DROSOPHILA INFORMATION SERVICE

67

June 1988

Material contributed by
DROSOPHILA WORKERS

and arranged by
P. W. HEDRICK

Department of Biology
Mueller Hall
The Pennsylvania State University
University Park, PA 16802

DROSOPHILA INFORMATION SERVICE

Number 67

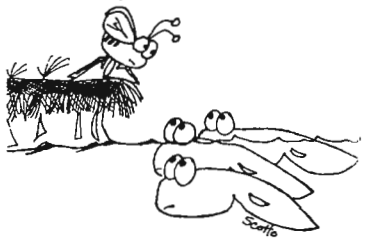
June 1988

Prepared at the
Division of Biological Sciences
University of Kansas
Lawrence, Kansas 66045 - USNA

Publication costs are partially funded by
NSF Grant BSR-8420293 to R.C. Woodruff.

For information regarding submission of manuscripts or other contributions to Drosophila Information Service: contact Philip W. Hedrick, Editor, Department of Biology, Pennsylvania State University, University Park, Pennsylvania 16802 - U.S.A.

Table of Contents



The Journal has moved ! Professor Hedrick has accepted a position at Pennsylvania State University beginning this fall of 1988. DIS will move with him; our new address (after August 15, 1988):

Drosophila Information Service
c/o Philip W. Hedrick, Editor
Dept. of Biology, Mueller Hall
Pennsylvania State University
University Park, PA 16802 USA

Tel: 814-865-3125
Dept.: 814-863-0278

CALL FOR MATERIAL FOR DIS 68

Contributions are now being accepted for DIS 68, June 1989. Please double-space all contributions, and otherwise follow the format of DIS 63 & 66. Make sure complete information is included with every section of submissions--which will be separated from one another.

Please provide good quality black & white photographs or drawings; do not mount photographs (unless the situation requires it)--the layout person will do it according to printing specifications. Be sure your name is on all pages, tables, figures, etc., which accompany manuscripts. Note: boldface will be used for species names and section headings; underlining is not used except in rare circumstances for clarity.

Sections you may contribute to are:

1. Announcements, requests, etc.
2. Research Notes
3. Technical Notes
4. Teaching Notes
5. Stock Lists, mel.
6. Stock Lists, spp.
7. Linkage Data
8. New Mutants
9. Directory
10. Special Reports

DEADLINE FOR DIS 68 IS APRIL 1, 1989.

ORDER COPIES OF DIS IN ADVANCE if possible. *Subscribers paying in advance with the enclosed form or by written request will pay $7.00 per copy.* The DIS office will add $1.00 to the cost of the current issue if invoicing is required; the current policy is to send an invoice at the time of mailing the current issue to those subscribers who must pay with invoices. We can only provide invoices in triplicate; any additional copies you must photocopy yourself. You may also request a standing order at this time. DIS will be sent air-mail book rate at subscriber's request and expense. Surface book rate postage is included in the cost of the journal. NOTE: Checks for DIS should be drawn on a **U.S. bank only** and in U.S. dollars. The office still has on hand the following volumes:

@ $5.00: 52, 53, 54, 55, 56, 58, 59, 60, 61, 63. @ $7.00: 57, 62, 64, 65, 66.

REPRINT POLICY: The DIS printer does not have the capability to provide reprints for DIS contributions. Instead, each author should use photocopy services at her/his own institution. If you request the DIS office to send photocopies, we will do so and bill you for the photocopying charge plus postage.

For Sale: Complete set of University of Texas **Studies in Genetics** series, Drosophila papers. Nearly impossible to obtain. They must be sold as a set. Full details are available from Dr. John D. Agnew, Box 1326, Toowong, Qld. 4066, Australia. Tel (07) - 277-8281.

GRAPHICS: Prof. Scott P. McRobert, Dept. of Biology, Swarthmore College, has once again submitted Drosophila cartoons. We appreciate very much his contributions to DIS in this form as well.

30th ANNUAL DROSOPHILA CONFERENCE
NEW ORLEANS

APRIL 26 - 30, 1989

For more information, contact: William Lee, Dept. of Zoology
Louisiana State University
Baton Rouge, LA 70803
Tel. (504-388-1132)

11th EUROPEAN DROSOPHILA RESEARCH CONFERENCE

MARSEILLE-FRANCE

5-8 SEPT. 1989

The 11th European Drosophila research conference will be held in Marseille-Luminy, France, September 5-8, 1989. Every two years, this meeting provides a unique opportunity for people working on Drosophila to present their research results. Approximately 250-350 scientists from more than 20 countries usually attend this meeting.

The 11th EDRC will consist of 6-9 scientific sessions comprising oral and poster presentations, each session being introduced by a plenary lecture given by an outstanding Drosophilist. During the conference, time will be available for informal workshops on specialized subjects.

The tentative program will include the following major topics: Gene-enzyme systems; Control of gene expression; Developmentally and hormonally regulated genes; Maternal, segmentation and homeotic genes; Cell interactions and morphogenesis; and Neurobiology and Behavior.

If you want to be on the mailing list of the 11th EDRC in Marseilles and so receive the official announcements, write to B. Jacq, Lab de Genetique et Biologie Cellulaires, C.N.R.S., Centre Universitaire de Luminay, Case 907, Marseille 13288 Cedex 9, France.

DROSOPHILA:
A LABORATORY HANDBOOK AND MANUAL

MICHAEL ASHBURNER

Two volumes
Due May 1989

In two volumes, DROSOPHILA: A Laboratory Handbook and Manual is the reference work for all who work with Drosophila as an experimental organism, and for all who wish to keep up with one of modern biology's most exciting fields of research.

The Handbook is a succinct summary, organized in a manner to make it handy for rapid reference, to most aspects of the biology of Drosophila. Its coverage ranges from mutagenesis to taxonomy, from tissue culture to aberrant chromosome segregation, from molecular and developmental biology to compound autosomes. Each of its 38 chapters give a summary of the present state of the subject, written with an historical perspective but with an emphasis on the practical use of genetic and other methods. Extensively referenced, these chapters will give you an up-to-date overview of Drosophila biology.

The manual, in the Cold Spring Harbor tradition includes over 130 detailed protocols for working with Drosophila in the laboratory, anything from the preparation of mitotic chromosomes to primary cell cultures, from in situ hybridization (chromosomes and tissues) to the dissociation of the central nervous system, from freeze-drying flies to making thin sections of polytene chromosomes. Many of these protocols have not been readily available before. Spiral bound, for easy use at the laboratory bench, The Manual also includes complete recipes for reagents, designs of special equipment and source supplies.

For information, contact: Cold Spring Harbor Laboratory
Fulfillment Department
Box 100
Cold Spring Harbor, NY 11724

Telephone: Continental US except NYS 1-800-843-4388
All other locations (516) 367-8423

SPECIAL NOTE

The following is a letter from Herman Spieth, University of California-Davis, to Ernst Mayr, printed by special permission from Prof. Spieth. A more formal report was published in the **Pan-Pacific Entomologist.**

Davis, California
November 15, 1983

Dear Ernst [Mayr]:

Finally, after more than three decades of searching, I have found the prime native larval substrate of **pseudoobscura.** As you know, during the past three years I have been systematically collecting **Drosophila** in the U.C. Blodgett Experimental Forest. This 3,000 acre forest located 11 miles east of Georgetown, California, is at the 4,000 ft. level. It is a "mixed" forest since it is in the transition zone between the low and high elevation forests. Four species of the **obscura** group are found here, i.e.: **azteca, miranda, persimilis** and **pseudoobscura**. For many years all non-native plants have been rigorously excluded and thus the four species that dwell and breed in the area are dependent upon native substrates.

During the three years I have systematically sought to find the larval substrates, starting each year while the snow still remains on the ground and until late in October when the flies no longer came to bait. Following Carson I sought out slime fluxes, especially those on oak. A number were found and regularly visited, but I was never able to find any larvae or pupae, and adults were never present. I checked out manzanita (**Arctostaphlos**), galls and berries, as well as fungi which produce fungus feeders but no **obscura** flies. I also investigated conifer cones, sap sucker holes, fermenting bark, etc. No luck.

Early in September of this year, I noticed a large number of acorns had been shed by a large black oak, **Q.kellogii,** located near one of my bait sites. Remembering that Dobzhansky had noticed that **pseudoobscura** was attracted to large oaks and also that my baiting at both Davis and Blodgett confirmed this observation, I decided to collect a fair quantity of these freshly fallen acorns. My intention was to break open the acorns, extract the acorn meats, allow them to ferment and then test the effectiveness of the rotting material as a larval substrate.

When I opened the acorns, to my surprise I found that the majority of the eggs had been parasitized by the weevil **Curculio occidentis** and a small number by a moth **Melissopus latiferreanus.** Fortunately, John Tucker of the UCD Botany Department devotes his research efforts to oaks and from him I learned that on the average 80% of all acorns in all parts of the United States are parasitized by species of **Curculio.** The female weevil inserts one or more eggs into the acorn two to three weeks before the acorn starts to ripen. The larvae feed on the nut meat and after the acorn drops to the ground and the larvae has matured, the weevil cuts a 1-2 mm circular hole in the shell of the acorn, then escapes and burrows into the ground and eventually pupates.

When the weevil larva exits from the acorn, it leaves behind a mass of soft, moist frass in the cavity of the acorn. I broke open a number of acorns and offered the frass to mature **pseudoobscura** females. They readily oviposited in the frass and eventually adults emerged. The frass has a pH of circa 4.0. So far, so good, but there is no organism in the field that breaks open parasitized acorns. In fact, birds and rodents that feed on acorns ignore parasitized acorns, and are attracted only to intact acorns.

There remained one possibility, i.e., that the **pseudoobscura** females would use the escape hole that the weevil larva had created, as an access portal for ovipositing into the parasitized acorn.

I therefore immersed 16 parasitized acorns from which the weevil larvae had exited into distilled water. I hoped thereby roughly to simulate winter rains and melting snow that the acorns experience in nature. After immersion for six days I removed the acorns and placed them on a layer of moist tissue in a quart jar. At the same time I introduced mature **pseudoobscura** adults. To provide food for the adults I prepared vials in the fashion which I had devised for shipping Hawaiian flies (DIS 41:196-197). You will remember that these vials are lined with blotting paper which has been impregnated with agar and karo syrup. The adult flies feed on the paper but will not oviposit into it. One of these vials was added to the quart jar.

Ten days later I broke open two of the acorns. Both contained robust **pseudoobscura** larvae. Thus the females can and will use the weevil exit holes to oviposit their eggs into acorns. Tim Prout was not surprised. Just recently he found that if he punctured small holes into the surface of laboratory food then the **pseudoobscura** females would oviposit all their eggs into the holes and totally ignore the smooth surface of the laboratory food.

Further observation shows that these **pseudoobscura** larvae, when they are ready to pupate, migrate to the parasite exit hole in the acorn shell, extend their heads into the hole and pupate. This results in the pupal spiracles extending outward while the remainder of the pupal body is inside the acorn. The adult,

when it emerges, wriggles out through the hole in the same manner as the parasite employed in leaving the acorn. Thus, the entire development of the **pseudoobscura** individual from egg laying to emergence of the adult can occur within the protective shell of the acorn.

The first sample of acorns that I had immersed in water was removed after six days. A second batch of 13 acorns was immersed for 21 days and then tossed into a jar which had a layer of two inches of wet sand. Seven of these acorns came to rest with all or a portion of the base of the acorn in contact with the wet sand. As you know, the base of an acorn has a circular structure which joins the acorn to the acorn cup. The xylem and phloem ducts run through this area. This basal region is histologically different from the thinner, smoother shell portion of the acorn. A shallow groove encircles the acorn at the junction of the shell portion and the bait. Twelve days after the **pseudoobscura** adults were introduced, I removed the acorns. Inspection showed that the 7 which had the base wholly or partially in contact with the wet sand had the groove filled with a mucous fluid and many **pseudoobscura** eggs had been laid in the groove. The eggs had hatched and many healthy larvae were in the grooves. Further, the base of some had partially pulled free from the remainder of the shell, and the larvae were therefore able to enter the acorn and feed on the frass that had been prepared by the parasites.

I think it can be validly assumed that during the fall rains, such as are now occurring at Blodgett, and during the spring thaw when the snow melts that any acorn embedded in the litter on the floor of the forest will be exposed to moisture for periods that equal or exceed the 21 days that these 7 acorns were subjected to in the laboratory. I will find out next spring when the snow melts.

Thus, in any area where oaks grow each fall an untold number of larval packets are prepared by the weevil, each packet enclosed in its own protective container. These food masses have a "long life span." Thus, I found several parasitized acorns that had been shed in 1982. These may well have served as larval substrates during the spring and summer of 1983, but when I opened them they still contained some frass and **pseudoobscura** females readily oviposited into the frass. The eggs hatched but the larvae apparently starved to death before they matured.

To date I have tested only **pseudoobscura** on the acorns. I have now developed techniques to the stage where I plan to test the other three species in the near future. There exist, however, two vexing considerations that probably will make it difficult to interpret the data.

(1) The existence of two parasites. By breaking open acorns which contain parasites in their last larval instar, I know that the Lepidoptera larva cuts an escape hole through the wall of the acorn that is identical in size and shape to that made by the weevil. Thus, when I experiment with a field collected acorn that has a hole in the shell I cannot tell which parasite made the hole. Further, the site at which the largest concentration of Lepidoptera larvae in the acorns was found is a site that has numerous oak trees and also is the site which has yielded the greatest number of all four **obscura** species adults.

(2) Acorns dessicate once they have fallen from the tree. Within two to four weeks a non-parasitized acorn desiccates to the point that the embryo dies. The foresters at Blodgett, when they rear oaks, always pick the acorns from the tree just before they are ready to fall in order to insure viability. Interestingly, the reverse is also true: acorns including those that have been parasitized can be quickly rehydrated by dropping them into a jar which has a couple of inches of wet sand on the bottom. It is not necessary to immerse them in water.

Although rodents and birds feed on the dead ("sound") acorns, some acorns escape these efforts and eventually the meats and dead embryo will decompose. I suspect that as a result of the fall rains and the spring snow melts and rains the base of these acorns will also separate from the remainder of the shell and thus allow them to be used as larval substrates. Such acorns may have a different yeast-bacteria flora than do the parasitized acorns. The number of such substrates will be much smaller, of course, than are the parasitized substrates. Perhaps **miranda** may utilize these.

Finally, some random observations:

(1) Normally we expect that the adults of **Drosophila** will feed on the substrates on which they oviposit. This is impossible, of course, with the acorns. In fact, if the adults are given only moist frass they quickly starve to death. What do they (the adults) feed upon in the Blodgett Forest during the period from April to October when rain fall is scant and infrequent at best and the forest is "dry as dust."

(2) The Blodgett data indicate that oak slime fluxes cannot be considered an important larval substrate, and that **pseudoobscura** should not be considered a sap feeder.

(3) Oaks are present in the region of Bogota, Colombia, and Tucker believes that the southern limits of oaks in South America is in Colombia. He does not know if oaks are found in the higher elevations of Ecuador and Venezuela. If oaks are there I wager that **D.pseudoobscura bogotana** will be there also.

With best regards to Gretel and yourself,

As ever,
Herman T. Spieth

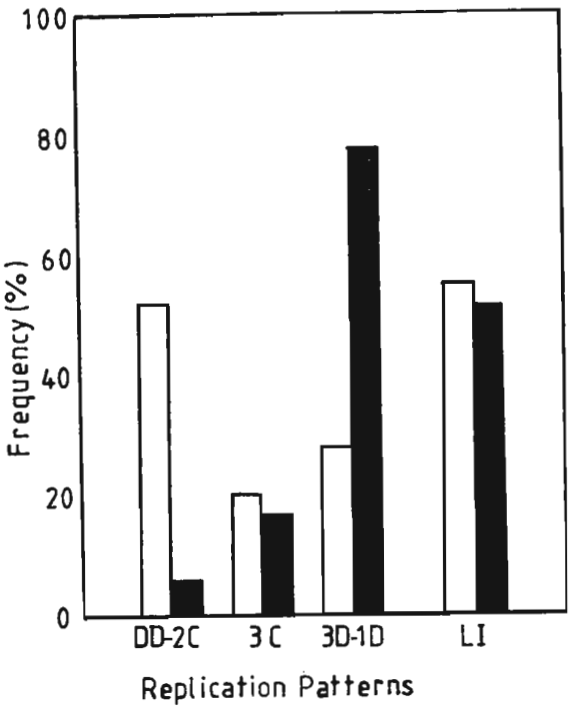
Achary, P.M. and A.K. Duttagupta. Calcutta University, India. Effect of novobiocin on the DNA replication of polytene chromosomes of **Drosophila melanogaster** in vivo.

Novobiocin, a sugar containing antibiotic produced by some strains of the fungus, **Streptomyces** which is known to be an effective inhibitor of bacterial DNA and RNA syntheses (Cozzarelli 1977), has been used in our present investigation for synchronizing DNA replication in the polytene chromosomes of Drosophila in vivo.

Early third instar giant mutant female larvae of **Drosophila melanogaster** (gt w^a / Df (1) 62 g^{18}) were fed on sucrose (1M) solution containing 3.2 mM novobiocin for 48 hr. During the treatment, the larvae showed stunted growth and did not pupate even after 72 hr. The salivary glands of such larvae were dissected out and H-TdR autoradiograms were prepared and the frequencies of different types of replicating nuclei were scored (for details, kindly see Achary et al. 1981).

Table. Mean frequencies (in %) of different types of ^{3}H-TdR replicating patterns of salivary gland polytene nuclei of **Drosophila melanogaster** larvae fed with novobiocin for 48 hr. tn = in total nuclei; ln = in labelled nuclei; LI = labelling index.

	Replicating patterns							LI
	DD	1C	2C	3C	3D	2D	1D	
Control:								
tn	20.99	5.76	2.06	11.11	8.23	2.47	4.53	55.14
ln	38.06	10.45	3.73	20.15	14.93	4.48	8.23	
		52.24				27.62		
Novobiocin:								
tn	0.49	1.62	0.97	8.75	13.45	10.70	16.53	52.51
ln	0.93	3.09	1.85	16.67	25.62	20.37	31.48	
		5.87				77.47		



The data show (Table and Figure) that novobiocin is effective in increasing the frequency of replicating nuclei of late S-phase (3D, 2D and 1D types) to 77.47% in contrast to the control where it was only 27.62%. The early patterns were about ten times less than the control frequencies in these series of experiments. The mean labeling indices, however, were more or less uniform in both control and novobiocin treated larvae.

These results suggest that novobiocin is more effective in synchronizing the cells during the latter half of the S-phase. This effect, we think, has been achieved by the specific property of this drug, alteration of some component of chromatin to change the supercoiling of the DNA and to block specifically the initiation of replication as has been suggested for the mammalian system by Mattern & Painter (1979).

References: Achary, P.M. et al. 1981, Chromosoma 82:505-514; Cozzarelli, N.R. 1977, Ann. Rev. Biochem. 46:641-668; Mattern, M.R. & P.B. Painter 1979, Biochem. Biophys. Acta 563:306-312.

Figure. Histogram showing frequencies (in %) of early (DD-2C), mid (3C) and late (3D-1D) patterns within the labelled nuclei. The open bars represent control and the solid bars represent experimental (novobiocin). LI indicates labelling index.

Aparisi, M.L. and C. Nájera. Universidad de Valencia, Spain. Mapping of new eye-color mutants from natural populations of **Drosophila melanogaster.**

One of the aims of genetical research is to represent the mutable genes on each chromosome as occupying positions on a linear map. But the map distance "x" is not equal to the recombination fraction "y" and this inequality results from two causes: the multiple crossing-over and the interference. Haldane (1919) related "x" and "y" with no interference, while Kosambi (1944) proposed his very useful formula assuming the effects both of multiple crossing-over and interference. The great advantage of Kosambi's mapping function is the introduction of the Kosambi coefficient K as an index of the intensity of interference; whenver the Kosambi formula is exactly satisfied, K is equal to unity.

The Haldane (1919) and Kosambi (1944) metrics were applied to relate the crossing-over frequencies to map distances in order to determine the final location of twelve new eye-color mutants coming from six natural populations of **Drosophila melanogaster**, captured by Nájera and Ménsua (1988) in a cellar, a vineyard and a pinewood, in spring and autumn. Identification of ten of these new mutants was reported by Aparisi & Nájera (1987), using only recombination values; the other two are described in this paper.

The method employed to map each mutation is summarized in Table 1, which uses the mutant chestnut (ches), located on the second chromosome, as an example. After estimating recombination frequencies, coincidence coefficient and interference were calculated. Trow's (1913) and Kosambi's (1944) formulas were used to calculate the recombination fraction (y_{1+2}) over the sum of the contiguous intervals with fractions y_1 and y_2, and choosing the real value of the recombination frequency between the markers (Lindsley & Grell 1972) as the value of y_2. Map distances were estimated using Haldane's (1919) and Kosambi's (1944) mapping functions and, finally, the Kosambi coefficient K was calculated. This coefficient was equal to unity in all experiments with the new mutants. For this reason, Kosambi's formula was chosen to determine the point of location of each mutable gene on its respective chromosome.

Table 1. Diagram of the general scheme used in constructing the map of all 12 new eye-colour mutations. The example corresponds to the mutant $ches^{ca}$ (chestnut), located in 2:94.4.

THREE-FACTOR CROSSING

Progeny phenotype	Number	Class	Class total	Class frequency
+ + +	365	parental	632	
ches b vg	267			
ches + +	172	Single I	261	$\alpha = 0.225$
+ b vg	89			
ches + vg	92	Single II	202	$\beta = 0.174$
+ b +	110			
ches b +	35	Double	64	$\delta = 0.055$
+ + vg	29			
		Total:	1159	

Calculations:

1) Recombination frequencies:

R(ches,vg) = $\alpha + \delta = 0.280 = y_1$

R(b,vg) = $\beta + \delta = 0.229 = y_2$

R(ches,b) = $\alpha + \beta = 0.399 = y_{1+2}$

2) C (Coincidence Coefficient) = 0.858
I (Interference) = 0.142

3) Recombination frequencies between the extreme markers:

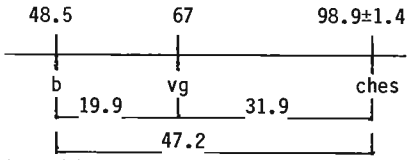
y_1	y_2	y_{1+2}^{obs}	y_{1+2}	y_{1+2}^{K}
0.280	0.229*	0.399	0.399	0.405
0.280	0.185**		0.376	0.385

* Experimental value of y_2
** Real value of y_2 (Lindsley & Grell 1972)
T: Following Trow's formula.
K: Following Kosambi's formula.

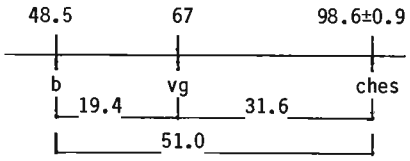
4) Map distances:

	x_1	x_2	x_{1+2}
Haldane:	0.319	0.199	0.472
Kosambi:	0.316	0.194	0.510

Haldane mapping:



Kosambi mapping:



5) K (Kosambi coefficient) = 1.00

TWO-FACTOR CROSSING

Progeny phenotype	Number	Class	Class total	Recomb. freq. between ches & px
+ +	697	Parental	1404	
ches px	707			
ches +	56	Recomb.	92	R(ches,px)=0.061
+ px	36			
		Total:	1496	

Calculations:

1)

y	y_H	y_K
0.061	0.062	0.061

2) Kosambi mapping:

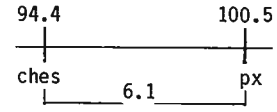


Table 2. Mapping location (in map units), description and key relating mutant name to mutant symbol of 12 new eye-colour mutants from natural populations of **Drosophila melanogaster.**

Map	Map distance	Eye-colour
almond (alm)*	3:17.6	light brownish darkening with age
chestnut (ches)*	2:94.4	chestnut
dark chestnut (dch)*	3:27.1	dark chestnut
dark red (dr)*	2:12.7	dark red
grape (gra)*	3:35.7	intense ruby
irregular facet (ifa)*	3:20.9	dark wine similar wild type w/irregular facets
jewel (jew)*	2:106.5	honey
ladybird (lad)	1:27.9	eyes small, narrow, rough, colour bright red w/black dotted about in it
mandarin (man)*	2:60.7	bright orange
mud (mud)	3:50.5	greyish brown
purplish ruby (pur)*	3:39.6	purplish ruby
soft brown (sb)*	3:37.7	soft brown darkening with age

(*): New mutants described by Aparisi & Nájera (1987).

The final location of each new mutation was defined using Kosambi's mapping function in two-factor crosses, since recombination frequencies give the map distances more accurately when crossing-over values are small.

The name, position and description of all new mutants appears in Table 2.

This analysis confirms that the most useful mathematical instrument contributed to the subject appears to be the Kosambi mapping function, which is largely an intelligent empiricism, but a very good approximate representation of the facts as they are found in several organisms, particularly Drosophila.

References: Aparisi, M.L. & C. Nájera 1987, DIS 66:13-14; Haldane, J.B.S. 1919, J. Genet. 8:299-309; Kosambi, D.D. 1944, Ann. Eugen. 12:172-175; Lindsley, D.L. & E.H. Grell 1972, Carnegie Inst. Wash. Publ. 627; Nájera, C. & J.L. Ménsua 1988, Génét. Sél. Evol. 20(1):25-36; Trow, A.H. 1913, J. Genet. 2:281-297.

Aparisi, M.L. and C. Nájera. Universidad de Valencia, Spain. Pigment patterns of eye-colour mutants from natural populations of **Drosophila melanogaster.**

Two classes of pigments contribute to the final colour of Drosophila eyes: the ommatins and pterins. The biosynthetic pathway of pteridines are not completely understood but the information available was summarized in a hypothetical pathway proposed by Ferré et al. (1986); the xanthommatin pathway, however, presents controversy only about the last step (Wiley & Forrest 1981; Ferré et al. 1986). Several investigators have used paper or thin-layer chromatography to study biochemical differences among eye-colour mutants taking advantage of the fluorescent properties of some compounds of the eye pigments of Drosophila (Hadorn & Mitchell 1951; Taira 1961; Narayanan & Weir 1964; Wilson & Jacobson 1977; Ferré et al. 1986).

The procedure of Ferré et al. (1986) was used to obtain the chromatographic pattern of several eye-colour mutants coming from six natural populations of **Drosophila melanogaster**, captured by Najera & Ménsua (1988) in a cellar, a vineyard and a pinewood in spring and autumn. A total of 44 strains were analyzed, from which 32 were previously described eye-colour alleles and 12 were new mutants. Identification of the new mutants was reported by Aparisi and Nájera (1987). All these were assayed for differences in concentration of eye pigments and metabolically related compounds. All the compounds were measured after separation by thin-layer chromatography.

Table 2. Description of the Chromatographic Spots according to Wilson & Jacobson (1977) and Ferré et al. (1986).

Name	Fluorescence	Colour
Neodrosopterin(NDP)	red	red
Drosopterin(DP)	orange-red	orange-red
Aurodrosopterin(ADP)	orange-red	orange-red
Isoxanthopterin(IXP)	purple	colourless
Sepiapterin(SP)	yellow	yellow
Acetyldihydrohomopterin(ADHP)*	--	brownish
Dihydrobiopterin*	blue	colourless
Pterin(PTE)	blue	colourless
Biopterin(BP)	blue	colourless
Xanthurenic acid(XTC)	light blue	colourless
Spot 18*	green	yellow
Spot 19*	blue	colourless
Spots 20, 21 and 22*	yellow	yellow
3-Hydroxykynurenine	orange	orange
Kynurenic acid*	pale blue	colourless
Xanthurenic acid 8-O-β-D-glucoside*	pale blue	colourless

(*): Spots described by Ferré et al. (1986).

In Table 1, the chromatographic pattern of each mutation is reflected, as well as the comparison with the pattern values of each standard mutation found by Ferré et al. (1986). Identification of some of the spots was reported by Wilson & Jacobson (1977); Ferré et al. (1986) identified the rest, completing the chromatographic pattern. The total of spots that we have analyzed is summarized in Table 2.

These analyses demonstrate the diversity of effects and the high degree of pleiotropy shown by the majority of the mutants, as well as the high biochemical variability that exists between the different alleles of the same mutable gene. With regard to the new mutants, there are features that permit grouping together some of these mutants in relation to the pathway that they seem

Table 1. Semiquantitative estimation of Pteridines and Xanthommatins in eye-colour mutants of **Drosophila melanogaster** from natural populations. **+** indicates an amount approximately equal to the Oregon strain; **1+**, **2+**, etc., increasing amounts; **1-**, **2-**, etc., decreasing amounts and **0**, not detected. The pattern values of each standard mutant found by Ferrè et al. (1986) are between parentheses.

Strain	NDP	DP	ADP	SP	ADHP	IXP	DHB	PTE	BP	XTC	Others
Or.R	+	+	+	+	+	+	+	+	+	+	
bw(cs)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1-(1-)	
bw(vs)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1-(1-)	
ca(vs)	3-(3-)	3-(3-)	3-(2-)	2+(1+)	1-(+)	1-(1-)	3-(1-)	3-(1-)	2+(1+)	4-(0)	
cd(ca)	+(+)	+(+)	+(+)	+(+)	+(+)	1-(+)	+(+)	+(+)	2+(+)	1+(+)	II(II)
cd(pa)	+(+)	+(+)	+(+)	1+(+)	+(+)	+(+)	+(+)	+(+)	1+(+)	+(+)	II(II)
cd(ps)	+(+)	+(+)	+(+)	+(+)	1-(+)	+(+)	1-(+)	1-(+)	1-(+)	1-(+)	II(II)
cn(cs)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	0(0)	I(I)
dke(ca)	1-(1-)	1-(1-)	+(+)	+(+)	+(+)	+(+)	+(+)	1-(1-)	+(+)	+(+)	
dke(cs)	+(1-)	+(1-)	+(+)	+(+)	+(+)	1+(+)	+(+)	+(1-)	+(+)	1+(+)	III(0)
g(cs)	1-(3-)	3-(3-)	2-(2-)	2+(1+)	+(+)	+(1-)	1-(1-)	0(0)	2+(1+)	3-(1-)	
g(va)	3-(3-)	4-(3-)	4-(2-)	+(1+)	1-(+)	1-(1-)	3-(1-)	0(0)	2+(1+)	4-(1-)	
Hnr3(ca)	2-(3-)	1-(2-)	0(0)	2+(3+)	2+(1+)	0(0)	1+(1+)	0(0)	2+(3+)	+(+)	II(II)
Hnr(cs)	2-(1-)	2-(1-)	1-(2-)	1+(2+)	+(1+)	+(1-)	1-(+)	3-(1-)	1+(+)	+(+)	II(II)
Hnr(ps)	1-(1-)	2-(1-)	2-(2-)	2+(2+)	1+(1+)	1-(1-)	+(+)	1-(1-)	+(+)	1+(+)	II(II)
Hnr(vs)	1-(1-)	1-(1-)	2-(2-)	2+(2+)	1+(1+)	1-(1-)	+(+)	1-(1-)	+(+)	1+(+)	II(II)
mah(cs)	+(1-)	+(1-)	+(+)	+(1+)	+(+)	+(+)	+(+)	+(+)	+(+)	1+(+)	
p(ps)	3-(3-)	2-(2-)	2-(2-)	+(+)	+(+)	1-(1-)	+(+)	2-(1-)	1+(1+)	3-(3-)	
pn(ca)	4-(2-)	4-(2-)	0(4-)	2+(+)	1+(+)	4-(+)	+(+)	3-(1-)	1+(+)	+(+)	
pn(cs)	4-(2-)	4-(2-)	4-(4-)	1+(+)	1-(+)	+(+)	1-(+)	3-(1-)	+(+)	3-(+)	
rb(vs)	2-(2-)	3-(2-)	3-(1-)	1-(+)	3-(+)	3-(1-)	4-(1-)	2-(1-)	2-(+)	3-(1-)	
rs2(vs)	2-(2-)	2-(1-)	2-(+)	+(+)	+(+)	+(+)	+(+)	3-(1-)	+(+)	2-(+)	
se(cs)	0(0)	0(0)	0(0)	5+(5+)	0(0)	+(+)	3+(3+)	3+(3+)	4+(4+)	1-(+)	IV(IV)
se(pa)	0(0)	0(0)	0(0)	5+(5+)	0(0)	+(+)	3+(3+)	3+(3+)	4+(4+)	1-(+)	IV(IV)
se(va)	0(0)	0(0)	0(0)	5+(5+)	0(0)	+(+)	3+(3+)	3+(3+)	4+(4+)	1-(+)	IV(IV)
sf(ca)	+(+)	+(1-)	+(+)	+(1-)	+(+)	+(+)	1-(+)	1-(+)	+(+)	+(+)	
sf(cs)	+(+)	+(1-)	+(+)	+(1-)	+(+)	+(+)	+(+)	+(+)	1+(+)	1+(+)	II(0)
sf(ca)	+(+)	+(1-)	+(+)	1+(1-)	+(+)	+(+)	+(+)	+(+)	1+(+)	+(+)	
sf(va)	1-(+)	1-(1-)	1-(+)	1+(1-)	+(+)	+(+)	1-(+)	3-(+)	1+(+)	+(+)	
sf(vs)	1-(+)	1-(1-)	1-(+)	1+(1-)	+(+)	+(+)	+(+)	1-(+)	+(+)	+(+)	
v(ps)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	0(0)	
v(va)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	0(0)	
vi(cs)	1-(2-)	1-(2-)	1-(2-)	2+(2+)	+(+)	+(+)	+(+)	3-(3-)	2+(2+)	3-(3-)	
alm(ca)	1-	1-	+	+	+	+	1+	1+	1+	+	
ches(ca)	+	+	+	+	+	+	+	1-	+	+	
dch(vs)	+	+	+	2+	+	+	1+	1+	1+	+	
dr(cs)	+	+	+	+	2-	+	1+	1+	1+	1-	
gra(ca)	1-	1-	1-	1-	2-	+	1-	1-	1-	+	
ifa(va)	1-	1-	1-	+	2-	+	+	+	+	2-	
jew(ca)	1-	1-	1-	1-	2-	+	1-	1-	2-	+	II
lad(ca)	1-	1-	1-	+	1-	+	1-	1-	+	0	
man(ps)	+	+	+	1+	1+	+	+	+	+	1-	
mud(ca)	+	+	+	1+	+	+	1-	1-	+	1-	
pur(ca)	+	+	+	1-	2-	1-	1-	1-	1-	+	
sb(ca)	+	+	+	1+	+	2-	+	+	+	1-	

I = kynurenic acid. II = xanthurenic acid 8-O-β-D-glucoside. III = I+II. IV = spots 18,19,20,21,22, and 26.

to affect. So, the alm, dch, dr, gra, mud and pur mutants are mainly affecting the biosynthetic pathway of pteridines; the man locus mainly affects the xanthommatin pathway and the ifa, jew, lad and sb genes seem to affect both pteridine and xanthommatin pathways. The ches mutant, however, presents a chromatographic pattern very similar to the wild type; that is to say, the dark colour eyes of this mutant cannot be explained by its effect on a specific pathway.

References: Aparisi, M.L. & C. Najera 1987, DIS 66:12-13; Ferre, J., F.J. Silva, M.D. Real & J.L. Mensua 1986, Biochem. Genet. 24:545; Hadorn, E. & H.K. Mitchell 1951, PNAS 37:650; Najera, C. & J.L. Mensua 1988, Genet. Sel. Evol. 20(1):25; Narayanan, Y. & J.A. Weir 1964, Genetics 50:387; Taira, T. 1961, Jap. J. Genet. 36:18; Wiley, K. & H.S. Forrest 1981, Biochem. Genet. 19:1211; Wilson, T.G. & K.B. Jacobson 1977, Biochem. Genet. 15:307.

Árnason, Einar. University of Iceland, Reykjavik, Iceland. ESTERASE-5 in **Drosophila pseudoobscura** is transferred from males to females during copulation.

The X-linked locus Esterase-5 (Est-5) in **Drosophila pseudoobscura** is one of the most polymorphic loci known in any Drosophila species with a large number of alleles and some in high frequency in natural populations (Coyne, Felton & Lewontin 1978; Keith 1983). The adaptive significance of this variation has been the focus of interest in a number of studies (e.g., Prakash, Lewontin & Hubby 1969; Yamazaki 1971; Árnason 1982; Keith 1983).

The Est-5 locus (map position 111.8 on the right arm of the X chromosome; Beckenbach 1981) encodes the ESTERASE-5 carboxyl esterase (EST-5, EC 3.1.1.1.). The locus is apparently homologous to the Est-6 locus of **D.melanogaster** (Abraham & Luchessi 1974). In **D.melanogaster** EST-6 is a component of the seminal fluid and the enzyme is transferred to the female within 2 to 3 minutes from the start of copulation (Richmond et al. 1980; Richmond & Senior 1981). The enzymes may have a role in reproduction in the hydrolysis of a pheromone cis-vaccenyl acetate (Mane et al. 1983), in sperm use (Gilbert et al. 1981; Costa, Rigoni & Schiavon 1987), or nutrition for females (Bownes & Partridge 1987).

Due to the homology between Est-5 and Est-6 and the evolutionary interest of the Est-5 locus, it is worthwhile to inquire the possible role of EST-5 in reproduction. This paper reports the finding that EST-5 is apparently transferred from males to females during copulation.

The genetic strains used were obtained from the laboratory of R.C. Lewontin, Harvard University. They are a null strain (FC51) which shows no EST-5 activity, a 104 allele strain (SCM20), a 106 allele strain (CN28), and a 112 allele (Allozyme Standard 112) (Coyne, Felton & Lewontin 1978; Arnason & Chambers 1987).

Homozygous null virgins were collected and aged for 3 days. They were then pair mated with males from one of the strains carrying an active enzyme. After completion of copulation, which took place very quickly, the individuals were frozen until they could be electrophoresed.

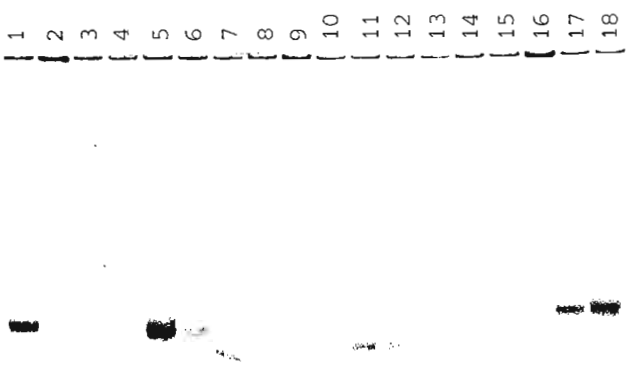


Figure 1. Results of electrophoresis of null females and their mates. Males of three genotypes encoding an active ESTERASE-5 copulated with homozygous null/null females which show no ESTERASE-5 activity. Subsequent to copulation activity is detected in some females. From left to right: **lane 1 and 6:** one 104/Y male each; **lane 2:** two null females which copulated with males of lanes 1 and 6; **lane 3:** a null female unmated control; **lane 4:** null female which copulated with male of lane 5; **lane 5:** one 104/Y male; **lane 7 and 12:** one 112/Y male each of which copulated with one of two females run in lane 8; **lane 8:** two null females which copulated with males run in lanes 7 and 12; **lane 9:** a null female unmated control; **lane 10:** null female which copulated with male run in lane 11; **lane 11:** one 112/Y male; **lane 13:** one 106/Y male; **lane 14:** null female which copulated with male run in lane 13; **lane 15:** a null female unmated control; **lane 16:** two null females which copulated with males run in lanes 17 and 18; **lanes 17 and 18:** one 106/Y male each.

Electrophoresis followed the methods of Keith (1983) and Arnason & Chambers (1987). The results are shown in Figure 1. The results are presented from left to right in 3 groups of 6 lanes, with the first group giving the 104 allele, the second the 112 allele and the third the 106 allele. The order of flies in the various lanes of the gel is given in the legend to the figure.

Males always showed an active enzyme. Lane 16 containing extract from two females which had copulated with the 106 males run in lanes 17 and 18, showed a faint EST-5 band with mobility identical to that of the males. This band shows up clearly in the photograph. Very faint bands could be observed in the gel in lanes 2, 8 and 14 but no traces were seen in lanes 4 and 10. None of this is evident in the photograph of the gel, however. The null control female, the third lane in each group (lanes 3, 9 and 15) never showed any EST-5 activity.

In conclusion, the EST-5 is transferred from males to females during copulation. There may be differences between the allozymes in the rate of transfer or activity in the female.

References: Abraham,I. & J.C. Luchessi 1974, Genetics 78:1119-1120; Arnason, E. 1982, Hereditas 96:13-27; ——— & G.K. Chambers 1987, Biochem. Genet. 25:287-307; Beckenbach, A.T. 1981, DIS 56:23-24; Bownes, M. & L. Partridge 1987, Acta X European Dros. Conf., Barcelona; Costa, R., F. Rigoni & L. Schiavon 1987, Acta X European Dros. Conf., Barcelona; Coyne, J.A., A.A. Felton & R.C. Lewonton 1978, PNAS USA 75:5090-5093; Gilbert, D.G., R.C. Richmond & K.B. Sheehan 1981, Evolution 35:21-37; Mane, S.D., L. Tompkins & R.C. Richmond 1983, Science 222:419-421; Prakash, S., R.C. Lewontin & J.L. Hubby 1969, Genetics 61:841-858; Richmond, R.C., D.G. Gilbert, K.B. Sheehan, M.H. Gromko & F.M. Butterworth 1980, Science 207:1483-1485; ——— & A. Senior 1981, J. Insect. Physiol. 27:849-853; Yamazaki, T. 1971, Genetics 67:579-603.

Asada, N. Okayama University of Science, Okayama, Japan. Genetic variation in reaction mass formation in Drosophila.

Reaction mass formation with the insemination reaction in Drosophila was first reported by Patterson (1946). In many Drosophila, various intensities of reaction mass formation was observed (Patterson 1946, 1947; Wheeler 1947; Takanashi 1983). Reaction mass formation seemed to play important roles in the post mating isolations caused by inhibition of fertilization and egg production.

Two related species of **Drosophila nasuta** and **D.pallidifrons**, belonging to the **D.nasuta** subgroup, **immigrans** species group showed pronounced reaction mass in both intra- and interspecific crosses. Genetic variations in **D.nasuta** males and **D.pallidifrons** females were analyzed. Effect of male nucleus and the association between duration of copulation and reaction mass formation by cross experimentation were also studied.

Intraspecific crosses among **D.nasuta** and interspecific crosses between **D.pallidifrons** females and **D.nasuta** males were made in order to clarify the genetic variations in **D.pallidifrons** females in ability of reaction mass formation. All flies were iso-female strains caught in nature and localities of **D.nasuta** and **D.pallidifrons** were Mahé, Seychelles and Ponape, Caroline Islands, respectively. No cross of **D.nasuta** females and **D.pallidifrons** males was made because no copulation had occurred (Asada & Kitagawa 1982).

Twelve strains of **D.pallidifrons** females were examined as to reaction mass formation in crosses to **D.nasuta** (SEZ-2, Seychelles strain) males. The SEZ-2 strain of **D.nasuta** males was the tester strain in the cross experiment because no significant differences among three strains of **D.nasuta** males (ADM-1, Andaman Islands, India; MYS-23, Mysore, India, and SEZ-2) were observed in reaction mass formation with the average ± standard error of 88.3±1.7% ranging from 85.0 to 90.0% and a X^2 value of 1.14 (df=2). Percent of reaction mass formation, given in Table 1, in the parental crosses showed various values with the average ± standard error of 79.7±3.3% ranging from 52.0 (PNI-74 strain as "low strain") to 94.0% (PNI-110 strain as "high strain") and heterogeneity X^2-value of 98.73 (df=11, $p<0.001$) showed a high significance among iso-female strains of **D.pallidifrons** females. Genetic variations in the ability of the reaction mass formation was evident in **D.pallidifrons** females which are distributing even in the same locality of Ponape, Caroline Islands. Genetic control for that phenomenon was suggested simultaneously. Females of two strains of **D.pallidifrons**, PNI-74 and PNI-110, screened by the above-mentioned experiment were presented to reciprocal crosses. As was shown in Table 2, the female's nucleus was substituted by the male's as followed by subsequent back-crosses. Percent of reaction mass formation of PNI-74 strain female (initially "low strain") turned significantly from 52.0% for parental cross to 84.0% for BC2 (back-crossed the second generation) with prolongation of duration of copulation from 9 min and 37 sec to 11 min and 49 sec. To the contrary, in reciprocal crosses, the percent of PNI-110 strain (initially "high strain") did highly significantly drop from 94.0% for parental cross to 28.0% for BC2 generation with shortening of duration of copulation from 14 min and 32 sec to 10 min and 3 sec. These results showed that, first, the reaction mass formation was controlled by multigenic systems involved in the male's nucleus, and second, the duration of copulation played important roles on the ability of reaction mass formation against the suggestion by Grant (1983). This result was co-confirmation of our past study that the threshold for quantity of secretions from the male's genital gland was necessary for reaction mass formation.

Table 1. Interstrain variations of **D.pallidifrons** females in reaction mass formation mated with **D.nasuta** males.

Strain	No. of mating pairs	No. of reaction mass	%R.M.
PNI-14	90	79	87.8
16	54	43	79.6
20	50	41	82.0
25	51	45	88.2
33	55	41	74.5
41	77	66	85.7
61	64	41	64.1
69	72	64	88.9
74	98	51	52.0
94	58	52	89.7
106	51	40	78.4
110	50	47	94.0
Average ± SE			79.7±3.3

R.M.=reaction mass; SE=std. error.

Table 2. Cross experiments and copulation duration of two strains of **D.pallidifrons**.

Cross type: Female **pallidifrons**	Male **nasuta**	n	%R.M.	Duration of copulation average ± SE
PNI-74	SEZ-2	50	52.0	9'37""±0'57""
PNI-74 x PNI-110 > F1	SEZ-2	50	44.0	-
F1 x PNI-110 > BC1	SEZ-2	50	68.0	-
BC1 x PNI-110 > BC2	SEZ-2	50	84.0**	11'49""±1'12""
PNI-110	SEZ-2	50	94.0	14'32""±1'15""
PNI-110 x PNI-74 > F1	SEZ-2	50	86.0	-
F1 x PNI-74 > BC1	SEZ-2	50	64.0***	-
BC1 x PNI-74 > BC2	SEZ-2	50	28.0***	10'03""±1'04""**

R.M. = reaction mass; ** = $p < 0.01$; *** = $p < 0.001$

Acknowledgement: I wish to express many thanks for invaluable constructive criticism to Prof. Emer. S. Ohba and Prof. O. Kitagawa, Tokyo Metropolitan University. I also wish to express my hearty thanks for reading the manuscript to Dr. D.M. Kimble, Okayama University of Science.

References: Asada, N. & O. Kitagawa 1982, Jpn. J. Genet. 57:658-659; Grant, B. 1983, Evolution 37:854-856; Patterson, J.T. 1946, PNAS 32:202-208; ——— 1947, Univ. Texas Publ. 4720:42-77; Takanashi, E. 1983, Jpn. J. Genet. 58:463-485; Wheeler, M.R. 1947, Univ. Texas Publ. 4720:78-115.

Balanya, J., C. Segarra and A. Prevosti.
Universidad de Barcelona, Spain.

A U chromosome duplication found in colonizing populations of **D.subobscura.**

During a study about the chromosomal polymorphism in **D.subobscura** colonizing populations of both North and South America, a duplication involving the 45 ABCDE region of the U chromosome was detected twice. The duplicated segment is not located in tandem, but reversed and inserted between the 39D and 40A subregions of a standard U chromosome.

This duplication had already been described by Götz (1965) in one individual captured near Zürich. He also pointed out that the duplication can be produced by crossover from a heterozygous U_{st}/U_{1+2+8} female in the segment comprised between the 40 and 44 regions both included. There are no more references of this duplication in the literature. Thus, it might be considered a rare sporadic rearrangement that could be detected by Götz due to the large sample examined.

For that reason, it is rather surprising that two heterozygous individuals for this duplication were detected in colonizing populations. It is even more surprising, if we consider that these two individuals were captured in two quite distant American localities: Viña del Mar (Chile) and Centralia (Washington).

A possible explanation for the presence of this duplication in the colonized area is the assumption that it was introduced in the New World by the founder individuals. However, this is most unlikely bearing in mind that this duplication is extremely uncommon in autochthonous populations. Then, it seems more reasonable to suppose that the duplication was originated during the colonization period by crossing over. In order to corroborate this idea, we have compiled and compared the frequencies of

Table 1. Expected frequencies of U_{st}/U_{1+2+8} individuals in European and American populations assuming Hardy-Weinberg distribution of genotypes. Only those European populations with expected frequencies higher than 0.05 are included.

Population	Expected frequency U_{st}/U_{1+2+8}	Reference
EUROPE		
Gröningen	0.071	4
Louvain	0.125	*
Tübingen	0.075	5
Zürich	0.053	1
Villars	0.096	*
Lagrasse	0.058	6
Montpellier	0.083	7
Bilbao	0.051	2
Eivissa	0.053	2
Menorca	0.072	7
SOUTH AMERICA		
Viña del Mar	0.179	8
Santiago de Chile	0.148	8
Chillán	0.181	8
Laja	0.196	8
Valdivia	0.170	8
Bariloche	0.176	8
Puerto Montt	0.198	8
Castro	0.133	8
Coyhaique	0.220	8
NORTH AMERICA		
Arlington	0.174	9
Centralia	0.160	9
Woodburn	0.196	9
Medford	0.221	9
Eureka	0.177	9
Davis	0.204	9
Gilroy	0.188	9

* = Prevosti (unpublished).

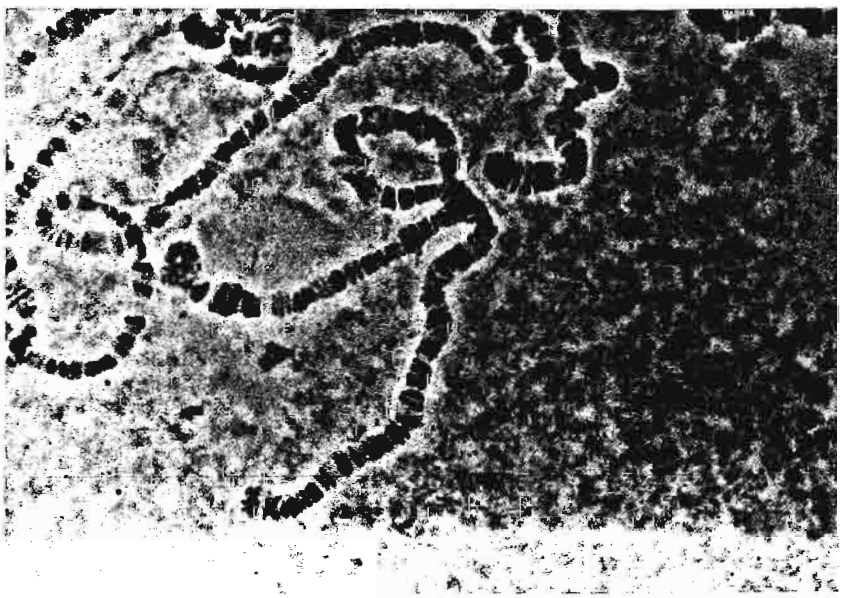


Figure 1. U chromosome of a U_d/U_d individual showing the pairing of the normal and duplicated 45 regions.

U_{st}/U_{1+2+8} individuals in both European and American populations (Table 1). As can be seen, the expected frequencies of U_{st}/U_{1+2+8} heterozygotes range from 0.133 to 0.220 with 0.183 as mean value in colonizing populations, while U_{st}/U_{1+2+8} frequencies above 0.05 are reached only in ten of the numerous analyzed Palearctic populations which always show lower frequencies than those found in the colonized area. These results seem to support that it would be more likely to detect the crossing over products carrying the U duplication in American than in European populations on account of the higher U_{st}/U_{1+2+8} heterozygote frequencies in the former than in the latter. It will be very interesting to follow how the U duplication frequencies change in the near future.

A homozygous strain for this U chromosome duplication has been obtained starting from the U_{1+2+8}/U_{σ} male captured in Viña del Mar (Chile). The homozygous individuals are fully viable and do not display any detectable phenotypic abnormality. In their polytene nuclei it can be seen, almost without exception, that the normal and the duplicated 45 regions, of the U chromosome, pair each other properly (Figure 1).

Acknowledgement: This work was supported by grant No. 2844 from the Comisión Asesora para la Investigación Científica y Técnica, Spain, and grant No. CCB-8504013 from the U.S.-Spain Joint Committee for Scientific and Technological Cooperation.

References: **1.** Burla, H. & G. Götz 1965, Genetica 36:83-104; **2.** Frutos, R. de 1972, Genet. Iber. 24:123-140; **3.** Götz, W.Z. 1965, Vererbungsl. 96:285-296; **4.** Krimbas, C.B. 1964, Z. Vererbungsl. 95:125-128; **5.** Pinsker, W. et al. 1978, Genetica 48:207-217; **6.** Prevosti, A. 1964, Genet. Iber. 16:1-19; **7.** ——— et al. 1984, Genet. Sel. Evol. 16:143-156; **8.** ——— 1985, Evolution 39:838-844; **9.** ——— 1988, PNAS USA (in press).

Banerjee, S.K., S. Banerjee and A.S. Mukherjee.
University of Calcutta, India. Silver nitrate stained non-ribosomal cistrons in **Drosophila hydei** polytene chromosome.

Figure 1. Silver stained polytene chromosome of **Drosophila hydei** showing three different silver positive sites. Arrow indicates the silver positive site in the chromosome.

Goodpasture & Bloom (1975) first described that Nucleolus Organizer Regions (NORs), which contain ribosomal cistrons (18s + 28s), can be visualized on metaphase chromosomes with the selective silver staining method. This technique is now routinely employed in different systems from fish to mammal to localize the NORs (Tantravahi et al. 1976; Hubbell & Hsu 1977; Buys et al. 1979; Hartung et al. 1979; Heneen 1979; Cheng et al. 1981; Schmid et al. 1982).

It has been established that silver staining procedure revealed nucleolar acidic proteins (Schwarzacher et al. 1978; Olert et al. 1979; Buys & Osinga 1980) which are located on the NOR sites (Hsu et al. 1975). These nucleolar proteins have been reported to be either the proteins C23 and B23 (Lischwe et al. 1979) or one specific protein (Hubbell et al. 1979) or a large sub-unit of the RNA polymerase I (Williams et al. 1982). Recently, Hernandez-Verdun et al. (1978, 1980, 1982) reported a nucleolar component by electron microscopy in which Ag-NOR proteins are located. Furthermore, it has also been found that these Ag-NOR specific proteins are to be related to NOR transcriptional activities (Miller et al. 1976a, b; Hofgartner et al. 1979; Hubbell et al. 1980).

We have employed the silver staining technique on **Drosophila hydei** polytene chromosomes to test the specificity of the technique in insect system. Polytene chromosomes were prepared using standard method. After preparing the chromosomes, slides were stained overnight in 50% silver nitrate aqueous soluton at 65°C in a moist chamber. Stained chromosomes were examined and photographed with a Zeiss photomicroscope. Figure 1 shows Ag-positive sites in the polytene chromosomes of **Drosophila hydei.** More than three sites in different chromosomes are silver positive. These three sites do not bear ribosomal cistrons. It is known that ribosomal cistrons in **Drosophila hydei** are located only on the X and Y chromosomes (Hennig et al. 1975).

The data presented here clearly demonstrate that besides impregnation of NOR, silver nitrate also stained other sites in the polytene chromosomes of **D.hydei,** which do not transcribe r-RNA. The result suggests that the silver stained protein(s) of the non-ribosomal sites of the polytene chromosomes of **D.hydei** are perhaps structurally similar to Ag-NOR proteins. However, the reason for this possible similarity is not yet clear.

References: Buys, C.H.C.M., J. Osinga & G.J.P.A. Anders 1979, Mech. Ageing 11:55-75; ——— & J. Osinga 1980, Chromosoma (Berl) 77:1-11; Cheng, D.M., T.E. Denton, S.L. Liem & C.L. Elliot 1981, Clinical Genet. 19:145-148; Goodpasture, C. & S.E. Bloom 1975, Chromosoma (Berl) 53:37-50; Hartung, M., C. Mirre & A. Stahl 1979, Hum. Genet.

2:295-308; Heneen, W.K. 1979, Genetica 50:31-39; Hennig, W., B. Link & O. Lebheini 1975, Chromosoma (Berl) 51:57-63; Hernandez-Verdun, D., J. Hubert, C.A. Bourgeois & M. Boutelle 1978, C.R. Acad. Sci. Paris 287:1421-1423; ———, ———, ——— & ——— 1980, Chromosoma (Berl) 79:349-362; ———, M. Derenzini & M. Bouteille 1982, Chromosoma (Berl) 35:461-473; Hofgartner, F.J., W. Krone & K. Jain 1979, Genet. 47:329-333; Hsu, T.C., S.E. Spirito & M.L. Pardue 1975, Chromosoma (Berl) 53:25-36; Hubbell, H.R. & T.C. Hsu 1977, Cytogenet. Cell Genet. 19:185-196; ———, L. Yun-Fai, R.L. Brown & T.C. Hsu 1980, Exp. Cell Res. 129:139-147; Lischwe, M.A., K. Smetana, M.O.J. Olson & H. Busch 1979, Life Sciences 25:701-708; Miller, O.J., D.A. Miller, V.G. Dev, R. Tantravahi & C.M. Croce 1976a, PNAS 73:4531-4535; Miller, D.A., V.G. Dev, R. Tanteravahi & O.J. Miller 1976b, Exp. Cell Res. 101:235-243; Olert, J., G. Sawatzki, H. Kling & J. Gebauer 1979, Histochemistry 60:91-99; Schmid, M., C. Loser, J. Schmidtke & W. Engel 1982, Chromosoma (Berl) 86:149; Schwarzacher, H.G., A.-V. Mikelsaar & W.J. Schnedl 1978, Cytogenet. Cell Genet. 20:24-39; Tantravahi, R., D.A. Miller, V.G. Dev & O.J. Miller 1976, Chromosoma (Berl) 56:15-27; Williams, M.A., J.A. Kleinschmidt, G. Krohne & W.W. Franke 1982, Exp. Cell Res. 37:341-351.

Barrio, E. and A. Latorre. University of Valencia, Spain. Conservation of the alternate Guanine-Cytosine sequences in the mitochondrial DNA of species belonging to the **Drosophila obscura** group.

The phylogenetic relationship among several Drosophila species that belong to the **Drosophila obscura** group (**obscura** and **affinis** subgroups) were studied using restriction analysis of mitochondrial DNAs (mtDNA) (Latorre et al. 1986, 1988). The species studied were **D.subobscura, D.obscura** and **D.ambigua**, three palaearctic species of the **obscura** subgroup; **D.pseudoobscura, D.persimilis** and **D.miranda**, three nearctic members of the **obscura** subgroup, and **D.azteca**, a nearctic species of the **affinis** subgroup. Ten different restriction enzymes were used to digest the mtDNA of the 39 isofemale lines studied, six of which recognize a sequence of six base pairs and the others, four base pairs.

All of the enzymes used in this study, that recognize four base pairs, cleave the DNA in a sequence formed by two Guanines and two Cytosines in different combinations: HaeIII, HhaI, HpaII and ThaI. Assuming a random sequence with a composition of Adenine of 32.3%, Thymine of 44.4%, Guanine of 12.2% and Cytosine of 11.1% (Clary & Wolstenholme 1985) and a standard size of 16.5Kb for the mtDNA of these species, the number of expected fragments is three for each of the enzymes. However, as it can be seen in the Table, the enzyme that recognizes the alternate composition Guanine-Cytosine (HhaI) gives two fragments in all the species and the enzyme that recognizes the alternate sequence Cytosine-Guanine (ThaI) gives one or two fragments. On the other hand, HaeIII gives from 1 to 5 fragments (3 on average) and HpaII from 3 to 7 (5 on average).

Undoubtedly, the most interesting result is the observed variability in the total number of restriction sites for all the species studied. Thus, HhaI and ThaI recognize one or two different sites and HaeIII and HpaII sixteen and twenty-three, respectively. This is so because the sites recognized by HhaI and ThaI are monomorphic, or practically so. HhaI gives a monomorphic pattern for all the species and ThaI gives two different patterns, one for all the isolines of **D.subobscura** and the other for the rest of the species. A high number of restriction patterns was obtained with the other two enzymes, giving rise to a high polymorphism; thus, HaeIII yields seven different patterns and HpaII ten.

Table 1. Characteristics of the mtDNA restriction analysis of seven species of the **Drosophila obscura** group with the restriction endonucleases HaeIII, HpaII, HhaI and ThaI.

Enzyme	Sequence*	Total Nr. Restriction Sites	Total Nr. of patterns	Restriction pattern	Sites per pattern	Species	Isofemale lines
HaeIII	GG/CC	16	7	A	2	**D.subobscura**	18
				B	3	**D.subobscura**	2
				C	1	**D.subobscura**	12
				C	1	**D.miranda**	1
				C	1	**D.azteca**	1
				D	2	**D.pseudoobscura**	1
				D	2	**D.persimilis**	1
				E	3	**D.pseudoobscura**	1
				F	4	**D.ambigua**	1
				G	5	**D.obscura**	1
HpaII	C/CGG	23	10	A	6	**D.subobscura**	30
				B	5	**D.subobscura**	1
				C	7	**D.subobscura**	1
				D	6	**D.pseudoobscura**	1
				E	5	**D.pseudoobscura**	1
				F	4	**D.persimilis**	1
				G	4	**D.miranda**	1
				H	3	**D.ambigua**	1
				I	3	**D.azteca**	1
				J	6	**D.obscura**	1
HhaI	GCG/C	2	1	A	2	All the species	39
ThaI	CG/CG	3	2	A	2	**D.subobscura**	32
				B	1	the other 6 species	7

* indicates the cleavage site.

The very strictly constrained variability of the HhaI and ThaI recognition sequences could be due to an important functional role of the sequences that alternate Guanine-Cytosine (or "vice-versa"). If this was so, these sequences would be conserved throughout the evoluton of Drosophila.

References: Clary, D.O. & R. Wolstenholme 1985, J. Mol. Evol. 22:252-271; Latorre, A., A. Moya & F.J. Ayala 1986, PNAS USA 83:8649-8653; ———, E. Barrio, A. Moya & F.J. Ayala 1988, Mol. Biol. Evol. (in press).

Bijlsma, R. University of Groningen, Haren, Netherlands. A preliminary note on the genetic structure of a **D.melanogaster** population from Death Valley (California).

Based on the observation of high rates of allelism of lethal chromosomes and annual changes in this rate in a **D.pseudoobscura** population at Furnace Creek (Death Valley, California), Bryant (1976) and Jones et al. (1981) argue that this population yearly goes through a severe bottleneck due to the extremely high summer temperature at this place (mean temperature in August is 44°C). Other Drosophila species also might be affected by this extreme environmental condition. In this paper, preliminary evidence is presented that this is the case for the **D.melanogaster** population present at this site.

In April 1980, a great number of **D.melanogaster** files were collected at the Furnace Creek date grove and over 150 isofemale lines were established. In the same month a **D.melanogaster** sample collected at an orange grove near Riverside (California) was obtained and from this population also a great number of isofemale lines were started. In December 1980, a sample of 94 Death Valley (DV) isofemale lines and 100 Riverside (RS) isofemale lines were taken to the Dept. of Genetics at Groningen and kept as vial populations at 25°C on normal food medium consisting of 1000 ml water, 54 g sucrose, 32 g dead yeast, 19 g agar and 13 ml nipagin solution (10 g nipagin in 100 ml ethanol 96%). Flies were transferred to new vials every 14 days. This has been done for 7 years and four times during this period the number of extinct lines was counted. The results are shown in Table 1. It is clear that the DV population shows a 2-3 times higher extinction rate than the RS population. This difference, when tested with a contingency chi-square, is highly significant for any given period. The extinction rate of the RS population was comparable to the one observed for a population collected at a local fruit market at Groningen in September 1982. Isofemale lines orginating from this population and maintained in the same way as those from the other populations, showed an extinction rate of 7% and 23% after 43 and 67 months, respectively. These rates are not significantly different from the rates observed for the RS population after 40 and 64 months. This indicates that the DV population is exceptional and has a relatively high extinction rate, suggesting a lower fitness of this population. This observation is in accordance with the idea that the DV population has gone through a severe bottleneck, causing a reduction in fitness of the population by the subsequent inbreeding. On the other hand, in an attempt to extract lines simultaneously isogenic for the 2nd and 3rd chromosome from the isofemale lines, it was observed that for the RS population in roughly 50% of the cases a lethal or semilethal was present for each 2nd and 3rd chromosome combination (unpubl. results), a frequency which seems normal for **D.melanogaster** (see e.g., Paik & Sung 1969). For the DV population the attempt to extract isogenic lines failed almost completely and the frequency of lethal and semilethal chromosome combinations was estimated to exceed 80% (Bundgaard, pers. comm.). This finding seems to contradict the assumption that the DV population has been subject to inbreeding, as it is generally accepted that, on the average, inbred populations show a lower frequency of lethals than outbred populations. It is possible, however, that the high frequency of lethals observed in the DV population is in fact due to a few lethals with a high frequency in the population. These lethals could have increased in frequency through genetic drift at the time of the bottleneck and thereafter remained at a high frequency either by the lack of selection pressure in the expanding population or by the heterotic properties of the lethals (see e.g., Ives 1970). This is in agreement with the results found by Bryant (1976) for **D.pseudoobscura**, who also observed an exceptional high allelism rate at Furnace Creek. Although the amount of data is still limited, the results so far indicate that the extreme environmental conditions at Furnace Creek significantly influence the genetic structure of natural Drosophila populations present at that site.

Table 1. Cumulative percentage of extinct isofemale lines together with the chi-square values that evaluate the difference between the Death Valley and the Riverside population.

months after establishment	DV lines (n=94)	RS lines (n=100)	Contingency chi-square	P
40	29.8	13.0	8.2	<0.005
64	34.0	16.0	8.5	<0.005
76	51.1	17.0	25.2	<0.001
88	60.6	23.0	28.3	<0.001

References: Bryant, S.H. 1976, Genetics 84:777-786; Ives, P.T. 1970, Evolution 24:507-518; Jones, J.S., S.H. Bryant, R.C. Lewontin, J.A. Moore & T. Prout 1981, Genetics 98:157-178; Paik, Y.K. & K.C. Sung 1969, Jpn. J. Genet. 44(suppl.1):180-192.

Birchler, J.A. Harvard University, Cambridge, Massachusetts USA. An inverse regulator of the Notch locus.

In the course of experiments for other purposes, the insertional translocation, T(Y;2)G44/SM1(44C-50B) was crossed as males to a stock containing the split, spl, allele of the Notch locus. The sons of this mating either have straight wings or Curly. The straight flies inherit a normal chromosome two from their mothers and the balanced T(Y;2)G44. The Curly flies receive a normal chromosome two from their mothers and the SM1 balancer plus the Y with the 44C to 50B insertion, which are therefore trisomic for this segment of chromosome two. In the above stated cross, it was found that the trisomic enhanced the mutant effect of split. To investigate this further, three other recessive alleles were tested in the same way. These alleles were fa, fa^{no}, and nd. Of these, fa and nd are enhanced in the mutant phenotype but fa^{no} is slightly suppressed. Finally, the Dp(1:1) Co, Confluens, which is a duplication of the Notch locus, was tested. In this case, the Confluens phenotype was suppressed. It is hypothesized that a locus resides within 44C to 50B that inversely influences the expression of the Notch locus. If the trisomic reduces the amount of functional product, by whatever means, then the phenotypes of the recessive alleles would be reduced further and create a more severe effect. The duplication phenotype would be suppressed, because the level of functional product would be returned to nearer the normal diploid level.

Chambers, G.K., S.L. Davies, M. Hodgetts, R.H. Moore and I.J. Pomer. Victoria University, Wellington, New Zealand. Drosophila species from the Wellington and Wairarapa districts of New Zealand.

The Drosophilid fauna of New Zealand has received very little attention. The most recent collections, made over thirty years ago (see Harrison 1959), suggest that New Zealand is depauperate in endemic Drosophila species compared to Australia (see Bock & Parsons 1981). In late 1985, we initiated a series of field collections intended (1) to provide baseline census information on Drosophila species distribution and (2) to collect large numbers of local **D.melanogaster** to test predictions of allele frequencies at allozyme loci from the studies of Oakeshott et al. (1982) in Australasia.

Our earliest collections from the KAR and BRO sites (see Table 1) indicated **D.melanogaster** was rather scarce around Wellington and we extended our coverage to include the vineyards and orchards of the Wairarapa district. Our collection sites are listed in Table 1. Up to 200 flies were taken on each visit to each site using an aspirator. They were brought back to the laboratory for identification and scoring. Flies belonging to the **melanogaster** subgroup were generally frozen and examined by electrophoresis for Adh (after Oakeshott et al. 1982 but using Helen Labs Titan III cellulose acetate sheets). This locus is fully diagnostic for this subgroup (see David et al. 1980).

The species collected and the dates of collections are given in Table 2 for Wellington and Table 3 for Wairarapa.

The later collections from PAU and UHU confirm that **D.melanogaster** are rather scarce in spring to midsummer around Wellington (see Table 2). However, **melanogaster** subgroup species can be relatively abundant at the Wairarapa sites. Interestingly some sites show rather marked differences: compare TAT which gave predominantly **D.melanogaster** and ATRA which gave predominantly **D.simulans** (see Table 3). These two sites are separated by no more than 20 km.

Overall we collected only those common cosmopolitan Drosophila species previously catalogued by Harrison (1959). One exception was the capture of several **D.pseudoobscura** specimens in the BRO and KAR collections. This report, therefore, represents an extension of the geographical range of **D.pseudoobscura**. Specimens have previously been reported from the Rotorua and East Cape districts of New Zealand (Lambert & McLea 1983; Parsons 1982). Based on karyotypic evidence this species was postulated to have become established following the recent introduction of individuals from the West Coast of N. America (D.Lambert, pers. comm.). Their discovery in Wellington only 2 years later suggests that either the species had become more widespread than previously thought or that they are colonizing the North Island quite rapidly (the distance from Rotorua to Wellington is 460 km).

In our Wellington collection **D.immigrans** or **D.hydei** were the most numerous species depending on collection site (BRO and KAR vs PUA and UHU: Table 2). **D.immigrans** was also abundant in Wairarapa collections but was sometimes outnumbered by **D.melanogaster** or **D.simulans** (e.g., ATRA and TAT, respectively: Table 3). **D.hydei** and **D.busckii** were comparatively rare in the Wairarapa district compared with Wellington and **D.pseudoobscura** was altogether absent. Despite those apparent general trends the most striking feature of these preliminary studies is the marked heterogeneity in species composition

Table 1. Drosophila Collection Sites

Wellington District:
BRO - Brooklyn: compost heap in suburban garden
KAR - Karori: permanent fruit bait in surburban garden
PAU - Pauatahanui: rubbish bin in market garden
UHU - Upper Hutt: fruit bait in suburban garden

Wairarapa District:
ATR - Ata Rangi Vineyard: Site A tomato dump
: Site B mixed fruit scrap bucket
CAR - Carmel: fruit shop
MAV - Martinborough Vineyard:
Site A on the 'mark' (rotting grape skins)
Site B inside the winery
TAT - Tates orchard: apples in rubbish bin

Table 2. Numbers and proportions of Drosophila species collected at Wellington sites.

Species	Site and Collection Date BRO 12/85	KAR 12/85	PAU 1/86	UHU 1/86
D.immigrans	0.94	0.69	0.02	0.04
D.melanogaster] **D.simulans**	-	0.07*	0.05	-
D.hydei	-	0.10	0.91	0.78
D.busckii	-	0.11	-	0.17
D.pseudoobscura	0.06	0.03	-	-
Total #	64	261	43	23

* No **D.simulans** males present. later collections show **D.simulans** < 1% total **D.melanogaster** and **D.simulans** at KAR 1985-87 (Chambers, unpubl.).

Table 3. Numbers and proportions of Drosophila species collected at Wairarapa sites.

Species	ATRA 3/86	ATRA 4/86*	CAR 3/86	MAVA 4/86*	TAT 3/86	TAT 4/86
D.immigrans	0.37	0.60	0.09	0.42	0.03	-
D.melanogaster] **D.simulans**	0.63[1]	0.36[1]	0.85[2]	0.51[3]	0.95[4]	0.98[4]
D.hydei	-	-	0.05	0.03	-	-
D.busckii	-	0.04	0.01	0.04	0.02	0.02
Total	117	260	114	80	102	42

* Pooled data for two collections 8/4 and 18/4/86.
1=mean proportion of **D.simulans** was 0.92 (total examined: 58 individuals). 2=mean porportion of **D.mel.** was 0.95 (total examined: 58 individuals). 3=no electrophoretic data. 4=mean proportion of **D.mel.** was 0.99 (total examined: 123 individuals).

between the sites. These observations may be due to the nature and/or location of the sites, to differences in dates of collections or to purely chance events.

To resolve these issues, we have since initiated a longitudinal study of the KAR site and repeat sampling at other sites which we will report later (Chambers & Moore, unpubl.). In the meantime we have established wild type reference lines of all local species including **D.pseudoobscura** from the KAR collections.

Cellulose acetate electrophoresis experiments indicated that Adh^F allele frequencies at CAR, ATRB and TAT were almost identical (mean frequency = 0.84 based on a pooled sample of 181 individuals). This is consistent with an extrapolation of the Southern hemisphere latitudinal cline in Adh allele frequencies reported by Oakeshott et al. (1982). This result supports the idea that these clines are maintained by natural selection in the face of considerable migration facilitated by human traffic (e.g., the accidental introduction of **D.pseudoobscura** to New Zealand). The mean allele frequency for α-$Gpdh^F$ at these sites was found to be 0.80 for a pooled sample size of 62 individuals. This is not as expected from the Oakeshott et al. (1982) data. Overall both of the results should be viewed as provisional until larger samples can be analysed.

One further interesting minor observation was made when a small sample (7 individuals) was examined from inside the Martinborough Winery (MAVB) and found to be fixed for the Adh^F allele. Thus one more datum is added to the Drosophila and wineries controversy (see Marks et al. 1980). We have little optimism about further studies of this type since all of the New Zealand wineries we have visited so far in the Wairarapa or Hawkes Bay districts seem to be exceptionally clean of flies.

We would like to point out that analysis is of Drosophila species collections including diagnostic electrophoresis makes an excellent 1 or 2 session practical exercise, for undergraduate students. We presently use our methods reported here in teaching a third year entomology class.

References: Bock, I.R. & P.A. Parsons 1981, The Genetics and Biology of Drosophila (M. Ashburner et al., eds.), v3a:291-308, Academic Press, N.Y.; David, J.R., M. De Scheemaeker-Louis & E. Pla 1980, DIS 55:28-29; Harrison, R.A. 1959, Acalypterate diptera of New Zealand, NZ DSIR Bulletin 128, Whitcombe & Tombs, Christchurch, N.Z.; Lambert, D.M. & M.C. McLea 1983, DIS 59:72-73; Marks, R.W., J.G. Brittnacher, J.F. McDonald, T. Prout & F.J. Ayala 1980, Oecologia 47:141-144; Oakeshott, J.G., J.B. Gibson, P.R. Anderson, W.R. Knibb, D.G. Anderson & G.K. Chambers 1982, Evolution 36:86-96; Parsons, P.A. 1982, Evol. Biol. 14:297-350.

Christensen, A.C. and J.C. Lucchesi. Thomas Jefferson University, Philadelphia, and Univ. of North Carolina, Chapel Hill, USA. Failure of the Triplo-lethal locus of **Drosophila melanogaster** to interact with sex-determining genes.

It has been suggested that the Triplo-lethal locus (Tpl) of **Drosophila melanogaster** may play a role in establishing or assessing the X/A ratio in the organism and determining sex (Denell 1976; Lucchesi 1977). The reasoning is as follows: a locus that represents the autosomes in the X/A ratio would be extremely dosage sensitive, Tpl is the most dosage sensitive locus in the autosomes (Lindsley, Sandler et al. 1972); hence, Tpl may be involved. This model also makes specific predictions which we have tested.

The first prediction is that females with two X chromosomes and a duplication of Tpl (3 doses total) will assess their X/A ratio incorrectly as less than one and switch the Sxl gene to the male mode (see Cline 1984). This will turn on male developmental pathways, initiate dosage compensation, thereby hyperactivating both X chromosomes, and cause death due to gene product imbalances. The Sxl^M allele is characterized by a constitutive expression of Sxl in the female mode. Therefore, a fly with 2 X chromosomes, one of them carrying the dominant Sxl^M allele, and bearing three doses of Tpl should survive. A cross between y Hw Sxl^M sn B / Binsinscy females and $Y^SX.Y^L$, IN(1)EN, y ;; Dp(3R)Tpl / Df(3R)Tpl, y^+ y^+ males should produce such flies if the rescue occurs. No survivors were seen indicating that constitutive expression of Sxl will not rescue flies bearing three doses of Tpl.

The second prediction is that males with one dose of Tpl will incorrectly assess their X/A ratio as one and switch Sxl to the female mode. This will result in female sex differentiation and a failure to dosage compensate the X chromosome, resulting in death. The Sxl^f mutations are loss of function alleles that prevent the female mode from being expressed. In addition, the maternal effect of the daughterless mutation is to prevent Sxl from being expressed in the female mode (Cline 1984). The model thus predicts that males with only one dose of Tpl should be rescued either by an Sxl^f mutation on the X chromosome, or by their mothers being homozygous for the da mutation. Again, several crosses were done and no such rescue was seen in either case.

The lack of interaction between Tpl and the sex determining genes Sxl and da as well as the existence of triploid intersexes and triploid females bearing four doses of Tpl (Denell 1976) suggests that Tpl does not function in measuring the X/A ratio in Drosophila, but unfortunately does not suggest what it is that Tpl does do.

Acknowledgement: Thanks to Tom Cline for Sxl and da mutant stocks. Supported in part by NIH grants 1-F32-GM09143 and 1-R29-GM38483 to A.C.C.

References: Cline, T.W. 1984, Genetics 107:231-277; Denell, R.E. 1976, Genetics 84:193-210; Lindsley, D.L., L. Sandler et al. 1972, Genetics 71:157-184; Lucchesi, J.C. 1977, Am. Zool. 17:685-693.

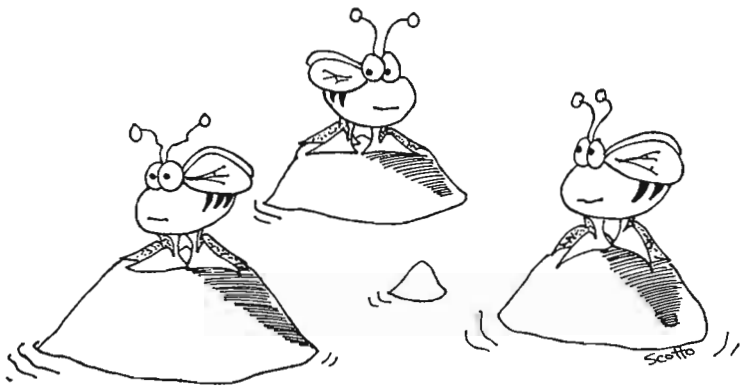


Table 1. Germ-line cell aneuploidy frequencies in females of **Drosophila melanogaster** treated with Nipagin.

Treat-ment[1]	Dose(M)	Treated females	ANEUPLOIDIES[2] TESTS														Total Aneuploidies[2]				Total tests
			Brood I				Brood II				Brood III				Brood I	Brood II	Brood III				
			(-)	%	(+)	%	(-)	%	(+)	%	(-)	%	(+)	%				(-)	%	(+)	%	
Control	--	328	10	0.261	5	0.131	17	0.420	16	0.395	27	0.270	33	0.330	3826	4049	9996	54	0.300	54	0.300	17871
Nipagin	6.5×10^{-2}	121	5	0.431	2	0.172	6	0.422	5	0.352	7	0.173	14	0.346	1160	1420	4050	18	0.270	21	0.320	6630
Nipagin	2.0×10^{-2}	326	7	0.222	25	0.794*	28	1.795*	48	3.077*	55	2.855*	43	2.233*	3150	1560	1926	90	1.360*	116	1.750*	6636

1=acute treatment: 6.5×10^{-2}M; Chronic treatment: 2.0×10^{-2}M. 2=(-) chromosome loss; (+) chromosome gain; Brood I=2 days; Brood II=3 days; Brood III=5 days. Statistical significance calculated by the conditional binomial test (Margolin et al. 1983); * = $p<0.0001$.

Costa, R., M. Zordan, A. Tavella and A.G. Levis. University of Padova, Italy.

Chronic exposure to Nipagin (p-hydroxy-benzoic acid methyl ester), a common additive of culture media, induces germ-cell aneuploidy in **Drosophila melanogaster.**

Nipagin, also known as Tegosept M (p-hydroxy-benzoic acid methyl ester), is a common additive of Drosophila culture media used to inhibit fungal growth (Christie 1935; Lakovaara 1969; Shorrocks 1971; Roberts 1986). No information concerning the possible genotoxic activity of Nipagin was available to us. Nipagin has been reported as able to increase both the time of emergence of Drosophila cultures as well as the frequency of abnormal-abdomen phenocopies (developmental abnormalities ?) (Wette 1954).

We evaluated the capacity of Nipagin to induce chromosomal malsegregation in female germ-line cells of **Drosophila melanogaster** using the Free Inverted X chromosome (FIX) system (Zimmering et al. 1986) to ensure that the use of this additive in our cultures would not affect the spontaneous rate of aneuploidies in control experiments. The FIX system was devised as a tool to study the capacity of chemical compounds to induce aneuploidy in meiosis and the system was adopted by our group to investigate the genotoxic properties of chemicals of environmental interest (Costa et al. 1987; Costa et al. 1988).

The FIX assay employs adult females (which are treated with the compound under study) homozygous for y, heterozygous for the complex $sc^{8}In49$ inverted X and for 2 (trans) X-linked lethals. Following treatment, the females are mated with attached XY w males not carrying a free Y chromosome. In the offspring all regular males die, regular females are wild-type while exceptional females (chromosomal gain) are y and exceptional males (chromosomal loss) are w. The adoption of a brooding scheeme provides useful information on the sensitivity, towards the chemical under study, of the different stages of germ cell maturation.

