DROSOPHILA INFORMATION SERVICE

80

July 1997

Material contributed by
DROSOPHILA WORKERS

and arranged by
James N. Thompson jr.

prepared at
Department of Zoology
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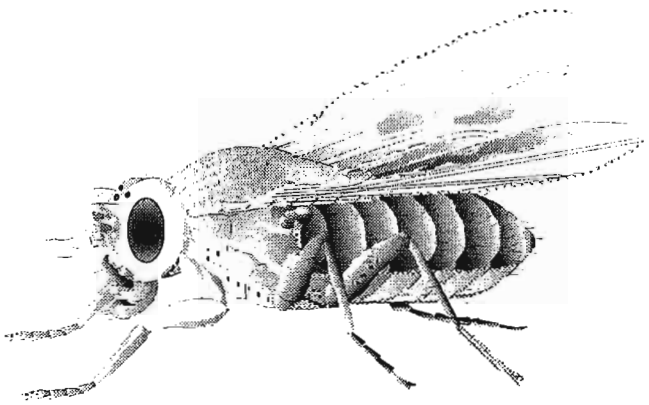
Norman, Oklahoma 73019

DROSOPHILA INFORMATION SERVICE



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Preface

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

The production of DIS 80 could not have been completed without the generous efforts of many people. Diane Jackson and Stanton Gray helped prepare and proof manuscripts; Gloria Stephens, Diane Jackson, Shalia Newby, and Caroline Tawes maintained key records; and Coral McCallister advised on artwork and computer graphics. Two special issues of Drosophila Information Service have appeared since DIS 77, the 1996 regular issue. DIS 78 and 79 were edited by William Gelbart on behalf of the FlyBase Consortium. Information on these two special issues can be obtained from Tatiana Murnikova (e-mail, dis78@morgan.harvard.edu). Because so much of the lengthy stock list and bibliographic data printed in previous DIS issues is now either available through electronic sources like FlyBase or these special issues, we expect that the regular annual issue will focus more exclusively on research, technique, new mutant, and teaching notes and on lists of supplementary or supporting data that cannot be included in research articles published elsewhere. Your contributions to any of these sections are warmly welcomed.

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

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Special issues of Drosophila Information Service are edited by William M. Gelbart, on behalf of the FlyBase Consortium. Ordering information is provided at the end of this issue.

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Research Notes

Babcock, C. S.[1], D.G. Baldwin[1], W. B. Heed[1], C. A. Istock[1], J. S. Russell[1], and W. J. Etges[2]. [1]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721. [2]Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701. Third chromosome inversion polymorphism in Baja California populations of *Drosophila pseudoobscura.*

The genus *Drosophila* was sampled throughout the Baja peninsula on March 19 - 26, 1996. Flies were collected by baiting with fermented bananas in twenty 2.5 quart plastic buckets. Dawn and dusk collections were made with buckets placed approximately 30 feet apart under trees, where available, shrubs, or cacti. Adult *D. pseudoobscura* were obtained from the three northern-most of seven sites baited (Fig. 1). The Observatory Road collection was made at about 6000' along the road to the Sierra San Pedro Martir National Park in a pine and oak forest along a dry creek bed similar to *D. pseudoobscura's* more northern habitats. The Punta Prieta collection was made in an open desert location in the Viscaino biotic province, characterized by several species of columnar cacti, agaves, boojums, and other desert vegetation. The small sample from San Ignacio was obtained in an irrigated date palm plantation near a permanent spring.

Chromosomes were analyzed from a single larva of each isofemale line to determine the karyotype. Sample sizes were moderate to small. The Observatory Road sample was found to be highly polymorphic for third chromosome gene arrangements (Table 1). This population shares similar gene arrangement frequencies with other northern Baja California populations, with the exception of TL which has not been observed in Baja until now. Flies from Punta Prieta were polymorphic for two gene arrangements and the sample from San Ignacio, while admittedly small, was also polymorphic.

When considering the frequency data from this and previous studies (Table 1), there appears to be an emerging geographic pattern of gene arrangements according to latitude in Baja California. The ST and AR inversions are common throughout the peninsula. CH and TL are present at low to moderate frequencies in the Northern Baja populations, but are absent from the Southernmost Baja populations. The SC gene arrangement is present at moderate to high frequencies in the Southernmost populations, but has only been found at low frequencies in the Northern populations. In contrast, the two mainland populations included in Table 1 appear to have different distributions of gene arrangements than the Baja populations.

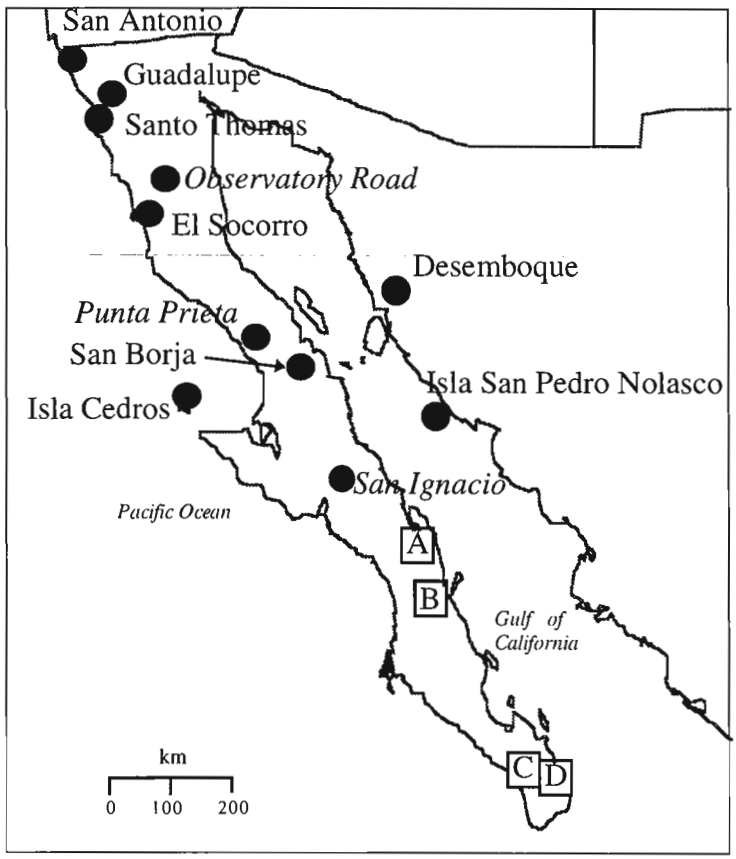

Figure 1. Collecting sites for *D. pseudoobscura* in the Sonoran Desert in and around Baja California. Sites in italics yielded *D. pseudoobscura* in this study, those indicated by a lettered box did not.

All males collected in this study were crossed to laboratory stocks to check for the presence of the Sex-Ratio phenotype (n = 25). Of these crosses, all yielded nearly equal numbers of male and female offspring, indicating that none of the wild-caught males carried the Sex-Ratio X chromosome. However, two females from Observatory Road and a single female from San Ignacio were determined to be heterokaryotypic for the three inversions associated with the Sex-Ratio X chromosome.

Four additional sites south of San Ignacio were sampled by baiting (Fig. 1, A-D). These sites yielded a total of 153 individuals of 8 species in the genus *Drosophila*, but no *D. pseudoobscura* (Table 2). Thus, it appears

from this study that central Baja California may be a boundary for the geographical range of *D. pseudoobscura*. However, one of us (W. B. Heed, unpublished) has records of a few *D. pseudoobscura* individuals collected from the Cape Region in the early spring of 1970, and the winter of 1981. Future collecting in the extensive date palm plantation in Mulegé and the higher elevations of the Sierra de la Giganta north of La Paz and the Sierra de la Laguna in the Cape Region will be required to determine if indeed central Baja has become a species boundary for *D. pseudoobscura*.

Table 1. Site locations and percentage of gene arrangements listed from Northernmost to Southernmost along Baja California and mainland Sonora.

Location	ST	AR	SC	CH	TL	PP	N[1]	Study
San Antonio Mesa	58	24	0	18	0	0	72	Dobz. and Epling, 1944
Santo Tomas and Guadalupe	60	28	9	3	0	0	32	*ibid*
Observatory Road	75	5	0	10	10	0	20	this study
El Socorro	93	0	7	0	0	0	28[2]	Anderson *et al.*, 1991
Desemboque (mainland)	50	33	0	17	0	0	24[2]	*ibid*
Punta Prieta	33	0	67	0	0	0	18	this study
San Borja	67	13	20	0	0	0	64	Anderson *et al.*, 1991
Isla Cedros	54	31	15	0	0	0	26	Dobz. and Epling, 1944
Isla S. Pedro Nolasco (main)	8	63	25	4	0	0	24[2]	Jefferson *et al.*, 1974
San Ignacio	25	0	75	0	0	0	4	this study

[1]Number of chromosomes.
[2] Fewer individuals were collected, but several were testcrossed to infer the chromosomes of *both* parents of captured adult.

Table 2. Number of individuals captured by baiting according to species[1] and site sampled.

Site	pse	mel	sim	bus	hyd	moj	nig	mai	ald	ari	spe	ham
Observatory Road	15	0	0	3	1	0	0	0	0	0	0	3
Punta Prieta	19	0	0	0	0	65	5	0	0	0	0	0
San Ignacio	12	0	11	0	0	0	0	0	0	0	0	0
A.Bahia Concepcion	0	0	0	0	0	15	0	0	0	0	0	0
B.Punta Agua Verde	0	0	0	0	0	11	4	6	2	0	0	0
C. Todos Santos	0	6	20	0	0	31	0	0	0	0	0	0
D. Santiago	0	2	4	0	0	43	0	4	3	1	1	0

[1]Species abbreviations are as follows: pse, *D. pseudoobscura*; mel, *D. melanogaster*; sim, *D. simulans*; bus, *D. buskeii*; hyd, *D. hydei*; moj, *D. mojavensis*; nig, *D. nigrospiracula*; mai, *D. mainlandi*; ald, *D. aldrichi*; ari, *D. arizonae*; spe, *D. spenceri* and ham, *D. hamatofila*.

Acknowledgments: Chromosomes were analyzed in the laboratory of Dr. M. G. Kidwell, C. S. Babcock was supported by a NIH Postdoctoral Training Grant, and this work was supported by an NSF grant to W. J. Etges and R. DeSalle.

References: Anderson W. W. *et al.*, 1991, PNAS 88: 10367-10371; Dobzhansky, Th. and C. Epling 1944. *Carnegie Inst. Washington Publ.* 554; Jefferson, M. C. *et al.*, 1974, Dros. Inf. Serv. 51:65.

Shivanna, N., and S.R. Ramesh. Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570 006, India. Intraspecific larval pupation site preference in *Drosophila melanogaster*.

The larval pupation site preference (PSP) is an important event in *Drosophila* preadult development; because the place selected by the larvae can have decisive influence on their subsequent survival as pupae (Sameoto and Miller, 1968). Investigations on PSP in various species such as *D. melanogaster, D. simulans, D. willistoni, D. pseudoobscura, D. nigrospiracula, D. mettleri, D. ananassae, D. bipectinata,* and *D. malerkotliana* (Sokal, 1966; Sameoto and Miller, 1968; DeSouza, *et al.*, 1968; Markow, 1979; Fogleman and Markow, 1982; Sokolowski, 1985; Singh and Pandey, 1991; Pandey and Singh, 1993) have been made. In most of these studies, the influence of various factors on PSP has been investigated by measuring the pupation height (the distance a larva pupates above the surface of the food medium). The PSP has also

been studied in different species namely, *D. melanogaster, D. simulans, D. gibberosa, D. mauritiana, D. yakuba, D. ananassae, D. rajasekari, D. hydei, D. nasuta nasuta, D. n. albomicans, D. n. kepulauana, D. sulfurigaster sulfurigaster, D. s. neonasuta, D. immigrans, D. rubida,* and *D. pararubida* by analyzing the percentage of pupae pupated at different sites viz., cotton, glass wall, and medium in the cultures (Barker, 1971; Shirk, *et al.*, 1988; Shivanna, *et al.*, 1996). These investigations have been focused on understanding the interspecific differences in the larval PSP by employing only one strain for every species analyzed. Further, the knowledge on intraspecific differences with regard to the pupation height in two species of *Drosophila* is only preliminary (Singh and Pandey, 1991). Present studies were undertaken to unravel the nature and extent of variations if any, with regard to the intraspecific larval PSP employing 5 wild type strains of *D. melanogaster* under constant environmental conditions. Oregon-K, Oregon-R, Berlin, Ithaca, and Canton-S were the wild type strains of *D. melanogaster* used in the present study.

In order to maintain uniformity with regard to age and density of larval populations during development, eggs of synchronized age were collected by modified Delcour technique (Ramachandra and Ranganath, 1986). Fifty first instar larvae were transferred into each culture vial (3" × 1") containing equal amounts of wheat cream agar medium. These cultures were raised at a constant temperature of 22 ± 1°C and a relative humidity of 80%. Further, 50 µl of fresh yeast solution per vial was added every alternate day to maintain moisture. After pupation of the larvae, the number of pupae at different sites were counted.

The mean and percentage of pupation in each case was calculated based on the data obtained from 10 replicates (Table 1). It is evident that the larvae of all wild type strains prefer to pupate to a greater extent on glass walls of the culture vial. Among different strains, maximum preference to pupate on the glass walls is observed in the case of Oregon-K strain; while the extent of preference to pupate on glass wall is minimum (89.6%) in the case of Canton-S larvae. Further, 9% of Oregon-R and Canton-S larvae are seen to pupate in the medium; while the percentage of pupation in the medium in Oregon-K is 5.4.

Table 1. Mean values and percentage of PSP in different wild type strains of *D. melanogaster.*

Strain*	Cotton Mean± SD	%	Glass Mean ± SD	%	Medium Mean ± SD	%
Oregon-K	0.3 ± 0.47	0.6	46.6 ± 1.23	94.0	3.1 ± 1.33	5.4
Oregon-R	0.6 ± 0.85	1.2	44.9 ± 1.74	89.8	4.5 ± 1.52	9.0
Berlin	0.5 ± 0.70	1.0	46.2 ± 1.49	92.4	3.3 ± 1.42	6.6
Ithaca	1.0 ± 1.17	2.0	45.1 ± 1.74	90.2	3.9 ± 1.74	7.8
Canton-S	0.7 ± 0.47	1.4	44.8 ± 1.33	89.6	4.5 ± 1.27	9.0
Mean	0.62 ± 0.73	1.24	45.52 ± 1.51	91.2	3.86 ± 1.46	7.56

* Stocks obtained from *Drosophila* Stock Centre, Dept. of Studies in Zoology, University of Mysore, Mysore-570 006, India.

The data on PSP was analyzed by two-way ANOVA to test the significance of variation in PSP among different wild type strains. Such an analysis revealed that the differences between strains at all three sites were insignificant, whereas the differences in preference between sites in all five strains analyzed were found to be significant ($df = 2$, $F = 4012.44$, $p < 0.001$) at 1% level.

Intraspecific variations in larval PSP has been studied by measuring to pupation height employing five strains of *D. bipectinata* and seven strains of *D. malerkotliana* (Singh and Pandey, 1991). Such a study has revealed significant intraspecific variation in pupation height only in the case of *D. bipectinata*; but not in the case of *D. malerkotliana.* Present study reveals absence of intraspecific variations in larval PSP in the case of *D. melanogaster* and confirms the findings of Barker (1971), Shirk, *et al.* (1988), as well as of Shivanna, *et al.* (1996) made by using only one wild type strain.

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References: Barker, J.S.F., 1971, Oecologia 8: 139-156; DeSouza, H.M.L., A.B. da Cunha, and E.P. dos Santos 1968, Amer. Nat. 102: 583-586; Fogleman, J.C. and T.A. Markow 1982, Southwest Nat. 27: 315-320; Markow, T.A., 1979, Behav. Genet. 9: 209-217; Pandey, M.B., and B.N. Singh 1993, Indian J. Exptl. Biol. 31: 912-917; Ramachandra, N.B., and H.A. Ranganath 1986, Chromosoma 93: 243-248; Sameoto, D.D., and R.S. Miller 1968, Ecology 49: 177-180; Shirk, P.D., P.A. Roberts, and C.H. Harn 1988, Roux's Arch. Dev. Biol. 197: 66-74; Shivanna, N., G.S. Siddalingamurthy, and S.R. Ramesh 1996, Genome 39: 105-111; Singh, B.N., and M. Pandey 1991, Indian J. Exptl. Biol. 29: 926-929; Sokal, R.R., 1966, Univ. Kansas Sci. Bull. 46: 697-715; Sokolowski, M.B., 1985, J. Insect Physiol. 31: 857-864.

Gupta, A., W. Tadei, A. Monzato, E. Maciel, and E. Godoi. Univ. Fed. da Paraíba, CCEN/DBM, 58.059-900 Joao Pessoa - PB, Brazil. A genetic study on gene regulatory systems between sibling species of *Drosophila*.

It is well established that *D. pseudoobscura* and *D. persimilis* are two sibling species. However, the two may have genetic differences in their gene regulatory systems underlying morphological characters. In order to answer this question, we have analyzed the data collected by Gupta, for the following meristic characters: 1) bristle number on 4th and 5th sternites in both sexes, 2) tooth number on proximal and distal sex combs on both legs, 3) genital arch tooth number on both sides (for details of materials and methods, see Gupta 1978, Evol. 32: 580-587). For each individual fly, average bristle number was computed using both sternites, both proximal as well as both distal sex combs, and genital arch tooth number on both sides. Such calculations were made for parental, F1 and backcross classes at two temperatures: 17.5° and 25.5°C. The results showed that the bristles on sternites are sex-linked. The transmission of such bristles from parental to F1's occurs in additive fashion and later segregate accordingly in their backcross classes. This holds true at both temperatures. A comparison (2x2) between the parental and backcross classes made by Tukey test showed that the backcross individuals, obtained from *D. pseudoobscura* Sc-h-11 x *D. persimilis* FC-46, gave higher number of significant classes than their parental classes at 17.5° than at 25.5°C, while the backcross classes obtained from *D. pseudoobscura* Sc-f-8 x *D. persimilis* FC-51B males gave the opposite results at these temperatures. In general, it clearly demonstrates that the temperature plays a significant role in the development of bristles analyzed in question (with the exception of bristles at distal sex combs where the backcross classes obtained from both the crosses were higher in number at 17.5° than at 25.5°C, when compared with the parental classes).

The ANOVA showed the existence of not only the effect of genotype and temperature but also a significant interaction effect, for each meristic character in question. This implies that the regulatory genes for the decrease or increase in bristle number are also influenced by such an interaction effect. That is to say, the increase or decrease in bristle number does depend upon the degree of an interaction that occurred during its development.

For each of the parental, F1's and backcross classes, the correlation analysis for the bristle number at two temperatures was made for the following: 1) sternites vs. proximal sex combs, 2) sternites vs. distal sex combs, 3) proximal vs. distal sex combs, 4) proximal vs. genital arch tooth number, and 5) distal vs. genital arch tooth number. The backcross individuals, in general, showed larger correlation values than their parental classes. It holds true at both the temperatures. Such observations indicate that there exists a common relationship among the gene regulatory systems for the development of the types of bristles studied. However, we do not know yet the degree of such relationship. It is interesting to note that specifically for bristle number on sternites vs. proximal sex combs, *D. pseudoobscura* Sc-h-11 males showed negative correlation at 17.5° but positive at 25.5°C. On the other hand, *D. persimilis* FC-46 males gave negative correlation value at both temperatures. The F1 classes showed positive or negative correlation value depending upon whether the parental males of the species had positive or negative correlation. However, the correlation values for each of the four backcross classes, obtained from *D. pseudoobscura* Sc-h-11 x *D. persimilis* FC-46, were found to be positive at 17.5° and negative at 25.5°C. Such results indicate that the developmental pathways for such bristles controlled by the gene regulatory systems are disturbed. That is to say that the gene regulatory systems of *D. pseudoobscura* are different from those of *D. persimilis* responsible for the development of bristles analyzed in question, and that such genes express their effects only in backcross individuals, and that clearly explains for the existence of very low viability in backcross classes. Thus, such a divergence in the structural as well as regulatory genes between the two species is the main cause for the occurrence of very low viability (specifically for males) in the backcross progeny. These results, therefore, imply that in addition to the reproductive characters such as spermatogenesis, the gene regulatory systems are also affected at the time of speciation. In other words, *D. pseudoobscura* and *D. persimilis* did diverge in their gene regulatory systems at the time of speciation (however, to what extent we do not know yet).

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Kekic, V. Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Yugoslavia. *Drosophila* community in barrels containing fermenting grapes.

It is common for fruit and wine growers in my country to distill homemade brandy, and it is still done in an old way: fruit is placed in barrels covered by cotton (or by plastic nowadays) cover, and stirred at

least once a day (in order to prevent molding), until the end of fermentation.

Above three such barrels, placed outdoors close to a distillery, containing 1,500 kg of fermenting grapes, I collected flies by sweeping on October 10 and 11, 1996, from 5 to 5:30 p.m., both days when there were plenty of flies, at a temperature of 18°C. It was in Sremska Kamenica (200 m a.s.l.) on Fruska gora Mt. (about 70 km north from Belgrade) in a habitat most closely described as a settlement with summer houses with vineyards, orchards and vegetable gardens. The habitat contains numerous trees (mostly coniferous), bushes and flowers around the houses, for decorative purposes.

Table 1 presents collected *Drosophila* species, as well as results of our previous study (Kekic *et al.*, 1983, 1985) which was performed in similar microhabitats (next to barrels with fermenting plums) and in the same season (during October and the beginning of November) on 29 geographic localities in Bosnia and Herzegowina (in a range of 90 to 1.031 m a.s.l.).

The general similarity in *Drosophila* species composition in both studies is obvious. But there are dissimilarities that can be explained by differences in habitats and, above all, in methods of collecting. Flies from Fruska gora were captured in barrels, while in Bosnia and Herzegowina they were captured near them (alcohol concentration in barrels is several times higher than by the barrels).

All captured *Drosophila* flies belong to domestic species (Dobzhansky, 1965; Parsons and Stanley, 1981), except *D. subobscura*. As it was expected, *D. melanogaster* was a dominant species. Someone can be surprised by a relatively minor presence of its sibling species *D. simulans*. This species is rather rare in Sremska Kamenica, and these were the first specimens captured at this locality after almost twenty years (Kekic, 1990). In studies of grape breeding *Drosophila* communities in vineyards of southern France, also in October, Capy *et al.* (1987) found the relative frequencies of the sibling cosmopolitan species highly variable: in the two places *D. simulans* was almost absent, and in one place, on the other hand, *D. simulans* was much more abundant than *D. melanogaster*.

Table 1. *Drosophila* species collected by sweeping over barrels containing fermenting grapes on Fruska gora Mt., Yugoslavia. For Bosnia and Herzegowina collections see text.

Species	Fruska gora Mt.	Bosnia & Herzegowina*
D. melanogaster	10,929	9,508
D. hydei	32	58
D. busckii	6	25
D. immigrans	6	54
D. simulans	9	48
D. subobscura	1	—
D. funebris	—	85
Total	10,983	9,778

* Kekic et al., 1983

The finding of *D. subobscura* is more interesting because of its relatively low tolerance of alcohol - if it is expressed by LC50 (the lethal concentration of ethanol killing 50% of the flies), then for the French population of *D. subobscura* the LC50 was 3.3%, while, for the sake of comparison, in the various French populations of *D. melanogaster* the LC50 was on average 19.8± 0.6% (Capy *et al.*, 1987). On the other hand, various studies demonstrate *D. subobscura* being rather widespread and ecologically versatile, e.g. it is dominant in most of "wild" (forest) habitats of ex Yugoslavia, very frequent in "semi-domestic" habitats (vineyards, orchards, city parks, etc.), and it can be scarcely found even in "domestic" habitats, sometimes inside the apartments (Kekic and Bächli, 1995). The recent colonization of the species in America, and its efficient spreading over the continent, also affirm the adaptability of *D. subobscura* (Krimbas, 1993). And, finally, Capy *et al.* (1987) found it emerges from grape, also, like *D. melanogaster* and *D. simulans*.

