prepared on buffer A without Mg^{++} ions and six layers of sucrose were overlayered as follows (from the bottom): 1.35 M, 1.2 M, 1.05 M, 0.9 M, 0.75 M and 0.6 M.

In Figure 2 one can see that after centrifugation for 20 min at 2500 rpm six layers of material are formed. Microscopic analysis of these layers has shown that layers 1-3 contain pure preparation of bulbuses (Fig. 3A); layers 4-5 contain bulbuses only slightly contaminated with eggs (Fig. 3B); and layer 6 and the pellet consist mainly of eggs (data not shown). As can be seen from the comparison of Figures 3A and 3B, bulbuses are distributed among the layers according to their size.

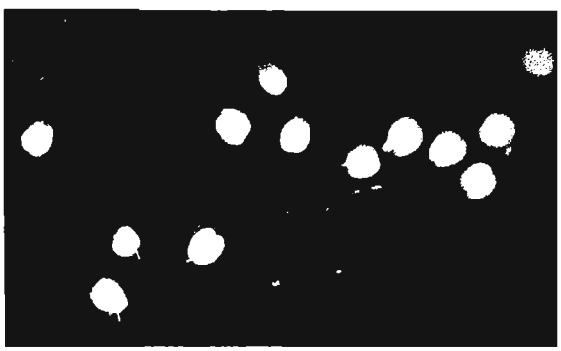
A   B

Fig. 3. Microscopic appearance of purified bulbuses. (A) Material from layer 1 (Fig. 2); (B) material from layer 5 (Fig. 2).

The efficacy of the method presented in this paper is 50-70%. It was employed by us with a very good result for isolation of bulbuses from flies 5-10 days after emergence.

It is necessary to note here that particular steps of the technique described in the present paper may be successfully employed for the isolation of several other Drosophila organs,

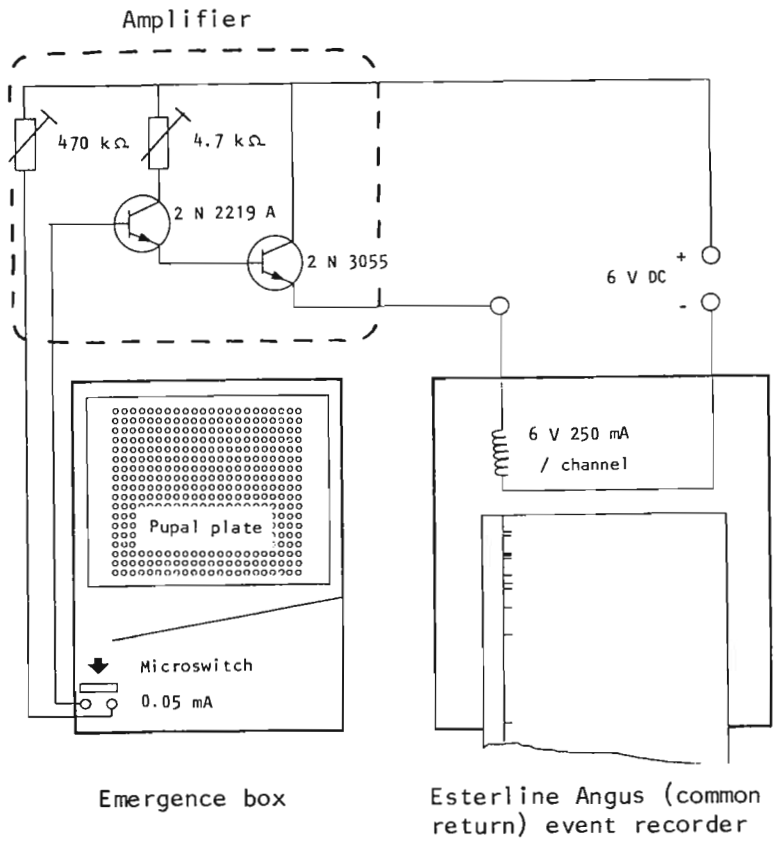


Fig. 3. The registration system of emergence. Only one of 20 channels is described in the figure.

mA (Fig. 3). It also allows long and thin cables to be used to connect the emergence boxes to a "recording center". Each channel needs its own amplifier circuit.

Instructions:

We rear larvae in two liter plastic jars covered by glass plates and ventilated through a net-covered 2 cm hole. When the pupariation has proceeded for several days, the puparia are rinsed away from the walls of the jar, washed and dried on absorbing paper. The pupal plate--all three pieces fixed together--is placed into a 4 cm deep carton frame, and the dry pupae are poured on the plate. When the plate is gently shaken horizontally, the bigger holes of the ball plate serve as funnels guiding the pupae into the pupal holes. It makes no difference whether the head is up or down. The carton frame is removed, as well as the ball plate, and the extra pupae are gently knocked away. The ball plate is put back, and the steel balls are poured into the holes.

The filling of a pupal plate according to the above instructions takes no more than a few minutes. After this the plate(s) is put to the emergence box, and the box is connected to the event recorder.

Each emerged fly is seen on the recording paper as a crosswise peak in the ink line. The eclosion rhythm is either directly visualized on the paper, or the peaks can be counted from the paper for further analysis. --If the pulses are counted by logical circuits, a word of caution is needed. The pulse of one falling ball can namely consist of several pulses due to bouncing of the ball in the microswitch. Thus for correct counting irregular pulses from our apparatus must be converted into uniform pulses by an additional pulse converter circuit (e.g., Block & Page 1977).

References: Block, G.D. and T.L. Page 1977, Comp. Biochem. Physiol. 58A:5-6; Lumme, J. and P. Lankinen 1973, DIS 50:190; Maier, R.W. 1973, J. interdiscipl. Cycle Res. 4:125-135; Truman, J.W. 1972, Z. Vergl. Physiologie 76:32-40.

Lindquist, S. L., S. Sonoda, T. Cox and K. Slusser. University of Chicago, Chicago, Illinois. Instant medium for Drosophila tissue culture cells.

Only one type of Drosophila culture medium (Schneider's) is commercially available. Unfortunately, it is expensive and not very reliable. Different batches support different rates of growth, shift the temperature maximum of the heat shock response, and induce changes in both the number of endogenous virus particles and in the number and size of vacuoles. Many of these problems can be eliminated by using a medium that is freshly made in the laboratory. Media preparation is tedious and time consuming, but certain formulas are easier to prepare than others. We tested the effects of several different insect culture media on the growth of Schneider's culture line #2 (Schneider 1972). The media tested were Schneider's revised formula (Schneider 1972), Shields and Sang (1975), Robb (1969), Poels (1972), Lengyel (1975), and D.20 (Eschalier & Ohanessian 1970). We were pleased to find that one of the simplest media to prepare, Shields and Sang, was also one of the best. It supported rapid growth, gave

a high temperature response profile during heat shock, and was of consistent quality from batch to batch.

To further reduce the time and effort involved in culturing, we decided to see if the dry ingredients could be premixed, ground to a fine powder, and stored for instant reconstitution as needed. We tested a few variations on the original formula and found one method which worked very well. Ingredients were mixed in the same proportions specified by Shields and Sang (3) with the following exceptions: yeast extract was added (in addition to bactopeptone); hydrated compounds were replaced by their anhydrous counterparts; choline and KCO_3 were omitted and added when the medium was prepared for use. We ground the quantities listed below in a Norton grinding mill for 48 hrs. This produced a very fine powder (with a consistency rather like that of Difco's Bacto Tryptone) which dissolved instantly in water. The quantities listed below were sufficient for 50 liters of medium. The grinder can easily accommodate much greater quantities.

Compound	Grams	Compound	Grams
α alanine	75.0	oxaloacetic acid	12.5
β alanine	12.5	phenylalanine	12.5
arginine·HCl	30.2	proline	20.0
asparagine	15.0	serine	17.5
aspartic acid	15.0	threonine	25.0
cysteine-HCl	10.0	tyrosine	12.5
glutamine	30.0	tryptophan	5.0
K glutamate	357.5	valine	20.0
Na glutamate	326.5	glucose	500.0
glycine	25.0	bactopeptone	125 gm
histidine	27.5	yeastolate	100 gm
isoleucine	12.5	$CaCl_2$	38.0
leucine	20.0	$MgSO_4$	107.5
lysine HCl	42.5	$NaH_2PO_4 \cdot H_2O$	39.0
methionine	12.5	BIS-tris	52.5

To make complete:
Dissolve 4.2 gm/90 ml of deionized H_2O.
Add 5 mg choline and 50 mg $KHCO_3$.
Adjust the pH to 6.8 with NaOH and then adjust the volume to 100 ml.
Add heat inactivated fetal calf serum to 10-12% and filter sterilize.
Complete medium is stable at 4°C for at least two weeks.

During the previous year, our cells had been continuously passaged in standard Shields and Sang medium. On four separate occasions we tested their ability to adjust to the reconstituted medium. Each time a certain percentage of the cells died. Culture viabilities varied from 60-95% 48 hrs after transfer. After a brief lag cells grew at normal rates although they did not attach to the surface of the flask during the first few days. Within two weeks, viabilities returned to greater than 97% and growth was again stable and rapid. After the initial adjustment period, cells grew to higher densities, were more uniform in appearance and had fewer vacuoles than in the preceding year. With 12% serum, the doubling times were approximately 20 hrs and cell densities reproducibly plateaued at 3×10^7/ml. Cells growing in Schneider's medium adapted to powdered Shields and Sang medium just as readily.

We also used the grinder to prepare medium for labeling studies. We omitted the bactopeptone, yeast extract, leucine and methionine. Leucine or methionine was added during reconstitution, depending upon the isotope used for labeling. The heat shock response profile produced with our initial batch of labeling medium had a maximum at 36.5°C the first day it was prepared. It gave exactly the same profile when retested after 4 and 9 months.

We find powdered medium a great convenience. Quantities sufficient for a year of culturing can be prepared in one day and stored in the space of a bread box. This method eliminates worries about the variability of media preparation and associated changes in cell growth and labeling properties.

References: Eschalier, G. and A. Ohanessian 1970, In Vitro 6:162-172; Lengyel et al. 1975, Methods in Cell Biology, Vol. 10; Poels, C.L.M. 1972, Cell Diffn. 1:63-78; Robb, J.D. 1969, J. Cell Biol. 41:876; Schneider, I. 1972, J. Embryol. Exp. Morph. 27:353-365; Shields and J. Sang 1975, DIS 52:161.

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Replacing Agar by Carrageenan in Drosophila medium.