A control series was set up in which the route of administration was by feeding of 1-3 day old adult females for 3 days with a sterile 5% sucrose solution. These females and their progeny were grown on a standard medium containing propionic acid (Mittler & Bennett 1962) instead of Nipagin as a fungicide. Treatments with Nipagin were performed in two ways: (1) acute treatment: Nipagin 6.5×10^{-2} M was added to the 5% sucrose solution and administered for 3 days as described above; (2) chronic treatment: adult females, which had been grown on a modified Mittler & Bennett medium with Nipagin 2×10^{-2} M added in place of propionic acid, were crossed with attached XY w males and allowed to lay eggs on the same medium. In all the above experiments a brooding scheme was adopted (see Table 1).

The results obtained are shown in Table 1. Chronic treatment with Nipagin (a condition which is normally present in laboratory Drosophila cultures) resulted in a highly significant increase both in chromosome gain and loss frequencies, in particular chromosomal loss was observed in the II and III broods while chromosomal gain was consistent in all three broods analyzed. No effects were observed in the case of the three day acute treatment with Nipagin at a concentration three times greater, of females previously grown in Mittler & Bennett medium (without Nipagin). These results are probably of relevance to laboratories in which Drosophila stocks (which are required to maintain stable chromosomal characteristics) are routinely maintained.

Acknowledgement: The authors are grateful to Prof. S. Zimmering, Brown Univ., Providence, Rhode Island USA, for helpful advice and for kindly supplying the FIX strains employed in the present study. This study was supported by the National Research Council (C.N.R.) of Italy (P.F. "Oncologia").

References: Christie, A.L.M. 1935, DIS 4:64-65; Costa, R., M. Zordan, A. Tavella, G. Strolego, A. Fastelli & A.G. Levis 1987, Atti Ass. Genet. Ital. 33:81-82; ———, A. Russo, M. Zordan, F. Pacchierotti, A. Tavella & A.G. Levis 1988, Envir. Mol. Mutagen. (in press); Lakovaara, S. 1969, DIS 44:128; Margolin, B.H. 1983, Envir. Mol. Mutagen. 5:705-716; Roberts, D.B. 1986, in: Drosophila a practical approach, IRL press; Shorrocks, B. 1971, DIS 46:149; Wette, R. 1954, DIS 28:166-167; Zimmering, S., J.M. Mason & C. Osgood 1986, Mutation Res. 167:71-87.

Crossley, S.A. Monash University, Clayton, Australia. Lek behaviour and its evolution in **Drosophila mycetophaga (Hirtodrosophila).**

Drosophila mycetophaga is a fairly large drosophilid (mean length, head to tip of wing: ♀ 4.5 mm, ♂ 4.0 mm). Male and females have picture wings (Fig. 1). The species is of particular interest because it is one of three Australian species reported to use a territory or lek for courtship and mating (Parsons 1977; Parsons & Bock 1977). According to these workers the courtship territory is the horizontal undersurface of a bracket fungus (**Ganoderma applanatum,** Polyporacea). This undersurface varies in colour according to the developmental stage of the fungus and flies are only found on it when it is in its white or grey stage. Courtship was briefly described as appearing "to conform with the general drosophiloid pattern" (Parsons 1977), and to include prominent wing display (Parsons & Bock 1977).

I attempted to confirm lek use by **Drosophila mycetophaga** in a field study (1981-1985). Approximately 32 hr of behavioural observations of **D.mycetophaga** were made each year. Five observers were used for this study, at least two in any year. In addition to behavioural observation and description, behaviour was also video recorded in the field.

Field work was in temperate rain forest in S.E. Australia. The fruiting bodies of **Ganoderma applanatum** were seen as hard wood-like brackets, growing out from the sides of fallen trees. Each year some brackets ripened and became covered ventrally with a pure white layer pierced by pores. Brackets occupied by **D.mycetophaga** were those which were ripe, discharging spores, and positioned where they reflected light penetrating the forest canopy.

To see whether individual flies selected and defended particular brackets, flies were aspirated from leks, sexed, marked with paint, and at dusk placed on a fern frond beside the capture site. Nine out of a total of ten marked individuals reappeared the next day, either on the same fungus, or on a neighbouring one that was in the same white phase of development. Therefore, flies did not return to exactly the same fungus from which they were taken, but on return they chose to land on a fungus which was equally conspicuous.

Field experimentation was hampered by difficult terrain, scarcity of **D.mycetophaga** and the fact that population density on accessible brackets was unpredictable from year to year. Eventually, success in culturing the species enabled detailed behavioural study and a re-interpretation of videotaped behaviors of flies on leks. Because of the difficulty of sexing and marking individuals in the field, any unmarked fly which wing displayed had been assumed to be male. This assumption was found to be wrong when flies of known sex were observed in the laboratory. The following account of behaviour is therefore based on laboratory studies and the behaviour of marked individuals of known sex in the wild.

The behaviour of males and females of **Drosophila mycetophaga** was more similar than for other drosophilids. Both sexes turned towards, oriented to, followed, bobbed the abdomen up and down, touched tarsi, and using the middle and fore legs stepped on (trampled) the fly oriented to. Females as well as males wing displayed by extending one wing to 90° and then turning the vane so that it was perpendicular to the substrate (wing extend, Fig. 2). Female wing display has only been reported previously for a "lesbian" mutant phenotype of **Drosophila melanogaster** (Cook 1975). Waving, which was a quick raising and lowering of one or of both wings alternately, was also shown by both sexes.

Behaviours unique to males were two additional wing displays in both of which the wings were vibrated, and chasing, which was distinguished from following by being faster. In vibration the wings separated 45° and in scissor the two wings were each held out to 90° to form a straight line across the body. Vibration occurred during approach and chase to both males and females, but scissor was only given, in long bouts at least, to females. Typically the male vibrated as he approached a female and scissored as he circled her. He paused and scissored in front, in long bouts, before moving behind and under her wings to make copulation thrusts. Copulation occurred when the male mounted the female and forced her wings apart. Copulation duration varied (17-22 min) and appeared to be terminated by female leg movements which dislodged the male.

Using a video-recording method (Crossley & McDonald 1980), sound emissions during courtship were matched to the displays which produced them. Wing extend was silent, vibration was accompanied by pulse song (interpulse interval 46 ms) and scissoring produced sine song, which had a carrier frequency of 180 Hz decreasing to 82 Hz as the wings closed in preparation for a copulation attempt.

In the laboratory at 22°C the life cycle (egg - adult) is 17 days. Six-nine day old virgin females elicit persistent courtships from the same aged virgin males; courtship amongst 1-3 day old virgins is rare. The eggs are conspicuous being surmounted by four long filaments which protrude from the substrate (Drosophila medium plus commerical mushroom **Agaricus**). Newly eclosed flies dehydrate rapidly unless they are placed on fresh food within a day. Presumably, because their natural habitat is wet and humid, they are specially susceptible to desiccation.

In the field, once a male landed on a lek it usually remained there all day. It patrolled the lek, bobbing, holding its wings slightly apart, and orienting to small blemishes as if they were mistaken for

Figure 1. Drosophila mycetophaga. The sexes are similar with picture wings (a) and sharply contrasting dark upper and white lower body colouring (b). Differences between the sexes are the downturned genitalia of males and their shorter body length but these differences are not useful for distinguishing the sexes on the lek.

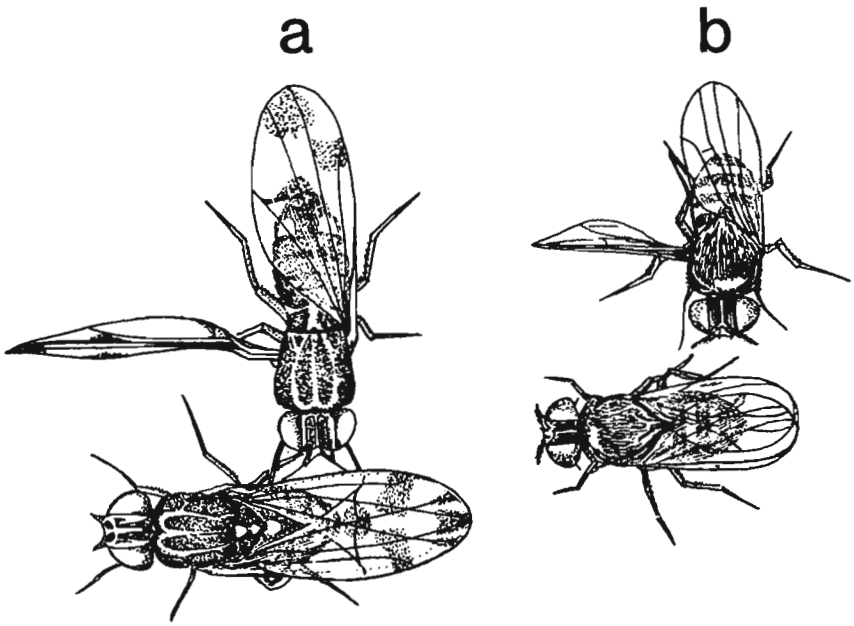


Figure 2. Wing extend in **D.mycetophaga** (a) compared with a similar display vibration in **D.melanogaster** (b). The wing vane does not extend into the partner's field of view in (b) as much as it does in (a).

another fly. Females alighted on the edge of the lek, stayed for shorter periods than males, and were outnumbered by males. The nearest male rushed to a newly arrived female and scissored. If a second male approached, the first male chased the intruder away. Such chases were short and usually successful in repelling intruders. After a chase the victor rushed back to the female and resumed courtship. Females were more passive than males in that they did not move around the fungus as much. However, they did wing extend to other flies as well as following them and during courtship they turned to bring themselves into the copulatory position or avoided copulation by abdominal raising. When females were not present on the fungus, chases were rare. Males moved sideways to avoid each other during patrols, as if spatial positions had been learned in earlier interactions. The same males tended to occupy the edge of the lek where females landed. Individual males, therefore, differed in their access to females and females had the opportunity to select a mate.

Nothing is known of the natural feeding and oviposition sites of this species but the conclusion, from laboratory study and dissections of **Ganoderma applanatum** (decaying as well as fresh), was that soft fungi, as distinct from hard bracket fungi, are used. Soft fungi grow on almost every surface of the wet forest and favour the dispersal of the species. Bracket fungi showed no evidence of larval damage and no pupae were found, despite intensive searches of brackets and their surrounding decaying wood. Kimura (1980) reports that adult members of the subgenus **Hirtodrosophila** prefer fresh mushrooms in contrast to their larvae which prefer decaying fungi. Adult **D.mycetophaga**'s presence on ripe bracket fungi in nature and the utilisation of decaying mushroom as the larval food source in the laboratory, therefore agrees with Kimura's conclusions.

The use of a lek for mating may have evolved because sexual displays on the food source were not visible in the dimly lit forest. On the lek, visual displays and picture wings, enhanced by the white background, were selected for. The male wing displays and their accompanying sounds may be sexually stimulating as well as serving a sexually isolating function in the evolution of **Hirtodrosophila**, whereas the bisexual wing extend and wave may advertise presence on the lek. In **D.mycetophaga** the wing is brought further into the partner's view by wing extend than it is in **D.melanogaster** males which lack picture wings (Fig. 2). Perhaps wing length is implicated in the evolution of wing adornment. Olfactory stimuli may also be important for aggregation on leks and during courtship but the evidence, as a whole, supports the view that visual stimuli provide the greater component of total stimulation.

Spieth (1974) suggested that bird predation was the significant factor in the evolution of lek behaviour in Hawaiian Drosophila, but no predators are known for **D.mycetophaga.** This species is camouflaged, however, when viewed on the lek from the side (Fig. 1) so that protection against terrestrial predators, such as spiders and lizards, may have featured in its evolution once it began to use fungal leks.

The behaviour of **D.mycetophaga** is consistent with the view that it is a "classical" lek species, as defined by Bradbury (1981), which evolved for different reasons from the Hawaiian lek species. Since the latter belongs to the subgenus Drosophila and **D.mycetophaga** to the subgenus **Hirtodrosophil**a this is a case of convergent evolution (Parsons & Bock 1976). Choice tests are in progress to determine the perceptual abilities of **D.mycetophaga** and the stimuli underlying lek use. By combining field and laboratory studies, it is ensured that hypotheses tested relate to the species' naturally occurring behaviour.

Acknowledgement: I thank Dr. J. McDonald, Miss L. Anderson, Mr. P.M. Crossley, and Mr. I. Taylor for research assistance in the field and in the laboratory. I am also grateful to Miss C. Cook for maintaining stocks.

References: Bradbury, J.W. 1981, in: Natural Selection and Social Behavior (Alexander & Tinkle, eds.), Chiron Press Inc. 138-169; Cook, R. 1975, Nature 254:241-242; Crossley, S. & J. McDonald 1980, DIS 5:150-151; Kimura, M.T. 1980, Evolution 34(5):1009-1018; Parsons, P.A. 1977, Evolution 31:223-225: ——— & I.R. Bock 1977, Nature 265:48; Spieth, H.T. 1974, in: Genetic Mechanisms of Speciation in Insects (White, ed.), Australian & New Zealand Book Co., 94-101.

Demakova, O.V. and E.S. Belyaeva. Inst. of Cytology & Genetics, Novosibirsk 630090 USSR.
Effect of mating direction on the position effect variegation of T(1;2)dor^{var7} in **D.melanogaster.**

The influence of some external and genotypic factors on variegation is known to be one of the characteristics of position effect variegation (see, for review, Spofford 1976). For some rearrangements influence of parental genotype on variegation was demonstrated. These so called parental effects have predominantly the maternal character (Hessler 1961; Cohen 1962; Spofford 1976; Khesin & Bashkirov 1979).

We started the investigation of position effect variegation of T(1;2)dor^{var7}, relocating the 1A-2B7-8 region of the X-chromosome to the 2L-chromosome 40A-F region (Zhimulev et al. 1986; Demakova et al., DIS:this issue). This note is devoted to revealing possible parental rearrangement source effect in this model system. The dor^{var7}/FM6 females or dor^{var7}/Y males from one stock were crossed to individuals, carrying different mutations in 1F3-4 - 2B7-8 region (see Demakova et al. DIS:this issue) at various temperatures (25°C, 18°C, 14°C). In F_1 the phenotypes and viability of daughters, heterozygous for rearrangement and mutations (B^+) were analysed.

The results of the analysis are given in Table 1. With the parental source of rearrangement variegation increases considerably, both in decrease of heterozygotes viability and in increase of abnormal flies number among B^+ females (Table 1, 25°C) or in stronger manifestation of mutant phenotypes:

swi - (a) Mosaics have crumpled, singed wings; (b) Most of mosaics have extremely reduced, singed wings. dor - (a) About 5% of B^+ females have dor-variegated eyes; (b) In dying pupae one can observe formed imago with eyes almost completely dor-coloured. Few surviving flies have eyes variegated in the same manner. sta - (a) Mosaics may have small rough eyes and some head macrochetes absent; (b) Besides these abnormalities mosaics may have roughly deformed heads, eyes, antennae and ocelli (3.3%). I(1)BA12 - (a) Some of B^+ females have reduced eyes or some have head macrochetes absent; (b) Abnormalities are numerous; about 8% of B^+ females have eyes and heads roughly deformed. I(1)BA11 - (a) Wings are slightly curled; (b) About 13% of B^+ females have curled and twisted wings. I(1)BA5 - (a) Mosaics have some macrochetes on heads and (rarely) palpuses reduced in size; (b) Reduction of macro- and microchetes can be found on heads, palpuses and toraxes; sometimes eyes are deformed.

After combining 2 modifying factors (low temperature and paternal source), variegation enhanced more considerably (Table 1, 18°C and 14°C). Only in such "extreme" conditions the variegation expression appeared to be significant for some loci, inactivation of which after position effect was questioned in the other conditions: cwi - crumpled wings; I(1)BA1 - viability reduced, rough small eyes; I(1)BA9 - viability reduced. Thus, the enhancement of variegation is expressed in some lengthening of its spreading distance also.

Table 1. Dependence of 2B region loci variegation on parental source of T(1;2)dor^{var7} and temperature.

Locus	Allele (m)	t°C	Ratio of B:B$^+$ females in progeny from mating (part of B$^+$ females with morphological changes, in %) ♀ dor^{var7}/FM6 x ♂ m/Dpy^{2}Y67g (a)		♀ m/FM6 x ♂ dor^{var7}/Y (b)	
cwi	St484	25	80:94	(0.0	118:146	(0.0
		18	92:89	(0.0)	68:72	(2.8)
		14	65:42	(0.0)	54:39	(17.9)
swi	lt467	25	103:107	(7.5)	105:95	(71.6)
		18	59:79	(0.0)	80:72	(63.9)
		14	61:54	(5.6)	51:42	(59.5)
dor	lt148	25	62:42	(95.2)	193:3	(100.0)
		18	41:36	(86.1)	127:0	--
		14	37:18	(83.3)	58:0	--
ecs	br^{lt336}	25	92:72	(56.9)	115:2	(100.0)
		18	62:38	(65.8)	106:0	--
		14	41:1	(100.0)	56:0	--
	rbp^{lt376}	25	87:100	(36.0)	78:13	(100.0)
		18	64:36	(100.0)	74:2	(100.0)
		14	60:3	(100.0)	85:0	--
sta	lt3	25	86:93	(4.3)	222:152	(12.5)
		18	63:79	(10.1)	83:48	(70.8)
		14	44:55	(56.4)	61:33	(87.9)
l(1)BA12	t27	25	107:111	(2.7)	116:52	(69.2)
		18	110:118	(16.9)	97:30	(86.7)
		14	113:37	(75.7)	71:2	(100.0)
l(1)BA11	t62	25	77:90	(4.4)	144:103	(62.1)
		18	101:114	(13.1)	155:36	(61.1)
		14	56:12	(58.3)	52:1	(100.0)
l(1)BA5	t233	25	81:88	(17.0)	103:113	(64.6)
		18	105:81	(18.5)	112:112	(95.5)
		14	91:81	(27.1)	62:44	(47.7)
l(1)BA1	t348	25	121:125	(0.0)	90:51	(3.9)
		18	101:100	(0.0)	76:64	(6.3)
		14	78:93	(1.1)	105:32	(3.1)
l(1)BA9	t471	25	118:141	(0.0)	180:184	(1.6)
		18	114:108	(0.0)	168:170	(0.0)
		14	64:72	(0.0)	151:68	(0.0)
l(1)BA6	t186	25	78:82	(1.2)	69:81	(3.7)
		18	85:83	(0.0)	64:54	(3.7)
		14	117:105	(0.0)	65:34	(2.9)

This fact correlates well with the cytological data of parental source influence on 2B-region compaction pattern in polytene chromosomes. Penetration and length of compaction zone were analysed in the dor^{var7}/-FM6 females at 18°C (20 nuclei from each larval salivary gland).

In 13 larvae with maternal dor^{var7} - chromosome about 28% of nuclei have compact blocks of chromatin. In most of these blocks only 2B1-2 - 2B7-8 region is compact, and only 3% of them include 2A1-2 region as well. In 19 larvae with paternal rearrangement about 58% of nuclei have compact blocks of chromatin. In 24%% of them blocks include 2A1-2 region and sometimes even 1E1-4 region.

One may discuss the minimal and maximal variegation spreading zones in T(1;2)dor^{var7}. The minimal zone is revealed under the maternal rearrangement source and 25°C and includes 7-8 loci: cwi (?), swi, dor, ecs, sta, l(1)BA12, l(1)BA11, l(1)BA5. The maximal one is revealed under paternal source of translocation and 14°C and includes 10-11 loci: cwi, swi, dor, ecs, sta, l(1)BA12, l(1)BA11, l(1)BA5, l(1)BA9, l(1)BA6 (?).

Now we can only speculate about the mechanisms of parental rearrangement source effect. Developing embryos might obtain different potentialities of compaction of relocated euchromatic region due to some unclear changes in oo - and in spermiogenesis of parents.

Acknowledgement: The authors thank Drs. I.F. Zhimulev and V.N. Bolshakov for help in preparing the manuscript.

References: Belyaeva, E.S. et al. 1982, DIS 58:184-190; Cohen, J. 1962, Genetics 47:647-659; Hessler, A.Y. 1961, Genetics 46:463-484; Khesin, R.B. & V.N. Bashkirov 1979, Genetica (USSR) 15(2):261-273; Spofford, J.B. 1976, in: Genetics and Biology of Drosophila (Ashburner & Novitski, eds.), v1c:955-1018; Zhimulev, I.F. et al. 1986, Chromosoma (Berl) 94:492-504.

Demakova, O.V., E.S. Belyaeva and I.F. Zhimulev.
Inst. of Cytology & Genetics, Novosibirsk USSR.

Genetical characteristics of loci in the 2B-region of **D.melanogaster** X-chromosome under position effect variegation in T(1;2)dor^{var7}.

The 2B-region of **D.melanogaster** X-chromosome is known to contain several loci, responsible for ecdysone induction (Belyaeva et al. 1987). Recently, it was shown that the T(1;2)dor^{var7} translocation, relocating the 1A-2B7-8 region in vicinity of the 2L-chromosome pericentric heterochromatin, led to the position effect variegation of these loci, resulting in different morphological changes and viability reduction (Belyaeva et al. 1982; Zhimulev et al. 1986.

In this report we give detailed description of the viability and phenotypes of mosaic females, heterozygous for dor^{var7} and different types of mutations of loci, mapped distal to the breakpoint in 2B7-8 (Figure 1).

The dor^{var7}/FM6 females and males, carrying the mutations were mated at different temperatures (25°, 18° and 14°C). F_1 females, heterozygous for dor^{var7} and mutation (B^+), and control females, heterozygous for mutation and FM6, were compared. Differences in B:B^+ ratios were estimated statistically with Fisher criteria. SEM microphotographs were taken on the JMS-35 for the most typical mutant phenotypes. Since no obvious morphological differences between nonmosaic dor^{var7}/m and wild Batumi-L stock females were revealed, the latter were used as a morphological control.

As seen in Table 1, for all the loci subjected to variegation in heterozygote T(1;2)dor^{var7} (at least for some of their mutations), the viability clearly depends on temperature conditions. Being usually normal at 25°C it decreases at lower temperatures, often more significantly at 14°C than at 18°C. As for swi and l(1)BA5 mutations, we perhaps may deal with the equilibrium of opposite-directed influences of low temperature on degree of normal genetical inactivation in rearrangement on the one hand, and the manifestation of mutation itself on the other. The former, as a rule, increases (Spofford 1976) and the latter decreases (Belyaeva et al. 1982) at the low temperatures.

The following abnormalities were found in the loci:

cwi - About 2-9% of B^+ females at different temperatures have crumpled, lowered wings (Fig. 2E). Because of uncertainty in exact position of cwi regarding the breakpoint of translocation, its possible localization proximal to dor^{var7} (2B7-8) cannot be excluded.

swi - The expression of variegation is weak enough: about 1-9% of the flies have crumpled, singed or reduced wings (Fig. 2F, G). Some females have deformed aristes at 14°C.

dor - One can find dor-variegated eyes of 3 types: (1) with dark single facets ("pepper and salt") or spots on the normal pigmented background; (2) with dor-coloured sectors; (3) with gradual transition from dark-pigmented area to the dor-pigmented one. The "pepper and salt" variegation can often be

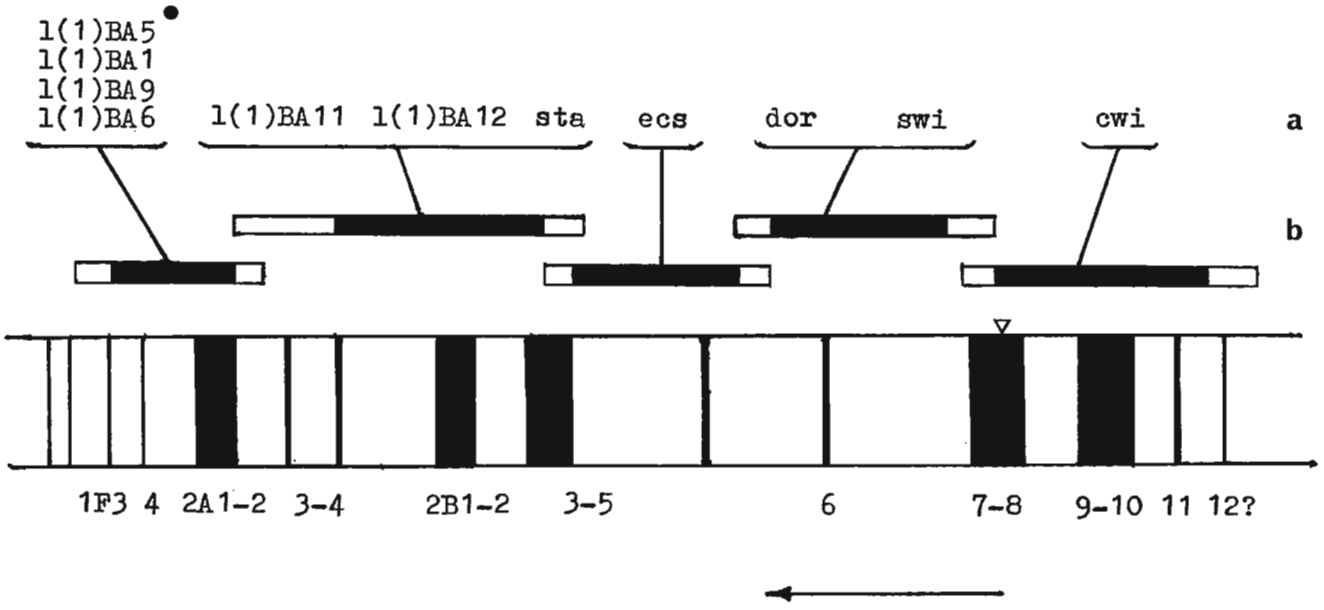


Figure 1. Cytogenetical map of the 2AB region (see Belyaeva et al. 1987). **a** - loci; **b** - EM map of the region and cytological limits of the loci. Arrow shows the direction of position effect variegation spreading; ● - linear arrangement of these loci regarding each other is not known yet; ∇ - breakpoint of T(1;2)dor^{var7} in 2B7-8.

Table 1. Viability of the females heterozygous for T(1;2)dor^{var7} and mutations located in the 2B region at different temperatures.

Locus	Complementation group	Allele	Ratio of B:B$^+$ females in progeny from mating: (part of B$^+$ females with morphological changes, in %) ♀ dor^{var7}/FM6 x ♂ m/Dpy^{2}Y67g					
			25°		18°		14°	
cwi	--	St484 h[a]	130:154	(0.0)[b]	159:131	(9.2)	169:179	(4.5)
			161:144	(2.1)	239:179	(0.0)	131:78	(0.0)*[c]
swi	--	t219 h(m)	37:38	(5.3)	112:91	(0.0)	102:77	(3.9)
			87:88	(0.0)	118:104	(0.0)	121:105	(2.6)
		t467 l	94:53	(9.4)*	161:177	(2.3)	166:155	(9.7)
			227:207	(1.0)	118:150	(1.0)	90:98	(5.1)
dor	--	1 h(m)	247:243	(78.2)	204:159	(91.8)	143:98	(80.6)*
			163:109	(91.7)*	108:77	(84.4)	71:43	(72.1)*
		t148 l	98:100	(91.0)	107:84	(86.9)	41:27	(92.6)
			180:163	(96.9)	90:102	(91.2)	113:41	(100.0)*
ecs	l(1)pp-2	t252 h	134:136	(3.7)	143:140	(49.3)	157:115	(37.4)*
			125:120	(12.5)	121:77	(23.4)*	112:86	(27.9)*
	l(1)pp-1	t10 l	114:70	(0.0)*	119:26	(0.0)*	128:1	(0.0)*
			137:110	(0.0)	123:18	(0.0)*	86:0	(0.0)*
	br	1 h(m)	152:157	(30.6)	157:55	(76.4)*	119:24	(62.5)*
			108:87	(35.6)	106:43	(55.8)*	95:8	(100.0)*
		t336 l	139:149	(40.9)	107:50	(56.0)*	142:4	(100.0)*
			158:117	(31.6)	100:68	(60.3)*	73:3	(100.0)*
	rbp	t132 h(m)	142:141	(43.3)	135:112	(48.2)	168:20	(75.0)*
			129:125	(34.4)	105:81	(71.6)	94:28	(92.9)*
		t376 l	150:161	(22.4)	128:67	(100.0)*	57:4	(100.0)*
			101:105	(15.2)	92:51	(66.7)*	71:3	(100.0)*
	"short overlapping" alleles	t143 l	92:67	(43.3)	132:21	(60.0)*	91:1	(0.0)*
			112:53	(58.5)*	135:10	(71.4)*	154:6	(100.0)*
	"long overlapping" alleles	t435 l	307:9	(100.0)*	267:0	(0.0)*	161:0	(0.0)*
			159:0	(0.0)*	194:0	(0.0)*	124:0	(0.0)*
sta	--	1 h(m)	91:100	(0.0)	153:34	(20.6)*	101:14	(57.1)*
			113:108	(0.9)	174:101	(8.9)*	63:12	(25.0)*
		t3 l	175:130	(3.1)	125:108	(6.5)	88:61	(60.7)
			114:84	(4.8)	104:101	(10.9)	56:53	(47.2)
l(1)BA12	--	t27 l	263:257	(17.1)	177:124	(10.5)*	102:24	(33.3)*
			67:71	(19.7)	90:64	(12.5)	176:52	(55.8)*
l(1)BA11	--	t62 l	236:216	(6.9)	164:101	(42.6)*	199:23	(39.1)*
			124:108	(0.0)	96:72	(20.8)	81:10	(20.0*
l(1)BA5	--	t233 l	81:88	(17.0)	105:81	(18.5)	91:81	(30.8)
			83:97	(6.2)	107:106	(12.3)	52:37	(40.5)
l(1)BA1	--	t348 l	135:147	(0.0)	128:129	(0.0)	63:74	(2.7)
			84:96	(0.0)	78:88	(0.0)	79:93	(1.1)
l(1)BA9	--	t471 l	109:120	(0.0)	45:47	(0.0)	143:115	(17.4)
			--		--		--	
l(1)BA6	--	t186 l	111:122	(0.0)	95:87	(0.0)	96:113	(0.0)
			--		--		--	

a = letters indicate different mutation types: h - hypomorph; h(m) - hypomorph with morphological abnormalities; l - lethal. b = in different lines, results of independent experiments are presented.
c = asterisks mean significant differences in B:B$^+$ ratios ($p > 0.95$ or higher).

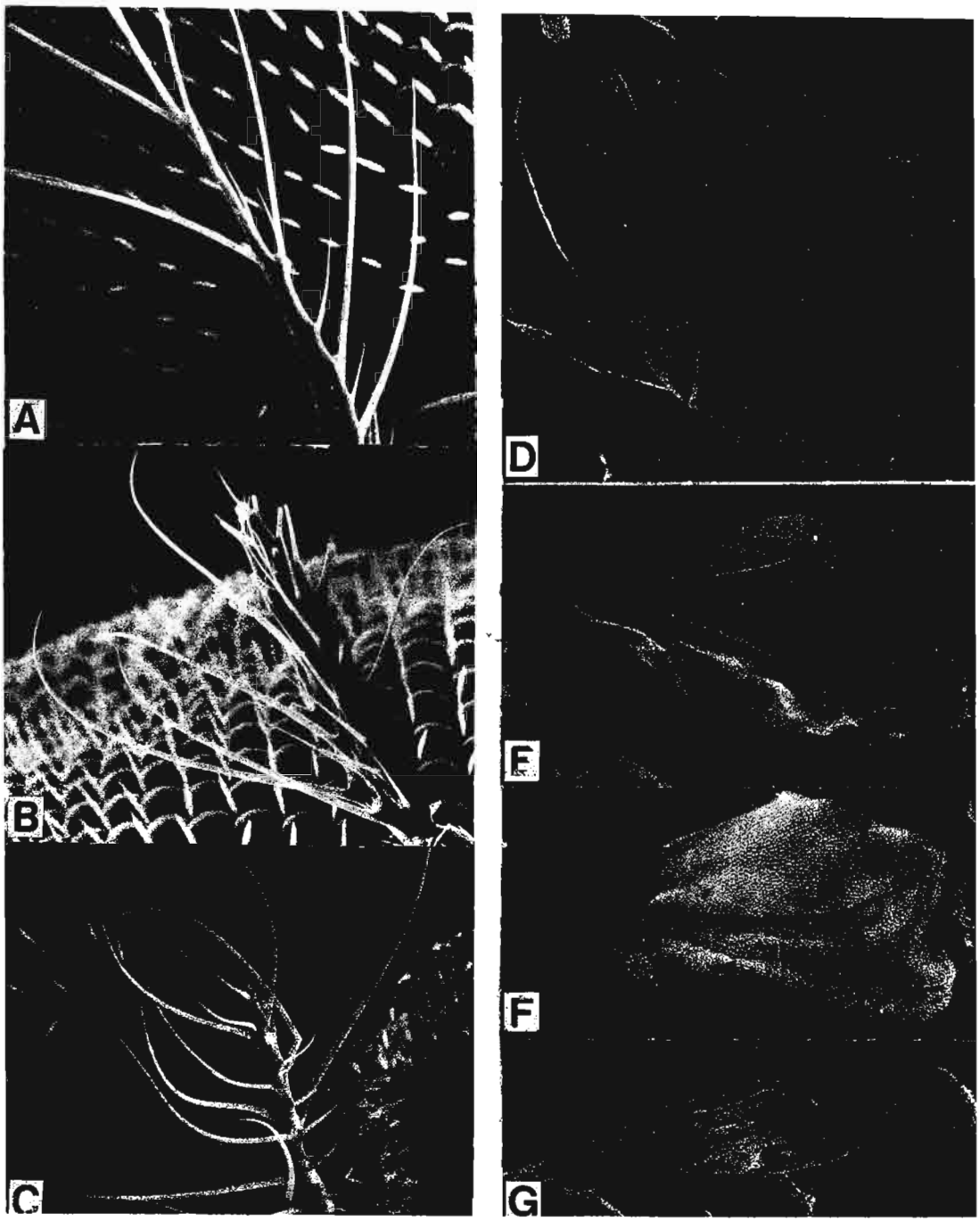


Figure 2. Morphological characters of cwi-, swi-, and dor-variegated females. **a,d** - Batumi-L wild type females, 25°; **b,c** - dor^{var7}/dor^{1t148}, 14°; **e** - dor^{var7}/cwi^{St484}, 25°; **f,g** - dor^{var7}/swi^{1t467}, 14°.

demonstrated on such bright areas. Besides eye phenotypes, the position effect variegation of the locus results in crumpled, singed wings (see swi) in 45-85% of B^+ females and at 14°C in deformed aristes as well (Fig. 2B,C). At low temperatures, we observed the increased number of flies having (i) both eyes variegated and (ii) bigger sectors (more than 1/3 of eye area) with dor colour.

ecs - Mutations from every complementation group of this complex locus were taken into analysis.

l(1)pp-2 - Depending on the temperature in 1-40% of B^+ females 1-2 bristles on palpuses are absent or reduced. The abnormality is weakly expressed at all temperatures.

l(1)pp-1 - Mutations of this group do not result in any distinct phenotypic abnormality under the variegation.

br and rbp - These mutations have a wide spectrum of morphological characters with penetrance of 30-100% and 15-100%, respectively. As for the br group of mutations, wing abnormalities are

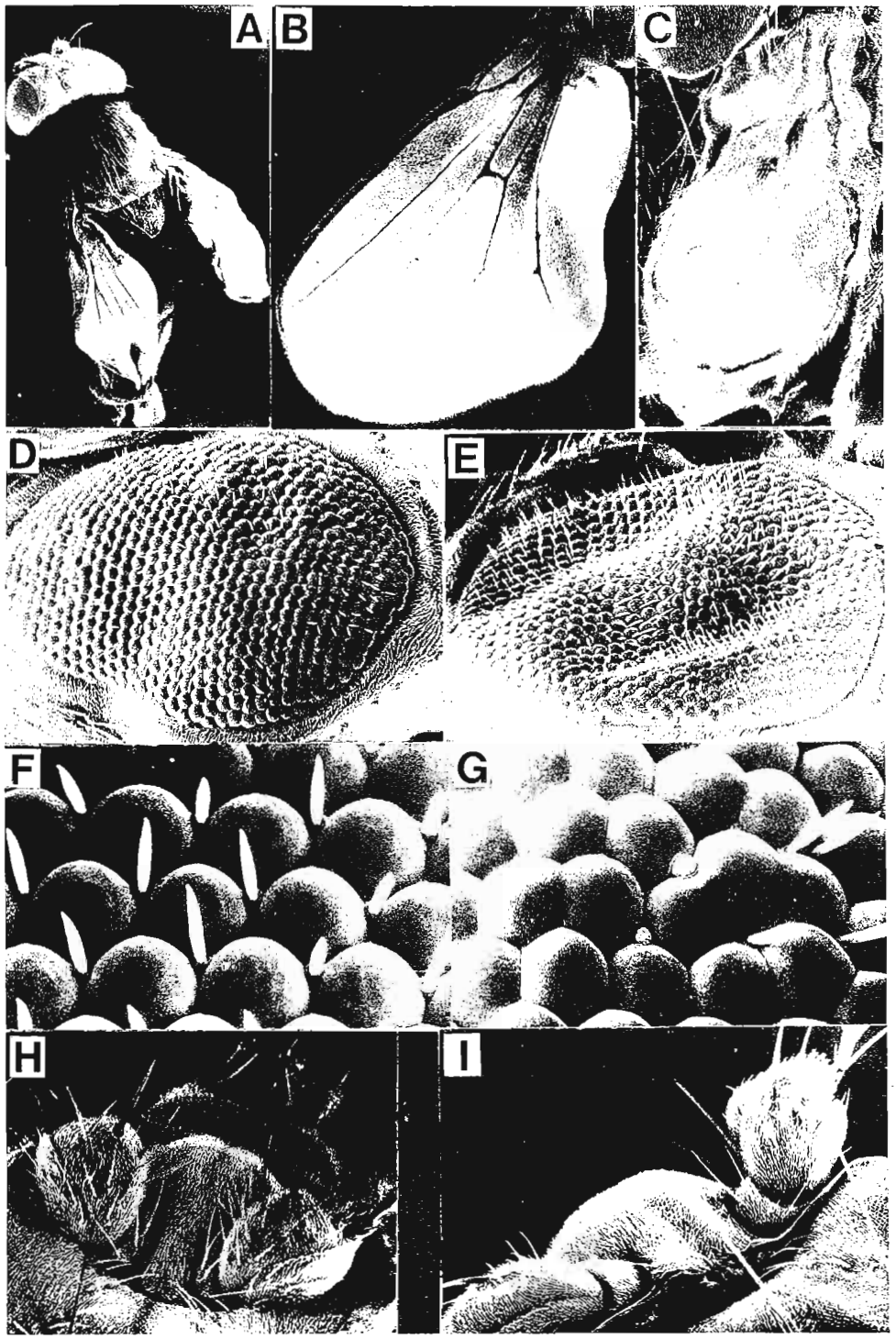


Figure 3. Morphological characters of ecs-variegated females. **a** - $dor^{var7}/-ecs^{lt143}$, 25°; **b,c** - dor^{var7}/ecs^{brl}, 18°; **d** - dor^{var7}/ecs^{lt336}, 18°; **e** - dor^{var7}/ecs^{brl}, 18°; **f,h** - Batumi-L wild type females, 25°; **g** - $dor^{var7}/-ecs^{br\ lt336}$, 18°; **i** - $dor^{var7}/ecs^{rbp\ lt376}$, 18°.

predominant, while for the rbp group bristle ones. Some mosaics (2-100%) have rough, small eyes because of the facet's fusion and irregular eye's hair arrangement between them; some (2-50%) have eye sectors without facets or crumpled surface (Fig. 3D,E,F,G). Flies with roughly deformed heads and eyes were also found.

Wing abnormalities vary from the wings slightly shortened from the norm to twisted and reduced ones. Short, broad or crumpled, sometimes blistered, wings appeared to be the most typical (Fig. 3A,B,C). For both groups curved, broken macrochetes off on the torax (2-100%) or absence of some macro- and microchetes (2-100%) on the heads, toraxes and palpuses are characteristic, with palpus abnormalities predominantly in rbp group (20-86%) (Fig. 4B,C,E). About 3-75% of mosaics occur with shortened, curved aristes (Fig. 3G). Only rbp variegated females (3-100%) may have antennae apart (Fig. 3I).

Short overlapping alleles - Phenotypic characters are the same as in br and rbp groups.

Long overlapping alleles - Morphological abnormalities for these alleles were not found because of strong lethality (Table 1). Some surviving B^+ females may have defects, typical for br group.

sta - Typical for sta-mosaics are the notches on wings (1-30%) (Fig. 5F), rough, small eyes (1-50%) and absence of some head macrochetes. At the low temperatures, some more "rough" defects were observed. In 1-8% of B^+ females some facet's groups were absolutely absent (Fig. 5C) and in 2-17% of them heads

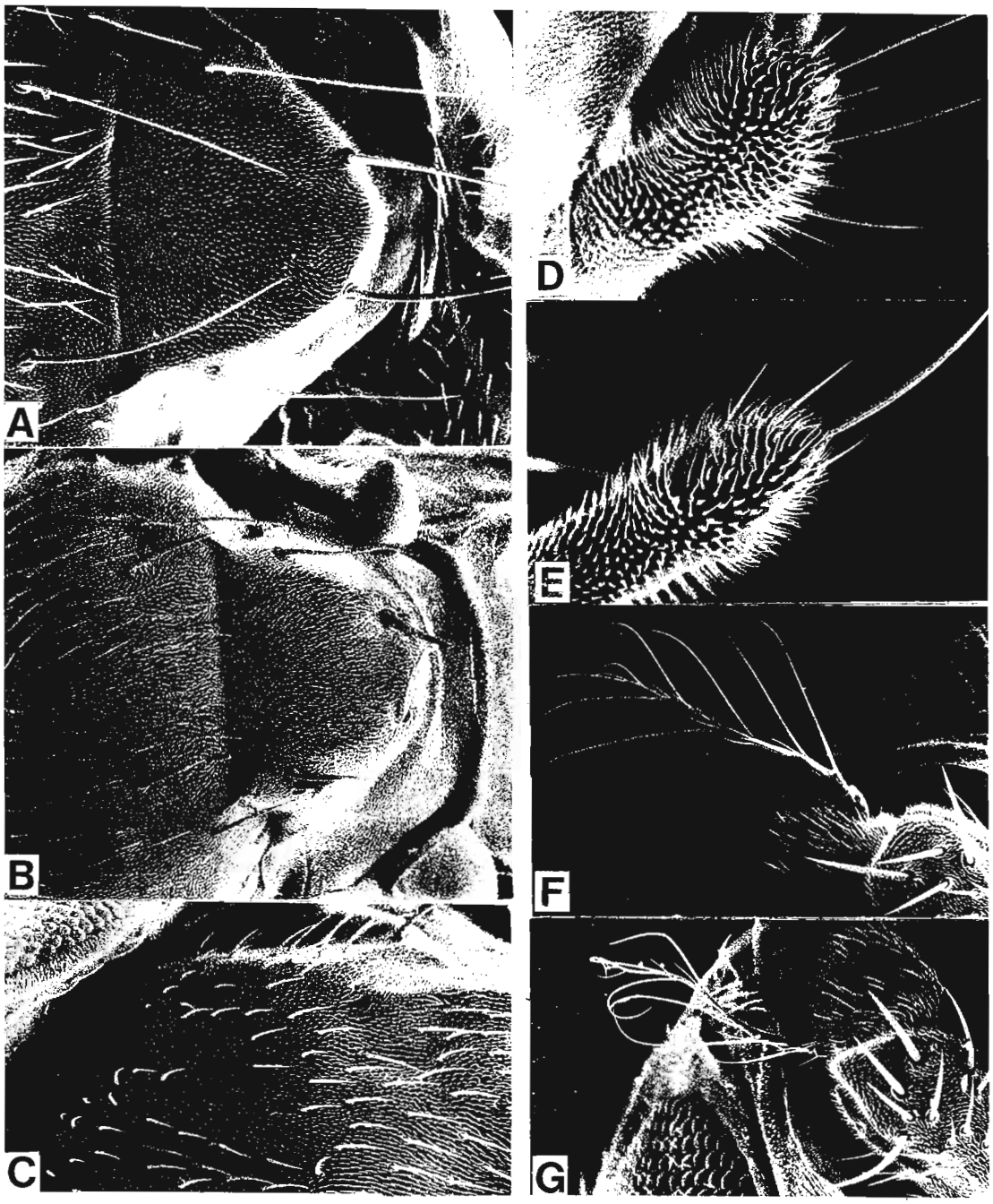


Figure 4. Morphological characters of ecs-variegated females. **a,d,f** - Batumi-L wild type females, 25°; **b** - $dor^{var7}/ecs^{1}t143$, 18°; **c,e** - dor^{var7}/ecs^{rbp} t132, 18°; **g** - $dor^{var7}/ecs^{1}t143$, 25°.

and eyes were deformed. Such flies may also have unusual morphology, arrangement and number of antennae, palpuses and ocelli (Fig. 5B).

I(1)BA12 - The pattern of morphological abnormalities is similar to that of the sta locus: reduced rough eyes (1-39%), notches on wings (2-5%) (Fig. 5E). Some macrochetes are absent on the heads and on the palpuses as well (6-14%). At 18° and 14°C some mosaics have roughly deformed head and some structures located on it (see sta) (Fig. 5D).

I(1)BA11 - About 7-43% of B^+ females have the wings curled in various degree (Fig. 6C). At the low temperature such wings may be simultaneously twisted or crumpled.

I(1)BA5 - Reduction in size of some macrochetes on the heads, toraxes and (more rarely) palpuses is characteristic for 6-40% of B^+ females.

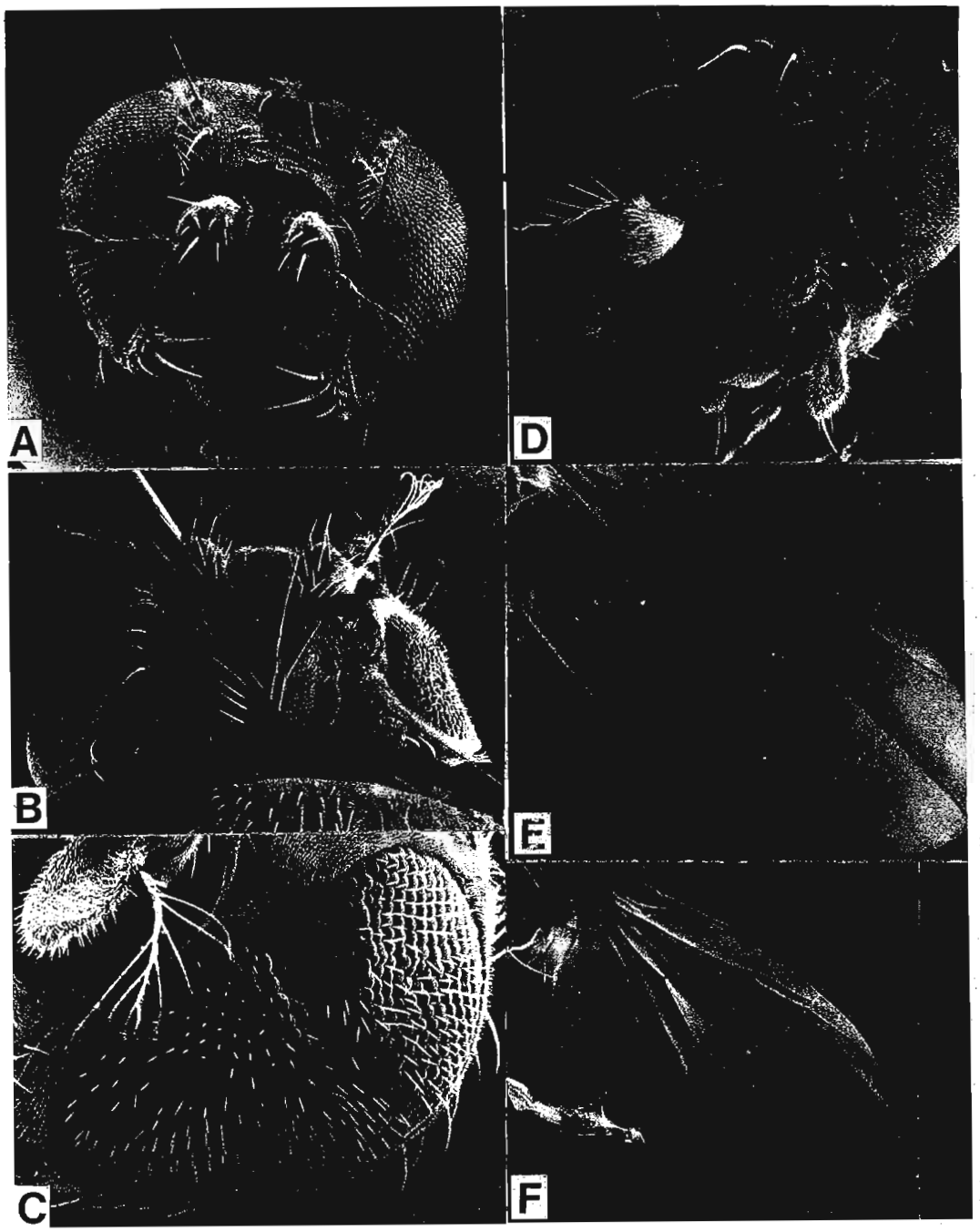


Figure 5. Morphological characters of sta- and l(1)BA12-variegated females. **a** - Batumi-L wild type females, 25°; **b,c** - dor^{var7}/sta^{1t3}, 14°; **d** - $dor^{var7}/l(1)BA12^{t27}$, 14°; **e** - $dor^{var7}/l(1)BA12^{t27}$, 25°; **f** - dor^{var7}/sta^{1t3}, 25°.

I(1)BA1 and I(1)BA9 - SInce at 14°C only a few females with reduced eyes [I(1)BA1] (Fig. 6D) or slight bristle abnormalities [I(1)BA] were found on the one hand, and the viability of such heterozygotes was normal on the other, we cannot take these loci to be subjected to variegation under these experimental conditions.

I(1)BA6 - No distinct morphological abnormalities were found.

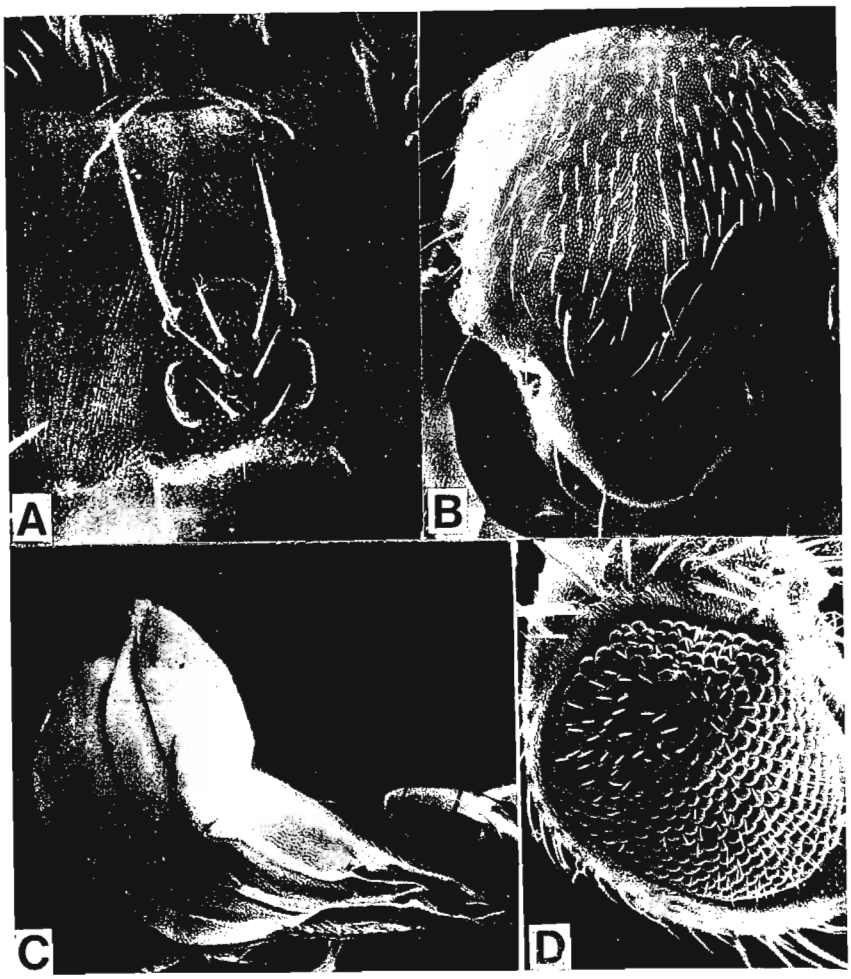


Figure 6. Morphological characters of 1(1)BA11-, 1(1)BA5-, and 1(1)BA1-variegated females. **a,b** - dor^{var7}/-1(1)$BA5^{t233}$, 25°; **c** - dor^{var7}/1(1)$BA11^{t62}$, 18°; **d** - dor^{var7}/1(1)$BA1^{t348}$, 14°.

Thus, enhancement of position effect variegation after lowering the temperature is expressed not only in the decrease of heterozygote viability, but in the increase of their variegation as well, when we observe more numerous and "rough" morphological abnormalities and an increased number of flies having mutant phenotype among B^+ females. In general, the manifestation of the mutations under variegation does not depend on their type resulting in heterozygotes mutant phenotypes of (and) their decreased viability.

Since different mutations, being under position effect variegation, affect phenotype at special conditions (at the background of partially inactivated normal allele), the morphological analysis of such mosaics is of great interest for further revealing of mutations manifestations and genetical organization of loci in question, as follows:

(1) Morphological abnormalities are more numerous than those which can be observed in mutations themselves. For example, ecs-variegated flies exhibit about 20 different abnormalities. At least 3 lethal loci [l(1)BA12, l(1)BA11, l(1)BA5] appeared to have morphological characters.

(2) Analysis of mosaics revealed pleiotropic effects of mutations, affecting wings, bristles, eyes and head, especially after lowering the temperature. Multiplicity of morphological characters in ecs-variegated females may reflect its activity in many tissues, confirming earlier data of its autonomous mode of action (Zhimulev et al. 1986) in many tissues of third instar larvae (Kish & Molnár 1980).

Obviously, similar patterns of abnormalities of l(1)BA12, sta, ecs, dor, swi loci-variegated flies can also confirm, at the morphological level, that they represent a cluster of genes with common function: participation in ecdysone induction.

Acknowledgement: The authors are indebted to Drs. V.N. Bolshakov and S.A. Demakov for help in preparation of this paper.

References: Belyaeva, E.S. et al. 1982, DIS 58:184-190; ——— 1987, Chromosoma (Berl) 95:295-310; Kish, I. & I. Molnár 1980, J. Insect Physiol. 26:391-401; Spofford, J.B. 1976, in: Genetics and Biology of Drosophila (Ashburner & Novitski, eds.), v1c:955-1018; Zhimulev, I.F. et al. 1986, Chromosoma (Berl) 94:492-504.

Dhingra, G., J.B. Chowdhury and P.K. Sareen.
Haryana Agricultural University, Hisar, India.

Effect of Sevidol on fertility and sex-linked recessive lethal frequency in **Drosophila melanogaster.**

Sevidol (1 carbaryl: 1 lindane), an insecticidal mixture comprising a carbamate and an organochlorine compound, respectively, was tested for its genotoxicity in Drosophila. The insecticide was dissolved in DMSO (E. Merck) and mixed in the cornmeal-yeast-agar medium. Three-to-four day old male flies were exposed to it for 15 hr and then were allowed to mate individually with Basc virgin females. The protocol for the Muller-5 test as given by Würgler et al. (1984) was adhered to. A total of three concentrations (LD_{50}, $4/5LD_{50}$, $3/5LD_{50}$), based on LD_{50} value in males (Dhingra 1985), were selected. The controls comprised: normal (untreated), negative (DMSO-2.0 ppm) and positive (EMS-0.5%).

For the effect on the fertility of the fly, the F_1 progeny of the Basc test was recorded. Data were transformed by angular transformation and Z-test was conducted (Table 1). Though a slight increase in percent sterility from 26.82 at 1.50 ppm to 27.71 at 2.50 ppm was observed, yet this was not found to be significant from the negative control value.

The findings of the Basc test after exposure of male flies to Sevidol are presented in Table 2. At the highest concentration (2.50 ppm), percent lethality was 0.62 and it decreased to 0.53 at 2.00 ppm and to 0.26 at 1.50 ppm. However, statistical analysis (Anova by CRD) showed that it was not different from the control value of 0.55 percent.

Table 1. Induction of sterility by Sevidol in **D.melanogaster.**

Conc. (ppm)	$F_1 \times F_1$ crosses	Sterile crosses	Sterility (%)	Transformed means
2.5	1043	264	27.71	30.18±5.53
2.0	1258	355	27.39	31.54±0.70
1.5	1192	379	26.82	29.18±4.53
D	837	216	21.37	24.58±5.55
C	783	188	21.23	25.45±5.48
E	433	116	25.25	29.34±3.40

D = 2.0% DMSO; C = untreated; E = 0.5% EMS.

Table 2. Sex-linked recessive lethal mutations induced by Sevidol in **D.melanogaster.**

Conc. (ppm)	Chromosomes tested	SLRL	Lethality (%)	Transformed means
2.5	779	4	0.62	2.94±1.44
2.0	903	7	0.53	2.54±1.25
1.5	812	3	0.26	1.37±0.91
D	853	4	0.55	2.26±1.21
C	757	10	1.09	4.16±1.23
E	317	45	15.18	22.82±1.11*

D = 2.0% DMSO; C = untreated; E = 0.5% EMS;
* = significant at $p \leq 0.05$.

Though reports on Sevidol in literature are not available, yet those on carbaryl and lindane may allow some tentative conclusions to be drawn for it. Lindane, at concentrations around LD_{50}, was not found to affect the fertility and SLRL frequency in treated Drosophila males (Dhingra & Vijayakumar 1987). Carbaryl in Drosophila showed only a slight increase in the SRLR frequency (Brzheskii & Vaskov 1971) and also no significant partial chromosomal loss was induced by carbaryl and carbofuran, another carbamate (Woodruff et al. 1983). Two other organochlorine insecticides, dieldrin and aldrin, also failed to increase the SLRL frequency in Drosophila (Ashwood-Smith 1981). Thus, it becomes somewhat apparent that the non-mutagenic nature of carbaryl, lindane and other carbamate and organochlorine compounds in Drosophila, is in accordance with the observations on Sevidol. However, the positive genotoxicity of Sevidol in mice (Dhingra 1985) and that of carbaryl and lindane in other test organisms (Wuu & Grant 1967; Lockard et al. 1982; Decloitre & Hamon 1980; Laxminarayana & Subramanyam 1985; etc.) indicate the genotoxic nature of the components of Sevidol. Hence, this calls for caution in the safe handling of Sevidol.

Acknowledgement: The senior author is grateful to D.A.E., Bombay, for the award of a senior research fellowship.

References: Ashwood-Smith, M.J. 1981, Mutation Res. 86:137-154; Brzheskii, V.V. & V.J. Vaskov 1971, Agnew Parasitol. 13:23-28; Decloitre, F. & G. Hamon 1980, Mutation Res. 79:185-192; Dhingra, G. 1985, PhD thesis, H.A.U., Hisar, India; ——— & N.K. Vijayakumar 1987, DIS 66:12; Laxminarayana, D. & S. Subramanyam 1985, in: Intl. Conf. on "Pesticides Toxicity, Safety and Risk Assessment", ITRC, Lucknow, India Oct. 27-31, p.6; Lockard, J.M, B.P. Schuette & P.S. Sabharwal 1982, Experientia 38:686-687; Woodruff, R.C., J.P. Phillips & D. Irwin 1983, Envir. Mutagenesis 5:835-846; Würgler, F.E., F.H. Sobels & E. Vogel 1984, in: Handbook of mutagenicity test procedures (Kilbey et al., eds.), Elsevier Science Publishers, Amsterdam, p555-601; Wuu, K.D. & W.F. Grant 1967, Cytologia 32:31-41.

Dhingra, G., J.B. Chowdhury and P.K. Sareen.
Haryana Agricultural University, Hisar, India.
Genotoxic effects of a synthetic pyrethroid insecticide on Drosophila.

Synthetic pyrethroid insecticides, though of recent origin, have been found effective against a variety of insect pests of different crops under field conditions. Deltamethrin (CAS registry no. 52918-63-5), one of these newer pesticides, has been recently released for agricultural use in Haryana after successful field trials. However, the impact of this compound on the non-target systems is now known. Hence, it was tested for its genotoxicity in the germ-cell line of **Drosophila melanogaster.**

Deltamethrin (99.9%; Roussel Uclaf, France) was dissolved in DMSO (E.Merck) and mixed in the standard cornmeal-yeast-agar medium. A total of four concentrations around the LD_{50} value for male flies, which had earlier been determined (Dhingra 1985), were studied. The Basc test-protocol as given by Würgler et al. (1984) was adhered to for studying the sex-linked recessive lethal (SLRL) frequency. Male flies were exposed for 15 hr to medium containing deltamethrin and then were allowed to individually mate with Muller-5 virgin females. The data for the progeny of the F_1 crosses were studied for the induction of sterility in the treated male while the F_2 progeny was scrutinized for sex-linked recessive lethals. Normal, negative and positive controls, comprising untreated medium, DMSO (2.00 ppm) and EMS (0.50%), respectively, were simultaneously conducted for comparison purposes.

The data on sterility induction are presented in Table 1. Only one concentration, i.e., 0.40 ppm, showed an increase in percent sterility (22.70) while others showed lesser sterility induction. However, statistical analysis by the application of Z-test, after angular transformation (Fisher & Yates 1948) of the data, showed these results to be non-significant from the negative control value.

In Table 2 is given the data for the induction of SLRLs. The induced lethality was observed not to show a particular trend with the increase in the concentration of the insecticide. Though an increase to 2.45 percent was seen at 0.80 ppm, yet statistical analysis (Anova by CRD) demonstrated a lack of significant lethality as compared to the control value.

Table 1. Induction of sterility by deltamethrin in **D.melanogaster.**

Conc. (ppm)	$F_1 \times F_1$ crosses	Sterile crosses	Sterility (%)	Transformed means
0.8	890	209	19.27	24.03±3.70
0.6	827	116	13.90	20.77±2.90
0.4	828	130	22.70	24.99±7.62
0.2	693	126	19.95	26.43±1.65
D	837	216	21.37	24.58±5.55
C	783	188	21.23	25.45±5.48
E	433	116	25.25	29.34±3.40

D = 2.0% DMSO; C = untreated; E = 0.5% EMS.

Table 2. Sex-linked recessive lethal mutations induced by deltamethrin in **D.melanogaster.**

Conc. (ppm)	Chromosomes tested	SLRL	Lethality (%)	Transformed means
0.8	682	9	2.45	6.16±2.13
0.6	722	7	0.63	3.00±1.20
0.4	766	7	1.67	4.87±1.97
0.2	830	6	0.72	3.52±1.37
D	853	4	0.55	2.26±1.21
C	757	10	1.09	4.16±1.23
E	317	45	15.18	22.82±1.11*

D = 2.0% DMSO; C = untreated; E = 0.5% EMS;
* = significant at $p \leq 0.05$.

Other reports in literature also depict the non-mutagenic nature of deltamethrin as it was not found to be positive in mammals for DL (Vannier & Glomot 1977), in bacteria and yeast for genotoxicity (Bartsch et al. 1980; Kavlock et al. 1979) and for chromosomal aberrations in mice (Polakova & Vargova 1983) and in CH cell cultures (Pluijmen et al. 1984). However, the mutagenicity of deltamethrin in the bone-marrow micronucleus assay in mice (Dhingra 1985) and the induction of chromosomal aberrations in onion root-tip cells (Chauhan et al. 1986) clearly depict the clastogenic nature of deltamethrin. Hence, more studies especially in higher organisms, are needed to elucidate the exact genotoxic potential of deltamethrin.

Acknowledgement: We are grateful to Roussel Uclaf, France, for the gift of deltamethrin and to D.A.E., Bombay, for financial assistance to G.D.

References: Bartsch, H., C. Malaveille, A.M. Camus, G. Martel-Planche, G. Brun, A. Hautefeuille, N. Sabadie, A. Barbin, T. Kuroki, C. Devon, C. Piccoli & R. Montesano 1980, Mutation Res. 76:1-50; Chauhan, L.K.S., T.S.S. Dikshith & V. Sundararaman 1986, Mutation Res. 171:25-30; Dhingra, G. 1985, PhD thesis, H.A.U., Hisar, India; Fisher, R.A. & F. Yates 1948, in: Statistical tables for biological, agricultural and medical research, Oxford & IBH Publ. Co., New Delhi, p3-42; Kavlock, R., N. Chernoff, R. Baron, R. Linder, E. Rogers, B. Carver, J. Dilley & V. Simmon 1979, J. Envir. Pathol. Toxicol. 2:751-765; Pluijmen, M., C. Drevon, R. Montesano, C. Malaveille, A. Hautefeuille & H. Bartsch 1984, Mutation Res. 137:7-15; Polakova, H. & M. Vargova 1983, Mutation Res. 120:167-171; Vannier, B. & R. Glomot 1977, Report Roussel-Uclaf Research Centre, Dept. Toxicol., France; Würgler, F.E., F.H. Sobels & E. Vogel 1984, in: Handbook of mutagenicity test procedures (Kilbey et al., eds.), Elsevier Science Publ., Amsterdam, p555-601.

Dhingra, G., J.B. Chowdhury and P.K. Sareen.
Haryana Agricultural University, Hisar, India.

Preliminary studies in Drosophila treated with deltamethrin.

Integrated pest management is one of the currently used practices used to combat the ever-increasing pests of crops. It includes chemical control whereby pesticide-usage, along with other control measures, are combined to decrease the pest population. However, resistance in a pest is a common problem. In order to overcome this, newer compounds are synthesized and made use of. Deltamethrin (52918-63-5), one such compound, is a synthetic pyrethroid, very popular with farmers in N. India. However, due to a lax in precautionary measures besides the tropical conditions of our country, its uninhibited use may result in deleterious effects on the non-target species. Hence, it is of great importance to test this insecticide for its toxicity so that any detrimental consequences can be prevented to the user. Our paper relates the toxicity and dominant lethality induced by deltamethrin (99%; Roussel Uclaf, France) in **D.melanogaster** (Or-K strain maintained at 25±2°C on the standard cornmeal-yeast-agar medium).

Table 1. Effect of deltamethrin on survival in the adult **D.melanogaster** (15 hr treatment).

Conc. (ppm)	Percent mortality		
	Females	Males	Combined
5.00	100.00	100.00	100.00
4.00	100.00	100.00	100.00
3.00	92.00	98.67	95.33
2.20	78.00	94.00	86.00
2.00	69.78	87.56	78.67
1.80	69.00	90.50	79.75
1.60	61.00	84.42	75.19
1.40	48.00	82.09	65.09
1.20	44.40	78.49	61.48
1.00	33.56	65.07	52.23
0.80	23.27	48.04	38.07
0.60	23.28	40.93	33.82
0.40	10.55	19.19	15.65
0.20	0.73	4.65	3.07
0.00*	0.00	0.44	0.24

* = 2.00% DMSO in the medium.

Sets of two-to-three day old flies, males and females separately, were exposed for 15 hr to medium containing varying concentrations of the insecticide (Table 1). At each concentration, 200-250 flies of each sex were given treatment. Mortality data were plotted on a probit-logarithm graph (Gupta 1980) to determine the lethal dose-50 values (LD_{50}). A total of 14 concentrations, varying from 0.20 to 5.00 ppm, were tested. A slow but steady increase in percent mortality was observed with the increase in concentration of deltamethrin. The females were found to be more resistant (LD_{50} = 1.40 ppm) as compared to males (LD_{50} = 0.80 ppm) while pooled LD_{50} was 1.10 ppm (Figures 1-3). Above 0.80 ppm, a sudden spurt in percent mortality was noticed and at 4.00 and 5.00 ppm,

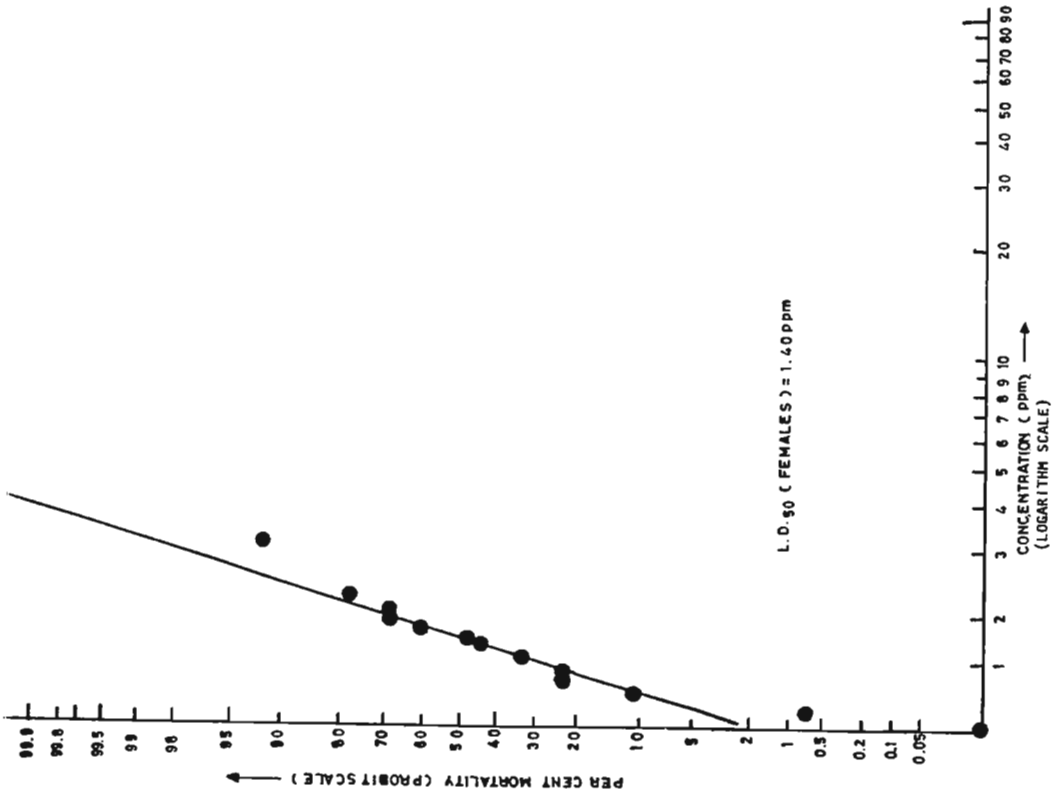


Figure 1. Effect of deltamethrin on survival in adults females of **D.melanogaster** (15 hr treatment).

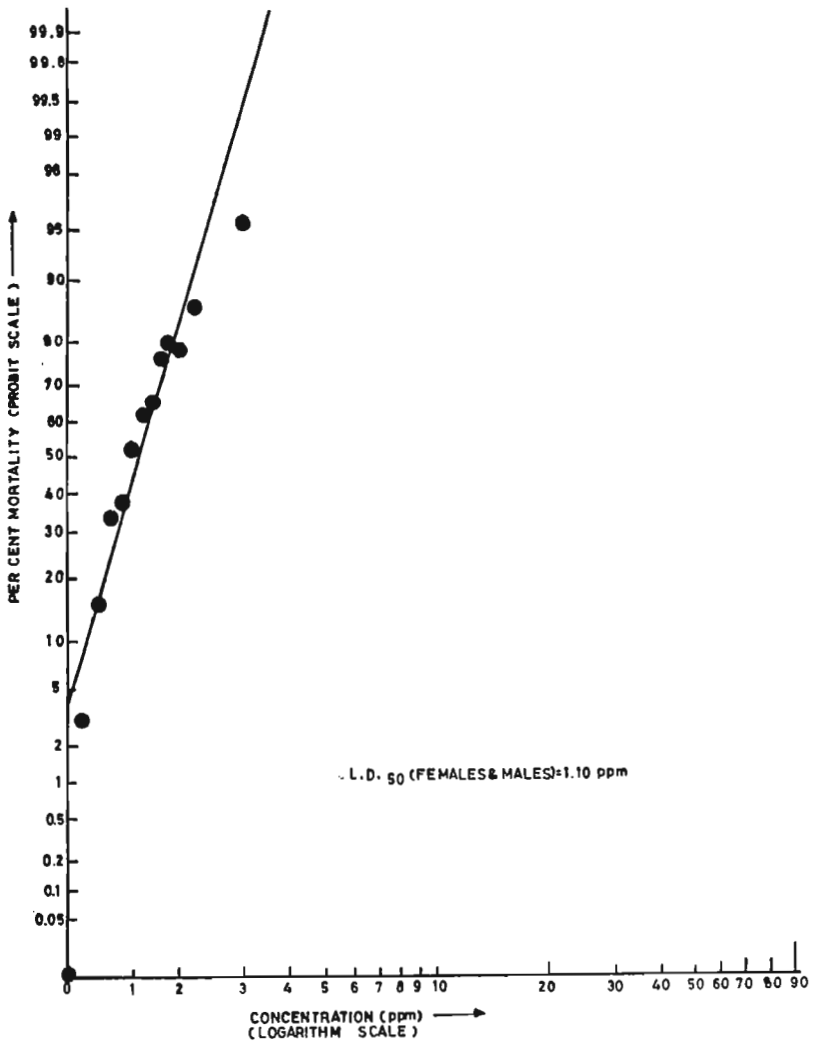


Figure 3. Effect of deltamethrin on survival in the adult flies of **D.melanogaster** (15 hr treatment).

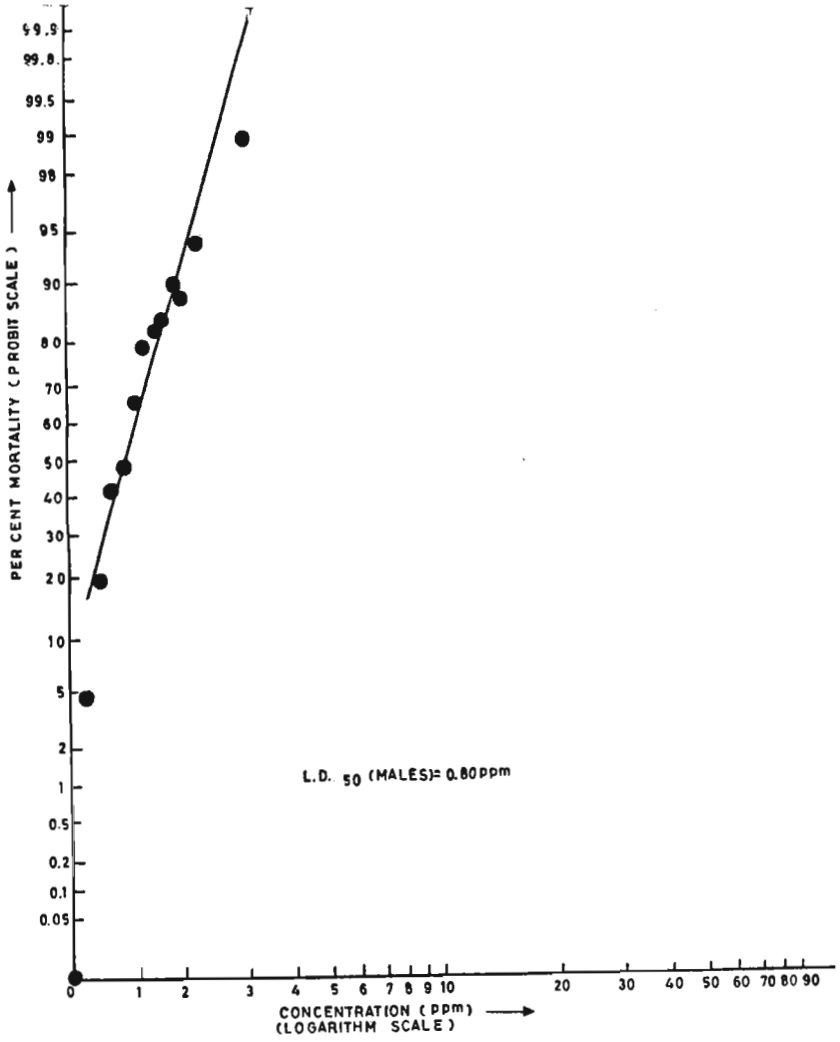


Figure 2. Effect of deltamethrin on survival in adult males of **D.melanogaster** (15 hr treatment).

Table 2. Dominant lethal mutations induced by deltamethrin in **D.melanogaster.**

Conc. (ppm)	Eggs tested	Unhatched eggs	Lethality (%)	Transformed means ± S.E.
1.0	1131	549	50.05	45.03±7.86
0.8	1713	735	43.02	40.97±2.38
0.6	1490	713	47.68	43.64±4.99
0.4	1436	533	44.22	41.25±8.42
0.2	1513	572	39.58	38.94±2.26
D	1382	599	47.82	43.69±3.77
C	1412	549	39.10	38.69±1.43
E	862	769	83.13	72.95±11.41*

D = 2% DMSO; C = untreated; E = 1.3% EMS;
* = significant at $p \leq 0.05$.

100 percent mortality was observed. This high order of toxicity in Drosophila is also reflected in other organisms: acute toxicity to bees was 0.51 µg/bee; LD_{50} for fish was 0.08-0.10 ppm (RAIS 1981); LD_{50} for house fly was 0.025 g/g (Roussel Uclaf 1982). In mammals also, varying dose levels of deltamethrin were found to be moderately toxic in different species (Roussel Uclaf 1982; Polakova & Vargova 1983; Dhingra 1985).

For DL studies, five concentrations (1/2, 2/5, 3/5/, 4/5 and approximately LD_{50}) were selected. Treated males were mated with virgin females and about 1000 eggs per concentration were screened for unhatchability (Table 2). Except for 0.80 ppm, in all other concentrations, increased lethality was noted. However, statistical analysis, ANOVA using CRD after angular transformation of the data (Fisher & Yates 1948), depicted the results to be not significant from normal (untreated flies) and negative (2% DMSO) controls, thereby indicating the non-incurrence of genetic damage by deltamethrin at the concentrations tested. The positive control (1.3% EMS) induced significant dominant lethality, hence, confirming the validity of the test-assay.

The non-mutagenic response of deltamethrin to Drosophila is akin to studies with this insecticide in yeast (Vannier & Glomot 1977), bacteria (Bartsch et al. 1980), mice (Polakova & Vargova 1983) and CH cells (Pluijmen et al. 1984). However, induction of chromosomal aberrations in **Allium cepa** root tip cells (Chauhan et al. 1986) and micronuclei in the bone-marrow cells of mice (Dhingra 1985) after treatment with deltamethrin, as examples of experiments conducted in our climatic conditions, clearly indicate the clastogenic nature of this pyrethroid, at least in the somatic cells. This calls for its judicious use so that excess chemical burden on the environment can be lowered.

Acknowledgement: We are thankful to Roussel Uclaf, France, for the generous supply of deltamethrin and to D.A.E., India, for the award of SRF to the senior author.

References: Bartsch, H., C. Malaveille, A.M. Camus, G. Martel-Planche, G. Brun, A. Hautefeuille, N. Sabadie, A. Barbin, T. Kuroki, C. Devon, C. Piccoli & R. Montesano 1980, Mutation Res. 76:1-50; Chauhan, L.K.S., T.S.S. Dikshith & V. Sundararaman 1986, Mutation Res. 171:25-30; Dhingra, G. 1985, PhD thesis, H.A.U., Hisar, India; Fisher, R.A. & F. Yates 1948, in: Statistical tables for biological, agricultural and medical research (3rd Ed.), Hafner Publ. Co., New York; Gupta, D.S. 1980, in: Residue analysis of insecticides (D.S. Gupta, ed.), H.A.U. Press, Hisar, India, p75-87; Pluijmen, M., C. Drevon, R. Montesano, C. Malaveille, A. Hautefeuille & H. Bartsch 1984, Mutation Res. 137:7-15; Polakova, H. & M. Vargova 1983, Mutation Res. 120:167-171; Rallis Agricultural Information Service, Bangalore, India 1981, No. 54:1-8; Roussel-Uclaf 1982, Deltamethrin Monograph, Roussel-Uclaf, France; Vannier, B. & R. Glomot 1977, Report Roussel-Uclaf Research Centre, Dept. Toxicol., France.

Di Pasquale Paladino, A., P. Cavolina, G. Romano and R. Ribaudo. University of Palermo, Italy.

The tu-pb melanotic tumor strain of **Drosophila melanogaster**: cytological analysis of salivary chromosomes.

Some features of the tu-pb melanotic tumor strain of **Drosophila melanogaster**, as penetrance variation (Di Pasquale Paladino et al. 1985), lack of response to selection (Di Pasquale Paladino et al 1985) and failure to obtain 2nd chromosome isogenic tu-pb lines (unpubl. observations) have prompted us to undertake a cytological analysis of tu-pb polytene chromosomes.

Temporary lacto-aceto-orcein squash preparations of larval salivary gland chromosomes were made by the usual procedure and were interpreted with the aid of the revised polytene chromosome map (Lefevre 1976). Synchronous 120 hr aged larvae reared at 23.5°C were used and at least 10 well analysable nuclei of 48 ♀♀ and 42 ♂♂ tu-pb larvae were studied. Oregon-R was used as control.

Previous genetic study (Di Pasquale Paladino et al. 1983, 1987) demonstrated that the tu-pb phenotype depends on at least two different loci, one recessive on the 3rd chromosome and the other one, semidominant, on the 2nd chromosome. The cytological observations revealed peculiar pairing irregularities affecting both 2nd and 3rd chromosomes. We considered the occurrence of the same site alterations in more than 20% of individual events deviating from randomness and therefore imputable to underlying chromosomal constitution irregularities. Inter- and intra-individual frequencies of the detected anomalies are presented in the histogram of Figure 1.