On the basis of 20 years continuing studies of *Drosophila* fauna in vineyards and orchards of Sremska Kamenica, it is to my knowledge that in this habitat live 16 *Drosophila* species (Kekic, 1990). They can all be captured by sweeping over a small quantity of fermenting fruit bait. The larger portion of fermenting fruit, producing high concentration of alcohol, is attractive only to several species (Table 1) probably only as a feeding substrate for most of them. At this moment I am not convinced if the found species lay eggs on this substrate - the only thing I am positive about is that only *D. melanogaster* can complete its development in it (maybe *D. simulans* and *D. hydei*, also, but it is only an assumption).

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Inhibition of *Drosophila melanogaster* and *D. hydei* by *Aspergillus niger*.

Introduction: In the laboratory, moulds can be a pest of *Drosophila* cultures, requiring the use of fungicides or the maintenance of high larval density to restrain fungal growth (Ashburner and Thompson, 1978). One common contaminant of laboratory *Drosophila* cultures is *Aspergillus niger*, which is also often found on natural *Drosophila* resources (Ashburner and Thompson, 1978; Sinha and Saxena, 1988; Hodge, 1996). In this paper we compare the effect of *A. niger* on the larvae of *D. melanogaster* and *D. hydei* and examine to what extent the observed effects can be explained by fungal 'metabolites' leached into the larval resource.

Methods: The *Drosophila* species used in the experiments were wild-type strains of *D. melanogaster* Meigen and *D. hydei* Sturtevant. The environment for all experiments described was an incubator maintained at 25±1°C and a 16:8 hour light:dark cycle. The relative humidity was not controlled but was generally between 45-55%.

To examine the effect of *A. niger* on *Drosophila*, forty first instar larvae were transferred to glass vials (75 x 25mm diameter). 0.5 g of Instant Drosophila Medium (IDM) hydrated with 3ml of distilled water was used as a resource. Half of the vials were then inoculated with *Aspergillus* by direct transfer of spores from a pure culture using a sterilized needle.

To prepare fungal 'extract' 6g of IDM were placed into each of six Petri dishes and hydrated using 36ml of distilled water. Three of the Petri dishes were inoculated with spores of *A. niger* from a pure culture and all six Petri dishes incubated for two weeks. After this time all three of the fungus-covered plates were emptied into a glass beaker with 200ml of distilled water and mixed thoroughly. The mixture was filtered twice using Buckner apparatus (10 μm pore size) to remove any fungal material and solid residues and the filtrate ('fungal extract') placed into sterile glass bottles for later use. The above procedure was repeated for the non-inoculated Petri dishes to obtain an 'IDM extract'. This was used to distinguish between the effects of fungal metabolites and the effects of other substances that may occur on two week-old IDM.

To examine the effect of the extracts on larval survival, 0.5g of IDM was placed into glass vials with 3ml of one of three liquids: (I) fungal extract, (ii) IDM extract or (iii) distilled water. Twenty-five first instar larvae were introduced into each vial.

To examine whether the larvae showed a behavioral response to the presence of fungal substances in the medium, the height from the base of the vial at which the larvae pupated was measured and a mean pupal height for each vial was calculated. For all three experiments replicate numbers are given in the results.

Results: *D. melanogaster* survival was not affected by the presence of *Aspergillus* fungus in cultures, whereas the survival of *D. hydei* larvae was significantly reduced (Table 1).

Table 1. Survival of *Drosophila* larvae (%; mean + SE (N)) in absence and presence of *Aspergillus niger*. Data were arcsine-root transformed before analysis.

	Aspergillus absent	*Aspergillus* present	F	P
D. melanogaster	40.5 ± 6.0 (8)	32.8 ± 9.2 (9)	0.88	> 0.35
D. hydei	56.0 ± 3.0 (8)	14.0 ± 10.0 (8)	18.3	< 0.001

Table 2. Survival of *Drosophila* larvae (%; mean + SE (N)) when using different extracts to hydrate the resource. Data were arcsine-root transformed before analysis.

	Distilled water	IDM extract	*A. niger* extract	F	P
D. melanogaster	90.0 ± 3.1 (12)	96.0 ± 1.2 (7)	88.0 ± 3.0 (12)	0.89	> 0.40
D. hydei	81.6 ± 2.4 (5)	56.0 ± 5.1 (5)	29.6 ± 5.7 (5)	29.3	< 0.001

Table 3. Pupation heights of *Drosophila* larvae (mm; mean ± SE, N = 5 for all treatments) when using different extracts to hydrate the resource.

	Distilled water	IDM extract	*A. niger* extract	F	P
D. melanogaster	21.4 ± 0.8	19.0 ± 1.2	25.9 ± 1.3	10.2	< 0.005
D. hydei	13.7 ± 0.8	3.8 ± 0.3	36.3 ± 1.6	324.1	< 0.001

With regard to 'extract' used to hydrate the medium, the liquid used had no effect on the survival of *D. melanogaster* larvae (Table 2). However, the number of *D. hydei* emergents was significantly affected, with larval survival being lowest when *Aspergillus* extract was used (Table 2).

The larvae of both species exhibited significant responses to the liquid used to hydrate the medium with regard to height

of pupation (Table 3). Both species pupated farther away from medium hydrated with *Aspergillus* extract, the response being much more extreme in *D. hydei*. (Tukey tests isolated fungus extract treatment for both *Drosophila* species; *D. mel.* $P < 0.05$, *D. hyd.* $P < 0.005$).

Discussion: Similar to the findings of Atkinson (1981) when investigating the effects of *Penicillium* on *D. immigrans* and *D. melanogaster*, we found that the inhibitory effect of *A. niger* on *Drosophila* was species-specific: survival of *D. hydei* larvae was reduced if *A. niger* or its extract were present on the resource whereas *D. melanogaster* larvae were unaffected. Many fungi, including species of *Aspergillus*, produced complexes of mycotoxins. This includes aflatoxin which is produced by *A. flavus* and has been shown to inhibit all parts of the *D. melanogaster* life-cycle (Matsumura and Knight, 1967). Other toxins produced by the *Aspergillus* group include ochratoxin A and sterigmatocystin and *A. niger* produces a number of bioactive substances, including enzymes such as amylases, invertases, pectinases and lipases (Jay, 1992). It appears that *A. niger* produces some water-soluble metabolite which significantly reduces the viability of *D. hydei* larvae. *A. niger* is used commercially to produce citric and oxalic acids (Collins *et al.*, 1989) and pH of the resource can affect *Drosophila* performance (Hodge *et al.*, 1996). However, the IDM buffered the pH of the *Aspergillus* extract from ~4.0 to ~6.0 so it is unlikely that pH *per se* produced the observed effects.

Differences in the life-history of the two *Drosophila* species may play a role in the specificity of the fungal effects. For example, the development time of *D. hydei* is longer than that of *D. melanogaster*, so *D. hydei* would tend to be exposed to toxins for a longer period. Also, *D. hydei* larvae tend to feed deeper in the medium than *D. melanogaster* which could influence the effects experienced if the distribution of the toxin(s) is not uniform.

In general, it is believed that dipteran larvae pupate further away from environments which may prove harmful to the developing pupae (Casares and Carracedo, 1987). In this experiment, the larvae of both species of *Drosophila* pupated at a greater distance from the medium if fungal extract was present. This suggests that they may be able to detect and respond to some potentially deleterious chemical in the resource.

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Joly, Dominique[1], **Christophe Bressac**[2] **and Daniel Lachaise.**[1] [1]Laboratoire Populations, Génétique et Evolution, C.N.R.S., 91198 Gif sur Yvette Cedex, France. [2] IBEAS, URA CNRS 1298, Faculté des Sciences et Techniques, Université F. Rabelais, Parc Grandmont, 37 200 Tours, France. Gradually Elongating Testis and 'Sperm Roller' in *Drosophila bifurca*.

The evolution of sperm of inordinate length has sporadically occurred in the arthropod phylogenetic tree: ostracods in crustaceans, *Scutigera* in millipedes, and waterbugs, ptiliid beetles, and fruitflies in insects (Sivinski, 1984). Within the Drosophilidae family, this trend has seemingly been magnified uniquely in the subgenus *Drosophila* (Joly *et al.*, 1989; Pitnick *et al.*, 1995a) and the most impressive lengths occur in the *hydei* species subgroup including *Drosophila hydei* and *D. bifurca*, of which sperm lengths are on average 16.9 (Joly and Bressac, 1994) and 58.37 mm (Joly *et al.*, 1995), respectively. The sperm of this last species was unambiguously determined both by direct measurements using a dissection technique described elsewhere (Pitnick *et al.*, 1995b) and by indirect measurements using the correlation curve between sperm and testis lengths established previously (Joly and Bressac, 1994).

During the pre-reproductive life, *D. bifurca* testes were shown to elongate gradually, growing two-fold every 5 days until the 20th day post-emergence (Figure 1). As a comparison, *D. melanogaster* testes reach their maximum size as soon as the first day after hatching. Testis and receptacle were measured from males or females at different ages from 24 hour until the age of sexual maturity in *D. melanogaster* Canton S and *D. bifurca* (from Bowling Green Stock Center, number 15085-1621.0). Flies were reared on a standard corn-meal medium at room temperature. In each case (that is 0, 1, 2 ,5, 10, 15 and 20 day-old flies), 25 testes and receptacles were dissected and spread out of the abdomen in a drop of saline solution on a microscope slide. Slides were then let dry at room temperature and organs from reproductive tracts were mounted in a drop of glycerol. The measurements were realized using a camera (Hitachi, model KP-C551) connected to a Macintosh 660 AV with the NIH-Image Program (written by W. Rasband at the U.S. National Institute of

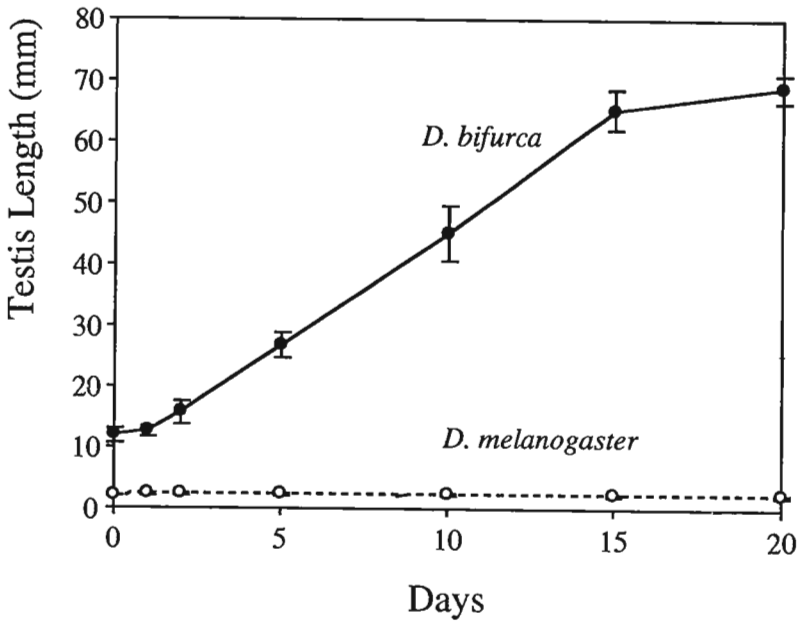

Figure 1. Enhancement of the testis length as a function of time in *D. bifurca* (giant sperm of more than 58 mm) and *D. melanogaster* (mid-sized sperm of less than 2 mm). Means are indicated with standard deviation for both species, but it is not visible in the case of *D. melanogaster* because of the Y scale.

Health) which can be obtained through anonymous ftp from zippy.nimh.nih.gov. One single testis was measured for each male. It is worth noting that sexual maturity in males (estimated by the occurrence of sperm in seminal vesicles) is reached when the testis elongation is nearly finished (e.g., 17 day-old in *D. bifurca*). This is consistent with Pitnick *et al.*,'s (1995a) data suggesting a relationship between the duration of the adult male nonreproductive phase and the sperm length in *Drosophila*.

Moreover, the most intriguing observation is that uniquely in *D. bifurca* the male tractus exhibits huge testes connected to the seminal vesicle via a special twisted and coiled structure, the 'sperm roller', absent from any other species previously described in the literature (Figure 2). This structure is seen as soon as the fly hatches and elongates and coils gradually during the testis development. The role of this peculiar structure seems to consist primarily in disentangling sperm issued from the same cyst, separating and rolling up them individually as a ball in the seminal vesicle. In the latter, the sperm are arranged in a rosary of huge separated monospermatic pellets of 80 μm wide (Figure 3) which are offered to females one after another at the rate of about 25 per mating (Joly *et al.*, 1995; Figure 4). The evolutionary significance of such unique reproductive tractus pattern and way of transferring sperm is controversial. A possibility, given as a working hypothesis, is that it could have some relevance with the multiple-mating behavior prevailing in both *D. hydei* and *D. bifurca*. It could for instance be a way to subdivide the input of synchronously-produced sperm in the seminal vesicles and thereby allow to 'control' the amount of sperm offered to each female. However, the fate of such giant sperm in females is still unclear (Bressac *et al.*, 1994; Joly *et al.*, 1995; Pitnick *et al.*, 1995b).

Figure 2. The 'sperm roller' structure localized between the testis (in the left part) and the seminal vesicle (not visible here).

How sperm pellets are manufactured in the reproductive tractus of *D. bifurca* males is documented in a forthcoming paper.

References: Bressac, C., A. Fleury, and D. Lachaise 1994, Proc. Natl. Acad. Sci. USA 91: 10399-10402; Joly, D., and C. Bressac 1994, Int. J. Insect Morphol. & Embryol. 23: 85-92; Joly, D., C. Bressac, and D. Lachaise 1995, Nature 377: 202; Joly, D., M.-L. Cariou, D. Lachaise, and J.R. David 1989, Genet. Sel. Evol. 21: 283-293; Pitnick, S., T.A. Markow, and G.S. Spicer 1995a, Proc. Natl. Acad. Sci. USA 10614-10618; Pitnick, S., G.S. Spicer, and T.A. Markow 1995b, Nature 109; Sivinski, J. 1984.

Sperm in competition. Pp. 85-115 *in* R. L. Smith, ed. Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York.

Figure 3. Giant sperm pellets arranged in a single file in the seminal vesicle.

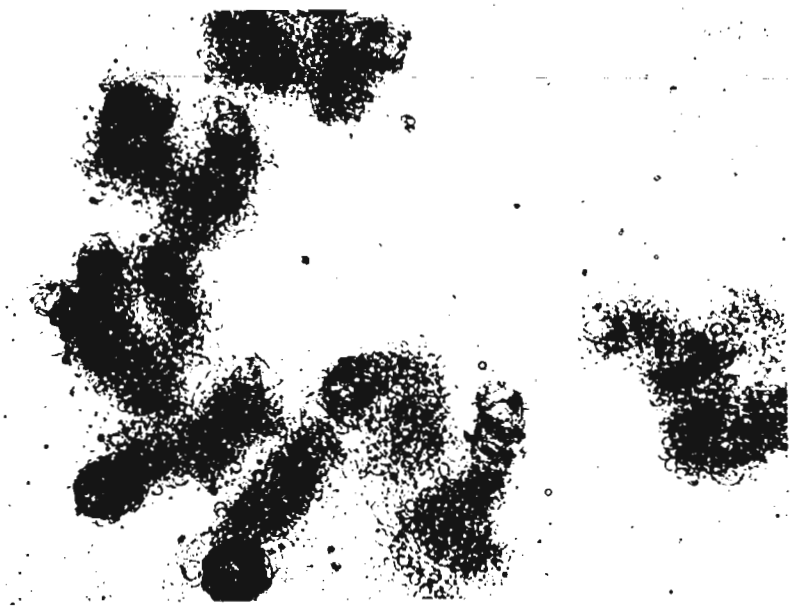

Figure 4. Giant sperm pellets released one after another into the female tractus.

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[1]University of Belgrade, Faculty of Biology, Belgrade, F.R. Yugoslavia. [2]Institute for Biology of Gene, Russian Academy of Science, Moscow, Russia. Genetic analysis of transgenic *D. melanogaster* flies after injection of the foreign gene of esterase S.

In several studies successful interspecific gene transfer has been achieved (Scavarda and Hartl, 1984; Daniels *et al.*, 1989). The system of DNA segment transfer by a P-element is widely used in cloning of different genes in eucaryotes (Spradling and Rubin, 1983). A very effective vector is CaSpeR, a P-element in bacterial plasmid (Pirrotta, 1986). In this experiment, the continuing part of esterase S gene sequence from *D. virilis* was inserted into the CASPER vector which is then injected into *D. melanogaster* embryos. The final goal was to determine whether the gene for esterase S maintains its tissue-specific expression when transferred into the genome of other species. The experiment in this paper deals with obtaining the transgenic individuals of *D. melanogaster*.

CASPER vector includes a *white* gene sequence which serves as a visible marker (white eyes) in detection of the transformed flies. As the P-element in the CASPER vector can not produce its own transposase, a mixture with a "helper" vector p 25.7 was used in 5:1 ratio. Embryos of *D. melanogaster* strain $Df(1)^{w67c23(2)}$, *y w* were injected with this mixture. The flies that developed from the injected embryos were each crossed with (y^+w; *Cy/L; D/Sb*) flies to obtain transformants with the markers on all major chromosomes (X, 2, 3). About 33.9% of the progeny of the injected flies were transformants. The transformed progeny, (all flies with any eye color) were back crossed with the flies form the above strain and their progeny represent the transgenic flies. An analysis of these transgenic flies shows the location and number of insertions on chromosomes, (*e.g.*, if the offspring is *Cy/L* with white eyes, the insertion of the white gene was on the 2nd chromosome). The results of segregation analysis indicate the existence of multiple simultaneous insertion of CASPER vector on autosomes and X-chromosomes and that the quantity of eye pigment might depend on the number of insertion sequences inherited in each individual.

It is very rare that unusual phenotypes are obtained from the above cross. From the reciprocal cross with $Df(1)^{w67c23(2)}$, *y w*, all progeny should have been y^+. However, we obtained *y* males. Their frequency was 0.0503% and they appeared both in families with and without transformants in segregations. In order to check whether this happened as a result of our experimental procedure, a control cross was done, without the injection treatment. No unusual flies were recorded in that progeny, which indicates that the experimental procedure might have caused the phenomenon.

Further on, we crossed the unusual *y* females with y^+w; *Cy/L; D/Sb* males. Only one female gave progeny and it comprised *yw* females and *yw* and y^+w males. One explanation could be that the appearance of the unusual female phenotype is the result of a mutation that suppresses the y^+ locus. Another would be that this is a consequence of the linked-X chromosome that was unstable and could divide, with high frequency, into free X-chromosomes. The latter assumption is in contrast with the obtained data, as there were no y^+ females together with *y* females. Thus, three different crosses of *yw* females, with three different types of males (y^+w; *w*; yw^a/w^+Y) were carried out. In the progeny of these crosses unusual flies were obtained, *y* males and y^+ females, with frequencies of 3.43% and 0.11% respectively. This means that in the initial lines used the linked-X chromosome was present, while still unstable and segregating with a frequency of about 10^{-3}. However, it can be concluded that a linked-X chromosome can be obtained from the cross with yw^a/w^+Y males which have a dominant w^+ marker on Y chromosome. If the daughters from our crosses got their fathers' Y chromosome, than we could expect pure segregation in daughters with w^+ and sons with *w* eyes. Our data show such segregation. In our case with unusual *y* females, we have a non-disjunction of X chromosome. The phenomenon of unusual flies in our crosses with individuals with the injected plasmid could be explained by a non-disjunction of sex chromosomes, which might be a result of the influence of the P-element that is injected together with the CASPER vector as a helper. Studies show that preference exists of the P element towards certain sites of the chromosomes. This can induce some loci to have the effect on chromosomal pairing (Tower and Kurapati, 1994). Understanding the target specificity of the P element transposition, and the behavior of homologous chromosomes in nuclei after injection helps in utilizing a P element as an insertional mutagen.

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Budnik, M., and L. Cifuentes. Departamento de Biología Celular y Genética, Facultad de Medicina - Universidad de Chile, Casilla 70061 - Correo 7 - Santiago - Chile, Fax: 56-2-7373158. Larval viability of *D. subobscura* competing with *D. pavani* and *D. simulans* at different initial frequencies of eggs.

Introduction: Since *Drosophila subobscura* was first detected in Chile (Brncic and Budnik, 1980), several experimental studies have been performed in order to investigate preadult competition between this species and the most common species found in the same collecting sites. All investigations showed *D. subscura* was a bad competitor (Budnik *et. al.* 1983, 1995).

In this note the authors wish to report the results of interspecific larval competition of *D. subobscura* with *D. simulans* and *D. pavani* in bispecific combination; but with different initial frequencies of eggs. The aim is to contribute further to the understanding of the colonization success of *D. subobscura* in Chile.

Materials and Methods: The following stocks were used: a) Chilean stock of *D. subobscura*, b) a stock of *D. pavani* an endemic Chilean species of the *mesophragmatica* group (Brncic and Koref-Santibañez, 1957) and c) a stock of *D. simulans*. All three strains came from La Florida, Santiago, Chile (in the southeastern zone of Santiago) and had been maintained in mass culture for several months before the experiments were started.

The methodology used to study the effect of preadult competition on the survival was similar to that reported by Budnik *et al.* (1983, 1995). Ten vials per group were used. Each vial contained 10 cc of basic cornmeal-yeast agar medium, into which either 10, 50 or 90 eggs of *D. subobscura* were put together with 90, 50 or 10 fertilized eggs of *D. pavani* or *D. simulans* (a total of 100 eggs per vial). As a control 10 vials were established with the same amount of medium, each sown with 100 fertilized eggs of either *D. subobscura*, *D. simulans* or *D. pavani*. The eggs represented a random sample of those laid by 150 inseminated females from each stock.

Table 1. Preadult viability of *D. subobscura*, under conditions of interspecific competition with *D. pavani* and *D. simulans* with different initial frequencies of eggs. In parenthesis, competitor (10 vials/group).

No. of eggs x vial		% of adults emerged	
D. subobscura	*D. pavani*	*D. subobscura*	*D. pavani*
10	90	7.00	59.70
50	50	33.80	57.20
90	10	51.60	72.00
100	—	53.10	—
—	100	—	48.70
		$\chi^2_3 = 101.70$	$\chi^2_3 = 36.60$
D. subobscura	*D. simulans*	*D. subobscura*	*D. simulans*
10	90	35.00	60.20
50	50	26.20	50.20
90	10	45.30	60.00
100	—	53.10	—
—	100	—	63.40
		$\chi^2_3 = 101.70$	$\chi^2_3 = 24.50$

For $\chi^2_3 = 7.81$ with DF = 3. $p = 0 < 0.05$

The eggs were allowed to hatch and to develop at 18°C; emerging adults were then counted and discarded.

Results and Discussion: Table 1 shows that the viability of the three species varies according to the initial frequencies of eggs of the competitor species; the differences are statistically significant. These findings should be taken into account when studying preadult competition. Regarding *D. subobscura*, these results once more show that this species is a bad competitor.

In face of the successful colonization of *D. subobscura* in Chile it is difficult to believe that the species could be subjected to competitive interaction such as those described above.

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Kekic, V. Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Yugoslavia. *Drosophila* fauna in habitats on the Danube bank in Yugoslavia.

Theoretical population geneticists, and laboratory experimentalists as well, who use *Drosophila* species as a model organism lament on their lack of knowledge on different life aspects of *Drosophila* in wild habitats.