Numerous products are authorized in human nutrition for producing gels. Carrageenan, extracted from red seaweeds, is widely used because of its lower price. It is chemically similar to Agar which is usually employed as reversible gel in Drosophila breeding medium: both are galactose polymers. Coded E-407 in the European Common Market nomenclature, Carrageenan contains about 20-35% of impurity, mainly sulfates, while gelose (or Agar-Agar, E-406) contains only 3-4%.

Various commercial products containing Carrageenan are available and distributed under different names according to their physical properties and composition (Aubygel, Satiagel, etc.). After several assays, one of them, Flanogene, which is a mixture of Carrageenan (E-407) and Carob-Bean meal (E-410) appeared most suitable for producing a hard and cohesive Drosophila medium at a similar concentration to that used for Agar (1.5-2%). Preparation of Drosophila medium is basically similar to Agar but the mixture can be prepared at cold temperature without any decantation of the components. Then, ingredients are heated together for 10 minutes at 115°C and gelification occurs after cooling. Because of its lower price (less than half the price of Agar) it seemed worthwhile to look for any detrimental effect of Flanogene upon Drosophila physiology. The basic medium used was a killed yeast medium according to David and Clavel (1965). A wild type, French strain of D. melanogaster was grown on the Flanogene containing medium for several successive generations. Comparisons of various quantitative characters with those of Agar grown flies were made after one and seven generations. Results of the comparison at the seventh generation are given in Table 1.

Table 1. Comparison of morphological or physiological traits in two strains of D. melanogaster raised for 7 generations on nutritive medium prepared respectively with Agar (A) or Flanogene (F). N = number of flies, statistical comparisons wee made either with t or χ^2 statistics; S = significant, NS = nonsignificant difference.

Characters		Results after seven generations	N	Statistical comparison
Development duration (hrs)	A	218.56 ± 0.56	92	NS
	F	218.80 ± 0.53	80	
Larval mortality	A	8%	100	S
	F	20%	100	
Male weight (mg x 10^{-2})	A	91.96 ± 0.45	30	S
	F	89.46 ± 0.54	30	
Female weight (mg x 10^{-2})	A	115.40 ± 0.88	30	NS
	F	117.57 ± 1.08	30	
Male wing length (mm x 10^{-2})	A	195.66 ± 1.02	30	NS
	F	197.53 ± 0.80	30	
Female wing length (mm x 10^{-2})	A	223.43 ± 1.22	30	NS
	F	227.86 ± 1.00	30	
Ovariole number	A	47.68 ± 0.89	30	NS
	F	47.70 ± 0.71	30	
Longevity (days)	A	42.83 ± 2.82	12	NS
	F	44.25 ± 2.30	12	
Fecundity (eggs)	A	40.25 ± 9.91	12	NS
	F	54.00 ± 17.27	12	

For all the traits studied, except two, non-significant differences were observed, even after several generations, showing a lack of detrimental effect and selective influence.

In another experiment, it was verified that, like Agar, Flanogene is deprived of any food value. Adults receiving only a gel containing Agar or Flanogene survived respectively 65.0 and 66.8 hours. Finally the oviposition behavior of females was compared using the technique of David (1959). At 1.5%, 60% of the eggs were laid on the Flanogene medium. It was supposed that this difference could be imputed to the softer consistency of the Flanogene medium since it is known that females prefer a soft surface. A harder gel was thus prepared with 2% of Flanogene and the preference was still increased (only 35% eggs laid on Agar). We can thus conclude that Drosophila females distinguish the two media not by their hardness but probably by some organoleptic properties. This could be interesting in favoring oviposition, especially for species difficult to grow under laboratory conditions.

In medicine, hydrolized Carrageenan was used to cure some digestive troubles. It was shown, however, that when added to drinking water (at high concentration) a delay of growth

and some intestinal injuries appeared in rats. Nothing similar seems to happen in fruit flies.

Flanogene seems able to replace Agar without any difficulty. The only possible drawback is that Flanogene is more easily "digested" by the larvae, so that the medium tends to become liquid under crowded conditions.

This work supported in part (gel production) by S.A.T.I.A.-C.E.C.A. 78140 - Velizy Villacoublay (France).

References: David, J. 1959, Bull. Biol. Fr. Belg. 93:472-505; David, J. and M.F. Clavel 1965, Bull. Biol. Fr. Belg. 99:369-378; David. J. 1979, Aquilo, Ser. Zool. 20:49-61; Carrageenan 1973, Journal of Food Science 38:367-368.

Mittler, S. and K. Schroeder. Northern Illinois University, DeKalb, Illinois.

Triethylamine as an anesthetic.

In a search for an anesthetic that would keep a large quantity of flies under anesthesia longer than could be done by ether, without harmful effects, triethylamine was selected. A concentration of 25% triethlamine after mixing with a 47.5% solution of ethyl alcohol will do this and can also be used when large numbers of flies have to be classified as to type of body bristles. The wings do not fold up to interfere with the bristle classification. Triethylamine is flammable.

Mogami, K. and Y. Hotta. University of Tokyo, Japan.

A sliding population cage for collection of clean and homogeneous population of flies.

We have developed a method to collect a large number of Drosophila thoraces from which myofibrils of indirect flight muscle were prepared (Mogami et al., in prep.). To obtain pure preparation of thoraces efficiently, we had to start from a clean, healthy and homogeneous population of flies. For this purpose, we devised a new cage which we name the "sliding" population cage.

Figure 1 (following page) shows the structure of the cage, which is made of transparent lucite. The blind plate (E) is made of 5 mm thick lucite plate (27 x 37 cm) to which four legs (5 x 5 x 0.5 cm) are attached. The ceiling from (A) is made by a 27 x 37 x 0.5 cm plate. Inner opening of the frame is 20 x 30 cm. Width of the frame is deliberately made unbalanced (3 cm on one side, and 4 cm on the opposite side). The upper chamber (B) is made by gluing lucite wall (3 mm thick) with frames which are identical to the ceiling frame. Inner dimension of the upper chamber is 20 x 30 x (20 or 30) cm. The lower chamber (C) also has a top frame which is identical to the ceiling frame. The height of the lower chamber is 5 cm. Cornmeal-agar medium is placed in the inner tray of the lower chamber (15 x 25 cm). The harvesting chamber (D) is similar to the lower chamber except the floor of the tray is slanted with an outlet at the lowest corner. To collect flies, the chamber is filled with CO_2 gas and a test tube is connected to the outlet with vinyl hose.

Figure 2 shows an assembled cage. A ceiling frame is wrapped with nylon cloth, and is clamped with a metal clip to the upper chamber. The bottom frame of the upper chamber opposes the top frame of the lower chamber so that the inner walls of the two chambers fit together, but the whole frames are staggered (arrow). When flies are transferred (Fig. 3), two lower chambers must be placed side by side with the stagger used to make the two chambers level.

Figure 3 shows typical procedure of transfer. a, a': Old cage assembly. b: New upper chamber. b': New lower chamber or harvesting chamber. c: Blind plate. 1: Before transfer. 2: Clips connecting lower chamber and upper chamber are removed, and the old lower chamber and new lower chamber are clamped with clips. Upper chambers are slid to the left. 3: Carbon dioxide gas is infused through ceiling cloth into the old upper chamber so that flies fall into the new lower chamber (or harvesting chamber). 4: Upper chambers are slid to the left in order to transfer residual flies. 5, 6: Flies are transferred to the new cage or the harvesting chamber. The old cage is either reassembled for further generations or washed. At least 70% of the live flies can be transferred by this procedure.

The minimum number of components are: 2 ceiling frames, 2 upper chambers, 2 lower chambers, 1 harvesting chamber, and 3 blind plates.

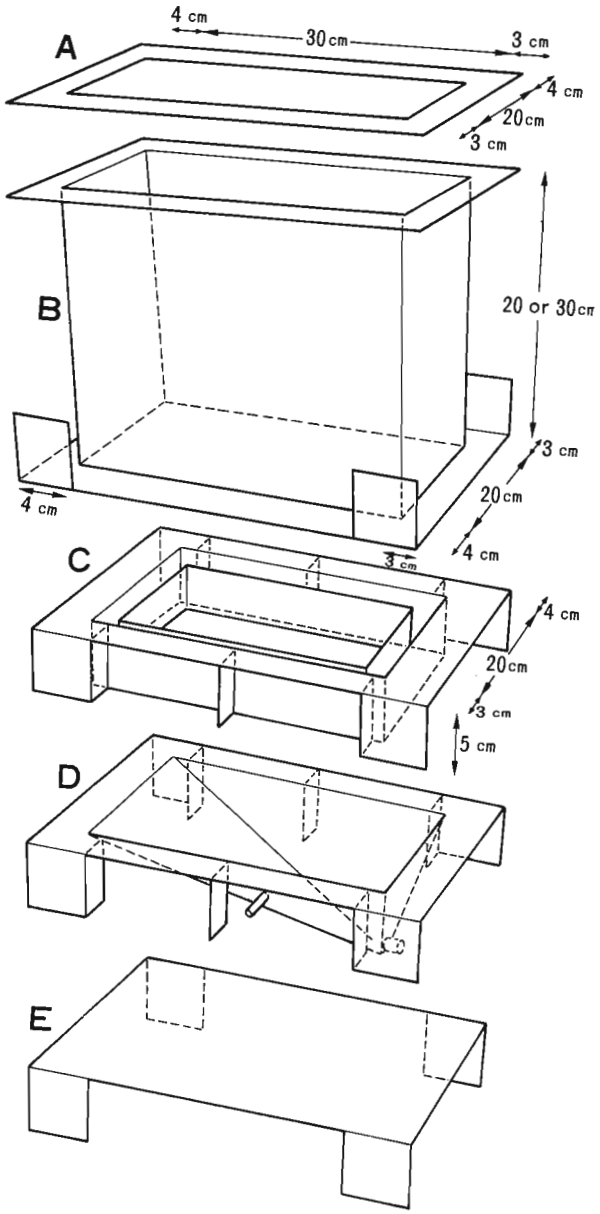


Fig. 1

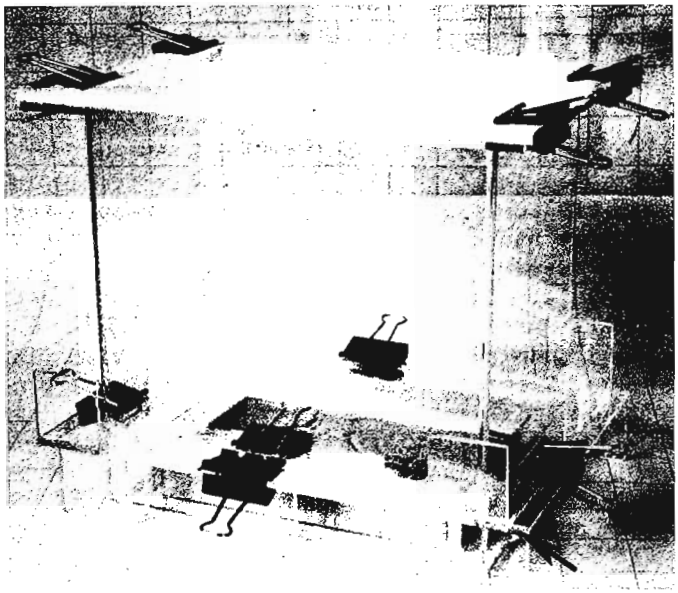


Fig. 2.