Chromosome arm 2L presents rings and hairpins binding regions 21-25, one or more extra inserted bands or band delection are also visible. Rings and hairpins appear in the regions 25-30, too. The binding

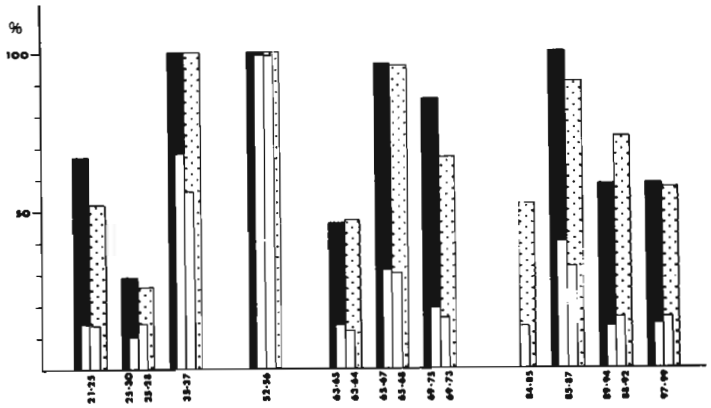


Figure 1. Incidence of chromosomal irregularities concerning female (filled bar) and male (dotted bar) populations or female and male (empty bar) individuals.

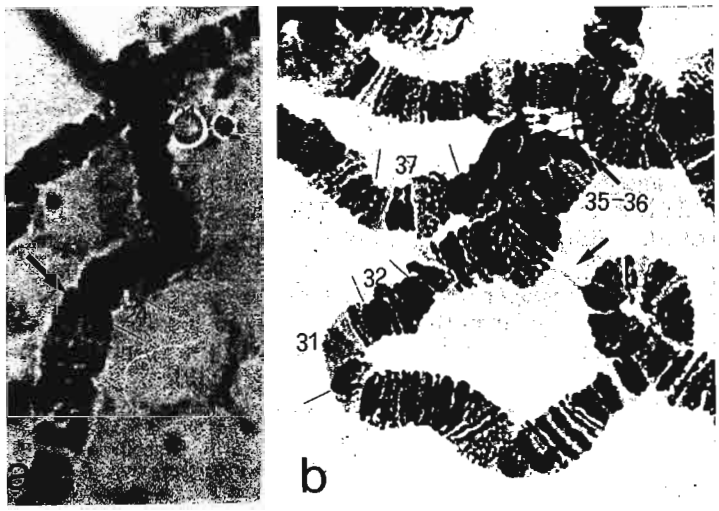


Figure 2. Chromosome arm 2L: (a) heterozygous extraband in region 24; (b) pairing irregularities in region 35.

Figure 3. Chromosome arm 2R: asynapsed omologous with heterozygous inversion in region 52-56.

pattern in regions 35-37 is always irregular (Figure 2). In chromosome arm 2R there is a heterozygous inversion including regions 52-56 (Figure 3); some genetic data are also consistent with this observation (Di Pasquale Paladino et al. 1987). In chromosome arm 3L rings, hairpins and extra-bands affect moderately regions 63-65, and more frequently regions 65-68. Rings and hairpins are common also in regions 69-75, ectopic threads bind these positions to heterochromatic ones on the same or other chromosomes (Figure 4). Chromosome arm 3R presents rings, hairpins and heterochromatic blocks affecting regions 84-85 (only in ♂♂), 85-87, 88-94 and 97-99 (Figure 5). The pairing chromosome patterns in heterozygous tu-pb/Oregon-R larvae are in agreement with these observations.

Considering the irregularities found, strong affinity sites along the chromosome can account for the occurrence of some of them. This may be the case of ring formation in absence of a heterozygous inversion, since clearly detectable heterozygous inversions recur in the same positions, ring formation might be an event preceding and favouring the chromosomal rearrangement. As regards hairpins, they are known to be due to the presence of inverted repeats. Amorphous heterochromatic masses, unexpected by Bridges's map may also create pairing affinities. Extra inserted bands and deleted bands are both indicative of movements of the genetic material throughout the genome. The recurrence of the irregularities, yet not at random, but with fluidity in relation to the inter- and intra-individual degree of manifestation may be due to the opportunity to assume the typical conformation as well as to the presence-absence of the alteration itself. As regards the nature of irregularities, some of their characteristics seem to account for the presence of transposable elements. In our analysis we find high concordance

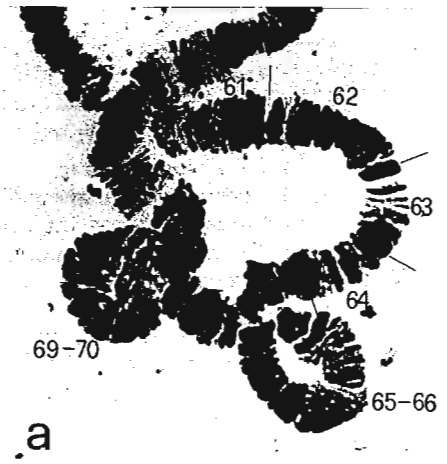


Figure 4. Chromosome arm 3L: (a) inversion rings in sections 65-66 and 69-70; (b) hairpin in region 70-71.

Figure 5. Chromosome arm 3R: ectopic thread and ring formation in region 86-87.

between the positions of the detected irregularities and the sites known to be the target of TEs; moreover, chromosomal morphology is in many cases very similar to that conferred by TEs insertions. This is the case of the region 35 for TE 146 insertion (Gubb et al. 1985) or the extrabands occurring in the region from 21 to 25 (TE 57, TE 30, TE 114, etc.) (Gubb et al. 1986). The fact that some of the TEs in the 2nd chromosome are associated with lethality (Ising et al. 1981) supports their presumed presence in the tu-pb strain (Di Pasquale Paladino et al. 1987). To confirm our hypothesis, we plan to perform in situ hybridization experiments to test the presence of TEs, or other transposable elements, in the tu-pb genome.

Acknowledgement: This work was supported by a grant from M.P.I. (Quota 60%, 1985).

References: Di Pasquale Paladino, A. & P. Cavolina 1983, DIS 59:31-33; ———, ———, G. Romano & R. Ribaudo 1987, DIS 66:47-48; ———, ——— & A. Vella 1985, DIS 61:66; Gubb, D., J. Roote, G. Harrington, S. McGill, M. Shelton & M. Ashburner 1985, Chromosoma 92:116-123; ———, ———, S. McGill, M. Shelton & M. Ashburner 1986, Genetics 112:551-575; Ising, G. & K. Block 1981, Cold Spring Harbor Symp. Quant. Biol. 45:527-544; Lefevre, G. 1976, in: Genetics and Biology of Drosophila (Ashburner & Novitski, eds.), 1a:31-66.

Duttagupta, A.K. and N. Manna. University of Calcutta, India. Hybrid dysgenesis in **Drosophila ananassae.**

When females of particular strains of a Drosophila species are crossed with the males of other strains of the same species, the hybrid progenies are sometimes found to be sterile or are observed to produce mutant progenies in F_2 generation. These traits viz. sterility, mutation induction along with some other anomalies in F_2 are collectively known as "hybrid dysgenesis" (Kidwell & Kidwell 1977). In **D.melanogaster** this phenomenon has been extensively studied by various workers (see review by Engels 1988). It has been established in **D.melanogaster** that the presence of 'P' transposable element in the genome of male parent causes such dysgenesis in the hybrid progenies if the maternal parent lacks this transposable element. Very little, however, is known about the genetic basis of hybrid dysgenesis.

We became interested in probing similar phenomenon in **D.ananassae**, a species that is endemic in this sub-continent.

For this, we used two parameters: (1) female sterility or gonadal dysgenesis and (2) mutation frequency in F_2. For the sterility test, we studied about 40-50 hybrid females from different crosses. Percentage of hatchability and survival was also estimated from the total number of eggs, pupae and adults. For complete sterility test, the hybrid females that lay less than 5-6 eggs were regarded as sterile. Gonads of such sterile females (about 10 days aged) were dissected out to study the morphology. Mutation frequency (visible) was calculated from the F_2 progeny.

Table 1. Showing the percentage of hatchability (Col. III), survival rate (Col. IV) and induced mutations in F_2 progenies (Col. V). Col. I represents the parents of cross 1A, 1B, 2A, 2B, 3A, 3B, 4A and 4B. Col. II represents the C_{1A}, C_{1B}, C_{2A}, C_{2B}, C_{3A}, C_{3B}, C_{4A} and C_{4B}, the F_2 progenies of respective crosses of Col. I. The maternal sources are shown in parentheses.

I Parental cross type	II F_1 X F_1 —> F_2(C)	III % of hatchability	IV % of survival	V Percentage and type of mutation
1A] Behala ♀ X Agartala ♂	C_{1A} (Behala cytoplasm)	66%	63.5%	Brown eye - 0.79% Curled wing - 0.17%
1B] Behala ♂ X Agartala ♀	C_{1B} (Agartala cytoplasm)	88%	79.4%	---
2A] Patna ♀ X Agartala ♂	C_{2A} (Patna cytoplasm)	66%	59.0%	Dysgenesis - 13.30%
2B] Patna ♂ X Agartala ♀	C_{2B} (Agartala cytoplasm)	67%	65.0%	---
3A] Agarpara ♀ X Agartala ♂	C_{3A} (Agartala cytoplasm)	78%	75.0%	Curled wing - 0.36%
3B] Agarpara ♂ X Agartala ♀	C_{3B} (Agartala cytoplasm)	82%	76.0%	---
4A] a^6Cal ♀ X Garia ♂	C_{4A} (a^6Cal cytoplasm)	70%	67.0%	Deep red eye - 1.90% Downey wing - 0.95%
4B] a^6Cal ♂ X Garia ♀	C_{4B} (Garia cytoplasm)	91%	81.0%	---

Figure 1. Ovaries in normal (b) and dysgenic (a) **D.ananassae** female.

Results (Table 1) reveal that in Cross 1A, the F_2 progenies show mutant features like brown eyes and curled wing. However, none of the F_1 progenies were sterile, whereas in the reciprocal crosses (1B) no such mutation could be recorded.

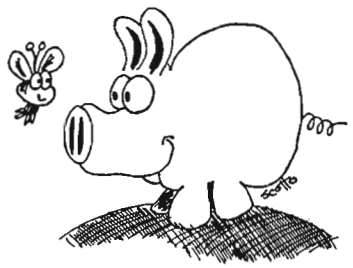
In Cross 2A, the hybrid females show about 13.3% complete sterility and the percentage of hatchability is quite low. Such females had dysgenic gonad (Fig. 1a & b). The reciprocal cross (2B), however, showed no such dysgenic traits.

Cross 3A is characterised by wing mutation curled, observed in F_2 (Table 1, column II) at a frequency of 0.36%. Interestingly again, here also the reciprocal crosses (3B) gave all normal progenies.

In Cross 4A, we have obtained an eye colour mutation (deep red) and a wing mutation (downey like) in the F_2 (Table 1, column II) with a frequency of 1.90% and 0.95%, respectively. In the reciprocal cross no such mutations were obtained.

It seems therefore that out of these tested strains of **D.ananassae**, viz., **Agartala** and **Garia** act like inducer (P ?) strains and **Behala, Patna, Agarpara** and **a^6Cal**, act as reactive (M ?) strains. The induction of mutation or sterility in the progeny of certain crosses and its absence in the reciprocal crosses led us to assume the presence of some insertional sequences that interfere with the structural regulation of the normal gene as is observed in **Drosophila melanogaster** (Rubin et al. 1982). We are now looking for different probes that may hybridize with polytene chromosome in a specific region to cause such anomalies. Until such experiments are done, any constructive suggestion on the cause of such dysgenesis in **D.ananassae** would only be speculative.

References: Engels, R.W. 1988, in: Mobile DNA (D. Berg & M. Howe, eds.), ASM Publication; Kidwell, M.G., Kidwell & Sved 1977, Genetics 86:813; Rubin, G.M., M.G. Kidwell & P.M. Bingham 1982, Cell 29:987.



Eberl, D.F., A.J. Hilliker and R.A. Voelker.*
University of Guelph, Ontario, Canada; *NIEHS, Research Triangle Park, North Carolina, USA.

Determination of lethal phases of mutations in the su(s) microregion.

A genetic saturation mapping of the suppressor of sable [su(s)] region of the X chromosome has been conducted (Voelker et al., in prep.). The region analyzed is that included within Df(1)su(s)^{83}, approximately 1B9-10 to 1E. Ten complementation groups (listed in Table 1 distal to proximal) were detected, su(s), twisted (tw) and eight loci that are lethal-mutable. To gain some insight into the biological time of action of the eight lethal-mutable genes in this region, their lethal phases were determined. The nomenclature of these loci follows the system of Lindsley & Zimm (DIS 64).

Most mutations studied are present on standard sequence X chromosomes marked with y and were balanced with Binsn. Eggs were collected from these balanced stocks by the method of Eberl & Hilliker (Genetics 118:109-120, 1988) and the hatch fraction was determined after 30 hr. All eggs which failed to hatch were dechorionated with bleach and examined as whole mounts using brightfield microscopy. Larvae with y mouthparts were transferred from the collection plates, placed in a 5 ml polystyrene tube containing a small plug of medium, and further observed for signs of lethality.

Males carrying the A89 allele of the multiple (mul^{5}) locus die at the late embryonic stages with no gross morphological abnormalities except the presence of some unmetabolized yolk in the gut. A few survive to hatching but die soon afterwards.

HM441 [=l(1)1Bk^{2}] causes most zygotes to die as early larvae. However, a few mutant embryos fail to hatch, as indicated by the increased frequency of unhatched fertilized eggs, some of which have y mouthparts.

The tested mutations at two loci l(1)1Bi^{6}(=A7) and l(1)1Da$^{1\&2}$ (=HM454 and A29, respectively) cause zygotes to die at early larval stages (first or second instar). No morphological abnormalities are visible in these mutant larvae under the dissecting microscope.

Lethal mutations at three loci cause pupal death. Pupae carrying l(1)1Bh^{8} (=A90) show extensive histolysis. Some pupae appear to have everted appendages, but these pupae are also histolyzed anteriorly. Mutant pupae of l(1)Ca^{5} (=A19) develop to the point of having pigmented eyes and everted appendages. brc^{6} (brachymachrochaetae) (=DCE12) pupae also show considerable pupal histolysis, especially at the anterior end.

A large number of su(s) alleles has been generated and none exhibit a lethal phenotype, although a few alleles have reduced viability. None of these alleles were studied in detail.

The final locus to be discussed, Minute (1)1B [M(1)1B] [formerly Minute (1) Blond], is represented by only one point mutation, B46, present on Dp(1;3)E1. Lethality of this mutation was studied by examining the offspring of the cross described in Table 1, footnote a. There are two lethal classes, one representing Df(1)su(s)^{83} and the other B46. These are distinguishable in late embryonic through adult stages with the y marker present in the Df(1)su(s)^{83} class, y^{+} in the B46 class. Df(1)su(s)^{83} causes lethality at the embryo-larval boundary, which is consistent with the earliest acting gene within it. B46 has a wide lethal phase, with most individuals dying as larvae, especially in the first two instars, several achieving the third instar, and a few individuals struggling to pupate 2-3 days later than sibs.

In summary, mutations at two loci are lethal at the embryo-larval boundary, two in the first or second instars, one variable through larval and early pupal stages, and three in pupal stages. Two are not lethal. Embryos carrying the deficiency which defines the region have the same lethal phase as the earliest mutant within it.

Table 1. Analysis of lethal phases.

locus/allele	Total eggs	Fert. eggs	%fert. unhatch	# y larvae collected	#pupae	#adults
l(1)1Bh^{8} (=A90)	82	77	2.6%	20	18(90%)	0
l(1)1Bi^{6} (=A7)	100	98	4.1%	20	0	0
M(1)1B (=B46)[a]	144	132	7.5%	111	84(76%)	77(92%)
su(s)	no lethal alleles					
l(1)1Bk^{2} (=HM441)	113	108	7.4%	20	0	0
l(1)1Ca^{5} (=A19)	110	98	5.0%	20	18(90%)	0
mul^{5} (=A89)	147	142	19.0%	b	--	--
tw	no lethal alleles					
l(1)1Da^{1} (=HM454)	81	77	2.6%	20	0	0
l(1)1Da^{2} (=A29)	100	94	4.0%	20	0	0
brc^{6} (=DCE12)[c]	55	52	0.0%	52	47(90%)	34(72%)

a=The B46 allele of M(1)1B was induced on Dp(1;3)E1,y^{+}, which is duplicated for the region deleted by Df(1)su(s)^{83}. Lethality of B46 was determined by observing the progeny of Df(1)su(s)^{83}, y/FM7, y^{2}; Dp(1;3), E1, y^{+} B46/+ virgin females crossed to FM7,y^{2}/Y males. Two of the eight classes of offspring are lethal. Df(1)su(s)^{83}, y/Y individuals die at the embryo/larval bounday, while Df(1)su(s)^{83}, y/Y; Dp(1;3), E1, y^{+} B46/+ individuals die as larvae, though a few are able to pupate 2-3 days after normal sibs pupate. b=A few larvae were able to hatch, but these were lethargic and died in first instar. c=DCE12 (=brc^{6}) is not marked with y, so the lethal class cannot be distinguished. Therefore all larvae were observed through development.

Furman, D.P. and S.A. Zabanov. Inst. of Cytology & Genetics, Novosibirsk USSR.

Influence of P element insertion on conjugated expression of scute, singed, forked mutations in **Drosophila melanogaster.**

Sixteen scute alleles were induced in P-M hybrid dysgenesis system to analyse the relationship between mutation process in the scute locus and varioius transposable element insertion in 1B3-4 region of **Drosophila melanogaster** X chromosome. The mutant stocks were maintained for 30-40 generations of mass-mating and their phenotypic expression with respect to bristle pattern remained quite stable during this period.

Seven mutations were examined for the presence in 1B3-4 region of the transposable elements P, copia, mdg 1, mdg 2, mdg 3, mdg 4 by in situ hybridization of salivary gland polytene chromosomes with the corresponding nick-translated, tritiated DNA sequences probes. The results obtained were negative in all but two instances when the sequences homologous to mdg 3 or P element found in the region of interest.

In the course of cultivation of the lines induced, spontaneous mutations were observed at the yellow, singed, raspberry, forked and other loci. In particular, a male with singed bristles has arisen within sc34P stock characterised by pv^{1-2} and h^{1-2} bristle pairs reduction (Table 1). This male was used as a founder to a new mutant stock. The mutation uncovered was shown to be allelic to the singed gene (1-21.0; 7D1-2), and the corresponding mutant stock was designated as sn34P. It should be noted especially that mutation at the singed locus as well as phenotypic normalization of the scute allele took place simultaneously (Table 1).

As to the hypothesis about the role of the male-founder of sn34P line in this reversion to wild type regarding the bristle reduction, it seems very implausible. Indeed, when single males from sc34P stock with different bristle pattern reduction were crossed with C(1)DX,ywf females, in F_1 offspring the whole spectrum of phenotypes typical for the initial sc34P stock appeared, although the ratio of the phenotypes varied for different males (Table 2).

Table 1. Percent of bristle reduction in sc34P and sn34P stocks (100 flies scored).

	♀♀ ++		♂♂	
Bristles	sc34P	sn34P	sc34P	sn34P
pv^1	55.0	--	59.0	--
pv^2	59.0	--	62.0	--
h^1	60.5	3.0	54.5	1.0
h^2	58.5	0.5	56.0	--

Table 2. Phenotypic variety among F_1 offspring produced from individual sc34P male with C(1)DX,ywf females cross.

	OFFSPRING PHENOTYPES			
	reduction	reduction	reduction	
wild type	of pv^{1-2},h^{1-2}	of pv^{1-2}	of h^{1-2}	total
wild type*				
46	13	16	25	100
reduction of h^{1-2}*				
31	14	2	53	100
reduction of pv^{1-2},h^{1-2}*				
5	45	27	23	100

*=male phenotype.

In addition, the sn34P mutant was revealed to be allelic to the forked gene (1-56.7; 15F1-3). All the heterogeneous females obtained in crosses sn34P males with tester lines ywsn or $ysc^{D1}f$ demonstrated the mutant bristle phenotype. Thus, sn34P line should be considered as the result of the simultaneous parallelic mutating of two genes, sn and forked, rather than of two subsequent mutational events.

Hybridization patterns of P element with salivary gland polytene chromosomes from sc34P and sn34P mutants were compared (Table 3).

The following conclusions may be drawn: (1) there were no hybridization sites in 1B3-4 and 7D1-2 bands in sc34P stock (Figure 1a); (2) in sn34P chromosomes the pattern of 11 hybridization sites typical for parental sc34P stock was conserved; (3) moreover, some new hybridization sites have been uncovered in sn34P line including 1B and7D regions corresponding to the scute and singed gene locations (Figure 1b); (4) 15F region corresponding to the forked gene was labeled in both strains.

Table 3. Sites of hybridization of P element with salivary gland X chromosomes in sc34P and sn34P mutants.

	1B	3A	3C	4D	5A	6B	6F	7D	8C	10A	10F	12D	13A	15F	16A	17C	19A
sc34P		+			+	+	+		+	+	+			+	+	+	+
sn34P	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Figure 1. In situ hybridization of sc34P (a) and sn34P (b) mutants with a P-containing probe. The arrows point to the hybridization grains located over 1B and 7D regions.

Galiana, A. and A. Moya. Universitat de Valencia, Burjassot, Spain. Isolation among ancestral and derived strains of **Drosophila pseudoobscura.**

Here we report preliminary results concerning isolation among ancestral and derived strains of Drosophila. The protocol used to derive lines is conceived to imitate under laboratory conditions the founder-flush speciation mechanism of Carson (1971), taking into account Templeton (1980) suggestions. This has been previously made in somewhat different ways by Powell (1978) and Ringo et al. (1985). Our own design is as follows.

Two ancestral populations of **D.pseudoobscura** were established in 1984: BCA from captures made in Bryce Canyon, Utah, and MA from Zirahuén, Michoacán, two places 2000 km apart. Each one is the result of combining isofemale lines and mass cultures from their unique geographic origin. After 6 months a number of derived lines were initiated, 27 from BCA and 18 from MA. Each line was first founded, and is re-founded after each flush-crash cycle, with n couples of flies, being n=1,3,5,7 or 9. A cycle consists of four generations of exponential growth -- flush -- and one of strong competition --crash. All populations are maintained in bottles. For the present work we chose all lines with n=1 or 3, plus one line per origin for the other n values.

Multiple choice mating tests between two strains were performed putting together 12 virgin flies of each sex-strain combination for 45 min in a cage. The derived lines used in tests were obtained from protocol populations after four flush-crash cycles. The wings were clipped and used to distinguish

Table 1. Results of multiple choice mating tests.

Strains			matings(x_{ij})					Strains			matings(x_{ij})				
a	d	(n)	x_{aa}	x_{ad}	x_{da}	x_{dd}	I	a	d	(n)	x_{aa}	x_{ad}	x_{da}	x_{dd}	I
BCA	BC2	1	15	10	19	19	0.079	MA	M3	1	26	26	22	29	0.067
	BC4	1	18	23	12	14	-0.044		M5	1	15	16	13	11	-0.035
	BC32	1	11	16	15	10	-0.192		M37	1	12	7	19	9	-0.106
	BC33	1	17	15	12	17	0.114		M7	3	27	21	22	16	0.000
	BC7	3	24	18	27	24	0.032		M8	3	24	12	12	16	0.250*
	BC9	3	12	16	23	21	-0.083		M10	3	13	11	9	13	0.130
	BC10	3	15	15	12	15	0.052		M11	3	13	12	8	15	0.166
	BC11	3	20	13	11	12	0.142		M12	3	20	12	3	7	0.285$
	BC12	3	8	19	18	12	-0.298*		M36	3	7	12	12	13	-0.090
	BC34	3	17	15	14	10	-0.021		M13	5	31	19	20	26	0.187$
	BC13	5	28	19	21	32	0.200*		M19	7	14	25	14	19	-0.083
	BC19	7	15	14	13	19	0.114		M25	9	22	15	18	20	0.120
	BC25	9	11	11	11	18	0.137								

i=male type; j=female type; a=ancestral; d=derived; n=number of couples of founders after each flush-crash cycle; *=p<0.05; $=p<0.10.

individuals of the same sex; the possible bias effect was discarded by chi-square tests. Four repeats were carried out for each cross.

Data are shown in Table 1, including the joint isolation index, I (see Gilbert & Starmer 1985). Several cases of significant deviation from random mating are detected. Only in one case (BCA x BC12) the result is significant heterogamy. This could be due to a particularly negative inbreeding of the BC12 strain. The remaining tests clearly indicate the potential of this experimental procedure to promote sexual isolation. There is an obvious chance component provided that only sporadically a derived line becomes more or less isolated.

References: Carson, H.L. 1971, Stadler Genet. Symp. 3:51-70; Gilbert, D.G. & W.T. Starmer 1985, Evolution 39:1380-1383; Powell, J.R. 1978, Evolution 32:465-474; Ringo, J.M., D. Wood, R. Rockwell & H.B. Dowse 1985, Amer. Nat. 126:642-661; Templeton, A.R. 1980, Genetics 94:1011-1038.

García-Vásquez, E. and F. Sánchez-Refusta.
Universidad de Oviedo, Spain.

Chromosomal polymorphism and macrochetae in a natural population of **D.melanogaster** studied by isofemale lines.

Chromosomal inversions generally show a high polymorphism in natural populations of insects (Ashburner & Lemeunier 1976). The correlation between this variability and morphological characters has been studied in different species by some authors (White & Andrew 1962; Prevosti 1967; Aquadé & Serra 1980; Butlin et al. 1982). Higher variation of dorsocentral and scutellar bristles was correlated in ten wild populations caught in Asturias (Spain) with a great frequency of In(3R)C inversion (Sánchez-Refusta & García-Vásquez 1986). We think this correlation is due to the presence of polygenes in the zone overlapping such inversion; these polygenes increase the expression of the extra bristles (Rubio & Albornoz 1982).

This correlation was found considering each population as unity; we address a question now: could this phenomenon repeat if we take as unity each isofemale line, and not each population?

In order to answer this question, a sample of 130 males and 62 females of **D.melanogaster** was captured in Villaneuva (Asturias, Spain), by means of banana and yeast traps. An isofemale line was founded with the progeny of each female. In such isofemale line the variation for the extra bristles, canalized in wild populations for the normal phenotype (Figure 1), can be studied. In the first generation in the laboratory, the inversions of 3 larvae and the dorsocentral and scutellar bristles of 50 males and 50 females from every line were analysed. The inversions were studied in the salivary gland chromosomes (Levine & Schwartz 1970), by direct analysis of the chromosomal pattern (Lefévre 1976)./ The studied cosmo-

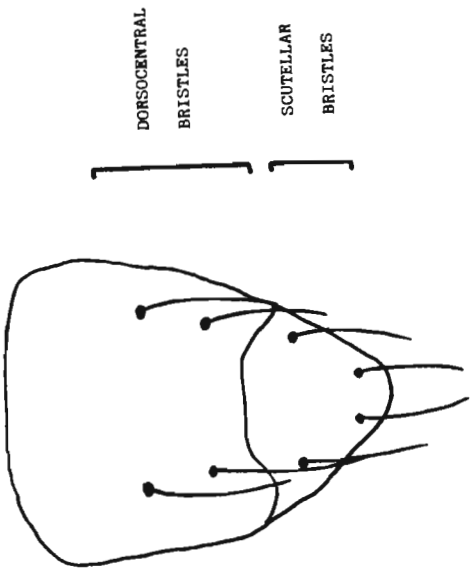


Figure 1. Normal phenotype canalized in wild populations.

Table 1. Analysis of population. (A) Inversions: frequency of heterozygotes for each inversion. N.A. = number of chromosome arms. (B) extra bristles: distribution of lines by their percent of individuals with extra bristles. N.L. = number of isofemale lines.

A):

Homozygotes	In(2L)t	In(2R)NS	In(3L)P	In(3R)P	In(3R)C	N.A.
72.3%	9.2%	4.1%	3.6%	4.7%	6.1%	752

B):

N.L.	less than 5%	5%-20%	more than 20%
62	24.2%	58.1%	17.7%

Table 2. Mean number of extra bristles of the lines with each frequency of inversion heterozygotes. N.I.=number of heterozygote chromosome arms. N.L.=number of lines with each N.I. M.E.B.=mean number of extra bristles of these lines. Test of the correlation between both characters: r coefficient and its significance.

N.I.	N.L.	M.E.B.
0	8	0.237
1	4	0.132
2	8	0.117
3	6	0.153
4	18	0.103
5	10	0.114
6	5	0.174
7	2	0.170
10	1	0.460

r = -0.0542, 6 d.f., N.S.

Table 3. Mean number of extra bristles of the lines with each frequency of In(3R)C heterozygotes. N.I.=number of In(3R)C heterozygote chromosome arms. N.L.=number of isofemale lines of each N.I. M.E.B.=mean number of extra bristles of these lines. Test of the correlation between both characters: b regression coefficent and test t for its difference with b=0.

N.I.	N.L.	M.E.B.
0	33	0.145
1	14	0.128
2	13	0.156
3	2	0.200

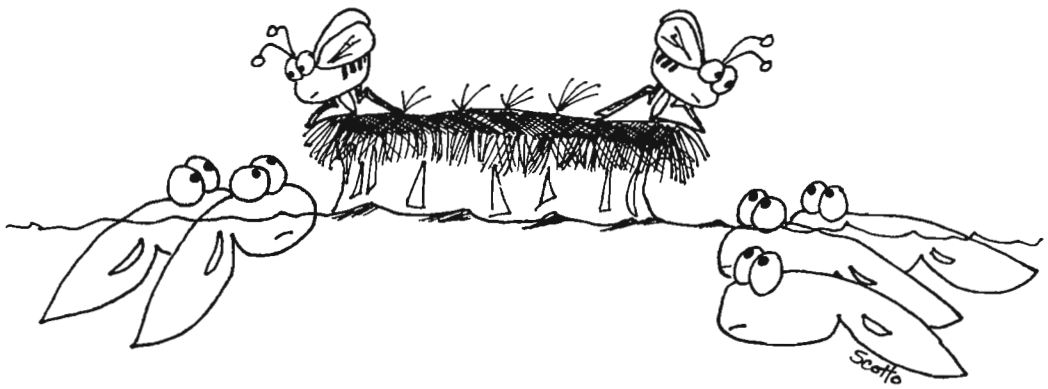
b = 0.0075; $t_{b=0}$ = 0.3225, 60 d.f., N.S.

politan inversions are In(2L)t, In(2R)NS, In(3L)P, In(3R)P and In(3R)C. The frequency of inversions is measured as the frequency of inversion heterozygotes. The isofemale lines are classified attending to the percentage of individuals with extra bristles.

In Table 1, the analysis of the 62 isofemale lines is presented. The variability level is high enough for the inversions (Inoué & Watanabe 1979) and for the extra bristles (Parsons 1980); nevertheless, there is no correlation between both characters (r = -0.054, N.S., see Table 2), and neither the number of bristles increase when the In(3R)C inversion has a high frequency (b = 0.007, N.S., see Table 3): the lines with more extra bristles do not have higher frequency of such inversion. The correlation obtained by taking population as unity was very high between both levels of variability (Sánchez-Refusta & García-Vásquez 1986); but in a single couple (the founder of each isofemale line), there is less variability than in a population (or a whole of lines), and this may be the reason a correlation is not found by taking lines as a unity. Nevertheless, lines with a high frequency of In(3R)C would have a high proportion of individuals with extra bristles, if our hypothesis is true.

Another explanation could exist for this absence of correlation: not all In(3R)C of the Asturian populations would carry the same factors for increasing the number of bristles. Although they have a common origin, each inversion could accumulate by mutation different variants in each locus determining bristles, and consequently in some populations, In(3R)C would be associated with the normal bristle phenotype. This could be the case of the studied population.

References: Aguadé, M. & L. Serra 1980, Genetika 12:111-120; Ashburner, M. & F. Lemeunier 1976, Proc. R. Soc. Lond. B 193:137-157; Butlin, R.K., I.L. Read & T.H. Day 1982, Heredity 49:51-62; Inoué, Y. & T.K. Watanabe 1979, Jpn. J. Genet. 54:69-82; Lefèvre, G. Jr 1976, in: Genetics and Biology of Drosophila, Academic Press, v1a:32-64; Levine, L. & N.m. Schwartz 1970, in: Laboratory Exercises in Genetics, C.V. Mosby Co. ed.: 65-70; Parsons, P.A. 1980, Evol. Biol. 13:175-217; Prevosti, A. 1967, Genet. Res. 10:81-93; Rubio, J. & J. Albornoz 1982, Rev. Real Ac. Cien. Ex. Fis. y Nat. LXXVI:773-802; Sánchez-Refusta, F. & E. Garcia-Vásquez 1986, DIS 63:118-119; White, M.J.D. & L.E. Andrew 1962, in: The Evolution of Living Organisms (G.W. Leeper, ed.), Melbourne Univ. Press, p94-101.



Gartner, L.P. University of Maryland, Baltimore, Maryland USA. Surface density of the rough endoplasmic reticulum in the aged Drosophila midgut. A morphometric analysis.

Introduction. Insects, particularly Drosophila, have been studied extensively in documenting alterations during the aging processes. Within the past two decades, the ultrastructural aspects of Drosophila aging have also been examined, and numerous subcellular alterations have been reported to occur in aged flies. These alterations accompany, or are accompanied by declines in vitality, reproductive capacity, metabolic efficacy (such as mitochondrial function and protein synthetic capabilities), as well as physiological activity, as measured by viability, fitness, and general activity. Moreover, it has been demonstrated that ultrastructural alterations may be influenced by various environmental agents, such as ionizing radiation, oxygen poisoning, and an increase or decrease in ambient temperature. Morphometric analysis of these subcellular changes provides credence to the close association between morphologic and biochemical age related changes (Gartner 1987a,b).

Materials and Methods. Wild type Canton S strain of **Drosophila melanogaster**, reared on standard cornmeal-agar-molasses medium were housed in shell vials (5 males and 5 females per vial) at 19±0.5°C in constant darkness. Flies were transferred to fresh food once per week. One day old and 71 day old males were lightly etherized, and their midguts were dissected in buffered (cacodylate) 3% glutaraldehyde. Dissected tissues were postfixed in 1% cacodylate buffered OsO_4 for one and a half hours, subsequent to which they were prepared for routine electron microscopy (Gartner 1987). Tissues, embedded in Epon 812 were sectioned on a Porter-Blum MT-2 ultramicrotome, and sections, floated onto copper grids, were doubly stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963). Morphometric analysis was performed according to Hecker et al. (1974) as adapted to the fruitfly (Gartner 1985).

Results. The normal histology and ultrastructure of the young and aged Drosophila midgut has been described (Miller 1950; Gartner 1985, 1987). The present study focused on the differences in the surface densities of the supranuclear and infranuclear rough endoplasmic reticulum in the parenchymal cells of the midgut. The surface density of the rough endoplasmic reticulum of the supranuclear region was 1.731±0.131 $\mu m^2/\mu m^3$ in the 1 day old and 0.908±0.134 $\mu m^2/\mu m^3$ (significance > 0.001) in the 71 day old fruitfly. The surface density of the rough endoplasmic reticulum in the infranuclear region of these cells was 1.180±0.055 $\mu m^2/\mu m^3$ in the one day old versus 0.599±0.040 $\mu m^2/\mu m^3$ (significance > 0.001) in the 71 day old fly.

Discussion. The cytoplasmic distribution of the rough endoplasmic reticulum in the parenchymal cell of the young fruitfly indicates that there must be a functional difference between the two cellular compartments. Moreover, the sharp (ca. 50%) age related reduction in the surface density of the rough endoplasmic reticulum is indicative of and corroborates the reported decrease in protein synthesis (Bauman & Chen 1968; Webster & Webster 1983; Bailey & Webster 1984).

Recent studies on calcium modulator proteins suggest that calmodulin may play an important role in controlling cellular activities by influencing several basic secondary messenger systems, such as cyclic AMP and Ca^{2+}, Mg^{2+} - ATPase, as well as the assembly of tubulin dimers during the formation of microtubules (Watterson & Vincenzi 1980; Hidaka & Hartshorne 1985). Since a calmodulin-like protein has been isolated from almost all organisms tested, including **Drosophila melanogaster** (Wandosell, Serrano & Avila 1986), this protein appears to be ubiquitous in the animal (and plant) kingdom, thus attesting to its importance in regulating cellular activities.

It is not known whether calmodulin directly controls protein syntheis; however, it is tempting to speculate that, since it directly controls plasma membrane Ca^{2+} ATPase and various protein kinases, it probably has an affect on protein synthesis. Consequently, it is possible that such an effect (or its absence) is responsible for the age related reduction in the surface density of the rough endoplasmic reticulum in the parenchymal cells of the Drosophila midgut.

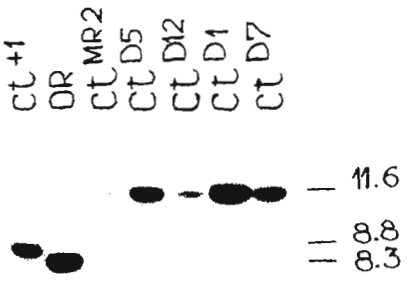
References: Bailey, P. & G. Webster 1984, Mech. Age. Dev. 24:233; Bauman, P. & P. Chen 1968, Rev. Suisse Zool. 75:1051; Gartner, L. 1985, Tissue & Cell 17:883; ——— 1987a, in: Aging in Drosophila. A selectively annotated bibliography, Jen House, Baltimore; ——— 1987b, Exp. Gerontol. 22:297; Hecker, H., R. Brun & C. Reinhardt 1974, Cell. Tiss. Res. 152:31; Hidaka, H. & D. Hartshorne (eds.) 1985, in: Calmodulin Antagonists and Cellular Physiology, Academic Press, NY; Miller, A. 1950, in: Demerec(ed) Biology of Drosophila, Wiley, NY; Reynolds, E. 1963, J. Cell Biol. 17:208; Wandosell, F., L. Serrano & J. Avila 1986, Arch. Biochem. Biophys. 247:147; Watson, M. 1958, J. Bioiphys. Biochem. Cytol. 4:457; Watterson, M. & F. Vincenzi (eds.) 1980, in: Calmodulin and Cell Functions, Annals of the New York Academy of Science, v356; Webster, G. & S. Webster 1983, Mech. Age. Dev. 22:121.

Gerasimova, T.I., V.A. Mitin, N.A. Tchurikov and E.Z. Kochieva. N.I. Vavilov Inst. of General Genetics, Acad. Sci. USSR, Inst. of Molecular Biology, Moscow USSR. Site-directed mutagenesis of the cut locus due to mdg4 injections.

Mdg reinsertions within loci and sites, where they had been integrated before, were observed earlier in the system of unstable stocks (ct^{MR2}) during transposition bursts (Gerasimova et al. 1985; Mizrokhi et al. 1985). The molecular mechanism of such a transposition memory depends on mdg4 (gypsy) solo LTR remaining in the cut locus of unstable ct^{+}-revertants arisen from the ct^{MR2} mutant which in turn was shown to be associated with the insertion of mdg4 within the cut locus. An attempt of artificially directed site-specific mutagenesis in the cut locus was made in this work using mdg4 and P-element microinjections. The ct^{+1} stock whose cut locus was chosen for experiments containing mdg4 solo LTR flanked with a target duplication was chosen for experiments (Mizrokhi et al. 1985).

The ct^{+1} stock homozygous with respect to the X-chromosome was unstable for 50 generations and gave rise to novel ct-mutations with a rate of 10^{-3}. However, the stock was stabilized and no ct-mutations were found among 20,000 chromosomes. The early embryos of the ct^{+1} stock were injected with the DNAs of two plasmids containing P-element (p25.1) and mdg4 or gypsy (p111). As control, the wild-type stock Oregon R was injected with these DNAs and also separately with either mdg4 or P-element. The mixture for injection contained the DNAs of plasmids at a concentration of 0.3 mg/ml (with mdg4) or 0.05 mg/ml (P-element). The flies obtained after the injection (F_0) were crossed individually with those of the parent stock. The offspring of F_0 females was analysed in F_1 and that of F_0 males in F_2. 31 fertile flies were obtained from 498 injected embryos. Two males in F_1 (D5, D1 2) and three males in F_2 (D1, D7, D8) gave rise to ct-mutants. The mutants appeared in clusters. Thus the injection activated locus-specific mutagenesis with a rate of 0.16. As a control, 420 embryos of the Oregon R stock and 600 embryos of the ct^{+1} stock were injected separately with the DNA of mdg4 and P-element. No ct-mutants were detected in the progeny of the injected flies. The phenotype of novel ct-mutants obtained after the injection was the same as that of the primary mutation ct^{MR2}. Stocks homozygous with respect to the X-chromosomes were obtained from mutant males. The same was done for non-mutant brothers from D7 and D8 families (ct^{+D7}, ct^{+D8}). The X-chromosomes of the parent stock ct^{+1} and of the seven novel ct^{D5}, ct^{D12}, ct^{D7}, ct^{D8}, ct^{+D7}, ct^{+D8} stocks were analysed by in situ hybridization with mdg4 ^{3}H-labelled DNA. An mdg4 site was revealed by hybridization in the cut locus 7B3-4 on the X-chromosomes of five mutants to indicate the reinsertion of mdg4 within this part of the locus.

The reinsertion was confirmed by blot analysis. The map of the cut locus is known and mdg4 was shown to be inserted into the 8.3kb EcoRI fragment (Tchurikov et al. 1987). The unstable ct^{+1} revertant



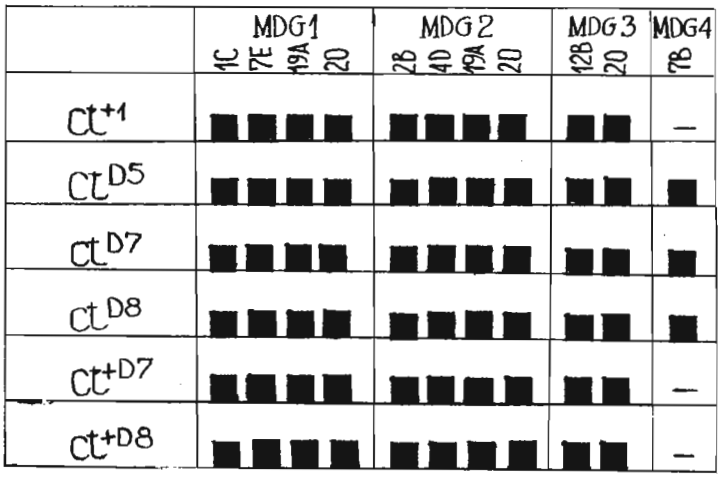


Figure 2. Distribution of in situ hybridization sites on X-chromosomes of the ct^{+1} strain and repeated mutant strains obtained after the injection.

Figure 1. Blot-hybridization of genomic DNA from the analysed strains treated by EcoRI endonuclease with an 8.3 kb fragment of the cut locus.

has a longer EcoRI fragment as compared with the wild-type due to the presence of mdg4 solo LTR. The 8.3 kb probe hybridizes to the EcoRI fragment of the ct-mutants and this fragment is the same as the primary ct^{MR2} mutant (Figure 1), i.e., mdg4 localization in the cut locus is identical in the repeated mutants in the primary ct^{MR2} mutant. The transposition memory in vivo is realised as usual in transposition bursts. Figure 2 shows that the hybridization sites for the mdg1, mdg2, mdg3 entirely coincide on the X-chromosomes of the analysed stocks. Therefore, mdg4 is inserted in the absence of transposition bursts and, most probably, is transferred to the cut locus directly from the injected plasmid, by means of homologous recombination between the genomic solo LTR and the injected mdg4 LTR.

The role of P-element remains obscure. However, when only mdg4 DNA was injected, no repeated mutants appeared. Thus the integration of a mobile element may cause an artificially directed mutagenesis which occurs with a high rate (16%). Therefore, our genetic system allows integration of any cloned DNA fragments at a particular site of the genome.

Acknowledgement: The authors are greatly indebted to Dr. G. Rubin and Dr. Y.V. Ilyin for providing us with the clones p25.1 and p.111. We thank Dr. M. Hatipova for assistance in some experiments described.

References: Gerasimova, T.I., L.V. Matyinina, Y.V. Ilyin & G.P. Georgiev 1985, The EMBO J 4:3773-3780; Mizrokhi, L.A. Obolenkova, A.F. Priimägi, Y.V. Ilyin, T.A. Gerasimova & G.P. Georgiev 1985, The EMBO J 4:3780-3787; Tchurikov, N.A., T.I. Gerasimova, A.L. Kenzior & N.V. Trubetskaya 1987, Genetika (Russ) 13:1149-1161.

Gimelfarb, A. and J. Willis. University of Chicago, Illinois USA. Etherizing parents reduces the weight of their offspring.

In our experiments aimed at estimating offspring-parent regression for body weight in **D.melanogaster**, we found highly significant differences in the mean weights of parents and offspring. Mothers were 0.76 heavier than daughters and fathers were 0.56 heavier than sons on the average. The procedures for producing offspring were exactly the same as those for producing parents, except that grandparents (parental parents) were never etherized, whereas parents were etherized twice before mating in order to take their measurements. Thus, parents were produced by unetherized flies, whereas offspring came from flies that underwent etherization.

An experiment was conducted to test the hypothesis that etherization of parents has an effect of reducing the body weight of their offspring. The experimental procedure closely followed the one employed in the experiments on offspring-parent regression. On Day 1, 30 virgin flies of each sex were collected under CO_2 from the laboratory stock. On Day 2, the flies were divided into two groups, referred to here as the Ether (E) and No Ether (NE) groups. Flies in the NE group were placed under CO_2 and then individually mated. In the E group, flies were also first placed under CO_2, but then each fly was twice etherized before being individually mated. On Day 8, all vials were cleared of mated pairs, and on Day 14, all vials were cleared of flies that had emerged by this time. On the next day, virgin females and males were collected from all vials using CO_2. No more than 2 flies of each sex were collected from each family. However, there were vials with fewer than 2 offspring of each sex (some did not have any flies emerge on this day). Altogether 15 females plus 16 males in group NE and 18 females plus 22 males in group E were collected. On the next day, these flies were etherized and weighed. The results are summarized in the Table.

The total number of offspring that had emerged by Day 21 was also recorded for each family and the results are presented on the bottom line of the Table (there were no offspring in one family in E group).

The weight of females in E group is significantly lower than in NE group ($t = 3.337$, $P<0.005$). The weight of males in E group is also lower than in NE group, but not significantly ($t = 1.297$, $P>0.1$). The total number of offspring in E group is smaller than in NE group (although not significantly). Therefore, the difference between body weights of mothers and daughters cannot be due to overcrowding in group E and is, probably, due to an effect of etherization of parents on the weight of their offspring.

	NO ETHER			ETHER		
	N	Mean	S.D.	N	Mean	S.D.
female weight	15	114.33	10.48	16	103.13	7.90
male weight	18	81.94	7.54	22	78.91	7.22
# offspring	15	56.40	33.31	14	45.71	28.65

Gupta, K.K. and J.P. Gupta. Banaras Hindu University, Varanasi, India. The family Drosophilidae in Nagaland, India.

Table 1. Drosophilid species collected from two different localities in Nagaland, India, during September 1987.

Species	Subgenus	Locality	Number ♂♂/♀♀
D.melanogaster	Sophophora	Medziphema, Kohima	503
D.ananassae	"	"	646
D.bipectinata	"	"	197
D.ficusphila	"	Medziphema	06
D.kikkawai	"	Medziphema, Kohima	93
D.takahashii	"	"	72
D.suzukii	"	"	04
D.malerkotliana	"	"	208
D.eugracilis	"	"	54
D.anomelani	"	Medziphema	13
D.biarmipes	"	Medziphema, Kohima	9
D.siangensis	"	Kohima	2
D.nepalensis	"	Kohima, Medziphema	5
D.immigrans	Drosophila	Kohima	320
D.nasuta	"	Kohima, Medziphema	215
D.siamana	"	Medziphema	10
D.synpanishi	"	"	3
D.lacertosa	"	Kohima	7
D.setitarsa	"	Medziphema, Kohima	72
D.trisetosa	"	Medziphema	17
D.penispina	"	"	13
D.pentafuscata	"	Medziphema, Kohima	46
D.busckii	Dorsilopha	"	229
D.bryani	Scaptodrosophila	"	276
D.paratriangulata	"	"	67
Z.multistriatus	Aprionus	"	4
H.guttata	Hypselothyrea	Medziphema	3
***Amiota sp.**	Amiota	"	2
***Leucophenga sp.**	leucophenga	"	2
***Leucophenga sp.**	"	"	3
***Liodrosophila sp.**	Liodrosophila	Kohima	20
***Liodrosophila sp.**	"	"	12
TOTAL			3133

* species yet to be identified.

India possesses a rich but still little known fauna of Drosophilidae. It is only in recent years has there been an increasing awareness of the need to study the geographic distribution of Drosophilid species inhabiting the subcontinent of India. As a result, several interesting areas of the Indian subcontinent with varied ecogeographic conditions could be surveyed, but a considerably vast area of the subcontinent still awaits exploration.

The northeast region of the Indian subcontinent comprises many though relatively smaller states of India. Our earlier studies in this region have yielded many interesting species of Drosophilidae (Gupta 1973; Singh & Gupta 1977; Gupta & Singh 1979; Diwedi & Gupta 1979; Diwedi et al. 1979, 1980; Singh & Gupta 1980, 1981; Gupta & Kumar 1986; Kumar & Gupta 1988, in press).

The present report deals with the results of our recent surveying studies carried out in the vicinity of Kohima (hilly area) and Medziphema (semi-hilly area), both situated in Nagaland, a previously unexplored state of India. The details pertaining to these species are shown in Table 1.

Acknowledgement: This work has been financially supported by the University Grants Commission, New Delhi.

References: Diwedi & Gupta 1979, Entomon 4:183-187; Diwedi et al. 1979, Oriental Insects 13(1-2):61-79; _____ 1980 Proc. Ind. Acad. Sci. 89:85-89; Gupta 1973, DIS 50:112; _____ & Kumar 1986, Senckenbergiana biol 67(1/3):43-49; _____ & Singh 1979, Entomon 4(2):167-172; Kumar & Gupta 1988, Annls. Soc. Ent. Fr. (in press); Singh & Gupta 1977, Ent. Mont. Mag. 113:71-78; _____ & _____ 1980, Oriental Insects 14(4):503-509; _____ & _____ 1981, Stud. Nat. Sci. 2(13):1-8.

Imasheva, A.G., L.A. Zhivatovsky and O.E. Lazebny. N.I. Vavilov Inst. of General Genetics, Moscow, USSR. The effect of directional and stabilizing selection on size of experimental Drosophila populations.

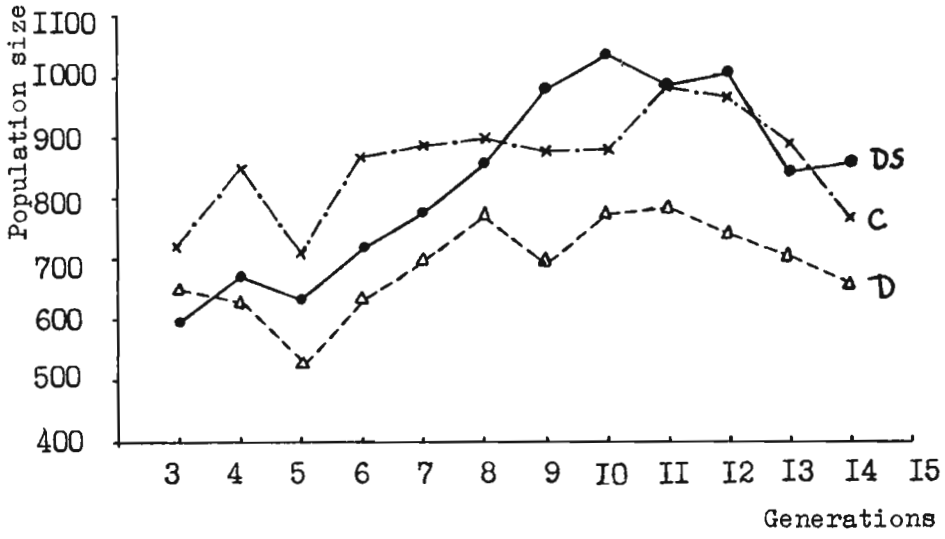
Experimental directional selection for quantitative characters is generally known to reduce the average population fitness (Falconer 1960) which is attributed to destruction of coadapted gene complexes with resulting segregation of low fitness phenotypes. On the other hand, from the theory of stabilizing selection (Schmalhausen 1949), it follows that genetic integration of populations and increase in population fitness are promoted by selection against such segregating phenodeviations. In view of this it seems likely that stabilizing selection might reduce the deleterious effects of directional selection.

With the aim to check this assumption we assayed population size as a measure of average population fitness in laboratory lines of **D.melanogaster**, subjected to directional selection for positive phototaxis and stabilizing selection for a set of metric characters of the wing. Phototaxis was measured by the countercurrent distribution technique (Benzer 1967); 20% of flies were selected out of 150 of each sex scored. Stabilizing selection was conducted only on females; the coefficient of selection was 50%: selected females had minimal distances from the "average" phenotype by 9 metric wing characters. Three experimental designs were used: directional + stabilizing selection (DS); directional selection (D); unselected controls (C). Each design was replicated 3 times. Selection was conducted for 15 generations. The details of selection procedure are given in a forthcoming paper (Imasheva & Zhivotovsky, in press).

The selection lines were set up using a large random mating laboratory population of **D.melanogaster** maintained for about 1.5 years prior to the beginning of the experiment. Each line was set up with 15 pairs of unselected flies taken from the above population. In each generation 15 males and 15 females selected according to the procedure described above were used as parents. The parents were left on fresh medium for oviposition for 3 days before being discarded. Each line was maintained in a $\frac{1}{2}$ pint milk bottle at 25±1°C. Population size was estimated as the number of progeny emerging in each bottle recorded for 8 days from the beginning of emergence. The population size or biomass is traditionally used as a measure of population fitness. This measure was introduced by Carson (1957) and used later by other authors (Ayala 1965; Van Delden & Beardmore 1968).

Change in population size in time averaged over replicate lines is presented in Figure 1, where running averages are plotted. Population size increases with time under all the experimental designs. This may reflect the increase in mean population fitness resulting from adaptation of populations to laboratory conditions (Hartl & Jungen 1979). The important point is that the size of D lines was on average substantially lower than that of controls and DS lines. This suggests that judging by population size, mean fitness of lines subjected to stabilizing selection was greater compared to lines where directional selection was conducted without stabilization. Furthermore, rate of population growth was higher in DS lines than in D lines; linear regression coefficients of logarithm of population numbers on generation number are equal to 0.041±0.010 and 0.017±0.008 in DS and D lines, respectively.

In an earlier paper (Zhivotovsky et al. 1987), we have shown that D and DS lines differ with respect to some parameters characterizing variation of phototactic response; in D lines these parameters showed a tendency to increase with time while in DS lines they remained constant. Lower population size of D lines as well as increase in variation might be associated with the low fitness phenotypes that arise in the population during directional selection. Stabilizing selection counteracting the disintegration of genetic systems could maintain the population fitness of DS lines at a higher level.



References: Ayala, F.J. 1965, Science 150:903-905; Benzer, S. 1967, PNAS USA 58:1112-1119; Carson, H.L. 1958, 44:1136-1141; Falconer, D.C. 1960, in: An Introduction to Quantitative Genetics, Oliver & Boyd, Edinburgh; Hartl, D.L. & H. Jungen 1979, Evolution 33:371-380; Imasheva, A.G., L.A. Zhivotovsky et al., Genetika (USSR), in press; Schmalhausen, I.I. 1949, in: Factors of Evolution, Blakiston, Philadelphia; Van Delden, W. & J.S. Beardmore 1968, Mutat. Res. 6:117-127; Zhivotovsky, L.A., A.G. Imasheva, O.E. Lazebny & D.B. Kholodenko 1987, Dokl. Akademii Nauk SSSR 297:982-985.

Islam, M.S., M.A.R. Khan, P.C. Barman and S.I. Ali. University of Rajshahi, Rajshahi, Bangladesh. Effect of copper and ferrous sulphates on the durations of developmental stages of **Drosophila melanogaster**.

The fruitfly, **Drosophila melanogaster**, is possibly the most reared laboratory insect employed for experiments of different disciplines. The salts, copper sulphate (Blue vitriol, $CuSO_4,5H_2O$) and ferrous sulphate (Green vitriol, $FeSO_4,7H_2O$) are very often used as laboratory reagents. Islam et al. (1986) determined the effect of these salts on the offspring production in **D.melanogaster** and Islam et al. (1987) assessed their effects on the hatchability of the same fly.

The wild type **D.melanogaster** was employed for the present work. The rearing medium contained 18 gm of Bacto agar, 125 cc of molasses dissolved in 1000 cc of water and 125 gm of cornmeal (fine-powdered maize) cooked until boiling. Nipagin was added to food as a mould inhibitor. The hatched out larvae were supplied with pastry yeast solution as supplementary diet on each alternate day. 0.05, 0.10, 0.15, 0.20 and 0.25% of copper sulphate and 0.10, 0.15, 0.20, 0.25 and 0.30% of ferrous sulphate were added to the rearing medium to get the requisite doses, while an untreated food medium was maintained as the control. To determine the incubation, larval and pupal durations on these salts, mated females were allowed to lay eggs on the treated and control media and consecutive 24-hr egg collections were taken in 10 cm X 4 cm culture tubes. The experiments were conducted in the incubator at 25±0.5°C.

The durations of incubation, larval and pupal stages were found to be 24, 100 and 116; 26, 110 and 128; 28, 114 and 146; 30, 118 and 152; and 34, 122 and 156 hr, respectively, on 0.05, 0.10, 0.15, 0.20 and 0.25% concentrations of copper sulphate. The durations of these developmental stages on 0.10, 0.15, 0.20, 0.25 and 0.30% concentrations of ferrous sulphate were recorded to be 22, 102 and 104; 24, 106 and 104; 24, 106 and 110; 28, 108 and 128; and 30, 112 and 146 hr, respectively. In the untreated food medium, 22, 96 and 98 hr of incubation, larval and pupal durations were recorded. Shakoori & Butt (1980) assessed the effects of different doses of thioacetamide on the development of **Musca domestica** and they noted that thioacetamide shortened the duration of various developmental stages. This contrasts with our results, which could be due to specific and chemical variations. Shakoori & Parveen (1983) reported that eggs of **M.domestica** treated with 0.56 and 0.64% thioacetamide do not hatch. Khan & Rahman (1983) showed that 64% formalin reduced hatchability in the Eri silkworm, **Samia cynthia ricini**. That both copper and ferrous sulphates reduce hatchability significantly and 30 and 64% copper sulphate inhibit the hatchability completely in **S.c.ricini** has been reported by Rahman & Khan (1984). These findings are in good agreement with our results. Therefore, it appeared from our observations that the effect of addition of copper and ferrous sulphates to the rearing medium of **D.melanogaster** is to lengthen the developmental periods; moreover, copper sulphate produced a more deleterious effect than did ferrous sulphate. Obviously, we should guard against any contamination of food with these salts to avoid unsolicited lengthening of the developmental periods of this important dipteran.

Acknowledgement: We are grateful to Dr. P.T. Ives, Dept. of Biology, Amherst College, Massachusetts (USA), for kindly supplying the stock of flies used in the present investigation; and to Prof. M.A. Hossain, the then Chairman, Dept. of Zoology, Rajshahi University, for providing necessary laboratory facilities.

References: Islam, M.S., M.A.R. Khan, P.C. Barman & S.I. Ali 1986, DIS 63:68; ____, ____, ____ & ____ 1987, Pakistan J. Zool. 19(1):85-87; Khan, M.A.R. & A.S.M.S. Rahman 1983, Univ. J. Zool. Rajshahi Univ. 2:75-76; Rahman, A.S.M.S. & M.A.R. Khan 1984, J. Asiatic Soc. Bangladesh (Sc.) 10:113-114; Shakoori, A.R. & N.Z. Butt 1980, Pakistan. J. Zool. 12(2):247-264; ____ & A. Parveen 1983, Bangladesh J. Zool. 11(2):53-74.

Islam, M.S. and A.B. Siddique. University of Rajshahi, Rajshahi, Bangladesh. The integrated effects of temperature-shock on pupae and selection for sex-ratio in **Drosophila melanogaster**.

The knowledge of the sex-ratio of a population is important because the size of the next generation is influenced by the proportion of females in that population. The ratio of the numbers of individuals of the two sexes in a species is often near that which might be expected from the mechanism of sex determination of that species. The ratios need not, however, be solely controlled by these mechanisms. There are numerous instances of genetic and other factors causing distortions of the sex-ratios in a species (Crow & Kimura 1970; Eshel 1975). Mechanisms of large effect have been found in meiotic drive in **Drosophila melanogaster** (Peacock & Miklos 1973) and the "sex-ratio" condition in Drosophila species (Poulson & Sakaguchi 1961). Lanier & Oliver (1966) described an unusual mechanism for sex-ratio condition in bark beetles, while Hickey & Craig (1966) observed the sex-ratio distortion in a mosquito, **Aedes aegypti**; Maynard-Smith & Stenseth (1978) in wood lemming and Mori et al. (1979) in **Sciara**. Moreover, that the normal commonly prevailing sex-ratio for some species may change under certain circumstances was studied by Anderson (1961) where the sex-ratio varied with population density, Nash & Thomas (1968) where there was a general tendency for sex-ratio in increase with increasing temperature in **D.melanogaster** and

Table 1. The observed sex-ratios and calculated significance test values for the male to female offspring in the control and temperature-treated lines of **D.melanogaster.**

Generations	CONTROL LINE sex-ratios (male:female)	CONTROL LINE sum of X^2 values	TREATED LINE sex-ratios (male:female)	TREATED LINE sum of X^2 values
Parental	1 : 0.97	0.4446(ns)	1 : 0.98	8.2607(ns)
F_1 - High	1 : 0.95	0.6035(ns)	1 : 0.90	9.5561(ns)
F_2 - "	1 : 0.91	6.6981(ns)	1 : 0.79	9.9733(ns)
F_3 - "	1 : 0.89	5.7444(ns)	1 : 0.75	18.7683(<0.05)
F_4 - "	1 : 0.88	9.3539(ns)	1 : 0.67	12.4693(ns)
F_5 - "	1 : 0.88	3.6440(ns)	1 : 0.66	17.2279(<0.05)
F_6 - "	1 : 0.84	10.0777(ns)	1 : 0.59	16.9974(<0.05)
F_1 - Low	1 : 0.97	0.5524(ns)	1 : 0.96	0.6079(ns)
F_2 - "	1 : 0.99	2.0992(ns)	1 : 0.92	2.6191(ns)
F_3 - "	1 : 1.05	11.9135(ns)	1 : 0.89	5.6919(ns)
F_4 - "	1 : 1.11	4.6486(ns)	1 : 0.74	19.7013(<0.05)
F_5 - "	1 : 1.10	4.2112(ns)	1 : 0.72	17.2614(<0.05)
F_6 - "	1 : 1.13	4.2152(ns)	1 : 0.69	21.5403(<0.01)

Figures in parentheses indicate probabilities at 9 d.f.; ns=not significant.

by Glasgow & Phelps (1971) where they showed that changes in stress may affect one sex more than the other. Colgan (1982) presented a model which allowed an examination of the effect of selection on the allelic frequencies at an autosomal locus which had an effect on the sex-ratio of offspring. The aim of the present investigation was to observe the effect of temperature-shock on the pupae and its consequent effect on selection for sex-ratio in **D.melanogaster.**

The wild-type **D.melanogaster** was used and the flies were reared in the common agar-molasses-corn-meal-yeast food medium in the incubator at 25±0.5°C except for the period of temperature-shock in the treated line. Single pair matings between the virgin females and males were exclusively used throughout the experiment. A set of 10 replicates (in culture tubes of 14 cm x 3 cm size) were maintained for each of the high and low selection lines for the untreated (control) and treated lines in every generation. A temperature-shock of 35°C for 30 min was applied to the pupae in the tubes showing the highest and the lowest male:female ratio in each generation of the treated line. After the treatment, of course, the tubes were taken out of the hot chamber and the adults were allowed to emerge at the rearing temperature. Excepting the parental generation, selection for high and low productivity of male:female offspring was made in all the four lines, two from the control and the other two from the treated, and the experiment was continued for 6 successive generations.

The results of the experiment have been summarized in Table 1. All the chi-square values for the observed and expected numbers of males and females were found to be insignificant in both the high low selection regimes of the control line. In contrast to these, in the treated line, significant deviations for sex-ratio were recorded in F_3, F_5 and F_6 generations and in F_4, F_5 and F_6 generations of the high and low selection regimes, respectively.

That physical factors like temperature and radiation can be utilized for the induction of genetic variability through artificial selection have been shown by many workers. Rasmuson (1955) adduced evidence that increased crossing-over by temperature treatment can result in an increased speed and, in some cases, level of response to selection for sternopleural bristles in **D.melanogaster.** Scossiroli & Scossiroli (1959) was able to almost double the number of sternopleural bristles in **D.melanogaster** by alternative selection and X-ray treatment. Grell (1978) reported that females of **D.melanogaster** exposed to elevated temperature for short periods show increased recombination. The filament length of **Bombyx mori** cocoons was found to increase by subjecting the eggs to gamma irradiation (Rahman et al. 1983). More recently, Islam & Hossain (1986) have shown that the temperature-shock on pupae of **D.melanogaster** resulted in an increased hatchability in both the high and low selection lines. In agreement with the findings of Rasmuson (1955), Grell (1978), Colgan (1982) and Islam & Hossain (1986), the present investigation reports that a significantly less recovery of females in the treated, high and low lines could be due to the effect of temperature-shock that produced increased crossing-over in addition to new mutations. While a significant excess recovery of males was due to the fact that no crossing-over takes place in male Drosophila. Owing to the absence of this situation, the control line showed insignificant deviations from the typical male:female sex-ratio.

References: Anderson, F.S. 1961, Oikos 12:1-16; Colgan, D.J. 1982, Aust. J. Biol. Sci. 35:95-101; Crow, J.F. & M. Kimura 1970, Harper & Row, New York; Eshel, I. 1975, Heredity 34:351-361; Glasgow, J.P. & R.J. Phelps 1971, George Allen & Unwin, London, p395-415; Grell, R.F. 1978, PNAS USA 75:3351-3354; Hickey, W.A. & G.B. Craig 1966, Genetics 53:1177-1196; Islam, M.S. & M.A. Hossain 1986, Abstr. 5th Nat. Zool. Conf. p12; Lanier, G.N. & J.H. Oliver 1966, Science 153:208-209; Maynard-Smith, J. & N.C. Stenseth 1978, Heredity 41:205-214; Mori, L., E.M.B. Dessen & A.L.P. Perondini 1979, Heredity 42:353-358; Nash, D.J.& V.C. Thomas 1968, Biol. Abstr. p2442; Peacock, W.J. & G.L.G. Miklos 1973, Ad. Genet. 17:361-409; Poulson, D.F. & B. Sakaguchi 1961, Science 133:1489-1490; Rahman, S., A.R. Khan & A. Haque 1983, Bangladesh J. Zool. 11(1):42-44; Rasmuson, M. 1955, Acta Zool. 36:1-49; Scossiroli, R.E. & S. Scossiroli 1958, Int. J. Radiation Biol. 1:61-69.

Jelisavčić, B. and M. Andjelković. Belgrade University, Yugoslavia. Adaptive significance of amylase polymorphism in Drosophila. V. Starch detection in the midguts of Drosophila adults and dynamics of its passage through the alimentary tract.

One of the methods to determine the type of nutrition of some organism is also the analysis of its intestinal contents. As the study of alpha-amylase polymorphism from the population-genetic and evolution-genetic aspect is rather frequent for some Drosophila species (see Andjelkovic et al. 1987), possibility of starch detection in the intestinal tract of its adults is of interest as an indicator for the presence of this carbohydrate in their nutrition.

Starch detection in the midguts of Drosophila adults is based on the common reaction between the starch and iodine at which the starch turns deep-blue in the presence of elementary iodine. Drosophila adults are dissected in Shen's solution. Separated midguts are transferred to a glass plate previously covered with a thin layer of albuminous glue (Mayr's albumin). Above the intestine two drops of KJ-J solution are dropped. After a two-minute incubation period at room temperature, intestinal starch (if present) will turn blue.

For the development of this method, **D.subobscura** adults have been used. Reliability of the method has been estimated from results shown in Tables 1 and 3. After a 24-hr fasting period, individual of **D.subobscura** have been transferred into small jars with standard maize-sugar-agar substrate for Drosophila. In successive time intervals, some individuals have been dissected to detect the presence of the starch in the midgut (Table 1). First starch detection is possible 15 min after the transfer to the substrate with the starch; that is, from the moment when there is a possibility to take food rich with starch. After 45 min in all dissected individuals starch was present, more or less, in the midgut. After this time the number of individuals both with some starch in the midgut and without it varies, which is by all means the consequence of the food taking rhythm, starch digestion and intestinal contents discharge.

Confirmation of results concerning the filling and emptying speed of the middle intestine was obtained in a similarly designed experiment (Table 2). Namely, after the fasting period individuals of **D.subobscura** have been transferred into small jars with filter paper soaked with 1% saccharose solution dyed with one of the dyes used in food industry. Intestinal filling and emptying dynamics (assessed on the basis of the presence of the dye in intestinal contents) is identical to that when using maize-sugar-agar substrate (x^2=5.880; df=5; p>0.05).

Table 1. Time of starch appearance in the midgut of **D.subobscura** adults after taking starch-rich food.

Time from start of food intake	Midgut completely filled with starch		Midgut partly filled with starch		Midgut without starch		Number of dissected flies
	n	%	n	%	n	%	
0'	--	--	--	--	30	1000.00	30
15'	4	13.33	19	63.33	7	23.33	30
30'	14	46.66	14	46.66	2	6.66	30
45'	23	76.66	7	23.33	--	--	30
60'	17	56.66	7	23.33	6	20.00	30
2 h	20	66.66	5	11.66	5	16.66	30
3 h	13	43.33	4	13.33	13	43.33	30
4 h	6	20.00	2	6.66	22	73.33	30
5 h	4	13.33	3	10.00	23	76.66	30
6 h	11	36.66	6	20.00	13	43.33	30
7 h	16	53.33	5	16.66	9	30.00	30
8 h	15	50.00	10	33.33	5	16.66	30

Table 2. Time of dyed intestinal contents appearance in the midgut of **D.subobscura** adults after the beginning of dyed 1% saccharose solution intake.

Time from*	Midgut completely filled		Midgut partly filled		Midgut empty		Number of dissected flies
	n	%	n	%	n	%	
0'	--	--	--	--	30	100.00	30
15'	5	16.66	16	53.33	9	30.00	30
30'	18	60.00	8	26.66	4	13.33	30
45'	17	56.66	8	26.66	5	16.66	30
60'	18	60.00	7	23.33	4	13.33	30
2 h	10	33.33	15	50.00	5	16.66	30

* = time from the beginning of saccharose solution intake.

Table 3. Time of starch elimination from the midgut of **D.subobscura** adults after cessation of taking the starch-rich food and the beginning of taking food without starch.

Time from*	Midgut completely filled with starch		Midgut partly filled with starch		Midgut without starch		Number of dissected flies
	n	%	n	%	n	%	
0 h	29	96.66	1	3.33	--	--	30
1 h	20	66.66	7	23.33	3	10.00	30
2 h	21	70.00	4	13.33	5	16.66	30
4 h	16	53.33	11	36.66	3	10.00	30
5 h	9	30.00	3	10.00	18	60.00	30
6 h	4	26.66	1	6.66	10	66.66	15
7 h	1	3.33	2	6.66	27	90.00	30
8 h	2	6.66	1	3.33	27	90.00	30
9 h	--	--	--	--	30	100.00	30

* = time from beginning of taking food without starch.

Table 4. Time of starch elimination from the midgut of **D.subobscura** adults after cessation of taking starch-rich substrate and the beginning of fasting.

Time after*	Midgut completely filled with starch		Midgut partly filled with starch		Midgut without starch		Number of dissected flies
	n	%	n	%	n	%	
24 h	14	46.67	10	33.33	6	20.00	30
25 h	14	48.27	9	31.03	6	20.69	29
27 h	10	33.33	12	40.00	8	26.67	30
29 h	15	50.00	6	20.00	9	30.00	30
32 h	7	35.00	11	55.00	2	10.00	20
48 h	--	--	2	6.67	28	93.33	30

* = time after starch taking cessation.

Confirmation of reliability of the suggested method for starch detection in the midgut of the Drosophila adults is obtained from results presented in Table 3. After a 24-hr cultivation period in small jars with maize-sugar-agar substrate, individuals of **D.subobscura** have been transferred to jars with yeast substrate without starch. Dissection performed in successive intervals showed that starch gradually disappeared from the midgut. It was practically completely absent 6 hr after cessation of taking substrate with starch. In the case when flies are submitted to fasting after a 24-hr period of taking the starch-rich substrate, starch will disappear from the midgut much slower (Table 4) than in the previous case.

On the basis of results obtained, we are convinced that the presented method for starch detection in the midgut of Drosophila adults is reliable and satisfactorily sensitive. It can be concluded that in most individuals starch has been taken in 30 min to 5 hr before dissection.

Johnson, S.A. and M.J. Milner. University of St. Andrews, St. Andrews, Fife, Scotland.

The ultrastructure of the peripodial membrane of Drosophila leg and wing imaginal discs during evagination.

Imaginal discs consist of two layers of epithelium enclosing a lumen. One of these layers, known as the columnar epithelium, is thick, pseudostratified and folded, and this layer is overlain by a much thinner layer of epithelial cells known as the peripodial membrane (pm). It has been known for some time that the proximo-distal elongation of the imaginal discs during evagination is driven by the rearrangement of cells in the columnar epithelium (Fristrom & Fristrom 1975). During the early stages of evagination, the pm stretches to accommodate the elongation of the appendage, while the latter stages of the process are effected by the contraction of the pm (Milner, Bleasby & Kelly 1984). We have therefore examined ultrastructurally the cells of the pm during contraction to see if we can determine how contraction occurs. Fixation and embedding procedures were as described previously (Tucker et al. 1986). The observations give some clues as to the mechanism of Drosophila pm morphogenesis.

A basal lamina overlay the entire pm prior to eversion, and this broke away probably during contraction (Figure 1). Some cell death was present at this stage. The central pm cells overlying the tip of the appendage were found to be extremely thin apico-basally as though stretched (Figure 2). However, peripheral pm cells were arranged in a columnar fashion with many septate junctions between them.

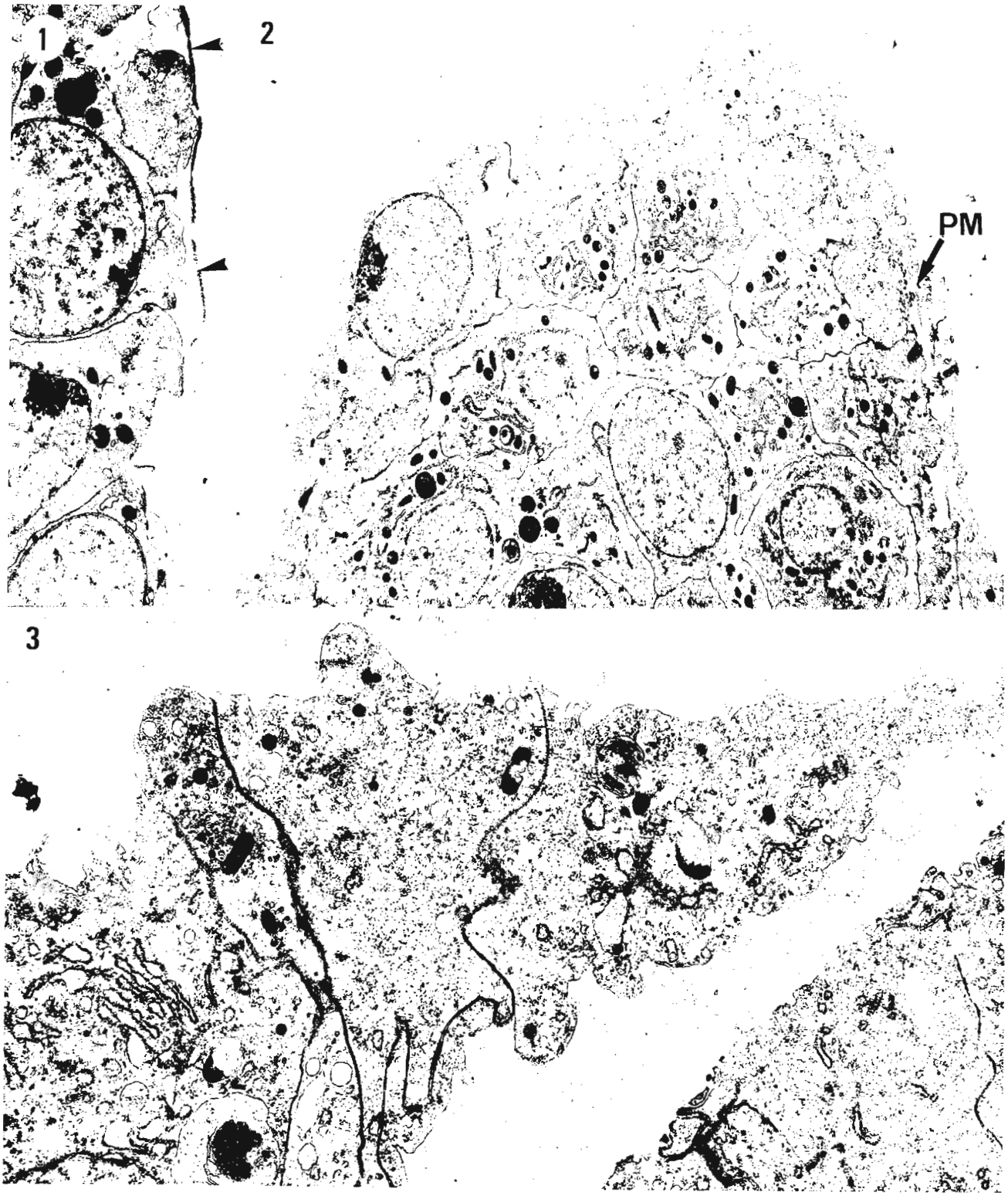
We examined leg and wing pm cells curing the curling stage to determine whether the deployment of microtubules was responsible for the cell shape changes involved in pm contraction. A few non-orientated microtubulues were seen in the pm cells during contraction, but it seems unlikely that they play an active part in the process. Figure 3 shows the edge of a wing peripodial membrane at the point where it is thickening, and a very few microtubules are present. This conclusion is supported by the observation that 10 ug/ml of the microtubule inhibiting drug nocodazole had no effect on wing disc evagination (n=24), this concentration being 10x higher than that which caused microtubule dissociation in early chick development (Mareel et al. 1984). Similarly, Fristrom & Fristrom (1975) found that the microtubule-inhibiting drug colcemid did not inhibit disc evagination.

We therefore feel that the possibility that microtubules can play a significant role in the morphogenesis of the peripodial membrane can be discounted. Most probably, an actin-based microfilament system (Fristrom & Fristrom 1975), and/or a change in cell adhesivity (Nardi, Norby & Magee-Adams 1987) are involved.

Acknowledgement: We wish to acknowledge the financial support of the Science Engineering Research Council (grant no. GR/D6104B) and the outstanding technical assistance of Mary Tuchel.

References: Fristrom, D. & J.W. Fristrom 1975, Devel. Biol. 43:1-23; Mareel, M. et al. 1984, J. Embryol. Exp. Morphol. 81:273-286; Milner, M.J., A.J. Bleasby & S.L. Kelly 1984, Roux's Arch. Dev. Biol. 193:180-186; Nardi, J.B., S.-W. Norby & S.M. Magee-Adams 1987, Devel. Biol. 119:20-26; Tucker, J.B. et al. 1986, Euro. J. Cell Biol. 41:279-289.

Figure 1. The basal lamina (arrowheads) breaking away from leg disc peripodial membrane cells during the contraction stage. x4,000. **Figure 2.** Highly stretched cells of the peripodial membrane (PM) overlying the distal tip of the leg during the elongation of the appendage. x7450. **Figure 3.** Cells at the edge of the peripodial membrane during contraction. x3160.



Kalisch, W.-E. and S.R. Ramesh. Ruhr-Universität Bochum, FR Germany. Individual empty pupal cases used for genotypic characterization of larval glue protein fractions in **Drosophila n. nasuta.**

The standard technique for obtaining homozygous strains of phenotypic visible mutations in Drosophila is by selection using single cultures. If non-morphological (biochemical) mutations are involved, marker chromosomes (which carry dominant mutations themselves) can be used to select homozygous flies as in **D.melanogaster** (Dickinson & Sullivan 1975). In Drosophila species for which, however, marker chromosomes and suitable marker genes do not exist so far, the selection for a homozygous biochemical mutant strain becomes difficult. This is especially so if the mutated allele is only expressed during the larval period and/or has a reduced fitness when compared with the wildtype allele. In such a case, one would have to test the same larva biochemically on the one hand and use it for a single culture on the other. This is of course impossible. Under these circumstances, the common method used so far is the setting up of a large number of single cultures, testing part of the F_1-progeny from each single culture, setting up again many single cultures from the F_1-progeny of the vial with the highest amount of heterozygote and homozygote mutant larvae, testing part of the succeeding progeny and so on, until (by chance) a homozygous strain is established.

We describe a simple new technique to characterize larval glue protein fractions from individual empty pupal cases. In the present note an analysis of the pupal case is used to obtain a homozygous strain of the **Sa** glue protein mutant, which is the first biochemical mutation to be found in **D.n.nasuta.**

Individual larvae of the mid-third instar were washed in distilled water and then transferred into fresh standard Drosophila food vials (one in each). After pupation and hatching of the fly (22±1°C), the empty pupal case was carefully removed from the wall. It was transferred into a 1.5 ml microfuge tube containing 30 µl of sample buffer [composed of 0.0625 M Tris-HCl (pH 6.8), 10% glycerol, 2% SDS and 5% β-mercaptoethanol] and allowed to stand at room temperature (22°C). After 6 hr, the sample solution was heated in a boiling water bath for 10 min and allowed to cool down to room temperature. The pupal case was then picked out of the sample solution and the solution was centrifuged at 5000 rpm for 2 min. These samples could be stored after that at -20°C. 15 µl of this sample solution was used for a 13.7% SDS polyacrylamide gel electrophoresis (Ramesh & Kalisch 1988b).

Glue proteins in **D.n.nasuta** are composed of several major fractions [labeled (*) in Figure 1] and of minor fractions. Details are given elsewhere of the glue proteins in **D.n.nasuta** and in the remaining members of the **D.nasuta** subgroup (kd-values; number of fractions; post translational modification of fractions; X-chromosomal linkage of glue protein genes; comparison with the glue proteins in **D.melanogaster**, etc.; Ramesh & Kalisch 1987-1989).