I have started faunistical researches of *Drosophila* along the Danube course with the belief that these results would improve our comprehension of the species

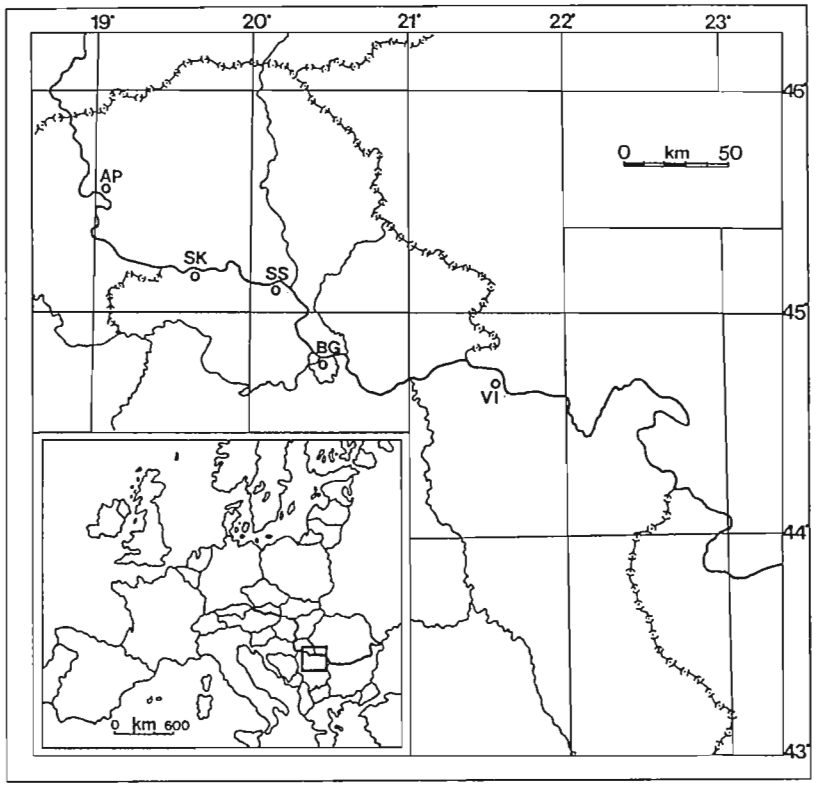

Table 1. *Drosophila* species captured in Apatin (AP), Sremska Kamenica (SK), Stari Slankamen (SS), Belgrade (BG) and Vinci (VI).

Species	Localities					Total
	AP	SK	SS	BG	VI	
Drosophila						
D. acuminata	1	—	—	—	—	1
D. ambigua	1	10	187	53	16	267
D. andalusiaca	—	—	—	—	2	2
D. bifasciata	12	1	66	321	1	401
D. busckii	4	1	—	—	—	5
D. confusa	2	—	—	1	1	4
D. cameraria	—	—	—	—	1	1
D. deflexa	—	2	—	—	—	2
D. fenestrarum	1	—	—	1	—	2
D. funebris	—	7	2	65	5	79
D. helvetica	5	1	—	2	24	32
D. hydei	—	5	7	1	2	15
D. immigrans	236	614	169	33	58	1110
D. kuntzei	367	7	18	7	4	403
D. limbata	17	—	—	4	1	22
D. littoralis	7	—	114	—	—	121
D. melanogaster	201	983	783	57	2621	4645
D. obscura	187	7	44	23	—	261
D. phalerata	250	284	165	505	392	1596
D. repleta	—	—	1	—	—	1
D. rufifrons	—	—	37	33	—	70
D. simulans	3	—	—	—	—	3
D. subobscura	1848	2598	604	767	817	6634
D. testacea	5	59	12	327	35	438
D. transversa	5	29	2	13	82	131
D. tristis	12	1	3	19	—	35
Total	3164	4609	2214	2232	4062	16281
Number of species	19	16	16	18	16	26

migration, provide some innovative consideration of their ecology, and offer more data on ecological conditions in certain habitats.

Danube river (2,857 km long) connects the North Sea with the Black Sea through the system of channels Rhine - Main, and Pannonian Plain on the north with the south part of Balkan peninsula through its tributaries (Tisa and Morava rivers) - it connects various biogeographic regions and habitats many of which are being altered by human activities, mostly negatively. Nevertheless, there are still numerous wild habitats (large forests along both banks, numerous islands and wetlands) where human impacts have mostly been indirect.

On each of five localities under the study (Figure 1) flies have been captured by fruit bait and net in two ecologically different habitats: in immediate vicinity of the Danube river, in the green belt along the river and in a nearby settlement (about 0.2 to 4 km far from the river, in orchards, vineyards and parks).

Table 1 presents *Drosophila* species collected only in habitats near the river. I believe that the richness of *Drosophila* fauna (26 found species) and the difference in species composition between localities suggest a possible sequel to this study along the Danube river. This is an invitation to all colleagues from the Danube countries to join this study.

Elaborate presentation of studies in question, with the detailed description of habitats, periods of collection, and the inclusion of other Drosophilidae species will be published in separate papers - results from the locality Stari Slankamen have been published already (Kekic *et al.*, 1996).

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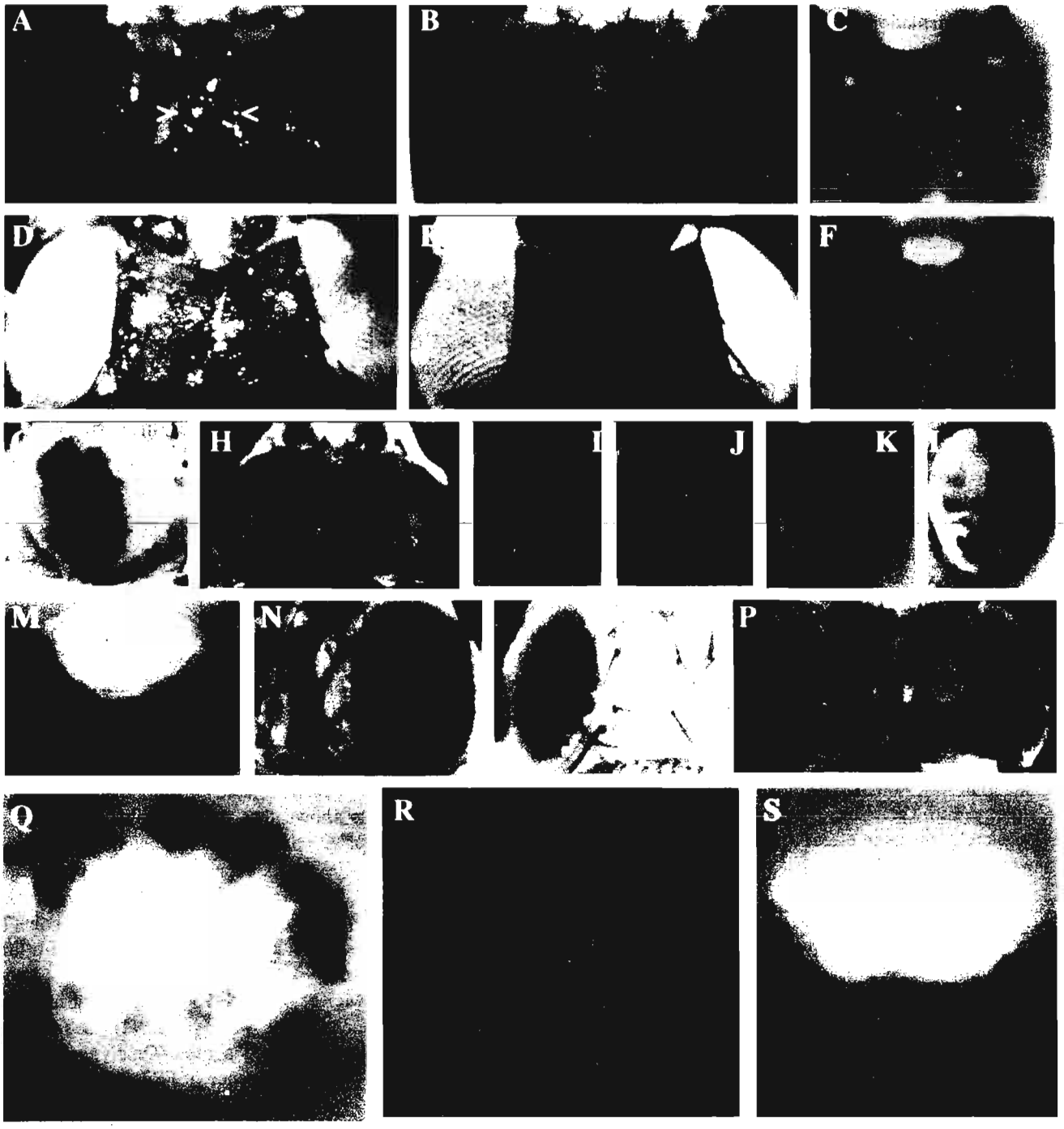
White,R.J., J.C.Eissenberg and W.S. Stark. Saint Louis University, St. Louis, MO USA. Eye color mosaicism in ocelli in variegating lines of *Drosophila melanogaster*.

The fly compound eye has been an important system to study structure, function and development. By contrast, the simple eyes (ocelli) have received considerably less attention. Papers on ocelli, few and far between, address electrophysiology (Hu *et al.*, 1978), input into phototaxis (Miller *et al.*, 1981), visual pigment (Feiler *et al.*, 1988; Pollock and Benzer, 1988) and ultrastructure (Stark *et al.*, 1989). The purpose of this study is to take advantage of newly-generated strains of *Drosophila* which exhibit position effect variegation in the compound



eye to demonstrate mosaicism in ocelli. A second purpose is to describe how to optimize viewing of ocellar pigmentation. Two lines of flies with "salt and pepper" *vs.* "large patch" mosaicism in the compound eye were described by Lu *et al.* (1996); they are respectively *In(3L)BL1*, here dubbed "BL1", and *Tp(3;Y)BL2* ("BL2"). Mosaicism has been useful in our studies of compound eye development and autonomy of gene expression (Antoine *et al.*, 1983; Harris and Stark, 1977; Stark *et al.*, 1988; Stark *et al.*, 1981); Ready *et al.* (1976) initiated use of eye color mosaicism in developmental analyses which has been widely applied since.

Traditionally, pigmentation in ocelli is visualized in a dissection microscope with incident illumination; pigmentation is subtle with such viewing in ocelli (between arrows) of wild type (A). An Optronics color CCD camera and an MCI black and white CCD 72 camera coupled with Image Pro Plus and Pagemaker operating on a PC (IBM clone) facilitated viewing and "photography." The same head viewed with transmitted light provided by a dark-field illuminator makes the pigmentation more obvious (B). Ocellar pigmentation is even better imaged in wild-type flies by fixing flies to a microscope slide, illuminating through the head with a narrow beam of transmitted light and viewing through the ocellar lenses with a 40× oil immersion objective (C). Ultrastructural studies (Stark *et al.*, 1989) showed that ocellar pigmentation is located proximally (but distal to the axon) within photoreceptor cells; this results in the cup-like appearance on the medial edges (in C). Corresponding images from white-eyed flies without ocellar pigmentation are shown (D-F). An example of the striking large patch mosaicism in the BL2 compound eye is shown (G). Ocellar pigmentation in BL2, if present, is not clear (H) even with the illumination used above for wild-type flies (B). An ocellus of a wild-type fly is shown (I and J) to show how we further improved our visualization of ocellar pigmentation. With a 100× oil immersion objective, the cup-shaped ocellar pigmentation is obvious with white light (I). However, the same ocellus appears uniformly dark when we optimized our viewing with a blue (480 nm) interference filter placed in the beam (J); eye color pigments absorb blue light well (Stark, 1973). Applied to the analysis of BL2, white light yields insufficient contrast (K) while blue light shows medium and large pigmented patches in BL2 reared at room temperature (L). When viewed from the side using blue light, large patch mosaicism in another ocellus is clear (M). The salt and pepper mosaicism in the compound eyes of BL1 is shown in several micrographs (N and O). As with BL2, ocellar pigmentation in BL1 is difficult to discern even with dark field illumination in a dissecting microscope (P). Mosaicism of ocellar pigmentation is obvious in the ocelli of BL1 flies reared at 25°C when viewed at higher magnification. Viewed with white light (Q) or blue light (R), many small pigmented patches are seen with straight-through viewing and a 100× oil immersion objective. In a different fly reared at room temperature and viewed from the edge with blue light, only a few pigmented patches are seen (S). Reared at 18°C, very few pigmented patches were seen in ocelli of BL1 or BL2. Thus, both stocks showed a direct correlation of numbers of pigmented patches with temperature, expected from the literature (Gowen and Gay, 1934). Our images of discrete pigmentation in a white background imply for ocelli what is well-known for the compound eye, namely that ommochrome pigmentation is autonomous to the cell.

Acknowledgments: Supported by NSF grant MCB 9506103 to JCE and NIH grant EY07192 to WSS.

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Fedorova, S., and L. Omelyanchuk. Institute of Cytology and Genetics, Novosibirsk 630090 Russia.
The interchromosome effect on nondisjunction of FRT-site insertion.

The genetic study of X-chromosome rearrangements shows that the nucleolus organizer is responsible for a correct X-chromosome disjunction (Gershenson, 1933; Cooper, 1964; Appels and Hilliker, 1982; McKee and Lindsley, 1987). The insertions of parts of ribosomal RNA genes in X -chromosome with deleted nucleolus organizer show that 240 bp of IGS contains almost all necessary information for this disjunction.

The autosome disjunction gives a more complex picture. The data of McKee *et al.* (1993) about the absence of heterochromatin participation in pairing is contrary to our results (Omelyanchuk and Volkova, 1994). Despite the noncompetitive nature of Dp(2;Y) element pairing (McKee *et al.*, 1993), we had found a case (Omelyanchuk and Volkova, 1994), where pairing does not obey this rule. Here we would like to give the evidence of FRT-site insertion in

III- chromosome influence on II-chromosome nondisjunction, that gives the additional complexity to the male chromosome behavior in meiosis.

The elements of yeast site-specific recombination system FLP-FRT contain FLP-recombinase construct inserted in X- chromosome under a heat shock promoter and the vector P[ry^+; hs-neo; FRT] 80B inserted in 80B region of the 3L chromosome were described in Xu and Rubin (1993). The results of crossing of individuals containing different combinations of those elements to *y; C(2)EN; ru ca* females is shown in Table 1. It could be seen that most low level of II- chromosome nondisjunction takes place in the absence of FRT-sites. FRT homo- and heterozygous individuals give a higher level of II- chromosome nondisjunction in comparison to wild type (0.92×10^{-3} in accord to Ashburner's Table 27.8 [1989]) despite the presence of FLP-chromosome. This gives the evidence that one III- chromosome FRT-site is enough to ensure increased level of the III-chromosome nondisjunction, *i.e.* FRT is dominantly acting. The heat shock induction (40 min. at 37°C) of FLP-recombinase has no effect on the nondisjunction. This means that the phenomena could not be due to the site-specific exchanges in the FRT area.

Table 1.

Sex of flies	Genotype of tested individuals	Heat shock treatment	Number of embryos	Number of alive progeny	Frequency of nondisjunction
Male	FRT /*ry e*	—	2224	5	2.2×10^{-3}
Male	FRT /FRT	—	1149	8	7.0×10^{-3}
Male	*w* FLP; *ry e* / TM3	—	2427	1	0.4×10^{-3}
Male	*w* FLP; FRT / FRT	+	3752	5	1.3×10^{-3}
Male		—	1252	51	40.7×10^{-3}
	w FLP; FRT / TM3	—	2629	57	21.7×10^{-3}
Female		—	2903	0	0
	FRT / FRT	—	2237	0	0
Female	*w* FLP; FRT / FRT	—	2574	0	0
Female	*w* FLP; FRT / FRT	+	2563	1	0.4×10^{-3}

Dramatic increase of II- chromosome nondisjunction could be seen when FRT-site presents together with III-chromosome balancer TM3.

The phenomenon described here clearly shows that the behavior of different pairs of homologous chromosomes in male meiosis are not independent. Some cellular structures such as a spatial organization of chromatin or a spindle structure may be mediators of the observed interaction.

Similar experiments with female meiosis (Table 1) show no FRT induced nondisjunction.

Acknowledgments: The work done was supported by CDA-National Institute Health grant, USA.

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Chubykin, V., S. Fedorova, and L. Omelyanchuk.
Institute of Cytology and Genetics, Novosibirsk 630090 Russia. Chromosome abnormality in new mutant allelic to *aar* gene.

Earlier we have described some P[1ArB] insertion mutants, which demonstrated an increasing of lethality and mitotic abnormalities in the third instar larvae (Omelyanchuk and Volkova, 1996). One of those insertions, named as *v158*, was mapped in the 85F region of 3R chromosome by *in situ* hybridization. In result of cytogenetic analysis we have found an abnormal mitotic chromosome condensation and appearance of chromosome-like bodies, the quantity of which did not coincide with chromosome karyotype; nondisjunction of the X and the IV-chromosome also took place. It was shown (C. Sunkel, personal communication) that the *v158* mutant is an allele of the *aar* gene (Gomes, 1993). Further detailed analysis demonstrated characteristic mitotic defects of the *v158* mutant, that distinguish it from already known aar^1 and aar^2 alleles.

Mitotic chromosome preparations of neuroblasts and its C-band staining were made by standard techniques (Ashburner, 1989).

In Figure 1 A, B, C a positive C-staining of heterochromatic blocks is shown at different and sequential stages of mitosis in wild type strain (normal). In *Drosophila*, pairing of homologous chromosomes and nonhomologous association of asynaptic paracentromeric region in the chromocenter are both in meiotic and mitotic cells. At the late stages the pairing of heterochromatic regions and transition from nonhomologous bounds to homologous ones that orient

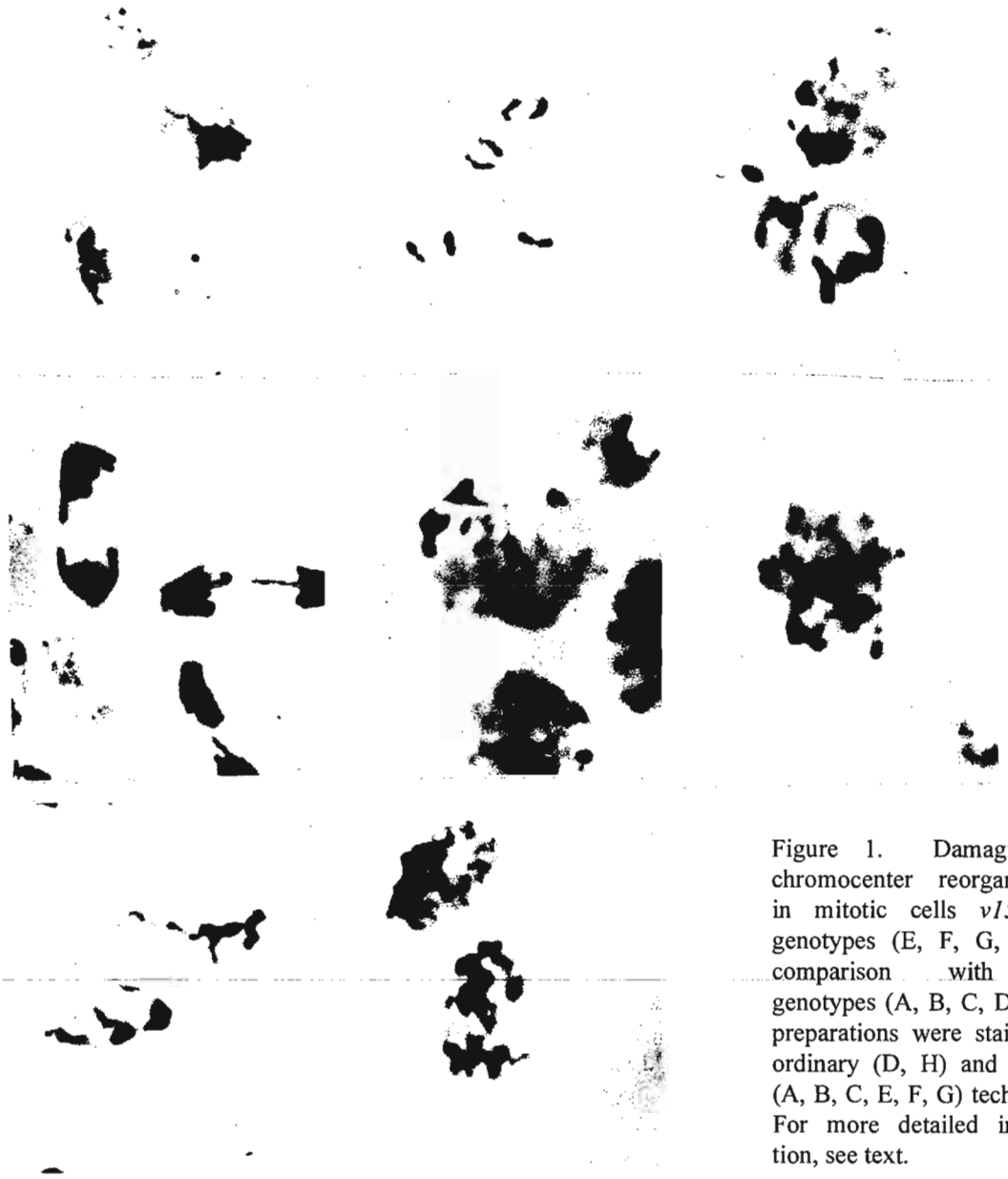

Figure 1. Damaging of chromocenter reorganization in mitotic cells *v158/v158* genotypes (E, F, G, H.) in comparison with +/+ genotypes (A, B, C, D). The preparations were stained by ordinary (D, H) and C-band (A, B, C, E, F, G) techniques. For more detailed information, see text.

the chromosomes in meiotic metaphase occur (Chubykin, 1996). In mitotic cells the reorganization of the chromocenter is terminated before microtubule attachment to kinetochores in prometaphase. The bounds between homologous heterochromatic regions in normal mitotic prometaphase are absent (Figure 1 B, C). Figure 1 E, F, G demonstrate heterochromatin in *v158/v158* individuals at similar mitotic stages which were determined by degree of compacting of chromosome euchromatic arms. In comparison with normal strain (Figure 1 A) the process of chromocenter restructuring in the *v158* homozygotes at the end of interphase is absent (Figure 1 E). Then, in prophase and prometaphase of the *v158* there are huge nonhomologous asynaptic heterochromatic blocks or associations which probably are formed by conservation of chromocentral bonds (Figure 1 F, G).

Table 1.

Genetic Constitution and Sex	Metaphase M	Anaphase A	Interphase I	M/A •10^{-2}	A/I • 10^{-3}
v158/v158					
females	41	19	4276	0.96	4.4
males	82	61	11334	0.72	0.54
v158/+					
females	62	56	2874	2.2	9.5
males	92	50	3664	2.5	13.6

Figure 2. Mitotic chromosomes containing isolated and more condensed chromatids without their division.

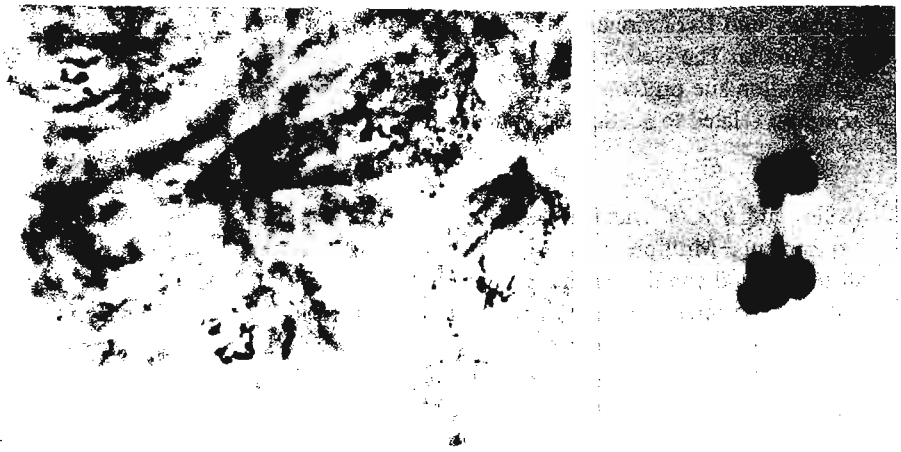

Figure 3. Asynchronous divisions (A, left) and nondisjunction of chromosomes (B, right) in meiosis of *v158/v158* male.