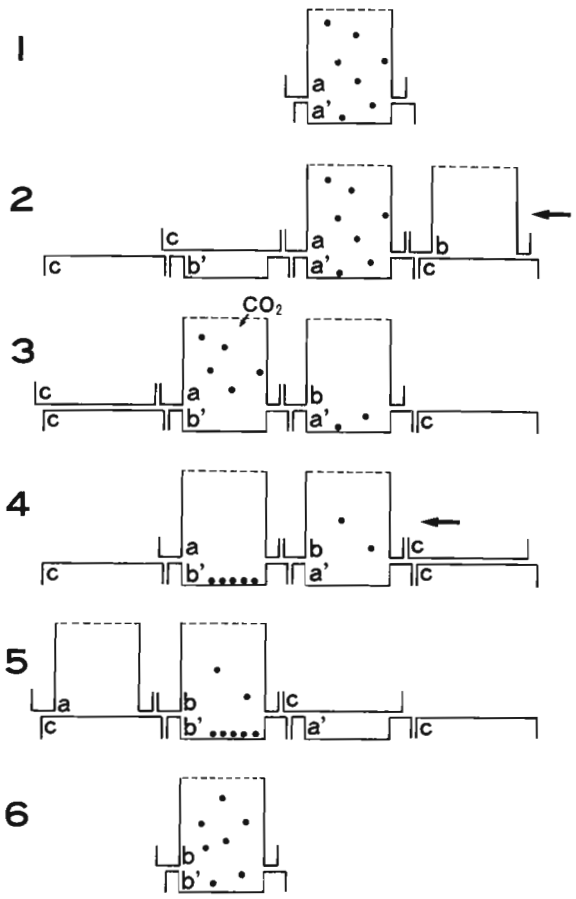


Fig. 3.

[Mogami and Hotta]

Paterson, R. and D. A. Hickey. Brock University, St. Catharines, Ontario, Canada. A simple method for the localization of Drosophila trehalase and sucrose isozymes on polyacrylamide gels.

Trehalase (α-α^1-glucoside-1-glucohydrolase, EC 3.2.1.28) cleaves the disaccharide trehalose (1 α-D-glucopyranosyl-α-D-glucopyranoside) into two glucose units. Many recent studies on trehalase have been motivated by the finding that trehalose is the principal sugar in the hemolymph of many insects (Wyatt 1967). A number of electrophoretic studies (Marzluf 1969; Oliver et al. 1978; Bargiello & Grossfield 1979) have addressed the question of the number of distinct trehalase isozymes which are present in insect tissues. Several methods have been described for the detection of trehalase enzyme-activity bands on electrophoretic gels. Gabriel and Wang (1969) used a general staining method, based on the formazan reaction, for the identification of reducing sugars on polyacrylamide gels. This method was modified by Oliver et al. (1978) for the visualization of trehalase bands after the electrophoresis of crude D. melanogaster homogenates. Alternatively, Marzluf (1969) coupled the trehalase reaction with glucose oxidase and used the nitro-blue-tetrazolium stain. Bargiello and Grossfield (1969) also used a coupled enzyme reaction (glucose oxidase and peroxidase) and the dye, o-dianisidine. The dinitrosalicylic acid reagent has long been used (Bernfeld 1955) as a standard method for the photometric quantitation of reducing sugars. Here we describe a simple modification of this method which allows for the localization of reducing sugars on polyacrylamide gels. This, in turn, indicates the position of any enzymes which produce such reducing sugars.

Several stocks of D. melanogaster and one stock of D. simulans were maintained under uncrowded conditions on a yeast-agar-sucrose food medium, at 25°C. The D. melanogaster stocks (30 in all) were derived from populations in North America, Europe and West Africa; the stock of D. simulans originated from Texas (USA).

Samples of eight flies each were homogenized in 60 µL of 0.06 M Barbiturate buffer, pH 8.6, containing 5% glycerol. Aliquots of 20 µL each were layered on 6% polyacrylamide slab gels (electrophoresis apparatus was obtained from Aardvark Instruments, Inc.). Vertical electrophoresis was carried out according to a modification of the procedure described by Marzluf (1969). A continuous buffer system was used (0.06 M Barbiturate buffer, pH 8.6). Electrophoresis was continued for 4 hr at 250 V and approximately 100 mA. Gels were cooled by a circulating coolant system.

The gel-staining procedure was as follows. Gels were rinsed in distilled water and incubated in 0.2 M acetate buffer, pH 5.5, containing 1% trehalose. After 2 hr incubation (on a shaking machine), gels were rinsed again in distilled water and 150 ml of dinitrosalicylic acid reagent (prepared according to the method described by Bernfeld 1955) was added. Gels were then heated gently to approximately 95°C. When brown bands appeared (after approximately 15 min), gels were cooled and photographed. Rapid heating or vigorous boiling causes the bands to diffuse rapidly.

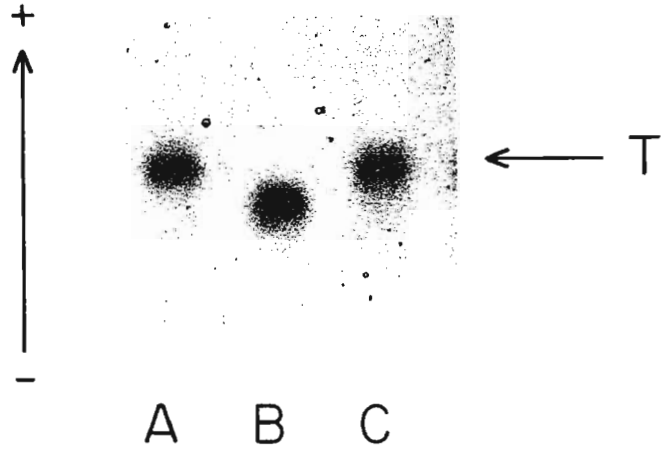


Fig. 1. Polyacrylamide slab gel stained with the dinitrosalicylic acid reagent to visualize areas of trehalase activity. Samples A and C are homogenates of D. melanogaster and the trehalase band is indicated by the arrow (T). The sample from D. simulans (B) has a slower-migrating trehalase band.

The dinitrosalicylic acid reagent has been used previously for the photometric quantitation of α-glucosidase activity, including trehalase activity (Marzluf 1969). However, this staining procedure has not previously been used for the localization of α-glucosidase activity on electrophoretic gels. The staining method described here is simple and rapid and yields reproducible results. Figure 1 shows the electrophoretic patterns obtained for trehalase isozymes in D. melanogaster and D. simulans. As already reported by Bargiello and Grossfield (1979), D. simulans is characterized by a relatively slow migrating trehalase isozyme. All strains of D. melanogaster showed identical electrophoretic patterns. A single main band of trehalase activity was observed in both species.

This method can be modified for the localization of sucrase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) activity, simply by incubating the gels in 5% sucrose rather than in 1% trehalose. Several zones of sucrase activity appear, as has been observed by Marzluf (1969). Incubation of gels in 1% starch allows for the visualization of α-amylase (EC 3.2.1.1) activity bands. Gels stained for amylase activity in this manner showed electrophoretic patterns which corresponded to the patterns visualized by the more usual iodine-potassium iodide staining procedure (Doane 1969).

One disadvantage of the technique described here is that the banding pattern diffuses rapidly (15 to 30 min) after its first appearance. Thus, a photographic record of the gel patterns should be made immediately.

This work was supported in part by an Operating Grant from NSERC, Canada, to D.A.H.

References: Bargiello, T.A. and J. Grossfield 1969, Insect Biochem. 9:323-329; Bernfeld, P. 1955, in: Methods in Enzymology (S. Colowick and N. Kaplan, eds.), Vol. 1, pp. 149-158, Academic Press, New York; Doane, W.W. 1969, J. Exp. Zool. 171:321-342; Gabriel, O. and S.-F. Wang 1969, Anal. Biochem. 27:545-554; Marzluf, G.A. 1969, Arch. Biochem. Biophys. 134:8-18; Oliver, M.J., R.E. Huber and J.H. Williamson 1968, Biochem. Genet. 16:927-940; Wyatt, G.R. 1967, Adv. Insect Physiol. 4:287-360.

Schubiger, M. University of Washington, Seattle, Washington. Technique for everted imaginal disc transplants.

One approach to understanding how axons of peripheral sensory neurons find their proper destinations in the central nervous system has been to study projection patterns from ectopic receptors. This can be accomplished either by analyzing the patterns in homeotic mutants (Ghysen 1978; Palka et al. 1979) or by grafting tissue to different places on the animal. For back-filling ectopically grafted receptors, it is advantageous if the graft everts with the host. Bhaskaran and Sivasubramanian (1969) have described a method for Musca in which transplanted discs can evaginate. I report here a modification of their method for Drosophila imaginal discs.

The transplantations are performed on tanned prepupae 2-3 hours after puparium formation. Younger prepupae are white, their cuticle is softer, and cutting the case is difficult. If we use older hosts, the appendages are already everted, the pupal cuticle apolysed and the transplanted discs no longer evert with the host. The animals are washed in water and rinsed in 70% ethanol. For the operation, the prepupae are placed in a groove made in a small block of plasticine and held in place by covering both ends of the prepupa with plasticine. Care is taken not to press the animal. A drop of Ringer is put over the prepupa and with a sharp glass needle an incision is made parallel to the long axis of the animal, usually on the dorsal lateral surface between abdominal segments 3 and 4. The cut is then extended transversely along the segmental folds at either end of the first cut to form a U-shaped flap which opens laterally. The size of the flap depends on the size of the discs to be transplanted. Discs from white prepupae are picked up with forceps and placed just under the flap made in the prepupal case. The edges of the discs are gently tucked in with tungsten needles. The Ringer solution is then removed with filter paper. No sealing of the wound is necessary. The operated prepupae are lifted from the plasticine and placed, operated side up, in a plastic petri dish containing moist filter paper. They are kept at 25°C until the flies emerge.