We have found a spontaneous biochemical mutation in one of our **D.n.nasuta** wildtype strains (Mysore I). It appeared in a protein extract from an individual salivary gland as two additional major glue protein fractions (approx. 37 kd and 34 kd) along with the common protein pattern from a salivary gland of a mid-third instar female larva in **D.n.nasuta** (Figure 1B). We tried to select for a homozygous strain of this

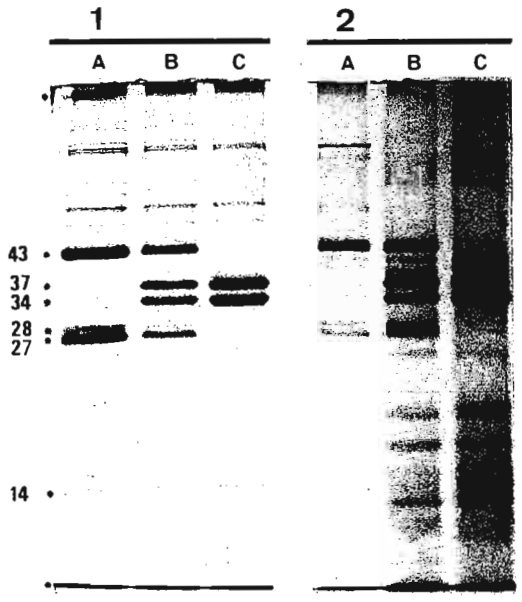


Figure 1. Patterns of protein fractions from individual salivary glands in **Drosophila n. nasuta.** Major glue protein fractions are labeled [*]. (A) +/+ female larva, (B) +/Sa female larva, (C) Sa/Sa female larva. 13.7% SDS polyacrylamide gel, Coomassie-blue staining. In earlier investigations (Ramesh & Kalisch 1987-1988b) the 28 kd and the 27 kd fractions were referred as 30 kd and 28 kd, respectively, according to methodological differences (Ramesh & Kalisch, in prep.).

Figure 2. Pattern variety of glue protein fractions from individual female pupal cases (after hatching of the flies) in the **Drosophila n. nasuta** wildtype strain in which the glue mutation Sa was encountered. (A) +/+ type, (B) +/Sa type, (C) Sa/Sa type. Same method used as in Fig. 1.

mutation by inbreeding and testing the progeny of single cultures (as described above). We tested 20 single cultures in each of seven generations but without any success.

The new technique we used after this failure is based on the following assumption: the larval glue (which is responsible for fixing the pupa to the substrate after its extrusion from the salivary gland) covers the bottom side of the pupal case as a dried but basically unchanged compound of the proteins. By this it should be possible to test the dried glue in the same manner one uses to analyze the protein fractions of the glue plug within the salivary gland of late-third instar larvae (Ramesh & Kalisch 1988b).

Figures 2A-C show the variety of patterns obtained from different pupal cases. As far as the major glue protein fractions between 43 kd and 27 kd are concerned, the patterns coincide with those in Figure 1. By this, we were able to analyze the genotype through the glue proteins from an individual pupal case and use this (the same) fly as one of the parents for a single culture. By the technique described, we could select a homozygous and so far genetically stable Sa strain (Figure 1C). Sa indicates codominance on the basis of the electrophoretic patterns. Both glue protein fractions of Sa are glycosylated but the 37 kd fraction shows a much stronger PAS reaction than the 34 kd one.

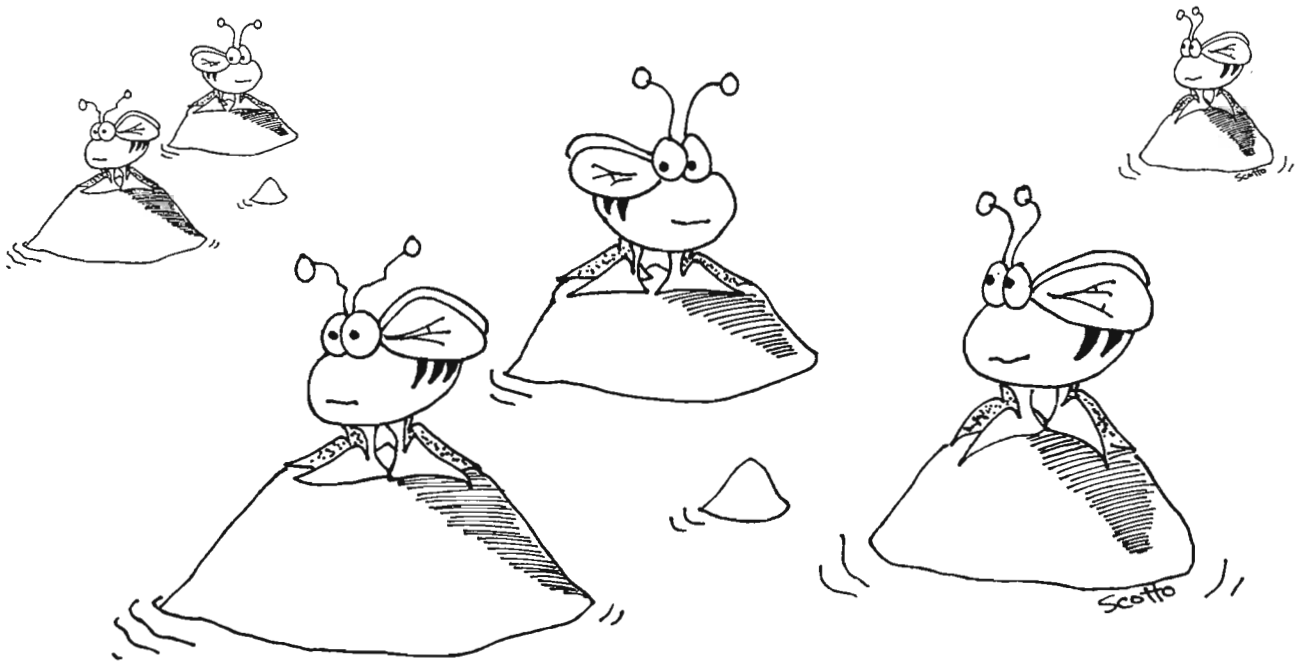
We have not analyzed so far the additional (minor) protein fractions to be seen in Figure 2 in comparison with Figure 1 nor have we compared these fractions with those known as being part of the procuticle proteins (Fristrom et al. 1978). Even the reason for the absence of the 14 kd glue protein fraction in the pupal cases is unknown so far.

Recently we could show that the 43 kd fraction itself is composed of a closely neighboured double band (Ramesh & Kalisch 1988b). By this, at least four fractions (the two 43 kd fractions, the 28 kd and the 27 kd fractions) are involved in changing into the 37 kd and the 34 kd fractions of the Sa mutation. Different explanations are possible: (1) all these fractions are coded by only one gene; (2) the 43 kd, the 28 kd, and the 27 kd fractions are coded by a cluster of linked genes; (3) the Sa mutation could be due to a chromosome mutation; (4) the Sa genotype could be based on several independent mutations which were already accumulated earlier in our **D.n.nasuta** wildtype strain.

So far, investigations have shown that all of the fractions between 43 kd and 27 kd are X-chromosomal. There is no crossing over between the fractions mentioned above (Ramesh & Kalisch 1988b). The 43 kd, the 28 kd, and the 27 kd fractions incorporate ^{3}H-Proline differently at 25°C as well as at 37°C. Sa/+ polytene chromosomes show a normal X-chromosomal band-interband pattern (Ramesh & Kalisch, in prep.).

Acknowledgement: This study is part of a project (Ka 309/9-1) which is financially supported by the Deutsche Forschungsgemeinschaft. S.R.R. is grateful to the Univ. Grants Commission, New Delhi, India, for sponsoring and to DAAD, FR Germany, for the award of a scholarship.

References: Dickinson, W.J. & D.T. Sullivan 1975, in: Results and problems in cell differentiation (Beermann, Reinert & Ursprung, eds.), Springer-Verlag, Berlin, 6:8-31; Fristrom, J.W., R.J. Hill & F. Watt 1978, Biochemistry 17:3917-3924; Ramesh, S.R. & W.-E. Kalisch 1987, DIS 66:117; ——— & ——— 1988a, DIS 67:this issue; ——— & ——— 1988b, Biochemical Genetics 26 (in press); ——— & ——— 1989, Genetica (in press).



Kamping, A. and W. van Delden. University of Groningen, Haren, Netherlands. Hybridization between **D.melanogaster** and **D.simulans** in nature.

In 1982, 1983 and 1987 Drosophila were collected during September in a fruitmarket in Groningen, The Netherlands. Isofemale lines both of **D.melanogaster** and **D.simulans** were set up. The founding females and part of their offspring were electrophoretically analysed for a number of enzymes. This allowed the detection of interspecific hybrids between the two species present in the original sample and in the F_1's. Table 1 gives the allele frequencies for the alcohol dehydrogenase (Adh), α-glycerophosphate dehydrogenase (α-Gpdh) and octanol dehydrogenase (Odh) loci in the wild caught flies. For **D.simulans** all flies were homozygous for an allele at the Adh locus which migrated in the direction opposite to that of the two Adh alleles in **D.melanogaster**. For α-Gpdh and Odh all **D.simulans** flies were homozygous for alleles identical in mobility with the F alleles in **D.melanogaster**. The only exception was a single individual, heterozygous for the α-Gpdh locus, which was identical with the SF heterozygote in **D.melanogaster**. It turned out that at the α-Gpdh locus in **D.melanogaster**, in addition to the regular S and F alleles, two rare alleles occurred, which were called Super-Slow (S^S) and Super-Fast (F^S) according to their migration distances.

Table 1. Allele frequencies in samples of **D.melanogaster** (♀♀) collected in 1982, 1983 and 1987. N is the number of flies tested.

Locus:		Adh		α-Gpdh				Odh	
Year	N	S	F	S^S	S	F	F^S	S	F
1982	240	0.19	0.81	0.002	0.383	0.615	--	0.07	0.93
1983	243	0.18	0.82	--	0.377	0.621	0.002	0.03	0.97
1987	138	0.16	0.84	0.004	0.424	0.572	--	0.04	0.96

Table 2. Frequencies of **D.melanogaster**, **D.simulans** and their hybrids among females caught in 1982, 1983 and 1987.

Year	Total no. females	Freq. **D.mel.**	Freq. **D.sim.**	Freq. hybrids
1982	267	0.899	0.101	--
1983	413	0.976	0.017	0.007
1987	159	0.868	0.132	--

The frequency of **D.simulans** was much lower than that of **D.melanogaster** and varied considerably among years (Table 2). In the original sample of 1983 three interspecific hybrids (females) were found. In the offspring of **D.melanogaster** founders of the isofemale lines hybrids were observed only once: among the 100 females tested in 1982, one gave both regular **D.melanogaster** offspring (both sexes) and hybrids (♀♀ only). Apparently this female had mated with both a **D.melanogaster** and a **D.simulans** ♂. **D.melanogaster** females caught in 1983 and 1987 only produced **D.melanogaster** offspring.

Two out of seven **D.simulans** females in the 1983 sample and one out of 21 in the 1987 sample gave hybrid offspring. The hybrid offspring contained both sexes. Their numbers were 11 ♀♀ + 14 ♂♂, 2 ♀♀ + 7 ♂♂ and 6 ♀♀ and 50 ♂♂, respectively. Though the number of ♂♂ exceeded the number of ♀♀, the results do not confirm the general belief that among the offspring of crosses between **D.simulans** ♀♀ and **D.melanogaster** ♂♂, females are either absent or very rare (Sturtevant 1920; Lee & Watanabe 1987). Thus a casual cheque for hybrids among the offspring produced by ♀♀ collected in nature, considering only the presence of both ♂♂ and ♀♀ as a proof for the absence of hybridization, without additional morphological or electrophoretic examination, may thus provide an unjustified negative or underestimated outcome. Crosses among hybrids and crosses between hybrids and either **D.melanogaster** or **D.simulans** in all possible combinations gave no offspring.

Our data prove that interspecific hybridization between **D.melanogaster** and **D.simulans** is not a unique event in nature. It is tempting to relate the relatively high frequency of **D.simulans** ♀♀ mating with **D.melanogaster** ♂♂ in 1983 (28.6%) to the low frequency of **D.simulans** in that year.

References: Lee, W.H. & K. Watanabe 1987, Jpn. J. Genet. 62:225-239; Sturtevant, A.H. 1920, Genetics 5:488-500.

Leicht, B.G.[1] and J.J. Bonner. Indiana University, Bloomington, Indiana USA. X-ray-induced generation of duplications and deletions along the third chromosome of **Drosophila melanogaster.**

Both the isolation and characterization of new mutations within the Drosophila genome are greatly facilitated by the availability of chromosomal deletions and duplications. Using a method originally described by Craymer (1981, 1984), we isolated a series of duplications and deletions spanning the 61A-72D and 87B-89E regions of chromosome 3 of **Drosophila melanogaster.** These duplications and deletions are described herein.

Experimental Design. The screen we carried out utilized a large insertional-tandem duplication of the third chromosome, Dp(3;3)S2 (Craymer 1981, 1984). This chromosome, which is duplicated for regions 61A2-72D11 and 87B4-89E2 (Figure 1), must be maintained over the corresponding deficiency [In(3LR)HR33^{L}bxd^{106R}]; i.e., it is lethal in combination with a standard third chromosome. However, if Dp(3;3)S2 males are irradiated with X-rays and then outcrossed to females with euploid third chromosomes, progeny carrying deletional derivatives of the duplication can be recovered. These X-ray-induced derivatives will include smaller insertional-tandem duplications, tandem duplications, and deletions along the length of the original duplicated region as well as other miscellaneous rearrangements.

Approximately 4000 Dp(3;3)S2/In(3LR)HR33^{L}bxd^{106R} males, aged 3 days at 22°C, were irradiated with 3000r of X-rays and mated en masse to Sb/TM3Ser virgin females in half-pint milk bottles (50 males and 50 females/bottle). Both marked versions of Dp(3;3)S2 (see Figure 1) were used. The mated adults were transferred to fresh bottles every other day for up to 6 days and then were discarded. Any surviving F_1 progeny (i.e., breakdown derivatives) were mated to CXD/TM3SbSer flies of the opposite sex to establish balanced lines. Where possible, the derivatives were balanced over TM3SbSer. Finally, each of the balanced lines was outcrossed to either gt^{1}w^{a} (for derivatives of Version 1) or red e (for derivatives of Q4Ly1) for cytological examination.

Results and Discussion. From the 4000 mutagenized Dp(3;3)S2 males, a total of 394 (193 females, 201 males) viable offspring were recovered. Roughly half of these (48 females, 146 males) were sterile, and lines could not be established from them. From the remainder, 164 separate lines, designated BK1-164, eventually were established. Cytological examination of 139 of these lines (the other 25 lines were lost prior to examination) revealed that 57 of the lines had visible rearrangements, whereas 82 of the lines appeared cytologically wild-type. Of the lines with rearrangements, the majority (50/57) were of the duplication class, bearing either tandem duplications or smaller insertional-tandem duplications. Eleven of the derivatives had deletions; 5 of these were interstitial, the other 6 were terminal deletions associated with insertional duplications of the 87-89 region. Eight lines carried other types of rearrangements (e.g., translocations, inversions). Figure 2 is a summary of the different duplications and deletions obtained, showing the breakpoints and extent of each. Table 1 lists those lines which are still available.

With regard to the types of breakdown derivatives recovered, several points are worthy of note. First, duplication-bearing derivatives were the predominant class of breakdown products recovered (discounting the cytologically normal lines). The ratio of duplication-bearing derivatives to deletion-bearing derivatives of Dp(3;3)S2 was 50 to 11 or approximately 4.5 to 1. It is known from existing duplications and deletions that, in general, a fly cannot tolerate deletions larger than one numbered division (on the polytene map), whereas duplications up to as many as 10 numbered divisions can be tolerated (Lindsley & Sandler et al. 1972). From such values, Craymer (1981) predicted that the screen described

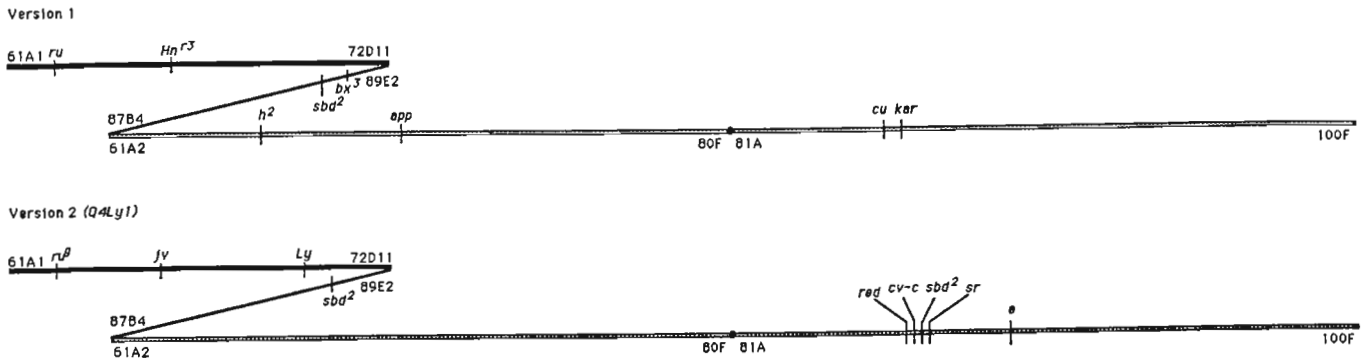
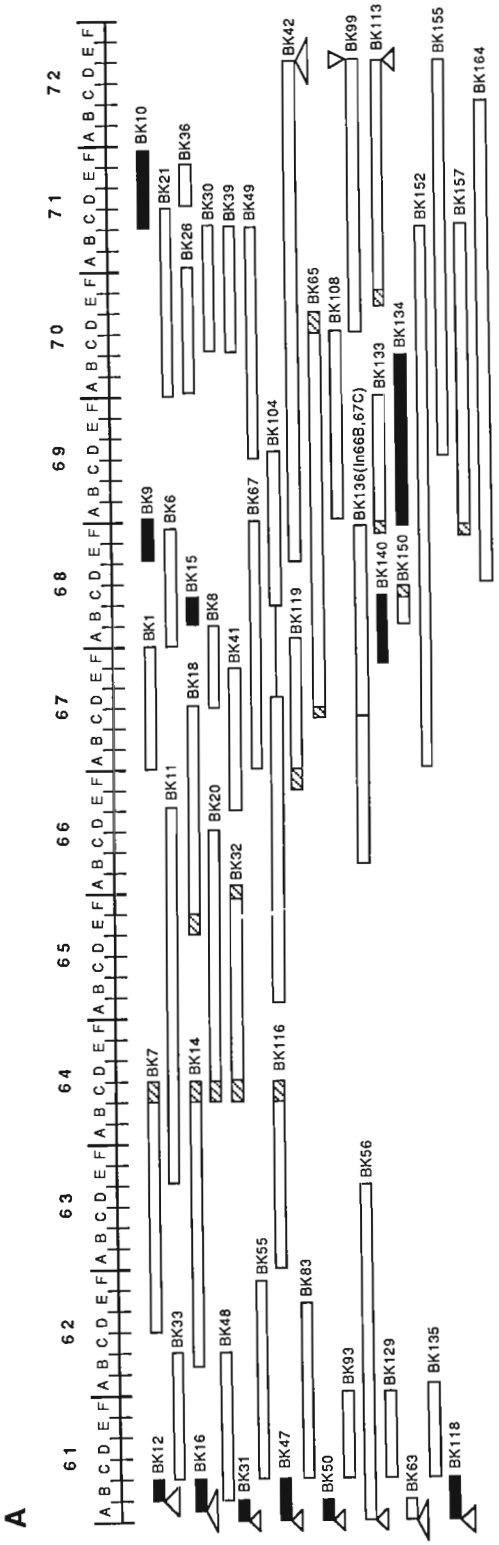


Figure 1. Diagrammatic representation of Dp(3;3)S2. Dp(3;3)S2 was derived, via recombination, from the two pericentric inversions In(3LR)HR33 and In(3LR)bxd^{106} (described in Craymer 1981, 1984). It carries either of two different marker combinations, denoted as Version 1 and Version 2.



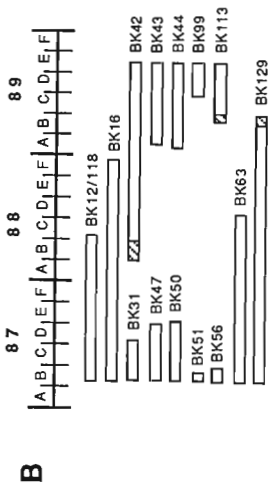


Figure 2. A: Distribution of duplications and deletions in the 61A to 72D region. Duplications are represented by open bars; deletions by filled bars. Cross-hatched regions indicate uncertainty in assignment of the breakpoint. Triangles extending from the bars indicate the insertion of material from the 87-89 region (see 87-89 region diagram; B). **B:** Insertional duplications of the 87B to 89E region.

above should yield on the order of one deletion to five duplications, which is very close to the value actually obtained. Second, tandem duplications, by virtue of their structure, can readily undergo intrachromosomal recombination. Thus, in the absence of selection for their maintenance, tandem duplications are frequently lost. Indeed, we have found this to be the case for many of our tandem duplication lines. Many of the cytologically normal lines we obtained may have carried duplications when first isolated but lost them before the cytological analysis could be done. Third, the distribution of breakpoints of duplication and deletion derivatives spanning the 61-72 region is clearly nonrandom. Out of over 100 breakpoints throughout this region, 32 are at one of only five sites (61A, 61C, 64C, 68F, and 71C). Chi-square analysis has revealed that the distribution of breakpoints deviates substantially from that expected for a Poisson (i.e., random) distribution ($P<0.001$). Similar nonrandom recovery of X-ray-induced rearrangements has been reported by several others (Bauer et al. 1938; Lefevre 1981; Mukhina 1981; Falk 1984).

Despite the aforementioned intrachromosomal recombination of tandem duplications and preferential recovery of breakdown derivatives in certain chromosomal regions, Craymer's approach for the generation of overlapping duplications and deletions along a chromosome arm is quite efficient and should be generally applicable. It offers several advantages over more conventional screening strategies in that large numbers of offspring can be screened, all progeny but the classes of interest die, and one does not need to rely on visible markers to identify the class of interest. Furthermore, this technique can readily be extended to the isolation of additional deletions in a chromosomal region for which one deletion already exists. For example, if one has a deletion spanning some interval between 61A and 72D and wishes to isolate smaller deletions throughout that inter-

Table 1. Existing Dp(3;3)S2 derivatives.

Line	Type of Rearrangement	Breakpoints	New Order*
BK9	interstitial deletion	68E2,3;69A1,2	61A-68E2,3/69A1,2-100F
BK10	interstitial deletion	71C1,2;71F4,5	61A-71C1,2/71F4,5-100F
BK12	terminal dupl/deletion	88C1,2;61B;61C	(87B4-88C1,2)/61A/61C-100F
BK15	interstitial deletion	68B1,2;68C6-8	61A-68B1,2/68C6-8-100F
BK31	terminal dupl/deletion	87D3,4;61A;61B	(87B4-87D3,4)/61B-100F
BK42	insertional-tandem dupl	88B;68E1,2	61A-72D11/89E2-88B/ 68E1,2-100F
BK43	insertional duplication	89A6,7;72D	61A-72D11/89E2-89A6,7/ 72D-100F
BK44	complex translocation/ insertional duplication	31A;33F;65E;66C 89A3-5;72D	21A-31A/66C-65E/33F-31A/ 66C-72D/89E2-89A3-5/72D-100F;61A-65E/33F-60F
BK47	terminal dupl/deletion	87D14;61A;61C	(87B4-87D14)/61C-100F
BK50	terminal dupl/deletion	87E1,2;61A;61B	(87B4-87E1,2)/61B-100F
BK51	terminal duplication	87B6	87B4-6/61A2-100F
BK56	insertional-tandem dupl	63D;87B15	61A-63D/87B4-15/61A2-100F
BK63	insertional-tandem dupl	61A;88D1,2	61A/88D1,2-87B4/61A2-100F
BK99	insertional-tandem dupl	89D3,4;70D1	61A-72D11/89E2-89D3,4/ 70D1-100F
BK113	insertional-tandem dupl	89B16-22;70E6-8	61A-72D11/89E2-89B16-22/ 70E6-8-100F
BK133	pericentric inversion	80A;89A	61A-80A/89A-80A/89A-100F
BK140	interstitial deletion	67F1;68C7,8	61A-67F1/68C7,8-100F
BK160	paracentric inversion	73F;75B	61A-73F/75B-73F/75B-100F

*Enclosure by parentheses indicates that the orientation is not established. The terminal duplications of 87-89 region material appear as "loops" attached to the end of 3L.

val, one can begin by irradiating Dp(3;3)S2 males, crossing them to balancer-carrying females, and collecting all F_1-progeny which survive to adulthood. These F_1-progeny (Dp*/Bal) are then crossed to the deletion-bearing stock (Df/Bal) and the F_2 progeny are scored for the absence of the Dp*/Df class as in any standard F_2 lethal screen. Because this scheme tests only those F_1-progeny known to have undergone some type of deletional event in the 61-72 region, it enables one to screen the progeny of a much larger number of irradiated males than is possible in conventional F_2 screens.

Acknowledgement: This work was supported by the National Institutes of Health grant GM26693 to J.J.B.; B.G.L. was supported by a National Institutes of Health Genetics Training Grant.

1 - Present address: B.G.L., Dept. of Molecular Genetics, 484 W. 12th Ave., Ohio State University, Columbus, OH 43210.

References: Bauer, H., M. Demerec & B.P. Kaufmann 1938, Genetics 23:610-630; Craymer, L. 1981, Genetics 99:75-97; ——— 1984, DIS 60:234-236; Falk, D.R., L. Roselli, S. Curtiss, D. Halladay & C. Klufas 1984, Mutat. Res. 126:25-34; Lefevre, G. 1981, Genetics 99:461-480; Lindsley, D.L., L. Sandler, B.S. Baker, A.T.C. Carpenter, R.E.Denell, J.C. Hall, P.A. Jacobs, G.L.G. Miklos, B.K. Davis, R.C. Gethmann, R.W. Hardy, A. Hessler, S.M. Miller, H. Nozawa, D.M. Parry & M. Gould-Somero 1972, Genetics 71:157-184; Mukhina, L.I., V.A. Kulitchkov & I.F. Zhimulev 1981, DIS 56:98-103

Loken, K. and M.J. Simmons. University of Minnesota, St. Paul, Minnesota, USA.

Further evidence for the somatic effects of P element activity in **D.melanogaster.**

In **D.melanogaster,** P transposable elements are normally active only in the germ line, where they cause mutations, chromosome breakage and gonadal dysgenesis (see Engels 1988, for a review). This restriction of movement has been shown to depend upon a step in the maturation of the primary transcript of complete (or autonomous) P elements (Laski, Rio & Rubin 1986; Rio, Laski & Rubin 1986). In the germ line, this transcript is processed by the removal of three introns, producing a messenger RNA that encodes the transposase of the P element family. However, in somatic cells the third intron is not removed, thereby preventing the synthesis of a bona fide transposase message.

Laski, Rio & Rubin (1986) have created an autonomous P element, called Δ2-3, that lacks this critical third intron. Flies transformed with this element produce the P transposase somatically, and therefore exhibit somatic P element movement. The biological effects of this somatic activity have been studied by utilizing a strain in which the Δ2-3 element was inserted in region 99B on the third chromosome (Engels et al. 1987). For unknown reasons, this particular insertion seldom excises or transposes, even though it produces a considerable amount of transposase (see also Robertson et al. 1988). Engels et al. (1987) crossed this stable Δ2-3 strain with an M′ strain called Muller-5 Birmingham, which carries only incomplete (or nonautonomous) P elements. None of the progeny of these crosses survived beyond the pupal

stage, indicating that the Δ2-3/Birmingham combination was lethal. This lethality was seen regardless of the rearing temperature (19-28°), and did not depend on the way in which the Δ 2-3/Birmingham hybrids were made. More refined analyses indicated that each of the Birmingham chromosomes contributed to the lethality, and that most of the deaths occurred in the pupal stage. Engels et al. (1987) noted that chromosome breakage engendered by somatic P element activity, particularly in the non-polytene cells of the imaginal discs, would be expected to cause lethality at this stage.

Engels et al. (1987) tested many strains for the pupal lethality phenomenon; however, only one strain, Muller-5 Birmingham, produced any positive results. This suggests that pupal lethality is peculiar to hybrids between the Δ2-3 and Birmingham strains, perhaps depending very specifically on the ensemble of P elements carried on the Birmingham chromosomes. Engels et al. (1987) noted that the frequency of pupal lethality was not correlated with the number of P elements, as determined by in situ hybridization, and suggested that some elements might be more likely to cause pupal lethality than others. Another possibility is that some strains possess repressors of P element activity (H. Robertson & W. Engels, pers. comm.). These repressors would be expected to reduce the amount of somatic P element movement, and thereby limit the frequency of pupal lethality. The Birmingham strain used in these experiments apparently lacks such repressors, but another subline of this strain, studied by Simmons et al. (1987), apparently possesses them.

Table 1. Mean proportion of eclosed pupae from reciprocal crosses between inbred lines and Δ2-3 or ry^{506} (control) strains.

	Cross 1		Cross 2		Cross 3		Cross 4	
	Inbred males x		Inbred females x		Inbred males		Inbred females	
Inbred line	Δ2-3 fem.		Δ2-3 males		ry^{506} fem.		ry^{506} males	
	N	P	N	P	N	P	N	P
B13.1 (I6)	141	0.75	102	0.94	211	0.95	95	0.99
B13.2 (I5)	106	0.89	143	0.97	202	0.98	108	0.98
B13.3 (L3)	106	0.87	48	1.00	198	0.98	90	0.99
B13.4 (I9)	146	0.89	76	0.99	207	0.98	161	0.88
B13.5 (L4)	108	0.59	64	0.98	157	0.98	74	0.99
B13.6 (L1)	140	0.91	29	0.97	141	0.98	71**	0.92
B13.7 (L2)	141*	0.80	83	0.99	133	0.91	49	1.00
B13.8 (I10)	103	0.50	81	0.98	134	0.99	57	1.00
B13.9 (H3)	160	0.90	118	0.99	182	0.95	105	1.00
B13.10 (H23)	130	0.92	53	0.98	237	0.96	111	0.99
B13.11 (H8)	147	0.87	32*	0.97	197	0.96	48	0.98
B13.12 (H15)	164	0.84	42	0.89	205	0.98	134	0.94
Mean		0.81		0.97		0.97		0.97
Sexi.1	145*	0.87	199	0.74	94	0.98	182	0.97
Sexi.2	201	0.95	159	0.75	75	1.00	56	0.98
Sexi.3	135	0.99	98*	0.92	93	1.00	55	1.00
Sexi.4	61	0.66	133	0.61	83	1.00	76	1.00
Sexi.5	122	0.85	131	0.65	7B	0.95	56	1.00
Sexi.6	108	0.99	71	0.97	103	1.00	62*	1.00
Sexi.7	122	0.91	120	0.68	107	1.00	93	0.99
Mean		0.89		0.76		0.99		0.99
UK4.1	69	0.93	54*	0.94	99	1.00	34*	1.00
UK4.2	74	0.96	57*	0.96	60*	0.99	96	0.99
UK4.3	--	--	78	0.94	154	0.97	101	0.99
UK4.4	91	1.00	88	0.97	108	0.99	59	1.00
UK4.5	96	0.98	45	0.96	95	1.00	32	1.00
UK4.6	188*	0.96	134	0.94	65	1.00	53	1.00
UK4.8	66	0.99	55***	1.00	75	0.98	50	1.00
UK4.9	91	0.99	60	0.99	118	0.99	60*	0.95
UK4.10	148	0.98	102	0.90	98	1.00	50	1.00
UK4.11	98	0.98	114	0.99	95	1.00	48	1.00
UK4.12	80	0.95	60	0.99	65	1.00	46*	1.00
UK4.13	--	--	102	0.98	156	0.98	116	0.97
Mean		0.97		0.96		0.99		0.99

N=number of pupae; P=unweighted mean proportion eclosed among 5 cultures. Asterisks indicate the number of cultures missing in each group.

Objectives, Materials and Methods: We have performed experiments to assess the generality of the pupal lethality phenomenon. These have utilized the same Δ 2-3 strain of Engels et al. (1987), in which Δ 2-3 is the only P element, and a set of inbred lines derived from three different strains, each containing P elements. One of these strains, called Sexi, was derived from a single inseminated female caught in Spain in 1975 (Kidwell, Frydryk & Novy 1983; Kidwell 1985). A second strain, called UK4, was derived by inbreeding and selection from a natural population in Madison, Wisconsin (Engels & Preston 1980), and a third strain, called B13, was synthesized by J. Curtsinger & Y. Hiraizumi by extracting an X chromosome from a natural population in Brownsville, Texas, and placing it in the genetic background of a cn bw laboratory stock. The Sexi, UK4 and B13 strains have been classified as M' strains, although other more refined tests indicate that each of these strains possesses some transposase-producing P elements (Jongeward, Heath & Simmons 1987; and unpublished results of M. Simmons & E. Heath). These strains also possess a limited ability to repress P element activity in crosses to standard P strains (Kidwell 1985; Jongeward, Heath & Simmons 1987; and unpublished results of M. Simmons & E. Heath). For each of these M' strains, sublines were made and propagated

by sib-mating for 20 generations. Thereafter, each subline was maintained by small mass matings.

Reciprocal crosses were made between each inbred line and the Δ2-3 strain; as controls, reciprocal crosses were made between each line and the ry^{506} strain from which the Δ2-3 strain had been derived by transformation. All the crosses were set up with several males and virgin females at 25°; after two days, single pairs were transferred from these mass matings to fresh vials, which were then incubated at 29° for 12 days. On the second day of incubation, the parents were removed in order to limit the period of egg-laying. Five replicate cultures were established for each cross. At the end of the incubation period, the pupae in each culture were scored for eclosion.

Results: The results of the experiment are shown in Table 1. This gives the average fraction of pupae that eclosed among all those that were scored. For most of the crosses, the eclosion rate was very high, between 97-100%. This is especially evident among the control crosses, where there was only one instance (out of 62) in which the eclosion rate was less than 90%. In contrast, among the crosses involving the Δ2-3 element, there were many cases in which the eclosion rate dropped below this value, indicating some pupal lethality. There was, however, considerable variation in the amount of lethality within and among the three sets of sublines.

None of the UK4 sublines generated much pupal lethality in either of the reciprocal crosses with the Δ2-3 strain. In contrast, the B13 sublines generated noticeable lethality in Cross 1, but not in Cross 2, while the Sexi sublines caused pupal lethality in both crosses. The significance of these results is evident when different sets of crosses are compared using the sign test. All 12 of the B13 sublines caused more pupal lethality in Cross 1 than in Cross 3 (the control) ($P<0.01$). Likewise, all 12 of these lines caused more lethality in Cross 1 than in Cross 2 ($P<0.01$). For the Sexi sublines, every comparison between Cross 1 and Cross 3 and between Cross 2 and Cross 4 showed more pupal lethality wherever the Δ2-3 strain was involved. Moreover, for each subline, the amount of pupal lethality was greater in Cross 2 than it was in Cross 1 ($P<0.01$).

The results with the sublines of B13 and Sexi clearly establish that pupal lethality is a general phenomenon. However, the amount of lethality seen in our experiments was very much less than that seen by Engels et al. (1987). This means that although other strains can produce pupal lethality, none seems to be as powerful as Engels' Muller-5 Birmingham. The reason for this difference is not readily apparent, but it probably involves the number and distribution of P elements on the Birmingham chromosomes and the complete absence of P element repressors in Engels' Birmingham strain.

These factors may also explain some of the differences that we observed among the UK4, B13 and Sexi sublines. For instance, the UK4 sublines, which did not produce pupal lethality, might have lacked sufficient numbers of P elements to cause dominant lethal mutations, or they might have synthesized repressors of transposase acticity in the somatic cells. Repressors might also explain the results with the B13 sublines, which caused pupal lethality in Cross 1 but not in Cross 2. In this case, the absence of lethality in Cross 2 might be due to X-linked or cytoplasmic repressors inherited from the B13 females. Among the Sexi sublines, pupal lethality was detected in both Cross 1 and Cross 2, but there was more of it in Cross 2. This difference cannot be explained by maternally inherited repressors, since it is in the wrong direction. However, P elements on the X chromosomes of the Sexi sublines might provide an explanation. Both the sons and daughters of Cross 2 possess an X chromosome from a Sexi subline, but only the daughters of Cross 1 have this chromosome. Assuming that in each subline, the X chromosome has P elements that contribute to pupal lethality, it is clear that more pupae would be at risk to die among the offspring of Cross 2. In situ hybridization experiments have demonstrated that the X chromosomes in the Sexi sublines are heavily populated with P elements (J.K. Lim & M.J. Simmons, unpubl. data), giving credence to this explanation for the reciprocal cross effect.

Our data reinforce and extend the conclusions reached by Engels et al. (1987). Pupal lethality is caused by the activation of P elements in somatic cells. Variation in the incidence of lethality is probably explained by variation in the numbers and types of P elements, and by interstrain differences in the location of these elements on the chromosomes. Repressors of P element movement also probably play a role.

Acknowledgement: This work was supported by the National Inst. of Environmental Health Sciences (ROI ES01960) and by the University of Minnesota's Summer High School Research Program.

References: Engels, W.R. 1988, P elements in Drosophila, in: Mobile DNA (Berg & Howe, eds.), ASM Publications; ———, W.K. Benz, C.R. Preston, P.L. Graham, R.W. Phillis & H.M. Robertson 1987, Genetics 117:745-757; ——— & C.R. Preston 1980, Genetics 95:111-128; Jongeward, G., M.J. Simmons & E.M. Heath 1987, DIS 66:77-80; Kidwell, M.G. 1985, Genetics 111:337-350; ———, T. Frydryk & J.B. Novy 1983, DIS 51:97-100; Laski, F.A., D.C. Rio & G.M. Rubin 1986, Cell 44:7-19; Rio, D.C., F.M. Laski & G.M. Rubin 1986, Cell 44:21-32; Robertson, H.M., C.R. Preston, R.W. Phillis, D. Johnson-Schlitz, W.K. Benz & W.R. Engels 1988, Genetics 118:461-470.

Mittler, S. and S. Garcia. Northern Illinois University, DeKalb, Illinois USA. The failure of the mushroom **Agaricus bisporus** to induce somatic mutation and recombination in wing hair test.

Table 1. Induction of wing spots on larvae fed mushroom mixture.

Number wings	1-2 mwh	2 < mwh	flr^3	Twin spots
Control: flr^3/TM3, Ser x mwh:				
218	48	0	6	1
Mushroom fed: flr^3/TM3 Ser x mwh				
260	48	0	4	3
Control: cn^{35} mus (2) 201^{D1} flr^3/TM1 x cn^{35} mus (2) 201^{D1} mwh; jv				
240	173	2	16	3
Mushroom fed: cn^{35} mus (2) 201^{D1} flr^3/TM1 x cn^{35} mus (2) 201^{D1} mwh; jv				
226	183	2	4	4

The most commonly eaten mushroom cultivated in the United States is **Agaricus bisporus.** This mushroom fed raw over a lifetime to male and female mice induced bone, forestomach, liver and lung tumors reported by Toth & Erickson (1986). In an attempt to induce somatic mutation and recombination in Drosophila in wing hair test as described by Graf et al. (1986), one gram of finely ground mushroom was mixed with 1 g of Carolina Biological Supply Co. finely ground Drosophila media to which 4 ml of water is added. Females flr^3/TM, Ser were mated to mwh, and in another group in which female cn^{35} mus (2) 201^{D1} flr^3/TM1, Ser were mated to cn^{35} mus (2) 201^{D1}, mwh; jv were permitted to lay eggs on this mushroom-food mixture and adult wings were examined. mus (2) 201^{D1} is a mutagen sensitive repair deficient mutant. Since this mixture was supposed to contain a weak mutagen if any, the larvae were permitted to feed their entire life cycle.

The mushroom mixture did not significantly increase the number of wing spots in repair proficient strain or in repair deficient mus (2) 201^{D1} mwh; jv strain. The 1-2 mwh hair spots were significantly spontaneously higher in the 201^{D1} strain.

References: Graf, U. et al. 1986, Envir. Mutagenesis 6:119-188; Toth, B. & J. Erickson 1986, Cancer Res. 46:4007.

Mohanty, S., S. Chatterjee and B.N. Singh. Banaras Hindu University, Varanasi, India. Variation in the expression of plexus mutation in **Drosophila ananassae.**

The plexus mutation in **Drosophila ananassae** was reported by Ray-Chaudhuri et al. (1959) from Calcutta population. Singh (1967) induced plexus mutation in **D.ananassae** by X-rays. Moriwaki (1968) reported the spontaneous occurrence of px^2 (px^{66} - Moriwaki & Tobari 1975), a semidominant allele of px in a wild stock from Madras. The px gene causes the formation of a network of extra veins at the distal end of wings. This gene has been located in 3R (Moriwaki 1968; Hinton 1970).

Hinton (1970, 1979) has utilized the px marker in his studies on mutability and crossing-over in **D.ananassae.** Mukherjee & Das (1971) have also used this marker in their studies on crossing-over. They have reported the occurrence of segregation distortion in px pc stock and the variability in the expression of px mutant phenotypes. A dot-like structure on both the wings of some heterozygous (+/px) flies was observed by these authors.

In our laboratory, the px stock which was supplied by Prof. C.W. Hinton, is being maintained. The px marker in combination with other markers is being used in recombination studies to detect the effects of heterozygous inversions on crossing-over in **D.ananassae.** We have observed considerable variation in the expression of px mutation. Different categories of wing venation in px/px flies have been observed. Females show better expression than males. Furthermore, some heterozygous (+/px) flies obtained by crossing wild females with mutant males also show a dot-like structure on both the wings. Nearly 15% of heterozygous flies show a dot-like structure on both the wings. Some heterozygous flies show a dot-like structure (size of dot varies) on only one wing. The heterozygous flies showing a dot-like structure on their wings can be easily identified and separated from px/px homozygotes. Figure 1 depicts the wings of **D.ananassae** showing variation in the expression of plexus mutation.

Acknowledgement: The financial assistance from the U.G.C. and the C.S.I.R., New Delhi, is thankfully acknowledged. We are indebted to Prof. C.W. Hinton who kindly supplied the stock. We also thank Dr. Kumar for his help in photography.

References: Hinton, C.W. 1970, Genetics 66:663-676; _____ 1979, Genetics 92:1153-1171; Moriwaki, D. 1968 DIS 43:79-80; _____ & Y.N. Tobari 1975, in: Handbook of Genetics (R.C. King, ed.), Plenum Press; Mukherjee, A.S. & A.K. Das 1971, Genetics 67:521-532; Ray-Chaudhuri, S.P., S. Sarkar, A.S. Mukherjee & J. Bose 1959, Proc. 1st All India Cong. Zool. Pt.2, add. i-xi; Singh, B.N. 1967, M.Sc. Diss., Banaras Hindu Univ.

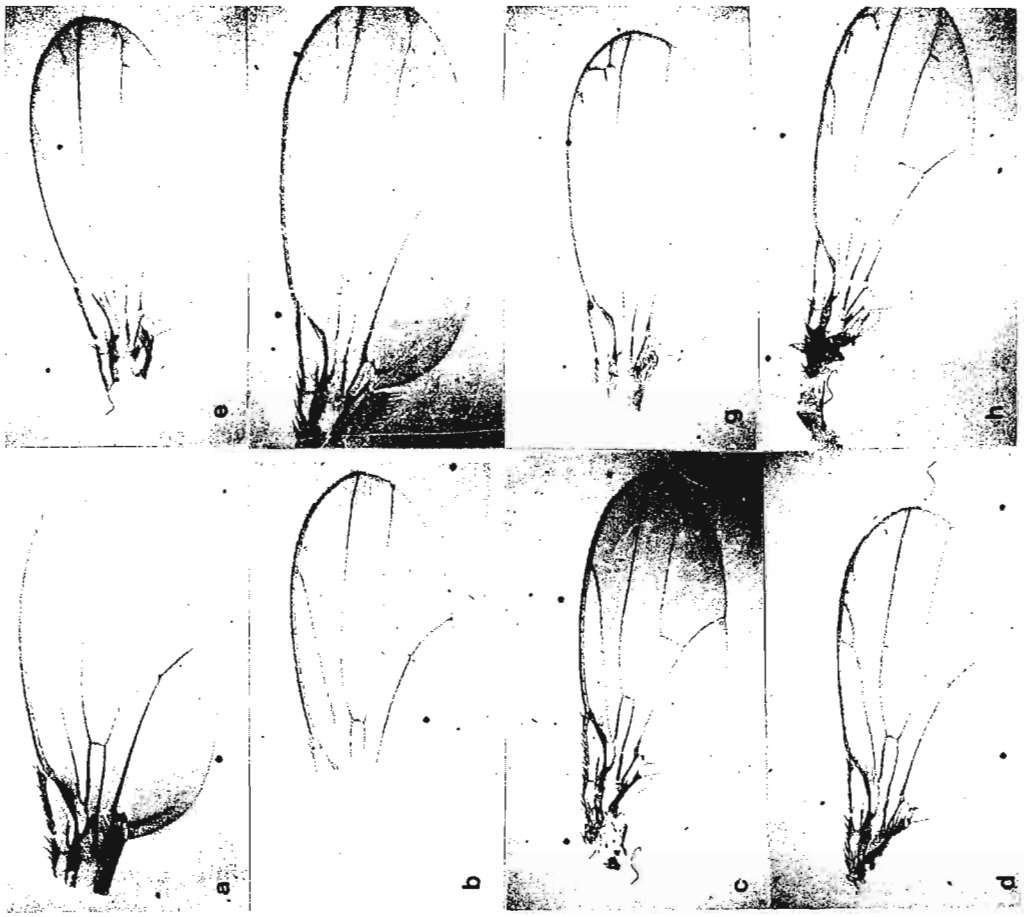


Figure 1. Wings of **D.ananassae** showing variation in the expression of px mutation. **a** - +/+; **b-c** - +/px; **d-h** - px/px.

Munoz, E.R. Com. Nac. de Energia Atomica, Buenos Aires, Argentina. Sex-linked recessive lethals induced in **Drosophila melanogaster** mature sperm by DES vapors.

Diethyl Sulfate (DES) is highly mutagenic (Pelecanos & Alderson 1964) and clastogenic (Munoz & Mazar-Barnett 1977) in **Drosophila melanogaster.** To administer DES we use the adult feeding method and owing to its high toxicity, at the end of 3.40 hr treatment most (0.032 mM DES solution) or all flies (0.048 mM DES solution) are motionless and die in high frequencies. Since shortly after placing the flies in the feeding chambers they become dizzy and notoriously uncoordinated, we suspected that most of the mutagenic action of DES was actually exerted through its vapors. To verify this, 7-day-old wild-type **Samarkand** males were fed DES or were exposed only to its vapors.

In the feeding experiments, the method of Lewis & Bacher (1968) was followed. The males were held in regular culture vials containing pieces of tissue paper soaked (0.15 ml) with DES (BDH) dissolved in ethanol, that was made up into 0.032 mM and 0.048 mM solutions in 5% sucrose. The toxic symptoms described above are independent of the use of a drop of ethanol to prepare the solutions.

The flies were exposed to DES vapors by means of a simple and useful devise (Munoz 1987): regular culture vials were cut 10 mm from the bottom and pieces of tissue paper were placed in the vials as in the feeding experiments. A piece of cheesecloth was stretched between the two parts of the vial and adhesive tape was used to rejoin the parts. The pieces of tissue paper in the lower chamber were soaked with 0.15 ml of the same DES solution used in the feeding experiments by means of a syringe with a long needle. The flies were placed in the upper chamber where additional pieces of tissue paper soaked with 5% sucrose solution were attached to the walls of the vial.

150 males were placed in each vial (direct feeding or vapors) and the flies were exposed to the chemical for 3.40 hr at 25°C. The treated males were allowed to recover for 2 hr before being individually mated for 24 hr to three 5-day-old Basc virgin females. The flies exposed only to the vapors of DES did

Table 1. Frequencies of sex-linked recessive lethals induced in **Drosophila melanogaster** mature sperm using the adult feeding method or exposing the males to the vapors of DES solutions.

DES concentration		VAPORS No. lethals/ No. tested	VAPORS % lethals	FOOD No.lethals/ No.tested	FOOD % lethals
0.32 mM	I	4/676	0.59	56/599	9.35
	II	5/655	0.76	48/615	7.80
	Total	9/1331	0.68	104/1214	8.57
0.048 mM		29/1892	1.53	383/1728	22.16
Control				2/2147	0.09

not show symptoms of intoxication. The males were discarded after this mating time and the inseminated females were allowed to lay eggs for an additional 48 hr period. F1 females were individually mated to three Basc males and their progeny was inspected for the occurrence of sex-linked recessive lethals. All suspected lethals were retested. The results obtained are shown in Table 1.

It can be seen that DES vapors are mutagenic in Drosophila strengthening the notion that manipulation of this chemical in a hood is mandatory. However, contrary to expectation the contribution of DES vapors to the yield of mutations detected when the feeding method is used is low. Actually, only 13-14% of the lethals induced can be imputed to the effect of vapors and this percentage is similar with both concentrations tested.

DES and Ethyl Methanesulfonate (EMS) have a close chemical reactivity (Hofmann 1980). It is interesting to mention that when the feeding method is employed to administer EMS at doses yielding 25% and 50% lethals, 50% of the total mutations scored are induced by its vapors (Munoz 1987).

References: Hofmann, G.R. 1980, Mutation Res. 75:63-129; Lewis, E.B. & F. Bacher 1968, DIS 43:193; Munoz, E.R. 1987, Envir. Mol. Mutagen. 10:307-309; ——— & B. Mazar-Barnett 1977, Mutation Res. 45:355-357; Pelecanos, M. & T. Alderson 1964, Mutation Res. 1:173-181.

Mutsuddi(Das), M., D. Mutsuddi and A.K. Duttagupta. University of Calcutta, India.

X-autosomal dosage compensation in Drosophila.

Equalization of X-linked gene products between sexes, despite different number of gene copies (1X vs 2X), constitutes the familiar phenomenon of dosage compensation. In Drosophila, such compensation is mediated via an upliftment of the level of X-transcription in males (Mukherjee & Beermann 1965). The X chromosomes in trisomic condition (3X;2A), however, exhibit level of transcription per cell equivalent to that of disomic one (2X;2A) (Lucchesi et al. 1974). A comparable finding in autosomal trisomies speaks the existence of autosomal dosage compensation also (Devlin et al. 1982, 1984). However, compensation in trisomic conditions is suggested to be accomplished by a mechanism distinct from male-female dosage compensation (Lucchesi 1983; Devlin et al. 1985).

The co-existence of an X chromosome (X_2 of **miranda**) and an autosome (3rd chromosome of **persimilis**) as two homologues of the C element in interspecific hybrids of **D.miranda** and **D.persimilis** (Figure 1) offered an interesting situation to study the compensatory mechanism in an X-autosome condition. In parental species, the chromosome complements were alike (possess three pairs of autosomes in both sexes, a pair of metacentric-X in females, but an X and a Y in males), barring the C element which was an autosome (3rd chromosome) in the ancestral species (**D.persimilis**) but an X chromosome in the derived one (**D.miranda**) (Sturtevant & Novitski 1941). In polytenized cells of hybrid females, while two homologues of the C element (X_2 and 3rd) represented a reasonably similar diameter and staining intensity, in hybrid males the X_2 was inflated and had a diameter wider than its autosomal counterpart (Figure 2; Mutsuddi et al. 1984). Thus the C element represented a higher template in hybrid males than in females.

Rate of transcription over the C element was measured by ^{3}H-uridine autoradiography after pulse labelling the larval salivary glands

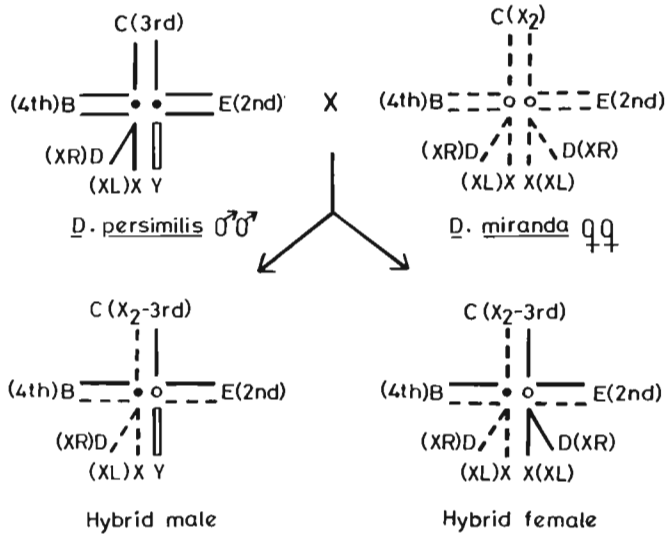


Figure 1. Interspecific hybrid generated from the cross between **D.miranda** females and **D.persimilis** males. Broken and solid lines represent the chromosomes of **D.miranda** and **D.persimilis**, respectively; hollow bar is the **persimilis** Y chromosome. Note that the C element comprises a sex chromosome (X_2 of **miranda**) and an autosome (3rd chromosome of **persimilis**) in hybrid nuclei.

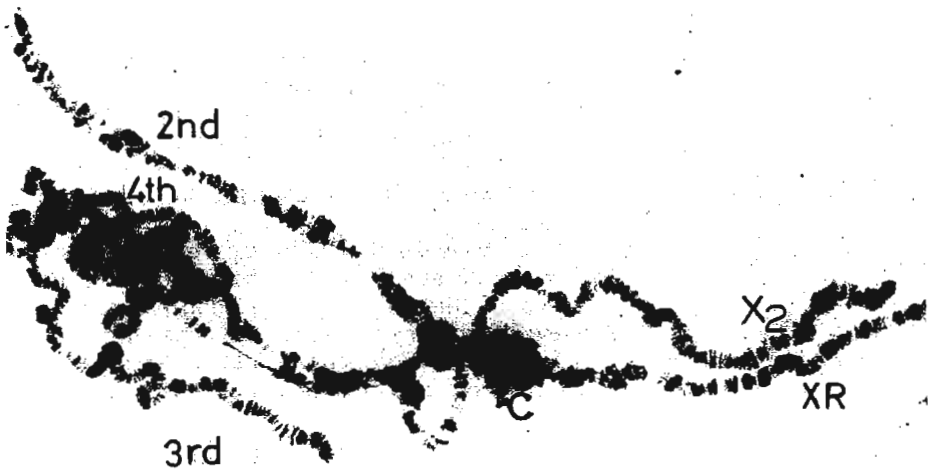


Figure 2. Salivary gland squashed preparation depicting morphology of different chromosomal arms in a hybrid male nucleus of **D.miranda** females and **D.persimilis** males. C = chromocenter.

Table 1. Data on ^{3}H-uridine labelling over 22A-34C subdivisions of the X_2 and 3rd chromosome (for chromosomal subdivisions, see Das et al. 1982 and Mutsuddi et al. 1984, resp.) in hybrid males and females in terms of mean grain and mean X_2/A and 3rd/A ratios, where the segment 35A-38C of 2nd chromosome has been used as reference

Chromosome arm	Hybrid Male		Hybrid Female		
	mean grain±S.E.	mean ratio±S.E.	mean grain±S.E.	mean ratio±S.E.	P
X_2	970.00±3.25 (25)	5.100±0.122	726.57±3.31 (26)	4.102±0.145	<0.01*
3rd	662.60±2.80 (25)	3.493±0.148 p<0.01*	705.14±2.67 (26)	4.017±0.173 p>0.05	<0.05*
C element (X_2+3rd)	1632.50±4.233 (25)	8.600±0.245	1432.00±4.188 (26)	8.119±0.332	>0.05

*=denotes significant variation. Values in () denote nr. of nuclei examined.

(^{3}H-UR activity : 12.7 Ci/mM, BARC, Trombay, India; cons. 400 µCi/ml, exposure time 14 days). Results (Table 1) revealed that the labelling density of incorporated grains over the C element, computed in terms of "sum of mean X_2/A and 3rd/A ratios" were nearly equal in both hybrid sexes ($p>0.05$). In short, the presence of inflated X_2 did not enhance the total transcription of the C element in hybrid males. However, a comparative analysis of ^{3}H-uridine distribution pattern over the X_2 (computed as mean X_2/A) and 3rd chromosome (mean 3rd/A) in both hybrid sexes revealed that in females both homologues of the C element (X_2 and 3rd) incorporated silver grains with equal efficiency ($p>0.05$), while in males the inflated X_2 showed an elevated level of activity over that of its autosomal partner ($p<0.01$). This enhanced activity of the X_2 in hybrid males was however accomplished at the expense of an almost proportional decrease of activity in the 3rd chromosome, that led the whole element to restore the constancy of total transcription in both hybrid sexes. Thus, while the X_2 showed an increased activity in hybrid males over females ($p<0.01$), the situation was the reverse in the 3rd chromosome ($p<0.05$; Table 1). Last, a comparison of activity between parental and hybrid males revealed that although the X_2 and 3rd chromosome conserved their respective "parental type" chromatin condensation (Mutsuddi et al. 1984), the mean ratios have been significantly reduced in hybrid males as compared to parental males ($p<0.01$ and $p<0.05$, respectively). All these observations lead us to support the contentions that (1) "all forms of dosage compensation simply reflect the existence of a homeostatic mechanism that operates at all times" (Devlin et al. 1982), and (2) the structural organization (state of chromatin compaction) of a chromosome does not always reflect the functional behaviour of the same (Devlin et al. 1984).

Acknowledgement: This work was supported by a UGC pre-doctoral fellowship to Mausumi Mutsuddi.

References: Das, M., D. Mutsuddi, A.K. Duttagupta & A.S. Mukherjee 1982, Chromosoma 87:373-388; Devlin, R.H., T.A. Grigliatti & D.H. Holm 1984, Chromosoma 91:65-73; ———, D.G. Holm & T.A. Grigliatti 1982, PNAS 79:1200-1204; ———, ——— & ——— 1985, Mol. Gen. Genet. 198:422-426; Lucchesi, J.C. 1983, Dev. Genet. p275-282; ———, J.M. Rawls & G. Maroni 1974, Nature 248:564-567; Mukherjee, A.S. & W. Beermann 1965, Nature 207:785-786; Mutsuddi, M., D. Mutsuddi, A.S. Mukherjee & A.K. Duttagupta 1984, Chromosoma 89:55-62; Sturtevant, A.H. & E. Novitski 1941, Genetics 26:517-541.

Ordoño, E., F.J. Silva and J. Ferré. University of Valencia, Spain.

Low dihydropterin oxidase activity in the "little isoxanthopterin" mutant of D.melanogaster.

Dihydropterin oxidase catalyzes in vitro the oxidation of 7,8-dihydropteridines into their fully oxidized derivates (Unnasch & Brown 1982). It is likely that this enzyme is involved in the biosynthesis of pterin and biopterin in Drosophila from their respective 7,8-dihydroprecursors. The aim of the present work was to screen mutants affecting pteridine biosynthesis with the hope to find some with altered dihydropterin oxidase activity.

The assay used was a modification of Unnasch & Brown's (1982) and consisted of incubating an extract enzyme (after Sephadex G-25) for 20 min at 44°C with 20 µM 7,8-dihydrobiopterin in 0.1 M potassium phosphate buffer (pH 6.4). The reaction was terminated by heating at 100°C for 1 min. After separating the precipitated proteins by centrifugation, the fluorescence of the synthesized biopterin was measured in an aliquot of the supernatant fluid after high-performance liquid chromatography in a C_{18} reverse-phase column.

The mutants analyzed for this enzyme activity were: bo, ca, car, cho, dke, g, Hn^{r3}, lix, ltd, mal, Me, rb, red, rs^2, snb and st. The dihydropterin oxidase activity was around normal levels (compared to the wild type Oregon R) in most mutants, with slightly increased or decreased values in some of them. However, we could not find any detectable activity in the lix mutant in the conditions of the assay. This finding corroborates early data by Hubby (1961) and Ferré et al. (1986) on the pteridine pattern in this mutant that led them to hypothesize that the lix gene regulated a step prior to the synthesis of pterin, in the biosynthetic pathway of isoxanthopterin.

References: Ferré, Silva, Real & Mènsua 1986, Biochem. Genet. 24:545-569; Hubby 1961, Genetics 47:109-114; Unnasch & Brown 1982, J. Biol. Chem. 257:14211-14216.

Oudman, L., A. Kamping and W. van Delden. University of Groningen, Haren, Netherlands.

Body size and survival at high temperature in Drosophila melanogaster.

In **Drosophila melanogaster** body size is negatively correlated with temperature under natural conditions, as well as in the laboratory (e.g., David et al. 1983). A question that can be raised is whether body size itself has adaptive value. In other words, are smaller flies more resistant to high temperature?

To manipulate body size flies were raised under different crowding conditions. The flies used were obtained from a laboratory strain founded in 1981 with females from a semi-natural greenhouse population in Haren, The Netherlands, and kept at 25°C. 50, 150 or 300 eggs were incubated at 25°C in glass vials of 80 x 23 mm with 10 ml medium of agar, sucrose and dead yeast (Van Delden et al. 1978). The eggs had been collected from medium on which females had laid for 8 hr. The moment of laying was different for each crowding group in order to synchronize the emergence of adults of each group (the difference between the 50 and 300 densities was two days). Flies for the experiment were collected within 24 hr on the day with the maximum number of emerging flies. After 3 days the flies were etherised and the sexes were separated and stored in new vials with 20 flies each. A number of flies of each group was weighed. Table 1 lists the mean weights of the groups of flies, and shows clearly that flies from the more crowded groups weigh less.

At the age of 8 days, the flies were placed in new vials and put in an incubator at 37°C with a relative humidity of 80% to prevent desiccation. After 3 and 4 hr, 5 vials (or 4, see Table 2) of each sex and crowding group were replaced to 25°C. After 20 hr the dead flies in each vial were counted. In control vials, which were kept at 25°C, virtually no flies died in this period. Table 2 lists the mean percentages

Table 1. Mean weight and standard deviation of each group of flies.

sex	crowding level	no. flies weighted	weight(mg) mean ± s.d.
male	50	10	0.88 ± 0.04
	150	20	0.77 ± 0.06
	300	20	0.67 ± 0.09
female	50	10	1.37 ± 0.12
	150	20	1.12 ± 0.11
	300	20	1.07 ± 0.13

Table 2. Angular transformed percentages of dead flies. Mean and s.d. per vial.

sex	crowding level	no. vials	Mortality after: 3 hr	4 hr
male	50	5	35±17	64±18
	150	5	41± 8	65± 9
	300	5	34±17	56± 8
female	50	5	30±11	64±17
	150	4	36±18	48±17
	300	5	34±20	50±12

Table 3. Analysis of variance of angular transformed percentages of dead flies per vial.

	SS	DF	MS	F	sign.
sex (s)	412	1	412	1.9	ns
time (t)	7827	1	7827	35.7	***
crowding (c)	301	2	150	0.7	ns
s x t	55	1	55	0.3	ns
s x c	197	2	98	0.4	ns
t x c	501	2	250	1.1	ns
s x t x c	208	2	104	0.5	ns
residual	10094	46	219		

(arcsin $\sqrt{p}$ transformed) of dead flies per vial. Table 3 gives an analysis of variance of this data.

These tables show that only the time of exposure to 37°C has, as expected, a significant effect on survival. There are no significant differences in survival between the sexes or between the crowding groups. So body size, when manipulated by crowding, does not affect the resistance to high temperature.

Levins (1969) found higher mortality of small flies at high temperature. McKechnie & Parsons (1974) found higher mortality of males at high temperature. In both experiments, however, desiccation was not prevented, while this character may be affected by body size in a different way than high temperature alone.

References: David, J.R., J. Van Herrewege & Y. Cohet 1983, in: Genetics and Biology of Drosophila (Ashburner et al., eds.), Academic Press, London, v3d:105-170; Levins, R. 1969, Am. Nat. 103:483-499; McKechnie, J.A. & P.A. Parsons 1974, Aust. J. Biol. Sci. 27:441-456; Van Delden, W., A.C. Boerema & A. Kamping 1978, Genetics 90:161-191.

Pegueroles, G., C. Segarra and A. Prevosti.
University of Barcelona, Spain. A new inversion of the E chromosome in **D.subobscura.**

The species **D.subobscura** has a very rich chromosomal inversion polymorphism that affects all its five acrocentric chromosomes (A, J, U, E, and O) although in different degrees. The E chromosome presents a rather marked polymorphism in the Palearctic distribution area of the species (Krimbas & Loukas 1980; Sperlich, Pinsker & Mitrofanov 1981). One inversion, E_{17}, has been found in low frequencies in two Chilean colonizing populations (Brncic et al. 1982).

We have found a new inversion, E_{18}, in a sample captured in Eureka (California). The inversion was detected after ten generations of sib mating in an inbred line started with a single inseminated female. Thus, we cannot be sure whether the inversion was present in the sample population or was originated in the laboratory, although the former assumption seems to be more likely.

E_{18} is an overlapping inversion of the E_{1+2+9} arrangement and is located at the distal end of the chromosome (Figure 1). Its breakpoints are located, approximately, in the 63C and 72D subsections and therefore the inversion comprises the sections 64 (A and B), 68 (C, D and E), 69, 70, 71 and 72 (A, B and C) described in the Kunze-Mühl and Müller map.

A homozygous strain for the $E_{1+2+9+18}$ arrangement has been obtained (Figure 2). This strain may be very useful in genetic experiments in order to prevent recombination in $E_{st}/E_{1+2+9+18}$ individuals because the described arrangement comprises nearly the whole length of the E chromosome.

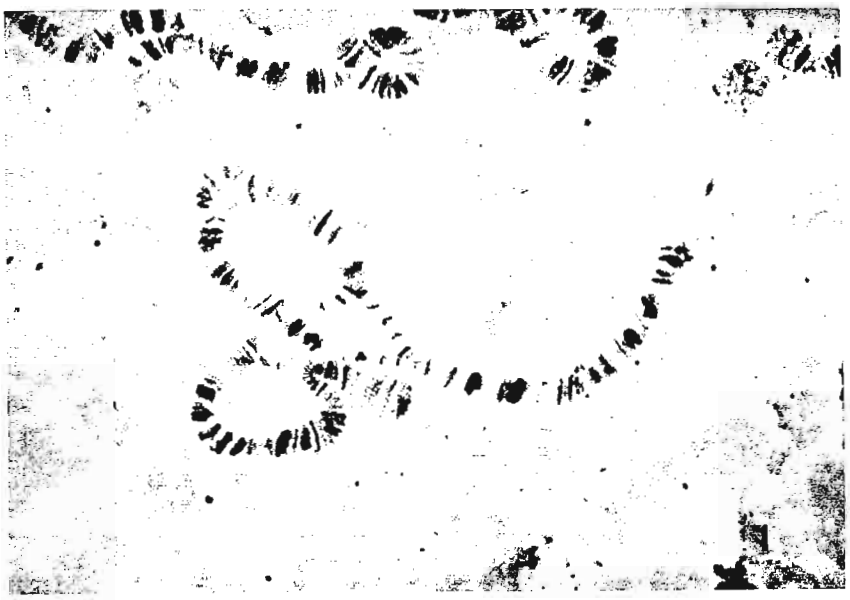


Figure 1. E chromosome configuration of an $E_{1+2+9+18}/E_{st}$ larva.

Figure 2. E chromosome of an $E_{1+2+9+18}$ homozygous larva. The arrowheads show breakpoints of the inversion E_{18}.

Acknowledgement: This work was supported by grant no. 2844 from the Com. Asesora para la Invest. Cient. y Tecnica, Spain, and grant no. CCB-8504013 from the U.S.-Spain Joint Comm. for Scient. and Tech. Cooperation.

References: Brncic, D., M. Budnik & A. Prevosti 1982, Medio Ambiente 6:23-32; Krimbas, C.B. & M. Loukas 1980, Evol. Biol. 12:163-234; Sperlich, D., W. Pinsker & V.G. Mitrofanov 1981, Genetica 54:329-334.

Real, M.D. and J. Ferré. University of Valencia, Spain. Screening of Drosophila species for the occurrence of xanthurenic acid 8-0-β-D-glucoside.

Xanthurenic acid 8-0-β-D-glucoside has been characterized as a tryptophan metabolite (via 3-hydroxykynurenine) in the pathway that leads to the synthesis of dihydroxanthommatin (brown eye pigment) in Drosophila (Figure 1) (Ferré et al. 1985). This compound was first discovered in heads of eye-color mutants of **Drosophila melanogaster** but not in the wild type (Ferré & Ménsua 1983). Based on a phylogenetic study carried out by Hubby & Throckmorton (1960) in species of the genus Drosophila, there was the possibility that a spot found in paper chromatograms of other species' wild types could be xanthurenic acid 8-glucoside.

The goal of this work was to survey other species closely related to **Drosophila melanogaster** for the occurrence of xanthurenic acid and its glucoside.

We have studied 29 Drosophila species (Table 1) using two-dimensional cellulose thin-layer chromatography. Eighty fly heads were homogenized in 0.1 ml of methanol/glacial acetic acid/water

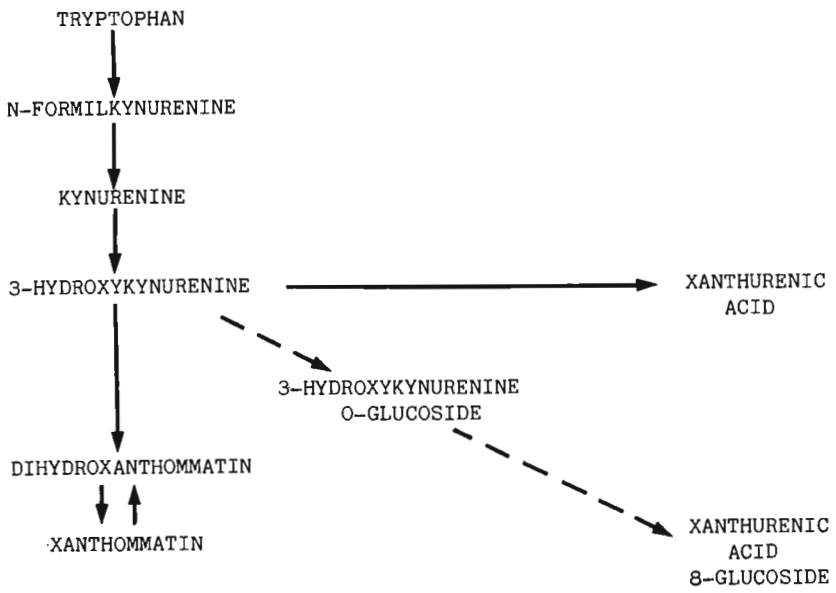


Figure 1. Biosynthesis of xanthommatin in Drosophila. Broken arrows show the proposed pathway that leads to xanthurenic acid glucoside (Ferré et al. 1985).

Table 1. Occurrence of xanthurenic acid and its glucoside in the wild types of species of the genus Drosophila (+ = detected; - = not detected).

SPECIES	Xanthurenic acid	Xanthurenic acid glucoside
SUBGENUS SOPHOPHORA		
obscura group		
D.azteca	+	-
D.ambigua	+	-
D.bifasciata	+	+
D.guanche	+	+
D.miranda	+	+
D.obscura	+	-
D.persimilis	+	-
D.subobscura	+	-
D.subsilvestris	+	+
D.tristis	+	-
melanogaster group		
D.melanogaster	+	-
D.simulans	+	-
willistoni group		
D.equinoxialis	+	+
D.nebulosa	+	+
saltans group		
D.emarginata	+	+
D.prosaltans	+	+
SUBGENUS SCAPTODROSOPHILA		
victoria group		
D.lebanonensis	-	-
SUBGENUS DORSILOPHA		
D.busckii	+	-
SUBGENUS DROSOPHILA		
virilis group		
D.littoralis	+	-
D.virilis	+	-
robusta group		
D.robusta	+	-
D.sordidula	+	+
repleta group		
D.buzzatii	+	-
D.hydei	-	-
D.mercatorum	+	-
D.repleta	+	-
immigrans group		
D.immigrans	+	-
funebris group		
D.funebris	+	-
quinaria group		
D.kuntzei	+	+

(4:1:5, by volume). After centrifugation at 15,600 x g for 2 min, 10 µl of supernatant was spotted on the plate and subjected to two-dimensional chromatography with isopropanol/2% aqueous ammonium chloride.

Xanthurenic acid and its glucoside were identified in the chromatograms by their characteristic blue fluorescence under UV light and their R_f values, as compared to those of the synthetic standards. The R_f values were 0.80 and 0.65 in the first dimension, and 0.26 and 0.55 in the second dimension for xanthurenic acid and its glucoside, respectively.

Results are summarized in Table 1. Xanthurenic acid glucoside was found in ten out of the 29 species analyzed, 9 of them belonging to the Sophophora subgenus. Xanthurenic acid was detected in 27 species and was present whenever its glucoside was found.

Hubby & Throckmorton (1960) studying 150 species for the occurrence of pteridines, found in 4 of them an unknown fluorescent compound which they reported not to be a pteridine. We have identified this compound as xanthurenic acid 8-glucoside. The present work adds a considerable number of species that accumulate this glucoside (6 new species) in relation to the species studied by Hubby & Throckmorton (1960).

This is the first time that the occurrence of xanthurenic acid glucoside has been demonstrated in an organism other than **Drosophila melanogaster.** The metabolic particularities that cause the accumulation of this side metabolite of dihydroxanthommatin biosynthesis in some but not other Drosophila species is currently being studied in our laboratory.

References: Ferré, J. & J.L. Mensua 1983, DIS 59:35-36; ———, M.D. Real, J.L. Mensua & K.B. Jacobson 1985, J. Biol. Chem. 260:7509-7514; Hubby, J.L. & L.H. Throckmorton 1960, PNAS 46:65-78.

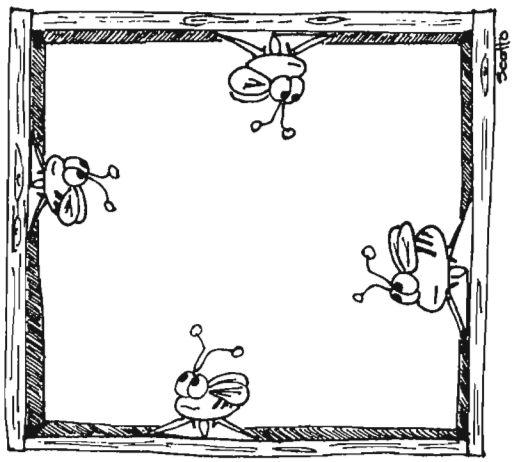


Figure 2. E chromosome of an $E_{1+2+9+18}$ homozygous larva. The arrowheads show breakpoints of the inversion E_{18}.

Acknowledgement: This work was supported by grant no. 2844 from the Com. Asesora para la Invest. Cient. y Tecnica, Spain, and grant no. CCB-8504013 from the U.S.-Spain Joint Comm. for Scient. and Tech. Cooperation.

References: Brncic, D., M. Budnik & A. Prevosti 1982, Medio Ambiente 6:23-32; Krimbas, C.B. & M. Loukas 1980, Evol. Biol. 12:163-234; Sperlich, D., W. Pinsker & V.G. Mitrofanov 1981, Genetica 54:329-334.

Real, M.D. and J. Ferré. University of Valencia, Spain. Screening of Drosophila species for the occurrence of xanthurenic acid 8-0-β-D-glucoside.

Xanthurenic acid 8-0-β-D-glucoside has been characterized as a tryptophan metabolite (via 3-hydroxykynurenine) in the pathway that leads to the synthesis of dihydroxanthommatin (brown eye pigment) in Drosophila (Figure 1) (Ferré et al. 1985). This compound was first discovered in heads of eye-color mutants of **Drosophila melanogaster** but not in the wild type (Ferré & Ménsua 1983). Based on a phylogenetic study carried out by Hubby & Throckmorton (1960) in species of the genus Drosophila, there was the possibility that a spot found in paper chromatograms of other species' wild types could be xanthurenic acid 8-glucoside.

The goal of this work was to survey other species closely related to **Drosophila melanogaster** for the occurrence of xanthurenic acid and its glucoside.

We have studied 29 Drosophila species (Table 1) using two-dimensional cellulose thin-layer chromatography. Eighty fly heads were homogenized in 0.1 ml of methanol/glacial acetic acid/water

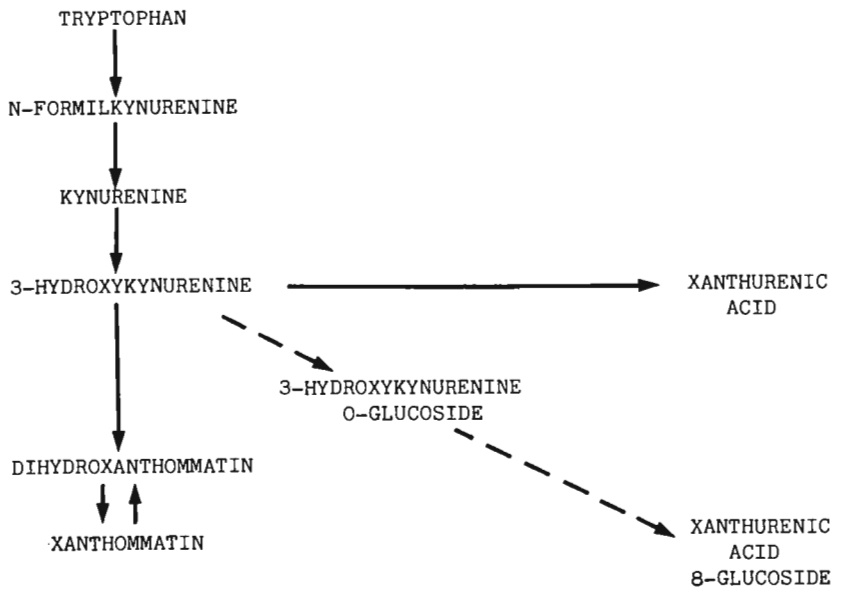


Figure 1. Biosynthesis of xanthommatin in Drosophila. Broken arrows show the proposed pathway that leads to xanthurenic acid glucoside (Ferré et al. 1985).

Table 1. Occurrence of xanthurenic acid and its glucoside in the wild types of species of the genus Drosophila (+ = detected; - = not detected).

SPECIES	Xanthurenic acid	Xanthurenic acid glucoside
SUBGENUS SOPHOPHORA		
obscura group		
D.azteca	+	-
D.ambigua	+	-
D.bifasciata	+	+
D.guanche	+	+
D.miranda	+	+
D.obscura	+	-
D.persimilis	+	-
D.subobscura	+	-
D.subsilvestris	+	+
D.tristis	+	-
melanogaster group		
D.melanogaster	+	-
D.simulans	+	-
willistoni group		
D.equinoxialis	+	+
D.nebulosa	+	+
saltans group		
D.emarginata	+	+
D.prosaltans	+	+
SUBGENUS SCAPTODROSOPHILA		
victoria group		
D.lebanonensis	-	-
SUBGENUS DORSILOPHA		
D.busckii	+	-
SUBGENUS DROSOPHILA		
virilis group		
D.littoralis	+	-
D.virilis	+	-
robusta group		
D.robusta	+	-
D.sordidula	+	+
repleta group		
D.buzzatii	+	-
D.hydei	-	-
D.mercatorum	+	-
D.repleta	+	-
immigrans group		
D.immigrans	+	-
funebris group		
D.funebris	+	-
quinaria group		
D.kuntzei	+	+

(4:1:5, by volume). After centrifugation at 15,600 x g for 2 min, 10 µl of supernatant was spotted on the plate and subjected to two-dimensional chromatography with isopropanol/2% aqueous ammonium chloride.

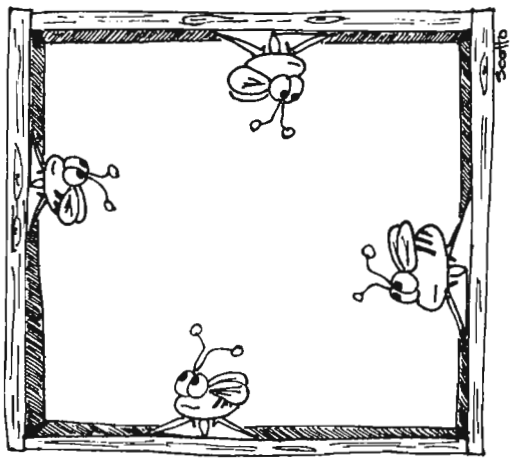
Xanthurenic acid and its glucoside were identified in the chromatograms by their characteristic blue fluorescence under UV light and their R_f values, as compared to those of the synthetic standards. The R_f values were 0.80 and 0.65 in the first dimension, and 0.26 and 0.55 in the second dimension for xanthurenic acid and its glucoside, respectively.

Results are summarized in Table 1. Xanthurenic acid glucoside was found in ten out of the 29 species analyzed, 9 of them belonging to the Sophophora subgenus. Xanthurenic acid was detected in 27 species and was present whenever its glucoside was found.

Hubby & Throckmorton (1960) studying 150 species for the occurrence of pteridines, found in 4 of them an unknown fluorescent compound which they reported not to be a pteridine. We have identified this compound as xanthurenic acid 8-glucoside. The present work adds a considerable number of species that accumulate this glucoside (6 new species) in relation to the species studied by Hubby & Throckmorton (1960).

This is the first time that the occurrence of xanthurenic acid glucoside has been demonstrated in an organism other than **Drosophila melanogaster.** The metabolic particularities that cause the accumulation of this side metabolite of dihydroxanthommatin biosynthesis in some but not other Drosophila species is currently being studied in our laboratory.

References: Ferrè, J. & J.L. Mènsua 1983, DIS 59:35-36; _____, M.D. Real, J.L. Mènsua & K.B. Jacobson 1985, J. Biol. Chem. 260:7509-7514; Hubby, J.L. & L.H. Throckmorton 1960, PNAS 46:65-78.



Rim, N.R., B.S. Lee and T.H. Lee. Chonbuk National University, Chonju, Korea.

Inversion behaviors corresponding to surrounding conditions from scores of Korean **D.melanogaster** populations.