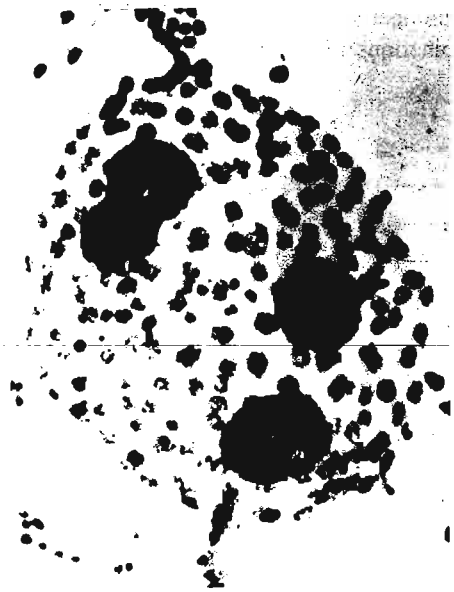

Figure 4. Damaging of ovarian development in *v158/v158* female.

Such abnormal associations are preserved during metaphase-anaphase and results in formation of fibrils, which connect the chromosomes (Figure 1 D, H).

Figure 2 illustrates C-banding of chromosome-like bodies in *v158/v158* individuals which are never observed in normal. Each such formation has a deeply stained heterochromatic region. Therefore we believe that a separate body can represent one or two paired chromatids: variations in its number can be explained to account for the difference in pairing ability.

Data of metaphase and anaphase indexes are presented in Table 1. Unlike the data of *aar* (Gomes, 1993), in *v158* these indexes are smaller in *v158/v158* than in *v158/+*. This corresponds to our data that the effect of *v158* allele is determined before metaphase in cell cycle.

The *v158* insertion is semilethal. Figure 3 illustrates asynchronous nuclear divisions (Fig. 3A) and nondisjunction of chromosomes (Fig. 3B) in meiosis of a homozygous male. Ovaries development are also defective. Figure 4 shows ovarian chamber with four nurse cells in the *v158*, while in normal there are 15.

The data obtained in this study demonstrate some unique mitotic abnormalities in the *v158* mutant, conditioned by heterochromatin behavior in prometaphase.

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Oviposition site preferences by *D. melanogaster* and *D. simulans* related to acetic acid concentrations.

Drosophila melanogaster and *D. simulans* encounter in their natural environment not only ethanol, but its oxidation product acetic acid as well (Hageman *et al.*, 1990; McKenzie and McKechnie, 1979). The significance of acetic acid for both *Drosophila* species has not been examined like ethanol. Three strains of *D. melanogaster*, homozygous for Adh^F (FFN), Adh^S (SSN) and *b* Adh^{n4}, and a *D. simulans* strain (Eisses and Den Boer, 1995) were used in oviposition site preference experiments. Multiple choice discs with 18 holes gave the possibility to present a number of patches with similar and different food conditions simultaneously (Eisses, 1991, 1997; Eisses and Bets, 1992). Two-way, three-way and multiple choice experiments have been performed with various combinations of concentrations of acetic acid, supplemented to standard medium. The experiments with *D. melanogaster* and *D. simulans* were on separate days, but all combinations of concentrations have been tested simultaneously and in duplicate. Flies from at least ten population bottles were collected, randomized, and distributed over the discs in approximately equal numbers, without anesthetizing the flies. The numbers of females per disc were counted after the experiment. Approximately 40 females per disc oviposited for about 2-4 hours.

Figure 1 shows the results of multiple concentration choice experiments. Experiment 1 lasted for 3.5 h with an egg production of 1.6 eggs/FFN fly/h and 2.1 eggs/SSN fly/h, whereas experiment 2 lasted for 2.5 h because of a higher egg production with 4.3 eggs/FFN fly/h and 4.6 eggs/SSN fly/h. In both experiments the oviposition preference of FFN females was more skewed to the lower concentrations than the oviposition preference of SSN females.

In three-way choice experiments, SSN and FFN flies choose in a different mode much more obviously (Figure 2). Again a much higher fraction of FFN eggs (33.4 and 54.2%, respectively) compared with SSN eggs (6.6 and 11.7%, respectively) were oviposited on non-supplemented medium in the two combinations of concentrations. In both cases SSN flies laid by far the majority of eggs (84.3 and 53.6%, respectively) on the highest concentrations of acetic acid supplemented medium (3 and 5% (v/v), respectively). FFN females deposited only 11.1% of the eggs on 5% (v/v) acetic acid supplemented medium.

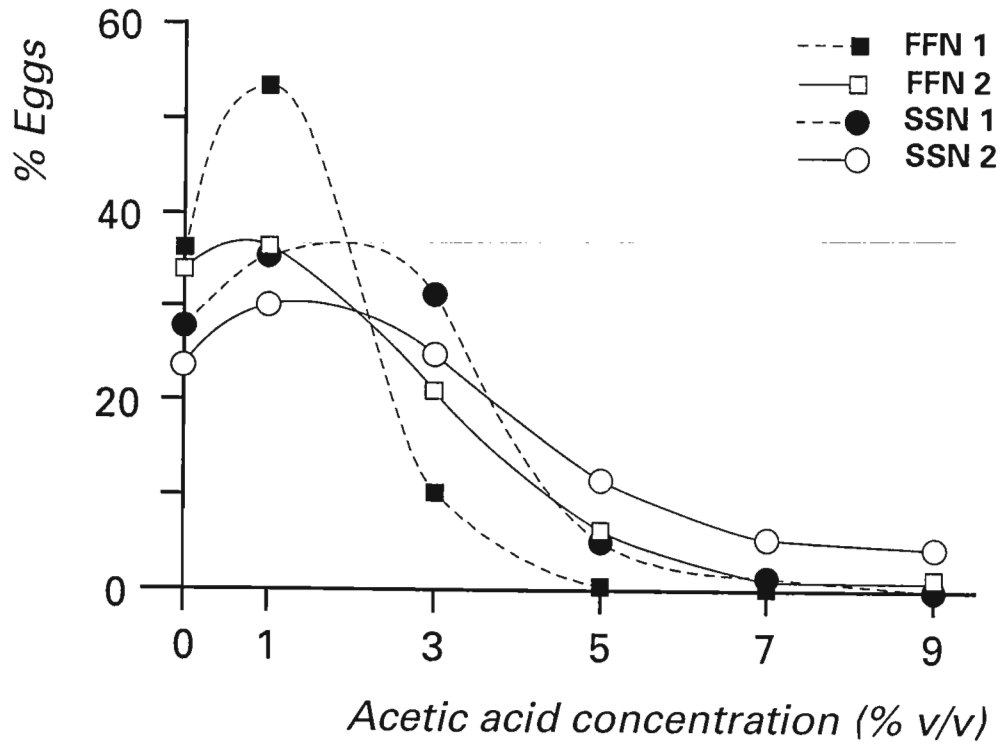

Figure 1. Oviposition site preferences of *D. melanogaster* strains, homozygous for Adh^F (FFN) and Adh^S (SSN) toward a range of acetic acid concentrations. The data are presented as fractions of the total number of eggs per experiment. Experiments 1 and 2 lasted 2.5 and 3.5 hr, respectively.

D. simulans showed yet another type of oviposition behavior. In two-way choice tests with standard medium and acetic acid supplemented medium the preference for 1% (v/v) acetic acid was much larger than for 3% (v/v). In a three-way test the presence of

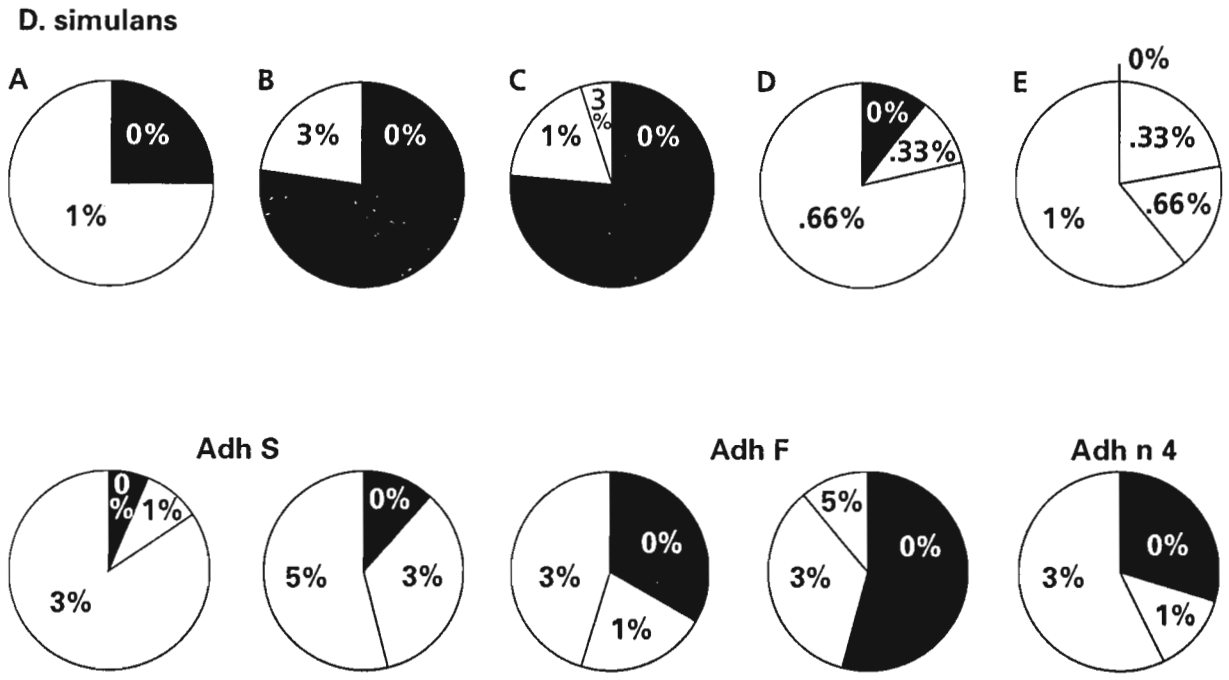

Figure 2. Oviposition site preferences of *D. simulans* and *D. melanogaster* strains, homozygous for Adh^F (FFN), Adh^S (SSN) and *b* Adh^{n4}, in two-way and three-way choice experiments with various combinations of concentrations of acetic acid, supplemented to standard medium.

patches with 3% (v/v) acetic acid negatively influenced the choice of patches containing 1% (v/v) acetic acid (compare Figure 2C with Figure 2A). In a concentration range below 1% (v/v), 78% of the eggs were laid on medium patches supplemented with 0.66% (v/v) acetic acid (Figure 2D).

Our *D. melanogaster* strains, homozygous for different *Adh* alleles, and *D. simulans* showed different adult tolerances toward acetic acid (Eisses and Den Boer, 1995), as has been shown before with other strains (McKenzie and McKechnie, 1979; Parsons, 1982). The same pattern has been repeated in the oviposition site preferences toward various concentrations of acetic acid. This phenomenon could indicate that flies search for oviposition sites they are pre-adapted for. Although flies prefer to lay eggs in medium patches consisting of acetic acid, newly-hatched larvae tried to escape these patches in a multiple choice situation and crawled into ethanol supplemented patches or into patches with standard medium (Eisses and Bets, 1992). The multiple choice situation probably mimics the situation in decaying fruit with inverse gradients of acetic acid and ethanol, respectively, perpendicular to the surface of the pile of rotting grapes (McKenzie and McKechnie, 1979).

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Weisman, Natalya Ya, and Ilya K. Zakharov. Institute of Cytology and Genetics, Russian Academy of Science, Siberian Division, Novosibirsk, 630090, Russia. Penetration of a new minus allele isolated from a wild population of *Drosophila melanogaster.*

The presented research is part of a scientific program on localization and identification of mutants isolated from wild populations (Weisman *et al.*, 1995; Weisman and Zakharov, 1995).

Isolation of mutation. The visible mutation #89381 was found and isolated from the offspring of a *Drosophila melanogaster* female fertilized in nature, from Uman in 1989. Mutants often have reduced body. Bristles vary in number and size. The hairs are also truncated and thinned in comparison with the norm. Some individuals have reduced number of hairs and their disposition is

disturbed. Besides, the order of eye facet disposition is broken; the breakage is better expressed in males rather than in females. In addition to the mentioned above visible characters, the homozygous #89381 females are sterile as a rule, *i.e.*, in crosses with the fertile males, they produce no eggs. The external genitals of the females do not differ from the norm. The internal organs are represented by under-developed spermatheca and parovarium, which are two-fold diminished in comparison with the genitals of normal females of the same age. The ovaries of #89381 females are filled with eggs in the early stages of development, but mature eggs are absent. The homozygous males are fertile.

The #89381 is a recessive mutation localized on chromosome 2. The chromosome #89381 is maintained in a balance with the chromosome *Cy In(2LR)*. In culture, the viability of #89381 homozygotes is reduced and the average share of #89381 homozygotes equals to 40% of the theoretically expected. However, life duration of adult homozygotes does not differ significantly from that of heterozygotes *Cy*/#89381.

Location of #89381 mutation. The location of #89381 mutation on the chromosome was made by using recessive mutations of the chromosome 2; such as dumpy (*dp*; 2-13.0); cinnabar (*cn*; 2-57.5); vestigial (*vg*; 2-67.0) and brown (bw; 2-104.0). The flies from the stock *Cy*/#89381 were crossed to those from corresponding marker stocks in order to set the compounds of #89381 with the marked chromosomes. The resulted heterozygous females *dp*/#89381, *cn*/#89381 and *vg*/#89381 were crossed to corresponding dihomozygous males *dp* #89381, *cn* #89381 and *vg* #89381.

The offspring of all three types of crossings were characterized by a decrease in homozygous #89381 mutation classes as opposed to heterozygous classes (see Table 1). On this basis, to calculate recombination frequency, the reciprocal homozygous class was considered equal in number to the corresponding heterozygous #89381 mutation class. This principle was applied both to recombinant and non-recombinant classes. The frequency of recombination between #89381 mutation and three marker genes, *dp*, *cn* and *vg*, was 49.1; 41.2; and 52.4 , respectively.

In analogous crosses with the marker mutation brown, amongst the offspring of females *bw*/#89381 out of more than 2500 flies, we did not observe any crossovers. This fact points out that #89381 mutation is located in the vicinity of *bw* locus at position 100.

Table 1. Segregation in crosses for #89381 localization (R.f. = recombination frequencies).

Female genotype	Progeny genotype	Number of progeny	
vg/#89381	*vg*/*vg* #89381	298	
	#89381/*vg* #89381	210	
	vg #89381/*vg* #89381	77	
	+/*vg* #89381	288	
			R.f. = 49.1%
cn/#89381	*cn*/*cn* #89381	385	
	#89381/*cn* #89381	178	
	cn #89381/*cn* #89381	63	
	+/*cn* #89381	271	
			R.f. = 41.2%
dp/#89381	*dp*/*dp* #89381	266	
	#89381/*dp* #89381	239	
	dp #89381/*dp* #89381	158	
	+/*dp* #89381	293	
			R.f. = 52.4%

Allelism test. The hybrid F1 from crosses of the flies from the stock *Cy*/#89381 with the stocks carrying mutations with the similar phenotypical expression and localization at the region of 100 units, in particular, abbreviated (*abb*; 2-105.5), morula (*mr*; 2-106,7) and minus (*mi*; 2-104,7), were analyzed. The heterozygous #89381/*shr bw*-2b *abb sp* and #89381/ *px bw mr sp* were phenotypically normal. The #89381 mutation was found to be allelic to the *mi* mutation. Heterozygotes #89381/*mi* have the phenotype of the minus mutation, characterized by reduced body size and bristles and by truncated and reduced number of hairs. Homozygous *mi* have low variable viability, and the hatching of flies occurs later than in normals. The females *mi*/*mi* are completely sterile, whereas the males are fertile (Lindsley and Zimm,1992).

The expression of the *mi*-#89381 allele from the Uman population differs from the previously known allele mainly by the influence on the eye structure and by varying phenotypic expression. It is not inconceivable that there are genetic modifiers which influence expression of the *mi*-#89381 allele. We have noticed an interesting peculiarity of recessive *mi*-#89381 mutation. In heterozygous condition with the chromosome from laboratory strain marked as Lobe 2 (*L*; 2-72.0) (Catalog number 2-23 in the fund of the laboratory of genetics of populations of the Institute of Cytology and Genetics, Russian Academy of Sciences), the *mi*-#89381 behaves as the dominant mutation. The heterozygous phenotype *L*-2/*mi*-#89381 is similar to homozygous phenotype *mi*-#89381/*mi*-#89381. The minus mutation from the fund of Umea *Drosophila* Stock Center is exhibited in heterozygote with *L*-2 (2-23) analogously to *mi*-#89381 mutation. In compounds of *mi*-#89381 chromosome with the chromosomes carrying the other Lobe alleles , *i.e.*, *L*-4, *L*-81 and *L*-2 (from the other laboratory stocks), the *mi*-#89381 is recessive. The phenomenon observed may be caused either by deletion or by interaction of *mi*-#89381 mutation with the gene *L*-2 or some other loci from this chromosome. An attempt to isolate by recombination the mutation with phenotypical expression similar to *mi*-#89381 from the chromosome Lobe-2 (2-23) of the laboratory strain gave no positive effect. To test the presence/absence of rearrangements at the chromosome Lobe-2 (2-23) in the region of minus location, the chromosomes from salivary glands of heterozygotes *L*-

2/+ were analyzed. The gene minus has a cytological location at the region 59 D9-C1 of the chromosome 2. The analysis by light microscope did not reveal any chromosomal rearrangements in this and close to this region. However, we can not exclude the microdeletion which is detectable only by molecular genetics methods. Mutations *abb*, *mi*, *mr* and slite (*slt*; 2-106,7) constitute a group of genes with the similar phenotypical expression, which are located within the limits of a small chromosomal region. Possibly, the genes responsible for the normal development of imaginal disks are located in this chromosomal fragment. The abnormalities of these genes may cause the arising of phenotypically similar mutations. On the other hand, the *mi*-#89381 allele described above is related by phenotypical expression to the gene morula. The minus and morula genes are probably the duplications of one and the same gene, which diverged in evolution. The analysis of functioning of such genes, the knowledge of their pleiotropic effect, peculiarities of expression of different genetic variants are necessary for solving the problems of genome evolution, chromosome structure and regulation of gene expression.

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Ryerse, J., J. Swarthout, and B. Nagel. Department of Pathology, St. Louis University Health Sciences Center, 1402 South Grand Avenue, St. Louis, M0 63104. tel 314-577-8480; fax 314-577-8489; e-mail ryersejs@sluvca.slu.edu Cloning and molecular characterization of a partial ATP citrate lyase cDNA from *Drosophila melanogaster*.

ATP citrate lyase (ATPCL) catalyzes the conversion of citrate and CoA to oxaloacetate and acetyl-CoA. Acetyl CoA is an essential intermediary metabolite in the biosynthesis of cellular fatty acids and cholesterol (Kornacker and Lowenstein, 1965; Sullivan *et al.*, 1973; Singh *et al.*, 1976). ATPCLs have been cloned from the rat (Elshourbagy, *et al.*, 1990; Kim *et al.*, 1994) and the human (Elshourbagy *et al.*, 1992). We report here a partial ATP citrate lyase cDNA from *Drosophila melanogaster*, denoted DmATPCL.

While screening a *D. melanogaster* Canton S 2-14 hour embryonic cDNA library (Stratagene) with antibodies to candidate gap junction proteins (Ryerse, 1993, 1995), a clone was isolated with sequence homology to ATPCLs from the human and rat. The nucleotide sequence of the *D. melanogaster* ATPCL cDNA is 1623 bps in length (including the polyA tail) and contains an open reading frame which codes for a protein of 391 amino acids (Figure 1).

GCG PileUp alignment of the human, rat and fly ATPCL amino acid sequences is shown in Figure 2. The fly sequence is proximally incomplete, beginning at amino acid 710 of the human sequence. In the region of overlap, DmATPCL has 75.5% and 75.7% identity with human and rat ATPCLs, respectively (GCG BESTFIT analysis).

Human and rat ATPCLs contain domains which are considered essential for enzyme function (Elshourbagy *et al.*, 1992), including a catalytic phosphorylation site at His759, two ATP binding domains (aa 700-720 and 751-777) and a potential acetyl-CoA binding site (aa 778-788). His759 is phosphorylated by nucleoside diphosphate kinase and ATP (Wagner and Vu, 1995). His759 is conserved in the DmATPCL sequence. DmATPCL has 64% identity with the first ATP binding domain, 74% identity with the second ATP binding domain and 50% identity with the putative acetyl-CoA binding region in the human sequence (Figure 2). ATPCL is thought to associate with the ATP conducting voltage dependent anion channel (VDAC) in the outer mitochondrial membrane (Brdiczka, 1990), presumably for ready access to newly synthesized ATP. DmATPCL has been assigned Genebank accession number U87317.

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Figures 1 and 2 on following two pages.