I transplanted 64 +/+ wing and 43 +/+ haltere discs into +/+ prepupae. 75% of the implants were recovered. Of these, 40% of the wing discs and 30% of the haltere discs were fully and an additional 20% partially everted. In 40% of the wing and 50% of the haltere disc transplants, the tissue did not evaginate with the host and formed a vesicle of differentiated adult structures in the abdominal cavity. The percentages of successful surface transplants are considerably lower than those reported for Musca. Bhaskaran and Sivasubramanian (1969) found distinctly higher frequencies of eversion when prepupal rather than late larval discs were used. I have also observed that for successful evagination, the donor discs should be only a few hours younger than the host.

The sensory axons differentiated by the ectopic wing tissue are able to connect to the central nervous system as revealed in $CoCl_2$ back-fills of the chemosensory fibers. These axons join an abdominal nerve bundle and enter the thoracicoabdominal ganglion where many of them terminate in the abdominal neuromeres. With this technique, any genetic combination between the sensory neurons and the central nervous system can be made, whereas in genetic mosaics, the combinations are limited. Moreover, we can also analyze the projection pattern of specific receptors entering the central nervous system through very different routes.

This work was supported by NIH grant NBO7778 to Dr. John Palka.

References: Bhaskaran, G. and P. Sivasubramanian 1969, J. Exp. Zool. 171:385-396; Ghysen, A. 1978, Nature 274:869-872; Palka, J., P.A. Lawrence and H.S. Hart 1979, Devel. Biol. 69:549-575.

Sharp, D., V. Gandhi, K. Kalumuck and D. Procunier. Rice University, Houston, Texas. A rapid method for determining rRNA gene numbers.

Saturation hybridization for estimating the number of 28 + 18S or 5S rRNA repeating units can be accomplished quickly while using a small quantity of flies. About 2.0 grams of frozen adult flies (-70°C) are homogenized in 20 ml of 10 mM $MgCl_2$, 50 mM Na acetate, 150 mM KCl, pH 6.0 at 4°C. To this mixture, 2.2 ml of 10X lysis solution (0.1 M EDTA, pH 8.0, 2% sodium dodecyl sulfate, 0.5 M sodium perchlorate and 0.15 M NaCl) and 20 ml of water-saturate phenol: chloroform (v/v = 1) containing 0.1% (w/v) 8-hydroxyquinoline are added. The homogenate is shaken for 10 minutes and centrifuged. The DNA in the aqueous layer is precipitated with 1 volume of 100% EtOH. The DNA is dissolved in 2 ml of MUP [8 M urea and 0.24 M phosphate buffer (P B), pH 6.8] and loaded onto a small HAP column (0.50 g Bio Rad HTP in 0.24 M P B). The column is washed with 15 ml of MUP and then with 10 ml of 0.14 M P B. With these washes, only the DNA remains bound to the column, and is eluted by 0.40 M P B.

The DNA is denatured in 0.5 M NaOH for 10 minutes at room temperature. The solution is neutralized with HCl and the DNA bound to nitrocellulose BA85 25 mm filters by gravity filtration. The filters are dried and baked at 80°C in vacuo for 2 hours. The amount of DNA bound per filter is determined after the hybridization procedure. Filters are incubated in 2X SSC (0.15 M NaCl, 0.15 M Na citrate, pH 7.0) at 60°C for 3 hours with saturation amounts of ^{3}H-28 + 18S rRNA or ^{32}P -5S rRNA. The filters are washed, RNAased (20 µg/ml, 1 hour at 37°C), washed, and counted in Beckman Ready-Solv™NA.

After counting, the filters are washed with chloroform, dried, and placed in 5% perchloric acid. The DNA on the filter is hydrolyzed for 30 minutes at 70°C, along with salmon sperm DNA standards treated in the same way. The DNA is quantified by reading the OD_{260} nm. There is no loss of DNA from the filter during the chloroform treatment. The advantage of this procedure is that we routinely experience DNA loss during the hybridization reaction. This error is circumvented by quantitating the DNA on the filter after the hybridization reaction.

This research was supported by NIH grant GM 28008-01.

References: Britton, R.J., M. Pavich and J. Smith 1970, Carn. Inst. Wash., Year b. 68: 400; Procunier, J.D. and K. Tartof 1978, Genetics 88:67.

Starmer, W. T. and D. G. Gilbert. Syracuse University, Syracuse, New York. A quick and reliable method for sterilizing eggs.

We have recently devised a quick, easy and reliable method for sterilizing eggs for experiments with axenic Drosophila. This method has the advantage of collecting all eggs which females lay, regardless of whether they are oviposited into the medium or onto the surface. Gravid females are placed in shell vials (8 dram) containing 3 ml of 10.0% gelatin. Depending on the species, additives may or may not be necessary to elicit oviposition. After the eggs have been laid, the adults are removed and the vial is placed in warm water (approximately 45-50°C) until the gelatin has gone into solution (approximately 1-2 minutes). The solution of gelatin and eggs is then poured into a 10-20 ml plastic syringe which is attached to a Delrin filter holder (Gelman 4320). The filter holder contains a metricel cellulose triacetate membrane (Gelman 60002, OD 25 mm, pore size 5 µm). The rubber O-ring accompanying the filter holder is excluded because eggs tend to stick to it. After the gelatin solution is in the syringe, the original vial is rinsed with warm water (5 ml) and this is added to the syringe. This solution is pushed through the filter with the syringe plunger. The eggs are retained on the filter and the gelatin and water pass through. Another rinse with warm water (5 ml) is recommended. Next, 10 ml of chlorox (1 part water to 1 part commercial chlorox) is added to the syringe and half of this is pushed through. The eggs are then allowed to stand 25 minutes (time will depend on the species), after which the remaining chlorox is pushed through with the syringe plunger. Two rinses with 5 ml sterile 0.7% NaCl completes the sterilization. The filter holder is then opened and the filter is removed with heat-

sterilized forceps and placed on sterile medium (vials or plates). After hatching, the larvae may be removed for experimentation, if necessary, with a sterile loop.

We have found the cellulose filters to be better than glass filters. We also find that a fine nylon mesh, such as that available for silk screening at art supply stores, works well if the mesh size is smaller than the eggs. This mesh is especially useful when coarse material, such as cactus homogenate, is added to the gelatin medium to elicit oviposition. The large pore size retains eggs but permits cactus fibers to be washed through. We have used this technique for producing axenic cultures as well as directly testing larvae for nutritional requirements and preferences such as yeasts.

Tompkins, L. and K. J. Barnhart. Temple University, Philadelphia, Pennsylvania.

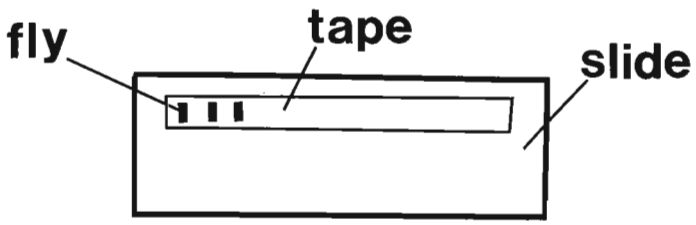
A new technique for the proboscis extension assay.

A hungry, water-satiated fly will extend its proboscis in response to sugar solutions applied to the tarsal or labellar chemoreceptors. To assay this behavior in the laboratory, it is necessary to restrain the fly, which is usually accomplished by embedding the wings in a lump of wax affixed to the end of a wooden applicator stick. This method has several disadvantages: it is time-consuming to mount the flies, tedious to label the wooden sticks, and inconvenient to handle large numbers of mounted flies. Furthermore, it is difficult to remove flies from the wax, which limits the use of this technique to flies which do not have to be mated or subjected to further experimentation after being assayed for proboscis extension.

To circumvent these difficulties, we have developed the following technique.

1. Tape a standard 1" x 3" glass microscope slide to a flat plate. We use a white ceramic tile which weighs 175 grams, but any similar object will be suitable. Attachment of the slide to the plate stabilizes the slide while the flies are being mounted and provides a light-colored background to facilitate observation.

2. Affix a strip of double-stick cellophane tape to the slide (see figure).



3. Lightly anesthetize the first fly. We use carbon dioxide.

4. Under a low-power dissecting microscope (7-10X magnification), use fine-tipped forceps to place the fly dorsal-side down on the double-stick tape. Orient the fly so that the head is close to the edge of the slide. Push the wings flat with the forceps to temporarily immobilize the fly. If it is difficult to attach both wings simultaneously, flatten one wing, then hold it down against the tape with forceps while flattening the second wing. If both wings are raised above the body, it is easier to position the fly so that the dorsal edges of the wings touch the tape. Then apply light pressure to the body, which will cause the wing surfaces to flatten against the tape.

5. Touch the tip of a micro soldering iron to a block of myristic acid (see below). Looking through the microscope, transfer the drop of molten acid which adheres to the tip to a position on the tape just behind the tip of each wing. The acid will run onto the wings, attaching them firmly to the taped slide. At this point, any or all of the tarsi can be attached to the slide with myristic acid if one wishes to limit the number of chemoreceptors stimulated. For these operations, we use an Ungar Princess soldering iron fitted with a 10-watt heat capsule and an Ungar microspade 1/8" thread-in tiplet, plugged into a variable transformer set at 40 volts. The size and shape of the tip are critical. Although the end of the tip must be small, pointed conical tips are not satisfactory, since the acid flows up the side of these tips rather than adhering to the end. The setting of the transformer is also important. If the tip of the soldering iron is too cold, it will not melt the myristic acid readily; if it is too hot, the acid will run onto the body of the fly and cause injury or death. Myristic acid is available from Sigma in flake form (M3253), which we melt in a shallow glass dish and allow to re-crystalize as a block.

6. Mount additional flies parallel to the first, keeping the heads in line. Up to 10 flies can be mounted on one slide, which is then removed from the white plate. An experienced person can mount 2-3 flies in a minute. Flies can easily be identified by writing on the slides with a glass marking pen.

7. After mounting, allow the flies to recover by placing the slides in a dessicator with a wet paper towel under the plate. We wait at least 30-45 minutes before the first test.