Relatively little study has so far been carried out on the relationships between inversion polymorphisms and environmental variables, although a direct correspondence may often have existed. Therefore the present work was focused on how polymorphic inversions respond to their surrounding conditions and what factors cause geographic differences in inversion frequencies. To examine the inversion behaviors with ecogeographical habitats, several techniques of multivariate analysis as canonical correlation, back-step multiple regression, principal component analysis (PCA), and canonical discriminant analysis were employed.

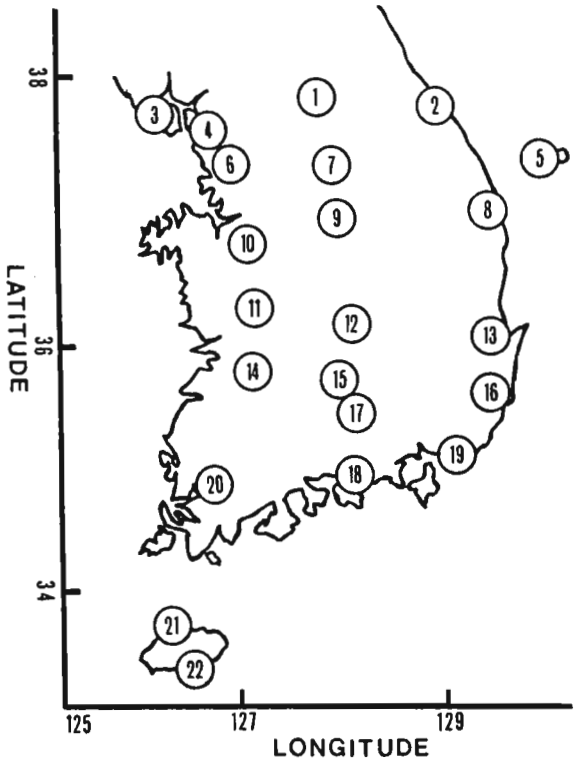


Figure 1. The Korean localities sample.

Table 1. Established environmental variables for the present study.

Abbreviation	Description
Mean	
MTAN	annual mean of average daily temperature
TRM	daily temperature range for a month
TRAU	daily temperature range for autumn
HAN	annual mean average relative humidity
HWI	average relative humidity for winter
SWI	duration of sunshine average over winter
PSU	summer rainfall
Cv (coefficient of variation)	
CMTAN	Cv of average daily temperature over year
CHTSP	Cv of daily maximum temperature for spring
CLTAN	Cv of daily minimum temperature over year
CHW	Cv of weekly average relative humidity
CHAN	Cv of average annual relative humidity
CWM	Cv of average monthly wind speed

A total of 9,260 fruit flies, 4,857 males and 4,403 females, were collected from 22 Korean localities shown in Figure 1, from late August through early October in 1985. Flies were captured when every population reaches its most prosperous size, by sweeping an insect net over fallen rotting fruits or trap cans containing yeasted fruit mash. For cytological chromosome preparations, the females inseminated in nature were separated individually into each vial placed culture medium. The smears of salivary chromosomes to detect inversion heterozygous were made from only one F_1 larva per vial. For males each was mated to several virgin females homozygous for standard chromosome sequence, and then the salivary gland cells of one F_1 larva from each mating were smeared to analyze gene arrangements in a haploid set of sperm chromosomes produced by wild males.

From the 22 South Korean populations, a total of 33 polymorphic inversions were found, among them 6 were identified as belonging to common and rare cosmopolitan types and the remnants to unique and/or recurrent endemic ones in a given locality where screened.

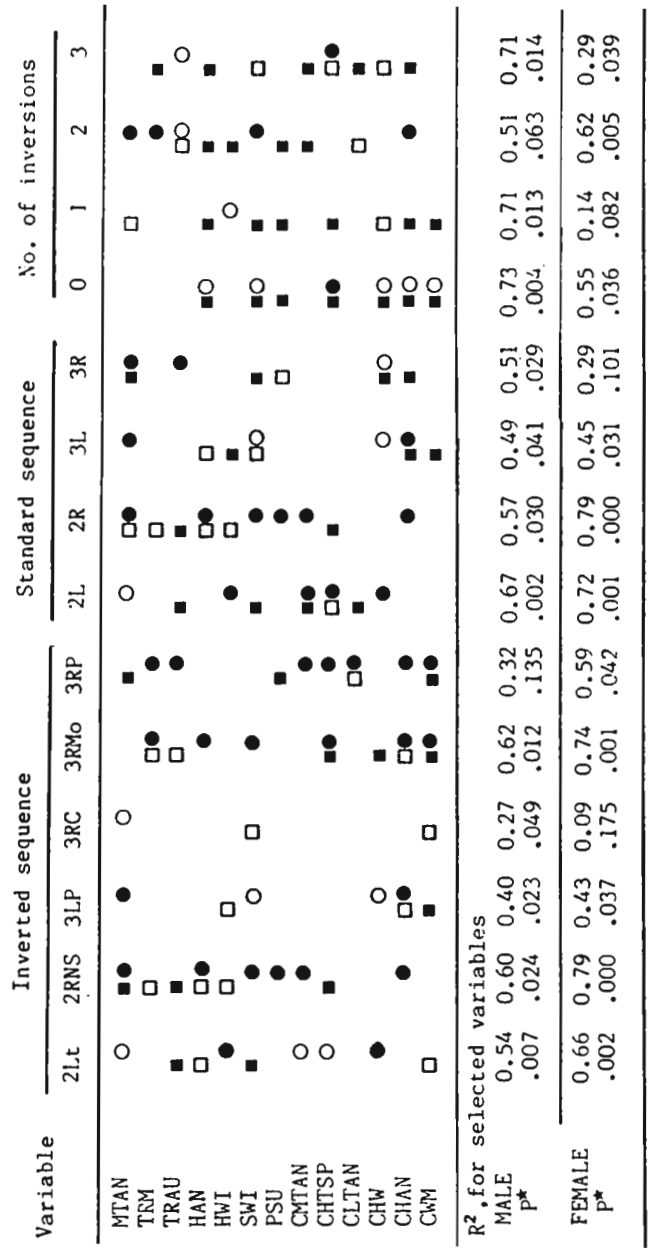
As shown in Table 1, the established 13 independent predictors have been selected from a total of 131 possible natural conditions through three steps of concentrating procedure. The two geographical conditions, latitude and longitude, were leached out by the reducing process.

Canonical correlation contrastive with multiple regression predicts multiple dependent variables from multiple independent variables; therefore, it is convenient to analyse the relationships between sets of multiple criteria and multiple predictors. The first canonical variate

Table 2. Canonical correlation analysis of the relationship between cosmopolitan inversions and environmental variables.

Variable	MALE Canonical loading	MALE Canonical weight	Female Canonical loading	Female Canonical weight
Environmental factors				
MTAN	0.28	0.22	-0.12	1.81
TRM	-0.04	1.23	-0.21	0.17
TRAU	-0.24	-0.84	-0.27	-0.40
HAN	-0.33	0.68	-0.21	0.09
HWI	-0.47	-1.54	-0.21	0.12
SWI	0.08	-0.53	0.20	0.30
PSU	0.40	0.47	-0.60	-0.97
CMTAN	-0.36	2.55	0.23	1.43
CHTSP	-0.55	-1.48	0.42	0.06
CLTAN	-0.24	-0.71	0.13	0.02
CHW	-0.05	0.07	0.48	0.23
CHAN	0.33	0.18	0.44	0.24
CWM	-0.13	-0.12	0.08	-0.40
Variance	9.66%		10.10%	
Inversions				
2Lt	0.42	1.03	0.34	0.44
2RNS	0.38	-0.46	-0.63	-0.89
3LP	0.26	0.28	-0.03	-0.16
3RC	0.02	-0.73	0.02	-0.10
3RMo	0.33	0.33	-0.47	-0.64
3RP	-0.47	-1.22	0.18	-0.07
Variance	11.90%		12.77%	
Canonical correlation (R)	0.996 (P=0.008)		0.989 (P=0.049)	
Redundancy (Ry| x)	0.12		0.12	

Table 3. Multiple regression analyses for inverted chromosome sequences, standard sequences, and number of inversions per fly with multiple environmental variables (symbols indicate square male and circle female, and open > 0.05, black < 0.05 for probability of the obtained partial regression coefficients). * = probability.



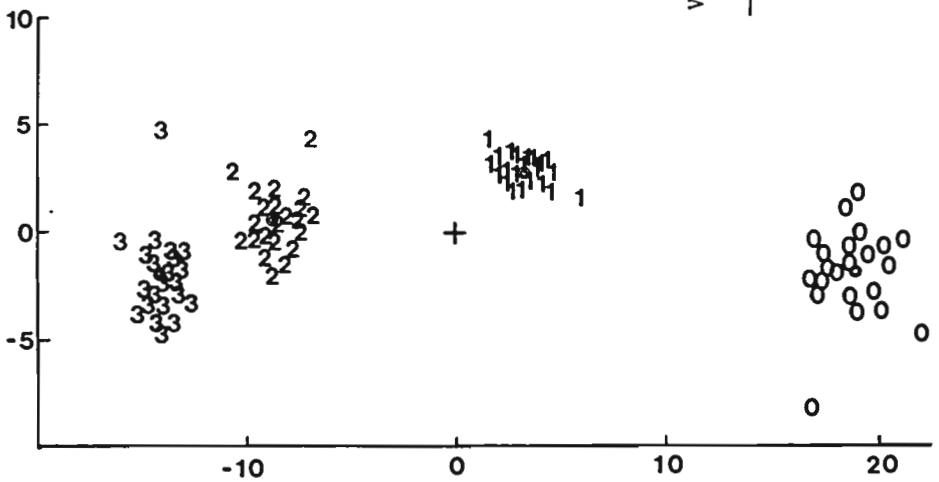


Figure 2. Canonical discriminant groups for thenumber of inversions carried by a single fruitfly based on the sample mean vectors.

only showed significant correlation in both male and female (Table 2). The association patterns of genetic variations with surrounding factors did not lay far from each sex; e.g., the environmental variabilities (coefficient of variation: Cv) might influence the inversion frequencies rather than the environmental mean (Mean) in both sexes.

The canonical loadings for each variable showed that in male (2L)t and (2R)NS negatively associated with environmental Mean TRAU and HAN, and negatively with environmental Cv CHTSP. Also (3R)P indicated a trend similar to that of (2L)t. In females, (2R)NS and (3R)Mo associated positively with Mean PSU, negatively with Cv CHW and CHAN.

Multiple regression scores are presented in Table 3. Here involved were all the multi-regression analyses together with inverted sequences and standard sequences in each arm of the 2nd and 3rd chromosomes, and with the number of inversions carrying by a single fly. Taking into account the results given in this Table, we could not show that particular environmental variables are heavily concentrated to any dependent criteria; however, there were some subtle differences among sexes. That is, while females in both the chromosome sequences, inverted and standard sequence, appeared to be sensitive to the changes of environmental conditions rather than males, in the number of inversions carried by a single fly, males showed more significant behavior to those changes. Especially note that although (2R)NS and 2R (standard: St) in male and group 0 (no. inversions per fly) in females correlated insignificantly to each of the several variables at 5% level, heavy correlations were found from the regression coefficients for the selected variables. In (3R)P and group 2 (no. inversions per fly) of males, conversely, though the selected variables were almost all closely correlated to the dependent variables, the P-values showed no significance statistically as seen in lower part of the Table. Therefore, the importance of the less effective variables cooperated with each other should be taken into consideration as a "poly-variable effect", even if it were so insignificant in respective.

Principal component analysis (PCA) was employed to determine what environments were more effective to the dependent variables. Among three PC factors we obtained, PC1 composed of MTAN and TRM of the Mean and CMTAN, CHTSP and CLTAN in the Cv. PC2 contained HAN only of the Mean and CHAN, and CWM of the Cv. Also PC3 included TRAU, HWI, and SWI of the Mean and CHW only from the Cv. Correlation analyses of the dependent variables with the three PCA scores were undertaken. In Male, (2L)t, 2L(St), group 0 and group 1 correlated significantly with PC1, and (3L)P and 3L(St) with PC2. Also in females there was a general similarity to those of males.

Canonical discriminant analysis in this study distinguished a relative adaptability of flies carrying none, one or many more inversion(s), in both males and females. As plotted in Figure 2, every group has independently separated from each other, where group 3 contains a few flies carrying 4 inversions. Also the null hypothesis was rejected in every pair of the groups, thus it is concluded that the four groups differ significantly from each other based on their mean vectors.

References: Bridge, C.B. 1935, J. Heredity 26:60-64; Hoffmann, R.J. 1981, Biochem. Genet. 19:145-154; Knibb, W.R., J.G. Oakeshott & J.B. Gibson 1981, Genetics 98:833-847; Mulley, J.C., J.W. James & J.S.F. Barker 1979, Biochem. Genet. 17:105-126; Watanabe, T.K., T. Watanabe & C. Oshima 1976, Evolution 39:109-118.

Rodell, C.F. Coll. of St. Benedict, St. John's Univ., Collegeville, Minnesota USA. Male age and mating success in **Drosophila melanogaster.**

Male mating success as related to age was investigated by female preference tests in two genetically modified stocks, w and w^a. In order to minimize genetic effects, the two stocks were made isogenic for chromosomes II, III, Y, and an unknown portion of X. The w stock was initiated with a single inseminated female and continued for 8 generations with a single, full-sib pair. The w^a stock was derived from a single w^a male and a single w male using females from the balanced lethal stock Cy/Pm; Sb/D. Virgin males were tested with 4-day-old, heterozygous (w/w^a), virgin females. For both males and females, flies eclosing over a 6 hr period were considered members of the same age cohort. To examine male mating competition between two different age groups, 20 females were placed in a $\frac{1}{2}$-pint culture bottle with 20 i-aged w males and 20 j-aged w^a males (i = 5, 10, 15, 20, 25, 35 and j = 5, 10, 15, 20, 25, 35 days). Combinations where i = j were used to indicate any selective differences between genotypes, while combinations where $i \neq j$ further indicate differences due to age. Males to be tested were placed in the same culture bottle 4 days prior to the introduction of females. Females were introduced into the male culture bottle without anaesthetization and left for 4 hr in constant light. I found that 4 hr allowed almost all females to mate while minimizing the frequency of double inseminations. At the end of the 4 hr period all flies were removed from the culture bottle, the males discarded, and the females individually cultured in shell vials. The progeny were

Table 1. Proportions of matings involving w males.[a]

Age w^a males(d)	Age of w males (d) 5	10	15	20	25	35
5	0.57 (60)	0.59 (59)	0.36 (59)	0.33 (55)	0.33 (58)	0.21 (57)
10	0.32 (53)	0.46 (57)	0.34 (53)	0.28 (60)	0.26 (57)	0.07 (57)
15	0.72 (53)	0.79 (56)	0.55 (60)	0.32 (59)	0.28 (54)	0.17 (60)
20	0.58 (60)	0.60 (58)	0.62 (52)	0.44 (57)	0.33 (54)	0.19 (59)
25	0.69 (48)	0.57 (60)	0.74 (50)	0.61 (49)	0.55 (58)	0.22 (50)
35	0.83 (54)	0.90 (51)	0.80 (56)	0.75 (55)	0.71 (52)	0.43 (49)

[a]number of matings is given in parentheses.

Table 2. Results of mate choice trials with males of different ages.

Age of tested males (d) i vs j	No. of matings	No. of matings by i males	X^2 (1 df); H_0: random mating
5 vs 10	112	41	8.04*
5 vs 15	112	76	14.29*
5 vs 20	115	72	7.31*
5 vs 25	106	72	13.62*
5 vs 35	111	90	42.89*
10 vs 15	109	79	22.03*
10 vs 20	118	78	12.24*
10 vs 25	117	76	10.47*
10 vs 35	108	99	75.00*
15 vs 20	111	72	9.81*
15 vs 25	104	76	22.15*
15 vs 35	116	95	47.21*
20 vs 25	103	76	23.31*
20 vs 35	114	89	35.93*
25 vs 35	102	76	24.51*

* = $p < 0.01$.

scored 15 to 18 days later. Insemination by a w male produces white and heterozygous daughters, while the daughters of a w^a male are either apricot or heterozygous. Heterozygous phenotypes are visually distinguishable from either homozygote. Double inseminations, indicated by both white and apricot daughters from the same female, were discounted. All tests were replicated three times and carried out at 25±1°C.

When the w and w^a males are of the same age, neither genotype appears to have a mating advantage. The number of females inseminated by any one genotype does not deviate significantly from what would be expected under random mating [X^2(5 df) = 2.29; $p>0.75$). These data are on the principle diagonal of Table 1 and represent combinations of i = j. Given no genetic differences in male mating success, mating success related to age was examined by combining complementary tests of $i \neq j$; i.e., the results of 5 days w vs 10 days w^a and 10 days w vs 5 days w^a, etc., were combined. The results, represented in Table 2, indicate that mating deviated significantly from random mating for all age group comparisons ($p<0.01$ for all comparisons). In general, the younger age classes are more successful, with the 10 day males having the greatest total success.

Geer & Green (1962) found that w^a males have greater mating success than w males in both constant light and an alternating light-dark environment. In constant dark they found mating to be random. They used males between 0 and 2 days of age. The difference between their results and mine, where I found mating to be random within any age class, might be due to genetic differences between the stocks or the older age of the males used in this study.

It has been demonstrated that female age can influence their choice of mates (Pruzan & Ehrman 1974; Klobutcher 1977). Age-dependent differential mating success among male Drosophila has not been directly demonstrated though several studies suggested it. For example, the number of females inseminated increases as male age increases from 12 to 144 hr (Bosiger 1953) and is greater in 3 days than in 28 day males (Kosuda 1985), the length of courtship latency is inversely related to age in males whose ages are between 14 and 66 hr (Eastwood & Burnet 1977), and the percentage of pairs mating within 1 hr increases with age in **D.persimilis** lines selected for fast and slow developmental speed (Spiess & Spiess 1966).

The results presented here are consistent with the se observations though it should be kept in mind that the mutant stocks used in this study were highly homozygous and, in general, considerably less vigorous than the wild type. Hence, the results are quantitatively valid only for the synthetic genetic system used in these experiments. At a qualitative level the results do demonstrate age-dependent mating success in male Drosophila and, as such, emphasize the need to control male age in mating experiments.

References: Bösiger, E. 1953, Experientia 9:221-222; Eastwood, L. & B. Burnet 1977, Behav. Genet. 7:359-372; Geer, B.W. & M.M. Green 1962, Am. Nat. 96:175-181; Klobutcher, L.A. 1977, Behav. Genet. 7:33-36; Kosuda, K. 1985, DIS 61:103-104; Pruzan, A. & L. Ehrman 1974, Behav. Genet. 4:159-164; Spiess, E.B. & L.D. Spiess 1966, Genetics 53:695-708.

Semionov, E.P. Institute of Molecular Biology, Sofia, Bulgaria. Multiplicity of nucleoli in larval polytene cells of **Drosophila melanogaster** Oregon R strain and its X/O derivative.

In **Drosophila melanogaster** the genes coding for (18S + 28S) ribosomal RNA (rRNA) are arranged as tandemly repeated units clustered as two nucleolar organizers (NOs) and located on X and Y chromosomes (Ritossa & Spiegelman 1965). The phenomenon of dose compensation of the rRNA genes (rDNA) in this organism was first described by Tartof (1971, 1973) who showed that in flies possessing one NO only, a compensatory increase in the quantity of these genes took place up to an almost normal level.

Table 1. Frequency of polytene nuclei with additional nucleoli in larvae of different genotypes.

Genetic constitution	No. of nuclei analysed	Freq. of nuclei with/additional nucleoli(%±2SEM)
X_{OR}/X_{OR}	398	43.7 ± 4.9
X_{OR}/Y_{OR}	350	47.1 ± 5.3
X_{OR}/O	435	48.5 ± 4.7
X_{CS}/X_{CS}	608	39.3 ± 4.0
X_{CS}/Y_{CS}	931	40.3 ± 3.2
X_{CS}/O	889	45.2 ± 3.4

In a number of works (Yedbovnick et al. 1980; Dutton & Krider 1984) by hybridisation of total adult flies or larval DNA to H^3-labelled rRNA it was shown that X-chromosome located NOs of two wild-type strains (Canton S and Oregon R) are different in their ability to compensate in the single-NO state: in X_{OR}/O males the compensation takes place, whereas in X_{CS}/O males it was not detected. According to our previous data (Seminov & Kirov 1986) in salivary gland polytene cells of X_{CS}/O males there are also no differences in the frequency of the additional nucleoli formation (rRNA genes not associated with the NO) compared to the normal X_{CS}/X_{CS} females and X_{CS}/Y_{CS} males. Nevertheless, in larvae of other single-NO genotypes carrying the Canton S X-chromosomes ($X_{CS}/Xsc^{4L}sc^{8R}$; X_{CS}/Y^{bb-}) the frequency of additional nucleoli formation was significantly higher. The results of in situ hybridisation experiments suggested that rDNA of the additional nucleoli appeared as a result of the excision of long replica from the polytene NO.

In this work we analysed the frequency of the additional nucleoli formation in females and males of the Oregon R strain and its X_{OR}/O derivative obtained by crossing the virgin Oregon R females with males from the stock $y^2su(w^a)\ w^a\ Y^LY^S/C(1)RM,y$ (similarly as in the case with X_{CS}/O). The analysis was performed on isolated polytene nuclei from salivary gland cells of third-instar larvae by a method described earlier (Semionov & Kirov 1986) using vital staining of the nucleoli with trypan blue. The results are shown in Table 1 together with the corresponding values for Canton S strain and its X_{CS}/O derivative taken from our previous work. According to these data the frequency of additional nucleoli formation in X_{OR}/O male polytene cells does not differ from these in females and males of Oregon R and Canton S strains and from X_{CS}/O males.

Acknowledgement: I thank Dr. Alexander Smirnov for providing the Drosophila stocks.

References: Dutton, F.L. & H.M. Krider 1984, Genetics 107:395-404; Ritossa, F.M. & S. Spiegelman 1965, PNAS 53:737-745; Semionov, E.P. & N.Kh. Kirov 1986, Chromosoma 93:477-482; Tartof, K.D. 1971, Science 171:294-297; ——— 1973, Genetics 73:57-71; Yedbovnick, B., H.M. Krider & B.I. Levine 1980, Biochem. Genet. 18:869-877.

Sharma, A.K. and G.S. Miglani. Punjab Agricultural University, Ludhiana, India. Drosophilidae collections from Ludhiana (Punjab, India).

Because of its luxuriant flora and varied climatic conditions, Punjab offers a rich abode for a variety of Drosophila species. Drosophilidae from Ludhiana is, however, less studied. In this communication we report a collection record of Drosophilidae present in and around the campus of the Punjab Agricultural University, Ludhiana (Punjab, India), during the period January 1984 to December 1984. Baits containing fermenting banana were used at 4 spots. Collections were made in the mornings at weekly/biweekly intervals.

Nine species of genus Drosophila, one species of genus Zaprionus and a few unidentified species were recorded (Table 1). Out of a total collection of 8916 flies, 400 could not be identified but seemed to belong to species **D.bifasciate, D.kikkwai** and **D.repleta.** Species existing in decreasing order of abundance can be arranged as follows: **Z.paravittiger** (3543), **D.busckii** (2288), **D.jambulina** (1721), **D.malerkotliana** (373), **D.punjabiensis** (170), **D.napalensis** (94), **D.takahashii** (94), **D.immigrans** (88), **D.melanogaster** (80) and **D.rufa** (65).

Among the winter species, **D.busckii** and **D.napalensis** were abundant in February, **D.immigrans** in March and **D.rufa** in January. In summer, **D.jambulina** was most prevalent in July, **D.takahashii** in May and September, **Z.paravittiger, D.malerkotliana** and **D.punjabiensis** in September. **Z.paravittiger, D.jambulina** and **D.melanogaster** were found in the natural populations almost throughout the year; the availability of

Table 1. Collection record of Drosophilidae from Ludhiana (Punjab, India) for the calendar year 1984.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
D.busckii	395	1381	422	61	2	0	0	0	0	8	4	15	2288
D.immigrans	2	28	33	15	0	0	0	0	0	10	0	0	88
D.jambulina	85	45	13	26	247	52	588	311	238	70	38	8	1721
D.melanogaster	6	13	2	14	18	0	6	2	4	13	1	1	80
D.malerkotliana	0	0	0	0	0	0	0	3	288	30	50	2	373
D.nepalensis	24	31	16	16	1	0	0	0	0	0	4	2	94
D.punjabiensis	14	28	0	4	0	0	0	2	66	13	23	20	170
D.rufa	35	9	12	0	0	0	0	0	0	0	0	9	65
D.takahashii	0	12	13	16	28	0	0	1	0	24	0	0	94
Z.paravittiger	6	1	2	79	294	62	965	134	1700	260	30	10	3543
unidentified	13	7	21	30	15	41	61	37	115	32	20	8	400
Total	580	1555	534	261	605	155	1620	490	2411	460	170	75	8916

the first two species being considerably low in January, February, March and December and that of the third one being very low throughout the year. There was a general observation made during the collection. In case of all the species, males started appearing in the baits one to two weeks earlier than did the females. The male fruitflies outnumbered the females approximately eight-fold in the entire collection.

Silva, F.J. University of Valencia, Spain.

pink and sunburst mutations of **Drosophila melanogaster** are alleles of the same locus.

pink (p) is a well characterized mutant located in the right arm of the third chromosome (position 48.0). Cytologically, it has been placed in region 86 A6-B3 (Ward & Alexander 1957). On the other hand, sunburst (snb) has not been precisely placed since it was located at 6.5 units from Dichaetae locus (3-34 or 3-47) (see Lindsley & Grell 1968). Both are eye-color mutants with some small differences (dull ruby [pink] and soft marron [sunburst]). Ferre et al. (1986) have quantitated the pteridines and ommochome metabolites in these strains finding slight differences (see Table 1 for some of these compounds).

We have found that the F1 offspring of a cross between p and snb strains is also mutant, presenting an eye-color difficult to distinguish from both kinds of parents. To avoid any mistake in our strains, we asked the Bowling Green and California Inst. of Technology for p and snb strains and made crosses between the four strains received. In all cases F1 was phenotypically eye-color mutant, indicating that they were probably alleles of the same locus.

In order to confirm that snb was not a delection including the pink locus, we dissected third instar larval salivary glands from the F1 of a cross between p and snb mutants, stained with lacto-aceto-orcein and observed the polytence chromosomes.

The region in which locus pink is located (85 A6-B3) did not show any kind of loop that would indicate the presence of a delection.

We conclude that pink and sunburst mutation are alleles and recommend the name of pink-sunburst (p^S) instead of the old name (sunburst).

References: Ferrè, Silva, Real & Mènsua 1986, Biochem. Genet. 24:545-569; Lindsley & Grell 1968, Carnegie Inst. Wash. Publ. 627; Ward & Alexander 1957, Genetics 42:42-54.

Table 1. Percentages of eye pigment and related metabolites (Oregon R has arbitrarily received the values of 100). XTM (xanthommatin or brown pigment), DP (drosopterin), SP (sepiapterin), ADHP (acetyldihydrohomopterin), IXP (isoxanthopterin), PTE (pterin), H2BP (dihydrobiopterin) and BP (biopterin).

Strain	XTM	DP	SP	ADHP	IXP	PTE	H2BP	BP
p	21	20	100	86	59	30	72	180
snb	16	9	84	91	71	22	73	200

Singh, Rita. Tenth Grade, Marion High School, Marion, Indiana USA. Pteridine establishment in **Drosophila melanogaster.**

The purpose of the experiment was to identify and separate pteridines in the eye color of **Drosophila melanogaster.** The research goal was to establish the combinations of pteridines that were responsible for the different eye colors. The eye colors worked with were wild, ebony, sepia, white, apricot, white-apricot, white-eosin, and white-coffee. Modification of the paper chromatography procedure developed by E. Hadorn and H. Mitchell was used in the experiment.

Eye color in the **Drosophila melanogaster** is the result of the interaction of two groups of pigments, the pteridines and brown ommochromes. The absence and overabundance of enzymes regulate the presence and amount present of each of the pteridines. This coincides with George Beadle's and Edward Tatum's one-gene-enzyme hypothesis. The conclusions of the experiment stated that the wild eye color contains all seven of the pteridines. The experiment also established that this white eye color contained no pteridines. Leitenburg and Stokes worked with this in 1964 and came upon the same results.

Table 1. The pteridines here are in the order found on the chromatogram with Drosopterins located at the bottom of the chromatogram and Isosepiapterin at the top. X = presence; 0 = absence.

PIGMENT	COLOR	wild	ebony	sepia	apricot	brown	white
Isosepiapterin	yellow	X	0	0	0	0	0
Biopterin	blue	X	0	0	0	0	0
2-amino-4-hydroxypteridine	blue	X	X	X	0	0	0
Sepiapterin	yellow	X	X	X	0	0	0
Xanthopterin	green-blue	X	X	X	X	0	0
Isoxanthopterin	violet-blue	X	X	X	X	0	0
Drosopterins	orange	X	X	0	X	0	0

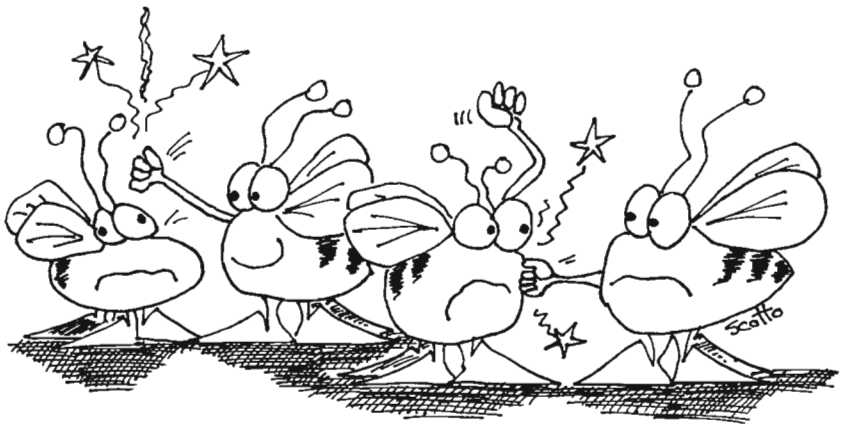
The ebony fly contained the pteridines drosopterines isoxanthopterin, xanthipterin, sepiapterin and 2-amino-4-hydroxypteridine. The apricot eye color contained the pteridines drosopterines, isoxanthopterin, and xanthopterin.

The sepia flies lacked the first pteridine, drosopterin, and showed an excess of the two pigments. Sepiapterin and 2-amino-4-hydroxypteridine. Hadorn (1962) reported an excess of the pteridine 2-amino-4-hydroxypteridine in his chromatograms. In addition, the sepia eye color contained the pteridines isoxanthopterin and xanthopterin.

No pteridine colors appeared on the chromatogram of the brown eye color. The same results occurred to Leitenburg & Stokes in 1964. The author's theory is that the brown ommochromes are solely responsible for the brown eye color. Since white-apricot, white-eosin, and white-coffee are all found at the same allele, paper chromatography is not accurate enough to distinguish the pteridine differences.

The conclusion drawn from the experiment is that the occurrence of various combinations of pteridines and amount of these pteridines present give fruitflies their eye color.

References: Hadorn, E. 1962, Scientific American, April:100-110; Mertens, T. & A.S. Bennet 1968, in: "Mutation: the Raw Material of Evolution", Laboratory Investigations in the Principles of Biology, Burgess Publ. Co., Minneapolis, Minn.; ———, E. Gardner & R. Hammersmith 1976, in: "Chromatographic characterization of **Drosophila melanogaster** mutants", Genetics (8th Ed.), Macmillan, NY; Ziegler, I. 1985, in: Advances in Genetics, Burgess Publ. Co., Minneapolis, Minn., p349-403.



Spicer, G.S. Linus Pauling Inst. of Science and Medicine, Palo Alto, California, and Univ. of Chicago, Illinois USA. The effects of culture media on the two-dimensional electrophoretic protein pattern of **Drosophila virilis.**

Two-dimensional electrophoresis has become a very powerful tool for surveying polypeptides in Drosophila. A variety of genetic studies on aging (Fleming et al. 1986), population genetics (Coulthart 1986), mutations (Kelly et al. 1985), development (Cheney et al. 1984), and phylogenetic reconstruction (Spicer 1988) have utilized this technique. Many of these studies depend on examining whole fly extracts as part of the research protocol. However, the sensitivity of the technique is such that it is quite possible the researcher is examining the protein pattern of various artifacts instead of the Drosophila proteins of interest. Several potential causes are present that could produce these artifacts. One is the bacterial fauna that live in the flies. **D.melanogaster** (Oregon R) is known to carry about 1,000 bacterial cells in their bodies and these increase in number with the age of the fly (J.E. Fleming, pers. comm.). In addition, various fungi often contaminate the culture and grow on the Drosophila. There is also the problem of the yeast that live in the cultures and are consumed by the flies. These are undoubtedly attached to the flies because they are carried from one culture to another during transfer. Finally, a major concern is that of the gut contents of the fly. Depending on the culture media that the fly has been raised on, it is possible that this could change the two-dimensional electrophoretic pattern as well. In order to test some of these potential problems, I have examined a strain of **D.virilis** under three different culture conditions to assess the effects of different media and microorganisms on the two-dimensional electrophoretic protein pattern.

The **D.virilis** strain that was used came from 16-Sapporo, Hokkaido, Japan, from Wheeler 1971. The flies were cultured on three different media at room temperature in half-pint bottles. The three culture conditions consisted of banana media without yeast, cornmeal media without yeast, and Carolina instant food with live brewer's yeast added. The flies were allowed to lay eggs and were then removed and discarded. One of the cornmeal bottles developed a severe fungal contamination, but enough flies were raised to be used in the experiment. About 20 male offspring raised from each of the cultures, aged 9-11 days, were homogenized in a urea mixture containing 9 M urea, 2% Nonidet P-40 detergent, 2% mercaptoethanol, and 2% pH 9-11 LKB ampholytes. Each sample was prepared with a concentration of 0.05 mg wet fly weight per 1 µl of urea mix. These samples were then centrifuged for approximately one and a half minutes at 10,000 x g. The supernatants from these samples were then centrifuged at 435,000 x g (maximum) for 5 min in a Beckman TL-100 ultracentrifuge.

Two-dimensional electrophoresis was performed as outlined by O'Farrell (1975) with the modifications of Anderson & Anderson (1978). The gels used in this study measured 20 x 25 cm. Isoelectric focusing was performed in the first dimension with a mixture of 3.6% pH 5-8 Pharmalytes, 0.2% pH 3-10 Pharmalytes, and 0.2% pH 3-10 Biolytes. This dimension was run in a tube gel 25 cm long. The amount of sample loaded onto each gel was 16 µl, which resulted in a concentration of 0.8 mg wet fly weight per gel. The first dimension was run at 30,000 volt-hours for an overall run time of 18.5 hr. The second dimension was a 9-18% gradient sodium dodecyl sulfate (SDS) gel run at about 100-150 volts (0.6 amperes) overnight. The gels were silver stained using a modified procedure described by Guevera et al. (1982).

A total of 12 two-dimensional electrophoretic gels were run and scored. An example of one of these gels is shown in Figure 1. From these gels 552 polypeptides were surveyed for qualitative differences among the treatments (different culture conditions). None of the protein spots varied in a qualitative fashion among the groups. That is, no spot either appeared or disappeared from the protein patterns. Because no densitometer measurements were performed, we are unable to assess any quantitative differences that might have occurred among the protein spots on the patterns, although by eye none were apparent. Therefore, it is concluded that most of the previously mentioned potential artifacts do not constitute a significant problem, at least at the qualitative level of the protein patterns.

References: Anderson, N.G. & N.L. Anderson 1978, Anal. Biochem. 85:331-340, 341-354; Cheney, C.M., K.G. Miller, T.J. Lang & A. Shearn 1984, PNAS 81:6422-6426; Coulthart, M.B. 1986, PhD thesis, McMaster Univ, Canada; Fleming, J.E., E. Quattrocki, G. Latter, J. Miquel, R. Marcuson, E. Zuckerkandl & K.G. Bensch 1986, Science 231:1157-1159; Guevera, J., D.A. Johnston, L.S. Ranageli, B.A. Martin, S. Capetillo & L. Rodriguez 1982, Electrophoresis 3:197-205; Kelley, M.R., I.P. Mims, C.M. Farnet, S.A. Dicharry & W.R. Lee 1985, Genetics 109:365-370; O'Farrel, P.H. 1975, J. Biol. Chem. 250:4007-4021; Spicer, G.S. 1988, J. Mol. Evol. 27:250-260.

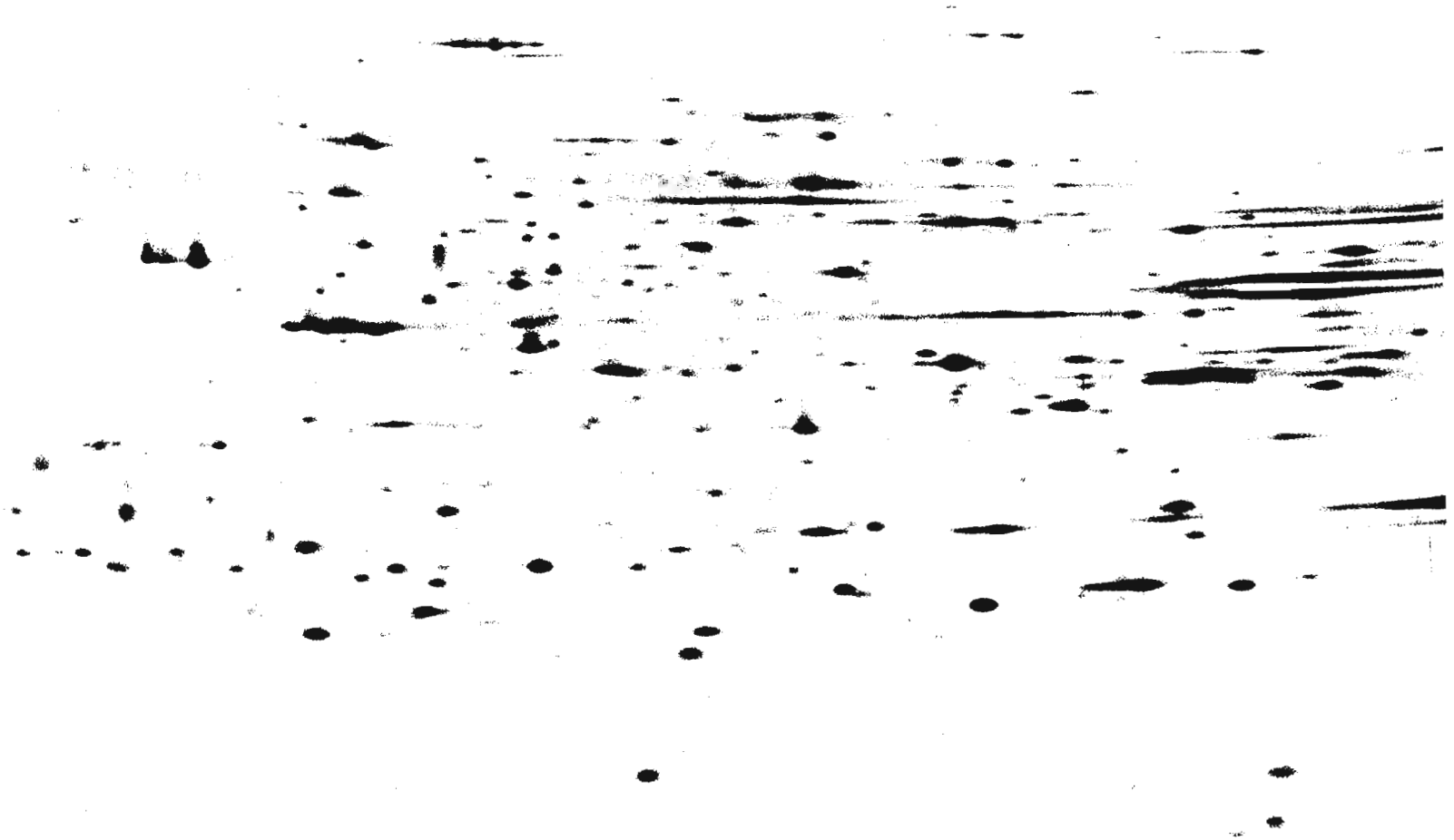


Figure 1. A silver stained two-dimensional electrophoretic gel of **Drosophila virilis** raised on banana media with no live yeast. The high molecular weight proteins are at the top of the gel and the basic proteins are on the right.

Syomin, B.V. and N.G. Schuppe. Inst. of General Genetics, Moscow B-333, USSR.

Intracellular distribution of sequences homologous to Drosophila retrotransposon changes with the age of culture.

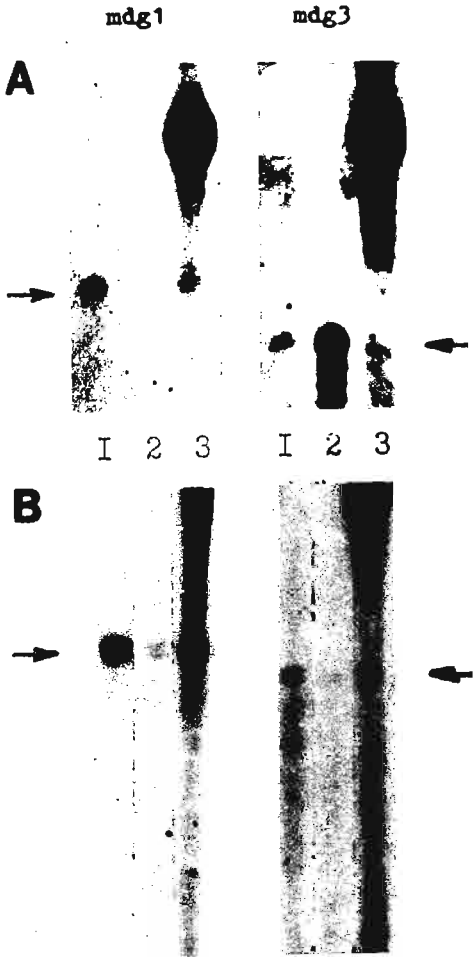


Figure 1. Southern blot analysis of reverse transcription intermediates of mdg1 and mdg3. **1** - membrane, **2** - cytoplasmic, and **3** - nuclear fractions. Arrows indicate full-sized molecules of mdg1 and mdg3.

Many different forms of sequences homologous to retrotransposons have been identified in Drosophila cells (Finnegan & Fawcett 1986; Arkhipova et al. 1984). Some of them are consistent with the reverse transcripton pathway of transposition and, therefore, are termed as reverse transcription intermediates of retrotransposons (Arkhipova et al. 1986; Baltimore 1985). The distribution of these intermediates throughout the cell may both reflect their biological role and throw light on the mechanisms which are regulating mobile genes expression.

In our previous report (Syomin & Schuppe 1988), we have described the intracellular distribution of reverse transcription intermediates of two Drosophila retrotransposons, mdg1 and mdg3. It was shown that their intermediates are distributed between subcellular fractions differently. The data presented in this paper show that intracellular distribution of intermediates changes with the age of cell culture line, and the changes in distribution of different retrotransposons are not coordinated.

The 67j25D cultured **D.melanogaster** cells were grown at 26°C in KSh-10 medium with 2% heat inactivated GIBCO foetal calf serum. On the seventh day of cultivation the cells were harvested and fractionated into nuclear, cytoplasmic and membrane fractions according to the method of Mayrand & Pederson (1983), modified for our cells. Nucleic acids were extracted from each fraction, and then poly(A)$^+$ - containing nucleic acids were selected by poly(U) - sepharose column chromatography. The same quantities of poly(A)$^+$RNA from all the fractions were separated on formaldehyde-formamide denaturing agarose gels, transferred onto nitrocellulose membranes and analysed by hybridization with nick-translated plasmids, containing full sequences of different Drosophila retrotransposons according to conventional techniques (Maniatis et al. 1982). Before blotting, the gels were treated with 0.5 N NaOH for 1 hr at 37°C, so only the DNA part of intermediates has been analysed. To obtain the distribution of intermediates in the same cells, the same filter was subsequently hybridized with different plasmids.

Figure 1a shows the distribution of intermediates obtained 18 months ago. The intermediates of both retrotransposons, mdg1 and mdg3, are detected in all fractions, although they are distributed differently. Most of mdg1 intermediates are localized in the membrane fraction, while mdg3 intermediates are distributed differently; most of them are detected in cytoplasmic fraction. However, in the course of cell cultivation, the intracellular distribution of some transposons changes (Fig. 1b). So, after 50 passages, the distribution of mdg1 intermediates remained unchanged, while the distribution of mdg3 intermediates turned out to be different. Now most of them were detected in the cytoplasmic fraction. The distribution of copia intermediates varied inversely to that of mdg3. If 18 months ago it was just the same as for mdg1, now it was similar to mdg3 distribution in our former experiments (data not shown).

To determine how quickly the distribution of intermediates can change, we analyzed it in the three consequent passages of cells. We have found that in this case the distribution of intermediates throughout the cell remains unchanged.

Now it is difficult to say, whether the changes in intracellular distribution of sequences homologous to retrotransposons reflect the change in the intracellular activity of various mobile elements or our results only show that different retrotransposons behave in the cell completely autonomously. On the other hand, it is known that in the course of cultivation there are significant instabilities in the rate of growth, morphology and ploidy of the cells. Simultaneously, the expression activity and metabolism of various genes (including transposable genes) might change. To elucidate this question, new experiments with different cell culture lines are required.

References: Arkhipova,I.R., T.V. Gorelova, Y.V. Ilyin & N.G. Schuppe 1984, NAR 12:7533-7548; ——, A.M. Maso, V.A. Cherkasova, T.V. Gorelova, N.G. Schuppe & Y.V. Ilyin 1986, Cell 44:555-563; Baltimore, D. 1985, Cell 40:481-482; Finnegan, D.J. & D.H. Fawcett 1986, Oxford surveys on eukaryotic genes 3:1-61; Maniatis, T., E.E. Fritsch & J. Sambrook 1982, in: Molecular Cloning (A laboratory manual), Cold Spring Harbor Laboratory; Mayrand, S. & T. Pederson 1983, Mol. Cell. Biol. 3:161-171; Syomin, B.V. & N.G. Schuppe 1988, DIS 66:139:140.

Szidonya, J. and G. Reuter.* Inst. of Genetics, Biol. Res. Ctr., Szeged, Hungary; *Martin Luther Univ., Halle/S., DDR. Cytogenetics of the 24D4-25F2 region of the **Drosophila melanogaster** 2L chromosome.

Table 1. Mutations and chromosomes used.

Mutations or chromosomes	Breakpoint/origin	Ref.
Df(2L)2802	25F2-3 ; 25F4-26A1	1
Df(2L)cl^{1}	25D2-4 ; 25F2-4	1
Df(2L)cl^{2}	25D2-4 ; 25F2-4	1
Df(2L)cl^{7}	25D5-6 ; 26A7-8	1
Df(2L)cl^{h1}	25D2-4 ; 25F1-2	2
Df(2L)cl^{h2}	25D5-6 ; 25F4-5	2
Df(2L)cl^{h3}	25D2-4 ; 26B2-5	2
Df(2L)cl^{h4}	25D6-E1 ; 25E5-F1	2
Df(2L)dp^{h19}	24E5-F1 ; 24F7-25A1	2
Df(2L)dp^{h24}	not visible	2
Df(2L)dp^{h25}	24E2-4 ; 25B2-5	2
Df(2L)dp^{h28}	24D7-8 ; 24F7-25A1	2
Df(2L)dp-cl^{h1}	24F7-25A1 ; 26A2-3	2
Df(2L)dp-cl^{h2}	24E4-F1 ; 26B9-C1	2
Df(2L)dp-cl^{h3}	24F7-25A1 ; 25E2-4	2
Df(2L)ed^{Sz-1}	24A3-4 ; 24D3-4	1
Df(2L)ed-dp^{h1}	24C3-5 ; 25A2-3	2
Df(2L)Gpdh75	25F2-3 ; 25F4-26A1	1
Df(2L)Gpdh78	25F2-3 ; 25F4-26A1	1
Df(2L)M11	24D3-4 ; 25A2-3	1
Df(2L)M-z^{B}	24D8-E1 ; 24F7-25A1	1
Df(2L)sc$^{19-1}$	24D4-5 ; 25C8-9	2
Df(2L)sc$^{19-3}$	24E2-3 ; 25A7-B1	2
Df(2L)sc$^{19-4}$	25A4-5 ; 25E5-F1	2
Df(2L)sc$^{19-5}$	25A4-5 ; 25D5-7	2
Df(2L)sc$^{19-6}$	24F1-2 ; 25C3-5	2
Df(2L)sc$^{19-7}$	24D2-4 ; 25C2-3	2
Df(2L)sc$^{19-8}$	24C2-8 ; 25C8-9	2
Df(2L)sc$^{19-9}$	24D4-5 ; 25F4-26A1	2
Df(2L)sc$^{19-10}$	25A4-5 ; 25B9-C1	2
Df(2L)sc$^{19-11}$	24D2-4 ; 25B2-4	2
Df(2L)sc$^{19-12}$	25A4-5 ; 26A6-B1	2
Df(2L)sc$^{19-13}$	24E2-4 ; 25B2-5	2
Df(2L)tkv^{Sz-2}	25D2-4 ; 25D6-E1	1
Df(2L)tkv^{Sz-3}	25A2-3 ; 25D5-E1	1
Dp(2;1)B19	9B/25F2 ; 24D4/9C	3
Dp(2;2)B3	23E2-3 ; 26E2-F1	3
Dp(2;2)B17	23A3-B1 ; 25C3-8	3
In(1)w^{m4h}	3C2-3 ; 20	4
T(Y;2)dp^{h14}	--	2
T(Y;2)dp^{h15}+Df(2L)ed^{-}dp^{-}	--	2
T(2L;3R)dp^{h27}	91D/24F4-7 ; 32B2/91E	2
dp^{hx}	X-ray induced dp mutations isolated over Dp(2;2)B3. X-ray induced lethal	2
h-x	and visible mutations isolated over	
a-x	Dp(2;2)B19. EMS enduced lethal and	2
b-x	visible mutations isolated over	
sz-x	Dp(2;1)B19.	2

1=Lindsley & Zimm 1985; 2=Szidonya & Reuter 1988; 3=Reuter & Szidonya 1983; 4=Lindsley & Grell 1968.

The 24-25 region contains several haplo-insufficient genes, first of all three Minutes [M(2)LS2, M(2)z and M(2)SI] and a haplo-sterile function. Therefore the usual genetic techniques for cytogenetic analysis of this region cannot be used. Thus duplications were isolated to cover these haplo-insufficient functions (Reuter & Szidonya 1983). Two of these duplications were used to generate deletions. With the help of Dp(2;2)B3, ed dp^{o2} cl (23E2-3 ; 26E2-F1) one ed^{-} dp^{-}, four dp^{-}, four cl^{-} and three dp^{-} cl^{-} deletions were isolated. They are symbolized by the superscript "h" followed by a number, e.g., Df(2L)ed-dp^{h1}. In another scheme the yellow marked Dp(2;1)B19, ed dp^{o2} cl chromosomes were used and we selected for an X-ray induced loss of the y^{+} marker of T(1;2)sc^{19}. In this way altogether 12 new deficiencies were recovered: Df(2L)sc^{19-n} series.

Recessive lethals and visibles were isolated for the region covered by Dp(2;1)B19 (24D4-25F2) after EMS and X-ray mutagenesis. In an F3 scheme from 10,148 chromosomes 100 lethals, 5 visibles and three Minutes were recovered. The a, b and sz mutations are EMS, whereas the h are X-ray induced. The new mutations were crossed to the deletions and those falling into the same subregion were complemented inter se to determine the number of complementation groups for the 20 subregions. In these studies the clot deletions isolated by Velissariou & Ashburner (1980) were also included. The cytological breakpoints of the deletions are shown in Table 1; the results of the complementation analysis are presented in Figure 1.

According to the summarized EM studies (Semeshin & Szidonya 1985; Semeshin et al. 1985), this region comprises 44 bands instead of Bridges's 60 bands, counting most of the doublets as single bands. We identified altogether 41 complementation groups covered by Dp(2;1)B19 which is in good accordance with the one band/one gene theory. The smallest dp deficieny [Df(2L)dp^{h24}] includes three complementation groups although no sign of any chromosomal deficiency can be seen even in EM analysis. Also most of the other dp deficiencies do not delete the 25A1.2 band. Therefore we located the dp between 24F6.7 and the distal most part of 25A1.2.

The dp deletions and point mutations were classified according to their o, v and lethal phenotype as well as to their suppressor effect on white position effect variegation with In(1)w^{m4h} (Table 2). According to our results, the dominant suppressor effect appears to be a function of the dp complex locus itself. The phenotypic distribution of the newly isolated dp mutations seems to be independent from the used mutagene. Using the duplications and deficiencies we identified three more genes modifying position effect variegation (Szidonya & Reuter 1988). A haplo-abnormal enhancer function can be placed between the proximal breakpoints Df(2L)cl^{h1} and Df(2L)cl^{h2} in 25F2-4. Since the deficiencies Df(2L)cl^{h3} and Df(2L)cl^{7} express a significantly stronger enhancer effect, another haplo-abnormal

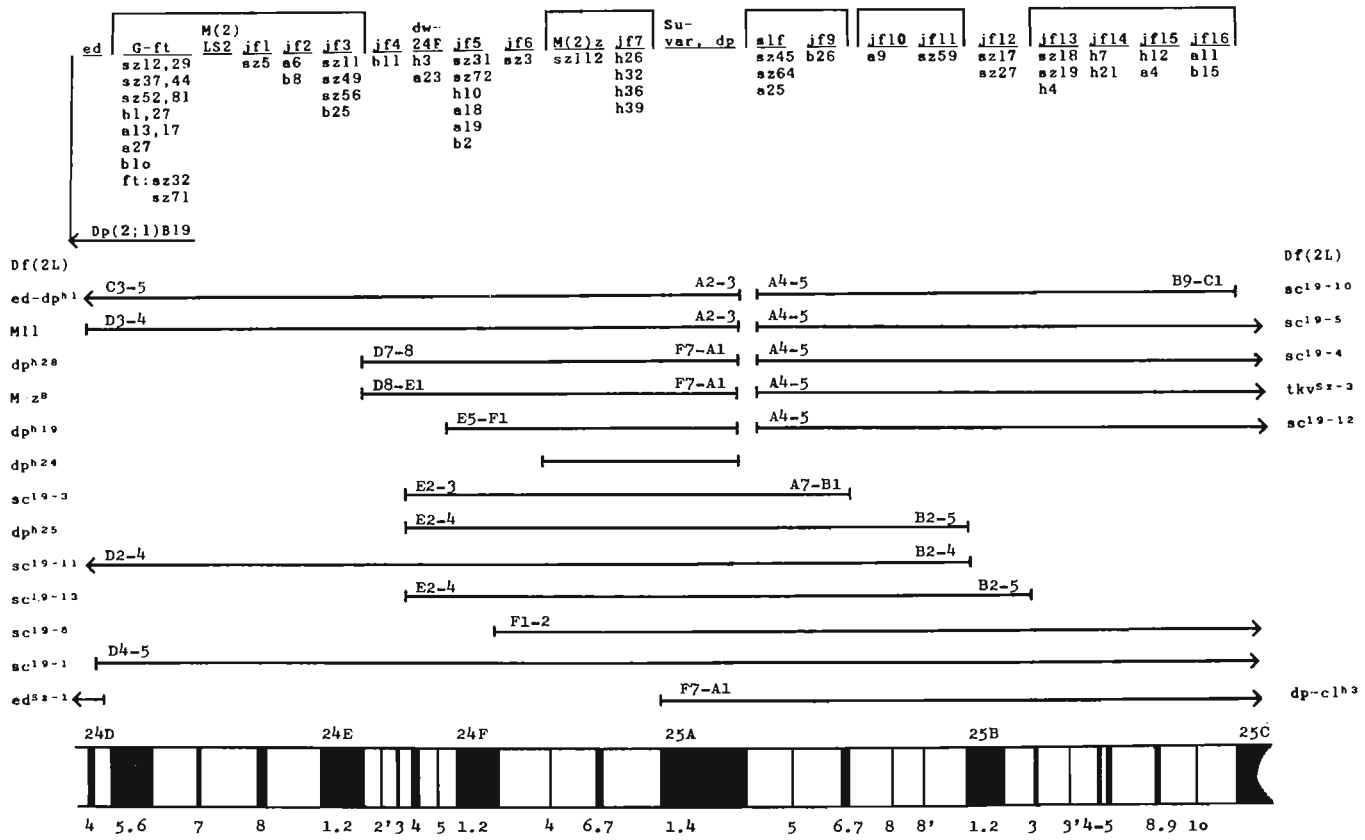


Figure 1. Complementation map of the mutations isolated over Dp(2;1)B19. Deficiencies are placed according to their complementation behavior with respect to the point mutations. The polytene chromosome bands have been placed to fit as many deficiencies as possible. The dp and DTS mutations are listed in Table 2 and Figure 2, respectively.

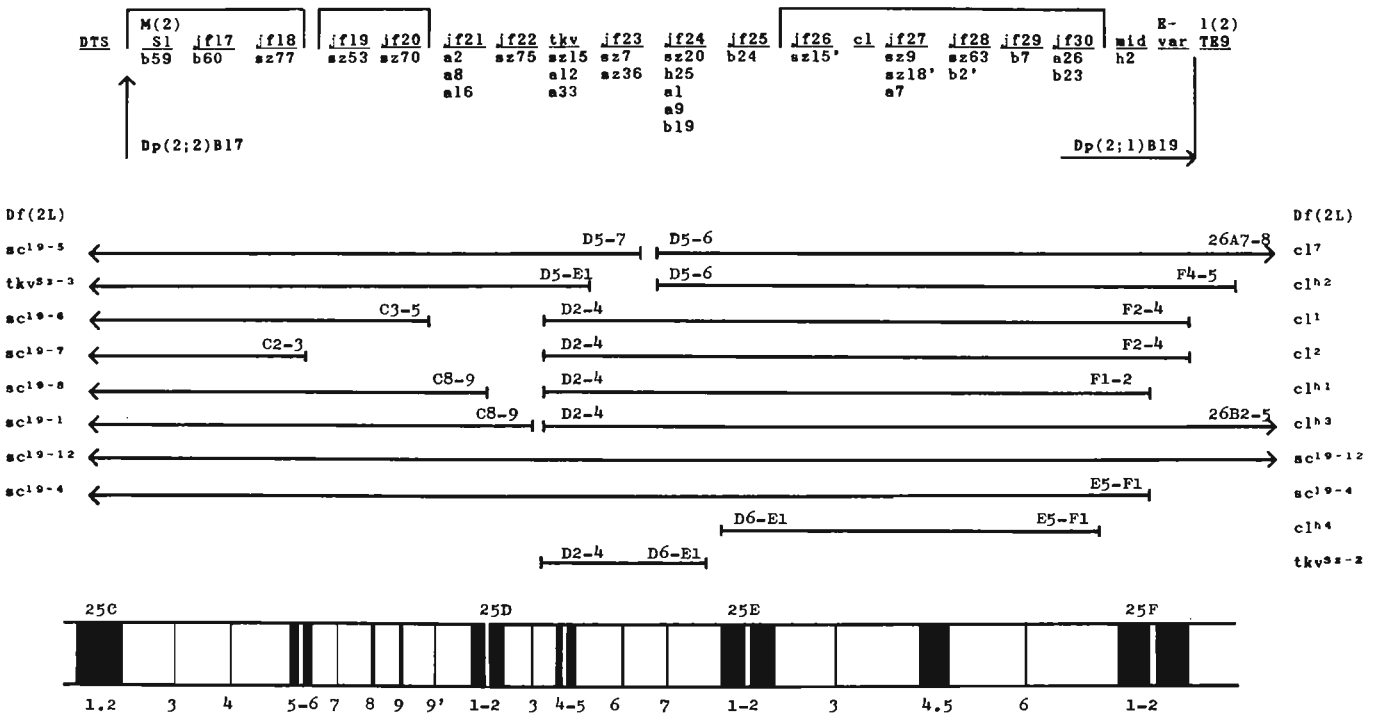


Table 2. Phenotypic distribution of the dp mutations.

Phenotype	Isolated in experiment Dp(2;2)B3	Dp(2;1)B19	Mutagene origin
olv, Su-var	Df(2L)dp^{h19,24,25,28}	--	X-ray
	dp^{h7,17,22,26}	--	X-ray
olv	dp^{h2,3,4,5,11}	--	X-ray
	dp^{h14,18,27,29}	--	X-ray
		b-12, sz-28	EMS
o^{D}(1)v	--	a-22	EMS
ol, Su-var	dp^{h6,8,9,10}	--	X-ray
	dp^{h12,20,21}	--	X-ray
	--	sz-24	EMS
(o)1	--	h-20	X-ray
o(1)	--	b-27, b-42	EMS
lv	--	h-37	X-ray
	--	b-21	EMS
l	--	b-1, sz-31, sz-46	EMS
	--	h-40, h-41	X-ray
o	dp^{h13}	--	X-ray
	--	sz-66	EMS
v	--	sz-4	EMS

enhancer can be assumed in the region between 25F4 and 26A7. With the independently isolated Df(2L)Gpdh75, Gpdh78 and Df(2L)2802 deficiences (Kotarski et al. 1983), this gene can be localized between 25F4 and 26A1. The already described triplo-abnormal suppressor function (Reuter & Szidonya 1983) is more proximal from these enhancers and it can be localized between the proximal breakpoints of Df(2L)cl^{h3} and Df(2L)dp-cl^{h2} from 26B2-5 to 26B9.

For the DTS locus (dominant temperature sensitive lethals) in the region 25C1.2, 13 new recessive lethals were isolated. Three of them (a-30, b-9 and b-17) show DTS phenotype while the others are non-conditional. The lethal complementation analysis was carried out at 22°C and the complementation map is shown in Figure 2. According to these results, the DTS lethals can be located in the middle of the DTS complex locus.

Lethal and visible mutations were isolated for the Gull-fat complex locus which is placed between the distal breakpoints of Df(2L)sc$^{19-1}$ and Df(2L)dp^{h28} in region 24D5-7 together with four other complementation groups. The dw-24F locus is represented by two new lethal alleles. Heterozygous combinations with dw-24F show dwarf phenotype. This gene can be placed in the bands 24E4-5. Lethal mutations were isolated also for the thick vein locus. Lethal/tkv heterozygotes show tkv phenotype. The proximal breakpoint of Df(2L)tkv^{Sz-3} and the distal breakpoints of four clot deletions overlap only in the tkv gene, placing it in 25D4.5 band.

The genetic techniques applied in the present work proved to be successful for cytogenetic fine structure studies in a haplo-insufficient region. These methods should be useful for other similar regions which contain interesting genetic functions which were poorly characterized until now.

References: Kotarski, M.A., S. Pickert & B.J. MacIntyre 1983, Genetics 105:371-386; Lindsley, D.L. & E.H. Grell 1968, Carnegie Inst. Wash. Publ. 627; ——— & G. Zimm 1985, DIS 62; Reuter, G. & J. Szidnya 1983, Chromosoma 88:277-285; Semeshin, V.G., E.M. Baricheva, E.S. Belyaeva & I.F. Zhimulev 1985, Chromosoma 91:210-233; ——— & J. Szidonya 1985, DIS 61:148-154; Szidonya, J. & G. Reuter 1988, Genet. Res. 51:197-208; Velissariou, V. & M. Ashburner 1980, Chromosoma 77:13-27.

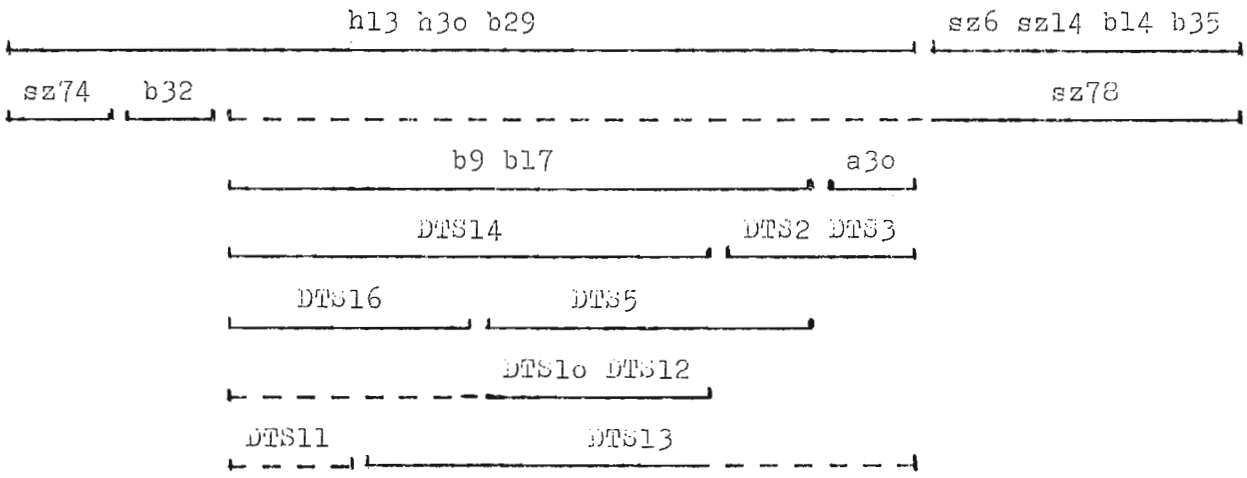


Figure 2. Complementation map of the DTS alleles. Dashed line means partial complementation (less than 75% of the full complementation).

Tarin, J.J. and J.L. Ménsua. University of Valencia, Spain. Comparison of mortality in **Drosophila melanogaster** with either sex together or isolated when sucrose is the only energetic resource.

Sucrose is an important energetic resource in **Drosophila melanogaster**, since it increases the mean longevity with an optimal concentration at 15%. Moreover, the response of both sexes has resulted comparably (Van Herrewege 1973).

In order to detect differences in the mortality between males and females and to see if either the presence or the absence of the other sex has an influence in the mortality of the individuals when the only existent energetic resource is sucrose, this work was undertaken.

The experiment was carried out with 22 isofemale strains coming from seven **D.melanogaster** populations: (a) four populations captured in nature (in a cellar of "Cheste", at 500 m from the cellar, at 1000 m from the cellar and in a pinewood of "La Canada", located 50 Km away from Cheste); (b) three laboratory populations captured 7 years ago in a cellar from "Requena", a vineyard of "El Pontón", 4 Km away from the cellar, and in a pinewood of "La Canada", located 70 Km away from "Requena".

In 10 x 2.5 cm vials (60 cm^3), either 10 males or 10 females or 5 males and 5 females together of 4-5 days old were placed, making two replicates for each of the three possible cases.

To each vial 5 strips of filter paper (5 x 2.5 cm) saturated with 1 ml of water solution of 0.3% sucrose were added. Vials were hermetically closed with parafilm. Flies were maintained at a temperature of 25±1°C in a thermoregulated chamber with constant lighting. The counts of dead flies were carried out at 5, 10, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264 and 288 hours.

In order to compare the mortalities of both sexes, alone as well as accompanied, the mortality percentage in the 12th day of the experiment was taken into account. A t-test between the possible comparisons of the four mortality percentage values in each of the seven analyzed populations was made.

The mortality percentage in the 12th day (Table 1) follows the pattern: females isolated > males accompanied > females accompanied > males isolated (Table 2). The t-test shows that all the comparisons are significantly different, except for the mortality percentage of males and females accompanied from the wild and laboratory cellar populations, males accompanied and females isolated from the 500 m and 1000 m wild populations and vineyard laboratory populations and females accompanied and males isolated from the 1000 m wild population that do not show significant differences.

When both sexes are isolated, males have a significantly lower mortality than females. However, when both sexes are together, males have a significantly higher mortality than females, except the wild and laboratory cellar populations. This behaviour pattern seems general, since the seven analyzed populations have had the same response.

The fact that males survive better than females when they are alone is likely due to the fact that they are smaller than females, and, for this reason, they have more available sucrose and/or oxygen quantity per individual than females.

When both sexes are together, males have less sucrose and oxygen per individual than when males are alone, while females have more sucrose and oxygen per individual than females isolated. For this reason, males accompanied have higher mortality than males alone and the females accompanied a lower mortality than females alone. If, in addition, we consider that males accompanied have a higher physical activity than males alone, due to males accompanied courting females, the inversion of the mortalities could be explained. That is, the fact that females have higher mortality than males when the sexes are isolated, but a lower mortality than males when the sexes are together.

References: Van Herrewege, J. 1973, C.R. Acad. Sc. Paris 276(Serie D):2565-2568.

Table 1. Mortality percentage of both sexes in each of the 7 populations analyzed, together and isolated.

	Population	MORTALITY PERCENTAGE: Together: males (1)	Together: females (2)	Isolated: males (3)	Isolated: females (4)
WILD	Cellar	70.46	68.18	54.32	86.59
	500 m	85.46	70.91	56.36	85.68
	1000 m	88.64	80.00	74.32	90.46
	Pinewood	82.73	68.18	59.77	94.01
LAB	Cellar	84.55	77.73	63.41	93.41
	Vineyard	83.64	72.73	54.32	88.86
	Pinewood	72.27	58.18	38.41	85.00

Table 2. T-test's statistical values of all possible comparisons between both sexes, together and isolated, in the 7 populations analyzed (see Table 1 for numbered groups). $p \leq 0.05$.

	Population	t-test statistical values (1)-(2)	(3)-(4)	(1)-(3)	(2)-(4)	(1)-(4)	(3)-(2)
WILD	Cellar	0.52	-10.89*	4.06*	-5.44*	-4.84*	-3.46*
	500 m	3.74*	- 9.89*	8.00*	-4.40*	-0.08	-3.68*
	1000 m	2.51*	- 6.45*	4.54*	-3.62*	-0.72	-1.64
	Pinewood	3.58*	-13.10*	6.26*	-8.57*	-4.43*	-2.13*
LAB	Cellar	1.84	-11.57*	5.95*	-5.62*	-3.50*	-3.83*
	Vineyard	2.79*	-11.92*	7.89*	-5.07*	-1.85	-4.67*
	Pinewood	3.12*	-14.97*	8.42*	-7.40*	-3.80*	-4.82*

Tarin, J.J. and J.L. Ménsua. University of Valencia, Spain. Oxygen consumption of males and females in **Drosophila melanogaster.**

Table 1. Number of replicates and means with their standard errors of metabolic rates expressed in µl O_2/mg fresh weight/hr of males (M) and females (F) of the 3 analyzed populations.

Population	Replicates M	Replicates F	Mean ± Standard Error µl O_2/mg fresh weight/h M	Mean ± Standard Error µl O_2/mg fresh weight/h F
Cellar	21	22	3.69±0.25	3.33±0.27
Vineyard	23	21	3.43±0.17	3.44±0.21
Pinewood	18	23	3.66±0.31	3.10±0.18

In order to find a possible explanation of the suggestive positive correlation that apparently exists between the beneficial effect of ethanol at low concentrations and the toxic effect at high concentrations, considering males and females separately within each of the three analyzed laboratory populations of **Drosophila melanogaster** (Cellar, Vineyard and Pinewood, see Tarin et al. 1988), the measure of oxygen consumption of males and females in the three populations was undertaken.

Conventional manometrics methods (Umbreit et al. 1972) were used to measure the oxygen consumption of the insect at rest. A Gilson's differential constant-pressure respirometer was used. In each flask were placed 50 flies wrapped with a gauze, 0.5 ml of 20% KOH and a 2 x 3 cm filter paper doubled in zigzag. The measure of the oxygen consumption was performed an hour and 15 min later so that the flasks were adjusted in the branches of the respirometer.

Table 1 shows the mean and standard error of males and females of three populations. The similarity of the means was confirmed by two way ANOVA, which showed non-significant differences, neither among populations nor between sexes. The positive correlation between the beneficial effect of ethanol at low concentrations and the toxic effect at high concentrations considering males and females separately could be explained by the effect that the ethanol fumes exerts in the flies within the hermetically closed vials. The different relation SURFACE/VOLUME between males and females could be important for explaining this phenomenon.

Males must incorporate relatively more ethanol fumes through their tracheas due to the higher relation S/V compared with females. This fact would give an advantage to males at low concentrations because both sexes have the same metabolic rate expressed in µl O_2/mg fresh weight/hr. On the other hand, at high concentrations females would incorporate relatively lower quantity of ethanol fumes; therefore, with equality of metabolic rate, they would be more tolerant than males.

References: Tarin, J.J., C. Najera & J.L. Mensua 1988, DIS 67 (this issue); Umbreit, W.W., R.H. Burris & J.F. Stauffer 1972, in: Manometric and Biochemical Techniques, 5th ed., Burgess Publ. Co., Minneapolis, Minn.

Tarin, J.J., C. Nájera and J.L.Ménsua. University of Valencia, Spain. Analysis of survival data on three laboratory populations of **Drosophila melanogaster** contrasting survival curves.

In order to compare the ethanol utilization and the ethanol tolerance inside and outside cellar populations of **Drosophila melanogaster**, three laboratory populations coming from a cellar, a vineyard and a pinewood have been studied. Nonparametric statistical methods for the survival data analysis have been introduced due to the limitations of calculating the LC50 (toxic concentration that kills 50% of individuals after a certain time of toxic exposure).

In 10 x 2.5 cm vials, ten 4-5 day old individuals were placed making four replicates for each of the seven tested concentrations (0, 2, 5, 7, 10, 12 and 15% ethanol), two for males and females in each of the 66 isofemale strains tested (22 from each population).

Five strips of filter paper (5 x 2.5 cm) saturated with 1 ml of a water solution with 0.3% sucrose and with 0, 2, 5, 7, 10, 12 and 15 ethanol proportions (v/v) were added to each vial. Vials were hermetically closed with parafilm to prevent the evaporation of alcohol. Flies were maintained at a temperature of 25±1°C in a thermoregulated chamber with constant lighting. Dead flies were counted at 5, 10, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264 and 288 hr. Table 1 shows the order of survival among the three populations. To establish this order the values of the statistical significances of the Lee-Desu's test (probability of obtaining a statistical value or a higher one) and the observation of the survival curves were taken into account.

The pattern of response of both sexes together is different from male and female patterns separately. This is indicative of the fact that the two sexes would not be group without having the security that both sexes have the same response. The survival curves and the Lee-Desu's test show that males have a higher survival rate than females from the 0 to the 5%. From 10% onwards, females have a higher survival rate.

Table 1. The upper part of each cell displays the significances of the Lee-Desu's statistical values (probability of obtaining a statistical or higher value) obtained by comparing in pairs the survival curves in the order: Cellar-Vineyard, Cellar-Pinewood, Vineyard-Pinewood. Under the significances is shown the order from higher to lower survival obtained by observing the survival curves together with the Lee-Desu's statistical value.

	TOTAL	MALES	FEMALES
0%	0 0 0 Pinewood > Vineyard > Cellar	0 0 0 Pinewood > Vineyard > Cellar	0 0 0 Pinewood > Vineyard > Cellar
2%	0 0 0 Pinewood = Vineyard > Cellar	0 0 0.003 Vineyard > Pinewood > Cellar	0.830 0.061 0.069 Pinewood = Vineyard = Cellar
5%	0 0 0.267 Pinewood = Vineyard > Cellar	0.040 0.011 0.549 Vineyard = Pinewood > Cellar	0 0 0.204 Pinewood = Vineyard > Cellar
7%	0.003 0 0.009 Pinewood > Vineyard > Cellar	0.186 0.004 0.311 Vineyard ‖ ‖ Pinewood > Cellar	0 0 0.027 Pinewood > Vineyard > Cellar
10%	0.019 0.001 0 Vineyard > Cellar > Pinewood	0.949 0.006 0.159 Vineyard ‖ ‖ Cellar > Pinewood	0.002 0.009 0 Vineyard > Cellar > Pinewood
12%	0.034 0 0 Cellar > Vineyard > Pinewood	0.179 0 0.026 Cellar = Vineyard > Pinewood	0.021 0 0.005 Cellar > Vineyard > Pinewood
15%	0.068 0 0.001 Cellar = Vineyard > Pinewood	0.469 0 0.003 Cellar = Vineyard > Pinewood	0.011 0 0.153 Cellar > Vineyard = Pinewood

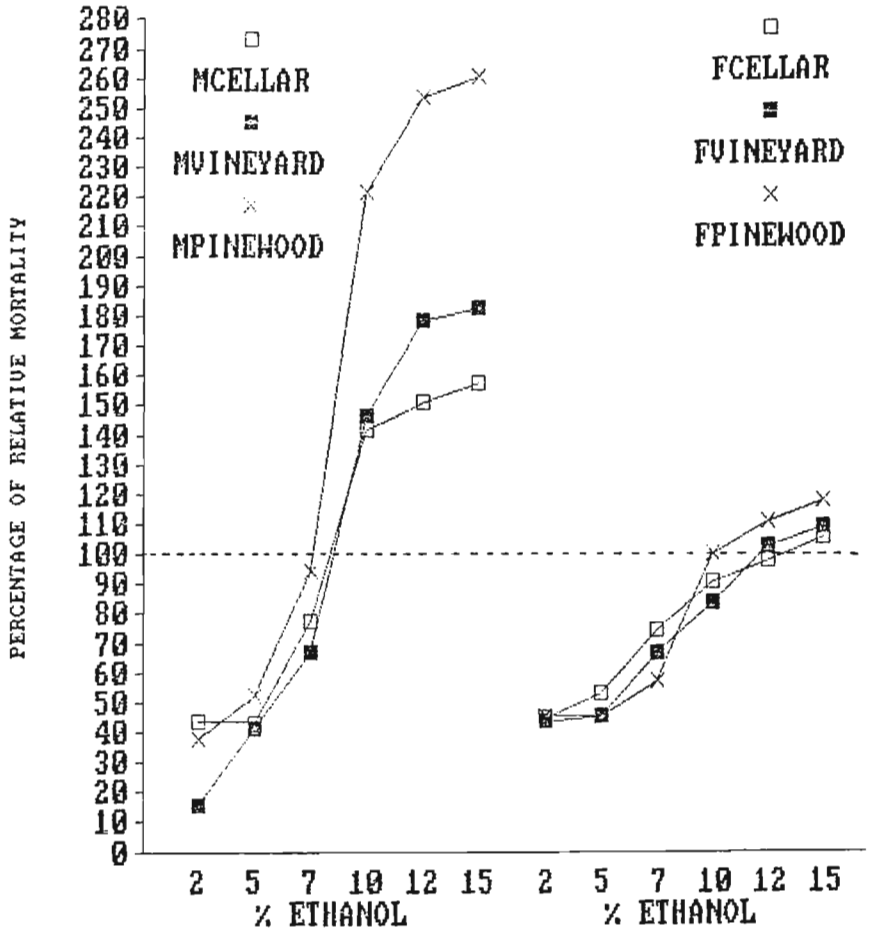


Figure 1. Curves of relative mortality percentage (percentage of the quotient between the number of deaths in a determined concentration during the 12 days and the number of deaths in the 0% concentration) of males (M) and females (F) of the three populations studied (Cellar, Vineyard, Pinewood) against the ethanol concentration. The toxic effect of ethanol is indicated by all the values higher than the 100% broken line, whereas the beneficial effect of ethanol is indicated by all the values lower than the 100% broken line.

These results show a positive correlation between the beneficial effect of ethanol at low concentrations and the toxic effect at high concentrations among the three populations and between the two sexes within each population, although this fact could be due to the different use of sucrose among the populations (Pinewood > Vineyard > Cellar) or to the fact that males use sucrose more efficiently than females.

Figure 1 seems to confirm this positive correlation between the two effects of ethanol among the three populations and between both sexes within each population, eliminating the possiblity that this correlation is due to sucrose and not to ethanol effects, although perhaps a statistical analysis of these data would be necessary to have complete surety, at least, in beneficial concentrations.

The positive correlation between the two effects of ethanol among the three populations may be explained by the action of a differential selection in each of the studied habitats while the positive correlation between males and females within each population may be explained by a different relation SURFACE/VOLUME between sexes.

References: Tarin, J.J. & J.L. Mensua 1988, DIS 67 (this issue).

Tarin, J.J., C. Nájera and J.L.Ménsua. University of Valencia, Spain. Use of the parametric statistic in the analysis of survival data.

Calculation of the LC50 (toxic concentration that kills 50% of individuals after a determined time of toxic exposure) is generally performed in studies on ethanol tolerance. The comparison of mean longevity is the method followed in studies of ethanol utilization.

The use of nonparmametrical statistical methods for survival data as an alternative to these methods has been carried out (Tarin et al. 1988). We use the parametrical statistical method for survival data to study tolerance and utilization of any substance and in particular ethanol in **Drosophila melanogaster.** For this reason, this work is only a methodological, and not a biological one.

Three isofemale strains (a, b and c), chosen arbitrarily, from each of the laboratory populations (Cellar, Vineyard and Pinewood) have been used. Tests of adult survival were made on adults in air-tight vials according to a previously described technique (Tarin et al. 1988). The parametric test used was the Cox's F test for exponential distributions (Gros et al. 1975; Lee 1980) which contrasts the equality of the lambda parameter between two exponential distributions. Prior to the application of this test, Gehan & Siddiqui's regression method was used to see which of the next four distributions our data fit and to estimate the appropriate parameters: Exponential, Weibull, Gompertz and Exponential linear. The distribution that gives the highest value of the ℓn-likelihood was considered the best fit. Nevertheless, considering that all these models reduce to the Exponential distribution, a Chi-square test was applied to see whether the likelihood of the data under this model was significantly greater than under the Exponential model. To decide if the best fitting model yielded a good fit to the data, a Chi-square test of goodness has been applied.

Table 2. (A) Values of Cox's F test statistics obtained by comparing in pairs the lambda values of Exponential distributions of aCellar, bVineyard, cPinewood strains in each conc. from 5% ethanol to 15% (*$p<0.05$).
(B) order from lower to higher instantaneous death tase (lambda).

		aCellar	bVineyard
A)			
5%	bVineyard	2.06*	
	cPinewood	0.27*	0.13*
7%	bVineyard	3.80*	
	cPinewood	2.42	0.64*
10%	bVineyard	3.39*	
	cPinewood	1.63*	0.48*
12%	bVineyard	1.96*	
	cPinewood	0.61*	0.31*
15%	bVineyard	1.61*	
	cPinewood	0.80*	0.51*
B)			
5%	cPinewood < aCellar < bVineyard		
7%	cPinewood = aCellar < bVineyard		
10%	aCellar < cPinewood < bVineyard		
12%	cPinewood < aCellar < bVineyard		
15%	cPinewood < aCellar < bVineyard		

Table 1 shows the results of applying Gehan & Siddiqui's regression method. In the 0% concentrations the survival times are fitted to a Gompertz distribution with the gamma parameter higher than zero (the Hazard function increases with time), which can probably be due to the fact that the flies are only supplied with sucrose, to an increased risk of infection or to a progressive anoxie.