Figure 1. Nucleotide and amino acid sequence of DmATPCL. * = stop codon.

```
5' 1  aaaccaagctgattgtccttttaggagaggttggtggaaccgaggagtac
   1      T  K  L  I  V  L  L  G  E  V  G  G  T  E  E  Y
  51 gacgtttgtgccgctctgaaggacggacgtattaccaagcctctggtggcc
  17    D  V  C  A  A  L  K  D  G  R  I  T  K  P  L  V  A
 102 tggtgcattggtacctgcgccagcatgtttacttcggaagtccagtttggc
  34    W  C  I  G  T  C  A  S  M  F  T  S  E  V  Q  F  G
 153 catgccggatcctgcgcgaactccgaccgagagacggctacggccaagaac
  51    H  A  G  S  C  A  N  S  D  R  E  T  A  T  A  K  N
 204 aagggtctgcgagatgccggcgcctacgttcctgattcgttcgacacgctg
  68    K  G  L  R  D  A  G  A  Y  V  P  D  S  F  D  T  L
 255 ggtgaactcatccaccacgtgtacggcgagctggttaagactggtcgagta
  85    G  E  L  I  H  H  V  Y  G  E  L  V  K  T  G  R  V
 306 gtgccgaaGgaggaggtgccaccaccaactgtgcccatggattattcgtgg
 102    V  P  K  E  E  V  P  P  P  T  V  P  M  D  Y  S  W
 357 gcccgcgagctgggtcttattcgcaagcccgcgtcattcatgacgtcgatc
 119    A  R  E  L  G  L  I  R  K  P  A  S  F  M  T  S  I
 408 tgcgacgagcgtggccaggagcttatctacgcaggaatgccgatcagcgag
 136    C  D  E  R  G  Q  E  L  I  Y  A  G  M  P  I  S  E
 459 gtccttagcaaggacgtcggcattggcggtgtcatctcactgctatggttc
 153    V  L  S  K  D  V  G  I  G  G  V  I  S  L  L  W  F
 510 cagcgctgcctgccttcatacgtgtgcaagttcttcgagatgtgcctgatg
 170    Q  R  C  L  P  S  Y  V  C  K  F  F  E  M  C  L  M
 561 gttactgcggatcacggtcccgcagtttctggggctcacaacaccattgtg
 187    V  T  A  D  H  G  P  A  V  S  G  A  H  N  T  I  V
 612 tgcgcccgtgctggcaaggacctggtgtcctcagtcgtgagcggtcttctg
 204    C  A  R  A  G  K  D  L  V  S  S  V  V  S  G  L  L
 663 actatcggggatcgatttggaggcgccctggacggatcggctcgacagttc
 221    T  I  G  D  R  F  G  G  A  L  D  G  S  A  R  Q  F
 714 tctgaggcatacgacaccaacctgcacccaatggagttcgtaaacaagatg
 238    S  E  A  Y  D  T  N  L  H  P  M  E  F  V  N  K  M
 765 cgcaaagagggcaagcttatcctgggtattggccaccgtgtaaagtccatt
 255    R  K  E  G  K  L  I  L  G  I  G  H  R  V  K  S  I
 816 aataaccccgatgtgcgcgtgaagatcattaaggaattcgtactggagaac
 272    N  N  P  D  V  R  V  K  I  I  K  E  F  V  L  E  N
 867 ttccctgcgtgtccacttctcaaatacgccttggaggttgagaagattacc
 289    F  P  A  C  P  L  L  K  Y  A  L  E  V  E  K  I  T
 918 accaacaagaaaccgaatcttatcctcaatgtggacggtgtgatagccacc
 306    T  N  K  K  P  N  L  I  L  N  V  D  G  V  I  A  T
 969 gcatttgtagacatgctgcgtaacagcggctcatttaccagtgaggaagca
 323    A  F  V  D  M  L  R  N  S  G  S  F  T  S  E  E  A
1020 caggagtacattaatgtcggcgcgatcaactcgttgttcgttctgggccgc
 340    Q  E  Y  I  N  V  G  A  I  N  S  L  F  V  L  G  R
1071 agcataggatttattggccattacatggatcagaaacgtctcaaacagggc
 357    S  I  G  F  I  G  H  Y  M  D  Q  K  R  L  K  Q  G
1122 ctgtaccgtcatccgtgggacgacatctcatacgtcattcccgagcagtac
 374    L  Y  R  H  P  W  D  D  I  S  Y  V  I  P  E  Q  Y
1173 aactaaggcgtgctgatgaagccgcgacgatgttatatatatatttagcta
 391    N  *
1224 tatacaagcatatatattattcggaagttgttgtagttaagcacagatgta
1275 tgatgttaggcaaagaggtcacccgctccactggatggaaggatccattga
1326 ggtctatccgcgcatgtgactctccccagatccctttccctctcacttatc
1377 ctgcatttaaagttcttaagttaccatcattaccctcgttgtttttgtaag
1428 cgctgcgtgtccccggagcccttaggggttttggaatctagacctagttaa
1479 atctagtgctccgagcggcacaacacaccagtacccggcataacattattt
1530 tgatacttttatactttagtttggatgctcacaattgtctagacaacaaga
1581 gaataaattgttgcaaaagcttaaaaaaaaaaaaaaaaaaaaa
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Figure 2. Comparison of human, rat, and fly ATP citrate lyase amino acid sequences.
* = identity, - = no amino acid.

```
Hum MSAKAISEQT GKELLYKFIC TTSAIQNRFK YARVTPDTDW ARLLQDHPWL   50
Rat ********** *******Y** ********** ********** *H********
Hum LSQNLVVKPD QLIKRRGKLG LVGVNLTLDG VKSWLKPRLG QEATVGKATG  100
Rat ***S****** ********** ******S*** ********** H*******K*
Hum FLKNFLIEPF VPHSQAEEFY VCIYATREGD YVLFHHEGGV DVGDVDAKAQ  150
Rat ********** ********** ********** ********** ******T***
Hum KLLVGVDEKL NPEDIKKHLL VHAPDDKKEI LASFISGLFN FYEDLYFTYL  200
Rat ********** *A****R*** ****E***** ********** **********
Hum EINPLVVTKD GVYVLDLAAK VDATADYICK VKWGDIEFPP PFGREAYPEE  250
Rat ********** ***I****** ********** ********** **********
Hum AYIADLDAKS GASLKLTLLN PKGRIWTMVA GGGASVVYSD TICDLGGVNE  300
Rat ********** ********** ********** ********** **********
Hum LANYGEYSGA PSEQQTYDYA KTILSLMTRE KHPDGKILII GGSIANFTNV  350
Rat ********** ********** ********** ********** **********
Hum AATFKGIVRA IRDYQGPLKE HEVTIFVRRG GPNYQEGLRV MGEVGKTTGI  400
Rat ********** ******S*** ********** ********** **********
Hum PIHVFGTETH MTAIVGMALG HRPIPNQPPT AAHTANFLLN ASGSTSTPAP  450
Rat ********** *******WA -PA******* ********** **********
Hum SRTASFSESR ADEVAPAKKA KPAMPQDSVP SPRSLQGKST TLFSRHTKAI  500
Rat ********** ********** ********** *********A **********
Hum VWGMQTRAVQ GMLDFDYVCS RDEPSVAAMV YPFTGDHKQK FYWGHKEILI  550
Rat ********** ********** ********** ********** **********
Hum PVFKNMADAM RKHPEVDVLI NFASLRSAYD STMETMNYAQ IRTIAIIAEG  600
Rat ********** K********* ********** ********** **********
Hum IPEALTRKLI KKADQKGVTI IGPATVGGIK PGCFKIGNTG GMLDNILASK  650
Rat ********** *****----- ---------- ---------- ----------
Hum LYRPGSVAYV SRSGGMSNEL NNIISRTTDG VYEGVAIGGD RYPGSTFMDH  700
Rat -******--** ********** ********** ********** **********
Hum VLRYQDTPGV KMIVVLGEIG GTEEYKICRG IKEGRLTKPI VCWCIGTCAT  750
Rat VLRYQDTPGV ********** ********** *********V **********
Dm             T KL**L***V* *****DV*AA L*D**I***L *A*******S
Hum MFSSEVQFGH AGACANQASE TAVAKNQALK EAGVFVPRSF DELGEIIQSV  800
Rat ********** ********** ********** ********** **********
Dm  **T******* **S***SDR* **T***KG*R D**AY**D** *T***L*HH*
Hum YEDLVANGVI VPAQEVPPPT VPMDYSWARE LGLIRKPASF MTSICDERGQ  850
Rat ******K*A* ********** ********** ********** **********
Dm  *GE**KT*RV **KE****** ********** ********** **********
Hum ELIYAGMPIT EVFKEEMGIG GVLGLLWFQK RLPKYSCQFI EMCLMVTADH  900
Rat ********** ********** ********** ********** **********
Dm  *********S **LSKDV*** **IS*****R C**S*V*K*F **********
Hum GPAVSGAHNT IICARAGKDL VSSLTSGLLT IGDRFGGALD AAAKMFSKAF  950
Rat ********** ********** ********** ********** **********
Dm  ********** *V******** ***VV***** ********** GS*RQ**E*Y
Hum DSGIIPMEFV NKMKKEGKLI MGIGHRVKSI NNPDMRVQIL KDYVRQHFPA 1000
Rat ********** ********** ********** ********** **F*K*****
Dm  *TNLH***** ***R****** L********* ****V**K*I *EF*LEN***
Hum TPLLDYALEV EKITTSKKPN LILNVDGLIG VAFVDMLRNC GSFTREEADE 1050
Rat ********** ********** *******F** ********** **********
Dm  C***K***** *****N**** *******V*A T********S ****S***Q*
Hum YIDIGALNGI FVLGRSMGFI GHYLDQKRLK QGLYRHPWDD ISYVLPEHMS 1100
Rat *V********V ********** ********** ********** **********
Dm  **NV**I*SL ******I*** ***M****** ********** ****I**QYN
Hum M 1101
Rat M     97.5% identity with human (aa 1-1101)
Dm  -     75.5% identity with human (aa 710-1100)
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Eisses, K.Th., and M. Santos. Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, 08193 Bellaterra (Barcelona), Spain. E-mail: eisses@cc.uab.es Depending on the stage of decay, banana traps attract *Drosophila melanogaster* and *D. simulans* in different ratios.

Four sites within a semi-abandoned *Opuntia ficus-indica* plantation near Carboneras (Almería, Spain) (Ruiz *et al.*, 1986) were sampled for coexisting *Drosophila* species from September 19 - 23, 1995, with mashed banana traps. The males of *D. melanogaster* and *D. simulans* were distinguished by their genital archs (Bächli and Burla, 1985; Coyne, 1983). The females were separated by their differences in pigmentation pattern of the sixth abdominal tergite (Gallo, 1973; Thompson *et al.*, 1979; Eisses and Santos, 1997) and by the differences in cheek widths (McNamee and Dytham, 1993). In cases of further doubt flies were also checked by means of gel electrophoresis and alcohol dehydrogenase staining (Eisses *et al.*, 1979). The distribution of the two species in the samples taken during five days were significantly different from expected ratios in a chi-square test (Table 1). The samples were lumped for the first three days and the last two days for all sites (Table 2). The proportion of *D. melanogaster* compared with *D. simulans* is significantly higher in the last two days than in the first three days. It appeared that the decaying banana traps became increasingly attractive toward *D. melanogaster*, and more so than *D. simulans*, assuming that the overall species ratio did not change during these five days. The traps did not become repellant toward *D. simulans*. In a release-recapture experiment McInnis *et al.* (1982) found repeatedly 2 to 4 times more *D. simulans* than *D. melanogaster* than their expectation based on the numbers of released flies. Given our data it might well be that "fresh" banana traps are less attractive for *D. melanogaster* than *D. simulans*. The latter species has a preference for low concentrations of acetic acid in a choice experiment, whereas *D. melanogaster* strains have a much broader range of attractive concentrations of acetic acid to deposit eggs (Eisses, 1997). The banana traps in our location had to compete with highly attractive decaying fruits of *O. ficus-indica* or prickly pears nearby. Sampling of natural populations with banana traps for only one or two days probably underestimates the number of *D. melanogaster*.

Table 1. Numbers of *D. melanogaster* and *D. simulans* trapped on five subsequent days in four sites A1, A2, B1 and B2, which represent two sites in two rows of cactus plants within a semi-abandoned *O. ficus-indica* plantation.

Day	A1 mel	sim	A2 mel	sim	B1 mel	sim	B2 mel	sim
19/7	1	21	2	2	1	6	0	2
20/7	6	5	1	6	1	1	2	6
21/7	11	36	13	36	7	35	2	2
22/7	27	51	19	15	52	34	11	3
23/7	13	20	41	40	21	5	27	13
Chi-square	12.99		12.04		35.85		10.28	
	0.010<p<0.025		0.010<p<0.025		p<0.001		0.025<p<0.05	

Table 2. Total numbers of *D. melanogaster* and *D. simulans* trapped in four decaying banana traps on the first three days and the last two days of the sampling period in four sites within an *O. ficus-indica* plantation.

Days	A1,2 + B1,2 D. mel.	A1,2 + B1,2 D. sim.
Sept. 19 - 21, 1995	47	158
Sept. 22 - 23, 1995	211	181
Chi-square	66.6; p < 0.001	

Acknowledgments: These data were collected during a project funded by Contract No. CHRX-CT92-0041 from the Commission of the European Communities. We thank Montse Peiró for help with electrophoresis.

References: Bächli, G., and H. Burla 1985, *Diptera:* Drosophilidae. Insecta Helvetica 7; Burla, H., 1951, Revue Suisse de Zoologie 58:23-175; Coyne, J.A., 1983, Evolution 37:1101-1117; Eisses, K. Th., 1997, Dros. Inf. Serv. 80:this volume; Eisses, K. Th., and M. Santos 1997, Entomol. News (in press); Eisses, K. Th., H. van Dijk, and W. van Delden 1979, Evolution 33:1063-1068; Gallo, A.J., 1973, Ciéncia & Cultura 25:341-345; McInnis, D.O., H.E. Schaffer, and L.E. Mettler 1982, Am. Nat. 119:319-330; McNamee, S., and C. Dytham 1993, Syst. Entomol. 18:231-236; Ruiz, A., A. Fontdevila, M. Santos, and E. Torroja 1986, Evolution 40:740-755; Thompson Jr., J.N., B.N. Hisey, and R.C. Woodruff 1979, Southwestern Naturalist 24:204-205.

Eisses, K.Th., and M. Santos. Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, 08193 Bellaterra (Barcelona), Spain. E-mail: eisses@cc.uab.es Short distance differences in ADH and alphaGPDH allozyme polymorphisms and linkage disequilibrium in *D. melanogaster* in Spanish desert populations.

Understanding why latitudinal clines exist for various characteristics requires studies in areas where these clines are particularly steep. Spain lies in the center of the so-called Mediterranean "instability" with respect to the *Adh* cline (David and Capy, 1988). Several Spanish studies on *Adh* allele frequencies within relatively short distances or short periods of time demonstrate their high variability (Malpica and Vassallo, 1980; Aguadé and Serra, 1980, 1987; Muñoz-Serrano *et al.*, 1985; Alonso-Moraga and Muñoz-Serrano, 1986).

We captured flies from natural populations in an arid area in southeast Spain without wine cellars nearby. Five locations (Figure 1) near Carboneras (37°00'N;1°53'W) were sampled during five days with banana traps. Agua Amarga was sampled with decaying *Opuntia ficus-indica* fruits (prickly pears). Flies emerging from *O. ficus-indica* fruits, which were collected in the Rambla location and transferred to the laboratory, were subjected to electrophoresis as well.

The data presented in Tables 1 and 2 reveal rather high frequencies of Adh^S compared with Adh^S frequencies in France (David *et al.*, 1986), but much lower compared with Central America (Van 't Land *et al.*, 1993). We made a subdivision of the data collected in the location "Rambla", a semi-abandoned *O. ficus-indica* plantation (Table 2). The frequencies of *alpha-Gpdh*^{F} and Adh^S from different locations covaried in similar directions. A linear regression on both allele frequencies gave a correlation coefficient of $R^2 = 0.982$. We found evidence for linkage disequilibrium between the two alleles. Estimates of D were calculated based on the maximum likelihood method as proposed by Hill (1974). Although we did not measure the presence of the *In(2L)t* or other inversions in our populations, Spanish populations contain this inversion in high frequencies in very different regions (Aguadé and Serra, 1980; Sanchez-Refusta *et al.*, 1990; Taberner and González, 1991). In our populations the inversion might be present as well and responsible for covarying frequencies of *alpha-Gpdh*^{F} and Adh^S. The two populations with relatively high Adh^S and *alpha-Gpdh*^{F} frequencies, Sopalmo and Mesa Roldan, were captured in locations above 100 m altitude. The MR site (Figure 1) is an abandoned *O. ficus-indica* plantation in a plain-like environment, whereas the Sopalmo sites were in a small village in a mountain pass with some *O. ficus-indica* and shadow-rich fig trees. Trapped flies in the Rambla location seemed to have systematically slightly higher Adh^S and *alpha-Gpdh*^{F} frequencies than the flies emerging from prickly pears. Whether these differences in allele frequencies are due to sampling errors or to natural selection processes related to microclimatic differences remains to be analyzed. Some additional empirical evidence from another location pointed in the same direction. A small number of *D. melanogaster* emerging from collected prickly pears in Sopalmo revealed a much lower frequency of Adh^S than obtained with the trapped flies, 0.269 (n = 26) and 0.568 (n = 37), respectively, whereas the frequency of *alpha-Gpdh*^{F} was only a little lower, 0.750 and 0.770, respectively. The minimum and maximum temperatures as measured in a cactus tree near site A1 in the *O. ficus-indica* plantation in the Rambla location (Ruiz *et al.*, 1986) were close to the long term average data from the Carboneras weather station shown in Table 3. Nevertheless there is a lack of information on real-temperature profiles over the day at different sites on and above the surface within a variety of locations. In the Rambla location we found a large difference in numbers of trapped *D. melanogaster* between the front rows A and B and the back rows C and D (Table 2), whereas *D. simulans* were present in the back rows in high numbers. The sites C and D were higher up the slope of the plantation and closer to the mountain ridges. A similar difference was found in another plantation at a distance of approximately 1 km (Eisses, Laayouni, Leibowitz, Santos, Galiana and Fontdevilla, unpublished results). It sounds reasonable to assume that different microclimates act differently at the species level. The same may hold for the interactions of microclimate and a set of major gene-protein arrangements. Sunny and shady sites or differences in surface texture of the environment can have a large influence on very local temperatures at short distances (Stoutjesdijk and Barkman, 1992). Seasonal fluctuations in *In(2L)t* frequencies have been reported in a Spanish population caught near Oviedo with the highest frequencies in September-October (Sanchez-Refusta *et al.*, 1990). Experimental populations in a greenhouse and in the laboratory showed similar covarying frequencies of *alpha-Gpdh*^{F}, Adh^S and *In(2L)t* with temperature (Van Delden and Kamping, 1989). Such data point to a temperature effect on a set of genes more or less closely linked to *alpha-Gpdh*^{F} through the inversion *In(2L)t* and dragging Adh^S.

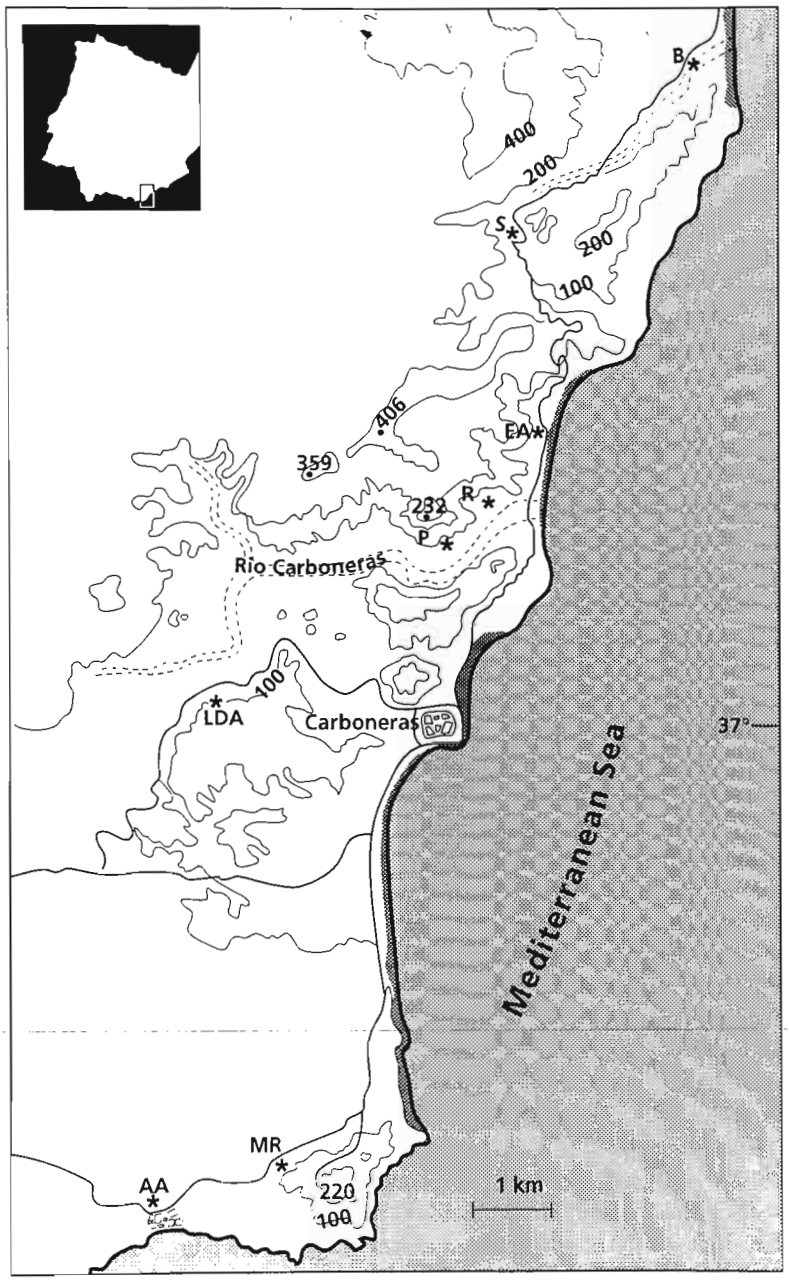

Figure 1. Locations of capture sites in the Carboneras area (37°00'N;1°54'W) is southeast Spain (see insert). B = Blanco, S = Sopalmo, R = Rambla, LDA = Llano Don Antonio, MR = Mesa Roldan, AA = Agua Amarga.

Some attention has been paid to the temperature constraint on body size of ectotherms as a consequence of the temperature dependency of biochemical reactions related to development and growth (Van der Have and De Jong, 1996). High temperature regimes result in small body sizes, short wings and high wingbeat frequencies, whereas low temperature regimes result in large body sizes, long wings and low wingbeat frequencies. As alpha-GPDH activity is directly coupled to flight muscle activity, it could well be that the presence of alpha-GPDH^{F} favours the mobility of small flies, because alpha-GPDH$^{F/F}$ activity was higher than alpha-GPDH$^{F/S}$ or alpha-GPDH$^{S/S}$ activity, especially in an ADH$^{S/S}$ background (Oudman *et al.*, 1991). Selection for short wings gave a quick response in the increase of the *alpha-Gpdh*^{F} and *Adh*^{S} allele frequencies, in spite of the absence of the *In(2L)t* inversion, whereas selection for long wings resulted in the increase of the *alpha-Gpdh*^{S} and *Adh*^{F} allele frequencies (Serra and Oller, 1984). Their results were discussed in the light of selection on a set of genes acting as a "supergene" surrounding the marker genes. However, these marker genes themselves might play a role as well. Comparison of wing lengths and *alpha-Gpdh*^{F}, *Adh*^{S} and *In(2L)t* frequencies of flies from an after-vintage population of *D. melanogaster* with an overwintering population showed longer wing lengths in the overwintering population in addition to considerably lower frequencies of these alleles and the *In(2L)t* inversion (Aguadé and Serra, 1980). Apart from the selection pressure favoring flies or larvae with the ADH-Fast allozyme due to high alcohol concentrations within a wine cellar, the temperatures inside a cellar are supposedly much more constant and lower than in the field, especially in the summer. In a wine cellar population near Montemayor at high altitude, the *Adh*^{F} frequency stayed above 0.8 all year, whereas in a field population nearby large but covarying fluctuations in *Adh*^{S} (0.3-0.9) and *alpha-Gpdh*^{F} (0.55-0.95) frequencies occurred (Muñoz-Serrano *et al.*, 1985).

Acknowledgments: Drs. Jan van 't Land and Albert Kamping from the Department of Genetics, Rijksuniversiteit Groningen are thanked for providing the values of D by means of their LINKDIS program. These data were collected during a project funded by Contract No. CHRX-CT92-0041 from the Commission of the European Communities.

Table 1. Alpha-GPDH and ADH allozyme combinations in trapped and emerged flies from samples taken in the Carboneras area (Almería, Spain) in September 1995. The first two characters point to alpha-GPDH dimers, and the other two to ADH dimers. D is an approximation of the linkage disequilibrium, based on gamete frequencies (Hill, 1974).