8. Test flies by inserting each slide into a plastic holder (Thomas 6707-G10) so that the flies' heads are up. We stimulate each fly in turn with either a cotton swab or a glass micropipet while observing the response under the microscope. Slides containing flies are returned to the dessicator between tests. We find that virtually all flies survive and reamin attached to the slide throughout the testing period.

9. If desired, remove flies after testing by scraping the myristic acid off the wings with fine-tipped forceps. If tarsi have been immobilized, free them by melting the myristic acid in which they are embedded with the soldering iron. Although a small amount of acid may remain on the appendages, flies almost always survive removal. Flies with myristic acid on their wings will readily mate if given the opportunity.

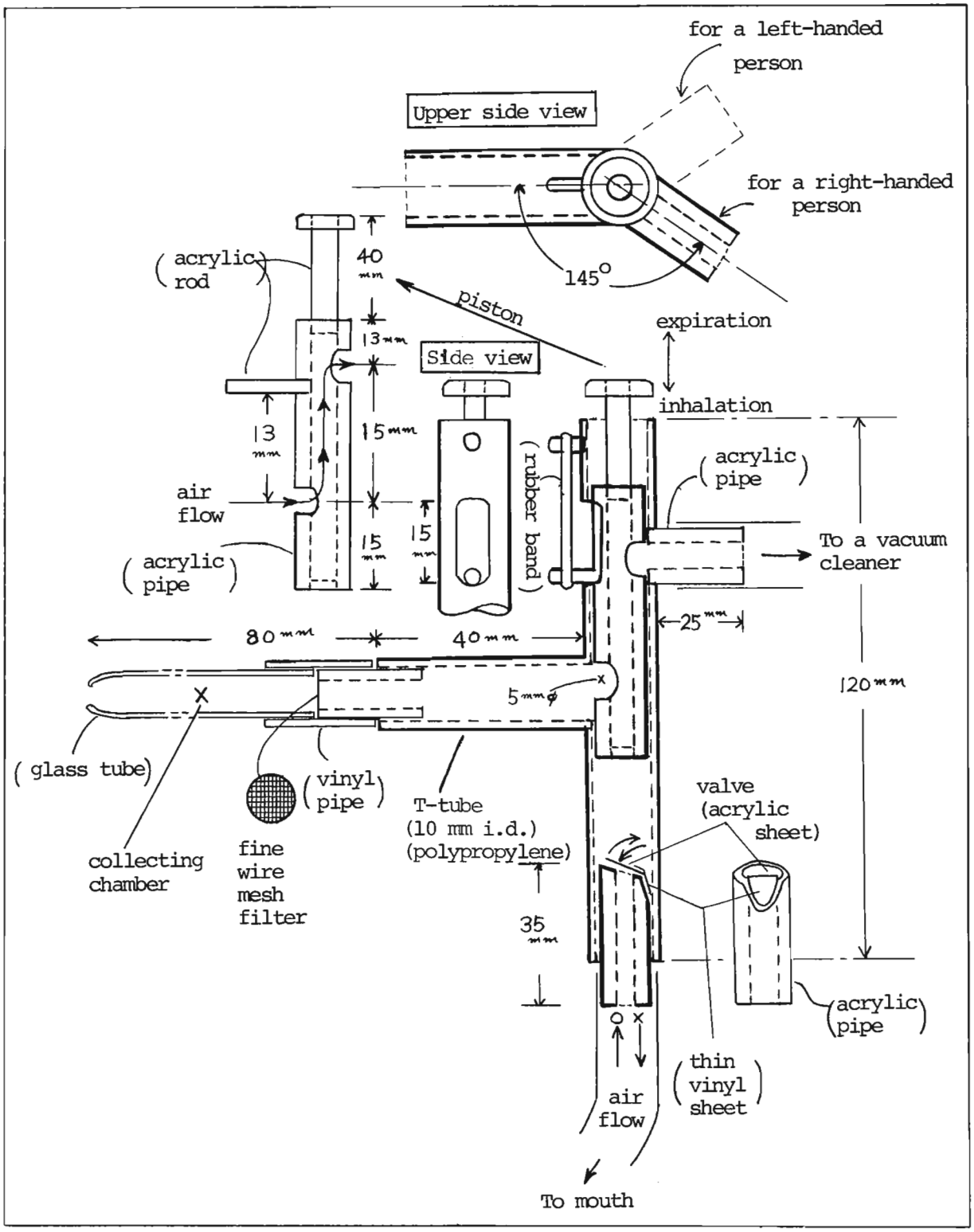
We thank V. Bakanauskas for technical assistance. This research was supported by a Grant-In-Aid from Temple University and GM 28998 from the Public Health Service, awarded to L.T.

Tsuno, K. Josai Dental University, Saitama, Japan. New aspirator design for prevention of toxic substance inhalation.

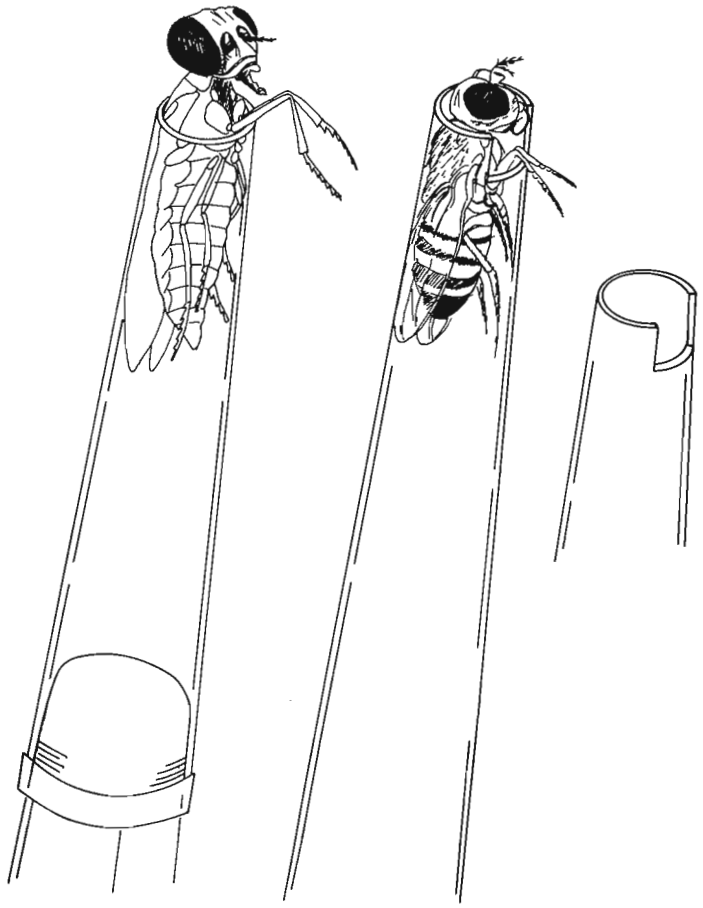
During routine handling of Drosophila fruit flies, many workers use a mouth aspirator equipped with a cotton or sponge stopper. Sometimes, however, they must deal with flies treated with some toxic chemicals such as fluorescent marking powder. Such chemicals can easily go through the cotton or sponge stopper. Additionally inhalation with the mouth aspirator brings discomfort when one has a sore throat. In order to circumvent these problems an apparatus has been devised which uses a vacuum cleaner to provide the necessary vacuum involved in aspiration.

The apparatus is made of acrylic pipe, T-shaped connecting tube, acrylic rod and a few materials as shown in the accompanying figure (following page). The piston consists mainly of an acrylic pipe (10 mm OD; 5 mm ID) containing two 5 mm diameter holes (15 mm apart) whose centers are twisted at a 145° angle. The inside diameter of the T-shaped connecting tube is slightly larger than the outside diameter of the piston so that the piston can freely move up and down in the T-tube. When the piston is pushed down, the flies are aspirated into the collecting chamber by means of the vacuum cleaner; and when it is released upwards, the flies may be expelled by the operator's breath. Accidental inhalation of noxious chemicals is prevented by a valve made of thin acrylic and vinyl sheet. The force of inhalation is controlled by a slide transformer connected to the vacuum cleaner. The piston can be easily operated with the index finger.

[Tsuno]


for a left-handed person
Upper side view
for a right-handed person
145°
acrylic rod
40 mm
piston
expiration
13 mm
Side view
inhalation
13 mm
15 mm
acrylic pipe
air flow
15 mm
15 mm
rubber band
To a vacuum cleaner
acrylic pipe
80 mm
40 mm
25 mm
120 mm
5 mm φ
glass tube
vinyl pipe
T-tube (10 mm i.d.) (polypropylene)
valve (acrylic sheet)
collecting chamber
fine wire mesh filter
35 mm
acrylic pipe
thin vinyl sheet
air flow
To mouth

Vargo, M. and J. Hirsch. University of Illinois, Urbana-Champaign, Illinois.
A mounting technique to observe proboscis extension.



McGuire and Hirsch (1977) developed and presented a technique for mounting blowflies (Phormia regina) in plastic micropipet tips to use in studying the proboscis extension reflex. At that time the major advance of the pipet tip technique over the previously used tacki-wax mounting was that flies were able to be retrieved undamaged after testing, to be used for breeding or other behavioral studies.

A mounting technique has now been developed for studying the Drosophila proboscis reflex. The major innovation in the preparation for mounting Drosophila is a notch cut into the pipet sheath (see figure). The notch is cut to compensate for the reversal in relative size relations of the prothorax and mesothorax in Drosophila as compared to Phormia. In Drosophila the prothorax is larger than the mesothorax, whereas in Phormia it is the reverse.

We are using Lancer Precision Pipette Tips (Lancer, Division of Sherwood Medical, St. Louis, Missouri, Catalog #8889-220003). These pipet tips are better suited for Drosophila because they have a constantly decreasing circumference throughout their length which allows one to cut any size opening desired. Most pipet tips are beveled at the end restricting the size of the desired opening.

To cut the pipet tips, a sharp razor blade and stereomicroscope are the only tools needed. The shape of the cut is similar to that in the figure. Actual dimensions of the cuts are difficult to suggest due to differences in sizes of flies from different stocks. A trial and error method is the best, using the basic design given above.

To mount the flies, first aspirate the flies into the pipet tip and hold it up to a light so that the positive phototactic response draws the fly head first to the cut end. The flies are then maneuvered into position with their ventral surface facing the notched end of the pipet. Flies are then carefully prodded into the end using a very thin paint brush.

References: McGuire, T.R. and J. Hirsch 1977, Proc. U.S. Natl. Acad. Sci. 74:5192-5197.