On the other hand, in the presence of ethanol, all strains are fitted to an Exponential distribution, except for the 15% concentration of the cCellar strain and the bPinewood strain in all concentrations which are fitted to a Gompertz distribution. Moreover, in the bPinewood strain there is a tendency to increase the gamma parameter value when the ethanol concentration is increased; that is, the Hazard function tends to have higher values and to grow faster with time when the ethanol concentration is increased.

In strains with an Exponential distribution in the presence of ethanol, the lambda parameter value (Hazard function) tends to increase when the ethanol concentration increases.

In Table 2a, it can be seen that all the comparisons of lambda parameter are significantly different, except for lambda values of the aCellar and cPinewood strains at the 7% ethanol concentra-

Table 1. Survival distributions with their parameter(s) and error(s) standard(s) to which the 14, 22 and 1 strains of each population in each of the 7 concentrations of ethanol have given a good fit.

Population: Strain	0%	2%	5%	7%	10%	12%	15%
Cellar:							
a			Exponential *	Exponential *	Exponential *	Exponential *	Exponential *
			=0.0211±0.0074	=0.719±0.0145	=0.0868±0.0184	=0.3689±0.0562	=0.6224±0.0930
b	Gompertz *		Exponential *	Exponential *	Exponential *	Exponential ****	Exponential *
	=-4.4687±0.5503		=0.0167±0.0063	=0.0174±0.0064	-0.1353±0.0233	=0.2742±0.0418	=0.3259±0.0501
	= 0.2963±0.0638						
c	Gompertz *		Exponential *	Exponential *	Exponential ****		Gompertz *
	=-4.8125±0.5561		=0.0151±0.0059	=0.0194±0.0068	=0.1277±0.0220		=-2.8753±0.6318
	= 0.4705±0.0662						= 1.1908±0.2718
Vineyard:							
a				Exponential ***	Exponential *	Exponential *	Exponential *
				=0.0605±0.0133	=0.1321±0.0241	=0.3982±0.0616	=0.6969±0.1047
b	Gompertz *	Exponential *	Exponential *	Exponential *	Exponential **	Exponential *	Exponential *
	=-4.3306±0.6611	=0.0177±0.0065	=0.0600±0.0150	=0.1336±0.0241	=0.3146±0.0501	=0.7317±0.1987	=1.0475±0.1485
	= 0.2151±0.0880						
c	Gompertz **		Exponential **	Exponential *	Exponential **	Exponential ***	Exponential *
	=-4.5777±0.5307		=0.0791±0.0153	=0.0456±0.0112	=0.1893½0.0325	=0.6403±0.0918	=1.4203±0.2214
	= 0.3665±0.0603						
Pinewood:							
a	Gompertz *	Exponential *	Exponential *	Exponential *	Exponential *	Exponential ***	
	=-4.7360±0.7286	=0.0165±0.0063	=0.0198±0.0069	=0.0470±0.0112	=0.1294±0.0252	=0.1975±0.0377	
	= 0.2135±0.0942						
b		Gompertz *		Gompertz *	Gompertz *	Gompertz *	Gompertz *
		=-4.0403±0.7583		=-4.7092±0.7256	=-2.9492±0.4795	=-2.2503±0.3998	=-1.3756±0.3834
		=-0.0259±0.1193		= 0.3610±0.0968	= 0.2735±0.0890	= 0.3477±0.1119	= 0.6841±0.2075
c	Gompertz	Exponential *	Exponential *	Exponential *	Exponential *	Exponential *	Exponential *
	=-4.4590±0.6814	=0.0154±0.0060	=0.0157±0.0061	=0.0189±0.0067	=0.1265±0.0242	=0.2673±0.0547	=0.5402±0.1371
	= 0.1808±0.0896						

* $\alpha = 0.05$; ** $\alpha = 0.025$; *** $\alpha = 0.01$; **** $\alpha = 0.005$.

tion. The order from lower to higher instantaneous death rate is indicated in Table 2b. These results differ from those obtained by means of the nonparametric analysis (Tarin et al. 1988), since in the last one, the whole of the 22 strains of each population have been considered; whereas in the present work, only one strain of each population has been considered with the purpose of showing the possibilities of parametric statistics in the analysis of survival data.

The parametric analysis of the survival data became more laborious and tedious than nonparametric analysis. For this reason, use of the parametric analysis is only advisable when the researcher wants to know the distribution of survival data and to compare parameters of different distributions. However, if this is not the case, nonparametric analysis of survival data is sufficient and less laborious in analyzing data and produces good results (Tarin et al. 1988).

References: Gros, A.J. & V.A. Clark 1975, in: Survival distributions: Reliability applications in the biomedical sciences, Wiley & Sons, New York; Lee, E.T. 1980, in: Statistical methods for survival data analysis, Lifetime Learning Pub., Belmont, Calif.; Tarin, J.J., C. Najera & J.L. Mensua 1988, DIS 67 (this issue).

Thomas, G.H. and S.C.R. Elgin. Washington University, St. Louis, Missouri USA. The use of the gene encoding the α-amanitin-resistant subunit of RNA polymerase II as a selectable marker in cell transformation.

The cloned gene for the α-amanitin-resistant subunit of RNA polymerase II (Biggs et al. 1985) was used as a selectable marker in cell transformations. pAM9400 is a 9.4kb Bst EII fragment containing the 7kb transcription unit for the mutant polymerase subunit, cloned into the Xba I site in Carnegie 4 (a gift from R.S. Jokerst & A.L. Greenleaf). A calcium phosphate-DNA coprecipitate of pAM9400 was prepared by standard procedures (Wigler et al. 1979). 2.5 ml of the coprecipitate containing 25 µg of plasmid was added to 10^6-10^7 Schneider 2 cells (RF13) in an 80 cm^2 tissue culture flask. The final volume was 10 ml in Schneider's medium (Schneider 1964), supplemented with 18% heat-inactivated fetal calf serum and 0.5% bactopeptone. The cells were incubated with the precipitate for 18-24 hr, washed twice in 5 ml of fresh media, resuspended in 7 ml of fresh media and put in an 80 cm^2 tissue culture flask at 25°C for two days. At this point the cells were split into three lots, placed in 25 cm^2 tissue culture flasks and made up to 3 ml and 0, 5 or 10 µg/ml α-amanitin (Calbiochem). A parallel experiment was carried out using the plasmid 88B13, which contains part of the small heat-shock protein gene cluster (Corces et al. 1980), as a non-selectable control.

After 7 days visible colonies of resistant cells were evident in the pAM9400 treated cultures; these could be harvested using a pasteur pipette. Cells in the 88B13 control culture were strongly, but not completely, inhibited and only underwent one or two cell divisions during this period in the presence of 10 µg/ml α-amanitin. After a further 14 days, the resistant colonies had grown to macroscopic floating clumps of cells.

Colonies were picked from both the 5 and 10 µg/ml selection flasks and grown up under selection with 5 µg/ml α-amanitin. In all cases analyzed these transformants contained high molecular weight tandem arrays of the pAM9400 DNA. A Southern blot analysis of three examples is shown in Figure 1. In each line (1,2,3) prominent restriction bands are visible which exactly match the bands seen in the digested plasmid (P). The undigested DNA samples (U) indicate that this is not free plasmid DNA but is incorporated into high molecular weight DNA. The copy number of the pAM9400 DNA can be estimated from the Kpn I digested DNA, where both the plasmid and endogenous gene produce one band (Figure 1, lanes K, bands a and b, respectively). In the examples in Figure 1, lines 1, 2 and 3 have approx. 19, 22 and 36 copies, respectively. It should be noted that these lines were never cloned from single cells and so there may be heterogeneity on a cell to cell basis in these lines.

Clearly it is possible to use this gene to produce α-amanitin-resistant cell lines. We have not tried relaxing selection to see if these sequences diminish or are lost. pAM9400 is somewhat large for routine cloning purposes, but could have utility for co-transformation with other sequences. We have also tried using soft agar for the initial selection process, and this greatly eases the task of picking out resistant colonies. It is interesting that growth is not completely inhibited in the control lines at this level of α-amanitin. This may indicate that the drug does not penetrate the cells efficiently and that greater concentrations should be used. However, the differential in the growth rates is such that sensitive cells should be quickly lost by dilution.

References: Biggs, J., L.L. Searles & A.L. Greenleaf 1985, Cell 42:611-621; Corces, V., R. Holmgren, R. Freund, R. Morimoto & M. Meselson 1980, PNAS USA 77:5390-5393; Schneider, I. 1964, J. Exp. Zool. 156:91-100; Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub & L. Chasin 1979, PNAS USA 76:1371-1376.

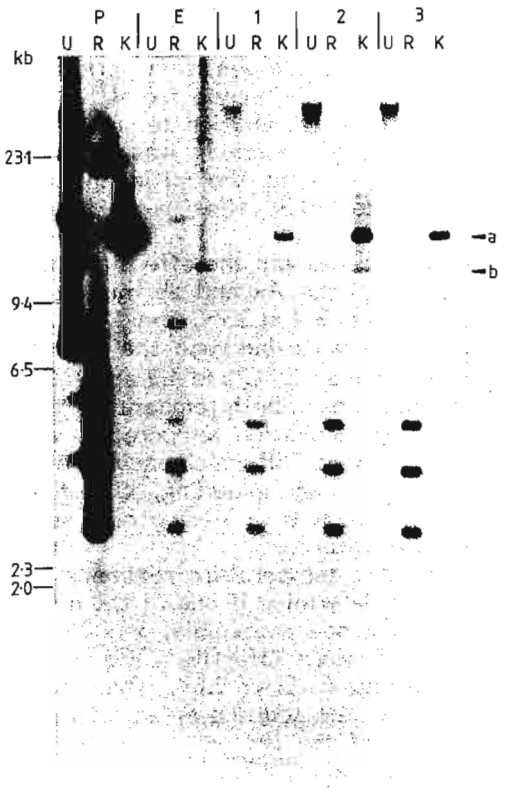


Figure 1. Southern blot analysis of transformed cell lines. Samples of DNA, either undigested (U), digested with Eco RI (R) or digested with Kpn I (K) were size-separated on a 0.5% agarose gel. Samples are P - 10 ng of pAM9400 DNA; E - 5 µg Drosophila embryo DNA; 1, 2 and 3 - up to 2 µg genomic DNA (not precisely quantitated) from each of three cell lines transformed with pAM9400. Numbers to the left are the positions and sizes in kilobases (kb) of marker DNA fragments. a and b are bands discussed in the text. The probe was nick-translated pAM9400.

Thompson, S.R., J. Bruni, D. Carbonaro and P. Russo. Ithaca College, Ithaca, New York USA.

Temperature sensitivity in Abruptex and abrupt.

Although the alleles at the Abruptex locus (Ax, 1-3.0) show variation in both viability and fertility, they all possess a dominant effect on wing venation. Homozygotes for Ax have a distal shortening of the L5 vein and usually exhibit interruptions in L4 and L2; heterozygotes normally show only a shortening of L5. The recessive mutants of the abrupt locus (ab, 2-44.0) are characterized by an extreme distal shortening of L5. In both of these loci, the venation phenotype is temperature sensitive, but the sensitivity is of opposite effect with ab having a more extensive vein interruption at low temperatures while Ax has a more extreme phenotype at higher temperatures. This study characterizes the temperature effect, the temperature sensitive period of the Ax and ab venation phenotype, and an interaction effect between these two loci.

Table 1. Temperature effects on Abruptex and abrupt.

Mutant: Temperature	Eclosion time*	Females n*	Females R-10*	Males n	Males R-10	Average n	Average R-10
Ax:							
20°C	190.1 ± 8.1	113	1.6	112	1.6	225	1.6
25°C	98.2 ± 3.5	114	3.3	114	3.5	228	3.4
ab:							
20°C	170.8 ± 6.7	110	5.8	142	4.3	252	4.9
25°C	97.6 ± 1.9	131	4.1	153	3.2	284	3.6

*Eclosion time in hr; n = no. of indiv.; R-10 = mean L5 rating.

Table 2. Temperature sensitive period in Abruptex and abrupt (average of females and males).

Mutant: Initial temperature	% Pupal life at initial temperature	n	R-10
Abruptex:			
20	5	40	3.4
	10	53	3.4
	15	60	2.3
	25	39	2.1
25	5	38	2.1
	10	60	1.9
	15	40	3.3
	20	60	3.7
	25	30	3.3
abrupt:			
20	10	44	3.4
	15	67	4.2
	20	27	4.5
25	10	50	4.2
	15	41	3.4
	20	36	3.2
	30	29	3.3

Cultures of Ax^1 and ab^1 mutant stocks were maintained in half-pint culture bottles on a cornmeal-agar-molasses-Brewer's yeast-tego-sept medium at 25±0.5°C. On the third or fourth day of mating, the culture bottles were cleared of adults and the medium was supplemented with a thick suspension of live yeast. White prepupae were collected from the cultures and placed on the walls of 25 x 95 mm shell vials which contained about 10 ml of the standard medium. These vials, containing the developing pupae, were aged at either 20±0.5°C or 25±0.5°C in constant temperature incubators. Whiteness of the prepupae indicates it is within one hour after the onset of puparium formation, the time of collection was taken as time zero in pupal development. The time to eclosion at these two temperatures was determined for both mutant strains. Following eclosion, the adult flies were rated as to the extent of missing L5. The rating system arbitrarily divides the segment of L5 from the posterior crossvein to the margin of the wing into fifths, so that a rating of zero indicates that this portion of L5 is present without interruptions in both wings, while a rating of ten indicates this entire segment is missing in both wings. The temperature sensitive period (TSP) was determined by switching developing pupae from the initial incubation temperature to the other incubation temperature to complete development. The time of switching was based on a percentage of the total eclosion time at the initial incubation temperature. After eclosion the adult flies were rated as described above.

Table 1 presents the eclosion times and the effect of the two temperatures on the expression of the Ax and ab mutants. As can be seen, Ax has a mean rating of 3.4 at 25°C and 1.6 at 20°C, and there was no significant difference between the expression in females and males. On the other hand, the mean ratings with ab were 3.6 at 25°C and 4.9 at 20°C, and in this case there were differences in the expression between females and males at both temperatures.

Table 2 illustrates the effect on the venation phenotype of switching incubation temperature during pupal development. The results clearly demonstrate that both Ax and ab have a TSP which occurs between 10 and 15% of pupal development time. That is, temperature switches up to, and including, 10% of pupal development exhibit the phenotype of the second incubation temperature, but if the switch occurs at 15%, or later, the phenotype is that characteristic of the initial incubation temperature.

Since the maximum expression of L5 interruption occurs with opposite temperature regimens in Ax and ab, we attempted to construct the double mutant, Ax;ab, in order to determine if one of the mutants acts epistatically or if some other interaction occurs. The double mutant is nearly inviable, only 26 adults (7 females and 19 males) were obtained in nearly 100 crosses, and all were infertile. The venation phenotype at 25°C was 9.57 in females and 9.67 in males, with an average R-10 of 9.64. These ratings are not only more extreme than either mutant alone, they are greater than the additive effect of the two mutants. All the double mutants possessed a deep middorsal furrowing of the thorax, a trait which is characteristic of some Ax alleles. It appears that ab acts as an enhancer of Ax.

Van Delden, W. and A. Kamping. University of Groningen, Haren, Netherlands. Test for viability differences among αGpdh genotypes in **D.melanogaster.**

In addition to the two naturally occuring electrophoretic alleles, Fast (F) and Slow (S) of the α-glycerophosphate dehydrogenase (α-Gpdh) locus in **D.melanogaster** (O'Brien & MacIntyre 1972) which occur in relatively high frequencies, we have regularly observed two additional mobility variants in a natural population in Groningen, The Netherlands (Kamping & Van Delden 1988). These alleles, Super-Fast (F^S) and Super-Slow (S^S) have electrophoretic mobilities on 5.5% polyacrylamide gels of 45 and 30 mm, respectively, to be compared with 40 and 35 mm for F and S alleles, respectively, when using a 0.041 M Veronal-HCl buffer (pH 8.4) and running the gels for 2.5 hr. The frequencies of the F^S and S^S alleles in the natural population are less than 0.01.

Table 1. Allele frequencies in F_2's. N is no. of individuals electrophoresed.

		Allele frequency			
Cross	N	F^S	F	S	S^S
F^S x F	108	0.50	0.50	--	--
F^S x S	100	0.46	--	0.54	--
F^S x S^S	108	0.47	--	--	0.53
S^S x F	108	--	0.48	--	0.52
S^S x S	108	--	--	0.52	0.48

Four different strains each homozygous for one of the four αGpdh alleles were obtained from the Groningen population. Crosses were made between the four strains as indicated in Table 1; F_1 flies were crossed and the resulting F_2's were analysed for deviations from the expected 1:2:1 ratio with the aim to ascertain differences in egg-to-adult survival, if any, between the genotypes. Regular laboratory conditions were applied (temperature 25°; standard medium). The allele frequencies are given in Table 1. No significant deviations from 0.50 were observed; neither did the genotype ratios differ from the expected Mendelian ratio. The conclusion must be that no viability differences occur among the F_2 αGpdh genotypes, at least under regular laboratory conditions. Genotypic differences may exist for other fitness components, which is presently under study.

References: Kamping, A. & W. van Delden 1988, DIS 67 (this issue); O'Brien, S.J. & R.J. MacIntyre 1972, Genetics 71:127-138.

Vikulova, V.K., E.J. Remisova and V.A. Mglinetz. Dept. of Devel. Genetics, Inst. of Med. Genetics, Moscow 115478 USSR. Induction of halter and metathoracic leg reduction in **D.melanogaster** by ether treatment of early embryos.

To induce early developmental anomalies 2.5 to 3.0 hr **D.melanogaster** embryos, strain M(2)S7/SM5, were treated with diethyl ether vapour. Most often the phenocopies of bithorax-mutants (bx) were found. Less frequently were the anomalies of abdominal segments and prothoracic (PT) to mesothoracic leg (MSL) transformations as already reported (Mglinetz & Ivanov 1987; Remisova et al. 1987) in some other strains of Drosophila. Beyond that, partial to total halter and/or metathoracic leg (MTL) reduction was sometimes observed (see Table).

Table. Disturbances in progeny of crosses ♀♀ M(2)S7/SM5 x ♂♂ Canton-S after treatment of ether vapour.

	Rate of anomalies, %			
	M(2)S7/+		+/SM5	
	females	males	females	males
bx phenocopies	8.3±2.2	11.0±2.4	10.3±2.5	20.5±3.2
Reduction of halters or/and MTL	5.1±1.8	4.9±1.7	6.8±2.1	1.9±1.1
Transformation of PTL to MSL	1.3±0.9	3.0±1.3	6.8±2.1	3.1±1.4
Fusion of PTL	4.5±1.6	0.6	0.6	0.0
Total abdominal anomalies in any of the segments	7.7±2.1	9.7±2.3	9.6±2.4	11.3±2.5
7-th tergite in males		11.0±2.4		17.9±3.0

In extreme cases total absence of metathoracic (MT) derivatives was found, which caused pupal death. In cases of incomplete anomalies the flies could develop either two halters and one MTL, or two legs and one halter; sometimes a halter and a leg were found (on the same body side). In 4 of the 31 flies, reduction in MT segment development was accompanied by similar anomalies in mesothorax, on the same body side. Beyond that, in 7 of the 625 flies PTL fusions (coxae, sometimes trochanters) were found. In none of these 7 flies were MT or MSL anomalies observed, thus suggesting independent origin of MTL and PTL anomalies, just as in cases of PT and MT transformations.

It seems that the MT anlagen are most sensitive to ether treatment, inducing both the bithorax-mutants phenocopies and limb reduction via common mechanisms. However, anomalies newly observed were peculiar for the Minute strain and never observed in 5 other strains tested. It may be suggested that both MT derivatives reduction and partial fusions in PTL are actually phenocopies of respective, not yet described mutants (some data on fusion mutants are available [Goldschmidt et al. 1951]).

References: Goldschmidt, R.B., A. Hannah & L. Piternick 1951, Univ. Calif. Publ. Zool. 55:67-294; Mglinetz, V.A. & V.I. Ivanov 1987, DIS 66:100; Remisova, E.J., V.K. Vikulova & V.A. Mglinetz 1987, DIS 66:118.

Chambers, G.K. Victoria University, Wellington, New Zealand. The use of beer as a Drosophila bait.

Table 1. Numbers and proportions of Drosophila species collected with fruit and beer baits.

	Collection Site and Bait			
	Trial 1		Trial 2	
Species	fruit	beer	fruit	beer
D.immigrans	0.64	0.32	0.85	0.08
D.melanogaster] **D.simulans**	0.02	0.24	0.06	0.92
D.hydei	0.12	--	0.01	--
D.busckii	0.22	--	0.07	--
D.pseudoobscura	--	0.44	--	--
Totals	209	34	135	38

D.melanogaster and **D.simulans** are relatively rare in the Wellington, N.Z., region (see Chambers et al. 1988: this issue). The use of selective baits to attract Drosophila species is a well established practice (see, for example, Bock & Parsons 1981, on the capture of endemic Australasian species). Therefore, I experimented with a beer-based bait in an effort (1) to attract increased numbers of **D.melanogaster** and (2) to sort them differentially from the more numerous **D.immigrans.**

The method was tested at the KAR collection site (Karori: see Chambers et al. 1988) in January 1986. The beer bait consisted of a plastic bucket half filled with wood shavings and soaked with well-oxidised beer from a failed fermentation experiment (Sprawley Dawg Assoc., unpubl.). The predominant aromas associated with this bait detectable by human subjects are acetic acid and brewers yeast. The beer bait was placed approximately one metre away from the KAR fruit bait and flies collected by aspirator the next day. To control for the effect of bait position, the buckets were switched and a second collection made.

The data from these two collections are shown in Table 1. It is readily apparent that **D.melanogaster** prefer the beer bait over the fruit bait regardless of position. Interestingly, in the first collection **D.pseudoobscura** showed the same preference, but regrettably the numbers of **D.pseudoobscura** are generally too low at the KAR site to make further tests worthwhile. A second experiment was run one month later to check if the beer bait increased the total number of flies taken. In this experiment one bait was set in place at each trial. From the beer I collected a total of 52 flies, 47 (90%) of which were **D.melanogaster** or **D.simulans** (probably all **D.melanogaster** since there were no **D.simulans** males in the collection). From the fruit bait I collected 129 flies of which 64 were **D.melanogaster** and 2 were **D.simulans**, i.e., 51% (identified by electrophoresis, Chambers et al. 1988). These data indicate that the fruit bait is a better overall attractant for Drosophila species but that beer selects for **D.melanogaster.**

In conclusion, I recommend this procedure for the enrichment of collections in regions where **D.melanogaster** is a minor component of the Drosophilid fauna.

Acknowledgement: I am indebted to Phil Debnam, Cam Falkner and Sue Marshall collectively known as Sprawley Dawg Associates (VUW, Biochemistry Dept.) for the provision of the beer used in this experiment and later physiological trials (unpublishable).

References: Bock, I.R. & P.A. Parsons 1981, in: Genetics and Biology of Drosophila (Ashburner et al., eds.), Academic Press, NY, v3a:291-308; Chambers, G.K., S.L. Davies, M. Hodgetts, R.H. Moore & I.J. Pomer 1988, DIS 67:this issue.

Gordesky-Gold, B., F.B. Schnee and L. Tompkins. Temple University, Philadelphia, Pennsylvania USA. An improved method for monitoring the responses of Drosophila larvae to chemicals in solution.

Several years ago, one of us (L.T.) developed a procedure for assaying the responses of Drosophila larvae to two repellents, quinine sulfate and NaCl (Tompkins 1977, 1979). We have recently modified this assay so that preparation of the apparatus and the assay itself take much less time. Instead of preparing a complex low-agar Drosophila culture medium, we simply boil 0.8 g agar, 20 g sucrose, and 180 ml distilled H_2O in a microwave, allow the solution to cool slightly, then fill one side of a 21.5 x 7 x 5.5 cm plexiglass box with it. After the agar block has solidified, we fill the other side of the box with a solution that is identical except that it also contains 11.68 g NaCl and a few drops of red food coloring.

After the second agar block has solidified, we immerse larvae in 0.6 M sucrose to separate them from the surrounding culture medium (Nothiger 1970), then wash the animals gently with distilled water in a Buchner funnel lined with a 290 micron nylon mesh. 100-200 of the clean larvae are transferred to the boundary between the two agar blocks with a damp artist's brush. After 30 min, we count the larvae in each block, those that have crawled up the sides of the assay box, and those that are on the boundary between the two blocks. We then calculate a "salt index" (SI), using the following formula: SI=larvae on NaCl/(total larvae - larvae on sides and boundary).

Salt indices for populations of wild-type (Canton-S or Oregon R) **D.melanogaster** larvae range from 0.08 to 0.18 (mean = 0.13).

Acknowledgement: This research was supported by a grant from the National Science Foundation (BNS-8615554), awarded to L.T.

References: Nothiger, R. 1970, DIS 45:177; Tompkins, L. 1977, PhD thesis, Princeton University, Dept. Biol.; ——— 1979, Devel. Biol. 73:174.

Hiebert, J.C. Harvard University, Cambridge, Massachusetts USA. A menu-drive computer database program with extended search capabilities for organizing Drosophila stocks: IBM pc/dBASE III compatible.

We solved the problem of managing laboratory fly stocks by writing programs to create a Drosophila database, accessible to anyone, regardless of his or her computer experience. This program uses the database manager dBASE III and is composed of accessory dBASE III command files. Since dBASE III alone is not sufficient to handle the special needs of the fly lab these programs must be employed to achieve heightened search capabilities as well as to make the program "user friendly". This database is highly specialized for Drosophila stock data. It is completely menu-driven, enabling the user to easily enter, edit, view, search, print and delete information. It provides a powerful search function for mutant and cytological characteristics.

The database consists of seven fields (columns): number, type, deficiency, duplication, translocation, inversions, and section.

NUMBER is the fly identification number. They can number anywhere from 0 to 9999. We use 1000's for chromosome #1 mutants, 2000's for chromosome #2, etc. Each fly stock has a number and all stocks are arranged numerically in the incubator. Locating and maintaining fly stocks has become simple.

TYPE is the description of the stock, i.e., its mutations. Superscripts designating alleles are bracketed. For example, w[sp55]; E(w[a]) / CyO is one of our entries designating the mutant w^{sp55}; $E(w^{a})$/CyO.

DEFICIENCY, DUPLICATION, TRANSLOCATION, and INVERSIONS are fields of cytological characteristics. SECTION contains the chromosome section of the cytological characteristics.

The search function permits the identification of any record (row) containing the specified character string in a field or any record containing the intersection of two or more character strings in a field. For instance, one might want to search for all the stocks having the rosy mutation. To do this one would enter "ry" and the program would return every record satisfying that condition, regardless of where in the record the character string "ry" appeared. Similarly one could search for all the records containing both "ry" and "Cy" by searching for the intersection of the two strings. The program would return all those stocks which contain both mutants. Any field may be searched in this way. This system of substring searching is particularly suited to Drosophila stock data.

The database is amenable to modification. One familiar with dBASE may add and delete fields, change field lengths, and alter many other parameters. The program is not compiled; this facilitates the modification of any part of the system. Any pc-compatible with at least 256K of memory is sufficient to handle dBASE III as well as this program. You must already have dBASE III or dBASE III Plus; I will send only the accessory fly programs. Also, I will send complete instructions and technical data on the software. Send two 360K diskettes (or one 1.2Mb diskette) to: J.C.H., The Biological Laboratories, Harvard Univ., Cambridge, MA 02138.

Lynch, D.V., S.P. Myers, S.P. Leibo*, R.J. MacIntyre and P.L. Steponkus. Cornell Univ., Ithaca, New York; *Rio Vista International, San Antonio, Texas USA. Permeabilization of Drosophila eggs using isopropanol and hexane.

We have developed a method for permeabilization of the eggcase of **Drosophila melanogaster** which allows for the flux of water and cryoprotective agents such as dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol (Lynch et al. 1988). Many procedures for permeabilizing eggs have employed organic solvents such as octane (Limbourg & Zalokar 1973; Arking & Parente 1980) or heptane (Mitchison & Sedat 1983) which dissolve the waxy layer of the eggcase. However, to date, the efficacies of many of these procedures have not been critically evaluated for both permeabilization and subsequent survival (hatching) of embryos. Of these methods, the best documented and most generally applicable appears to be that described by Limbourg & Zalokar (1973) for small numbers of mechanically-dechorionated eggs. During initial experiments using large numbers of hypochlorite-dechorionated eggs, we found that the direct addition of organic solvent was of limited effectiveness. This was attributed to the immiscibility of organic solvent and water, preventing penetration of the thin aqueous film remaining on the eggs. To overcome this we incorporated an intermediate rinsing step with isopropanol to remove surface moisture and increase miscibility of solvent and water. Subsequently, we tested the efficacies of different organic solvents and postpermeabilization treatments (see Lynch et al. 1988, for complete details and discussion). The resulting optimized procedure for permeabilization is described below.

Eggs (12-13 hr old) were dechorionated using a 2.6% sodium hypochlorite solution (50% Clorox) for 2 min as described by Hill (1945) and rinsed with copious amounts of distilled water. Eggs were transferred to a clean, dry nylon screen filter (see Widmer & Gehring 1974) and rinsed for 20 sec with a continuous stream of isopropanol from a squirt bottle. The eggs were then rinsed for 30 sec with a continuous stream of n-hexane. Residual hexane was blotted from the nylon screen using a Kimwipe and the eggs were rinsed immediately and dispersed using a pulsing stream of Drosophila Ringer's solution (128 mM NaCl, 5 mM KCl,

2 mM $CaCl_2$) from a squirt bottle. The treated eggs were transferred to modified Drosophila cell culture medium (9 mM $MgCl_2$, 10 mM $MgSO_4$, 3 mM NaH_2PO_4, 68 mM glutamic acid, 67 mM glycine, 4 mM malic acid, 0.2 mM Na acetate and 5 mM $CaCl_2$, pH 6.8, and containing 0.1% bovine serum albumin to prevent clumping of eggs). A paint brush was used to disperse any remaining aggregates of eggs and to submerge any eggs floating on the surface. Embryos were held in cell culture medium for at least 15 min prior to plating or other subsequent manipulation.

The percentage of embryos permeabilized was determined by transferring 100-200 eggs to 1 M sucrose and counting the number of eggs in the sample population exhibiting plasmolysis/contraction. To determine the incidence of hatching, permeabilized eggs were transferred to a watch glass containing light paraffin oil (using a paint brush and taking care to remove any excess cell culture medium) and incubated at 25°C in a humid atmosphere for 24-48 hr to allow for development to the larval stage.

Using this two-step method, we routinely obtain 80-95% of the eggs permeabilized and 75-90% hatching. In addition, we found that permeabilized embryos could be held in modified cell culture medium at 4°C for up to 9 hr with little loss of viability.

References: Arking, R. & A. Parente 1980, J. Exp. Zool. 212:183-184; Hill, D.L. 1945, DIS 19:62; Limbourg, B. & M. Zalokar 1973, Dev. Biol. 35:382-387; Lynch, D.V., T.T. Lin, S.P. Myers, S.P. Leibo, R.J. MacIntyre, R.E. Pitt & P.L. Steponkus 1988, Cryobiology, submitted; Mitchison, T.J. & J. Sedat 1983, Dev. Biol. 99:261-264; Widmer, B. & W.J. Gehring 1974, DIS 51:149.

Tidon, R. and F.M. Sene. University of Sao Paulo, Ribeirao Preto, SP, Brazil.

A trap that retains and keeps Drosophila alive.

We have developed a simple and efficient method for Drosophila collection in tropical environments. This trap can retain and keep alive all of the drosophilids attracted by the bait, is resistant and can be easily built with plastic bottles.

The trap basically consists of two coupled plastic bottles (Figure 1A). The lower bottle (A) is shaped like a glass, is black and has openings that permit the flies to enter to reach the bait inside. The upper container (C) is transparent and shaped like a bottle whose bottom is removed and replaced with a funnel (B); this part also has a series of small perforations for ventilation. The trap hangs on a permanently attached copper wire which is affixed at collection sites.

The drosophilids attracted by the bait enter the trap through the openings in the lower compartment (A), which should be of a size that will not permit the entry of larger insects. The flies then go to the upper compartment (C) attracted by its clarity and also because they intrinsically tend to fly upwards under these conditions. The funnel (B) at the base of the upper compartment does not prevent the ascent of the flies but hampers their return to the darker compartment. A few strips of filter paper may be placed in the upper compartment to absorb any possible moisture present and to act as a support on which the flies can alight. Under these conditions, the flies stay alive for several days.

Collection is done by separating the upper compartment (B+C) from the lower compartment (A) and by replacing the cover of the upper compartment (D) with a collection bottle. The orifice of the funnel (B) should be closed with a cottonwool stopper. The upper compartment of the trap (B+C) coupled to the collection bottle is turned upside down to transfer all the flies from the trap to the bottle. This procedure is rapid and safe, since fly transfer does not involve contact with the bait and therefore there is no risk of losing insects sticking to the moist fermenting fruit. Furthermore, this technique permits the collection of all the flies attracted by the bait as long as the operator has a minimum of experience with the procedure.

These traps have proved to be resistant to attack by animals and to adverse environmental conditions such as strong rains and winds. In addition, they can be hung at a height of several meters directly on tree branches or can be suspended using bamboo sticks or a pulley system. We were able to collect about 850 drosophilids in a single trap containing only one fermented banana.

Acknowledgement: Research supported by FAPESP, CNPq, CAPES and FINEP.

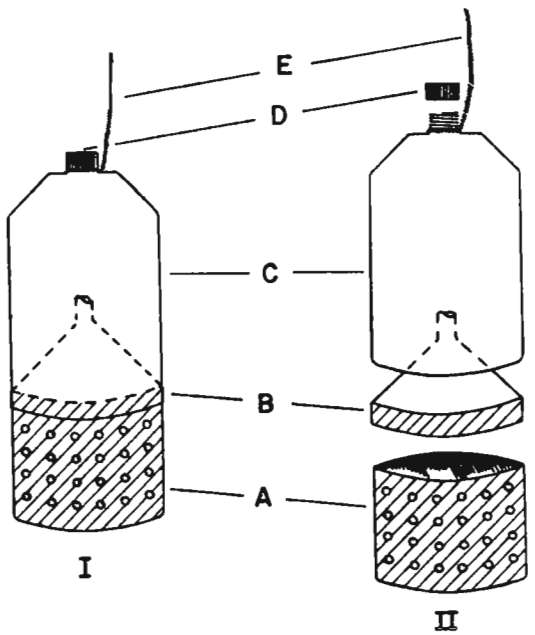


Figure 1. I. Fully mounted trap. **II.** Separate components of the trap: **A** lower compartment painted black; **B** transparent funnel; **C** transparent upper bottle; **D** cover; **E** wire.

Warn, R.M. and J. Geeson. University of East Anglia, Norwich, England. Prevention of fungal infections in Drosophila cultures with o-hydroxybiphenyl.

Fungal contamination is an occasional hazard with Drosophila cultures. The traditional remedy has been the regular addition of Nipagin to the culture medium and, if necessary, treatment of contaminated stocks with propionic acid. Recently, we have had several infections resistant to these forms of treatment. However, the addition of o-hydroxybiphenyl (also referred to as o-phenylphenol) has successfully overcome these infections without harm to the cultures. A 0.5% w/v solution of o-hydroxybiphenyl (Sigma) is made up in alcohol and 2.5 ml of the stock added to 20 bottles worth of the cornmeal-treacle-agar medium described by Roberts (1986). One or two passages through medium containing o-hydroxybiphenyl is usually sufficient to stop an infection.

Reference: Roberts, D.B. 1986, in: Drosophila: a practical approach, IRL Press, Oxford & Wash. DC.

Weber, K.E. Univ. of Minnesota, Minneapolis, Minnesota USA. An apparatus for measurement of resistance to gas-phase agents.

The "inebriometer" is a system for the measurement of resistance of flies to any bottled gas or to the vapor of any volatile liquid. The parameter of resistance that is measured is the exposure time required to incapacitate flies at a given concentration of gas or vapor. The flies are anaesthetized inside a vertical glass cylinder, 122 cm tall and 7 cm inside diameter (Figure 1). The concentration of gas inside this chamber is maintained at a constant level throughout by continous replacement of the contents. A sample of flies to be measured is introduced at the top of the cylinder. In fresh air, flies do not venture downward from the top. In anaesthetic gas or vapor, the flies gradually lose their ability to cling, and as they succumb more and more to gravity, they sift downward over a series of sloping mesh baffles toward the exit funnel at the bottom. Fully incapacitated individuals tumble through, and out into the advancing tubes of a fixed-point fraction collector. The exposure to the drug is therefore self-limiting and all flies recover from the measurement. The tubes in the fraction collector are coated inside with Fluon (Northern Products, Inc., Woonsocket, R.I.), which leaves a nearly transparent film that insects cannot climb; and the tubes are too deep and narrow for them to fly out. The tubes are also covered by a transparent lid to prevent migrants in or out. The flies can be reanaesthetized with CO_2 and counted inside the tubes. The distribution is spread over many classes. Population differences as small as 15 seconds can be resolved. Up to 1500 flies at a time can be measured within an hour, and the presence of an operator is not required for most of the time. The system has been used to select for resistance to ethanol, CO_2, and ethyl acetate, and measurements have been made with N_2. In the longest-running selection experiments, ethanol resistance has been increased to six times control resistance.

To generate a flow of vapor, air is diffused through an airstone in an evaporation chamber containing a volatile liquid (Figure 2). The air flow rate (4.7 liter/min) is controlled by a regulator valve and flowmeter which allow control to an accuracy of approximately 0.05 l/min. The evaporation rate is a function of the temperature of the liquid, which is controlled by a water jacket completely surrounding the evaporation chamber. The water jacket is connected to a circulating water bath (Haake, Model FK). The temperature within the evaporating liquid is monitored by an enclosed precision thermometer and any desired temperature can be maintained with variation of less than 0.05°C. The level of the evaporating liquid is kept constant by automatic replacement from a reservoir over the evaporation chamber. The reservoir is connected to the evaporation chamber by a buret tube. When the level of the evaporating liquid in the lower chamber falls, bubbles entr the buret and travel up into the reservoir, allowing an equivalent volume of liquid down into the evaporation chamber. A teflon disk inside the chamber is perforated everywhere except below the replacement tube, so bubbles will not enter unless the level falls. When the stopcock between the reservoir and the buret is closed, the descending meniscus in the buret allows determination of real-time evaporation rates. At 20°C the evaporation rate of 95% ethanol is about 0.6 ml/min in this apparatus, and this is constant within about 0.02 ml/min. The actual percent ethanol within the evaporation chamber is monitored by a built-in ethanol hydrometer, which is protected from the rising bubbles by an enclosing sleeve.

Measurement of resistance to ethyl acetate requires modified procedures. Because this agent acts extremely rapidly on flies, the bubbling liquid must be cooled to 6°C to reduce the concentration of the vapor, and the fraction collector must be set to advance on 12 sec intervals. By these means it is possible to spread out the distribution and select for resistance. It was necessary to build a separate small insulated evaporation system with an all glass and teflon interior to handle this agent. To select for CO_2 resistance, parallel flowmeters and regulators are employed to mix CO_2 and air at the ratio of 60:40. Flies must be

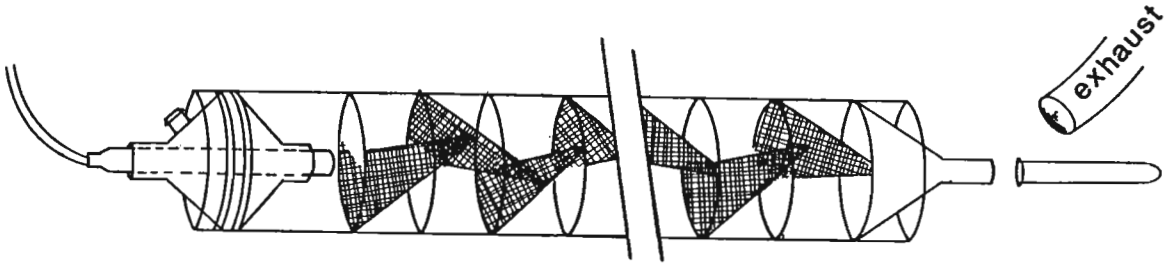


Figure 1. Anaesthetization chamber. Baffles are made of nylon mesh, stapled to tight-fitting polypropylene rings. Insertion is facilitated by glycerin which is then washed out. Introduction enclosure is made of two funnels, cut and drilled to fit inner and outer tubes precisely.

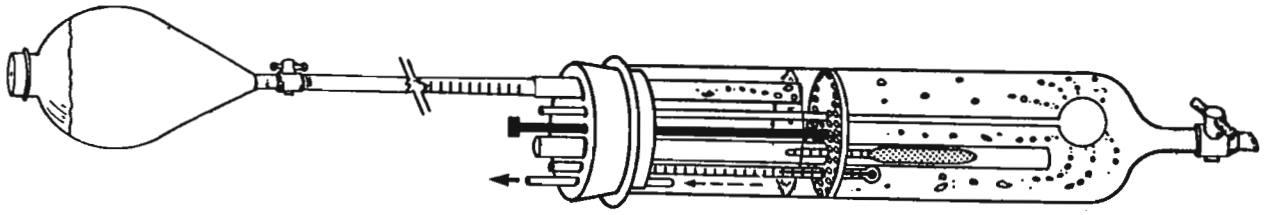


Figure 2. Evaporation chamber, with water jacket removed.

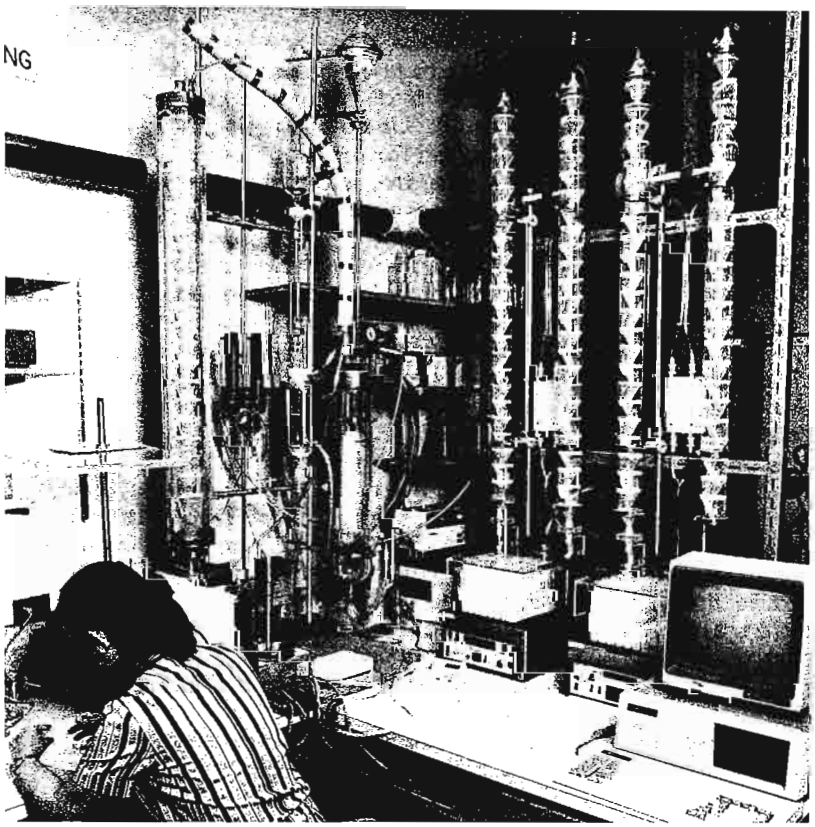


Figure 3. Installation of 5 inebriometers over 3 fraction collectors.

placed in a totally dry environment for one hour before measuring CO_2 resistance, in order to get consistent results.

To begin a run, a sample of flies is first introduced into a small enclosure at the top of the anaesthetization chamber. This enclosure contains fresh air; a sliding tube through center transmits the flow of gas into the chamber below. When this tube is withdrawn upwards, the introduction chamber immediately fills with gas and becomes confluent with the lower chamber. At the same moment, the fraction collector is activated.

In the original inebriometer system the entire anaesthetization chamber is enclosed by a water jacket connected to a Fisher Thermomix circulating water bath. In the present installation at the University of Minnesota (Figure 3), four additional chambers have been constructed without water jackets. These are installed over two additional fraction collectors, for selection on large populations. An exhaust system for all 5 chambers is powered by a 3/4 horsepower explosion proof motor, with a spark-proof in-line duct fan which pulls in all exhaust gases via small openings adjacent to the outlet funnels of the individual anaesthetization chambers. The components of the system are on timer clocks so that the system can be started up an hour early in the morning to allow the dose rate and temperature to equilibrate before use. Electric eyes (Keyence Corp. of America, Model PG-602) are currently being installed in the outlet funnels, to convey counts directly to a computer. Tests of these eyes in typical runs show that they consistently underreport the number of flies falling in one minute by a constant percent, so that the eyes function as analogue detectors. Total counts must be corrected, but the apparent means and selection cutoff points are virtually identical to their true values.

Resistance is measured in time, but resistance time is shorter at higher concentrations. As flies evolve greater resistance in the course of selection, it eventually becomes necessary to increase the vapor concentration, by raising the temperature of the evaporating liquid, so that the duration of individual runs can be kept under one hour. For ethanol, tests showed that as the temperature is varied, the ratio of mean resistances between any two lines is conserved over the entire range of temperatures used. Therefore the resistance phenotypes of selection lines, and all other statistical parameters, are expressible as a ratio to control means in each generation.

Several simpler versions of this system have been adopted in other labs, and have already been used in a variety of applications (Cohan & Graf 1985; Cohan & Hoffman 1986; Hoffman & Cohan 1987; Whitaker & Nash 1987; Frankham et al. 1988). For a more detailed description of the construction of the original system, as well as further discussion of operating techniques, Weber (1986) can be consulted.

References: Cohan, F.M. & J.D. Graf 1985, Evolution 39:278-293; ——— & A.A. Hoffmann 1986, Genetics 114:145-163; Frankham, R., B.H. Yoo & G.L. Sheldon 1988, T.A.G. (in press); Hoffmann, A.A. & F.M. Cohan 1987, Heredity 58:425-433; Weber, K.E. 1986, Thesis, Harvard U.; Whitaker, J. & H. Nash 1987, in: Molecular Neurobiology of Drosophila (Ganetzky & Hall, eds.), Cold Spring Harbor, N.Y. p113.

Weber, K.E. University of Minnesota, Minneapolis, Minnesota USA. An apparatus for selection on flying speed.

The "antianemometer" (Figure 1) is a 1.5 meter long wind tunnel, divided into 40 sequential compartments. The compartments are connected by circular openings forming a single straight tubular path, through which a beam of light passes in the same direction as the wind. Flies are introduced at the downwind end and are attracted by the light to fly upwind. They cannot walk upwind because the downwind compartment walls are coated with Fluon, and tests with dead flies in the airstream show that flies cannot be drawn upwind passively by local turbulence. At the top of every compartment a small screened hole permits a fraction of the air to escape through an adjustable light-proof valve. Thus headwind can be locally tuned so that it declines along a desired gradient from a maximum in compartment 40 to zero at the point where flies are introduced.

Before introduction flies are preloaded into a cylindrical cartridge which then locks into a lightproof airtight port aimed at the light. The flies are released from the cartridge by retraction of a plunger. From the upwind end a microscope illuminator focussed at infinity shines through a sealed optically flat glass disk down the axis of the tunnel into the mouth of the introduction cartridge. All surfaces toward the light are flat black. The wind stream enters laterally, near the light, and enters the terminal compartment of the wind tunnel through a fine transparent screen. The initial wind velocity is controlled by a valve and flowmeter with a range of 1 to 9 CFM.

The body of the wind tunnel has the form of a box within a box: the inner box stands on six legs and comprises the introduction port, the illuminator port, and the 40 compartments, all open at the top. The outer box fits down over the inner box to provide a light-proof shroud around the compartments. The top of the shroud is lined inside with foam gasket material, and it is fastened down with a clamp at each of the six legs. The pressure applied by each clamp can be adjusted by a vertical screw inside the leg, so that an

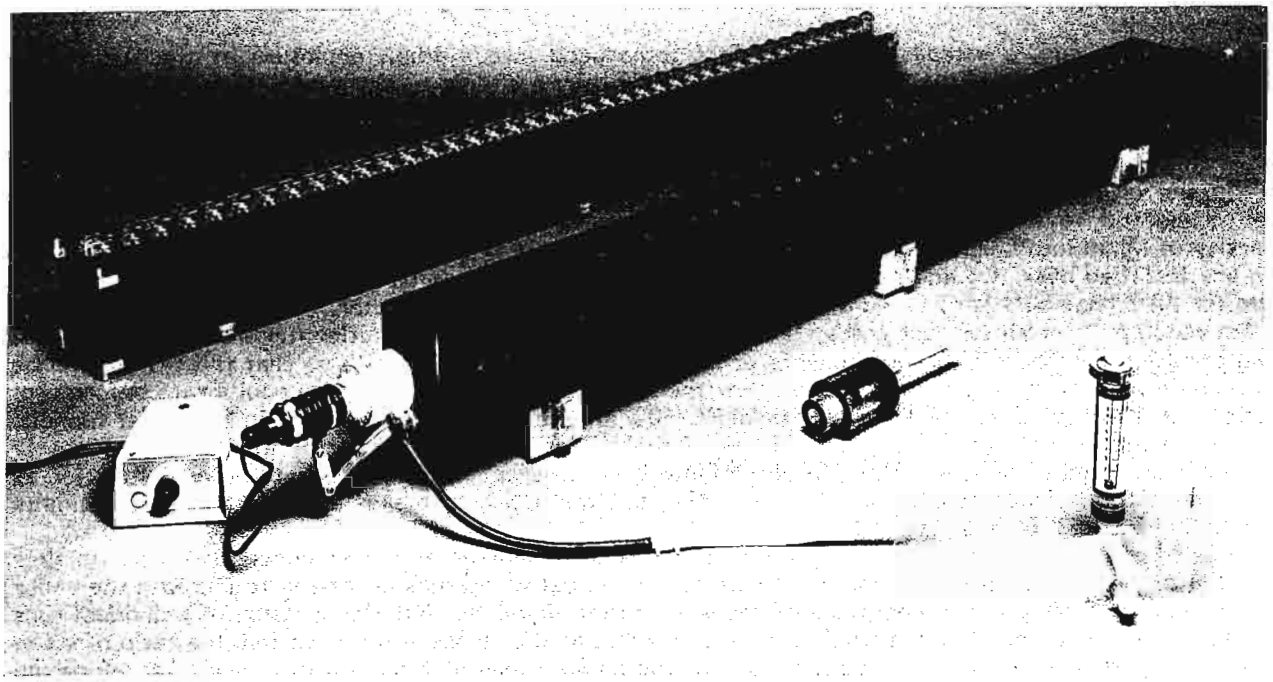


Figure 1. Outer box removed, in rear. Illuminator and one cartridge are installed. Flowmeter and extra cartridge to right.

air-tight seal is obtained around the tops of all compartments. Each air-release valve in the top of the cover is made of a short length of black latex tubing which coils in a complete circle, after emerging between a pair of opposing shut-off screws.

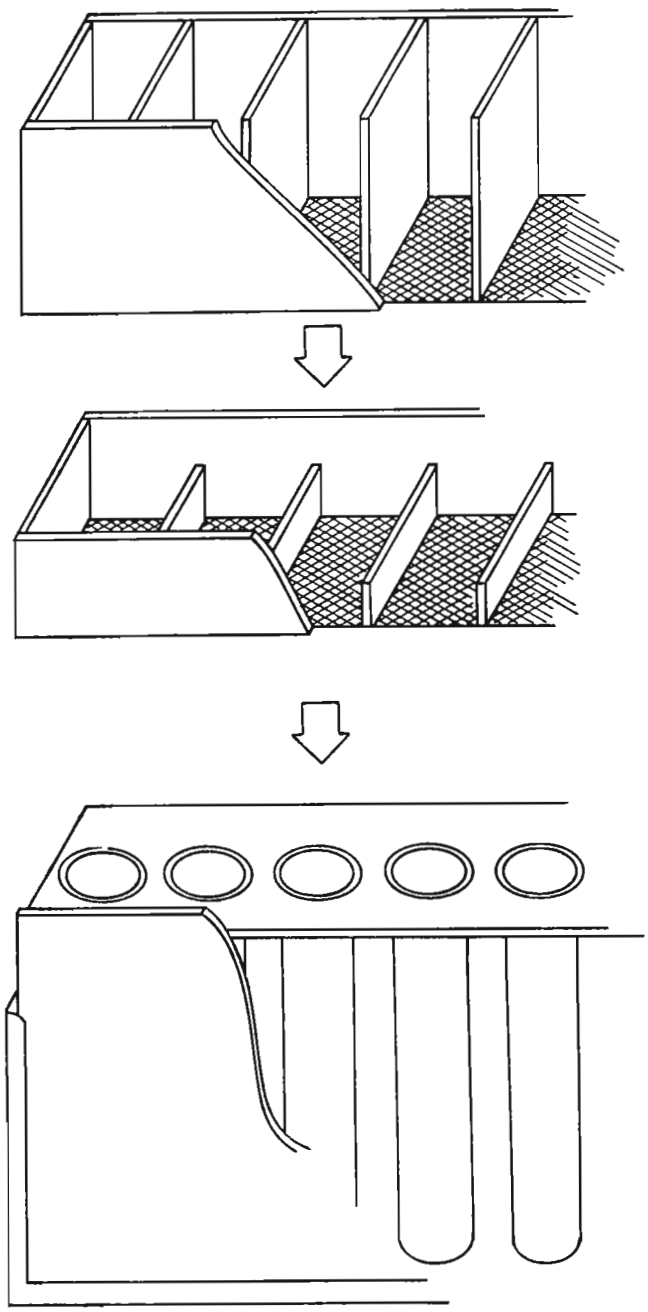
The clear plexiglass of which both top and bottom parts are built is painted black everywhere, except for clear strips left along both sides to form windows. These windows are normally curtained by tight black neoprene rubber strips, held in place by their own tension between clips at either end of the tunnel. To view the flies in the tunnel unobtrusively, the laboratory is darkened and a curtain is detached.

A run is terminated by switching the input from air to CO_2, so that the flies are rendered unconscious and fall down into the compartments. Then the whole cover is unclamped, lifted off and set aside, revealing the distribution of flies. The bottoms of the compartments are painted white on the inside to facilitate counting. The flies remain anaesthetized in the compartments even with the cover removed, since the incoming CO_2 is heavier than air and flows in as fast as it diffuses.

Samples which are used to obtain full distribution measurements are limited to about 1000 flies for convenience. For selection runs, however, much larger samples are used, and only the selected individuals at the top of the distribution are actually counted. The total number of flies in these large selection runs is estimated volumetrically before the run, using a graduated plastic vial with a scale derived from actual counts of up to 15,000 flies. A maximum of 15,000 flies can be loaded into a cartridge at one time, so runs larger than this have not been attempted. At present, selection is being carried out on populations of 60,000 to 80,000 flies, of which the top 2000 are selected, averaging around 3% selection. With typical runs of 15 min, a generation of this size can be selected and founded in 6 hr. Response has been rapid. After 25 generations of selection, the mean scores attained in the apparatus are around 20 times the mean of unselected controls. It is anticipated that the earliest major components of this response would be positive phototaxis and anemotaxis, followed by distinguishable changes in physiology and form to bring about an actual increase in maximum flight speed.

Weber, K.E. Univ. of Minnesota, Minneapolis, Minnesota USA. Creation of an odor gradient to measure olfactory preference.

In the "olfactofractometer", flies are fractionated according to their relative attractions to different concentrations of two competing odorants. The scale of preference corresponds to a row of 20 test tubes, 3 cm wide and 25 cm deep, at 4.5 cm intervals. Typically the ten on the right contain one liquid odorant, and the ten on the left another. Above the row of tubes is a compartmented runway made of plexiglass, its screened bottom resting directly on the mouths of the tubes (Figure 1). Several hundred flies are introduced at the midpoint of the runway, and the nesting lid of the runway is hoisted 0.5 cm, allowing the flies to roam freely from compartment to compartment, without being able to escape from the runway. The lid of the runway is also screened, so that the odorants can diffuse up through the runway, and the lid also has walls to retard local air movement across or along the row. The whole apparatus is enclosed inside a wooden case with a gasketed lid to exclude all random light while olfactory preferences are being measured. A small bulb in the bottom of the case, behind a white plastic screen, illuminates the interior indirectly and symmetrically.



The closer the surface of any volatile liquid is to the mouth of its container, the more rapid is its instantaneous rate of evaporation. Therefore the concentration of an odorant will be stronger in the air over the mouth of a fuller container, if there is protection from air currents. Nothing else is needed to establish a linear gradient of odor. This principle is utilized here by increasing the depths of the odorants in the tubes progressively toward both ends of the row. With pure chemical odorants, the levels at the ends of the gradient can be raised until the concentrations are actually repellent. The flies in the runway can move through the alternative gradients freely while the runway lid is up. At the end of a run, the wooden case is opened and the runway lid is depressed, trapping the flies in their last locations. The runway is transferred to a CO_2 diffuser where the flies can be anaesthetized, scored, and selected.

This is a probabilistic (i.e., sloppy) measurement. If the flies are observed in the runway, they seem to be in constant motion between compartments, changing apparent phenotype. A retest of flies on one pair of odorants (ethanol & ethyl acetate) showed exactly zero repeatability of phenotype. Yet by about the tenth generation of divergent selection for preference with the same two odorants, the behavior of the two lines could be distinguished in the apparatus. Selection was continued for 60 generations, and there was continued divergence. Selection was also carried out for divergent preferences between ethanol and acetic acid, with similar results.

Figure 1. Schematic drawing of olfactofractometer. The lid fits down into the runway, which rest directly on the row of tubes. Not shown are: the props on the lid which hold it up when the runway is open; the introduction port at the runway midpoint; and the lightproof case around the apparatus.

Weber, K.E. Univ. of Minnesota, Minneapolis, Minnesota USA. A system for measurement and selection of pupation height.

It is readily observable that at the end of third-instar development, the normal larval attractions to food and moisture change, and the larvae seek a place of low humidity to pupate, if necessary far from the medium. In typical culture containers this leads to a degree of zonation in pupation level on the vertical surfaces. This behavior can be converted into a conveniently scorable trait.

A "pupaltimeter" (named by Dick Frankham) is a 1" (2.54 cm) ID transparent plexiglass tube, 36" (91.4 cm) long, encircled by scribed calibration lines at 1" (2.54 cm) intervals. About 200 larvae, ready to pupate, are deposited at the tube's midpoint with a long-handled spoon. One end (the bottom) is sealed with a serum cap and injected with 0.2 ml of water. The top is then covered with a cloth cap and a rubber O-ring, and the tube is leaned against a wall at 30 degrees from vertical, in the dark. The water at the bottom of the tube slowly evaporates out the top, creating a humidity gradient. The slanted tube allows falling larvae to stick again. The system approximates a very deep culture vial. By the next day the number of pupae in each interval can be scored through the clear sides of the tube. To select, pupae can be retrieved undamaged with a wet piece of sponge attached to a rod.

To obtain synchronized larvae, parents are placed on fresh high yeast medium each day for two or three days. Whatever the age of the flies, the second and third days usually yield the most eggs. The formula is 1 gm killed yeast: 1 gm Carolina Biological Supply Instant Drosophila Medium: 6 ml cold water. This is mixed rapidly and pours as a liquid; it hardens in about a minute. Very dense cultures do best on this medium, but it is also possible for the density to be too high for the available medium, which will decrease the synchrony. When many mature larvae crawl up the sides the containers are moved to 16-18°C for another day. This seems to retard pupation more than development, and the walls become blanketed with larvae which will pupate at room temperature. Larvae are extracted from the medium by rinsing first with tap water, then floating on 15% sucrose solution.

Larvae can be measured volumetrically. An open plastic tube is pushed into a mesh-bottomed cup, and larvae in sucrose are funneled through the tube, so that the tube fills with packed larvae. When larvae reach the right level, the cup is lowered and the larvae are flushed out into it. The tube should have a slit or pinholes on one side to help liquid pass when the bottom of the tube is occluded by larvae. Further refinements include a large notch at the 200-larva level for correction of overfilling, and a rotating, partially cut away outer cuff which allows the notch and the pinholes to be exposed or covered as needed. For a tube, the trimmed barrel of a 1 ml tuberculin syringe works well.

Measured larvae must be thoroughly rinsed of sucrose at a tap; at the same time they can be concentrated into a ball at the center of the mesh-bottomed cup. The cup is then placed on a hand-towel briefly to drain. The mesh between the larvae and the towel should only permit the transfer of excess water; it should not allow the towel to dry out the larvae. Thick nonwettable plastic mesh is the best material. All operations with the larvae will succeed best when cool water is used, which leaves larvae stiff, contracted, and immobile for a short time.

In an hour 30 tubes can be set up; the 6000 pupae can be scored and selected in about three hours. The tubes can be washed in a few minutes with a plunger made with a tight-fitting rubber cork. Some factors measurably affecting pupaltimeter score are ambient humidity, starting position in the tube, exact age of larvae, the sample size, and the wetness of larvae, but the amount of time floating in sucrose solution has no effect. In pupaltimeters, pupation height can be selected in both directions until the up and down line distributions overlap only by a few stragglers in the tails.

Weber, K.E. Univ. of Minnesota, Minneapolis, Minnesota USA. A system for measurement and selection of time until mating.

The "copulometer" (named by Eric Post) is a system for measuring the times to mating of 200 pairs of flies at once, with optional prevention of sperm transfer, so that virginity of females can be preserved. Time until mating can be measured, remeasured, and selected on, in either sex, without committing any fly to procreation with the partner with which it is tested.

Males and females are retained in individual neighboring cells which are simultaneously united in pairs when the run begins. This array of cells is created from plastic "egg-crate" lattice commonly used in suspended ceiling systems below lighting, which is available from most building supply outlets. The cells in this material are about 1.5 cm on a side, so that 200 of them take up an area of 15 x 30 cm, allowing easy observation of all cells simultaneously. The array is built of three identical 10 x 20 cell pieces of this material which stack upon each other, on top of a white plastic backing of the same size. First one piece

of the "egg-crate" is placed on the backing, and a cold-anaesthetized female is placed in each cell. Then the cells are covered with a sheet of tough transparent plastic such as Mylar, with a wide margin protruding on one side. Then a second piece of "egg-crate" is placed over the first, the cells are filled with individual cold-anaesthetized males, and these cells are covered with a sheet of Saran Wrap. Then the third piece of "egg-crate" is placed on the stack and the whole stack is fastened together tightly with clips on the sides. Soon the flies recover and begin to walk around in their cells.

After the flies have all recovered from the cold anaesthesia, the Mylar sheet is gripped by the protruding margin and pulled slowly out of the stack, so that female and male cells are united in pairs. This begins the measurement of time to mating for all 200 pairs.

The most convenient way to record the times of mating is with a microcomputer. The array of cells can be fastened in an upright position beside the computer and each onset of mating can be recorded by depressing a key. The computer clock is used to record the elapsed time from the beginning of the run to each event, and the same program provides statistics at the end.

As soon as each pair of flies begins to mate, both flies are vacuumed out of their cell. This is done with an aspirator powered by a small vacuum pump. The intake nozzle of the aspirator is long and straight and tipped with a piece of aluminum tubing, which is cut obliquely to give a sharp point. This can be pierced through the Saran Wrap directly into any cell to suck out the occupants. If it is desired to prevent sperm transfer, a flask filled with ice water is used as the body of the aspirator. Then the mating flies will be sucked in through the nozzle and thrown down onto the surface of the ice water. The flies are anaesthetized instantly by the ice water and can float on its surface for many hours without any apparent ill effects. Most of the mating pairs soon become disconnected. Later they can be poured out and caught in a sieve. As soon as they reach room temperature they recover at once.

By this method the fastest or slowest flies to begin mating in any population can be selected, by changing ice-water flasks when the desired percentile or selection cutoff point has been reached. The same sample can be tested against one strain and mated with another. In tests this method was 100% effective in preventing sperm transfer, with 100% survival of flies.

Weber, K.E. Univ. of Minnesota, Minneapolis, Minnesota USA. A system for rapid morphometry of whole, live flies.

With the "planomorphometer" system the image of a live fly can be projected onto a digitizer pad. All visible morphology is reduced to a plane and measured in two dimensions. Detailed external morphology can be measured on all parts which transmit light well (Figure 1). Parts which are less translucent can be measured only along their outlines. Among the appendages, resolution of surface detail is clear for wings and legs, and lower on mouthparts, while antennae and halteres can be measured precisely only in profile. Although some light is transmitted in areas of the head, neck, thorax, and abdomen, only their profiles are distinct.

The system (Figures 2 & 3) includes the following elements: a holding device which secures an anaesthetized fly in the desired viewing orientation with the aid of adjustable currents of air; a set of hand- and knee-operated valves which control the air currents used in picking up, positioning, and ejecting the fly; a microprojector which is modified to receive the fly holder; two mirrors to transfer the projected image; and a digitizer pad linked to a computer to record and process the measurements.

The key technical development is the hand-held vacuum-operated holding device. There are two kinds of holding device, with different applications. The main or full-body holding device (Figure 4a) allows the entire fly to be viewed in profile. The mouth of this holder (Fig. 4b), which receives the body of the fly, consists of two transparent sheets of 0.1 mm Mylar or acetate, sandwiched within a window in the tips of two plates. The plates are made of 1/16" (1.6 mm) tool steel stock, milled to half of that thickness in the area around the window, and are clamped between two plexiglas blocks which form the body of the holder. In the center of the sandwich, the transparent sheets are held apart, with a gap of 0.6 mm, by compressed gasket strips on either side of the window. The edges of the transparent sheets are cut to an empirically developed contour, which has been preserved by filing a steel template. This contour has two 60° notches, one about 0.3 mm deep for the thorax, and another about 0.2 mm deep for the head. The notches stabilize the position of the fly. The mouth is bridged across one end by three short pieces of hair-thin nickel-chromium wire, held in tiny notches with small droplets of superglue. A fourth wire may be used to bridge the other side. The job of cutting, emplacing, and glueing a new pair of windows is accomplished using a small vise under a dissecting microscope, and with appropriate tools takes about one hour.

Flies are loaded and positioned in the holder as follows. First, the flies are anaesthetized briefly on a circular CO_2 diffuser, which rotates freely so that the flies can be brought into convenient

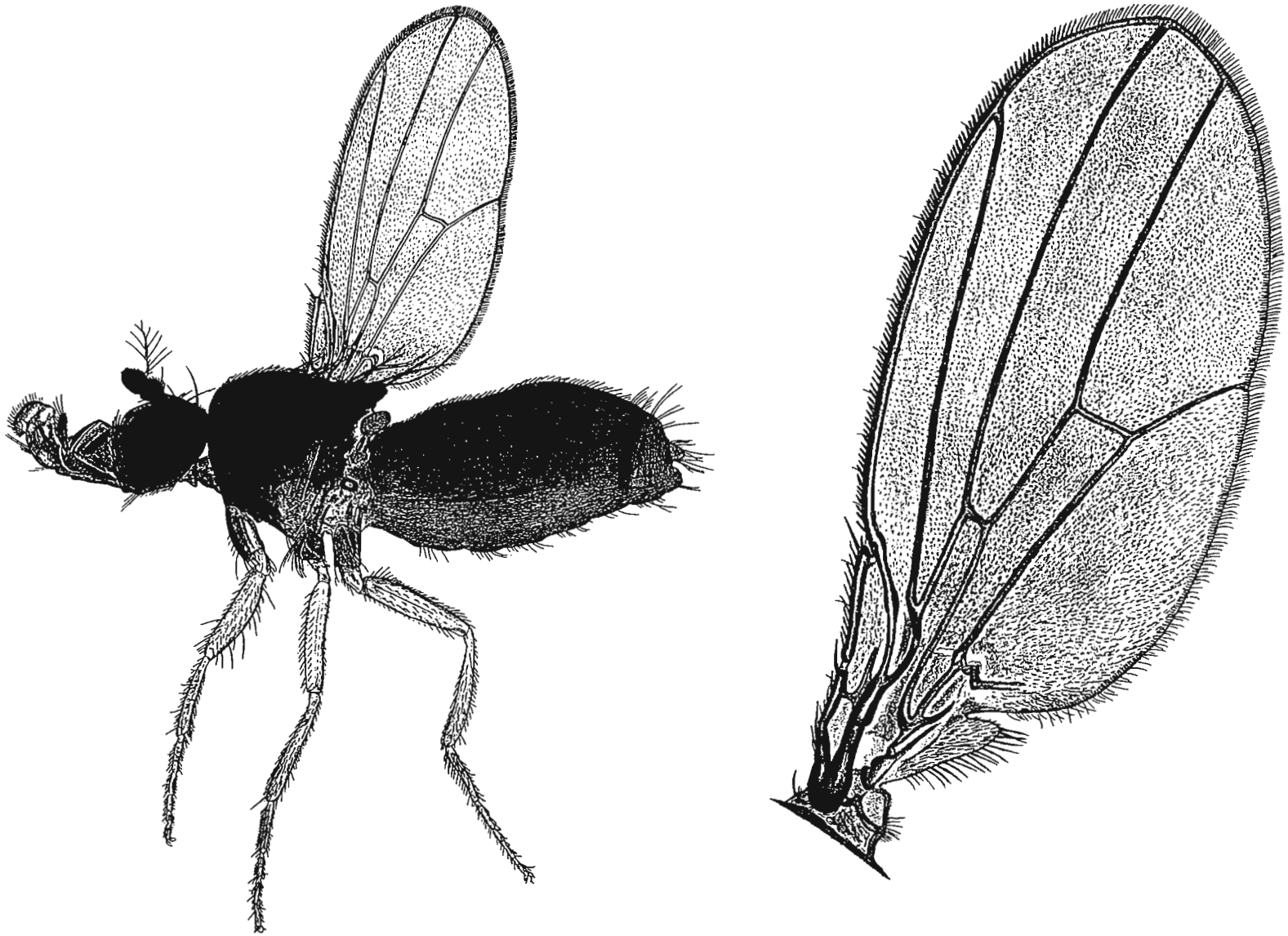


Figure 1. Drawings of whole-body and full-wing projected images as they appear on the digitizing pad. Visibility of surface detail depends on local opacity. Arrangement of body parts is determined by air currents and contours of holding devices.

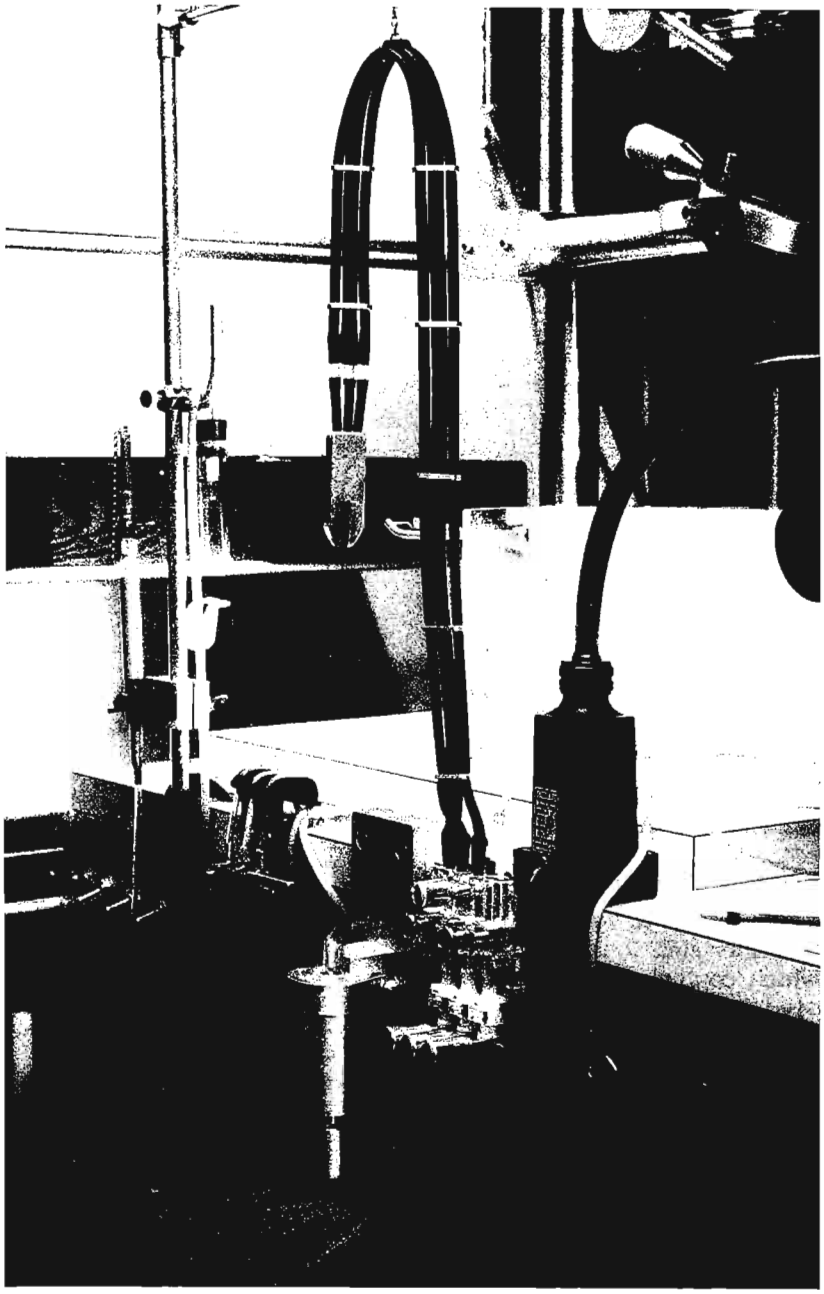


Figure 2. Three latex tubes (black) transmit vacuum and pressure from a set of adjustment valves out to the holding device, which hangs from a spring-supported swinging arm. A fourth latex tube on right (grey in photo) transmits vacuum from the valve set to a capillary tube. The large pressure on-off valve is operated with the knee.

orientations. By briefly interrupting and restoring CO_2 flow, while lightly agitating the flies on the diffuser, the wings of the majority soon are made to snap up into a V position faciltating loading. A fly is then picked up by the tip of its abdomen using the fire-polished end of a capillary tube attached to a latex vacuum line. The holding device is held in the operator's left hand, and the fly is transferred with the right hand from the capillary tube tip to its proper position in the holding device.

Since air is being drawn into the window mouth, a fly can be drawn in by both wings so that the wings are dorsally erect and parallel between the transparent sheets. The thorax and the abdomen are cradled by the contoured edges of the window. The three bridging wires prevent the soft abdomen from being sucked between the plastic sheets, while the head and thorax are too wide and rigid to enter. The head is pulled upward into a notch, so that the mouthparts are pulled out anteriorly and pushed upwards by the passing air stream. The crown of the head appears inside the edge of the plastic, and the antennae are pulled out in parallel. The fly is retained very securely in this position, so that the capillary tube tip, by which it was manipulated, detaches easily from its abdomen.

At this point, all parts of the fly are in their proper positions except the legs, which are held in random orientations, usually crossed and partially retracted. The legs can now be blown straight out from the fly into fully extended positions by means of jets which emerge from the body of the holder above and below the window. These jets are focussed inward so that they merge on the fly's thorax and legs. As long as the maximum suction and pressure levels are adjusted to the proper balance, the body of the fly continues to rest securely in the mouth of the holder and is partially shielded from the reverse air stream by the mouth itself. Even a strong jet of air on the legs will not dislodge the body of the fly unless the suction is released. While the legs are held in extended positions by the reverse jets, a pair of retaining jaws is brought together by the thumb and forefinger of the right hand, to close over the legs (Fig. 4c). Since the reverse jets are activated with the left knee, the right hand is free to deal with the jaws. These jaws contain another pair of transparent sheets in steel windows. When the jaws come together they retain the legs in this window, in the extended position. The upper and lower jaws are

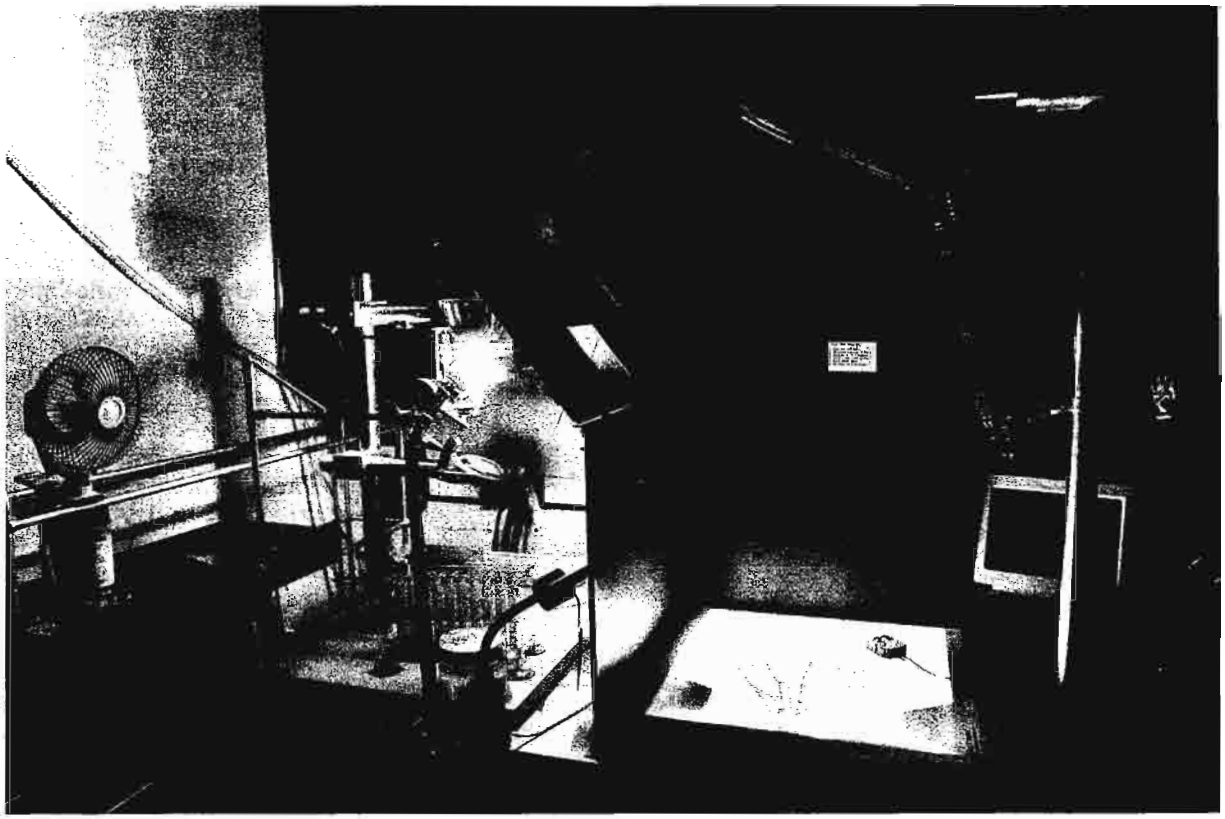


Figure 3. Full system in operation.

fastened to each other by latex bands on either side so that they snap bistably into either the open or the closed position. Each jaw has screws which control the point where it stops on the inward swing. This allows the jaws to be individually adjusted to work together so as to hold the legs in the central plane, without crushing them. After the legs are captured in the viewing window, the reverse jets can be eliminated by dropping the left knee.

There is also a second, much simpler holding device which can be used if it is only desired to view the wings. This device (Fig. 4d) has a one-piece aluminum body in which two spring steel plates are compressed together with set screws. A window containing transparent plastic sheets is located in the tip of the plates. The plastic edges are cut straight, and the sheets are held apart by pieces of brass shim foil, with a gap of 0.05 to 0.1 mm. The sides of the steel plates are sealed with silicone rubber sealant. There are no leg-retaining jaws on this device, but it has air jets for ejecting flies from the window. With this device, flies can be picked up directly from the CO_2 diffuser by one wing. The wing is drawn immediately up into the window by suction. For viewing only wings, this holding device is faster and better, particularly since it makes the entire wing visible right up to the junction of the wing base with the thorax. In the whole body holder, the base of the wing is obscured by the upper thorax, but in the wing holder the fly rolls sideways as it goes in. In this "alarometer" mode (named by Anna Haynes) it is also possible to view the full length of the balancer (haltere) on one side, since it extends from the thorax at about the same level as the wing. Also with this holder it is a simple matter to turn the fly perpendicularly to the plane of the window, using the capillary tube again, so that both wings can be captured in the window and viewed at the same time side by side without any superposition. For these last two applications (halteres, both wings), the gap between the transparent sheets must be slightly widened. Flies are currently being measured in all orientations.