Location Trapped Flies	Number	FF_FF	FF_FS	FF_SS	FS_FF	FS_FS	FS_SS	SS_FF	SS_FS	SS_SS	Adh^S	$Gpdh^F$	D
Blanco	154	8	42	20	20	28	8	16	8	4	0.461	0.636	-0.07011*
Sopalmo	37	3	12	8	1	8	2	2	0	1	0.568	0.770	-0.05111
Rambla	259	23	50	35	30	69	22	11	15	4	0.494	0.651	-0.04438*
LlanoDonAntonio	82	5	7	6	21	22	6	7	8	0	0.372	0.518	-0.05879
Mesa Roldan	97	16	17	18	6	19	9	3	7	2	0.521	0.701	-0.00673
Agua Amarga	78	5	18	9	8	20	5	5	7	1	0.481	0.622	-0.05812
Total Trapped	707	60	146	96	86	166	52	44	45	12			
alpha-GPDH			302			304			101			0.642	-0.05311*
ADH		190	357	160							0.479		
Emerged Flies from *O. ficus-indica* fruits in Rambla location													
Total Emerged	1094	107	189	125	145	284	89	74	63	18			
alpha-GPDH			421			518			155			0.622	-0.04804*
ADH		326	536	232							0.457		

*significantly different from D = 0

Table 2. Alpha-GPDH and ADH allozyme combinations in samples of trapped and emerged flies from the Rambla location near Carboneras (Almería, Spain) in September 1995. The Rambla location has been subdivided in smaller areas (Ruiz *et al.*, 1986). Between brackets the number(s) of the sites are given.

Location Trapped Flies	Number	FF_FF	FF_FS	FF_SS	FS_FF	FS_FS	FS_SS	SS_FF	SS_FS	SS_SS	Adh^S	$Gpdh^F$	D
Subdivisions													
Rambla A (1-2)	129	6	25	21	14	36	12	6	8	1	0.531	0.643	-0.07601*
Rambla B (1-2)	113	13	22	11	16	29	7	5	7	3	0.442	0.637	-0.01802
Rambla C-D (1-2)	17	4	3	3	0	4	3	0	0	0	0.559	0.794	+0.09083
Total Rambla	259	23	50	35	30	69	22	11	15	4	0.494	0.651	-0.04438*
Emerged Flies													
Subdivisions													
Rambla A (13)	506	39	90	60	55	143	53	27	31	8	0.500	0.622	
Rambla A (E1)	30	5	1	6	2	7	0	3	3	3	0.483	0.550	
Rambla A (E2)	174	11	34	26	24	37	18	11	11	2	0.500	0.635	
Rambla A (E3)	38	2	2	5	8	10	2	1	4	4	0.500	0.500	
Total A (13-E3)	748	57	127	97	89	197	73	42	49	17	0.499	0.616	-0.04704*
Rambla B (14)	182	36	28	15	32	39	6	21	5	0	0.315	0.646	
Rambla C (15)	14	0	3	0	3	6	2	0	0	0	0.464	0.607	
Rambla C (E4)	41	7	3	7	5	11	4	2	2	0	0.463	0.670	
Rambla D (16)	109	7	28	6	16	31	4	9	7	1	0.404	0.610	
Total B-D (14-16)	346	50	62	28	56	87	16	32	14	1	0.366	0.634	-0.05731*

* significantly different from D = 0

Table 3. Temperatures in °C at the Carboneras weather station "Central Termica" as measured from 1986 - 1995. Data from January are given, because it is the coldest month on the average.

Month	Mean temp.	Mean Max.	Mean Min.	Absol. Max.	Absol.Min.
June	22.3	25.6	18.9	34.5	9.5
July	25.5	28.5	22.4	41.6	12.5
August	26.5	29.6	23.3	39.3	16.3
September	23.8	26.9	20.7	33.6	9.4
October	19.8	23.1	16.5	32.5	9.4
November	16.6	19.8	13.3	28.6	5.3
December	14.0	17.1	10.8	23.9	5.0
January	12.4	15.8	8.9	24.4	-4.5

The mean year temperature over ten years was 18.7 °C.

References: Aguadé, M., and L. Serra 1980, Genetika (Beograd) 12:111-120; Aguadé, M., and L. Serra 1987, Genetica 75:3-9; Alonso-Moraga, A., and A. Muñoz-Serrano 1986, Experientia 42:1048-1050; David, J.R., and P. Capy 1988, TIG 4:106-111; David, J.R., H. Merçot, P., Capy, S.F. McEvey, and J. Van Herrewege 1986, Génét. Sél. Evol. 18:405-416; Hill, W.G., 1974, Heredity 33:229-239; Malpica, J.-M., and J.M. Vassallo 1980, Nature 286:407-408; Muñoz-Serrano, A., A. Alonso-Moraga, and A. Rodero 1985, Genetica 67:121-129; Oudman, L., W. van Delden, A. Kamping, and R. Bijlsma 1991, Heredity 67:103-115; Ruiz, A., A. Fontdevila, M. Santos, and E. Torroja 1986, Evolution 40:740-755; Sanchez-Refusta, F., E. Santiago, and J. Rubio 1990, Genet. Sel. Evol. 22:47-56; Serra, L., and J.M. Oller 1984, Genetica 63:39-47; Stoutjesdijk, Ph., and J. J. Barkman 1992, *Microclimate, vegetation and fauna.* Opulus Press; Taberner, A., and A. González 1991, Heredity 67:307-316; Van Delden, W., and A. Kamping 1989, Evolution 43:775-793; Van der Have, T. M., and G. de Jong 1996, J. Theor. Biol. 183:329-340; Van 't Land, J. Van Delden, and A. Kamping 1993, Dros. Inf. Serv. 72:102-104.

Eisses, K.Th., and M. Santos. Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, 08193 Bellaterra (Barcelona), Spain. Hybrids between *Drosophila melanogaster* and *D. simulans* in a Spanish natural population.

The sibling species *Drosophila melanogaster* and *D. simulans* coexist in natural conditions in various abundances. The siblings are so closely related that it took a while until the two species were distinguished as such (Sturtevant, 1919). Interspecific matings can easily be provoked in laboratory conditions and used as a tool for studying processes of speciation (*e.g.*, Davis *et al.*, 1996). We screened field populations for these species because of ecological and genetical reasons and used gel electrophoresis as a tool, which method revealed an approximation of the frequency of interspecific hybrids between *D. melanogaster* and *D. simulans*. Data about frequencies of such hybrids in natural populations are very scarce.

Flies were captured with mashed banana traps during five days in Carboneras (Almería, Spain; 37°00'N; 1°53'W) and other locations up to 10 km north and south (Eisses and Santos, 1997). *Opuntia ficus-indica* fruits (prickly pears) were put in an experimental design for almost seven days in a semi-abandoned *O. ficus-indica* plantation near Carboneras. After recollection of the fruits emerging flies were aspirated. Captured and emerged flies were checked for *D. melanogaster* morphology and frozen at -29°C until gel electrophoresis and staining for alcohol dehydrogenase (ADH; EC 1.1.1.1.), which is a diagnostic enzyme between *D. melanogaster* and *D. simulans* (Eisses, Van Dijk and Van Delden, 1979). Gel buffer and electrophoresis buffer were according to the system of Poulik (1957).

Figure 1 shows the dimeric hybrid enzyme band between the parental ADH-S band of *D. melanogaster* and the ADH-simulans band in the lane labelled with *. However, the hybrids resulting from a cross between *D. melanogaster* ADH-Fast and *D. simulans* did not show a dimeric hybrid enzyme band. In these cases only the parental ADH-bands were visible on the gels (not shown on photograph). We do not know whether these hybrid dimers do not exist or are inactive. Four female hybrids were found, three of them were ADH-Fast/ADH-simulans. One of the hybrids was found among 137 captured *D. melanogaster* flies in Llano de Don Antonio. One natural hybrid on a total of 425 captured females in the wider Carboneras area means a frequency of 2.4×10^{-3}. Three interspecific hybrid females among 1096 females, emerging from prickly pears in the Carboneras *O. ficus-indica* plantation in September 1995, means a frequency of 2.7×10^{-3}. *D. simulans* was approximately three times more abundant than *D. melanogaster* in this area of Spain in this time of the year (Eisses, Laayouni, Leibowitz, Santos and Fontdevila, unpublished results).

The frequency of hybrids in the Carboneras population with 2.7×10^{-3} was about 2.5 times lower than the frequency reported in a Barcelona population (Mensua and Pérez, 1977). Much higher frequencies have been reported for natural populations and ranged from 1 to 40% (Casares and Carracedo, 1985; Inoue *et al.*, 1990; Sperlich, 1962).

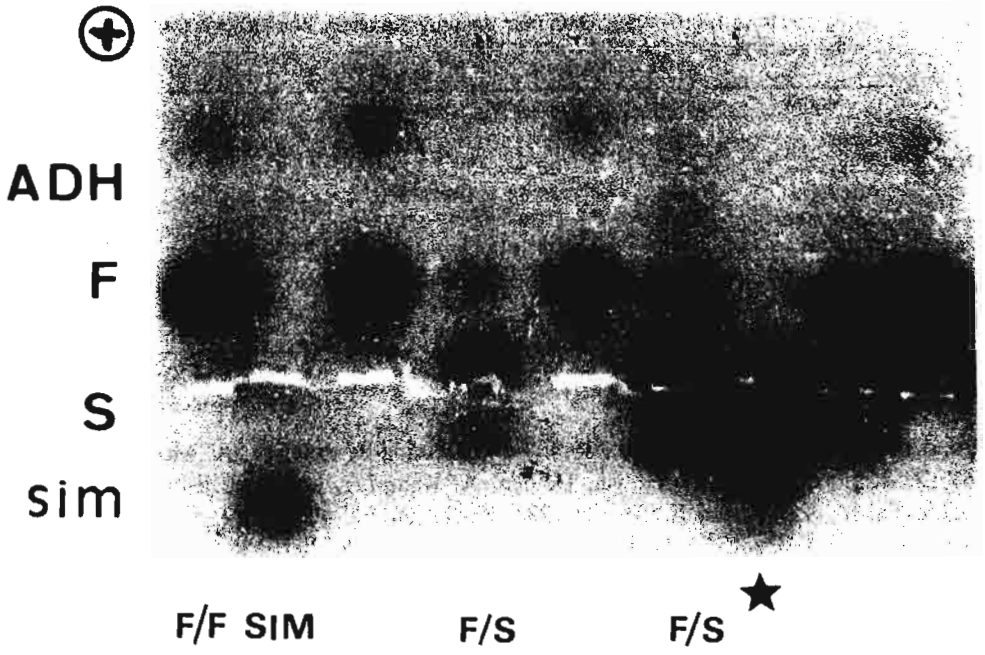

Figure 1. Electrophoresis of alcohol dehydrogenase isozymes ADH-F/F, ADH-F/S of *D. melanogaster* and *D. simulans* adults. The ADH pattern from the hybrid between *D. melanogaster* ADH-S and *D. simulans* is in lane labelled (*).

However, these data were based on the number of hybrid progeny of *D. melanogaster* females, inseminated by *D. simulans* males. The capture of hybrid flies in nature has not been reported often. Kamping and Van Delden (1988) found three hybrids among a total of 406 females caught in a fruit market. The frequency of hybrids in this natural population was 7.3 x 10^{-3}, which is three times higher than in our location. The Groningen fruit market population consisted less than 2% *D. simulans*. A much larger frequency of hybrids (1.4%) was found by Capy *et al.* (1987) among flies emerging from rotting grapes with relatively low alcohol contents. In that area *D. simulans* was three times more abundant than *D. melanogaster*.

Electrophoresis unequivocally reveals hybrids between the two species, so our data signify a good approximation for natural populations, although interspecific mating barriers seem to vary a lot. Sperlich (1962) described a population with hardly any barriers at all. As our estimated frequency of natural hybrids captured in the field is not essentially different from the frequency of hybrids emerging in the laboratory from fruits collected in the field, 2.5 x 10^{-3} seems a reasonable lower limit of interspecific hybrids in nature. However, this figure might be an underestimation of the hybrid frequency, because potential hybrids with a *D. simulans*-like phenotype were not involved in electrophoresis.

Acknowledgments: Dr. Mariano Labrador is thanked for helping with preparing photographs and Montse Peiró for help with gel electrophoreses. This work was funded by Contract No. CHRX-CT92-0041 from the Commission of the European Communities.

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Kuhn, D. T., and M. L. Sawyer. Department of Biology, University of Central Florida, Orlando, FL 32816. Distribution pattern of spinules and hairs on the dorsal surface of the 3rd instar *D. melanogaster* cuticle.

Morphological specializations on the *D. melanogaster* larval cuticle have provided valuable clues as to the function of many genes required during early development. Most of the focus has been on mutant transformations in the 1st instar stage. However, for mutants that survive to later larval stages, cuticle analysis can provide further insights into developmental fate since the morphology is quite complex. We detail here the specializations found on the dorsal surface of the 3rd instar larval cuticle, which allow one to distinguish between all abdominal segments. The analysis emphasizes differences between compartments and parasegments. Features not addressed here are the sensory pits and hairs in the naked cuticle regions.

The anterior compartment of the 3rd thoracic segment (aT3) is characterized by 5 to 6 rather horizontal rows of very small triangular, posterior pointing spinules tightly compacted (Figure 1A). In parasegment 6 (PS6) pT3 lacks features conferring unique identity to this compartment while aA1 is frequently marked by 5 relatively horizontal rows of somewhat larger posterior pointing spinules. However, parts of posterior rows tend to angle in a slightly posterior direction (Figure 1B). The pA1 of PS7 lacks spinules needed for identification. aA2 has continuous rows of larger, posterior pointing spinules that are not as straight as in aA1 (Figure 1C). However, the rows are discontinuous in the middle of this spinule belt, with the exception of the posterior most row. Spinules in the anterior most row are larger, less dense, and wider spaced than more posterior rows. In the middle of this spinule belt, there is a conspicuous constriction due a lack of spinules in the center of the anterior most rows. For PS8, pA2 displays a half row of anterior pointing spinules medially, while aA3 (Figure 1D) exhibits roughly 5 rows of larger, posterior pointing spinules. A gradient exists with larger spinules becoming smaller progressing posteriorly. The aA3 spinules are not as compact or dense as those of A2. The pA3 compartment of PS9 has 2/3 a row of anterior pointing spinules, with 3 spinules on the lateral edge of the belt (Figure 1E). aA4 displays roughly 4 rows of posterior pointing spinules, with rows generally less dense, wider spaced and more disorganized than in A3 (Figure 1E). The pA4 of PS10 contains a complete row of anterior pointing spinules (1F) that are V-shaped. The aA5 generally contains only 3 rows of posteriorly oriented spinules that are widely spaced, with the most posterior rows found in A4 absent (Figure 1F). In PS11, pA5 displays one complete row of anterior pointing spinules and a second very incomplete row (Figure 1G). The aA6 varies; normally not containing many posterior pointing spinules. Sometimes 2 rows of spinules form with Figure 1G showing more spinules than normally encountered. The PS12 pA6 generally possesses 2 complete rows of spinules, plus an anterior pointing medial row (Figure 1H). The aA7 generally consists of slender, circular based spinules. Although wide-based, triangular spinules do appear in this region, showing split tips. Figure 1H shows an unusual number of spinules, yet the type and shape of them are typical of A7. In PS13, pA7 displays 3 horizontal rows of anterior pointing spinules, which are long, slender, and round based. Usually a tiny 4th row of short thin spinules appears anterior to the 3 complete rows. Also, unlike A6, the larger, triangular, wide-based spinules are conspicuously absent in the most posterior row. Spinules appearing in the aA8 are long, slender and almost wispy in appearance (Figure 1I). An analysis of other features in A8-A10 can be found in Kuhn *et al.* (1992) and Kuhn *et al.* (1993).

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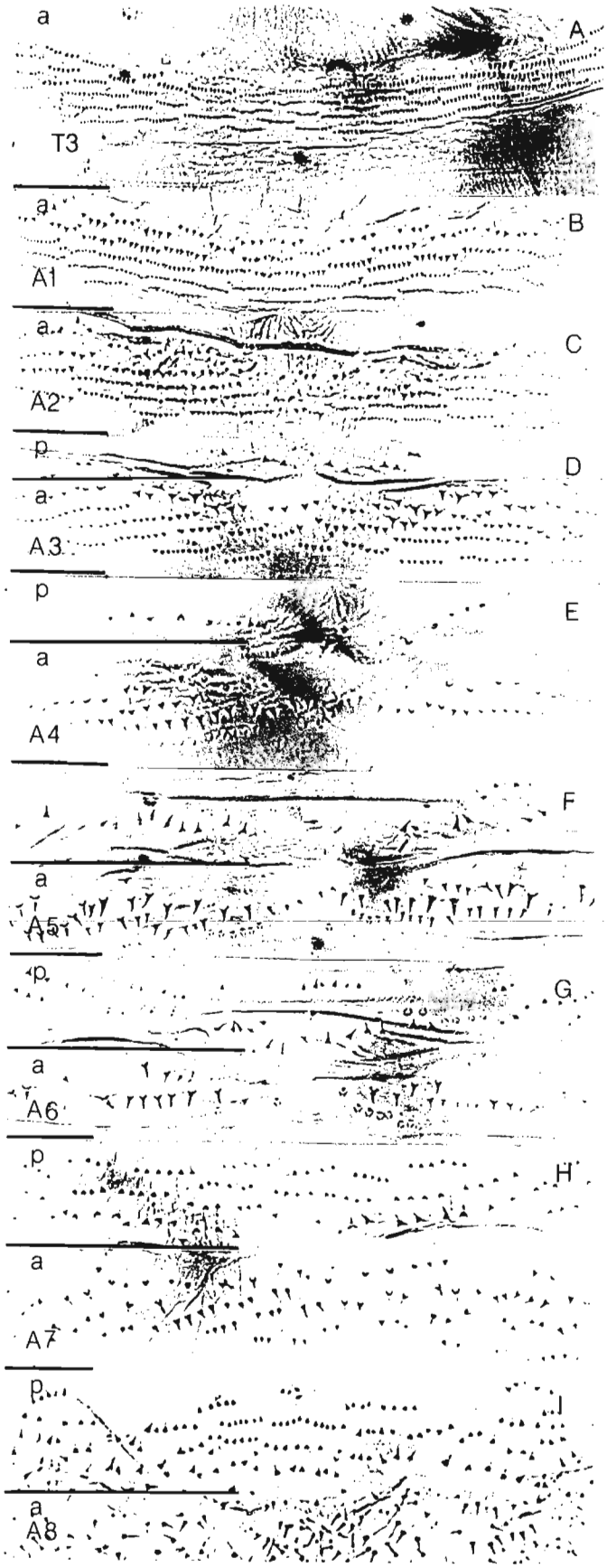
References: Kuhn, D.T., M. Sawyer, J. Ventimiglia, and Th. E. Sprey 1992, Dros. Inf. Serv. 71: 218; Kuhn, D.T., M. Sawyer, G. Packert, G. Turenchalk, J.A. Mack, Th. E. Sprey, E. Gustavson, and T.B. Kornberg 1993, Development 116: 11.

Figure 1 (next page). Dorsal spinule belts of a Canton-S 3rd instar larva. Short lines mark parasegment borders, while long lines mark segments. Naked cuticle regions, pits and hairs have been omitted. A) aT3. B) aA1. C) aA2. D) PS8 (pA2-aA3). E) PS9. F) PS10. G) PS11. H) PS12. I) PS13 (pA7-aA8). a, anterior compartment; A1-A8, abdominal segments 1 through 8; p, posterior compartment; T3, thoracic segment 3


a
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T3
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B
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Acharyya, M., and R.N. Chatterjee. University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019 India. In situ transcription analysis of chromatin template activity of the X chromosomes of sex-transformed flies of *Drosophila melanogaster*.

In *Drosophila melanogaster* sex is determined by the ratio of number of X chromosomes (X) to the sets of autosomes (A). Several mutants including *tra, tra-2, ix* and *dsx* have phenotypes which also suggest that the wild type functions of the loci play important roles in somatic sexual differentiation in *D. melanogaster*. The *transformer* (*tra*), *transformer-2* (*tra-2*) or *intersex* (*ix*) mutants affect somatic sex differentiation only in females. The null mutations at *double sex* (*dsx*) affect both XX and XY individuals causing them to develop as phenotypically intersex. Smith and Lucchesi (1969) claimed that altered sexual physiology does not have any role in dosage compensation of X-linked gene in *D. melanogaster*. However, it is not clear from the data whether the altered sexual physiology of the sex transformed flies has any role to set and /or maintain the transcription of the X-chromosome by altering the chromatin template activity of the X-chromosome or not. In view of this reason, the present investigation was undertaken to examine the relative template activity of X chromosome in sexually transformed flies of *Drosophila melanogaster* with the use of *E. coli* RNA polymerases.

For the present experiments, *in situ* transcription assay was carried out on the fixed cytological preparation of salivary gland chromosomes of third instar larvae of the sexually transformed flies as described by Chatterjee (1985) using DNA dependent *E. coli* RNA polymerase holoenzyme obtained from Sigma Chemicals. Reaction of *in situ* transcription was carried out on the fixed polytene chromosome preparation by addition of 3 units of purified RNA polymerase holoenzyme on each slide in combination with assay mixture (Chatterjee, 1985). Reactions were terminated by placing the slide in 5% cold TCA w/v containing 0.01 M $Na_4P_2O_7 \bullet 10\ H_2O$. Unincorporated nucleoside triphosphate was removed by repeated washing in 5% cold TCA and the preparations were then processed for autoradiography. Exposure time was 15 days.

Table 1 gives the grain number (together with statistical analysis) over the X chromosome and autosomal arm (2R). Data reveal that the relative template activity as measured by the X/A ratio of grain number in wild type male and *tra/tra* male is very close to that in female. Thus, the ratio of the number of silver grains on the X chromosomal segment (1A-10F) to that on autosomal segment (56F-60F) in XX; *tra/tra* transformed flies is fairly concordant with that of XY; *tra/tra* flies. In fact, the male phenotype produced by *tra* mutation had no recognizable influence on chromosomal organization of the paired X's. Similarly, when the relative template activity of the X chromosome of either XX; *dsx/dsx* or XX, *ix/ix* flies was compared to normal females, no appreciable change in the X chromosomal organization was noted. On the other hand, in XY, *dsx/dsx* and XY; *ix/ix* flies, the relative template activities were comparable to normal males. The statistical analysis of the data further shows a positive correlation between the grain number over the X chromosome to that of the autosome (2R) in all individuals.

On the basis of these observations it appears that it is the X:A ratio and not the sexual phenotype of the adult flies that sets the level of template activity of the X chromosome. Thus it has been observed that when the X:A ratio is

Table 1. Data on ^{3}H-UMP labeling pattern of an X chromosomal segment (1A-10F) and an autosomal segment (56A-60F) of the normal and sex determination mutant strains of *D. melanogaster* under *in situ* transcription condition with *E. coli* RNA polymerase.

Genotype	X:A ratio	Phenotypic sex	Number of nuclei examined	Mean grain no. with ± S.E.		X/2R with S.E.	Correlation coefficient (r)
				X chromosome (1A-10F)	2R (56A-60F)		
XX; +/+	1.00	Female	18	542.81 ± 47.38	305.30 ± 29.53	1.76 ± 0.11	0.96
XY; +/+	0.50	Male	19	515.83 ± 38.10	297.11 ± 28.17	1.73 ± 0.19	0.94
XX; tra/tra	1.00	Male	17	531.24 ± 44.96	291.92 ± 25.51	1.81 ± 0.18	0.93
XY; tra/tra	0.50	Male	19	421.95 ± 36.26	266.93 ± 24.29	1.58 ± 0.12	0.82
XX; ix/ix	1.00	Intersex	19	331.38 ± 38.14	184.43 ± 25.01	1.79 ± 0.09	0.91
XY; ix/ix	0.50	Male	20	301.07 ± 42.15	171.16 ± 29.21	1.75 ± 0.11	0.85
XX; dsx/dsx	1.00	Double-sex	17	437.21 ± 34.91	249.17 ± 27.12	1.75 ± 0.12	0.86
XY; dsx/dsx	0.50	Double-sex	18	401.61 ± 31.19	241.94 ± 19.81	1.65 ± 0.12	0.92

1.0 (as in the case of XX; *tra/tra* males) irrespective of sexual phenotype, the template activity of the X chromosome is set at a female level. Similarly, in the case of XX; *dsx/dsx* individuals, the X chromosome is transcribed at female level. On the other hand, in XY; *dsx/dsx* individuals, the level of template activity is set at a male level. In summary, our data clearly indicate that the sex determining mutants, *tra*, *ix* and *dsx* have no role in regulating the template organization of the X chromosome(s) (see Table 1) for dosage compensation.