Wirtz, R. A. Toxicology Services Group, Letterman Army Institute of Research, Presidio of San Francisco, California.
Handling and containment procedures for use with Drosophila.†

Mutagenicity and genetic tests using Drosophila often require the transfer of individual or small numbers of insects. The use of anesthetics in these procedures is a time consuming process with several inherent disadvantages which may include induction of sterility in sensitive insects, fire hazards, and possible direct contact with flies treated with known mutagens (Ashburner & Thompson 1978).

The following procedures permit the transfer of flies without anesthetics while minimizing the possibility of escape and reducing exposure of personnel to test and positive control treated insects.

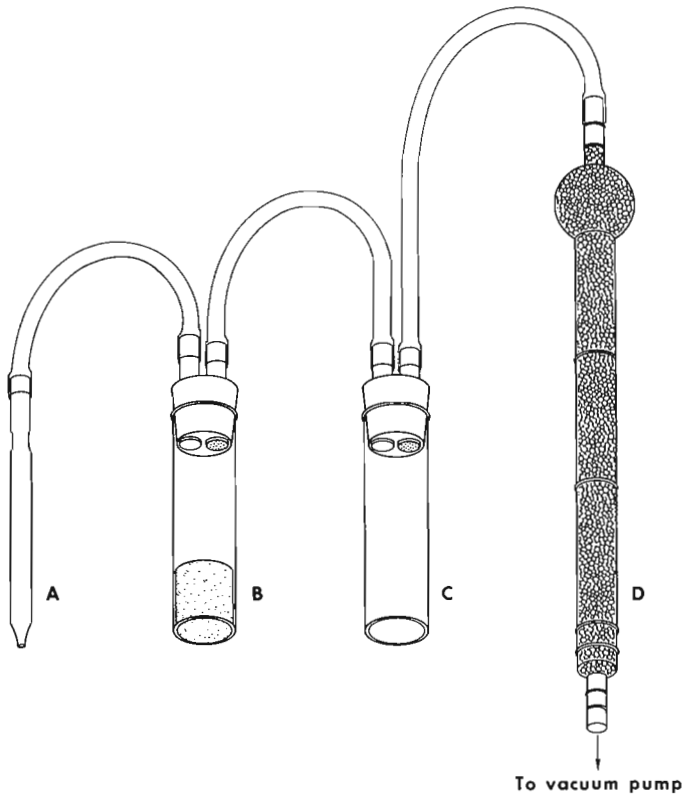


Figure 1. DROSOPHILA TRANSFER APPARATUS : A-Transfer tube. B-Receiving vial. C-Insect trap. D-Volatile chemical trap.

The vacuum transfer system (Fig. 1) consists of a glass transfer tube (constructed from Pasteur pipets), receiving and trap vials, a volatile chemical trap, and a foot-actuated vacuum pump. Two-holed rubber stoppers (size #4 for 25x95 mm vials) are used in the system with glass wool plugs or fine mesh fabric over the openings, to insure that the transfer flies remain in the receiving tube. The volatile chemical trap consists of a drying tube (Nalgene #6201-0080, size 8, Nalge Co., Rochester, New York) filled with 4-12 mesh charcoal. The vacuum pump (1/6 to 1/3 hp) should be equipped with needle valve adjustments and be connected to an electrical outlet through an automatic off foot switch. Individual insects can be transferred to a receiving vial by positioning the opening of the glass transfer tube near the insect and activating the vacuum pump. The transfer system depicted in Fig. 1 is disposable and is replaced after the mutagenicity testing of an experimental compound. Parts of the system coming in direct contact with treated insects are changed between the transfer of flies treated with negative and positive controls and test compounds to prevent possible cross contamination. The exit port of the vacuum pump can be vented into a fume hood in lieu of or in addition to the charcoal filter.

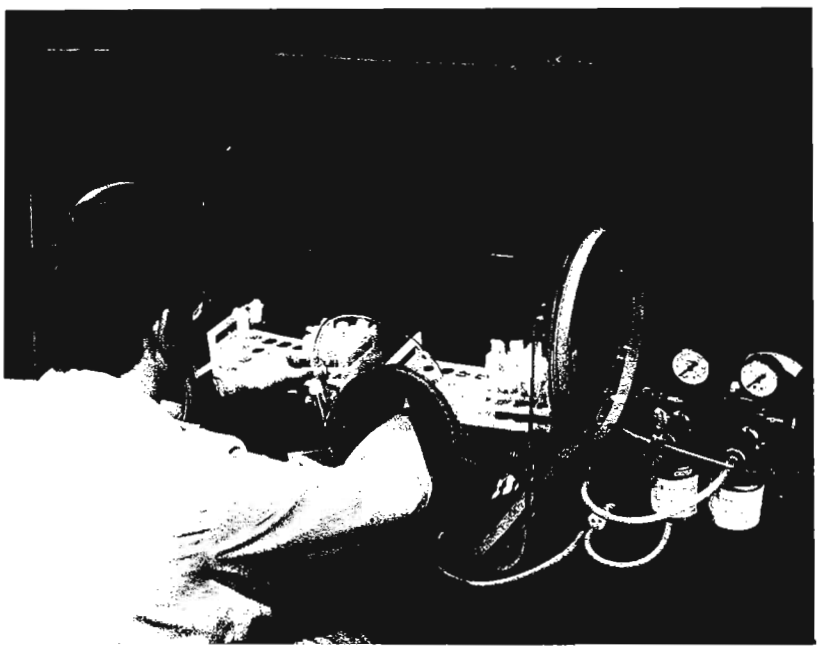


Fig. 2. Drosophila transfer-containment glove box with iris closures.

All transfers are conducted in a Plexiglass^{R} glove box (Fig. 2, #32981-003, $530.00, VWR Scientific, San Francisco, California) to insure that treated flies do not escape into the work area. Insects which escape into the glove box during the transfer procedure are easily recaptured using the vacuum system. In addition to the iris closures shown in Fig. 2 (VWR

#32981-058, $125.00/pr) which allow hand entry, the glove box can be equipped with gas-tight neoprene gloves (medium gloves, VWR #32981-105, $54.00/pr plus glove holders #41-905-320, $12.00/pr) if desired. The glove box can be modified to insure a negative working pressure by venting it into an evacuation system.

I would like to express my sincere appreciation to Drs. S. Abrahamson and R. Valencia, University of Wisconsin, Madison, Wisconsin, for their assistance in establishing our D. melanogaster mutagenicity testing capability. The basic transfer technique described in this technical note was developed by Dr. Abrahamson. The upport of LTC J. T. Fruin and MAJ H. G. H. Eisenberg for the Drosophila program is also greatly appreciated.

Reference: Ashburner, M. and J.N. Thompson, Jr. 1978, in: The Genetics and Biology of Drosophila, Vol. 2a, pp. 1-109 (M. Ashburner and T.R.F. Wright, eds.), Academic Press, NY.

†The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

Wirtz, R. A. and H. G. Semey*. Toxicology Services Group and *Division of Cutaneous Hazards, Letterman Army Institute of Research, Presidio of San Francisco, California. The Drosophila kitchen - equipment, media preparation, and supplies.†

Tasked with establishing a large D. melanogaster insectary for mutagenicity testing we found the information in the articles by Merriam (1973) and Ashburner & Thompson (1978) helpful. The following is an update and expansion of Merriam's article, with emphasis on media preparation and sources of equipment and supplies used in our laboratory or recommended by laboratories visited.

Equipment (Table 1): Media cookers and mixers are available in a variety of sizes, styles and power sources (Table 1). The Agarmatic Bench-top Agar Sterilizer is ideal for small volume (1-3 l) preparation. This self-contained unit requires only a 110 volt outlet and a suitable tap water supply (40 psi) and drain. Sterilization and mixing capabilities, as well as separately adjustable cooking and dispensing temperatures, make this a versatile unit. However, the accessory peristaltic pump (Model 1062) was not effective in dispensing corn meal-based medium.

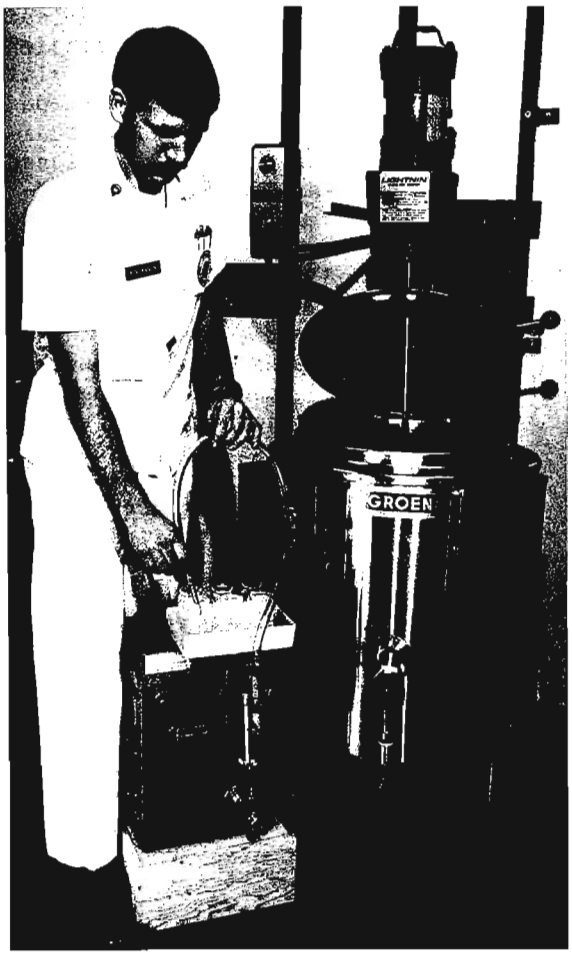


Fig. 1. Steam kettle (20 gal), variable speed mixer and syringe-type dispenser unit used for Drosophila media preparation.

Steam-jacketed kettles (Table 1) are used for large volume media preparation in many Drosophila kitchens. We, as well as several other laboratories, have used the Groen FT or EE models (20 gal) with excellent dependability and results. These are stainless steel kettles equipped with 1-1/2" draw-off valves and hinged covers (Fig. 1). The EE model is an electrically operated, self-contained unit. The FT model requires an external steam source of 5-20 psi. This model has the capability of being attached to a cold water source, through the steam system, for rapid cooling of media before addition of temperature-sensitive material.

Self-contained mixers are available on some models of kettles; however, many laboratories purchase the mixer separately. This usually requires modification of the kettle cover for installation. Mixing volume and medium consistency determine the horsepower rating of the motor required.