The second element of the system is the air supply. Vacuum and pressure are supplied by a Gast diaphragm-type vacuum/pressure air pump (1.2 CFM free air capacity, 60 psi pressure, 24" Hg vacuum). Both input and output of the pump are completely integrated into the system. The vacuum line goes first into a 20-liter tank. This tank provides a cushion so that the beating of the pump diaphragm is not transmitted into the suction lines; this gives a completely smooth flow of air so that appendages do not

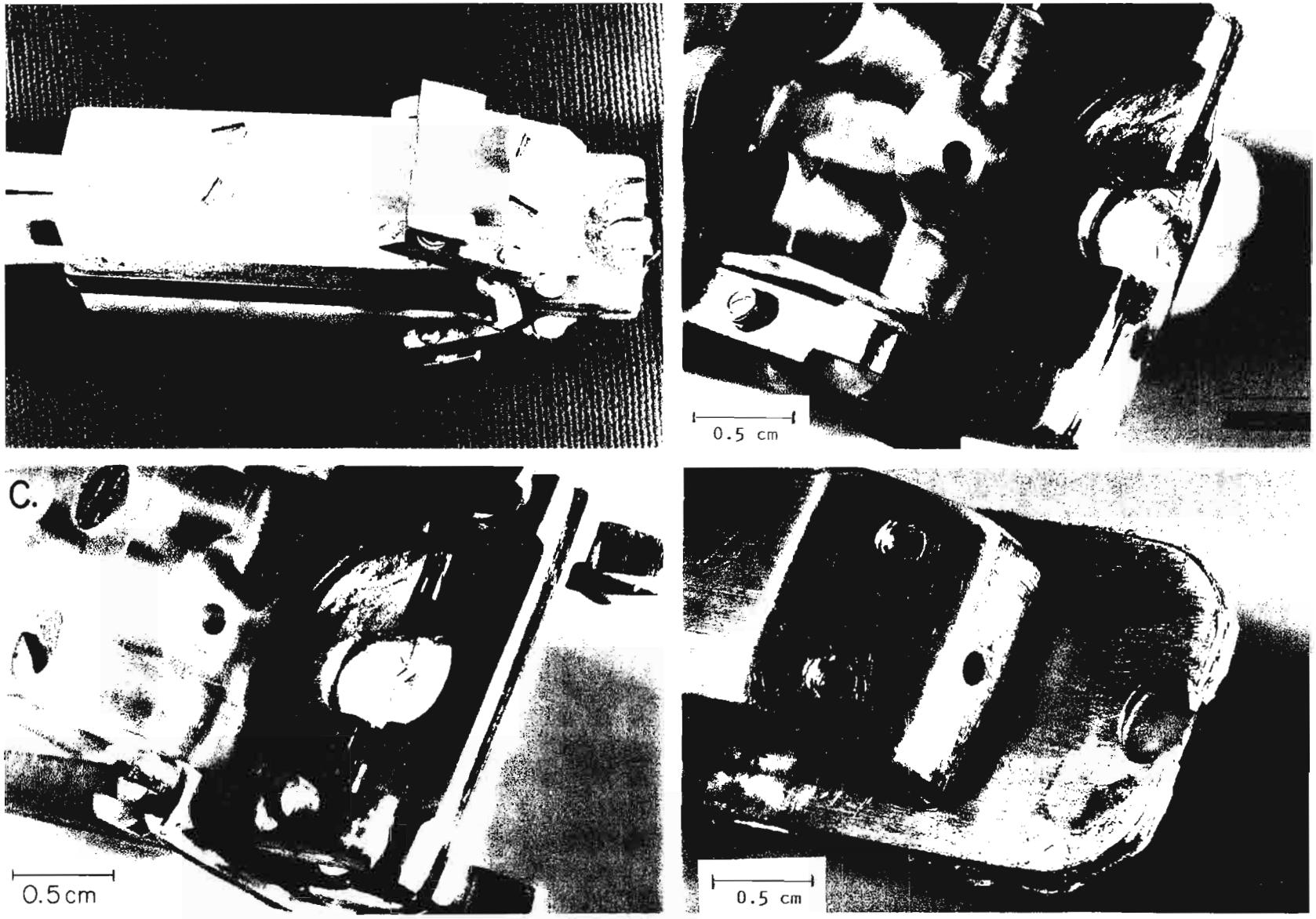


Figure 4. **A:** Whole-body holding device, with jaws open. **B:** Enlarged view looking into mouth of window, jaws open. **C:** Same view with jaws closed. **D:** Wing holding device.

flutter. From the tank the suction is divided into two lines, one for the capillary tube manipulator and one for the holding device. Each of these two lines has its own screw-in bleeder valve, to set absolute and relative base vacuum level, and its own lever-operated bleeder valve, which allows sensitive adjustments during operation, ranging between the base level and complete disconnection. The pressure output of the pump goes directly from the pump to the knee-valve. The knee-valve is normally held down in the open position by a strong spring, so that the pressure escapes. When the valve plunger is pushed up with the knee (or by hand) the pressure is shunted into the line to the holding device. On its way it first goes through screw-in and lever-operated bleeder valves like those for the vacuum, then it splits into two lines, one for each of the side jets. Then there is one more lever which can be moved from side to side to shunt all the pressure through either one jet or the other. The knee-operated valve provides the sudden full pressure needed to blow the legs into the extended position, or, if the suction to the holder is disconnected, to shoot the fly out of the holder into a numbered Fluonized test tube corresponding to its phenotype. The side-to-side valve is sometimes useful in disentangling legs during extension. A full discussion of the available maneuvers would be lengthy.

The projection system is based on a remodeled microprojector (Ken-A-Vision, Model X1000-1). The transformer was removed from the base and placed in a separate box so that the projector itself, with stage and lenses, could be mounted on a new, more stable stand. The reflector was removed and replaced by a 10 cm circular front-surface mirror attached to the same stand, and provided with a large knurled handle for convenience since the angle is frequently adjusted during measurements. Also for operator convenience the small focus knob was replaced with a large wheel that fills the hand. Two swing-out infra-red-absorbing glass plates were interposed between the lamp and the fly. The microprojector stage was altered so that it can be lowered until the objective barrel protrudes up through it. Finally, a teflon disk was attached to the stage; the disk has a hole in the center for the objective and a slot which receives the holding device containing the fly. The position of the fly over the lens can be adjusted by moving the teflon disk or by sliding the holder along in the slot. The sides of the teflon slot can be adjusted with screws to obtain the precise pressure on the holder that allows it to move easily but not slip. The operator slides the holder into the slot until the image is centered on the digitizer pad, then moves the left hand to the focus knob, while the holder is retained in the slot. The right hand is used to operate the valves if required, and to digitize the points.

The image of the fly is reflected from the circular mirror up to a second mirror, then down to the digitizer pad. The second mirror is a 34 x 34 cm square front-surface mirror, enclosed in a cabinet with a dust-proof door which opens downward while the system is in use. The mirror-cabinet is mounted solidly on a 1.7 m stainless steel post welded to a heavy steel base, and is universally adjustable. The image beam passes from the first mirror to the second through an aperture in a black light-shield between the microprojector and the pad. Black overlapping curtains can also be drawn to form a completely light-proof booth around the system and the operator. The digitizer pad is a Jandel Scientific with a 20" x 20" active surface, attached to a microcomputer system. All operations of measurement and selection are handled by original software, program MORPHIT. During selection, the program estimates the percentile score of each fly's phenotype from the mean and standard deviation of the previous generation. Each measured fly is ejected into one of twenty numbered, Fluonized test tubes. Flies estimated to be in the top 57% are distributed over the top 19 tubes, with each tube assigned a class width of 3 percentiles. This method usually allows good resolution of the 20% cutoff point at the end of a typical sample of 50 to 100 flies.

Weber, K.E. Univ. of Minnesota, Minneapolis, Minnesota USA. Systems for measurement and selection of wing-tip height.

The "dorsoventrometer" (Figure 1) consists of a glass platform, above which is suspended a parallel adhesive surface which can be lowered and raised by a hand crank. Flies homozygous for the gene **raised** (**rsd**), which causes elevated wings, are placed on the glass platform. The apparatus is operated in a 6°C cold room, where flies are too cold to move. The glass platform is prewarmed slightly from below by four 25-watt light bulbs on rheostat control, to obtain an empirically-determined local temperature at which the flies on the platform will be able to stand but not to walk. When the adhesive surface is lowered to some preset height above the platform, flies above that height become stuck by their wing tips, and are lifted from the platform when the adhesive surface is raised again. The adhesive plate locks automatically in the elevated position, and the attached flies are collected from it by means of an aspirator connected to a suction pump.

The adhesive surface is a sheet of coated Mylar, stretched tightly across two polished parallel straightedges at the sides of a stout wooden frame. The ends of the Mylar sheet go around behind the frame and are attached to steel bars that can be drawn together by turning a turnbuckle. This develops

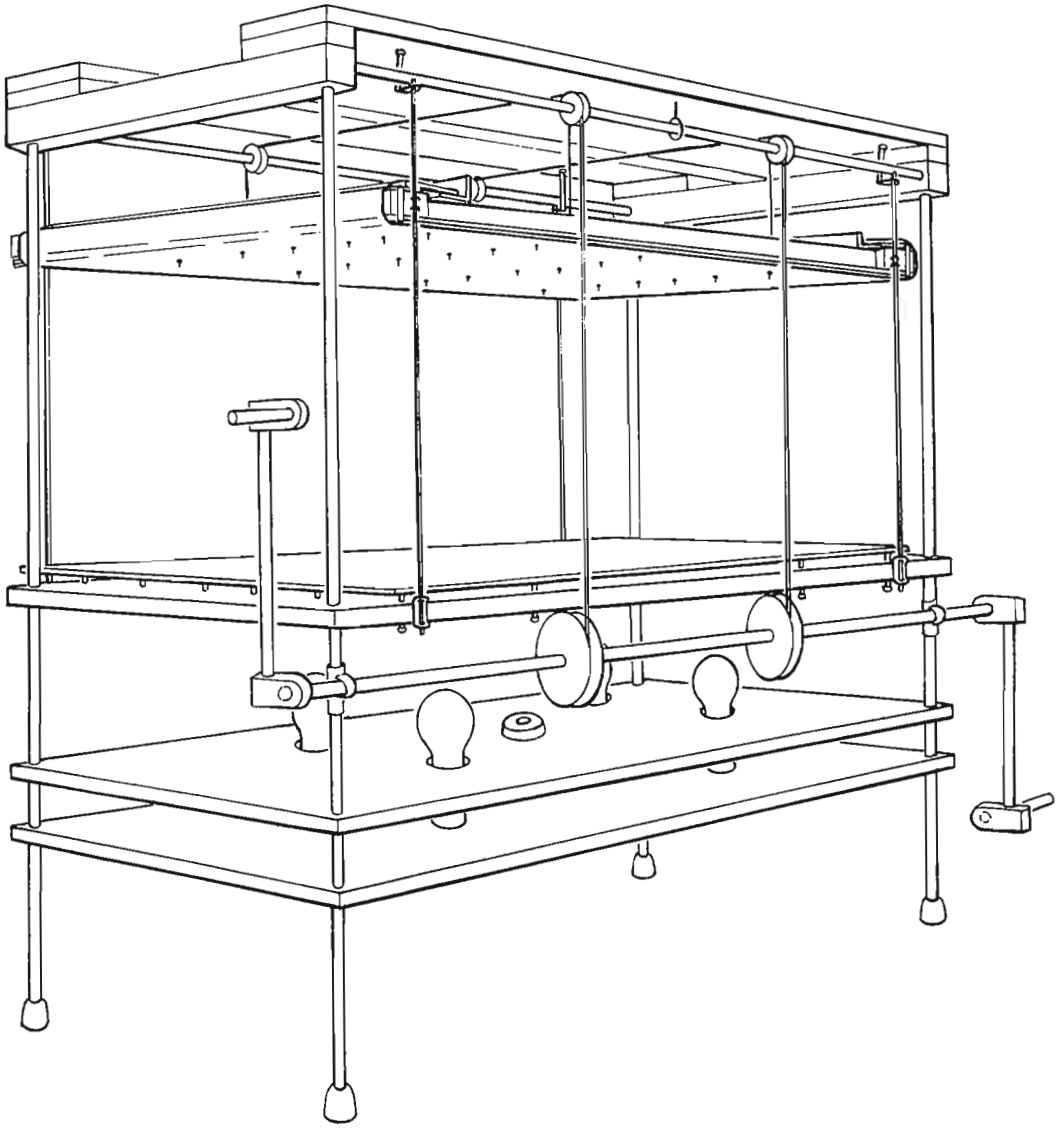


Figure 1. Dorsoventrometer. The suspended frame with adhesive underside can be lowered to the glass platform, guided by rods at the corners.

high tension in the Mylar sheet, which pulls tight like a drumhead across the straightedges, and stands about 3 mm from the frame. Mylar does not elongate appreciably under tension, so it can be pulled in one direction without being thrown into ripples. The sheet is coated with a thin, even film of Tangle-Trap (Tanglefoot Co., Grand Rapids, MI), which is a sticky, salve-like substance used in insect control. It can be spread smoothly with a straightedge and settles into a glossy, uniform surface. It remains quite tacky when it is cold.

The glass platform, on which the flies stand, is a square pane of 3.2 mm glass about 60 cm on each side. It is mounted above an open wood and metal framework with 18 leveling screws on which the glass rests. At the sides of the frame are plastic clips which retain the edges of the glass plate. The underside of the glass is painted white.

Height intervals above the platform are measured in mils (0.001 inch or 0.025 mm). The Mylar sheet sags about 16 mils due to gravity, so the exact interval between the adhesive and the glass is controlled by 30 small aluminum spacers. These are shaped like thumbtacks, but are smaller, and stick to the adhesive surface with points downward. They are not detached by gravity when the adhesive surface is raised, but they can be plucked off and replaced with tweezers, in order to change the size of the gap. When the adhesive plate is down all the way, the ends of the straightedges rest on four feeler gauges which are held in clips at the corners of the platform (not shown). The feeler gauges are set to match the height of the aluminum spacers. By these means the height of the gap can be set over an area of 3600 cm^2, with a total range in value cf 3 mils.

There are 8 sets of 30 aluminum spacers -- a set for each multiple of 5 mils from 105 to 140 mils. To select the tallest 20% of flies, a height interval is chosen at which nearly 20% will stick in 30 sec. After collecting and counting these, more flies can be picked up by lowering the adhesive again, until a total of 20% is obtained. There is always some minimum time required for a fly to stick, which is a function of its height.

The distribution of the heights of flies is not measured during the process of selection, but is measured by a different device with a separate sample of flies. This device is an 18 x 20 cm plate of glass which stands upon four short legs at its corners. The legs are blocks of aluminum milled to exact heights. On one side of the plate the legs are 160 mils high, and on the other side they are 70 mils high, so that the plate forms an inclined plane which spans the range of observed fly heights. The underside of this plate is coated with a film of adhesive which is exactly 2 mils thick, being applied with a straightedge resting on strips of shim stock. The plate is moved slowly across a sample of flies which are standing on the glass platform, with the high side leading. Each fly sticks to the adhesive at its own exact height and is dragged along. The plate is then inverted and placed over a ruled paper, from which the heights of flies can be read off to a resolution of 0.5 mil, corresponding to 1 mm on the paper. About 100 flies can be picked up on one plate before the plate becomes too crowded. Twenty-four of these plates were constructed.

The purpose in building this system was to select on very large populations for a morphological trait. Up to 1500 flies can be placed on the plate at a time without crowding. The size of the sample is estimated by its weight, by reference to a smaller counted sample that is weighed from each line each generation to get the mean weight. Since the flies are flightless, they can be poured about in open Fluon-coated cups, and conveniently weighed on a Mettler balance. About 5000 flies can be selected on in one hour. Females are taller than males, making up about 70% of selected flies; they have all already mated at random before selection, so selection is almost entirely on females. After 55 generations of selection, the highest response was around 20 mils of added wing-tip height. Further details of construction and operation can be found in Weber (1986).

Reference: Weber, K.E. 1986, Thesis, Harvard University.

Wolfner, M.F.[1,2] and D.J. Kemp.[1,3] 1-Stanford Univ. Sch. of Medicine, California USA; 2-Cornell Univ., Ithaca, NY USA; 3-Royal Melbourne Hosp., Victoria, Australia. A method for mass-isolating ecdysterone-inducible tissues of **D.melanogaster.**

We describe a procedure for mass-isolation of salivary glands and other tissues of **D.melanogaster** larvae and pupae. This procedure, which is a modification of ones developed by Zweidler & Cohen (1971) for **D.melanogaster** and Boyd (1978) for **D.hydei** offers several advantages. First, salivary glands can be isolated fairly rapidly, and side fractions of the procedure are enriched for gut, malpighian tubules, fat body or imaginal discs, which can be further purified manually. Second, salivary glands purified by this procedure are able to respond to ecdysterone in a normal way following isolation. This is desirable because it allows the simultaneous induction of hundreds of salivary glands in vitro, so that all are at the same stage of response to the hormone at the same time.

Animals for tissue isolation are raised in mass culture as described in Elgin & Miller (1978). Under these conditions, at approximately 130±8 hr after egglaying, third instar larvae have climbed up the sides of their cages, and about 10-20% have pupariated. 30-100 g of animals are scraped off the sides of the cages, suspended in 300-500 ml of aerated 25°C 30% sucrose and floated free of food. Larvae are further purified by placing the animals in a 14.5 cm diameter watchglass, and collecting the larvae from the sides as they crawl away from pupae and dead larvae, which remain in the center of the dish. Salivary glands purified as described below from these animals are predominantly in puff stage (PS) 1 as defined by the criteria of Ashburner (1972a), with fewer than 10% in PS 3-11. For purification of salivary glands from prepupae and pupae, animals are harvested at 143±12 hr after egglaying, when 50-80% of the animals have pupariated. Animals are floated away from their food in aerated 30% sucrose in a separatory funnel. Aerated 25°C water is slowly added and stirred until a density is reached (approx. 15% sucrose) at which white and tanning prepupae, larvae, and pupae form three layers; pupae float on the surface as they contain a gas bubble (Mitchell 1964). Prepupae are collected onto Nitex 3-400-37 mesh and hand-picked free of contaminating larvae. Such prepupae can be used directly for the isolation of salivary glands at PS 11. For isolation of salivary glands at later puff stages, the prepupae are incubated at 25°C on moistened filter paper prior to isolation of tissues and then collected by virtue of flotation on 10% sucrose. In our hands, the salivary glands reach PS 14 at approximately 4 hr post-isolation and PS 18 at approximately 10 hr post-isolation.

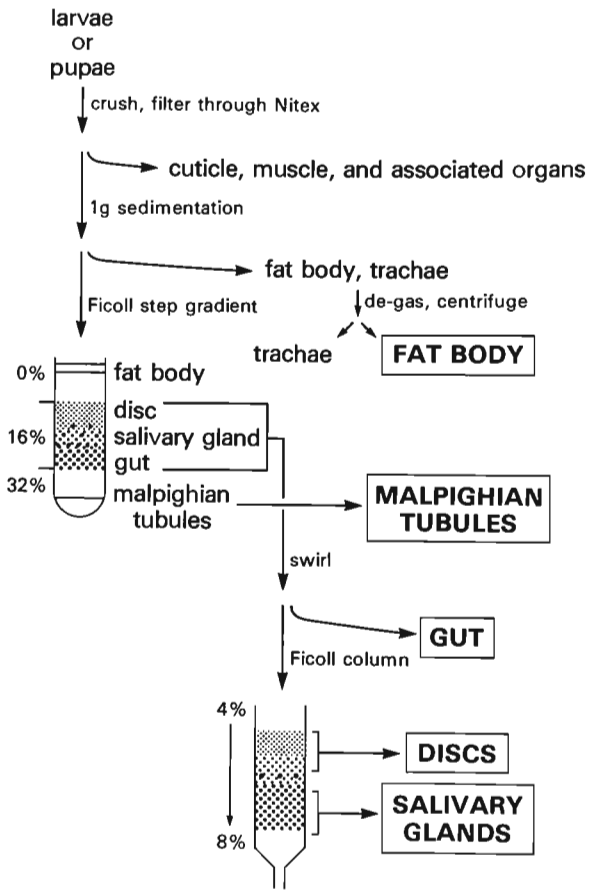


Figure 1. Method for purification of larval or prepupal organs. See text for description.

The steps in tissue isolation are summarized in Figure 1. If no further incubation of the tissues is required following isolation, all steps are carried out at 4°C. If the tissues are to be incubated with ecdysterone following isolation, all purification steps are performed rapidly in aerated media at 25°C to avoid inducing anoxic shock (Ashburner & Berendes 1978). All manipulations are performed using plastic or siliconized glassware. Animals are suspended in Grace's medium modified by the addition of 1 volume of 10% ethanol to 5 volumes of medium (Ashburner 1972b) or in Robb's saline (Robb 1969), and crushed between metal rollers spaced 38 microns apart. The mixture is filtered successively through Nitex 850 and Nitex 600 screens to remove cuticle and large gut fragments and collected by sedimentation at 1 x g for 5 min. If purification of gut is desired as well, filtration through the Nitex 600 screen is omitted. The tissues are suspended in 200 ml of the same medium and collected again in the same manner to remove fine debris. Fat body floats in this procedure (Boavida & Roberts 1975).

The concentrated mixture of organs is then resuspended in 30 ml of 16% w/v Ficoll in Robb's saline. This mixture is layered over a 32% w/v Ficoll shelf (10 ml) in a 60 ml centrifuge tube, overlaid with 5 ml Robb's saline and centrifuged at 3000 rpm for 5 min in a swinging bucket rotor. In this density step, malpighian tubules, muscle and mouthparts sediment to the bottom of the tube and fat body floats to the top, whereas salivary glands, imaginal discs and pieces of gut collect at the 16%/32% Ficoll interface or just above it. Organs at and just above this interface are diluted 20-fold with Robb's saline and collected by sedimentation at 1 x g for 5 min.

Salivary glands are further purified by a modification of the procedure of Boyd (1978). Organs in Robb's saline are slowly swirled by hand in a plastic petri plate, and the gut fragments rise to the top of the solution and are removed with a pasteur pipette. At this point, salivary glands can be picked out by hand, or the partially purified salivary glands, contaminated mostly by imaginal discs and small gut fragments, can be further purified by fractionation by sedimentation for 25 min through a 4-8% Ficoll gradient at 1 x g (Zwiedler & Cohen 1971) in a 2.8 cm diameter, 40 cm long cylindrical plexiglass column. The salivary glands comprise the fastest-sedimenting fraction. This fraction is diluted 10-fold with Robb's saline, collected by sedimentation at 1 x g and hand-picked to purity under a dissection microscope with a siliconized glass pipette. The resulting salivary glands are at least 95% pure, by morphological criteria. Major contaminants are gut and imaginal discs. Most salivary glands thus isolated are split into lobes.

The procedure for isolation of salivary glands from prepupae is identical to that described for larvae, except that the swirling step to remove gut is not necessary as prepupal gut sediments differently from larval gut and is not a major contaminant of the salivary gland fractions.

At least four other tissues can be mass-isolated relatively easily as side fractions of the procedure described above, thus permitting comparisons of gene expression between different tissues from preparations of animals of the same developmental state. Fat body, in pieces, can be isolated by slight modifications of the procedure of Boavida & Roberts (1975). The material which floats upon 1 x g sedimentation is collected and de-gassed to remove air from trachae (which contaminate fat body) and centrifuged for 5 min at 3000 rpm. Fat body (more than 90% pure), contaminated by small pieces of salivary glands surrounded by fat body, forms a band at the top of the tube. If fat body is to be incubated with ecdysterone, the de-gassing should be delayed until after the incubation to prevent heat shock. Greater than 95% pure gut is isolated during the swirling step. The isolated tissue is primarily midgut, but foregut is also observed. The morphological criteria used do not permit a distinction to be made between midgut and hindgut, so it is not known if hindgut co-purifies in this step. The major contaminant of the

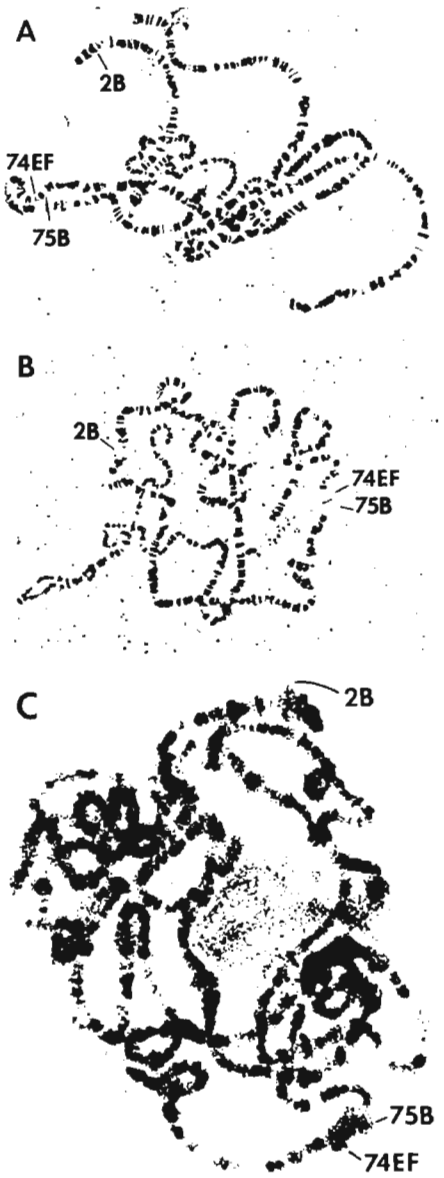


Figure 2. Induction of puffing and transcription at early puff sites during mass-incubation of larval salivary glands in vitro. **A & B:** Salivary glands were mass-isolated from third instar larvae and incubated with ecdysterone as described in the text. Chromosomes were squashed and stained with aceto-orcein as described in Ashburner (1967). **Panel A** shows chromosomes, in PS 1, of salivary glands squashed prior to induction. **Panel B** shows chromosomes of salivary glands which were incubated for 2 hr with ecdysterone in vitro as described in the text. Chromosomes are in PS 3. In addition to induction of the early puffs (three, those at 2B, 74E/F and 75B, are indicated), induction of in vitro incubation puffs (e.g., 50 C/D), and regression of intermolt puffs (e.g., 3C) can also be observed. **Panel C:** Oregon-R larval salivary glands were mass-isolated at room temperature and incubated in ecdysterone plus 7×10^{-5}M cycloheximide in Robb's saline. Nine hours after the addition of cycloheximide, ^{3}H-uridine was added to a final concentration of 25 µCi/ml. After 10 min of incubation at 25°C, the glands were fixed, and cytological preparations were made as in Bonner & Pardue (1977). Slides were dipped into Kodak NTB-2 emulsion, stored, developed and stained as described by these authors. Extensive incorporation can be seen at early puff sites including the three indicated. The amorphous area in the center containing many silver grains is the nucleolus. 16 day exposure. The average width of the chromosomes is 5 µm.

purified gut is salivary gland, but small amounts of malpighian tubules and imaginal discs are occasionally present. 50-70% pure malpighian tubule, in pieces and contaminated primarily by muscle and to a lesser extent by gut and mouthparts is isolated from the bottom of the 16-32% Ficoll gradient. Additional purification can be obtained by swirling the tissue in a petri plate and by hand-picking. The extent of muscle contamination depends upon the degree of crushing of the larvae. Imaginal discs are isolated either from the 4-8% Ficoll column, or following additional swirling of the gut-gland-disc mixture to remove salivary glands from them. They are approximately 70% pure, the major contaminant being salivary gland pieces. These discs may not represent all types of discs, as many remain in the carcass which is discarded.

There is no change in puff stage in the batch of glands between the time that the glands are first placed in chilled Robb's saline and the time that pure glands are obtained. Salivary glands purified at room temperature are still capable of responding to ecdysterone by forming puffs, and by incorporating high amounts of ^{3}H-uridine at ecdysterone-responsive loci (Figure 2). The response to ecdysterone is somewhat slower than that for hand-isolated glands (Ashburner 1972b), but otherwise is indistinguishable from it. In 4×10^{-6} M ecdysterone, isolated salivary glands reach PS 3 in 1-2 hr at room temperature, and PS 7 at approximately 4-5 hr of incubation.

References: Ashburner, M. 1967, Chromosoma 21:398-428; ——— 1972a, in: Results and Problems in Cell Differentiation (W. Beerman, ed.), v4:101-151; ——— 1972b, Chromosoma 38:255-281; ——— & H.D. Berendes 1978, in: The Genetics and Biology of Drosophila (M. Ashburner & T.R.F. Wright, eds.), v2b:315-395; Boavida, M.G. & D.B. Roberts 1975, J. Insect Physiol. 21:1587-1596; Bonner, J.J. & M.L. Pardue 1977, Cell 12:219-225; Boyd, J. 1978, in: The Genetics and Biology of Drosophila (M. Ashburner & T.R.F. Wright, eds.), v2a:127-135; Elgin, S.C.R. & D.W. Miller 1978, in: The Genetics and Biology of Drosophila (M. Ashburner & T.R.F. Wright, eds.), v2a:112-121; Mitchell, H.K. & A. Mitchell 1964, DIS 39:135-137; Robb, J.A. 1969, J. Cell Biol. 41:876-885; Zweidler, A. & L.H. Cohen 1971, J. Cell Biol. 51:240-248.

Ramesh, S.R. and W.-E. Kalisch. Ruhr-Universität Bochum, FR Germany. SDS-PAGE technique for demonstrating sex linked genes.

Classical experiments to demonstrate the inheritance of X-chromosomal genes involve reciprocal crosses to compare heterozygous F1-female and hemizygous F1-male flies phenotypically, the latter ones getting their sex linked genes exclusively from their mothers. Here, we describe a simple technique involving the use of a 13.7% SDS-PAGE (Sodium Dodecyl Sulfate - PolyAcrylamide Gel Electrophoresis) to demonstrate electrophoretically the sex linkage of several genes coding for major glue protein fractions in the larvae of **D.n.nasuta** and **D.n.albomicans.** Interspecific crosses of these two Drosophila species belonging to the **nasuta** subgroup of the **immigrans** group are used for the following reasons: (1) The **nasuta** subgroup members show species-specific glue protein patterns. The major glue protein fractions are very prominent; therefore, one pair of salivary glands (from a single individual) is sufficient for obtaining the electrophoretic pattern (Ramesh & Kalisch 1987). (2) The major glue protein fractions in **D.n.nasuta** and **D.n.albomicans** show different kd-values, as a result of which the patterns of protein fractions can easily be analyzed in the heterozygous F1-females (Ramesh & Kalisch 1988).

We crossed the species **D.nasuta albomicans** (stock number: 15112-1751.0, obtained from the National Drosophila Species Resource Center, Bowling Green, Ohio, USA) and the **D.nasuta nasuta** [stock Mysore I from our lab (Ramesh & Kalisch 1988), but the 15112-1781.0 stock from Bowling Green can also be used]. Standard cornmeal medium was used; cultures and crosses were maintained at 22±1°C.

The advantages of this experiment for teaching purposes are the little amount of tissue required (i.e, only one larva is needed) and the clear identification of the prominent glue protein fractions, by which, an unequivocal interpretation of the results is possible even by beginners.

Preparation of samples: Samples from third-instar larval salivary glands from parental and F1-hybrid cultures are prepared. Well-grown larvae are washed, separated according to the sex, and dissected in a 0.03 M phosphate buffer (pH 6.8) with 0.04 M KCl, 0.011 M NaCl, 0.003 M $CaCl_2$, and 0.021 M $MgCl_2$ (Ashburner 1970), at 20±1°C. The glands are transferred for 20 min into a 1.5 ml microfuge tube filled with cold 10% TCA. The tissue is washed with the help of a Pasteur pipette (each time for 20 min) in 95% ethanol, a mixture of methanol and chloroform (1:1); finally it is dried at 37°C (which requires about 15 min). To the dried tissue, 30 µl of sample buffer [0.0625 M Tris-HCl (pH 6.8), 1% SDS, 1% β-mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue] is added and the lid of the microfuge tube is tightly closed. After 2-3 hr, it is heated in boiling water for 10 min, cooled to room temperature and then centrifuged for 3 min at 1,000 rpm. These extracts can be stored at -20°C for 8-10 days.

Table 1.

Solutions	lower gel	upper gel
Lower gel buffer	7.5 ml	--
Upper gel buffer	--	4.0 ml
Acrylamide solution	18.0 ml	4.0 ml
Dist. water	4.5 ml	7.76 ml
1% Ammonium persulfate	200.0 µl	160.0 µl
20% SDS	225.0 µl	240.0 µl
TEMED	20.0 µl	16.0 µl

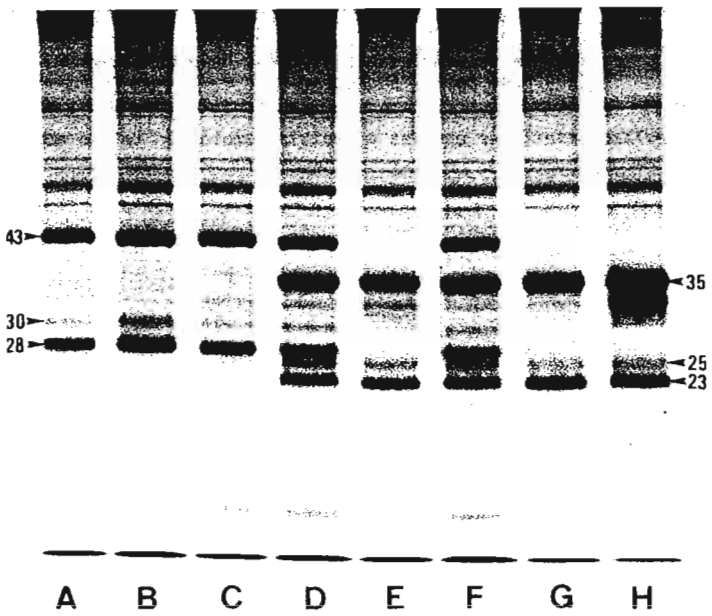


Figure 1. X-chromosomal linkage of major glue protein fractions in **D.n.nasuta** and **D.n.albomicans.** SDS-PAGE patterns of protein extracts from salivary glands of third-instar larvae. **(A)** male and **(B)** female of **D.n.nasuta.** **(C)** F1-male and **(D)** F1-female from a **D.n.nasuta** mother and a **D.n.albomicans** father. **(E)** F1-male and **(F)** F1-female from the reciprocal cross. **(G)** male and **(H)** female of **D.n.albomicans.** Note that in (C) as well as in (E) the F1-male shows the pattern of its mother, whereas in (D) as well as in (F) the F1-female shows a heterozygous pattern of both parents. Each protein extract is from one pair of salivary glands by the use of an individual larva. Differences in protein staining is based on gland size as well as species- and stage-specific development. Arrow-labeled bands indicate major glue protein fractions in both species which are coded by X-chromosomal genes. The approximate kd-values are given according to Ramesh & Kalisch (1988). Coomassie-blue staining.

Solutions required for preparation of the gels: (1) **Lower gel buffer:** 18.15 g Tris dissolved and made up to 100 ml with dist. water, pH adjusted to 8.8 with conc. HCl; (2) **Upper gel buffer:** 6.0 g Tris dissolved and made up to 100 ml with dist. water, pH adjusted to 6.8 with conc. HCl; (3) **Acrylamide solution:** 22 g acrylamide and 0.8 g bis acrylamide dissolved and made up to 100 ml with dist. water; (4) **Ammonium persulfate:** 1% solution (should be prepared fresh); (5) **Sodium dodecyl sulfate:** 20% solution.

Stacking gel and separating gels are prepared by mixing different proportions of solutions as shown in Table 1. The amount of gel solution mixtures are enough for casting 15 x 10 cm gels of 1 mm thickness. For details with regard to gel casting, see Ostermann (1984). It is preferable to cast the gels in cold. The prepared gel may be used immediately or may be stored at 8-10°C for 24-48 hr.

Electrophoresis: 15 µl of the samples prepared is injected into each slot of the gel, which are then carefully layered with running (tray) buffer [0.05 M Tris, 0.384 M glycine buffer (pH 8.3) containing 0.1% SDS]. The electrophoresis is performed with 165 volts (35 mA max.) for $3\frac{1}{2}$ hr at 20±1°C. The gel is treated overnight with a prestaining solution [50% TCA and isopropanol (1:1)]. 0.175% Coomassie brilliant blue R-250 (in 50% methanol and 10% acetic acid) is used for staining the gel for 2 hr, and a different mixture (25% methanol and 7.5% acetic acid) is used for destaining.

Figure 1 shows the SDS-PAGE patterns of protein fractions from the salivary glands. The prominent bands between 43-23 kd belong among others to the glue proteins (Ramesh & Kalisch 1987). It is evident from Fig. 1, that the three labeled glue protein fractions in both species are produced by genes located in the X-chromosome, since the F1-hybrid males (C and E) show the phenotype of the P-females used for the cross; while the F1-females (D and F), irrespective of the direction of the cross, are heterozygous for the maternal and paternal patterns. Chromosomal linkage of the remaining major and minor glue protein fractions will be published elsewhere (Ramesh & Kalisch, in prep.).

Acknowledgement: S.R.R. is grateful to the Univ. Grants Commission, New Delhi, India, for sponsoring and to the Deutscher Akademischer Austauschdienst, FR Germany, for the award of a scholarship. This study is part of a project (Ka 309/9-1) supported by the Deutsche Forschungsgemeinschaft.

References: Ashburner, M. 1970, Chromosoma 31:356-376; Osterman, L.A. 1984, in: Methods of protein and nucleic acid research, Springer-Verlag, Berlin & N.Y., p21-25; Ramesh, S.R. & W.-E. Kalisch 1987, DIS 66:117; ____ & ____ 1989, Genetica (in press).

SUBMITTED STOCK LISTS D.melanogaster

University of Wisconsin. Doris Ursic and Barry Ganetzky, Laboratory of Genetics, 445 Henry Mall, Madison, WI 53706.

A previously unknown **Drosophila melanogaster** gene has been isolated, mapped and sequenced. This gene has a strong homology to a gene in mouse, encoding a much studied protein in the mouse t-complex.

Genomic clone	: M221p	cDNA clone	: 5c
Vector	: Charon 4	Vector	: Lambda gt10
Strain	: Canton S	Strain	: Oregon R
Source	: Maniatis library	Source	: Yedvobnick & Artavanis-Tsakonas head cDNA library
Insert size	: 15 kb	Insert size	: 1929 base pairs, sequenced
In situ mapping	: 94B1-2	In situ mapping	: 94B1-2

Reference: Ursic, D. & B. Ganetzky 1988, "A **Drosophila melanogaster** gene encodes a protein homologous to the mouse t complex polypeptide 1", Gene 68:267-274.

University of Wisconsin. D. Ursic, R. Kreber and B. Ganetzky, Laboratory of Genetics, 445 Henry Mall, Madison, WI 53706.

Five genomic clones have been isolated and mapped by in situ hybridization to the Drosophila polytene chromosomes. They are:

Vector : Charon 4
Strain : Canton S
Source : Maniatis library

Clones	in situ mapping
1-2	22B (extended exposure also 90C)
6-1	100DF (extended exposure also 70DE)
7-1	70DE
19-2	2B

Vector : gt11
Strain : Oregon R
Source : L. Goldstein

Clone	in situ mapping
4-1-2a	53AB

Report of P. Aguado*, F.Galán-Estella** and J. González-Julián.**
University of Salamanca, Spain: *Dept. of Genetics; **Laboratory of Biology.

The 'dark carmine' gene of **Drosophila melanogaster.**

The recessive and sex-linked 'dark carmine' mutant of **Drosophila melanogaster** appeared spontaneously in one of the culture flasks used for studying the 'scarlet' phenotype (st, III, 44.0) of our collection. The specimens belonging to this new phenotype are characterized by the presence of dark carmine eyes.

The new allelic pair (d-cm$^+$, d-cm) and the scarlet allelic pair (st$^+$, st) correspond to the same group of allelomorph phenotypes, the gene interaction not being epistatic; the double recessive genotype corresponds to a new 'orange' phenotype.

The dark carmine locus is situated in the first linkage group between the 'yellow' (y, I, 1.00) and 'white' (w, I, 1.5) loci, the calculated genetic distance between 'yellow —— d-cm' being 0.5491 centimorgans, and the genetic distance 'd-cm —— white' being 0.9594 centimorgans.

Report of P. Aguado*, F. Galán-Estella** and J. González-Julián.**
University of Salamanca, Spain: *Dept. of Genetics; **Laboratory of Biology.

The 'divergent II' gene of **Drosophila melanogaster.**

The recessive and autosomic 'divergent II' mutant of **Drosophila melanogaster** appeared spontaneously in one of the culture flasks of our collection. The morphological characteristic of the individuals belonging to the new phenotype refers to the wings; although they have a normal configuration, they adopt a divergent orientation with respect to the longitudinal axis of the body when in the resting state.

The corresponding locus is situated in the second linkage group between the 'cinnabar' and 'brown' loci, the 'cinnabar-divergent II' genetic distance being 13.96 centimorgans and the 'divergent II-brown' distance being 32.97 centimorgans.

Report of C.B.C. Bonorino and V.L.S. Valente.
Dpto. de Genetica, Inst. Biociencias, Univ. Fed. do Rio Grande do Sul, Porto Alegre, Brazil.

Mutant "ebony" in **Drosophila nebulosa.**

A spontaneous mutant of **Drosophila nebulosa** for dark coloration of the exoskeleton was obtained in the F_1 of flies ecloded from a sample of rotten fruits (**Arecastrum romanzoffianum**, Palmae) deriving from nature (Agronomic Experimental Station, Guaiba County - 30°05' S, 51°39'W) (Figure 1).

Trying to identify the mechanism of inheritance of the mutation, 90 crosses have been made. The pairs were distributed in individual bottles with culture medium (Marques et al. 1966) and kept under a constant temperature chamber (25±1°C). A wild stock was chosen from Porto Alegre (30°10'S, 51°05'W) in order to be crossed with the mutant strain as follows: 30 crosses male M x female M; 30 crosses male W x female M and 30 crosses male M x female W (M=mutant; W=wild). Each pair has been observed three times after the distribution, and each observation was made at an interval of seven days. The F_1 were counted, and the ratios in F_2 calculated for the pairs that left offspring. The results of the crosses are summarized in Table 1.

Due to the fact that a large number of pairs did not present F_1 (although in some cases eggs were observed in the culture medium), the females were dissected for examination of the spermathecae after the third observation. No insemination was observed (Table 1, last column). In this procedure, we could verify that the mutant females spermathecae have a darker coloration than those of the wild females. All F_1 showed phenotype, except in the cross male M x female M, which was all mutant. In the F_2 the ratios found fell between 3:1 and 16:1, favoring the wild phenotype (Table 1).

It was also observed that the mutant is pronouncedly less active than the wild, which strongly contrasts with the characteristic behavior of this species (extremely active). Due to the lack of initiative of the mutant males and the absence of response to courtship by the mutant females, test-crosses could not be accomplished. Polytene chromosomes of the salivary glands of third instar larvae from F_1 of three kinds of crosses have been examined, and no apparent alteration at the chromosomal level was observed.

There was no evidence of sex linkage, and so our results suggest it is an autosomic recessive mutation, implying in reduction of fitness. This reduction of fitness probably explains the deviations of expected ratio 3:1 in F_2, once the low viability of several mutants as the ebony mutant of **Drosophila melanogaster** is well known (Kalmus 1945; Elens 1957, 1958; Jones & Barker 1966; Jones 1967). Its characteristics resemble the ones observed in the ebony mutation, which in the **willistoni** group has yet been mapped at the third chromosome of the sibling **Drosophila willistoni** and **D.pau-listorum** (Malagolowkin & Ehrman 1960; Ehrman

Figure 1. Wild and mutant flies of **Drosophila nebulosa.** Left: mutant. Right: wild.

Table 1. Summary of results of crosses between the wild stock and the mutant strain of **Drosophila nebulosa.**

Cross	Number	Nr.with F_1	Phenotype of F_1	Nr.with F_2	F_2 ratio W:M	Nr. of spermathecae observed
M x ♀ M	30	1	100% M	--	00	29
W x ♀ M	30	5	100% W	5	3:1;3:1; 4:1;9:1; 16:1	25
M x ♀ W	30	2	100% W	2	3:1;6:1	28
Total	90	8		7		72

M = mutant; W = wild.

chromosome of the sibling **Drosophila willistoni** and **D.paulistorum** (Malagolowkin & Ehrman 1960; Ehrman & Powell 1982). Besides the dark coloration, "but not nearly as dark as ebony in **D.melanogaster**" as Malagolowkin & Ehrman (1960) describe for **D.paulistorum,** the low activity resembles the pattern also yet described in literature (Magalhaes et al. 1970) for the ebony mutant in **D.melanogaster.** This could be an explanation for the low rate of insemination and, consequently, the few number of pairs with offspring.

Under these observations, we propose the mutant to be called "ebony", for its characteristics are similar to those described in literature for other species. The elucidation of the doubts raised at the present work shall serve as object for further studies.

Acknowledgement: Thanks are due to CNPq, PROPESP-UFRGS, FAPERGS and FINEP by grants and to Luciana P. Regner, Walter A. Zanette, Alice K. Oliveira and Neua Morales for the help given.

References: Elens, A.A. 1957, Experientia 13:293-294; ____ 1958, Experientia 14:274-276; Ehrman, L. & J.R. Powell 1982, in: The Genetics and Biology of Drosophila (M. Ashburner & H.L. Carson & J.N. Thompson, eds.), Academic Press, v3b:193-225; Jones, L.P. 1967, DIS 42:79; ____ & J.S.F. Barker 1966, Genetics 53:313-326; Kalmus, H. 1945, J. Genet. 47:58-63; Magalhaes, L.E., M.A. Querubim, C.R. Vilela & C.A.B. Pereira 1970, Ciencia e Cultura (Supl):135; Malagolowkin, C. & L. Ehrman 1960, Evolution 14:266-270; Marques, E.K., M. Napp, H. Winge & A.R. Cordeiro 1966, DIS 41:187.

Report of B.H. Huettner and P.J.Bryant.
Developmental Biology Center, University of California, Irvine, CA 92717 USA.

l(2)ey: lethal(2)eyeless.

A recessive EMS-induced mutation located at 2-0.65 and uncovered by Df(2L)al, indicating a cytological location of 21B8-CI to 21C7-8. About 50% of homozygotes die as larvae, 50% as pharate adults with an eyeless phenotype. The pharate homozygotes break open the operculum, but usually fail to eclose. The few that do eclose remain attached by the wings to the pupal case and live for as long as 6 days. They show uncoordinated movement and fall over and flail around wildly. The homozygotes show massive head defects including lack of one or both eyes, duplications and triplications of antennae, and greatly increased numbers of bristles in the postorbital, postgenal, and antennal areas. Other defects include missing or swollen leg segments, increased leg bristle numbers, and poorly developed and non-expanded wings. Eye-antennal imaginal discs from homozygous larvae show abnormalities including duplications and triplications of the antennal discs, and reduced eye discs and optic lobe connections. Although the lethality is not temperature sensitive, the head defects are much more severe after larvae are raised at lower temperatures. At 18°C, defects were seen in the eyes, antennae, legs, and palps, including duplicated palps. At 25°C, a similar range of head defects was produced, but there were no leg or palp defects. At 29°C the only defects were reduced or missing eyes. The frequency of totally eyeless flies was 80% at 18°C, 75% at 25°C and 16% at 29°C.

THE DROSOPHILA CLONE LIST BY CHROMOSOME LOCATION

August 10, 1988

John Merriam
Department of Biology
University of California
Los Angeles, CA 90024
213-825-2256

Explanatory Notes. The format of this list is what we call a "pocket guide". In the column called "Table Information" are included the names of the clones or other entries. Clones are listed by gene names where they are known. Unidentified clones are labelled "anon." for anonymous. In addition, this column lists rearrangement names for which at least one breakpoint has been identified on a Southern blot, and lists P element insertions. By convention, the P element insert names are identified within square brackets [ ].

In the column called "Tab#" are listed various numbers from 1 to 6 which key the kind of information available in the reference. In this key, 1 = a clone, 2 = a rearrangement, 3 = a P element insert, 4 = a walk, generally over 20 kb, 5 = transcription units (TU) identified, and 6 = a sequence reported.

The last column, "KBsze", represents a start to determining how much of the genome has been cloned, and is incomplete at this printing.

Corrections and additions should be sent using a copy of the page or the Report Form located at the end of the list.

Acknowledgement: This publication is supported by a grant from the National Library of Medicine.

GENE INFORMATION FOR TABLE NUMBER <> 8/10/88

Location	Table Information	Ref#	Tab#	KBsze
		361	1,5	0
	repeat of 31C	333	1	0
	[swallow]	343	3	0
1A	anon.	71	1	0
1A	telomeres	156	1	0
1A6-B1	In(1)elavG3	206	2	0
1A8-B1	yellow	242	1,5	6
1A8 to B1	erect wing	392	1,4,6	80
1B	[10-2]	360	3	0
1BC	[26.1]	298	3	0
1B	[27X-F]	200	3	0
1B	yellow	74	1	0
1B	anon.	34	1	0
1B	[g71:1]	114	3	0
1B1,2 to 1B4,5	yellow, achete, scute In(1)y3P, T(1;2)sc19, Dp(1;3)scJ4	152	1,2,4,6	113
1B1,2	yellow, achete In(1)y3p	8	1,2	70
1B1,2 to 4,5	AS-C	371	5,6	0
1B4,5 to 8,9	elav In(1)elav63	206	1,2,4	70
1B5,8	anon.	57	1	0
1B11,13	suppressor of sable su(s)	15	1	24
1B14-C1	In(1)ac3	105	2	0
1CD	[Bs2.71-2]	115	3	0
1E to 2A	anonymous	418	1	0
1EF	[23.3]	298	3	0
1E2,3	In(2)sc260-22	105	2	0
1E2,3	[Strain 23-2]	186	3	0
1F	T(1;3)Uab5	110	2	0
1F	[DA24-14]	114	3	0
1F	[R704.2, R702.1]	115	3	0
2A	[27P X/X-F]	200	3	0
2A	[P [(w,ry) E] 5]	176	3	0
2B	early ecdysome puff, ecs, dor swi Df(1)sta, Df(1)St472, Dp(1;U)67g^24.2, In(1)br^et103, In(1)Hw^49C	374	1,2,4	200
2B	[P (ry,hsp0-1) 8]	137	3	0
2B	[Tf(1)Gr304-1]	111	3	0
2B1,2 to 2B5,6	occ, 1(1)BA11, sta, Dp(1;f)101, T(1;3)sta	108	1,2,4	230
2B1,2	1(1)BA11 Dp(1;f)101	108	1,2	0
2B3,4 to 2B11	ecdysone sensitivity, early ecdysone T(1;Y)G20, T(1;Y)B131, T(1;3)sta, In Hw^49c, In br^ 1t103	418	1,4	160
2B5	br, rbp, 1(1)pp-1, early puff	380	1,4,6	120
2B13-18	[g711:2]	114	3	0
2B14,17	armadillo (segment polarity)	317	1	0
2C	[Pc [ry(delta0-1)] 48]	137	3	0
2C1 to 2E1	vsp,csw,pgd,kz,11lethals,polyhomeotic ph Df(1)pn38(2D1), DfJA52(2D3), DfPdg-kz(2D3), Dfpu3, DpdorY18T	161	1,2,4	200
2DE	EGF like repeats	310	1	0
2E	anon.	57	1	0
2E2 to F3	fs(1)K10, crooked neck, pcx, kurz, DF278,4B-1a, DF2F1-3A4	98	1,2,4	200
2EF	fs(1)pecanex,pcx1	80	1	0
2F5,6	raf oncog. homo.; pos. ser/thr kinase	129	1	8
2F5,6	Draf-1, proto oncogene	129	1,5	8
2F6 to 3A1	1(1) ph DfX12	348	1,2,4,	200
3,4	anon.	34	1	0
3A	[7-1;7-2]	140	3	0
3A1,4	zeste	295	1,5	0
3A1 to 4	zeste In(1)e(bx); Df(1)64C4; Df(1)w258-11; Df(1)wrj1; Df(1)62g18	166	1,2,4	200
3A3 to 4	zeste Df(1)62g18, Df(1)w-rJ1, Df(1)K95, Df(1)64c4, Dp(1;Y)y-z	225	1,2,4	170
3A4-4B	Df(1)2F1-3A4, Df278.4B1a	98	2	0
3A6,8	Df(1)64j4	107	2	0
3B3,4	homology with AS-c, Tu	373	1,5,6	11
3B	anon.	217	1	0
3B	[Tf(1)GR420-3]	111	3	0
3B	anon.	1	1	0
3B1,2	per	9	1,5	40
3B1,2	per, L(1)BA11 Df(1)w-64D, In(1)3B1, 2-20F, Df(1)3B1,2-3C2,3, Dp(1;f)101, Df(1)62d18, T(1;4)JD43	107	1,2	0
3B2 to C2	white	62	1,4	200
3B3,4	fs	330	1	35
3BC	anon.	84	1	0
3C	intracellular follicle cell, 2 genes	359	1	0
3C	anon.	198	1	0
3C	distal to white	95	1	25
3C1,2	white -blood (Wbl)	235	1,5	0
3C1,2	white	3	1	48
3C1,4	Df(1)64j4	107	2	0

Location	Table Information	Ref#	Tab#	KBsze
3C1,3	Df(1)wNfm20, Df(1)Nfm21, Df(1)N10	109	2	0
3C6,8	Notch	107	1,2	0
	In(1)N76b8, Df(1)62d18			
3C6	Df(1)N541g	109	2	0
3C7	Notch	310	1	0
3C7	Notch	261	1,5	0
3C7	intermolt I RNA	34	1	0
3C7	Notch	46	1,4	80
3C7	notch-epidermal growth factors	177	1	40
3C7,8	Notch	9	1,2	0
3C9,10	In(1)N76b8	107	2	0
3C11	Sgs-4	134	1	3
3C11,12	Sgs-4	45	1	0
3C11,12	Sgs-4	109	1,2	0
	Df(1)WNfm20, Df(1)Nfm21, Df(1)N10,			
	Df(1)N541g, Df(1)dm75elg			
3C11	flanking sgs4	379	1,4,6	20
3C11 to 3D4	dunce, pig-1, Sgs-4 and sam	417	1,4,5,6	100
3D1	intermolt I RNA	34	1	0
3D4	dunce, sam	416	1,2,6	0
	Df(1) N^64j15, Df(1)N^71h24.5, Df(1)N^64i16			
3D4	dunce (phosphodiesterase)	276	5	25
3D5,6	Df(1)N62b1	107	2	0
3E	CA/GT Z DNA probe	367	1	0
3E	[delta Sr 4-6]	411	3	0
3E4	Df(1)dm75elg	109	2	0
3F	[g71dx:2]	114	3	0
4A	polycomb homology	182	1,6	0
4B	[cHB delta -59]	118	3	0
4B3,4	mei-9	368	1	0
4BC	anon.	1	1	0
4BC	no receptor potential	90	1,4	40
4C	unn	263	1	0
4C	anon.	84	1	0
4C5,6	[Strain 23-2]	186	3	0
4D	T(1;3)bxd111	110	2	0
4D	[R405.1]	115	3	0
4F-5A	anon.	34	1	0
4F-5A	late IV RNA	34	1	0
5AB	anon.	34	1	0
5B	T(1;3)Ubx21560.8A complex	110	2	0
5C	[HSAd010]	65	3	0
5C	actin	179	1	0
5C	anon.	12	1	0
5D	ribosomal protein 7/8	85	1	0
5D3,6	In(1)sc7	105	2	0
5E6,7	swallow	343	1,5	0
5EF	anon.	34	1	0
5F (prox)	maternal restricted TU	69	1,6	0
6AB	[P [(w,ry) H] 4]	176	3	0
6E1,2 to 4,5	1(1)ogre	363	4	100
6F	[S6.9-2]	115	3	0
6F5	Sex-lethal (sxl)	223	1	0
6F5	sex lethal	66	1	0
6F5	In(1)sx1-af, Df(1)sx1-ra	223	2	0
7A	[C2.1]	218	3	0
7A	anon.	66	1	99
7B	cut	299	1	7
7B3,4	cut	29	1,4	100
7D	CA/GT Z DNA probe	367	1	0
7D	anon.	198	1	0
7D	[R403.1]	115	3	0
7D	anon.	84	1	0
7D	[SIR3]	411	3	0
7D5,6	fs(1) homeotic;l(1) B104 leth. myospher.	202	1,2	0
	T(1:3) N72			
7D5,6	fs(1)h (ss nucleic acid binding protein)	313	1	0
7E10 to 7F3,4	s36,s38 chorion genes, ocelliless	255	1,2,4,6	90
	In(1)ocelliless, In(1)7F1,2-8A1,2			
7EF	[plus 15 rb]	413	3	0
7F1	ovarian tumor (otu)	204	1,4	85
7,8	anon.	34	1	0
8A1,2	In(1)ocelliless	255	2	0
8BC	[tAP-25,3.2]	112	3	0
8D	lozenge	2	1	0
8E	[cHB delta-59]	118	3	0
8F-9A	Yolk proteins 1 and 2	2	1	0
9A	yolk polypeptide genes Yp1 & Yp2	273	1,5	0
9AD[R404.2]	115	3	0	
9B	[SB2.1-5]	115	3	0
9B	[tAP-24B,3.2]	112	3	0
9B	[unn]	117	3	0
9C	[S6.9-9]	115	3	0
9D	[P (ry,hsp0-1) 22]	137	3	0
9D4'-E1,2	[Bg9.61 (19Kb)]	240	3	0
9E	collagen probe homology	376	1	0
9E	[R701.1]	115	3	0
9E	[cHB lambda -23]	118	3	0
9E3,4	raspberry	138	1	20

Location	Table Information	Ref#	Tab#	KBsze
9E3,4	gus 1-pur1- ras complex	357	1,2	19
	In(1)123, ras-1			
9F	[1.9A2]	415	3	0
10ABC	collagen probe homology	376	1	0
10A	vermilion: tryptophan oxygenase	79	1	0
10A1 to B2	sevenless	173	1,2,4,5	110
	Df(1)rasv17Cc8 at 66Kb; Df(1)v64f29 at 72Kb			
10A1,2	sevenless(sev)	292	1,4	120
10A1,2	vermilion (tryp oxygenase)	217	1,5	18
10B	anon.	91	1	0
10B8,11	glutamine synthetase GSII	243	1	15
10BC	[tAP-20,3.2]	112	3	0
10BC	polycomb homology	182	1,6	0
10C1,2	RpII215	258	1,5	0
10C1,2	RNA polymerase II largest subunit	51	1	0
10C1,2	Df(1) GA112; Df(1) HA85; Df(1) M259-4	147	2	0
10C2,3	near m-dy complex	268	1	0
10C2,3	Df(1)m259-4	268	2	0
10D	[AR4-038]	126	3	0
10E	andante clock locus	364	1,2,4	0
	Df(1)m-259-4			
10E1,2	Df(1)m259-4	268	2	0
10EF	late V RNA	34	1	0
10F	minor heat shock cDNA from Kc cells	39	1	0
10F	[RB10-18]	422	3	0
11A	LSP-1 alpha	238	1	0
11A	LSP1 alpha	130	1,4	200
11A	gastrulation defective	8	1	0
11A2 to 4	gastrulation defective	174	1,4	100
11A7-B9	Larval Serum Protein 1 (LSP1)-alpha	308	1	0
11C	PRD gene5 (paired homology)	284	1,6	35
11D3,8	In(1)sc260-14	105	2	0
12	anon.	198	1	0
12	anon.	34	1	0
12AD	Yp3 (yolk polypeptide)	273	1,5	4
12A	[tAP-17,4.8]	112	3	0
12A	[3.8D]	415	3	0
12B	[P [(w,ry) E] 2]	176	3	0
12B,C	yolk protein 3	2	1	0
12B,C	[SRS3.9-1]	115	3	0
12BC	[SRS3.9-1]	115	3	0
12BC	[AR4-032(X)]	126	3	0
12D	[R301.2]	115	3	0
12DE	ser 7,4,4-7 tRNA, respectively	27	1	0
12E	[HSAd006]	277	3	0
12E	[E 7-10]	134	3	0
12E	[cHB delta-73]	118	3	0
12F-13A	[26.2]	298	3	0
12F	tRNA	58	1	0
12F1,2	tandem repeated 2L1 sequence	75	1	0
13A2,5	In(1)sc29	105	2	0
13AC	[BS.27-5]	115	3	0
13CD	[SB2.1-6]	115	3	0
13EF	c-myb (proto-oncogene)	178	1,5	30
13EF	[P[(w,ry)E]3g;P[(w,ry)G]4]	176	3	0
13F	[26.3]	298	3	0
13F	Glyceraldehyde-3-phosphate dehydrogenase		135	1
13F	Dmyb	320	1	0
13F	6 Protein beta subunit	395	1,5,6	0
14A	[21-1]	140	3	0
14B3,4	Disco	398	1,4,6	40
14BC	anon.	34	1	0
14C4 to 6	mei-41	369	1,4	60
14D	anon.	84	1	0
15AB	head specific RNA	31	1	0
15A1	rudimentary, CPSase, ATCase, DHOase	30	1,2,4	90
	In(1)r-70b26			
15A1	alpha subunit PS2 antigen	409	1,5,6	0
15B	ribosomal protein S18	85	1	0
15DE	[BS2.7-10]	115	3	0
15E	[P15-1]	0	3	0
15EF	GC rich dispersed repeat	414	1	0
15F	forked	158	1,4	40
15F	forked	77	1	0
16B3,5	anon.	2	1	0
16BC	[S6.9-11]	145	3	0
16C	[H1]	221	3	0
16D	[unn]	117	3	0
16E	[27 N/P-A]	200	3	0
16EF	shaker	40	1,4	50
16F	shaker	338	1,2,4,5,8	50
	T(1;Y)B55, T(1;Y)W32, T(1:3) Sh^1c			
16F	[27C X/X-A]	200	3	0
16F	[(delta 2-3) 10-1]	410	3	0
16F17	anon.	34	1	0
17A	[unn]	225	3	0
17AB	anon.	58	1	0
17B	[23.3]	298	3	0
17C to E	fused	331	1,4	100
17C	[tAP-5]	181	3	0

Location	Table Information	Ref#	Tab#	KBsze
17DE	[B1-2]	113	3	0
17EF	EGF like repeats	310	1	0
18A	PRD gene10 (paired homology)	284	1,6	36
18A	[R704.3]	115	3	0
18A	[In(1)34A]	342	2	0
18A3,4	In(1)y4	105	2	0
18AB	[1.9C]	415	3	0
18B8,9	In(1)sc9	105	2	0
18CD	maternal restricted TU	69	1,6	0
18D	G6PD	28	1	0
18D	anon.	201	1	0
18D	[16-3]	140	3	0
18D	[BS2.7-3]	125	3	0
18D	[RB18]	422	3	0
18E	G6PD	165	1	13
19,20AF	collagen probe homology	376	1	0
19A	[cHB delta-89]	118	3	0
19B	CA/GT Z DNA probe	367	1	0
19B	[detla SR 4-6]	411	3	0
19DE	runt	342	1,2,4	50
	In 34A, T(1;4)17-169			
19E	[+65]	118	3	0
19E	[tAP-1]	181	3	0
19E8	unc	160	1	0
19EF	collagen-like gene	25	1	0
19F	Arg rTNA locus	27	1	0
19F	[P [(w,ry) E] 1]	176	3	0
19F3-4	flightless, l(1)wz	397	1,6	18
	Df(1)16-129, Df(1)17-257			
20	In(1)y3P	152	2	0
20	Dp(1;f)101	108	2	0
20	T(1;3)CbxrvR17.49A,T(1;3)P115,Dp(3;1)P68	110	2	0
20A	[2]	123	3	0
20A	[Adh hs20A]	116	3	0
20AB	collagen-like gene	25	1	0
20BC	[23.1]	298	3	0
20C	In(1)scL8	105	2	0
20C1	In(1)sc4	105	2	0
20CD	[P (ry,HsAFP)]	141	3	0
20D	[AR4-024]	126	3	0
20D1	In(1)sc8, In(1)y3P	103	2	0
20D1	In(1)scS1	105	2	0
20F	Df(1)w-64D= In(1)3B1,2-20F+,	107	2	0
	Df(1)3B1,2-3C2,3			
21 tip	T(3;2)bxd-D36 complex	110	2	0
21A	telomere	71	1	0
21A	telomeres	156	1	0
21A	lethal(2) giant larvae	131	1,4	40
21A1,2	l(2)gl	252	1	0
21A1,2	T(2;3)Ubx16160.18	110	2	0
21B	anon.	34	1	0
21B	[P [(w,ry) G] 1]	176	3	0
21C	double sex cognate	88	1	0
21D	LSP1 beta	130	1	0
21D	LSP-1 beta	219	1	0
21D	anon.	136	1	0
21D	U1 snRNA	151	1,5	0
21D	[P [(w,ry) F] 4-2]	176	3	0
21D	[R602.1]	115	3	0
21D2-22A1	Larval Serum Protein 1 (LSP1)-beta	308	1,5	0
21DE	[tAP-10,4.8]	112	3	0
21E	anon.	310	1	0
21F	anon.	34	1	0
22A	anon.	34	1	0
22A	[R604.1]	115	3	0
22AC	4S RNA	193	1	7
22B	anon.	1	1	0
22B	[w20.2,w20.10]	120	3	0
22B1,2	T(2;3)Cbx rvR17.175	110	2	0
22B,C	anon.	34	1	0
22F1,2	dpp (decapentaplegic gene)	290	1,5	0
22F1,2	tyr tRNA	189	1	0
22F1,2 to 23A	decapentaplegic complex	55	1,4	130
22F-23A	[P13-1]	0	3	0
23AB	[T13.5X]	271	3	0
23A	[+65]	118	3	0
23A3 to 7	anon.	59	1,4	70
23BC	maternal restricted TU	69	1,6	0
	[g5:2]			
23D	homology with AS-c, Tu	373	1,6	9
23E to 24B	odd skipped	346	1,4	25
23E	ser 7 tRNA	27	1	0
24B	0-1 hrs.	84	1	0
24AB	[P [(w,ry) D] 4]	176	3	0
24C	anon.	1	1	0
24CD	[AR4-24]	126	3	0
24E	anon.	310	1	0
25	[26.4]	298	3	0
25A	bsg25A	256	1	0
25A	T(1;2)sc19	105	2	0

Location	Table Information	Ref#	Tab#	KBsze
25A	[6-1]	140	3	0
25B	[alpha T3.21]	82	3	0
25BC	anon.	1	1	0
25C	[P [(w,ry) D] 1]	176	3	0
25C	collagen-like gene	25	1	0
25C	[R401.3]	115	3	0
25D	type-IV-related collagen	376	1,5	0
25D	blastoderm specific	310	1	0
25D3	blastoderm specific gene(bsg)	293	1,5,6	16
25D1,4	anon.	6	1	0
25F	mst 323, testis-specific tu	264	1,6	0
25F	[27P X/X-B]	200	3	0
25F5	glycerol-3-phosphate dehydrogenase	139	1,5	1
26A	position effect variegation (PEV) T(Y;2) D222	335	1,2	0
26A	egg shell proteins, 4 genes	359	1	0
26AB	Kr homol. DNA finger protein coding gene	289	1,5	0
26A	GDPH: glycerol-3-phosphate dehydrogenase	246	1	11
26A	[23.5]	298	3	0
26A	vitelline	72	1	0
26A7,9	beta galactosidase	6	1	0
26AB	anon.	69	1	0
26B	acetylcholine receptor subunit	340	1	0
26B	new H box TU	355	1,6	28
26B	[D1 and D4]	119	3	0
26F-27A	[8.0:4 (period gene DNA)]	307	3	0
27A	[2-2 Icarus-neo]	278	3	0
27AC	[icarus-neo]	185	3	0
27C	Pupal cuticle protein	86	1	0
27C	Gar transformylase,synthetase,AIR synth.	7	1	0
27C	[33-4]	140	3	0
27C to 28BC	gart	326	1	0
27D	anon.	58	1	0
27DE	E6F-like homology	365	1	0
27F	anon.	34	1	0
28	int oncogene homologue	279	1	0
28A	head specific TU	31	1	0
28A	[BS2.7-11]	115	3	0
28A1,3	wingless(wg) (segment polarity) In(2L)wg-P	234	1,2	30
28C	urate oxidase	239	1	28
28C	head specific TU	31	3	0
28C	anon.	58	1	0
28D2	[S-Ica]	278	3	0
28D5	[E 7-1]	134	3	0
28D9,12	CDNA, Kc cells	8	1	0
28EF	anon.	310	1	0
29	T(2;3)Hm complex	110	2	0
29A	C1A9 antigen - nuclear protein	267	1,5	0
29A	lys 5 tRNA locus	27	1	0
29AC	T(2;3)P10	110	2	0
29A	heterochromatic specific	323	1	0
29B	[R308.1]	115	3	0
29B1,4	CDNA, Kc cells	8	1	0
29C	SRC homologous	61	1	0
30A	[28C-B (lost?)]	121	3	0
30A	[cHB delta-89]	118	3	0
30A9-B1 to 2	P6	219	1,4	55
30B	anon.	58	1	0
30C	[23.5]	298	3	0
30C	[A4-N22]	126	3	0
30C	[S6.9-3]	115	3	0
30DE	anon.	34	1	0
30EF	anon.	58	1	0
31	T(2;3) Ubx 18264.1	110	2	0
31A	anon.	84	1	0
31A	anon.	1	1	0
31B	[cHB delta-89]	118	3	0
31C	anon.	34	1	0
31F	anon.	34	1	0
31B-D	trunk and 31C	333	1	0
32	T(2;3)Hm complex	110	2	0
32AB	head specific TU	31	1	0
32BC	[BS2.7]	115	3	0
32CD	myogenic cell RNA	63	1	0
32CD	[cp70 delta B]	118	3	0
32C to 32F	oocyte-specific TU	210	1,4,6	200
32EF	In(2L) wg-P	234	2	0
32EF	vitelline membrane	72	1	0
32F	[g6:5]	124	3	0
32F to 33B	esc	218	1,2,4,6	700
33	anon.	198	1	0
33A1,2	spalt (sal)	386	1,5	16
33A to 33B	extra sex combs	70	1,4	250
33B	[26.3]	298	3	0
33B	0-1,2-5,3-5 TU	84	1,6	0
33B	anon.	34	1	0
33B to 33E2,3	paired (prd) with H box	300	1,4,6	300
34EF	[P1/23]	283	3	0
34	T(2;3) Ubx4.3 (madrid)	110	2	0

Location	Table Information	Ref#	Tab#	KBsze
34AB	anon.	69	1	0
34C	vitelline, Oregon R ovaries, cDNA	72	1	0
34D	[g7:4]	124	3	0
34E	[P(w)11P]	136	3	0
34EF	[g711:1]	124	3	0
34F	head specific TU	31	1,6	0
35A	[15-1;15-2]	140	3	0
35A4 to B1	Adh, outspread, no ocelli	132	1,4,5	165
35AB	anon.	133	1	0
35B	Adh	301	1	0
35B	noc, osp, Adh, Scutoid	325	1,5	175
	In(2LR)ScoR+1, T(2;3)ScoR+13			
35B1,3	Adh, noc, osp, l(2)br22	132	1	0
35B1,2	no-ocelli	167	1	0
35B3,5	alcohol dehydrogenase	52	1	0
35B9,C1	crinkled locus	149	1	0
	[TE 36]			
35C36	anon.	34	1	0
35D	snail	375	1,2,4,5,6	0
	Df(2)A48, Df(2)fn^27			
35DE	[14.6:21 (period gene DNA)]	307	3	0
35DE	[S11.4-1]	115	3	0
36A	[26Z-36A]	280	3	0
36A	[tAP-3]	181	3	0
36A	[unn]	118	3	0
36B	myosin heavy chain	13	1	0
36B	walked from myosin heavy chain	20	1	0
36C	dorsal	172	1	75
	In(2L)dlT; In(2L)dlH			
36C	[tAP-8C,4.8]	112	3	0
36D1-E1	Df(2L)VA18	35	1,4	160
36E1	fasciclin III	272	1	0
36E4,6	Df(2L)hk18	35	2	0
36EF	Na Channel homology	401	1	0
36F	anon.	1	1	0
37A	[26Z-37A]	280	3	0
37A	[P [w,ry) G] 2]	176	3	0
37A	[unn]	118	3	0
37B13 to C5	dopa decarboxylase	35	1,2,4	100
	Df(2L)hk18			
37BC	Oregon R ovaries	72	1	0
37BC	[unn]	122	3	0
37C	dDC and 3' flanking gene	288	1,5	0
37C	Df(2L)hkUC2	249	2	0
37C	Cc	249	1	0
37C	Ddc (dopa decarboxylase)	237	1,5	0
37C	amd (alpha methyldopa hypersensitive)	248	1,2	1
	Df(2L)NST			
37C	Ddc and amd	250	1,5	0
37C	Ddc	259	1,5	0
37B13 to C5	Ddc	35	1,2	0
	Df(2L)VA18, Df(2L)VA17, Df(2L)TE42-1, Df(2L)VA12			
37D3 to 5	SD tandem duplication	356	1,4,6	200
37F5-38A1	Df(2L)VA17	35	2	0
38A6	anon.	35	1	0
38B	[P (ry, HsAFP) 2]	141	3	0
38B	[cHB delta-59]	118	3	0
38B1,2-C1,2	Df(2L)VA12	35	2	0
38BC	[tAP-19,4.8]	112	3	0
38C1,2-D1,2	Df(2L)TE42-1	35	2	0
38D	[1]	123	3	0
38EF	caudal (S67)	180	1,5	16
38E	caudal (cad)	143	1,5	0
38E	[unn]	117	3	0
38E	caudal (cad)	324	1,4,5,6	45
38E5,6	caudal	303	1	0
38F4	Df(2L)TE42-1	35	2	0
39	T(2;3)Ubx19286.8m	110	2	0
39B	[28P-C (lost?)]	121	3	0
39BC	[E 5-5]	134	3	0
39BC	[S6.9-8]	115	3	0
39D	anon.	198	1	0
39DE	H1	257	1,5	0
39DE	histone	54	1	0
39DE	[H5]	221	3	0
39E	chromocenter	34	1	0
39E	[B4]	119	3	0
39EF	[AR4-2]	113	3	0
39F	anon.	34	1	0
40	chomocenter	34	1	0
40A	[R30b polo]	390	3	0
40A	[26Z-84D, 278-40A]	280	3	0
40A	[AR4-3]	113	3	0
40A	[1.9A1]	415	3	0
41	chromocenter	34	1	0
41	T(2;3)AntpNS+RC8	102	2	0
41A	T(2;3)Ubx17756.180, T(2;3)Ubx18136.147	110	2	0
	T(2;3)Ubx19649.18, T(2;3)UbxD1, T(2;3)bxdB231			
	T(2;3)CbxrvR17.22x, T(2;3)rvR17.34			
41A	T(2;3)bxd22044D	110	2	0

Location	Table Information	Ref#	Tab#	KBsze
41F	T(2;3)Ubx16160.36	110	2	0
42A	[R301.1]	115	3	0
42A	cluster of asn, arg, lys, ile tRNAs	190	1,4	94
42A	[+411]	118	3	0
42A	anon.	1	1	0
42A	[tAP-13,4.8]	112	3	0
42AB	[46]	391	3	0
42AB	[R303.1]	115	3	0
42BC	anon.	83	1	0
42BC	T(2;3)bxd x22290.11x	110	2	0
42CD	[14.6:63 (period gene DNA)]	307	3	0
42DE	collagen probe homology	376	1	0
42DE	[unn]	117	3	0
42EF	In (2R)man	344	2	0
42E	tRNA-lys-2	115	1	0
	[R305.1]			
42EF	anon.	34	1	0
42F	[2BX-C (lost?)]	121	3	0
42F	[S6.9-4]	115	3	0
43	[R704.1]	115	3	0
43A	anon.	58	1	0
43A2,5	raf oncogene homologous	129	1	7
43A2,5	Draf-2, proto oncogene	129	1	0
43AB	head specific TU	31	1,6	0
43BC	maternal restricted TU	69	1,6	0
43C	[R304.1]	115	3	0
43CD	[+411]	118	3	0
43DE	maternal restricted TU	69	1,6	0
43E	Glyceraldehyde-3-phosphate dehydrogenase	135	1	3
43E	[S6.9-7]	145	3	0
43E	[g7:7]	124	3	0
44A	T(Y;2;3)Mcp rvc10 complex	110	2	0
44C	anon.	71	1	0
44C	[S6.9-7]	145	3	0
44C	[2.5A]	415	3	0
44C4	T(2;3)P75Ubx 5T17.14-17	110	2	0
44CD	patch	381	1	30
44CD	head specific TU	115	1,6	0
	[S6.9-7]			
44D	anon.	71	1	0
44D	larval cuticle protein	36	1	50
44D	LCP1-4	224	1	0
44E	[R3.9-4]	115	3	0
44EF	anon.	84	1	0
44F	[cp70ZT]	118	3	0
45A	anon.	1	1	0
45A	[S11.4-1]	115	3	0
45AB	[F4]	119	3	0
45B	0-3.5 hrs	84	1	0
45CD	[swallow]	343	3	0
45D	[AR4-020(11)]	126	3	0
45E	[DR-18]	114	3	0
45E	[cHB delta-73]	118	3	0
46B	maternal restricted TU	69	1,6	0
46C	even-skipped (eve) with H box	304	1,5,6	20
46C	eve H box	187	1	0
46C	[27 X/A-2-A;27 X/A-2-B;27 X/A-2-C;	200	3	0
	27 X/A-2-D]			
46C	FMRF amide neuropeptide homology	403	1,5	2
46C	[A4-N21]	126	3	0
46C	DPKQDFMRF amide	396	1,5,6	0
46DF	myogenic cell TU	63	1,6	0
46E	head specific TU	31	1	0
47A	mst 325, testis specific transcript	264	1	0
47A	mst 325, testis specific tu	264	1,6	0
47A	[A1-1]	264	3	0
47A	[tAP-18,4.8]	112	3	0
47A	[1.9B]	415	3	0
47C	[cHB delta-89]	118	3	0
47D	[27N/P-B]	200	3	0
47D	[P [(w,ry) E] 8]	176	3	0
47EF	[P1/14]	283	3	0
47E	head specific TU	31	1,6	0
47F	anon.	14	1	50
47F48D	myogenic cell TU	63	1,6	0
48A to B	engrailed ecomplex	306	1,2,4	225
	T(2;3)enSF37;In(2R)enC2;In(2R)enSF49;			
	Df(2R)en30;T(2;3)enSF50;T(2;3)enSF52;			
	T(2;3)enSF42;T(2;3)enSF37;T()enSF50;T()enES			
48AB	[g7:3]	124	3	0
48B	[26Z-48B]	280	3	0
48B	Met 2 tRNA	27	1	0
48B	[hsp26-lacZ]	118	3	0
48B	[tAP-6]	181	3	0
48C	elongation factor F1	407	1,5	0
48C	anon.	34	1	0
48D	F1, female enriched RNA (all stages)	188	1,5	0
48D	[DR-9]	114	3	0
48E	anon.	34	1	0
48EF	Deb-A, Deb-B	198	1	0

Location	Table Information	Ref#	Tab#	KBsze
48EF	[tAP-4]	181	3	0
48F-49A	[13-2]	360	3	0
48F	his tRNA genes	196	1	0
48F	head specific TU	31	1,6	0
49A	calmodulin	12	1	0
49A	Calmodulin protein	314	1	18
49A12-B3	possible site aristapedioid	73	1	0
49B	[26Z-49B]	280	3	0
49B	[unn]	118	3	0
49CDE	[23.4]	298	3	0
49C	anon.	1	1	0
49C	[P(w)24S]	136	3	0
49CD	anon.	1	1	0
49D	[-51]	118	3	0
49D	[A1]	119	3	0
49D	[AR4-042]	126	3	0
49D	[S38M-5]	115	3	0
49DE	anon.	34	1	0
49DEF	vestigial	214	1	0
49E5-F1	possible site aristapedioid	73	1	0
49EF	[S3.8-6]	115	3	0
49F	aristapedioid sup. zeste	329	1,4	80
49F	muscle specific Troponin C(ca++)	22	1	35
49F	[tAP-9,4.2]	112	3	0
50A	[P [(w,ry) F] 1]	176	3	0
50AB	cluster of leu and ile tRNAs	191	1	2
50B	anon.	31	1	0
50B	[R306.1, S6.9-6]	115	3	0
59BC	cluster of lys tRNAs	192	1	0
50CD	Mastermind	344	1,2,4	85
	In, P Insert			
50C	mastermind (mam)	310	1	200
50C	anon.	198	1,4	0
50C	[13C-1 hsp prom-P (Icarus)]	278	3	0
50C	anon.	1	1	0
50CD	anon.	34	1	0
50DE	GC rich dispersed repeat	414	1	0
50EF	[plus 15 r]	413	3	0
50F	double sex cognate	88	1	0
51A	anon.	198	1	0
51A	[2.5A]	415	3	0
51B	head specific TU	31	1,6	0
51CD	maternal restricted TU	69	1,6	0
51D	anon.	34	1	0
51DE	anon.	01	1	0
51E	T(2;3)Cbx rvR17.6F	110	2	0
51F	paragonia (male) specific TU	385	1,6	1
51F-52A	E6F-like homology	365	1	0
52A	[tYPb]	297	3	0
51A1,2	anon.	87	1	0
52A	[DR-12]	114	3	0
52A3,6	anon.	84	1	0
52AC	T(2;3)Ubx X6000.78A4	110	2	0
52B	anon.	1	1	0
52B	[cHB delta-89]	118	3	0
52B	[tAP-21,3.2]	112	3	0
52BC	[P(w)5P]	136	3	0
52CD	[H3]	221	3	0
52D	neurogenic notch homol.	365	1,5	0
52D	not double sex cognate	88	1	0
52D	[SRS3.9-1]	88	3	0
52D	flight muscle TU, alpha GPO	322	1,6	0
52D6,15	07, 0-6	84	1	0
52DF	anon.	34	1	0
52E	2 epidermal segment genes	310	1	0
52F	[S6.9-10]	115	3	0
53A	Kinesin	232	1	0
53A	kinesin heavy chain	370	1	0
53A	[S38M-6]	115	3	0
53BC	[swallow]	343	3	0
53BC	[E 7-3]	134	3	0
53C	T(2;3)Ubx 105	110	2	0
53CD	amy pseudogene	33	1	0
53CD	ribosomal protein A1	17	1,5,6	20
53DE	[23.5]	298	3	0
53E	protein kinase C (PKC)	286	1,5	0
53E	[R3,9-1]	115	3	0
53E	C-Kinase homology	402	1	0
53EF	[SB2.1-1]	115	3	0
53F	[27B-53F]	280	3	0
53F	anon.	58	1	0
53F	[g4:1]	124	3	0
54-55	[23.2]	298	3	0
54AB	[P1/30]	283	3	0
54A	[2]	123	3	0
54A1B1	amylase duplication	33	1	0
54B	[K10;p(cos9)]	98	3	0
54C	anon.	198	1	0
54C	[Adh,hs54c]	116	3	0
54D	PRD gene11 (paired homology)	284	1,6	15