Acknowledgments: This work was partially supported by CSIR research grant to R.N.C. M.A. is a fellow of Lady Tata Memorial Trust.

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Alatortsev, V.E. Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Sq.46, Moscow, 123182, Russia. Genetic loci in the *Pgd-K10* region of the *Drosophila* X chromosome.

The *Pgd-K10* region of the X chromosome is one of the most genetically investigated areas of the *Drosophila melanogaster* genome. The fine genetic structure of this region has been determined in several independent studies based on saturation of this region by lethal mutations and by rearrangements and complementation analysis (Gvozdev *et al.*, 1973; Perrimon, *et al.*, 1984; Alatortsev and Tolchkov, 1985). For the descriptions of the individual complementation groups, see Lindsley and Zimm (1992).

There are some additions to the information about earlier described complementation groups. First, our complementation analysis showed that group *N2* (Gvozdev *et al.*, 1977) coincides with group *l(1)C204* (Perrimon *et al.*, 1985), as well as groups *N7* and *l(1)JA127*. Thus, two pairs of groups were correctly jointed (Lindsley and Zimm, 1992). They were designated as *wapl* and *l(1)2Ea*, respectively. Second, the *l(1)90* mutation representing separate complementation group complements the *JC105* deletion and must be situated to the left of *wapl*, between the *Pgd* and *wapl* loci.

Contiguous and overlapped DNA fragments from the *Pgd-K10* region were cloned in several laboratories in the course of chromosomal walks along the Canton (Haenlin *et al.*, 1985), Oregon (Dura *et al.*, 1987), and *gt* w^a (Alatortsev, 1987) X chromosomes, and the physical map for the region was constructed. Molecular approaches allowed to expand our knowledge about genetic structure of the region. Thus, cluster containing four *Cytochrome P450* genes was found in the interval between *wapl* and *pn* loci (Gandhi *et al.*, 1992; Frolov and Alatortsev, 1994). Recently the *Vinculin* (*Vinc*) gene was described between the *2Ea* and *pcx* loci (Alatortsev *et al.*, 1997).

Current arrangement of genetic loci in the *Pgd-K10* interval is shown in Figure 1.

Acknowledgment: This work was supported by an RBRF grant.

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- Pgd - l90* - wapl* - P450* - pn - 2Ea* - Vinc* - pcx - kz - K10 -

Figure 1. Arrangement of genetic loci in the *Pgd-K10* region of the *Drosophila X* chromosome. The orientation is from centromere-distal (left) to centromere-proximal (right). Added or changed loci are marked by asterisks (see text).

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Mutations in the *DHR39* orphan receptor gene have no effect on viability.

The *DHR39* gene (also known as *FTZ-F1ß*) encodes an orphan member of the nuclear receptor superfamily (Ayer *et al.*, 1993; Ohno and Petkovich, 1992). A 3.5 kb *DHR39* mRNA is present in early embryos as a maternal component while a 5 kb mRNA is expressed at later stages of development. The 5 kb mRNA is significantly induced in late third instar larvae and prepupae as a direct response to the steroid hormone ecdysone (Horner *et al.*, 1995). DHR39 is highly related to the *Drosophila* FTZ-F1 orphan receptor, with 63% identity in the DNA binding domain. Consistent with this sequence similarity, both proteins can bind to the same regulatory sequences in the *ftz* zebra element and the *Adh* adult distal enhancer (Ayer *et al.*, 1993; Ohno and Petkovich, 1992; Ohno *et al.*, 1994).

The ecdysone-induced expression of *DHR39* during the onset of metamorphosis suggested that this gene may perform a critical function during this stage in development. To test this hypothesis, we set out to identify mutations in *DHR39*. Two lethal P element insertion stocks that mapped to the 39B4 region were obtained from the Berkeley Drosophila Genome Project, designated *P[11226]* and *P[13215]* (Spradling *et al.*, 1995). Inverse PCR was used to amplify genomic DNA flanking each P element insertion and these fragments were used as probes for Southern blot

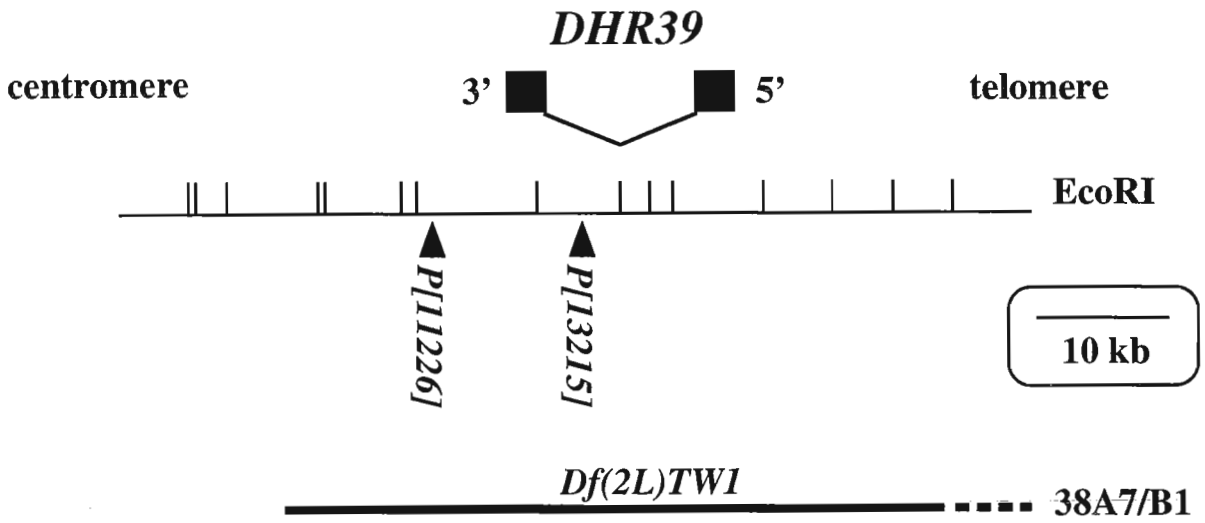

Figure 1. A map of the *DHR39* locus. The *DHR39* gene is depicted at the top with the large first intron represented by a line (intron A in Ohno and Petkovich, 1992). The two P element insertions discussed in the text are marked below an EcoRI restriction map of genomic DNA. Shown at the bottom is a deficiency that removes the *DHR39* locus. One endpoint of *Df(2L)TW1* appears to lie approximately 10-20 kb downstream from *DHR39* by genetic criteria.

hybridization to fragments of genomic DNA surrounding the *DHR39* region. In this manner, *P[11226]* was localized downstream from the 3' end of *DHR39* while *P[13215]* was mapped to the first intron of the *DHR39* gene (Figure 1). *P[13215]* thus lies upstream from the second exon, which contains the beginning of the *DHR39* protein coding region (Ohno and Petkovich, 1992). Curiously, however, only the lethality associated with *P[11226]* failed to complement the *TW1* deficiency that removes the *DHR39* locus (Figure 1). The lethality associated with *P[13215]* mapped outside of the region defined by this deficiency. The lethal mutation associated with the *P[13215]* chromosome was easily dissociated from the P element insertion by recombination. Henceforth, we will use the name *P[13215]* to refer to the stock from which the lethal mutation was removed by recombination.

Because *P[13215]* mapped within the *DHR39* gene, it seemed likely that it would effect *DHR39* transcription. To test this possibility, we isolated RNA from two control stocks, Canton S and w^{1118}, as well as *P[13215]* homozygotes. Equal amounts of RNA were analyzed by northern blot hybridization using three different radioactive probes derived from either the 5' or 3' ends of *DHR39*, or from the *white* gene that is carried by the *P[13215]* insertion. As expected, the 5 kb *DHR39* mRNA can be detected using both the 5' and 3' *DHR39* probes, in both Canton S and w^{1118} animals (Figure 2). In contrast, almost undetectable levels of *DHR39* mRNA are present in *P[13215]* homozygotes. A truncated *DHR39* mRNA could, however, be detected in these animals which showed strong cross-hybridization to the *white* probe.

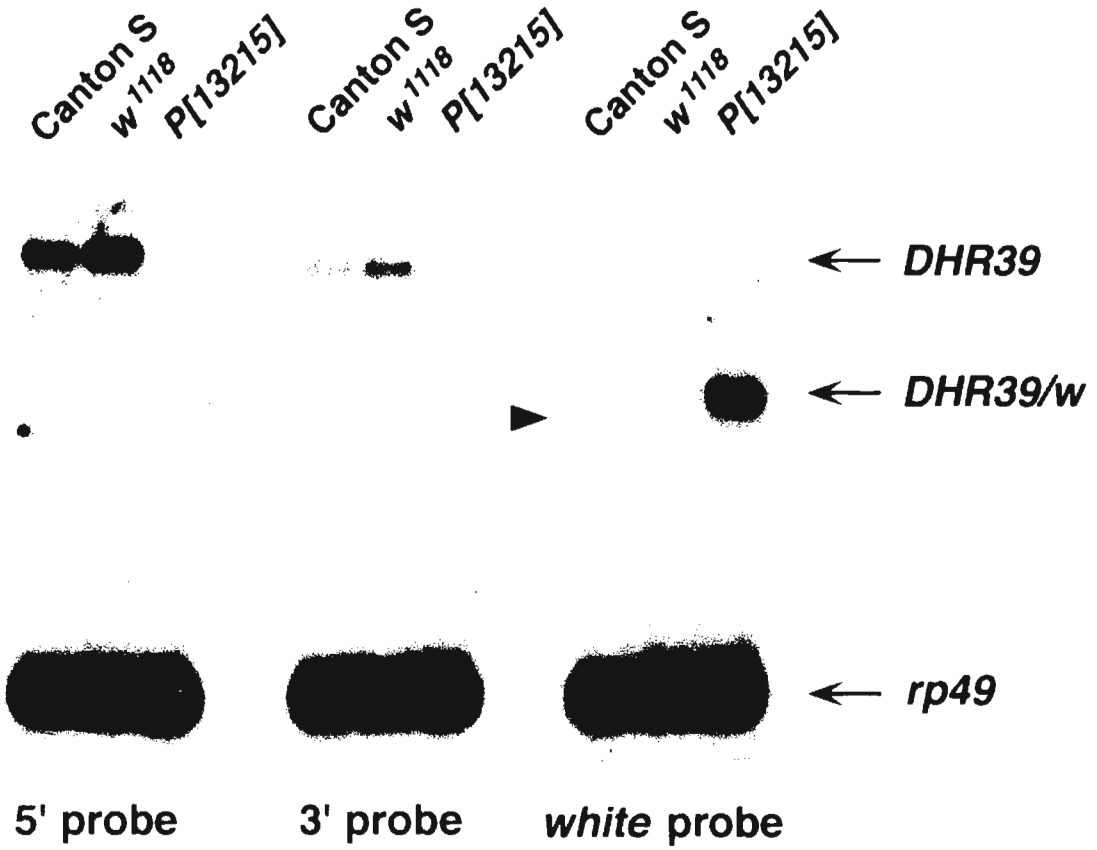

Figure 2. Northern blot hybridization analysis of RNA isolated from control and *DHR39* mutant prepupae. RNA was isolated from newly-formed prepupae of two control strains, either Canton S or w^{1118}, and *P[13215]* homozygotes. Equal amounts of RNA were loaded on a gel, fractionated by formaldehyde agarose gel electrophoresis, and hybridized with one of three radioactive probes. The 5' probe was derived from an EcoRI fragment that spanned the 5' end of the c10 cDNA clone (Ayer *et al.*, 1993). The 3' probe was derived from a HindIII fragment that spanned the 3' end of c10, and the *white* probe was derived from a SalI fragment isolated from pCaSpeR. The *DHR39* transcript and *DHR39/white* fusion mRNA are marked by arrows, as is *rp49* that was used as an internal control for loading and transfer. The arrowhead marks the wild-type *white* mRNA in Canton S animals.

This transcript migrates slightly slower than the *white* mRNA, which is expressed in Canton S but not w^{1118}, as detected with the *white* probe (arrowhead in Figure 2). These observations are consistent with a *DHR39/white* fusion transcript that contains a short length of the *DHR39* 5' untranslated region joined to the *white* coding region. Densitometric scans of the full-length *DHR39* mRNA expressed in *P[13215]* homozygotes revealed that this level is approximately 0.1% of wild-type levels. Similar results were obtained by northern blot hybridization using RNA samples isolated from *P[13215]* over a deficiency, or from 0-3 hour *P[13215]* homozygous mutant embryos (data not shown). This mutation thus appears to severely reduce both maternal and zygotic *DHR39* activity.

Surprisingly, the *P[13215]* insertion could be easily maintained as a homozygous viable stock. This suggests that there is no essential function for either the maternal or zygotic functions of *DHR39*. Furthermore, when both parents carried *P[13215]* over a balancer, the *P[13215]* homozygous offspring comprised the expected one-third of the population (30.9%; n = 313). In order to test for embryonic *DHR39* function, embryos were collected from *P[13215]* homozygotes and followed to hatching. Of these, 91% hatched on time (n = 625), as compared to 95.2% from a w^{1118} control stock (n = 666). Furthermore, no effects on *ftz* expression could be seen in *P[13215]* embryos by *in situ* hybridization, and no defects were evident in the cuticle (data not shown).

These results indicate that *DHR39* does not play an essential role during *Drosophila* development. Similar results have been obtained with mutations in the *E78* orphan receptor gene, although these mutations did lead to subtle changes in the puffing patterns of the polytene chromosomes (Russell *et al.*, 1996). Further insights into *DHR39* function will require a screen for second-site interacting mutations in *P[13215]* homozygotes.

Acknowledgments: We thank P. Taghert for freely sharing information regarding their studies of the 39BC interval, and T. Laverty and G. Rubin for providing the P element stocks used in this study.

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Alatortsev, V.E. Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Sq. 46, Moscow, 123182, Russia. An indication on overlapping functions of the *Vinculin* and *α-catenin* loci in *Drosophila melanogaster*.

Vinculin, a conservative protein of the cellular cytoskeletal and anchorage system, was localized in adherent contacts of cells (Geiger *et al.*, 1980; Burridge *et al.*, 1988; Geiger *et al.*, 1990). Vinculin is homologous to the other peripheral cytoplasmic protein, α-catenin, in vertebrates (Kemler, 1993). Recently the *Vinculin* and *α-catenin* genes were described in *Drosophila melanogaster* (Alatortsev *et al.*, 1997; Oda, *et al.*, 1993). Structures of the corresponding *Drosophila* proteins are compared in this note.

Alignment of the vinculin (962 amino acids) and α-catenin (935 amino acids) sequences revealed that internal repeats and proline-rich domain are unique to the *Drosophila* vinculin. However, vinculin and α-catenin contain three extended regions of homology which occupy greater parts of their sequences (Figure 1). These regions lie within the highly conservative N- and C-domains of vinculin, as well as in the central part of the vinculin sequence. Given this multiple homology, it is possible to suggest that vinculin and α-catenin have some functions in common.

Interestingly, sequence of the central part of vinculin is variable in different vinculins (Weller *et al.*, 1990). High level of similarity between vinculin and α-catenin found for central region (71.5%) reflects co-evolution of two proteins in *Drosophila* and represents a special indication on overlapping functions of the *Vinculin* and *α-catenin* genes.

Acknowledgment: This work was partly supported by an RBRF grant.