Table 1. Equipment and sources (approximate costs are 1980-81 prices).

Item description	Sources	Approximate cost
Agarmatic Bench-top Agar Sterilizer	New Brunswick Scientific, Inc. P.O. Box 986; 44 Talmadge Rd. Edison, NJ 08817 (201) 287-1200	$2,575.00
Steam Kettles (also check local commercial kitchen supply companies)	Groen Division/Dover Corp. 1900 Pratt Blvd. Elk Grove, IL 60007 (312) 439-2400	EE Model, $2,945.00 FT Model, $1,535.00
Mixers (available on many models of steam kettles)	Mixing Equipment Co., Inc. 221 Rochester Street Avon, NY 14414 (716) 226-6136	Model ND-1 SCR, $1,535.00
Reduction Fittings (also check local dairy equipment suppliers)	Duhig, Inc. 14375 Wicks Blvd. San Leandro, CA 94557 (415) 352-6460	see text
Media Dispensers (prices do not include costs for syringe units)	National Instrument Co. 4119 Fordleigh Road Baltimore, MD 21215 (301) 764-0900	Model AB, $795.00 Model AB-5, $870.00
	Brewer Pipetting Machine BBL, Division of BioQuest Cockeysville, MD 21030 (301) 666-0900	Model 40-SS $630.00
Vacuum Pump, hand operated	Fisher Scientific Co. 711 Forbes Avenue Pittsburgh, PA 15219 (412) 562-8300	Nalge 6131, $23.50

Table 2. Drosophila medium recipes for use with the steam kettle (5-70 liter capacity).

Ingredients	Volume	or	Weight	Approx. weight percent[1]
Water (ml)	8,000		20,000	80.0
Agar (gm)	100-150		250-375	1.0-1.5
Molasses (ml)	800		2,000	8.0
Corn meal (gm)	700		1,750	7.0
Yeast (gm)	400		1,000	4.0
Propionic acid (ml)	50		125	0.5
20% Methyl p-hydroxybenzoate (ml)[2]	125		315	0.25
Approximate total volume (ml)	10,200		25,490	100.75-101.25%

[1]Recipes for total volumes other than those listed can be computed using the given approximate weight percentages. Changes in the volume/weight of ingredients used may be required because of the age of the ingredients or variation between batches. These changes will be recorded on the "Drosophila Medium Record Sheet". Some variation in the final composition of the medium will occur due to moisture change during cooking. These variations have not affected performance in previous tests.

[2]Stock solution: 20% methyl-p-hydroxybenzoate (100 gm q.s. 500 ml 95% ethanol). Final concentration in the medium is 0.25%.

We use the Lightnin Model ND-1 SCR (1/3 hp) and feel that the convenience of the variable-speed mixer is well worth the additional cost. One laboratory we visited hand-mixes the ingredients during the initial cooking, thus eliminating the need for a mechanical mixer.

Stainless steel reducing adapters, required for the attachment of dispensing equipment to a kettle, are usually carried by dairy equipment suppliers. The following are required to reduce the 1 1/2" sanitary draw-off valve on the Groen kettles to 1/4": 1 1/2x1" taper reducer (#31-19F1 1/2x1", $72), 1 1/2" hex nut (#13H-1 1/2", $12), 150 lb screwed reducing coupling (#1x1/4", $7), and a 1/4x1" nipple (# schedule 40304 nipple, $1). Order numbers and approximate prices are those of Duhig, Inc. (Table 1). We routinely use Teflon® tape on the connections to prevent leaks and allow easy disassembly for cleaning. A 1" hose can be clamped directly to the taper reducer for rapid draining after cleaning.

Sources of syringe-type media dispensers are given in Table 1. We have found that stainless steel syringe units (#FUS-60ml, $415; #FUS-130ml, $480, National Instrument Co.) are excellent for dispensing corn meal based media, especially when used in conjunction with an adjustable suck-back filling unit (#1886 ASB-130ml, $73) to prevent dripping. An air-actuated spool valve dispensing attachment, recommended by National Instrument Co. for use with corn meal based media, is also available for approximately $1700; however, none of the laboratories contacted were using this unit.

Media preparation (Table 2): A standard Drosophila medium is used for most mutagenicity testing and routine colony rearing. When small volumes of medium are required, or when special larval feeding procedures are needed, Carolina Biological Supply Instant Drosophila medium formula 4-24® may be used. The following is the procedure used for preparation of our standard medium: (1) Record the sequential batch number, date and starting time on a standardized "Drosophila Medium Record Sheet" (Fig. 2). (2) Weigh the 10, 100, and 500 gm standard weights on the balance used for weighing the medium ingredients. Record the weight readings to the nearest 10th of a gram on the record sheet. (3) Fill in the record sheet as to the weight/volume of ingredients required for the desired batch size (Table 2). As ingredients are used, check the item off the designated area of the record sheet. ($) Add the designated volume of deionized water, minus 1000 ml, to the kettle. Turn on the mixer and set the temperature on the steam kettle to 95±5°C (setting of 7.0 on the model EE-20). The setting light will go out when the kettle reaches the cooking temperature. (5) Slowly add the following to the water in the order listed: (a) agar, (b) molasses, and (c) premixed corn meal plus yeast. When adding these materials increase the mixer speed to prevent settling. Wash out the molasses container with 1000 ml of deionized water and add it to the mixture. (6) Cook for 10±3 minutes at 95±5°C. Reduce the temperature to 45±5°C (setting of 3.0) and cook for an additional 10±3 minutes. (7) After approximately 20 minutes of cooking add the propionic acid and/or methyl p-hydroxybenzoate, cook for an additional 5-10 minutes and dispense the medium. (8) Record all pertinent information on the record sheet, sign and place the sheet in the record log notebook. (9) Thoroughly clean the steam kettle and mixer.

1. Batch No:_____ 2. Date:_____ 3. Agarmatic:_____
4. Time:_____ 5. Steam Kettle:_____

6. Check Ingredients	Volume/Weight	7. Std. Weight (gm)	Weight (gm)
____ a. Water	_____ml	10	____.____
____ b. Agar	_____gm	100	____.____
____ C. Molasses	_____ml	500	____.____
____ d. Corn meal	_____gm		
____ e. Yeast	_____gm		
____ f. Propionic acid	_____ml		
____ g. 20% MHB	_____ml		

8. Cooking Time:_____ minutes (before adding mold inhibitors).
9. Cooking Temperature:_____°C/Kettle setting:_____.
10. Mold inhibitor mixing time:_____ minutes.
11. Label medium as to batch number, date mixed, and expiration date.
12. Clean up the equipment and work area.
13. Notes:_____

Signature:_____

Fig. 2. Drosophila Medium Record Sheet.

Granular baker's yeast (Table 3) is currently used because of the ease of handling. When we used brewer's yeast the material was sifted, if necessary (using a large tea strainer)

Table 3. Sources and approximate costs of Drosophila media ingredients and rearing supplies. (Approximate costs are 1980-81 prices.)

Item Description	Source	Approximate cost
Agar, fine ground powder form "A"	Moorehead & Co. 14801 Oxnard Street Van Nuys, CA 91401 (231) 873-6640	$8.90/lb (less than 25 lb) $8.45/lb (more than 25 lb)
Corn meal, ground, yellow (#901411)	ICN Nutritional Biochemical Co. 26201 Miles Road Cleveland, OH 44128 (216) 831-3000	$24.00/100 lb $8.00/25 lb
Molasses, unsulfured, "Home Made Molasses"	Saroni Total Foods, Inc. P.O. Box 96 Oakland, CA 94604 (415) 428-2662	$41.05/5 gal
Yeast, brewer's, powdered (#103312)	ICN Nutritional Biochemical Co.	$104.00/100 lb $30.00/25 lb
Yeast, baker's, active dry (#ADY-12/2 lb cans)	Fleischmann 921 - 98th Avenue Oakland, CA 94603 (415) 562-7677	$48.50/24 lb
Methyl p-hydroxybenzoate (EKC 2844)	American Scientific Products 255 Caspian Drive Sunnyvale, CA 94086 (408) 743-3100	$25.07/500 gm
Propionic acid (MAL 7179)	American Scientific Products	$7.80/pt
Instant Drosophila Medium (#67-5003)	Carolina Biological Supply Co. Gladstone, OR 97027 (503) 656-1514	$28.80/16 liters
Bottle, glass, urine specimen, 6 oz (#B 7925)	American Scientific Products	$7.76/pkg 12
Bottle, polypropylene, urine specimen, 6 oz (square base, #B7928)	American Scientific Products	$70.95/cs 500
Bottle, polypropylene, urine specimen, 6 oz (round base, #11500)	Superior Plastics Products Cumberland Industrial Park Cumberland, RI 02864 (401) 333-6061	$22.60/cs 250
Bottle lids, paper (#B7926)	American Scientific Products	$5.24/pkg 500
Bottle lids, paper (#11900)	Superior Plastics Products	$8.40/cs 500
Burco Drosophila anesthetizer (#F14300)	Bell-Art Products Technilab Instruments, Inc. Pequannock, NJ 07440 (201) 694-0500	$4.00 each ($50.00 min. order)
Ball, absorbent, cotton, 2" (#1404A)	Chaston Medical & Surgical Products Lake Road Melville, NY 06241 (800) 243-1172	$35.77/cs 2000
Ball, absorbent, rayon, 2" (#6890)	Kendall Co. 1 Federal Street Boston, MA 02101 (617) 432-2000	$13.80/cs 2000
Vials, shell, 95x25 mm (#66020-188)	VWR Scientific P.O. Box 3200 San Francisco, CA 94119 (415) 469-0100	$27.70/cs 144

and added to the dry corn meal. Adding this yeast-corn meal mixture eliminated the necessity of making a yeast slurry to prevent caking.

Molasses is easily transferred to a 1000 ml Fleaker™ (Corning) flask using a vacuum system. A 40 x 1.2 cm glass tube, run through a hole of the same size in the lid of the molasses pail, is connected to a #10 2-hole stopper with clear tubing. The second hole in the stopper is attached to a hand-operated vacuum pump (Nalgene Co., Table 1). This allows transfer of the molasses, without opening the container, by placing the stopper into the Fleaker™ and pulling a vacuum on the system.

Medium ingredients are purchased from several suppliers (Table 3). Check with the manufacturer as to the shelf life of ingredients before large volume purchases. Yeast, molasses, and agar can deteriorate with age when stored at room temperature. Unless all materials are used rapidly, insure that they are kept in cold storage to maintain viability and reduce the possibility of microbe or arthropod contamination.