Location	Table Information	Ref#	Tab#	KBsze
54E	minor heat shock cDNA	39	1	0
54E	In(3LR)89/75+T(2;3)54/75, Ubx6-26, Madrid	110	2	0
54F-55A	anon.	69	1	0
54F-55A	anon.	34	1	0
55BCD	anon.	221	1	0
55F	maternal restricted TU	115	1	0
56AB	[H2]	221	3	0
56C	beta tubulin	25	1	0
56D	[R3.9-6]	115	3	0
56D4,12	alpha tubulin	22	1	0
56EF	anon.	34	1	0
56F	[DR-15, DR-5]	114	3	0
56F	tRNA Gly	58	1	0
56F	5S RNA	194	1	0
56F	[5]	391	3	0
57	[HB4/Sc1]	82	3	0
57A	[alpha T3.21]	82	3	0
57A	[plus 15 or]	413	3	0
57AB	[27P X/X-A;27P X/X-E]	200	3	0
57B	homeobox homology	389	1,5,6	3
57B	[27S-B]	200	3	0
57B	anon.	12	1	0
57B	[P [(w,ry) E]6;P [(w,ry)) F] 4-1]	176	3	0
57B5,6	H box homol.	354	1	0
57BC	GC rich dispersed repeat	414	1	0
57C	head specific TU	31	1	0
57C	punch	92	1,4	85
57C	tudor	93	1	200
57D	paragonia (male) specific TU	385	1,5,6	1
57E	[3.8'A]	415	3	0
57F	EGF receptor homolog (DER)	285	1,5	0
57F	c-erbB, EGF receptor protein	101	1	0
57F	[unn]	117	3	0
58A	[26.4]	298	3	0
58C	en-like homeobox	208	1	0
58D	[27 X/A-1-B]	200	3	0
58D	[AR4-043]	126	3	0
58EF	[R3.9-5]	115	3	0
58F	anon.	34	1	0
58F	[cp70 delta B]	118	3	0
58F	[tAP-7A,4.8]	112	3	0
59-60	T(2;3)Mcp rvC1 complex	110	2	0
59B	[A3-1]	115	3	0
59C	T(2;3)bxd 29315.46	110	2	0
59C	[tAP-7A,4.8]	112	3	0
59C5	GC rich dispersed repeat	414	1	6
59CD	twist zygotic dorsal-ventral gene Df(2R)twi*S60	412	1,2,4,6	300
59D	[27C X/X-B]	200	3	0
59EF	bw(?)	244	1,4	120
59E	anon.	201	1	0
59E	[S11.4-1]	115	3	0
59F	[3.8'B]	415	3	0
60A	maternal restricted TU	69	1	0
60A	anon.	34	1	0
60A	[R302.1]	115	3	0
60A11-B4	[gl(60AB) (8Kb)]	240	3	0
60AB	[gl]	124	3	0
60B	beta 3 tubulin locus	71	1	0
60B	T(2;3) Mcp rv26105A	110	2	0
60B	[BS2.7-4]	115	3	0
60BC	maternal restricted TU	69	1	0
60BC	[27P X/X-B]	200	3	0
60C	beta tubulin	377	1,5	0
60C	Na channel?	339	1	0
60C	beta tubulin locus	25	1	0
60C	[C2]	119	3	0
60C	[unn]	117	3	0
60C1,3	T(1;2)scS2	105	2	0
60C6,8	beta tubulin locus	22	1	0
60E	[S38Z-1]	115	3	0
60E	[tAP-15A,4.8]	112	3	0
60E	ion channel?	394	1	0
60E5,6	distal-less	347	1,4	180
60E9-F2	gooseberry (gsb), Kruppel (Kr)	287	1	0
60F	anon.	71	1	0
60F	telomeres	156	1	0
60F	[S3.8-4]	115	3	0
60F3	Kruppel	97	1,4	50
61A	Dp(1;3)scJ4	152	2	0
61A	[23.2]	298	3	0
61A	Bg9.61]	240	3	0
61A	LSP1 gamma	130	1	0
61A	LSP-1 gamma	219	1	0
61A	telomeres	156	1	0
61AB	[tAP-2]	181	3	0
61A	[Bg61]	118	3	0
61A	[S38Z-5]	115	3	0
61A	[27C+G-B]	200	3	0

Location	Table Information	Ref#	Tab#	KBsze
61A1,6	Larval Serum Protein 1 (LSP1)-gamma	308	1,5	0
61A1,3	anon.	1	1	0
61C	PRD gene2 (paired homology)	284	1,6	25
61C	[Adh hs61C]	116	3	0
61C	[neo R]	421	3	0
61C5-8	extra machochaete (emc)	341	1,4	50
61D	[tYPc]	297	3	0
61D	[28P-A (lost?)]	121	3	0
61D	[cp70 delta B]	118	3	0
61D	[3.8C[	415	3	0
61D	[neo R]	421	3	0
61D	[neo R]	421	3	0
61D3,4	[3.8A]	415	3	0
61E	[SRS3.9-4]	115	3	0
61F	double sex cognate	88	1	0
61F	[P(ry,HsAFP)3]	141	3	0
61F	[30]	391	3	0
61F-62A	In(3LR)Ubx 300	110	2	0
62A	cluster of glu tRNAs	195	1	0
62A	anon.	34	1	0
62A	tRNA locus	58	1	0
62A	[P [(w,ry) D] 2]	176	3	0
62A	[neo R]	421	3	0
62A	[neo R]	421	3	0
62A	[neo R]	421	3	0
62AB	anon.	34	1	0
62AB	[tAP-27]	112	3	0
62B	ras 3	291	1,5	0
62B	ras3	316	1	0
62B	[neo R]	421	3	0
62B7,12	apr+	211	1	0
62B9	apr+	211	1	0
62CD	myogenic cell TU	63	1,6	0
62D	anon.	34	1	0
62E	ribosomal protein L12 locus	85	1	0
62F	[neo R]	421	3	0
63-66	anon.	16	1	0
63AB	[HB4/Sc2]	82	3	0
63AC	myogenic cell TU	63	1,6	0
63B	hsp 83	39	1	0
63BC	hsp 82	227	1,5	0
63BC	hsp 83	10	1	0
63BC	[28C-A (lost?)]	121	3	0
63C	[tYPg]	297	3	0
63C	[S38Z-6]	115	3	0
63C	[neo R]	421	3	0
63C	[neo R]	421	3	0
63CD	[unn]	225	3	0
63E	20-hydroxyecdysone inducible	164	1	15
63E	[23-3]	140	3	0
63EF64F	collagen probe homology	376	1	0
63F	Ubiquitin	282	1,5	0
63F	minor hsp	39	1	0
63F-64A	anon.	34	1	0
64B	ras 2	291	1,5	0
64B	src kinase domain	254	1,5	7
64B	[P [(w,ry) F] 3]	176	3	0
64B	20-hydroxyecdysone inducible	164	1	15
64B	anon.	84	1	0
64B	ras oncogene	76	1,5	0
64B	SRC homologous	60	1	0
64B	ras-2	316	1	15
64B	ras2	316	1	0
64BC	anon.	1	1	0
64C	RAS homologous	60	1	0
64C	[R405]	115	3	0
64C	[tAP-7B,4.8]	112	3	0
64CD	polycomb homology	182	1,6	0
64D	In(3L)HR15	110	2	0
64D	[2.5B]	415	3	0
64E	In(3LR)3 ry64	110	2	0
64F-65A	PRD gene1 (paired homology)	284	1,6	25
64F	anon.	58	1	0
64F	anon.	1	1	0
64F	[SB2.1-3]	115	3	0
64F	[cHB delta -73]	118	3	0
64F	anon.	34	1	0
65A	[SB2.1-3]	115	3	0
65A	Tp(2;3)enSF37	306	2	0
65A	anon.	34	1	0
65A	[neo R]	421	3	0
65AB	[w47.1 N8]	120	3	0
65B	20-hydroxyecdysone inducible	164	1	15
65BC	tyrosine hydroxylase homology	400	1,5,6	4
65C	anon.	34	1	0
65D	PRD gene3 (paired homology)	284	1,6	30
65D	[278-65D]	280	3	0
65D	[28A-B (lost?)]	121	3	0
65D-66B	[S38Z-7]	145	3	0
65F	[g711:3]	124	3	0

Location	Table Information	Ref#	Tab#	KBsze
66A	[28X-D (lost?)]	121	3	0
66A	[plus 2 or]	413	3	0
66A	[neo R]	421	3	0
66B	Tp(3)P47	110	2	0
66B	leu tRNA	216	1	0
66C	Tp(3)bxd 100	110	2	0
66C	20-hydroxyecdysone inducible	164	1	15
66C	anon.	34	1	0
66CD	anon.	34	1	0
66D	mst 349, testis specif.+2 non sex-specif	264	1,6	0
66D	head specific TU	31	1,6	0
66D	[DR-17]	114	3	0
66D	[neo R]	421	3	0
66D9,10	anon.	32	1	0
66D10,15	anon.	57	1	85
66D11,15	chorion protein genes	44	1,4	100
66D12,15	gene s18-1, s15-1, s19-1	148	1,5	0
66D/E	[neo R]	421	3	0
66E1,2	[Tf(3L)Ga6.0-1]	111	3	0
66EF	[26.2 delta]	298	3	0
66E	[HSAd001]	277	3	0
66E	[AR4-012]	126	3	0
66F	anon.	58	1	0
67A	[neo R]	421	3	0
67A5,7 to B1,2	anon.	22	1,4	0
67B	includes hsp 28,23,26	42	1	0
67B	hsp22, hsp23, hsp26, hsp27, 1, 2, 3	171	1	0
67B	hsp23	298	1	0
67B	anon.	198	1	0
67B	anon.	233	1	0
67B	hsp 23	247	1,5	1
67B	loci of hsp 22,23,26, and 28	233	1	0
67B	hsp and flanking TU	43	1,6	0
67B	[neo R]	421	3	0
67BC	[27P X/X-D]	200	3	0
67C	collagen probe homology	376	1	0
67C	alpha tubulin	25	1	0
67C4,5	0-1 hrs.	84	1	0
67C4,6	alpha4 tubulin	281	1	0
67D8	[gl(67D)(8Kb)]	240	3	0
67DE	anon.	83	1	0
67E	[27 C X/X-A]	200	3	0
67F	polycombeotic Tp(3)P20	209	1,2	0
68A	[R7.7-1]	115	3	0
68A	[unn]	197	3	0
68A	Tp(3)Ubx P20	110	2	0
68A	GC rich dispersed repeat	414	1	0
68B	[plus 2 r]	413	3	0
68B5-C2	Df(3L)vin 7	1	2	0
68C	rotated abdomen	352	1	0
68C	intermolt IV, III, II RNA	34	1	0
68C	glue gene cluster	309	1,5	0
68C	[28X-A (lost?)]	121	3	0
68C	[g7:5, g71:2]	124	3	0
68C		410		0
68C1,5	anon. In(3L)HR15	1	1	0
68C3,7	Sgs proteins	153	1	0
68C3,7	Sgs protein genes Df(3L)vin 3	1	1	0
68C4,6	sgs 3,7,8	1	1	0
68C7,15	anon.	1	1	0
68C10,12	Df(3L)vin 7	1	2	1
68D	[S38Z-3]	115	3	0
68D/E	[neo R]	421	3	0
68E	LSP-2	219	1	0
68E	Tp(3)Ubx7 P20	110	2	0
68E	[g6:4]	124	3	0
68E3,4	Df(3L)vin 3	1	2	0
68EF	anon.	34	1	0
68F	[neo R]	421	3	0
69	anon.	198	1	0
69AB	anon.	310	1	0
69CD		354	1	0
69C	fst 241 avary, germ-line specif. transc.	264	1	0
69C3,4	In(3LR)bxd 113	110	2	0
69CD	[tAP-12,4.8]	112	3	0
69D	anon.	84	1	0
69F	myogenic cell TU	63	1,6	0
70A	asp tRNA genes	196	1	0
70A	20-hydroxyecdysone inducible	164	1	15
70A	anon.	34	1	0
70A	[neo R]	421	3	0
70AB	anon.	34	1	0
70AB	[S3.8-3]	115	3	0
70BC	maternal restricted TU	69	1,6	0
70BC	Val 4 tRNA locus	27	1	0
70C	anon.	34	1	0
70C	[S11.4-2]	115	3	0

Location	Table Information	Ref#	Tab#	KBsze
70C2	glued	419	1,6	30
70C2	glued from MISC_INFO-region of exons 1-3	420	5	0
70D	[26.3]	298	3	0
70D	In(3LR)Cbx rvR17.42	110	2	0
70D1,3	P1	219	1	85
70D4,5	frizzled locus	81	1,4	60
71-73	[12-1]	360	3	0
71AB	[26.3]	298	3	0
71A	gastrula differential poly(A) RNA	47	1	0
71AB	[-51]	118	3	0
71AB	anon.	34	1	0
71B	non comp # 16 of beta ta 6 (nc16)	353	1,4	0
71C	[S38M-1]	115	3	0
71C to F	EIP 28/29 locus	26	1	220
71CD	T(1;3)sc260-15	105	2	0
71DE	late II,III TU	34	1,6	0
71DE	late I TU	34	1,6	0
71E	ecdysone induce puff:late intermolt gene	24	1	0
71F	In(3LR)AntpPW	102	2	0
71F	[g7:2]	124	3	0
71F	[tAP-11,4.8]	112	3	0
72	[unn]	225	3	0
72B	brahma	350	1,4	60
72BC	head specific TU	31	1,6	0
72D11-E1	In(3LR)bxd 106	110	2	0
72DE	anon.	58	1	0
73A	H box homol	354	1	0
73A	transformer	87	1,5,6	0
73A	double sex cognate, not transformer	88	1	0
73A2-3 to 73B5-6	st, tra, In(3LR) st a 27 87D12-14	422	1,2,4,6	260
73A3,4	[unn]	197	3	0
73A3,4	scarlet	197	1,5	35
73A3-B1,2	anon.	201	1	0
73A3,4 to B1,2	tra, Dash	215	1,4	200
73B	abelson, proto-onvogene, st	349	1,4	152
73B	Dash, abl oncogene	296	1	9
73B	Abelson SRC homologous	106	1	0
73B	[27S-D]	200	3	0
73B	[Pc[ry(delta0-1)] 2]	137	3	0
73B	[plus 60 or]	413	3	0
73B	[plus 63 or]	413	3	0
73B	[neo R]	421	3	0
73B	[neo R]	421	3	0
73D	minor heat shock locus	39	1	0
73DEF	head specific TU	31	1,6	0
73E	[P(ry,HsAFP)6]	141	3	0
73F	[neo R]	421	3	0
74	In(3)Ubx 130 (TM2) complex	110	2	0
74E to F	early ecdysone responding puff	56	1,4	300
74F-75A	In(3LR)Antp NS+RC4	102	2	0
74F/75A	[neo R]	421	3	0
75	anon.	198	1	0
75A	PRD gene8 (paired homology)	284	1,6	15
75B	[plus 63 r]	413	3	0
75B	[neo R]	421	3	0
75C	paragonia (male) specific TU	383	1,5,6	3
75C	[23.5]	298	3	0
75C	anon.	34	1	0
75C	In(3LR)89/75+T(2;3)54/75,Ubx6.26 Madrid	110	2	0
75C	[P [(w,ry) H] 2-2]	176	3	0
75C	[neo R]	421	3	0
75CD	[R502.1]	115	3	0
75D	[R706.1]	115	3	0
75D	anon.	201	1	0
75EF	naked? H box homol.	354	1	0
76A	anon.	34	1	0
76A	[A38M-4]	115	3	0
76A	[neo R]	421	3	0
76DE	anon.	310	1	0
76DE	maternal restricted transcript	69	1	0
76F	anon.	1	1	0
77A	[cHB delta-73]	118	3	0
77B	[neo R]	421	3	0
77DE	[P(ry, HsAFP)1]	141	3	0
77E	[26.2]	298	3	0
77E1,2 to 78A1,2	knirps	405	1,4,6	250
78B	[278-78B]	280	3	0
78BC	[R603.1]	115	3	0
78C	[neo R]	421	3	0
78CD	[P [(w,ry) H] 1]	176	3	0
78D	[cHB delta-89]	118	3	0
78D7 to 8	polycomb	182	1,4,5,6	200
78EF	[26.2]	298	3	0
79-80	ribosomal protein-21	222	1	0
79B	anon.	12	1	0
79B	GC rich dispersed repeat	414	1	0
79CE	In(3LR)Cbx rvR17.44V	110	2	0
79C	[(delta 2-3) 10-1]	410	3	0
79D	[neo R]	421	3	0
79E	[+411]	118	3	0

Location	Table Information	Ref#	Tab#	KBsze
79E1,2	anon.	16	1	0
79EF	[plus 60 r]	413	3	0
79F	[8-1]	140	3	0
80	chromocenter	34	1	0
80A	[B1,F2]	119	3	0
80B	In(3LR)Cbx rvR17.16R	110	2	0
80C	Kc cells	8	1	0
80F	chromocenter	34	1	0
80F	In(3LR)bxd 19409.2X	110	2	0
81	chromocenter	34	1	0
81	In(3)Hab rvFC51, In(3)Ubx 125	110	2	0
	In(3)Mcp rvC1 complex			
	In(3R)ry 54, In(3R)ry PS11136			
82AB	[23.4]	298	3	0
82A	anon.	22	1	0
82A	[w47.4L]	120	3	0
82B	[Adh hs82b]	116	3	0
82BC	[g4:3]	124	3	0
82BC	[tAP-8B,4.8]	112	3	0
82C	CA/GT Z DNA probe	367	1	0
82C	[tYPa]	297	3	0
82C	[28X-E (lost?)]	121	3	0
82C	[SIR3]	411	3	0
82E	2 SnRNA U1 pseudogenes (inverted)	220	1	0
82E	U1 snRNA	151	1,5	0
82E	[neo R]	421	3	0
82F	head specific TU	31	1,6	0
82F	[5-1]	140	3	0
82F	[AR4-025]	126	3	0
82F	[3.8B]	415	3	0
83A	anon.	34	1	0
83A	[unn]	117	3	0
83AB	anon.	34	1	0
83AB	Lys 5 tRNA locus	27	1	0
83B	anon.	34	1	0
83B	[27S-E]	200	3	0
83BC	[SB2.1-2]	115	3	0
83C	[neo R]	421	3	0
83CD	[23.1]	298	3	0
83C	anon.	1	1	0
83CD	maternal restricted TU	69	1,6	0
83D4,5	Tp(3;3)Dfd, Tp(3;3)DfdTRX1	69	2	0
83F	anon.	34	1	0
83F	[R3.9-3,9/ S38M-2]	115	3	0
84,85	[plus 32 or]	413	3	0
84A	bicoid(bcd), PRD gene6 (paired homology)	284	1,6	25
84A1,2	labial (F90-2)	180	1	12
84A1	zen	213	1	0
84A1,2	Df(3R)JA99, Df(3R)Scr	102	2	0
84A4,5-C1,2	Antp	253	1	0
84A4,5	deformed (Dfd)	305	1,5	24
84A to C	antennapedia complex, labial,zen,bicoid	49	1,2,4	330
	In(Antp)s2			
84A4,5	Tp(3;3)Dfd	69	2	0
84AB	Lys 5 tRNA Ylocus	27	1	0
84B	alpha tubulin	25	1	0
84B1	Df(3R)ASCB	102	2	0
84B	[27P X/X-E]	200	3	0
84B1	Antp, Scr, Dfd	226	1,2	60
	Df(3R)9A99, Df(3R)45cb, In(3R)Msc-1			
84B1,2	S60 (zen)	266	1,5	0
84B2	Antennapedia	265	1	0
84B2-C1	hu	102	3	0
	Df(3R)ASCB, In(3R)Hu, Df(3R)Antp NS+RC7			
	In(3R)AntpB, In(3R)Antp LC, In(3LR)AntpPW			
	In(3LR)Antp NS+RC4, Df(3R)JA99, In(3R)Antp73B			
84B2	Antp T(2;3)AntpNS+RC8, Df(3R)Scr	102	3	0
84B3,6	delta tubulin	22	1	0
84B3,6	alpha1 tubulin	281	1	0
84B3-C1,2	double sex	48	1	75
84BC	anon.	34	1	0
84BC	[S3.8-1]	116	3	0
84BD	male germ specific TU	384	1,5,6	4
84C	[hsPneosev-15]	173	3	0
84C	[unn]	117	3	0
84C1,8	stk,hat,rue:1(3)84Cb,ted:Cc1(3)84Ce,Gld	4	1,4	160
84C1,8	InAntp73b, DfdsxMas+R29, DfA41	260	2	0
	T(2;3)XaD5(GldXaD5)			
84C5,6	In(3R)Antp73b	274	2	0
84C8	glucose dehydrogen., eclosion genes A-D	4	1	152
84DF	[14-1]	360	3	0
84D	alpha tubulin locus	25	1	0
84D	tRNA	58	1	0
84D	anon.	1	1	0
84D	Val 3b tRNA locus	27	1	0
84D	Df(3R)Antp NS+RC7, T(2;3)bxd DB6	110	2	0
84D	[28X-B (lost?)]	121	3	0
84D	[unn]	118	3	0
84D	[neo R]	421	3	0
84D1,2	responsible for dominant phenotype(rfd)	270	1	0

Location	Table Information	Ref#	Tab#	KBsze
84D1,2	In(3R)Antp 73b	102	2	0
84D3,4	overlaps Val 3b tRNA	19	1	30
84D4,8	delta tubulin	22	1	0
84D4,8	alpha3 tubulin	281	1,5	0
84DE	[neo R]	421	3	0
84E	20-hydroxyecdysone inducible	164	1	15
84E	[cHB delta-194, g6:2]	118	3	0
84E	[g6:2]	124	3	0
84E1,2	double sex locus and flanking	48	1	105
84E11,12-F4,5	anon.	50	1	240
84F	cluster of arg and asn tRNAs	196	1	18
84F	maternal restricted TU	69	1,6	0
84F	In(3)Mcp rv29175.10	110	2	0
84F	[1]	123	3	0
84F	[A3]	119	3	0
84F	[g71:3]	124	3	0
84F2,3	In(3R)Hu	102	3	0
85	anon.	198	1	0
85	anon.	34	1	0
85A	Dhod	336	1,4	0
85A	hunchback	366	1	15
85A	tRNA locus	58	1	0
85A	[27 X/A-1-A]	200	3	0
85A	[R309.1]	115	3	0
85A	[18]	391	3	0
85A3 to B1	hunchback	294	1,4,5	40
85AB	[814,CH8]	119	3	0
85C	[plus 2 r]	413	3	0
85BC	[24-1]	140	3	0
85C-D1,2	PRD gene7 (paired homology)	284	1,6	20
85C	tRNA	58	1	0
85C	anon.	6	1	0
85C	Arg tRNA	27	1	0
85C	[neo R]	421	3	0
85D	ras 1	291	1,5	0
85D	RAS homologous	60	1	0
85D	alpha tubulin locus	022	1	0
85D	anon.	198	1	0
85D	Dyps-clps homologue	319	1	0
85D	alpha 2 tubulin locus	71	1	0
85D	[P(ry,HsAFP)5]	141	3	0
85D	[P [(w,ry) H] 3]	176	3	0
85D	[g6:1]	124	3	0
85D	[BS2.7-7]	115	3	0
85D,E	double sex cognate	88	1	0
85D6,12	beta tubulin locus	25	1	0
85E	1(3)c43	328	1	0
85E	Dp(3;3)MtnH22	183	2	0
85E	[26.4]	298	3	0
85E	alpha tubulin	9	1	0
85E	0-1 hr. TU	0	1,6	0
85E	anon.	1	1	0
85E	In(3R)AntpB	50	2	0
85E6,10	alpha tubulin locus	22	1	0
85E6,10	alpha2 tubulin	281	1,5	0
85E10,15	metallothionein	162	1,5	0
85E10,15	metallothionein (MT)	163	1,5	13
85E10,15	metallothionein (Mtn), Dp(3;3)MtnH22	183	1,2,5	5
85F	In(3R)Hu	50	2	0
85F	[P [(w,ry) H] 2-1]	176	3	0
85F	[tAP-15B,4.8]	112	3	0
86	anon.	198	1	0
86	anon.	198	1	0
86	anon.	34	1	0
86A	[20-1]	140	3	0
86B4-C1	In(3R)Hu	50	2	0
86C	[P [(w,ry) F] 2]	176	3	0
86C	[(delta 2-3) 10-1]	410	3	0
86D	[R311.1]	115	3	0
86D	[g7:6]	124	3	0
86DE	[plus 32 r]	413	3	0
86E	[S38Z-2]	115	3	0
86F	[278-86F]	280	3	0
86F	[neo R]	421	3	0
87AB	[P1/35]	283	3	0
87A	hsp 70 and flanking	41	1	0
87A	hsp70, Sn cell DNA	42	1	0
87A	[R307.1]	115	3	0
87A	[neo R]	421	3	0
87A7	hsp70 subclone	39	1	0
87A7	hsp70	10	1	0
87AB	anon.	198	1	0
87AB	[28A-C (lost?)]	121	3	0
87B	In(3)Ubx 882	109	2	0
87C	hsp70, Sn cell DNA	42	1	0
87C1	hsp70 and flanking	41	1	0
87C1,3	Df(3R)ry 81	110	2	0
87C7,8	Df(3R)kar SZ11	110	2	0
87C10	hsp 70	10	1	0
87CD	[C1-1]	113	3	0

Location	Table Information	Ref#	Tab#	KBsze
87CD	[plus 74 or]	413	3	0
87CF	anon.	34	1	0
87D	hsp70	42	1	0
87D	rosy Ace, 1512, In(3LR)3 ry64,	144	2	0
	In(3R)ry54, In(3R)ryPS11136			
87D	[RB87]	422	3	0
87D1,2	Df(3R)ry75	144	2	0
87D2,4	Df(3R)ry614, Df(3R)ry1301, Df(3R)ry1402	144	2	0
87D3,4	Df(3R)ry 1607	144	2	0
87D5 to E5	rosy and Ace	5	1,4	315
87D5,6	Df(3R)ry 1608	144	2	0
87D6,8	rosy Ace, Df(3R)ry 619	144	2	0
87D7 to E6	mesA,B,G9,S12,rosy,snake,hsc2,pic,...m32	275	1,4	320
87D8,10	rosy Ace	144	2	0
	Df+In(3R)kar 1g27			
87D10,12	snake(snk+)	271	1,5	0
87D12,14	In(3)Cbx rv21988B	144	2	0
87D12,13	rosy Ace	144	1	0
	T(3;4)ryP51149			
87D14-E3	4 scaffold attachment regions(SARs)	275	1	0
87D14	rosy Ace, Df(3R)ry75	144	2	0
87D17	rosy Ace	144	1	0
87D/E	[neo R]	421	3	0
87E	anon.	12	1	0
87E	[unn]	117	3	0
87E	[neo R]	421	3	0
87E1,5	Ace (proposed structural gene for AChE)	302	1,5	0
87E1,2	Df(3R)126c	110	2	0
87E1,2	rosy Ace	110	2	0
	Df(3R)ry 81, Df(3R)karSZ11,In(3)Cbx+R1			
	Df(3R)ry1402, Df(3R)ry1301, Df(3R)126c			
	Df(3R)ry1607, Df(3R)ry1608			
87E5,6	Df(3R)1C4a	110	2	0
87E11,F1	Df(3R)1C4a	110	2	0
87E12,F1	Df(3R)ry 619	110	2	0
87EF	In(3)Cbx rv21987A	110	2	0
87F	Mst(3) g1-9	383	1,5,6	1
87F	In(3)Cbx wt, T(2;3)Cbx rvR17.6F	110	2	0
87F	[DRI-15]	114	3	0
87F	male germ cell specific transcript	408	1,5,6	0
87F	[R308.2, R404.1]	115	3	0
87F11,12	Df(3R)126c	110	2	0
87F-88A	In(3)Ubx80	110	2	0
88	anon.	198	1	0
88A	homeobox homology, TU	389	1,6	3
88A3-10	empty spriracles? H box homol.	354	1	0
88A	l(3)k43, imaginaldisk and chorion amp.	362	1,4	320
88A	[cHB delta-23]	118	3	0
88A	[tYPe]	297	3	0
88AB	RNA pol II 140kd subcent (Rp11-140)	337	1	0
88B	In(e)Ubx 12.5 (Madrid)	110	2	0
88B	minor heat shock cDNA	39	1	0
88C	anon.	1	1	0
88C	[S6.9-5]	115	3	0
88C	[neo R]	421	3	0
88C4	In(e)56A62Y	110	2	0
88D	double sex cognate	88	1	0
88D	[neo R]	421	3	0
88E	CA/GT Z DNA probe	367	1	0
88E	hsp70	42	1	0
88E	[BS2.7-9, R401.2]	115	3	0
88E	[SIR3]	411	3	0
88E	[neo R]	421	3	0
88E4,5-F	actin,tropomyosin,+ myofibril proteins	170	1	250
88F	tropomyosins 1 & 2	262	1,5	0
88F	tropomyosin	58	1	0
88F	muscle specific tropomysin	169	1	0
88F	actin	63	1	0
88F	[S6.9-1]	115	3	0
88F	[cp70 delta B]	118	3	0
88F2,5	3 tropomyosin loci	22	1	0
89	pic	110	2	0
	In(3)Cbx rv21987A, In(3)Cbx rv21988B			
89A	[Q39b]	390	3	0
89AB	In(3)bxd27830.C5A	110	2	0
89A	In(3)Cbx 3 (Cbx-like)	110	2	0
89A	[+204]	118	3	0
89A	[B1-1]	113	3	0
89A	[neo R]	421	3	0
89BC	[26.3]	298	3	0
89B	Val 4 Phe 2 tRNA	27	1	0
89B	In(3)Camel	110	2	0
89B	[27P X/X-C]	200	3	0
89B	[28-2]	140	3	0
89B	[BS2.7-6]	115	3	0
89B	[g7:8]	124	3	0
89B	[neo R]	421	3	0
89B	[neo R]	421	3	0
89B4	Sb	207	1	0
89B21	T(1;3)sta	108	2	0

Location	Table Information	Ref#	Tab#	KBsze
89BC	anon.	23	1	0
89C	In(3)bxd 183	110	2	0
89C	[neo R]	421	3	0
89C1,2	T(2;3)P10	110	2	0
89D	fasciclin I	399	1,5,6	0
89E	anon.	110	1	0
89E	In(3)89E/97F-97A, Cbx rv21560.60 Tp(3)Vno, Tp(3)abx, In(3)Ubxx, In(3)Mcp rvB315	110	2	0
89E	In(3LR)Ubx130TM2 complex In(3LR)bxd106, In(3LR)bxd19409.2x	110	2	0
89E	T(1;3)CbxrvR17.49A,T(2;3)Ubx16160.18 T(2;3)Ubx18264.1,T(2;3)Ubx4.3,T(2;3)Ubx4.3 Mc T(2;3)CbxrvR17.175, T(2;3)Hm complex, T(2;3)P10	110	2	0
89E	In(3)Cbx rvR17.5E, Tp(3)P47, In(3)Ubx130(TM2)complex, Tp(e)Vno, Tp(3)McpB277, In(3)Ubx19286.76	110	2	0
89E	T(2;3) Mcp rv26105A	110	2	0
89E	In(3)bxd27830.C5A, In(3)Ubx5.12 Madrid, T(1;3)bxd111, In(3)Camel, In(3)bxd183 In(e)56A62 (szedged)	110	2	0
89E	In(3)Mcp rv29175.10, In(3)Cbx wt In(3)Ubx80, In(e)Ubx12.5 Madrid, In(3)Cbx3 (Cbx-like)	110	2	0
89E	In(3)Cbx +R1	110	2	0
89E	In(3)Ubx 130 (TM2) complex	110	2	0
89E	In(3)Sab Mcp rv29340.8	110	2	0
89E	In(3)Ubx 882	109	2	0
89E	T(2;3)bxdB231, T(2;3)P75Ubx5T17.14-17 T(2;3)CbxrvR17.6F, T(2;3)UbxX6000.78A4 T(2;3)Ubx105	110	2	0
89E	In(3LR)89/75+T(2;3)54/75,Ubx 6-26 Madrid T(1;3)Ubx21560.8A Complx,T(1;3)Uab5 T(1;3)bxd111,Dp(3;1)P68,T(1;3)P115	110	2	0
89E	T(3;2)bxdD36 complx,T(2;3)Ubx18136.147 T(2;3)Ubx19286.8m,T(2;3)Ubx17756.180 T(2;3)Ubx19649.18,T(2;3)UbxD1	110	2	0
89E	In(3)Hab rvFC51, In(3)Ubx125, In(3)Mcp rvC1 complex, In(3LR)Cbx rvR17.44V In(3LR)Cbx rvR17.16R	110	2	0
89E	Tab (Transabdominal)	315	1	0
89E	T(2;3)Cbx rvR17.6F	110	2	0
89E	T(2;3)McprvC1complex, T(2;3)bxd29315.46 Tp(3)bxd100, Tp(3)UbxP20, Tp(3)Ubx7P20, Tp(3)P47, In(3LR)bxd113,In(3LR)CbxrvR17.42	110	2	0
89E	T(2;3)P10, In(3)Ubx961.29, In(3)Tab, In(3)Ubx3966.30, In(3)cbx2, Tp(3)bxd110, Tp(3)bxd110	110	2	0
89E	In(3)Ubx 1928.16N, T(3;4)bxd101, T(3;4)UbxA complex, T(3;4)Cbx rv R17.40R	110	2	0
89E	T(2;3)CbxrvR17.34,T(2;3)rvR17.22x T(2;3)bxd22044D,5(2;3)UbxUbx16160.36 T(y;2;3)Mcprvc10 complx,T(2;3)bxd x22290.11x	110	2	0
89E1 to 4	bithorax complex, T(1:3)Uab T(1;3)bxd111, T(1;3)Ubx21560.8A complex	110	1,4	400
89F	T(1;3) P115, Dp(3;1) P68	110	2	0
90A	[11-3]	360	3	0
90A	In(3)Ubx 5.12 (madrid) In(3)Sab Mcp rv29340.8	110	2	0
90A	[plus 74 r]	413	3	0
90A	[jump starter]	421	3	0
90B2	T(1;3)bxd 111	110	2	0
90B3,8	Sgs-5	236	1	21
90BC	anon.	24	1	0
90BC	Val tRNA, val 4 tRNA, Cal 3b, pro tRNA	27	1	0
90BC	intermolt V RNA, sgs	34	1	0
90C	tRNA	58	1	0
90C	In(3)Ubx 961.29	110	2	0
90CD	[S3.8-2]	115	3	0
90CD	[neo R]	421	3	0
90E	In(3)Tab	110	2	0
90E	[P [(w,ry) D] 3]	176	3	0
90E	[neo R]	421	3	0
90EF	[DR-1]	114	3	0
90F	H box homol	354	1	0
91A	[26.3]	298	3	0
91AB	[AR4-01]	126	3	0
91A/B	[neo R]	421	3	0
91BD	ChA (choline acetyltransferase)	245	1,5	2
91B	In(3)Ubx 3966.30	110	2	0
91B	[cHB delta-194]	118	3	0
91C	anon.	58	1	0
91C	In(3)cbx 2	110	2	0
91C	[A2-1]	113	3	0
91D	anon.	1	1	0
91D	[g4:2]	124	3	0
91D	[neo R]	421	3	0
91D1,2	Tp(3)bxd 110	110	2	0
91F-92A	In(3R)Antp LC	102	2	0
91F-92A	[S3.8-5]	115	3	0
92	anon.	198	1	0

Location	Table Information	Ref#	Tab#	KBsze
92	Tp(3)P47	110	2	0
92AB	[23.3, 26.2]	298	3	0
92A	anon.	1	1	0
92A	In(3)Cbx rvR17.5E	110	2	0
92A	[R3.9-2]	115	3	0
92A	Delta, T(3;3)DIII13	232	1,4	200
92A	[neo R]	421	3	0
92A1,2	Tp(3)bxd 110	110	2	0
92A2	Delta	310	1,4,5,6	180
92B	[AR4-032(111);AR4-01(111)]	126	3	0
92B	[neo R]	421	3	0
92B	[neo R]	421	3	0
92B8,11	opsin	159	1,5	0
92B8,11	opsin (ninaE)	269	1	0
92B8,11	rhopdopsin (nina E)	99	1,5	0
92BC	[B2-1]	113	3	0
92BC	[H4]	221	3	0
92CD	R7 specific opsin gene	269	1,5	0
92E	anon.	34	1	0
92F	[BS2.7-2]	115	3	0
93A	[P1/36]	283	3	0
93AB	[R310.1]	115	3	0
93B	homology with ASE, Tu	373	1,6	10
93B	rudimentary-like (r-1)	78	1,4	80
93B	In(3)Ubx 130 (TM2) complex	110	2	0
93B	Na pump (NA^+/K^+ Atpase) alpha subunit	393	1,6	14
93C to 93D	ebony, In(3R)e^AFA, In(3;3)e^D12, In(3R)e^N24, (continuation) T(2;3)e^D8	372	1,2,4,	100
93CD	ebony, 93D heat shock locus	127	1	300
93D	heat shock	251	1,5	0
93D	[26Z-93D]	280	3	0
93D	heat shock	146	1	0
93D	anon.	78	1	60
93D	[SB2.1-4]	115	3	0
93D	[neo R]	421	3	0
93D5,7	heat shock (3 transcripts)	205	1	0
93D6,7	[g5:1]	124	3	0
93E	DIR-insulin receptor homologue	318	1	0
93E1,2	PRD gene9 (paired homology)	284	1,6	18
94A	anon.	198	1	0
94A	anon.	34	1	0
94A	Tp(3)Vno, Tp(3)McpB277	110	2	0
94B	[28P-D (lost?)]	121	3	0
94D	[23.2]	298	3	0
94D	anon.	34	1	0
94D	[P [(w,ry) E] 7]	176	3	0
94D	[(delta 2-3) 10-1]	410	3	0
94D/E	[neo R]	421	3	0
94EF	[swallow]	343	3	0
94E	anon.	58	1	0
94E	[AR4-020]	126	3	0
94F	oocyte TU	41	1,6	16
95A	HMG Co A reductase	358	1,5,6	40
95A	[R601.1]	115	3	0
95AB	en-like homeobox	208	1	0
95AB	[27S-C]	200	3	0
95B	anon.	1	1	0
95C	blastoderm-differential poly(A) TU	47	1,6	0
95C	U1 snRNA	151	1,5	0
95C	[D1]	119	3	0
95D	hsp68	10	1	0
95D	[27S-A]	200	3	0
95D	[BS2.7-8]	115	3	0
95D	[unn]	117	3	0
95E	mst 316, mst(3)ag-3	264	1	0
	access. gland trans.+2 others			
95EF	mst 345a, mst 345b, 2 testis-spec. trans	264	1	0
95F	possibly crumbs (crb)	310	1	0
95F	[28A-A (lost?)]	121	3	0
95F	E6F-like homologoy	365	1	0
96	[DR-2]	114	3	0
96A	U-6 sn RNA	378	1,5,6	6
96A	neural nicotinic recptor subunit homology	404	1,5,6	50
96A	anon.	34	1	0
96A	In(3)Ubx 19286.76	110	2	0
96AB	[P [(w,ry) G] 3]	176	3	0
96B	[S3.8-1]	116	3	0
96B	[S38M-3]	115	3	0
96B	[neo R]	421	3	0
96D	anon.	1	1	0
96F	Tp(3)Mcp B277	110	2	0
96F-97A	anon.	34	1	0
96F-97C	anon.	34	1	0
96F 8 to 13	E(spl), gro	387	1,4,6	150
96F-97A	Tp(3)Vno,In(3)89E/97F-97A,Cbxrv21560.60	110	2	0
97A	anon.	58	1	0
97A	[28N]	200	3	0
97A	[tAP-16,4.8]	112	3	0
97AB	[+65]	118	3	0
97AB	[28-term (lost?)]	121	3	0

Location	Table Information	Ref#	Tab#	KBsze
97B	[28P-B (lost?)]	121	3	0
97B	[P [(w,ry) F] 4-3]	176	3	0
97C	anon.	34	1	0
97CD	In(3)Ubx 3798.68	110	2	0
97C/D	[neo R]	421	3	0
97D	[L2a]	390	3	0
97D	Tp(3)abx	110	2	0
97D1,2	toll	203	1	50
97EF	[P1/25]	283	3	0
97EF	beta tubulin	25	1	0
97E/F	[neo R]	421	3	0
97F	beta tubulin	22	1	0
97F	E6F-like homology	365	1	0
97F	[unn]	117	3	0
98 (+ or -)	T(1;3)scKA8	105	2	0
98A	[BS.27-1]	115	3	0
98B	[cHB delta-89]	118	3	0
98BC	In(3)Ubx-x	110	2	0
98C	male germ cell specific TU	384	1,5,6	1
98CE	mst 336a,336b,plus non sex-specif.transc	264	1	0
98C	[R602.1, R705.1]	115	3	0
98D1 to 98E1	forked head (fkh)	406	1,4,6	120
98D3,7	anon.	310	1	0
98DF	In(3)Mcp rvB315	110	2	0
98E	anon.	310	1	0
98E	[23.2]	298	3	0
98E	maternal restricted TU	69	1,6	0
98EF	homology with sry "fingers"	388	1	0
98F	anon.	198	1	0
98F	0-2.5 hrs. TU	84	1,6	0
98F3-10	maternal Tu, yema cluster of material	175	1,4,6	39
98F	myosin alkali light chain	168	1	0
98F1	polycomb homology	182	1,6	0
98F1,2	Tp(3;3)DfdTRX1	69	2	0
98F to 99A	collagen probe homology	376	1	0
99	In(3)Ubx 1928.16N	110	2	0
99A	[g6:3]	124	3	0
99A	[neo R]	421	3	0
99AB	[DA24-44]	114	3	0
99B	en-like H box homology	382	5,6	0
99B	[delta 2-3]	351	3	0
99B	Homeo box	50	1	0
99C	acid phosphatase, Dpca-74	334	1,2	0
99C	Head specific RNA	31	1	0
99C5,6	transient receptor potential	68	1	45
99CF	anon.	34	1	0
99D	blastoderm-specific poly(A) TU sry	47	1,6	0
99D	serendipity alpha, beta, delta TU	142	1,6	0
99D	ribosomal protein, Minute	53	1	0
99D	[SRS3.9-3]	115	3	0
99E	anon.	34	1	0
99E	myosin light chain-2	229	1,5	0
99E	myosin light chain	22	1	0
99E	[B 25]	134	3	0
99E	myosin light chain 2	47	1	0
99E1,3	blastoderm differential poly(A) TU	47	1,6	0
99E1-F1	Df+In(3R)kar 1g27	110	2	0
99F	anon.	34	1	0
99F	[neo R]	421	3	0
100	anon.	198	1	0
100CD	Killer of prune (k-pn), awd	327	1	0
100AB	anon.	16	1	0
100B	head specific TU	31	1,6	0
100B	Ag24B10	150	1,5	0
100B	anon.	1	1	0
100B	anon.	58	1	0
100C	double sex cognate	88	1	0
100C1,7	anon.	1	1	0
100CD	cell death-5 locus	94	1	0
100D	anon.	1	1	0
100D	[unn]	117	3	0
100DE	GC rich dispersed repeat	414	1	0
	from MISC_INFO-not transposable element			
100E	F2, pupal mTU in both males and females	188	1,6	0
100EF	205K MAP gene	83	1	0
100F	anon.	71	1	0
100F	microtubule associated protein locus	83	1	0
100F	[A4-4]	113	3	0
100F	telomeres	156	1	0
100G	T(3;4)Cbx rvR17.40R	110	2	0
100G	T(3;4)ryP51149	110	2	0
101-102	T(1;4)scH	105	2	0
101F	T(3;4)bxd101	110	2	0
102	T(3;4)Ubx A complex	110	2	0
102	T(1;4)JC43	107	2	0
102C	homology to ribosomal spacer	264	1	0
102C	anon.	1	1	0
102CD	anon.	3	1	0
102EF	anon.	58	1	0
102F	telomeres	156	1	0

REFERENCES

1 Meyerowitz & Hogness (1982) Cell 28: 165-176
2 Barnett et al. (1980) Cell 21: 729-738
3 Bingham et al. (1981) Cell 25: 693-704
4 Cavener, Corbett, Cox & Whetten (1986) EMBOJ 5(11): 2939-2948
5 Bender, Spierer & Hogness (1983) J Mol Biol 168: 17-33
6 Knipple, D., T. Fuerst & R. MacIntyre, Genetics Dept, Cornell U, Ithaca NY 14853
7 Henikoff et al. (1986), PNAS USA 83: 720-724 [Genetics]
8 Biessmann (1985), PNAS USA 82: 7369-7373 [Genetics]
9 Bargiello & Young (1984) PNAS USA 81: 2142-2146
10 Holmgren et al. (1979) Cell 18: 1359-1370
11 Fyrberg et al. (1983) Cell 33: 115-123
12 Tobin, S.L., Mol Biol Dept, U Oklahoma, Oklahoma City, OK 73190
13 Mogami et al. (1986) PNAS USA 83: 1393-1397 [Genetics]
14 Poole et al. (1985) Cell 40: 37-43
15 Chang, D-Y et al. (1986) Mol & Cell Biol. 6(5): 1520-1528
16 Goldstein, E., K. Brogan, W. Vincent & K. Schultz, Zool Dept, Arizona State U, Tempe AZ 85287
17 Qian, Zhang, Kay & Jacobs-Lorena (1987) Nucleic Acids Res 15:987-1003
18 Bender, W. et al. (1983) Science 221: 23
19 Kerridge, S. & R. Griffen-Shea, LGBC CNRS Case 907, Univ Luminy, 70 Route Leon Lachamp, 13288 Marseille, France
20 George, E.L. & C.P. Emerson Jr, Biol Dept, U of Virginia, Charlottesville VA 22901
21 Lis, J.T., J.A. Simon & C.A. Sutton (1983) Cell 35: 403-410
22 Mischke & Pardue (1982), J Mol Biol 156: 449-466
23 Devlin, R. & V. Finnerty, Biol Dept Emory U, Atlanta GA 30322
24 Restifo, L.L. & G.M. Guild (1986) J Mol Biol 188: 517-528
25 Natzle, J.E. & B.J. McCarthy (1984) Devel Biol 104: 187-198
26
27 Hayashi, S., D.L. Cribbs, I.C. Gillam, T.A. Grigliatti, C. Newton, J. Leung, B. Rajput et al., Biochem Dept, U of Brit Columbia, Vancouver BC Canada
28 Ganguly, R., N. Ganguly & J. Manning (1985) Gene 35: 91-101
29 Jack, J. NIEHS, Research Triangle Park NC 27514
30 Segraves et al. (1984) J Mol Biol 175: 1-7
31 Levy, L.S., R. Ganguly, N. Ganguly & J.E. Manning et al. (1982) Devel Biol 94:451-464
32 Ish-Horowicz, D., R. Howard, P. Ingham, A. Leigh-Brown & S. Pinchin, Imp Cancer Res Fund, Burton Hole Lane, Mill Hill, London NW7 1AD
33 Lev, Gemmill & Doane (1985) Genetics 110: 313
34 Wolfner, M.F., PhD Thesis, Stanford U 1980
35 Hirsh & Davidson (1981) Mol & Cell Biol 1:475-485
36 Snyder, Hirsh & Davidson (1981) Cell 25:165
37 Fristrom, J. & D. Kimbrell, Dept Genetics, U Calif-Berkeley CA 94920
38 Mahowald et al. (1981) Chrom 82: 205
39 Lis, Mechameyer, Dubonsky & Costlow (1981) Gene 15: 67-80
40 Cabrera, C.B., J. Casal & A. Ferrus, Centro de Biol Mol, Consejo Sup. de Invest, Cint. & Univ Auto de Madrid, Spain
41 Shedl et al. (1978) Cell 14: 921-929
42 Craig & McCarthy (1980) Nucleic Acid Res 8: 4441
43 Sirotkin & Davidson (1982) Devel Biol 89: 196-210
44 Spradling et al. (1980) Cell 19: 905-914
45 Muskavitch & Hogness (1982) Cell 29: 1041-1051
46 Artavanis-Tsakonas, M. Muskavitch & B. Yedvobnick (1983) PNAS USA 80: 1977-1981 [Genetics]
47 Roark, Mahoney, Graham & Lengyel (1985) Devel Biol 109: 476-488
48 Baker, B. & M. Wolfner, Biol Dept, U Calif-SD, LaJolla CA 92093
49 Scott, M., T. Kaufman et al. (1983) Cell 35: 763-776
50 Garber, Kuroiwa & W. Gehring (1983) EMBOJ 2: 2027-2036
51 Searles, Jokerst, Bingham, Voelker & Greenleaf (1982) Cell 31: 585
52 Goldberg, D. (1980) PNAS USA 77(10): 5794-5798 [Biochemistry]
53 Vaslet, O'Connell, Izquierdo & Rosbash (1980) Nature 285: 674
54 Lifton, Goldberg, Karp & Hogness (1978) CSHQB 42: 1047-1051
55 Hoffman, F.M., D.St. Johnson & W. Gelbart, Biol Dept, Harvard U, Cambridge MA 02138
56 Jones, C.W., K. Burtis & D. Hogness, Biochem Dept, Stanford U, Palo Alto CA 94305
57 Carramolino et al. (1982) EMBOJ 1(10): 1185-1191
58 Davidson, N., S. Falkenthal, N.D. Hersey, W.W. Mattox & V. Parker, Div Chem & Chem Engg, CIT, Pasadena CA 91125
59 Hoffmann, F.M. & W. Gelbart, Cell & Devel Biol, Harvard U, Cambridge MA 02138
60 Hoffman-Falk, Enat, Shilo & Hoffmann (1983) Cell 32: 589
61 SImon, Kornberg & Bishop (1983) Nature 302: 837
62 Pirrotta et al. (1983) EMBOJ 2: 927-934
63 Storti & Szwatz (1982) Devel Biol 90: 272-283
64 Rozek & Davidson (1983) Cell 32: 23
65 Rubin, G.M. & A.C. Spradling (1982) Science 218: 348-353
66 ?????
67 Spradling, Carnegie Institute, Baltimore MD 21210
68 Wong, Hokanson & Chang (1985) Invest. Ophthalmol. Vis. Sci. 26: 243-246
69 Stephenson, E., Biol Dept, U Rochester, Rochester NY 14627
70 Struhl
71 Renkawitz-Pohl & Bialojan (1984) Chromosoma 89: 206; Bialojan et al. EMBOJ in press
72 Mindrinos, Scherer, Garcini, Kwan, Jacobs & Petri (1985) EMBOJ 4: 147-153
73 Adler, P. & B. Brunk, Genetics Lab, U Virginia, Charlottesville VA 22901

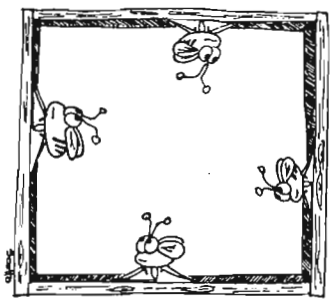
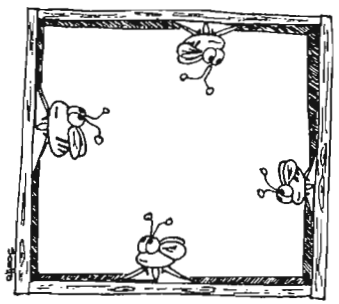
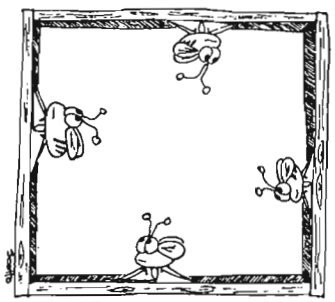
74 Eggleston, W., Genetics Lab, U Wisconsin, Madison WI 53706
75 Livak (1984) Genetics 107: 611-634
76 Mozer, Marlor, Parkhurst & Corces (1985) Mol & Cell Biol 885-889
77 Parkhurst & Corces (1985) Cell 41: 429-437
78 Rawls, J., Biol Sci, U Kentucky, Lexinton KY 40506
79 Searles, NIEHS, Research Triangle Park NC 22709
80 LaBonne, S. & A. Mahowald, Dev Genet & Anat, Case Western Reserve U, Cleveland OH 44106
81 Vinson, C. & P. Adler, Biol Dept, U Virginia, Charlottesville VA 22901
82 Davies, J., C. Addison, S. Delaney, C. Sunkel & D. Glover, in prep.
83 Goldstein, Laymon & McIntosh (1986) JCB 102: 2076-2087
84 Underwood, E. & J. Lengyel, Mol Biol Inst, UC-Los Angeles CA 90024
85 Burns, D.K., B.C. Stark, M.D. Macklin & W.Y. Chooi (1984) Mol & Cell Biol 4: 2643
86 Henikoff, S., M.A. Keene, K. Fechtel & J.W. Fristrom (1986) Cell 44: 33-42
87 Boggs, Gregor, Idriss, Belote & McKeown (1987), Cell 50:739-747
88 Andrews & B. Baker, Biol Dept. U Calif-San Diego, LaJolla CA 92903
89 Kaufman, Biol Dept, Indiana U, Bloomington IN 47401
90 Brodberg, R. & W. Pak, Genetics, U Calif, Davis CA 95616 & Biol Sci, Purdue U, W. Lafayette IN 47907
91 Sponaugle & Woods, Dev Biol Ctr, U Calif, Irvine CA 92717
92 Paulus & J. O'Donnell, Biol Sci, Carnegie-Mellon U, Pittsburgh PA 15213
93 Boswell, Genetics Lab, NIEHS, Research Triangle Park NC 22709
94 Dearolf, Biology, John Hopkins U, Baltimore MD 21218
95 Goldberg, Genet & Devel, Cornell U, Ithaca NY 14850
96 Tartof, Inst Cancer Res, Philadelphia PA 19111
97 Preiss et al. (1985) Nature 313: 27
98 Haenlin, Steller, Pirrotta & Mohier (1985) Cell 40: 827-837
99 O'Tousa et al. (1985) Cell 40: 839
100 Zuker et al. (1985) Cell 40: 851-858
101 Livneh et al. (1985) Cell 40: 599
102 Garber, A. Kuroiwa & W. Gehring, Genetics Dept, U Washington, Seattle WA 98195
103 Biessmann, Biochem & Biophys, U Calif, San Francisco CA 94143
104 Brittnacher & Ganetzky (1983) Genetics 103: 659-673
105 Carramolino et al. (1982) EMBOJ 1: 1185-1191
106 Hoffmann, Fresco, Hoffman-Falk & Shilo (1983) Cell 35: 393-401
107 Reddy et al. (1984) Cell 38: 701-710
108 Chao & Guild (1986) EMBOJ 5: 143-150
109 McGinnis & Beckendorf (1983) Nucleic Acid Res 11(3): 737
110 Bender
111 Meyerowitz, Biol Dept, CIT, Pasadena CA 91125
112 Posakony, Biochem & Mol Biol, Harvard U, 7 Divinity Av, Cambridge MA 02138
113 Hazelrigg, Levis & Rubin (1984) Cell 36: 469-481
114 Scholnick, B.A. Morgan & J. Hirsh (1983) Cell 34: 37-45
115 Spradling & Rubin (1983) Cell 34: 47-57
116 Bonner, Bonner, Parks, Poster-Thornburg, Martin & Pelham (1984) Cell in press
117 Chovnick, Genetics & Cell Biol, U Conn, Storrs CT 06268
118 Simon et al. (1985) Cell 40: 805
119 Kongsuwan et al. (1985) Nature 317: 555
120 Gehring, Roman Klemenz, Ursula Weber & Urs Kloter (1984) EMBOJ 3: 2077-2085
121 Hoffman & Corces (1986) Mol & Cell Biol 6(2): 663-673
122 Viglianti, Biochem, Harvard U, Cambridge MA 02138
123 Mahaffey et al. (1985) Cell 40:349-357
125 should contain info about transformation at location 18D; T-strain = BS2.7-3; markers = chorion+, ry+; cross refs = 66D11-15 & 87D12
126 Levis, R., T. Hazelrigg & G.M. Rubin (1985) Science 229: 558-561
127 Walldorf et al. (1984) EMBOJ 3:2499-2504
128 Benyajati, Place, Powers & Sofer (1981) PNAS USA 78: 2717-2721
129 Mark, MacIntyre, Digan, Ambrosio, Perrimon (1987) Mol & Cell Biol 7: 2134-2140
130 Smith, A. McClelland, B. White, C. Addison & D. Glover (1981) Cell 23: 441-449
131 Mechler, McGinnis & Gehring (1985) EMBOJ 4(6): 1551-1557
132 Chia, Karp, McGill & Ashburner (1985) J Mol Biol 186: 689-706
133 Scherer et al. (1981) Devel Biol 86: 438-447
134 Hofmann, Korge in prep
135 Sullivan et al. (1985) J Biol Chem 260: 4345-4350
136 Levis (unpubl) FHCRC M616, 1124 Columbis St, Seattle WA 98104
137 Laverty (unpubl), Biochem Dept, UC-Berkeley CA 94720
138 Nash (unpubl), Genetics Dept, U Alberta, Edmonton, Alb., Canada
139 von Kalm (unpubl), Biol Dept, Syracuse U, Syracuse NY 13210
140 Krumm, Roth & Korge (1985) PNAS USA 82: 5055-5059
141 Rancourt, Walker & Davies (unpubl) Biochem & Biol Dept, Queen's U, Kingston, Ont. Canada
142 Vincent, Colot & Rosbash (1985) J Mol Biol 186: 149-166
143 Mlodzik, Fjose & Gehring (1985) EMBOJ 4: 2961-2969
144 Spierer, Spierer, Bender & Hogness (1983) J Mol Biol 168: 35-50
145 Wakimoto, Kalfayan & Spradling (1986) J Mol Biol 187: 33-45
146 Ryseck, Walldorf & Hovemann (1985) Chromosoma (Berl) 93: 17-20
147 Voelker, Wisely, Huang & Gyurkovics (1985) Mol Gen Genet 201: 437-445
148 Wong, Pustell, Spoerel & Kafatos (1985) Chromosoma (Berl) 92: 124-135
149 Gubb, Shelton, Roote, McGill & Ashburner (1984) Chromosoma (Berl) 91: 54-64
150 Zipursky, Venkatesh & Benzer (1985) PNAS USA 82: 1855-1859
151 Kejzlarova-Lepesant et al. (1984) Nucleic Acid Res 12(23): 8835-8846

152 Campuzano et al. (1985) Cell 40: 327-338
153 Meyerowitz & Martin (1984) J Mol Evol 20:251-264
154 Mechler, McGinnis & Gehring (1985) EMBOJ 4: 1551-1557
155 Parker, Falkenthal & Davidson (1985) Mol & Cell Biol 3058-3068
156 Young, Pession, Traverse, French & Pardue (1983) Cell 34: 85-94
157 Gilbert & Hirsh, in "Developmental Biology Using Purified Genes" (1981) Academic Press
158 McLachlan (1985) Mol & Cell Biol 1-6
159 Zuker, Cowan & Rubin (1985) Cell 40: 851-858
160 Miklos, Healy, Pain, Howells & Russell (1984) Chromosoma (Berl) 89: 218-227
161 Brock (unpubl) Biol Dept, U Brit Columbia, 6270 Univ Blvd, Vancouver, BC Canada
162 Lastowski-Perry, Otto & Maroni (1985) J Biol Chem 260: 1527-1530
163 Maroni, Otto & Lastowski-Perry (1986) Genetics 112: 493-504
164 Natzle, Hammonds & Fristom, J Biol Chem in press
165 Hori, Akasaka, Ito, Hanaoka, Tanda, Ohtsuka, Miura,Takahashi & Tang (1985) Japan J Genet 60: 455-463
166 Mariani, Pirrotta & Manet (1985) EMBOJ 4:2045-2052
167 Chia, McGill, Karp, Gubb & Ashburner (1985) Nature 316: 81-83
168 Falkenthal, Parker, Mattox, Davidson (1984) Mol & Cell Biol 4: 956-965
169 Karlik & Fyrberg (1985) Cell 41:57-66
170 Karlik, Mahaffey, Coutu, Fyrberg (1984) Cell 37:469-481
171 Ayme & Tissieres (1985) EMBOJ 4: 2949-2954
172 Steward, McNally & Schedl (1984) Nature 311: 262
173 Hafen, Basler, Edstroem & Rubin (1987) Science 236: 55-63
174 Goralski, Konrad & Mahowald (unpubl), Biol Sci, Stanford U, Stanford CA 94305
175 Ait-Ahmed, Thomas-Cavallin & Rosset (1987), Devel. Biol. 122:153-162
176 Levis, Hazelrigg & Rubin (1985) EMBOJ 4: 3489-3499
177 Wharton, Johansen, Xu & Artavanis-Tsakonas (1985) Cell 43: 567-581
178 Katzen, Kornberg & Bishop (1985) Cell 41: 449-456
179 Fyrberg et al. (1980) Cell 19: 365-378
180 Hoey et al. (1986) PNAS USA 83: 4809-4813 [Devel Biol]
181 Goldberg, Posakony & Maniatis (1983) Cell 34: 59-73
182 Zink, Franke & Paro (1987), Heidelberg
183 Otto, Young & Maroni (1986) PNAS USA 83: 6025-6029
184 Bautch et al. (1982) J Mol Biol 162: 231
185 Steller (unpubl) Biochem Dept, UC-Berkeley CA 94720
186 Samson, 27th Annual Drosophila Conf, April 10-13, 1986, Asilomar Conf Ctr, Pacific Grove, CA
187 Harding, Rushlow, Doyle, Hoey & Levine (1986) Science 233: 953-959
188 Walldorf, Hovemann & Bautz (1985) PNAS USA 82: 5795-5799
189 Gelbart (pers comm), Biol Dept, Harvard U, Cambridge MA 02138
190 Yen & Davidson (1980) Cell 22: 137-148
191 Robinson & Davidson (1981) Cell 23: 251-259
192 Gergen, Loewenberg & Wensink (1981) J Mol Biol 147: 475-499
193 Schedl & Donelson (1978) Biochimica et Biophysica Acta 520: 539-554
194 DeLotto, Louis, Wormington & Schedl (1982) Mol Gen Genet 188: 299-304
195 Hosbach, Silberklang & McCarthy (1980) Cell 21: 169-178
196 Dudler, Egg, Kubli, Artavanis-Tsakonas, Gehring, Steward & Schedl (1980) Nucleic Acid Res 8(13): 2921-2937
197 Howells (in prep), Tearle et al. (in prep)
198 Goldstein et al. (1986) Biochem. Bioiphys. Acta 867: 209-219
199 Kidd, Lockette & Young (1983) Cell 34: 421-433
200 Hoffman & Corces, EMBOJ (submitted)
201 McKean et al. (1985) Molec Devel Biol Symposium
202 Digan et al. (1986) Devel Biol 114: 161-169
203 Hudson & Anderson (pers comm), Molec Biol Dept, UC-Berkeley CA 94720
204 Champe & Laird (pers comm), Zool Dept, U Washington, Seattle WA 98195
205 Garbe & Pardue (1986) PNAS USA 83: 1812-1816 [Genetics]
206 Campos, Rosen, Robinow & White (1987) EMBOJ 6(2): 425-431
207 Hammonds & Fristrom (pers comm), Genetics Dept, UC-Berkeley, CA 94720
208 Gustafson (pers comm), UC-San Francisco CA 94143
209 Phillips & Shearn (pers comm), Genetics Dept, U Melbourne, Melbourne, Australia
210 Graziani et al. (pers comm), Genetica e Biofisica, Istituto Internazion, 80125 Napoli, Italy
211 Johnson, Friedman & Edstrom (pers comm), Hutchinson Cancer Res Ctr, Seattle WA 98104
212 Garfinkle et al. (1983) J Mol Biol 168: 765
213 Doyle et al. (pers comm), Biol Dept, Columbia U, NY NY 10027
214 Williams & Bell (pers comm), Genetics Dept, U Alberta, Edmonton, Alb. Canada T6G 2E9
215 Butler, Pirrotta, Irminger-Finger & Nothiger (1986) EMBOJ 5(13): 3607-3613
216 Glew et al., Gene (in press)
217 Walker, Howells & Tearle (1986) Mol Gen Genet 202: 102-107
218 Frei, Baumgartner, Edstrom & Noll (1985) EMBOJ 4: 979
219 Moreau et al. (1985) Mol Gen Genet 199: 357-364
220 Lepesant et al. (1984) Nucleic Acid Res 12: 8835-8846
221 Maschat et al. (1986) EMBOJ 5(3): 583-588
222 Jacobs-Lorena (pers comm), Case Western Reserve U, Cleveland OH 44106
223 Maine, Salz, Cline & Schedl (1985) Cell 43: 521-529
224 J.W. Fristrom et al. (1986) Archives of Insect Biochem. & Physiol. 119-132
225 Gunaratne, Mansukhani, Lipari, Liou, Martindale & Goldberg (1986) PNAS USA 83: 701-705 [Genetics]
226 Kuroiwa, Kloter, Baumgartner & Gehring (1985) EMBOJ 4(13B): 3757-3764

227 Blackman & M. Meselson (1986) J Mol Biol 188: 499-515
228 Meyerowitz, Biol Div, CIT, Pasadena CA 91125
229 Pardue, Biol Dept, MIT, Cambridge MA 02139
230 Hirsh, Biol Chem Dept, Harvard Med Sch, Cambridge MA 02115
231 Hoffman, Harvard U, Cambridge MA 02138
232 Muskavitch, Alton, Fechtel, Kopezynshi, Shepard, Meikle & Terry (1987) 28th Drosophila Conf
233 Corces et al. (1980) PNAS USA 77(9): 5390-5393 [Genetics]
234 Baker (1987) EMBOJ 6(6): 1765-1773
235 Bingham, P. & C. Chapman (1986) EMBOJ 5(12): 3343-3351
236 Shore, E. & G. Guild (1986) J Mol Biol 190: 149-158
237 Eveleth et al. (1986) EMBOJ 5(10): 2663-2672
238 Lepesant, J.-A. et al. (1982) J Mol & Appl Gen 1: 371-383
239 Kral, L., Johnson, Burnett & Friedman (1986) Gene 45: 131-137
240 Semeshin, V.F. et al. (1986) Chromosoma (Berl) 93: 461-468
241 Spradling & Mahowald (1981) Cell 27: 203-209
242 Geyer, Spana & Corces (1986) EMBOJ 5(10): 2657-2662
243 Caggese, C. et al. (1985) Mol Gen Genet 204: 208-213
244 Johnson, Daniel, Thomas Dreesen & Steven Henikoff (unpubl)
245 Itoh, N. et al. (1986) PNAS USA 83: 4081-4085 [Neurobiology]
246 Cook, J. et al. (1986) J Biol Chem 261(25): 11751-11755
247 Mestril, R. et al. (1986) EMBOJ 5(7): 1667-1673
248 Marsh, Erfle & Leeds (1986) Genetics 114(2): 453-467
249 Eveleth & Marsh, Nucleic Acid Res in press
250 Eveleth & Marsh (1986) Genetics 114(2): 469-483
251 Hovemann, Walldorf & Ryseck (1986) Mol Gen Genet 204: 334-340
252 Lutzelschwab et al. (1986) Mol Gen Genet 204: 58-63
253 Schneuwly, Kuroiwa, Baumgartner & Gehring (1986) EMBOJ 5(4): 733-739
254 Lev & Segev (1986) Biochim et Biophy Acta 867: 144-151
255 Parks, Wakimoto & Spradling (1986) Devel Biol 117: 294-305
256 Lengyel, Judith (pers comm)
257 Murphy & Blumenfeld (1986) Nucleic Acid Res 14(13): 5563
258 Searles, Greenleaf, Kemp & Voelker (1986) Mol & Cell Biol 6(10): 3312-3319
259 Morgan, Johnson & Hirsh (1986) EMBOJ 5(12): 3335-3342
260 Cavener, Otteson & Kaufman (1986) Genetics 114: 111-123
261 Kidd, Kelley & Young (1986) Mol & Cell Biol 6(6): 1965-1973
262 Karlik & Fyrberg (1986) Mol & Cell Biol 6(6): 1965-1973
263 Jackson (unpubl)
264 DiBenedetto et al. (1987) Devel Biol 119: 242-251
265 Stroeher, Jorgensen & Garber (1986) Mol & Cell Biol 6(12): 4467-4675
266 Doyle, Harding, Hoey & Levine (1986) Nature 323(6083): 76-79
267 James & Elgin (1986) Mol & Cell Biol 6(11): 3862-3872
268 Elliot & Jackson (unpubl)
269 Fryxell & Meyerowitz (1987) EMBOJ 6(2): 443-451
270 Schneuwly, Kuroiwa & Gehring (1987) EMBOJ 6(1): 201-206
271 DeLotto & Spierer (1986) Nature 323: 688-692
272 Patel, Snow & Goodman (1987) Cell 48: 975-988
273 Yan, Kunert & Postlethwait (1987) Nucleic Acid Res 15(1): 67-85
274 Frischer, Hagen & Garber (1986) Cell 47: 1017-1023
275 Mirkovitch, Spierer & Laemmli (1986) J Mol Biol 190: 255-258
276 Chen, Denome & Davis (1986) PNAS USA 83: 9313-9317 [Biochemistry]
277 Eissenberg & Elgin (1986) Mol & Cell Biol 6(11): 4126-4129
278 Steller & Pirrotta (1986) Mol & Cell Biol 6(5): 1640-1649
279 Kiss, Istvan (pers comm)
280 Glaser, Wolfner & Lis (1986) EMBOJ 5(4): 747-754
281 Theurkauf, Baum, Bo & Wensink (1986) PNAS USA 83: 8477-8481 [Biochemistry]
282 Arribas, Sampedro & Izquierdo (1986) Biochim et Biophys Acta 868: 119-127
283 Baylies, Bargiello, Jackson & Young (1987) Nature 326: 390-392
284 Frigerio, Burri, Bopp, Baumgartner & Noll (1986) Cell 47: 735-746
285 Schejter, Segal, Glazer & Shilo (1986) Cell 46: 1091-1101
286 Rosenthal et al. (1987) EMBOJ 6(2): 433-441
287 Bopp, Burri, Baumgartner, Frigerio & Noll (1986) Cell 47: 1033-1040
288 Spencer, Gietz & Hodgetts (1986) Nature 322: 279-281
289 Schuh et al. (1986) Cell 47: 1025-1032
290 Padgett, Johnston & Gelbart (1987) Nature 325: 81-86
291 Neuman-Silberberg et al. (1984) Cell 37: 1027-1033
292 Banerjee, Renfranz, Pollock & Benzer (1987) Cell 49: 281-291
293 Boyer, Mahoney & Lengyel (1987) Nucleic Acid Res 15(5): 2309-2325
294 Tautz et al. (1987) Nature 327: 383-389
295 PIrrotta et al. (1987) EMBOJ 6(3): 791-799
296 Telford, Burckhardt, Butler & Pirrotta (1985) EMBOJ 4(10): 26099-2615
297 Tamura, Kunert & Postlethwait (1985) PNAS USA 82: 7000-7004 [Genetics]
298 Pauli, Spierer & Tissieres (1986) EMBOJ 5(4): 755-761
299 Modolell, Bender & Meselson (1983) PNAS USA 80: 1678-1682 [Genetics]
300 Kilchherr, Baumgartner, Bopp, Frei & Noll (1986) Nature 321: 493-499
301 Savakis, Ashburner & Willis (1986) Devel Biol 114: 194-207
302 Hall & Spierer (1986) EMBOJ 5(11): 2949-2954
303 Macdonald & Struhl (1986) Nature 324: 537-545
304 Macdonald, Ingham & Struhl (1986) Cell 47: 721-734
305 Regulski, McGinnis, Chadwick & McGinnis (1987) EMBOJ 6(3): 767-777
306 Kuner et al. (1985) Cell 42: 309-316
307 Hamblen et al. (1986) Jrl Neurogenetics 3: 249-291
308 Delaney, Smith, McClelland, Sunkel & Glover (1986) J Mol Biol 189: 1-11
309 Martin & Meyerowitz (1986) PNAS USA 83: 8654-8658
310 Knust et al. (1987) EMBOJ 6(3): ....
311 Levis, Bingham & Rubin (1982) PNAS USA 79: 564-568

312 Coleman et al. (1987) Genes & Development 1: 19-20
313 Haynes et al. (1987) PNAS USA 84: 1819-1823
314 Yamanaka et al. (1987) Nucleic Acid Res 15(8): 3335-3348
315 Celniker & Lewis (1987) Genes & Development 1: 111-123
316 Bishop & Corces (1987) 28th Drosophila Conf
317 Weischaus, Eric (pers comm), Biol Dept, Princeton U, Princeton NJ 08544
318 Rosen, cited in Shilo (1987) Trends in Genetics 3: 69
319 Bishop, cited in Shilo (1987) Trends in Genetics 3: 69
320 Katzen, Kornberg & Bishop (1985) Cell 41: 449-456
321 Laughon, Boulet, Bermingham, Laymon & Scott (1986) Mol & Cell Biol 6: 4676-4689
322 Davis & MacIntyre (1987) Genetics 116: 249
323 Eissenberg, Elgin & James (1987) Genetics 116: s4
324 Mlodzik & Gehring (1987) Cell 48: 465-478
325 McGill, Chia, Karp, Ashburner (1987) Genetics
326 Tiong & Nash (1987) 28th Drosophila Conf
327 Biggs, Tripoulas & Shearn (1987) 28th Drosophila Conf
328 Miner, Burn, Lang & Cheney (1987) 28th Drosophila Conf
329 Brunk, Wu & Adler (1987) 28th Drosophila Conf
330 Lin, Goutte & Wolfner (1987) 28th Drosophila Conf
331 Steinhauer & Kalfayan (1987) 28th Drosophila Conf
332 Saxton, Porter, Scholey, McIntosh & Raff (1987) 28th Drosophila Conf
333 Tucker, Valoir, Bolikoff & Bechingham (1987) 28th Drosophila Conf
334 Shaffer & MacIntyre (1987) 28th Drosophila Conf
335 Davis, Grieg, Nero & MacIntyre (1987) 28th Drosophila Conf
336 Jones & Rawls (1987) 28th Drosophila Conf
337 Nortinb, Weeks, & Greenleaf (1987) 28th Drosophila Conf
338 Kamb, Iverson & Tanouye (1987) Cell 50: 405-413
339 Salkoff, Butler, Wei, Scavarda, Goodman & Mandel (1987) 28th Drosophila Conf
340 Kammermeyer, Rosenthal, Potter & Wadsworth (1987) 28th Drosophila Conf
341 Ellis, Spann & Posakony (1987) 28th Drosophila Conf
342 Gergen (1987) 28th Drosophila Conf
343 Stephenson & Chao (1987) 28th Drosophila Conf
344 Yedvobnick, Smoller, Mills & Young (1987) 28th Drosophila Conf
345 Ismard Lamour, Boucham-Limbourg, Busson, Mariiol & Preat (1987) 28th Drosophila Conf
346 Coulter (1987) 28th Drosophila Conf
347 Cohen, Di-Biao, Jergens & Jackle (1987) 28th Drosophila Conf
348 Ambrosio, Perrimon & Mahowald (1987) 28th Drosophila Conf
349 Henkemeyer, Gertier, Goodman & Hoffmann (1987) 28th Drosophila Conf
350 Kennison & Tamkun (1987) 28th Drosophila Conf
351 Laski, Rio & Rubin (1986) Cell 44: 219
352 Cooley (pers comm)
353 Fuller (pers comm)
354 Dalton, Chadwick, McGinnis & McGinnis (1987) 28th Drosophila Conf
355 Barad, Jack & McGinnis (1987) 28th Drosophila Conf
356 Powers & Ganetzky (1987) 28th Drosophila Conf
357 Nash, Leonard, Filips & Tiong (1987) 28th Drosophila Conf
358 Chin, Ricter-Mann, & Gertler (1987) 28th Drosophila Conf
359 Popodi, Burke, Minou & Waring (1987) 28th Drosophila Conf
360 Tansey, Mikus, Dumoulin & Starti (1987) EMBOJ 6(5): 1375-1385
361 Hursh, Andrews, Mortin, Hunt & Raff (1987) 28th Drosophila Conf
362 Kelley (1987) 28th Drosophila Conf
363 Watanable & Kankel (1987) 28th Drosophila Conf
364 Jackson, Elliott, Newley-Kew (1987) 28th Drosophila Conf
365 Rothberg, Hartlry & Artavanis-Tsakonas (1987) Yale
366 Margolis, Shim, Seavello & Posakony (1987) 28th Drosophila Conf
367 Pardue, Lowenhaupt, Rich & Nordheim (1987) EMBOJ 6(6): 1781-1789
368 Brodberg, Harris, Yamamoto, Boyd & Mason (1987) 28th Drosophila Conf
369 Banza, Yamamoto, Mason & Boyd (1987) 28th Drosophila Conf
370 Yang, Saxton & Goldstein (1987) 28th Drosophila Conf
371 Villares & Cabrera (1987) Cell 50: 415-424
372 Caizzi, Ritossa, Ryseck, Richter & Hovemann (1987) Mol Gen Genet 206: 66-70
373 Beamonte, Campuzano & Modolell (1987) 10th Euro Dros Conf, Barcelona
374 Belyaeva, Protopopov, Baricheva & Zhimulev (1987) 10th Euro Dros Conf, Barcelona
375 Boulay, Dennefield & Alberga (1987) 10th Euro Dros Conf, Barcelona
376 Cecchini, Knibiehler, Mirre & LeParco (1987) 10th Euro Dros Conf, Barcelona
377 Chapel, Sobrier, Coaderc, Micard, Bruhat & Dastugue (1987) 10th Euro Dros Conf, Barcelona
378 Dauwalder & Kubli (1987) 10th Euro Dros Conf, Barcelona
379 Furia, Cavaliere, Artiaco, Digilio & Polito (1987) 10th Euro Dros Conf, Barcelona
380 Galceran, Sampedio, Ilanos & Izquierdo (1987) 10th Euro Dros Conf, Barcelona
381 Hidalgo, Guerrero, Whittle & Ingham (1987) 10th Euro Dros Conf, Barcelona
382 Jacq, Fjose & Gehring (1987) 10th Euro Dros Conf, Barcelona
383 Schafer (1986) Mol Gen Genet 202:219
384 Kuhn, Borsch, Huvermann, von Eynery, Schafer & Schafer (1987) 10th Euro Dros Conf, Barcelona
385 Schafer, Weinert, Simmerl, Rosenkranz, Senftleben & Schafer (1987) 10th Euro Dros Conf, Barcelona
386 Schuh, Frei, Rentes, Baumgartner, Burri, Noll & Jackle (1987) 10th Euro Dros Conf, Barcelona

387 Tietze, Knust & Campos-Ortega (1987) 10th Euro Dros Conf, Barcelona
388 Vincent, Lepesant, Yanicosta & Lepesant (1987) 10th Euro Dros Conf, Barcelona
389 Walldorf & Gehring (1987) 10th Euro Dros Conf, Barcelona
390 Fasano & Kerridge (1987) 10th Euro Dros Conf, Barcelona
391 O'Kane & Gehring (1987) PNAS
392 Fleming, DeSimone & White (Dec 1987) Brandeis
393 Lebrovitz & Fambrough (1987) Johns Hopkins
394 Iverson, Kamb, Ramaswami, Tseng-Crank & Tanouye (1987) Cal-Tech
395 Yarfitz, Prevost & Hurley (1987) Biochem., U of Wash., Seattle
396 Nambu, Murphy-Erdosh, Andrews, Feistner & Scheller (1987) Stanford
397 deCouet, Yamamoto, Davies, Pirrotta & Miklos, RSBS, Canberra
398 Steller, Heilig, Fischbach & Rubin (1987) Berkeley
399 Zinn, McAllister, Snow, Elkins & Goodman (1987) Stanford
400 Neckameyer & Quinn (1987) MIT
401 Okamoto, Taka-Ishihawa, Sakai, Goto, Nakao, Suzuki & Hotta (1987) Tokyo
402 Schaeffer, Choi, Mardon & Quinn (1987) MIT
403 Schneider & Taghert, Washington Univ, St. Louis
404 Bossy, Ballivet & Spierer (1987) Geneva
405 Nauber, Lehman & Jackle (1987) Tübingen
406 Weigel, Jurgens & Jackle (1987) Tübingen
407 Richter & Hovemann (1987) Heidelberg
408 Schafer, Molsberger, Kuhn & Schafer (1987) Düsseldorf
409 Bogaert & Wilcox (1987) MRC Cambridge
410 Engels (1987) Madison
411 Delidakis & Kafatos (1987) J Mol Biol 197:11-26
412 Thise, El MEssal & Perrin-Schmidt (1987) Nuc Ac Res 15:3439-3459
413 Klemenz, Weber & Gehring (1987) Nuc Ac Res 15:3947-3960
414 Flavell, Dyson & Ish-Horowicz (1987) Nuc Ac Res 15:4035-4048
415 McNabb & Beckendorf (1986) EMBOJ J 5:2331-2340
416 Davis & Davidson (1986) MCB 6:1464-1470
417 Chen, Malone, Beckendorf & Davis (1987) Nature 329:721-724
418 Belyaeva, Protopopov, Baricheva, Semeshin, Izquierdo & Zhimulev (1987) Chromosoma 95:295-310
419 Swaroop, Sun, Paco-Larson & Garen (1986) MCB 6:833-841
420 Swaroop, Swaroop & Garen (1987) PNAS 84:6501-6505
421 Cooley, Kelley & Spradling (1988) Science 239:1121-1128
422 McKeown, Belote & Baker (1987) Cell 48:489-499

REPORT FORM

THE DROSOPHILA CLONE LIST BY CHROMOSOME LOCATION

Corrections and additions may be sent using this report form or by using a copy of the page from the list.

New fields RECORD NO _____ REF NO. _____
version 7/24/87

4. Date _____

5. Location _____

6. Table Num____0____1____2____3____4____5____6_____

7. Clone Name _____

8. Genes Info _____

9. Stock Orgn _____

10. KB Size _____

11. Cross Ref _____

12. Misc Info _____

_____

13. Reference _____

14. Breakpoint _____

15. BPCont1 _____

16. BPCont2 _____

17. T Strain _____

18. Markers _____

19. DIS Vol _____

20. Comments _____

21. KB Sequenced _____

22. Coordinates _____

_____

_____

key to Table num for this record: 0 = new data and ref on clone previously entered, 1 = clone, 2 = rearrangement breakpoing, 3 = insert, 4 = walk, 5 = sequenced, 6 = transcription unit. multiple entries ok (ie 1,2,4,5,6)