Rearing supplies (Table 3): Glass urine specimen bottles or disposable polypropylene urine bottles are excellent replacements for the scarce 1/2-pint milk bottles traditionally used for stock colony rearing. These bottles can be capped with disposable paper closures or reusable foam plugs (27-35mm diameter). Burco anesthetizers fit into the cap seats of the half-pint milk bottles and glass or plastic urine specimen bottles for easy collection and etherization of flies. Glass shell vials (25x95mm) with 2" rayon or cotton plugs may also be used. Medium volumes for the containers are: bottles - 50±10 ml, and vials - 10±2 ml.

Bottle trays (10 1/4 x 12 1/4 x 2 1/2" inside dimensions) were constructed from exterior "A" grade 1/2" plywood. However, these were difficult to clean and invited microbial and mite infestations. Trays constructed of non-porous materials are therefore highly desirable. Rubbermaid® drawer organizers are excellent, inexpensive vial and bottle trays. The 15x6" (#2918, $1.29 each) hold approximately 85 standard 95x25mm vials, 10 round-bottomed or 12 square-bottomed plastic urine bottles. The 9x6" (#2916, $0.79 each) hold 50 vials or 6 bottles (Fig. 3). The vial trays are available in several colors for coding of colonies and are easily equipped with sliding dividers cut from 1/8" acrylic plastic or made from 4x6" index cards.

We would like to express our sincere appreciation to Drs. S. Abrahamson and R. Valencia, R. Burns, K. Houtchens and D. White, University of Wisconsin; Dr. G. Schmolesky, Raltech Scientific Services, Inc., Madison, Wisconsin; and Dr. B. Evans and J. White, SRI International, Palo Alto, California, for their assistance in establishing our Drosophila insectary and mutagenicity testing capability. We also acknowledge the support and assistance of LTC J.T. Fruin and MAJ H.G.G. Eisenberg of Letterman Army Institute of Research.

References: Ashburner, M. and J.N. Thompson, Jr., 1978, in: The Genetics & Biology of Drosophila, Vol. 2a (M. Ashburner and T.R.F. Wright, eds.), pp. 1-109, Academic Press, NY; Merriam, J.R. 1973, DIS 50:196-197.

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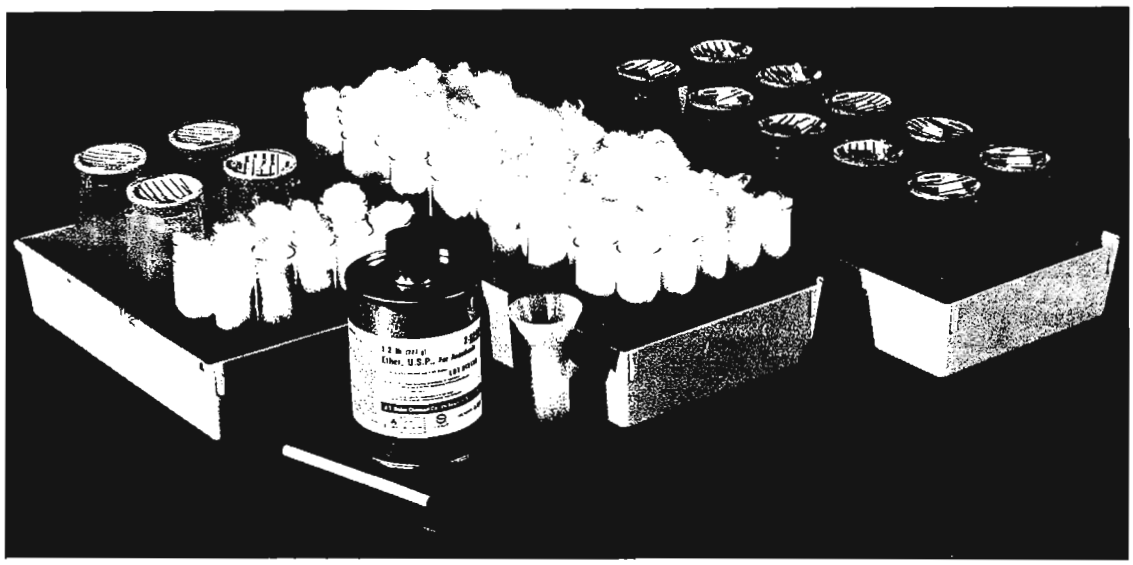

Fig. 3. Bottle and vial trays and ether anesthetizer used for Drosophila rearing.

Coyne, J. A. and T. Prout. University of California at Davis, California. Abduction of Drosophila virgins in introductory genetics classes.

A recent occurrence in the undergraduate genetics lab at UC Davis has led us to issue this note as a warning to those who may be in similar situations. In this course students are asked to identify an unknown Drosophila mutant by crossing it to various tester strains. This work requires the regular collection of virgin females for the crosses. Several students have reported the mysterious disappearance of virgin females from their collection vials, presumably by theft. Those who have trouble collecting virgins or who are otherwise inept at Drosophila husbandry may be tempted to surreptitiously acquire virgins from their classmates. Victims of such theft have been forced to sequester their cultures in secret hiding places. We suggest that teachers of similar courses advise their students of this possibility, especially if there is a high proportion of pre-medical students.

Huber, I. Fairleigh Dickinson University, Madison, New Jersey. A search for pleiotropic effects of a mutant gene: An exercise in ecological genetics.

In studying a population, ecologists often make the simplifying assumption that all members of a population are genetically identical. A large body of recent studies in population genetics, especially with electrophoretic techniques, indicates that this assumption is unrealistic. Ecological genetics is a field that has developed at the boundary of ecology and population genetics and is concerned with the effects of genes (such as those for eye color or body color in Drosophila) on biologically important parameters, such as longevity and fertility, as well as aspects of physiology and behavior which may affect viability.

From the viewpoint of the organismic geneticist, there is a major phenotypic effect by which the presence of a gene is identified and a multiplicity of minor effects on many aspects of the anatomy, physiology and behavior of an animal. Collectively, these effects are referred to as pleiotropic. As an example, the sickle-cell gene in homozygotes, in addition to causing sickling of erythrocytes, has an effect on virtually all organ systems (Neel & Schull 1954). Both phenomena, namely the existence of pleiotropic effects of a mutant gene and the fact that these effects may be important in population dynamics, can be demonstrated with Drosophila melanogaster.

Materials and Methods: Three groups of D. melanogaster will be tested: wild-type, a recessive mutant, and their F_1 hybrid. Students are asked to select a trait unrelated to the major phenotypic effect and test samples of all three groups. Examples of adult traits which have been studied with interesting results are: dry weight, wet weight, longevity (for faster results, deprive flies of food but not water), longevity at elevated temperatures, resistance to standard dose of insecticide, number of eggs laid/female/day, O_2 consumption/gram body weight/hour. Other traits which could be investigated include behavioral characteristics such as walking speed, frequencies of grooming movements and components of courtship and rates of maze-learning ability.

Means for each group will be determined and (depending on the amount of statistical knowledge of the class) statistical tests run. For weights of flies, it is best to group them. For example, weigh four groups of 25 flies of each genotype, expressing the results as mean weight/25 flies.

Interpretation: Several kinds of outcomes are possible for this exercise. All of the mutants studied are recessive.

1. All three genotypes have the same mean. In this case, the mutant has no pleiotropic effect on the trait measured. This is entirely possible, though not as interesting, as the other kinds of results.
2. The heterozygote has a mean not significantly different from the wild-type. Since the mutant is completely recessive, the pleiotropic trait parallels the expression of the major phenotypic effect. This is the extreme of the range of values mentioned in #3 (below).
3. The heterozygote mean is closer to wild-type than to the mutant.
4. The heterozygote has a mean intermediate between that of the wild-type and the mutant. Therefore, the trait shows incomplete dominance in contrast to the recessive inheritance pattern of the mutant.
5. The heterozygote is closer to the mutant than to the wild-type.
6. The heterozygote mean is identical with the mutant mean. This is the extreme of the range of values mentioned in #5 (above).

7. The heterozygote mean is more extreme than either homozygote, a phenomenon called overdominance.

Whether a gene is regarded as dominant, overdominant, incompletely dominant or recessive depends entirely on the criterion by which an individual is classified and is thus relative. The criterion may be the major phenotypic effect, a pleiotropic effect (or amounts of protein products of the genes). For example, by appropriate choice of criteria, the sickle-cell gene can be shown to resemble most of the dominance relationships listed above (see Mange & Mange 1980: 189-193 for a useful discussion).

References: Mange, A.P. and E.J. Mange 1980, Genetics: Human Aspects, Saunders, Philadelphia; Neel, J.V. and W.J. Schull 1954, Human Heredity, Univ. Chicago Press, Chicago.

Johnson, D. A. Concordia College, Moorhead, Minnesota. A computer program to illustrate selection and random drift.

Computer simulation of populations is a valuable means of reinforcing concepts taught in introductory biology and genetics courses. A new program has been written (POPULA.GEN) to illustrate some of the dynamics of basic population genetics in populations with discrete, non-overlapping generations. Given population size, initial allelic frequency and selection coefficients, the program will indicate genotypic frequencies before and after selection and print out the new allelic frequency for each generation. Drift and the effects of various types of selection are easily demonstrated with this program. The program is written with optional steps that will store the allelic frequencies in a separate file for retrieval and plotting using an SPSS (Statistical Package for the Social Sciences) program called PLOT.ITT. POPULA.GEN is written in BASIC-PLUS for the Resource Time Sharing (RSTS system of the PDP-11 computers and is similar to EVOLUT (Chelsea College, London), but has several advantages. First, selection (and not just mating) is a randomized function (based upon the given selection coefficient); second, any selection coefficient can be assigned to each genotype; and third, its use with PLOT.ITT provides an easily visualized illustration of changes in allelic frequencies. Listings of PLOT.ITT (for use with the SPSS package) and POPULA.GEN are available upon request.

SUBMITTED STOCK LISTS - Other Species

University of Swansea, Dept. of Genetics, Swansea, West Glamorgan, U.K.

D. simulans

wild type stocks
Bebek (Istanbul)
Canary Islands
Peramola (Spain)

D. hydei
D. erecta
D. Teissieri
D. yakuba

D. funebris
D. virilis
D. subobscura

Università di Milano, Istituto di Genetica, Milano, Italy.

D. simulans

wild type stocks
Aspra
Giannutri
S. Antioco

D. pseudoobscura AR
D. pseudoobscura TL
D. virilis
D. hydei