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Figure 1 (next page). Alignment of amino acid sequences of the *Drosophila* vinculin (Dmvincp) and α-catenin (Dmcatp) produced with the help of the GENEBEE program (Brodsky *et al.*, 1995). Standard parameters were used. Only regions with reliable homology are shown. The meaning of signs at the top of the alignment is following: ' ' - the average weight of column pair exchanges is less than weight matrix mean value; '.' - is less than mean value plus one SD; '+' - is less than mean value plus two SD; '*' - is more than mean value plus two SD.

```
                                                    + +..  . ++  + ++* .++. +
Dmvincp (  46)                                  ... VGRDTINSSDDKILRQDMPSALHRV
Dmcatp  (  86)                                  ... IQKGEQIAYENPDITQEMLTAVDEV

                . ..+ +. +.   ..+*.+.  * .++ ..*.+* ....**+  +  +++ ++.. . +
Dmvincp (  81) EGASQLLEEASDIVRSDPYSGPARKKLIEGSRGILQGTSSLLLCFDESEVRKIIQECKRV
Dmcatp  ( 121) KKTGDAMSIAAREFSEDPCSSLKRGNMVRAARNLLSAVTRLLILADMVDVHLLLKSLHIV

                 + *.  .  .. ++*++ ++++..  ..+ ++.. *+.+*...  .+ *.   . +*.
Dmvincp ( 141) LDYLAVAEVINTMEQLVQFLKDLSPCLSKVHREVGAREKELTHQVHSEILVRCLEQVKTL
Dmcatp  ( 181) EDDLNKLKNASSQDELMDNMRQFGRNAGELIKQAAKRQQELKDPQLRDDLAAARAMLKKH

                ..+*+ . *+*++  + +
Dmvincp ( 201) APILICSMKVYIHIVEQQ ...
Dmcatp  ( 241) STMLLTASKVYVRHPELD ...

                           ...*. ..+ .++ .*  +*+ .+..++++++ +.*++..+** +
Dmvincp ( 449)             ... GSGPAAKQAAKQLTQKLYELKAAIQNALVNRIVQDFMDVSTPLKQ
Dmcatp  ( 380)             ... DNSPGLSRAIDQMCRKTRDLRRQLRKAVVDHVSDSFLETTTPLLD

                +.++.   ...  *+.. .  ..+ + + + +. ...* .....    +       .+++
Dmvincp ( 494) FTEAVLQPEGTPGREQNfNQKSNNLQAFSDRASKTSRMVAAGGACGNKKIaeillsSAAQ
Dmcatp  ( 425) LIEAAKSGNEKKVREKS-EIFTKHAEKLVEVANLVCSMSNNEDGVKMVRY------AAAQ

                +++* *++*.+..*    *.+*.+++++ . ++...  +  + .  ++ +. .+*+ .++
Dmvincp ( 554) VDSLTPQLISAGRIRMNYPGSKAADEHLQNLKQQYADTVLRMRTLCDQATDPADFIKTSE
Dmcatp  ( 478) IESLCPQVINAASILTVRPNSKVAQENMTTYRQAWEVQVRILTEAVDDITTIDDFLAVSE

                .*+   .. *  +++. +...+ .....*.   .*+  +...+ ++ +  +*+.*+ .+.
Dmvincp ( 614) EHMQVYAKLCEDAIHARQPQKMVDNTSNIARLINRVLLVAKQEADNSEDPVFTERLNAAA
Dmcatp  ( 538) NHILEDVNKCVMALQVGDARDLRATAGAIQGRSSRVCNVVEAEMDNYEPCIYTKRVLEAV

                . *
Dmvincp ( 674) NRL ...
Dmcatp  ( 598) KVL ...

                     .+. ++ .*....++**  +*+* +++  +.+  +. .**.   ..++*..+**
Dmvincp ( 799)    ... GLHQEVRQWSSKDNEIIAAAKRMAILMARLSElvLSDSRGS---KRELIATAKK
Dmcatp  ( 728)    ... TFDSEVAKWDDTGNDIIFLAKHMCMIMMEMTD--FTRGRGPlktTMDVINAAKK

                *.++...+.+*.++++ +*.+...+.+**.  +**.    ++.* +.+*+.+ .  *.
Dmvincp ( 850) IAEASEDVTRLAKELARQCTDRRIRTNLLQVCERIPTIGTQLKILSTVKATMLGAQGS--
Dmcatp  ( 780) ISEAGTKLDKLTREIAEQCPESSTKKDLLAYLQRIALYCHQIQITSKVKADVQNISGEli

                 .. +... *+ .+.+**..+  +++ .  ++.* .* . .+.. + *
Dmvincp ( 908) DEDREATEMLVGNAQNLMQSVKETVRAAEGASIK-IRSDQTSNR-LQW ...
Dmcatp  ( 840) VSGLDSATSLIQAAKNLMNAVVLTVKYSYVASTKyTRQGTVSSPiVVW ...
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Singh, S.R., and B.N. Singh. Department of Zoology, Banaras Hindu University, Varanasi, India. Female remating in *Drosophila ananassae*.

Drosophila females store large amounts of sperm after mating and mate repeatedly, sometimes before sperm already stored are exhausted. Female remating results in sexual selection on males. Thus it is of considerable evolutionary significance. Female remating has been reported in several species of *Drosophila: D. melanogaster, D. pseudoobscura, D. silvestris* and *D. subobscura* (Dobzhansky and Pavlovsky, 1967; Anderson, 1974; Milkman and Zeitler, 1974; Cobbs, 1977; Gromko and Pyle, 1978; Craddock and Johnson, 1978; Loukas *et al.*, 1981). The phenomenon of multiple mating is important because it is associated with sperm usage (Parker, 1970). In *D. melanogaster*, studies on sperm competition in relation to sperm displacement have been carried out and interesting results have been reported by various investigators (Prout and Bundgaard, 1977; Gromko and Pyle, 1978; Griffiths *et al.*, 1982; Harshman and Prout, 1994).

Drosophila ananassae is a cosmopolitan and domestic species. This species occupies unique status in the whole of the genus *Drosophila* due to certain peculiarities in its genetic behavior (Singh, 1985). Extensive work on population genetics, behavior genetics, male recombination and mutagenesis have been carried out by numerous investigators in *D.*

ananassae (Tobari, 1993; Singh, 1996). We have initiated studies on multiple mating in *D. ananassae* and report the results of preliminary study on female remating in this species.

Virgin females and males were collected from a wild laboratory stock of *D. ananassae* and aged for seven days. One female and one male were transferred to a food vial without etherization and the pair was observed. Courtship time and duration of copulation were recorded for each mated pair. After termination of copulation, the male was aspirated out and a fresh virgin male was transferred to the vial containing the mated female. The pair was observed and if the remating did not occur until evening, female and male were separated. Again next day in the morning, the mated female was kept in a food vial with a virgin male and the pair was observed. Out of 8 mated females, remating was observed in 5 females. In 3 females, remating was observed on the 6th day and in 2 females remating occurred on the 2nd day. Duration of copulation was recorded for all the females in both the matings. Interestingly, the duration of copulation was shorter in the second mating as compared to the first mating for all the five remated females.

Experiments will be conducted to study the phenomenon of multiple mating in *D. ananassae* with particular reference to its frequency in different populations, reproductive consequences and genetic basis.

Acknowledgments: Financial assistance from the UGC, New Delhi in the form of a Research Project to BNS is gratefully acknowledged.

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Pérez, M.; N. Castillo-Marín, and L. A. Quesada-Allué. Instituto de Investigaciones Bioquímicas, Fundación Campomar and University of Buenos Aires, Patricias Argentinas 435, Buenos Aires (1405), Argentina. ß-alanyl-dopamine synthase in *Drosophila melanogaster* and *Ceratitis capitata* melanic mutants.

Melanic cuticle mutants have been described and genetically characterized in a number of insects (Czapla *et al.,* 1990; Roseland *et al.,* 1987) including *Drosophila* (Hodgetts and Konopka, 1973; Hodgetts and Choi, 1974). We recently studied a *Ceratitis capitata* mutant, *niger*, that shows a melanic puparium cuticle and was found to be defective for the enzyme conjugating ß-alanine and dopamine to synthesize ß -alanyl-dopamine [NBAD] (Wappner *et al.,* 1996a). This molecule seems to be the main sclerotizing and pigmentation agent in brown insect cuticles (Kramer and Hopkins, 1987). When the NBAD synthase is not functional (Wappner *et al.,* 1996a; this note) or when ß-alanine is not available, like in the *Ceratitis* mutants *Dark pupa* and *Black pupa* (Wappner *et al.,* 1996b), the redundant dopamine substrate (that cannot be conjugated with ß-alanine) enters the melanine pathway, thus giving raise to a black cuticle. *Drosophila* e^1 (*ebony*) (FlyBase FBgn0000527) shows a shining black adult cuticle and is unable to use ß-alanine for tanning the puparium. *ebony* was postulated to be defective in NBAD synthase (Wright, 1987; Hodgetts and Konopka, 1973) but no proof was available, since the data on the direct measurement of the enzyme activity in the wild type were not further substantiated in a publication. Based on both the melanic phenotype and abnormal behaviour (Kyriacou *et al.,* 1978) we previously suggested that *Drosophila* e^1 and *Ceratitis nig* (*niger*) might be mutants corresponding to related genes (Wappner *et al.,* 1991). Here we report confirmative preliminary results related to these questions.

Wild type (Oregon R), *black* and *ebony* strains of *Drosophila melanogaster* were grown in Carolina's blue food. Wild type (Arg.17), *Dark pupae* and *niger* strains of *C. capitata* were grown in carrot-corn-yeast medium (Quesada-Allué *et al.,* 1994). The flies were maintained at 21°C in a D/L regime of 8/16 hs.

The Standard assay for NBAD synthesis contained 1.0 mM ATP, 10 mM $MgCl_2$, 0.1 mM dopamine, 10 µM ß-alanine and 0.01 µCi of [^{14}C]-ß-alanine in 50 mM Na-tetraborate-Boric acid buffer, pH 8.3. The reaction was started with 10 µL of the enzymatic preparation (see Wappner *et al.,* 1996) in a final volume of 50 µL. Radioactive [^{14}C]ß-alanine-containing catecholamines were isolated and measured as previously described (Mason and Weinkove, 1983; Wappner *et al.,* 1995). The different reaction products were separated from substrates and identified in C_{18}-reverse phase HPLC or in silica gel-TLC (Solvent I = Methyl-ethyl-ketone:propionic acid:Water [40:13:11]).

Table 1 shows that crude extracts of *Drosophila melanogaster* wild type and *Ceratitis* w.t. were able to synthesize a [^{14}C]-ß-alanine-containing substance behaving in acid alumina as a diphenolic cathecolamine and further co-

chromatographing in reverse-phase HPLC (not shown) and TLC (R_{fl} = 0.63; see Figure 1) with a standard of ß-alanyl-dopamine. This is the first report on the *in vitro* biosynthesis of this substance in *Drosophila*. The enzymatic activity of NBAD synthase in recently emerged *Drosophila* w.t. adults was found to be similar to that in pupae (Table 1) in spite of the fact that the wild-type puparium of *D. melanogaster* shows a very pale brown coloration whereas the adult cuticle shows the typical brown color generated by NBAD-dependent tanning. In contrast, a strong reddish-brown color develops in *Ceratitis* w.t. puparium. This probably indicates that the synthesis of NBAD in the latter is comparatively higher than in *Drosophila* puparium. This seems to be confirmed by the specific activity data in Table 1. However we cannot discard that, in addition, low levels of one or both of the substrates, dopamine and ß-alanine, might also contribute to the *Drosophila* pale puparium phenotype. Hodgetts and Konopka (1973) reported lower levels of dopamine in *Drosophila* than in other insects at the beginning of pupariation.

The crude extract of the mutants *Dark pupa* of *Ceratitis* and *black* of *Drosophila,* known to be deficient in ß-alanine (Wappner *et al.,* 1996b; Jacobs,1985), were also able to synthesize NBAD (Table 1 and Figure 1), thus showing

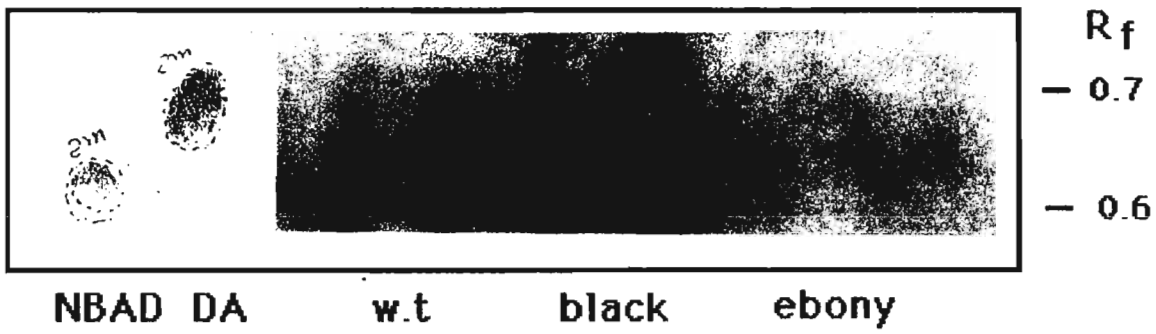

Figure 1. TLC analysis of β-alanyl dopamine synthesized by *Drosophila* extracts (duplicates). Ninhidrin-visualized standards = DA: Dopamine; NBAD: β-alanyl dopamine. Rf: relative mobility in solvent I.

Table 1. Activity of NBAD synthase in wild type and melanic mutants

INSECTS	Specific activity ($pmol.min^{-1}.mg^{-1}$)
Drosophila melanogaster	
- wild type (pupae)	3.26
- wild type (adults)[a]	4.96
- black (pupae)	5.18
- ebony (pupae)	0.56
- ebony (adults)[a]	0.00
Ceratitis capitata (pupae)	
- Arg 17 (wild type)	29.00
- Dark pupa	36.26
- niger	0.72

(a) just emerged exarate adults

that, as expected, the synthase was not affected. Table 1 also shows that the extract of the *Drosophila* mutant *ebony* was unable to synthesize NBAD, thus behaving as the extract of the NBAD synthase-deficient *niger* of *Ceratitis* (Table 1; see also Figure 1). This result represents a direct confirmation of the accepted theory postulating, on the basis of indirect data and unpublished results, that the *ebony*$^+$ locus encodes NBAD synthase (Wright, 1987; Hopkins and Kramer, 1992). It is noteworthy that similar levels of ß-alanine were found in w.t. and in *ebony* pre-pupae as well as in *Ceratitis* w.t. and *niger* prepupae (not shown). Moreover, the enzymatic activities hydrolyzing NBAD to give ß-alanine and dopamine were measured and no differences were found between wild-types and melanic mutants, thus discarding an enhanced hydrolysis of NBAD as explanation for the apparent lack of NBAD synthesis in *ebony* and *niger* mutants (not shown).

From the above data, it can be concluded that the *ebony* mutant of *Drosophila* and the *niger* mutant of *Ceratitis* are deficient for the activity of the NBAD-synthase and therefore seem to be homologous mutants.

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Costas, J.[1], **E. Valade**[1], **and H. Naveira**[2]. [1] Dpto. Bioloxia Fundamental, Facultade de Bioloxia, Universidade de Santiago de Compostela, E-15703 A Coruna, Spain. [2] Dpto. Bioloxia Celular e Molecular, Facultade de Ciencias, Universidade de A Coruna, E-15071 A Coruna, Spain. A preliminary study on the relationship between the blood transposable element and the *Drosophila* genome.

Genetic and molecular studies of the coevolution between transposable elements and the host genome can provide important clues for the elucidation of gene regulation, identifying new loci involved in different aspects of gene expression, from chromatin insulation (*Su(Hw)*, Gerasimova *et al.*, 1995) to RNA stability (*su(f)*, Mitchelson *et al.*, 1993).

In this context, we have initiated an analysis of the relationship between the blood transposable element and the *Drosophila* genome. blood was first described as an insertion into the *white* gene of *Drosophila melanogaster* (Bingham and Chapman, 1986). This mutant, called *white-blood* (w^{bl}), has been included in several studies of modifier genes of the white locus: w^{bl} is partially suppressed by *Inr-a* (Rabinow *et al.*, 1991) and *Mow* (Bhadra and Birchler, 1996), partially enhanced by *Wow* (Birchler *et al.*, 1994) and *Lip* (Csink *et al.*, 1994b) and not affected by *Doa* (Rabinow and Birchler, 1989), $E(w)^a$ (Birchler and Hiebert, 1989), *Msu* (Csink *et al.*, 1994a) and *mw* (Birchler *et al.*, 1989). Nevertheless, a direct interaction between the modifier gene and the blood transposable element has only been demonstrated in the case of *Lip*, which modifies the total transcript abundance of blood.

We tried to search for another modifier of w^{bl} in order to find genes that interact with the blood element. We chose our candidate genes among that than meet these two requirements: i) they modify spontaneous mutations at other loci, but ii) have not been characterized at the molecular level. The selected genes were: $su(t)^1$, $e(dp^v)$, $Su(ss)^2$ and $su(pr)^B$ (Lindsley and Zimm, 1992). We also included $Su(Hw)^3$ because of the proposal of the existence of two potential *Su(Hw)* binding sites in the blood element (Wilanowski *et al.*, 1995).

All of these genes are on the third chromosome. So, males bearing the putative modifier gene were crossed with w^{bl} females over the TM3 *Sb Sr e* balancer chromosome. The F1 males (heterozygous for the tested gene) were screening for modification of the w^{bl} phenotype. The F2 generation allowed us to test for modification of the w^{bl} phenotype in homozygous condition of the tested gene.

w^{bl} is a temperature sensitive allele and the critical period for the w^{bl} eye phenotype is during the first half of pupal development (Ephrussi and Herold, 1945). So, pupae were subjected to two different developmental temperatures during these tests, 24° and 28°C.

No evidence of modification was found in any of these cases, suggesting that these genes do not interact with blood.

When you begin to search for coevolution between a transposable element and the host genome, you must take into account the possibility of a recent horizontal transfer, thus reducing the coevolution period (Kidwell, 1993). The importance of coevolution is clearly shown in the case of the copia transposable element. A transgenic copia element shows 100-700 fold increased expression in cell lines derived from *D. hydei*, which lack copia elements, relative to *D. melanogaster* cells (Cavarec *et al.*, 1994). These data have been interpreted as a result of coevolution between copia and the *D. melanogaster* genome to limit the mutagenic potential of copia.

Using the blood sequence submitted to the Genbank data base by the Berkeley Genome Group (AC: L49394) we designed a PCR to search for the presence of the blood element within the genome of the species of the *D. simulans* complex (*D. simulans*, *D. mauritiana* and *D. sechellia*). We amplified around 800bp comprising the integrase domain of blood in each of these species, using primers:

CAAAAGCCGGAATGCATAAAA and TCTGGGTAGTCTGCCAAATACT.

The comparison of the sequences we obtained revealed eight variable sites. In each site, only one of the species was different from the others. In four cases, *D. melanogaster* was the different one, in two cases, *D. mauritiana*, and in one case each, *D. simulans* and *D. sechellia*.

These data confirm the presence of blood within the genome of the four species of the *D. melanogaster* complex. Besides, they suggest vertical transfer of blood between these species. blood was present in the genome of the common ancestor of these species, thus allowing enough time to expect coevolution between blood and the *D. melanogaster* genome.

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Kozlova, A., and L. Omelyanchuk. Institute of Cytology and Genetics, Novosibirsk 630090. Y-chromosome factor controls transcription of fertility genes in *Drosophila melanogaster*.

Three transposants of P[lArB] showing sterility of homozygous males were induced and mapped by *in situ* hybridization (ms (3) P50 - 67A4-B13, ms (3) P122 - 92A2-14 and ms(3) P115 - 75D). The lacZ reporter gene in P[lArB]construction is under the control of a weak P-element promoter. In chromosome, nearby enhancer can activate lacZ, and β-galactosidase encoded by this gene is registered by X-gal staining of tissues. We use this approach to reveal an influence of Y aneuploidy on the activity of male fertility genes.

The presence of β-galactosidase in testes was detected after fixation in 0.1M PIPES pH6.9, 2mM EDTA, 1mM $MgSO_4*H_2O$, 9.2% formaldehyde for 20 min and incubation in staining solution (10mM $NaH_2PO_4*2H_2O$/ $Na_2HPO_4*2H_2O$ pH 7.2, 150 mM NaCl, 1mM $MgCl_2*6H_2O$, 0.3% Triton X-100, 0.2% X-gal) overnight.

The results of X-gal staining in male testes with different sets of sex chromosomes (Table 1) show that the presence of Y-chromosome is neccessary for the expression of male fertility genes. Our next step was to test the effect of Y-chromosome arm aneuploidy on the LacZ expression (Table 2).

Table1.

Genotype	Staining
X / Y; ms(3) P50 / TM3	+
X / Y; ms(3) P122 / TM3	+
X / Y; ms(3) P115 / TM3	+
X / O; ms(3) P50 / +	_
X / O; ms(3) P122 / +	_
X / O; ms(3) P115 / +	_
X / O; TM3 / +	_

Table 2.

Genotype	Staining
X/Y; ms(3) P50/TM3	+
X/Y; ms(3) P122/TM3	+
X/Y; ms(3) P115/TM3	+
X/O; ms(3) P50/+	_
X/O; ms(3) P122/+	_
X/O; ms(3) P115/+	_
YSX., y cv v f /O; ms(3) P50/+	+/-
YSX., y cv v f /O; ms(3) P122/+	+/-
YSX., y cv v f /O; ms(3) P115/+	+/-

Table 3.

Genotype	Staining
X/Y; ms(3) P50/TM3	+
X/Y; ms(3) P122/TM3	+
X/Y; ms(3) P115/TM3	+
X/R(Y)L; ms(3) P50/+	_
X/R(Y)L; ms(3) P122/+	_
X/R(Y)L; ms(3) P115/+	_

Table 4.

Genotype	Heat-shock 37°C 30 min.	Staining
X/Y ; +/+	_	_
X/Y; Bg 9.61/+	+	+
X/Y; Bg 9.61	_	_
y w/O; P103/+	_	+
y w/Y; P103/CyO	_	+
X/O; Bg9.61/+	+	+

The LacZ expression in flies which genotypes contain YS was registered, but its intensity was weaker than in X/Y. An analogous experiment was performed with YL.

The data obtained lead to the conclusion that the YS but not the YL contains factors that control the expression of male fertility genes. However the possibility must be excluded that the Y-chromosome aneuploidy interrupts in a nonspecific manner the expression of the majority of genes involved in spermatogenesis by disruption of this process *per se*.

To test this assumption we use P[lArB] transposant stocks P103 (described earlier, Omelyanchuk, 1995) and Bg 9.61 (Lis *et al.*, 1983). In P103 individuals, staining was observed in neural ganglia, imaginal disks, ovaries and testes. The Bg 9.61 stock contains insertion of a construct where the LacZ gene is under control of heat-shock promoter. All tissues were stained after heat-shock in this stock.

The relults of testes staining in males containing different sets of sex chromosomes and insertions P103 and Bg 9.61 are shown in Table 4. The expression of LacZ in flies of X/O and X/Y genotypes is similar. So the possibility that the Y chromosome aneuploidy nonspecifically interrupts gene expression in testes may be excluded. And we can conclude that the YS arm contains factors responsible for the transcription of male fertility genes in *D. melanogaster*.

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Singh, B.K., and R.S. Fartyal. Cytogenetics Laboratory, Department of Zoology, Kumaun University, Nainital-263002, India. A list of Drosophilid species so far described and recorded from Kumaun region, India.

The great importance of Drosophilidae in genetic and evolutionary studies evoked most of the countries of the world to study the Drosophilid fauna thoroughly. However, the Indian subcontinent still remains a virgin field to be explored.

The Kumaun region, a wild hilly area is located at an elevation of about 6,000 ft. (1828 meters) from the sea level on the north east periphery of the state Uttar Pradesh. This region includes four border districts of the state, Nainital, Almora, Pithoragarh and Udham Singh Nagar.

Although more than 300 species of Drosophilidae have been described and recorded so far from different parts of Indian subcontinent (Gupta, 1981, 1985), a vast area of great ecological interest still awaits exploration. Our knowledge in this field seems scanty in comparison with other countries of the world. Due to the above situation an extensive study of the Drosophilidae of Kumaun region was done, which is almost a virgin field for the above mentioned study. The following table shows the results of surveying studies of Drosophilids of Kumaun region since 1984 to 1996. The present surveying studies shows that the members of the Drosophilidae are fairly distributed in Kumaun region, particularly the members of the subgenus *Drosophila* of the genus *Drosophila*.

List of Drosophilid species so far described and recorded from Kumaun region:

GENUS *Amiota* Loew
Subgenus *Phortica* Schiner
1. *bandes* Singh and Negi, 1992

GENUS *Gitona* Meigen
2. *distigma*, 1830

GENUS *Leucophenga* Mik
3. *bellula* (Bergrowth, 1984)
guttiventris (de meijere, 1908) Syn. ref. Bock, 1979, Aust. J. Zool. Suppl. Ser. 71:25
4. *neolacteusa* Singh and Bhatt, 1988
5. *angulata* sp. nov. (In press)
6. *Okhalkandensis* sp. nov. (In press)
7. *Clubiata* sp. nov. (In press)

GENUS *Paraleucophenga* Hendel
8. *neojavanaii* Singh and Negi, 1992
Lissocephala Malloch
9. *parasiatica* Takada and Momma, 1975

GENUS *Scaptomyza* Hardy
10. *himalayana*, Takada, 1970
11. *quadruangulata* Singh and Dash, 1993

GENUS *Zaprionus* Coquillette
12. *indianus* Gupta, 1970 (for *indiana*)

Subgenus *Dorsilopha* Sturtevant
13. *busckii* Coquillette, 1901

Subgenus *Drosophila* Falle'n Str.
14. *analspina* Singh and Negi, 1995
15. *bishti* Singh and Negi, 1995
16. *bageshwarensis* Sp. nov. (In press)
17. *immigrans* Sturtevant, 1921
18. *lacertosa* Okada, 1956
19. *nainitalensis* Singh and Bhatt, 1988
20. *nasuta* Lamb, 1914
21. *paunii* Singh and Negi, 1989
22. *painaii* Singh and Negi, 1995
23. *repleta* Wollaston, 1858
24. *sulfurigaster* Duda, 1923
25. *trizonata* Okada, 1966
26. *serrata* Sp. nov. (In press)
27. *paramarginata* Sp. nov. (In press)
28. *hexaspina* Sp. nov. (In press)
29. *surangensis* Sp. nov. (In press)
30. *paharpaniensis* Sp. nov. (In press)
31. *khansuensis* Sp. nov. (In press)
32. *elongata* Sp. nov. (In press)

Subgenus *Scaptodrosophila*
33. *coracina* Kikkawa and Peng, 1938
34. *chandraprabhiana* Gupta and Ray-Chaudhury, 1970
35. *hirsuata* Sp. nov. (In press)

Subgenus *Sophophora* Sturtevant
36. *bifasciata* Pomini, 1940
37. *jambulina* Parshad and Paika, 1964
38. *kikkawai* Burla, 1954
39. *malerkotliana* Parshad and Paika, 1964
40. *melanogaster* Meigen, 1830
41. *nepalensis* Okada, 1955
42. *suzukii indicus* Parshad and Paika, 1964
43. *takahashii* Sturtevant, 1927
44. *neobaimaii* Sp. nov. (In press)
45. *neokhaoyama* Sp. nov. (In press)
46. *saraswatii* Sp. nov. (In press)

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References: Gupta, J.P., 1981, Dros. Inf. Serv. 56: 50-53; Gupta, J.P., 1985, Dros. Inf. Serv. 61: 86-88.

Additional Information:

Drosophila Stocks wild :

1. *Drosophila melanogaster*
2. *Drosophila jambulina*
3. *Drosophila kikkawai*
4. *Drosophila malerkotliana*
5. *Drosophila immigrans*
6. *Drosophila nepalensis*
7. *Zaprionus indianus*

Nongthomba, U., and N.B. Ramachandra. Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570 006, India. Induction and isolation of chromosome specific indirect flight muscle mutations in *Drosophila melanogaster*.

The indirect flight muscles (IFMs) of *Drosophila melanogaster* provide a unique model system to genetically dissect muscle structure and function (Sparrow *et al.*, 1991). These are the bulk of thoracic muscles consisting of two groups, namely dorsal longitudinal muscles (DLMs) which are composed of six fibers from dorsal to ventral and dorso-ventral muscles (DVMs), DVM I, DVM II, DVM III which are formed by 3, 2, and 2 fibers, respectively. The wild type IFM development has been studied (Crossley, 1978; Fernades *et al.*, 1991). *Drosophila* is also a suitable system because mutations that affect IFMs development can readily be isolated and these mutations do not affect much on the viability of the flies. Hence, the genetic analysis of mutants has greatly advanced the understanding of muscle development.

A number of mutations affecting the IFMs have been reported (Crossley, 1978; Lindsley and Zimm, 1992). Most of these mutations are quite general in their expression which affect all the flight muscles. However, the genes involved in the development of IFMs have not been identified systematically. So far no other chromosome 2 specific mutation has been reported which affects the IFMs development except *Mhc* gene. In view of this, investigations are made to identify and characterize genes involved in IFM development which reside on chromosome 2 by using ethyl methanesulfonate (EMS) mutagenesis. Here we report the induction and isolation of 16 new viable mutations on the second chromosome in *Drosophila melanogaster* which affect the indirect flight muscles.

Table 1. Scheme for Ethyl Methanesulfonate Mutagenesis

Generation	Cross		
	Females		Males
Parental	CyO / Tft	x	+ / + (EMS treated)
F_1	CyO / Tft	x	* / #
F_2	* / CyO	x	* / CyO
F_3	* / * males and females scored for abnormal wing and IFMs		* / CyO males and females females retained for stocks

CyO / Tft = Chromosome 2 marker; * = mutagenised chromosome # = CyO 0r Tft male.

The Canton-S strain and Curly Oster/Tufted (*CyO/Tft*) mutant strain of *Drosophila melanogaster* were used as wild type and dominant markers of chromosome 2, respectively (Lindsley and Zimm, 1992). All the stocks were cultured on standard wheat cream agar medium at 24 ± 1°C. 25mM of EMS was administered to the Canton-S male flies following the procedure of Grigliatti (1986). The protocol used for induction and detection of mutation on chromosome 2 is presented in Table 1. Control experiments for EMS mutagenesis were made using X^XY stocks. For muscle analysis, thoracic whole mounts were prepared following the procedure described by Fyrberg *et al.* (1995). Complementation analysis was done by crossing the virgins of each of the newly-isolated mutants reciprocally and analyzing the progenies for the defects in wings and IFMs.

The summary of the EMS mutagenesis on chromosome 2 which affect IFMs phenotypes in *D. melanogaster* is given in Table 2. A total of 3283 mutation induced lines were screened. Of these, 70.5%, 2.2% and 27.3% mutations were lethal, sterile and viable, respectively. Out of the 27.3% viable homozygotes scored, 3% were of wing mutants, of

Table 2. Summary of the Ethyl Methanesulfonate mutagenesis

Particulars	1st EMS	2nd EMS	3rd EMS	4th EMS	5th EMS	Total	%
Lines screened	623	686	582	493	900	3283	—
Lethal lines	424	557	442	308	583	2314	70.5
Sterile lines	10	13	19	22	08	72	2.2
Viable homozygotes	189	116	120	163	209	897	27.3
Viable wing mutants	05	05	01	04	14	29	0.9
Viable muscle mutants	05	—	—	03	10	16	0.5

which nearly 60% had shown IFM defects. Not all the wing mutants showed defects in the muscle phenotypes indicating that the wing phenotype is not completely penetrant. However, all the muscle mutants showed defects in wing phenotypes. Similarly, Cripps *et al.* (1994) have reported that a number of flightless mutants display abnormal wing positions but all have not shown the muscle defects. The complementation analysis revealed that, of all the 16 mutants, only one has 9 alleles and all other mutants complement to each other. Thus, these 16 newly-isolated mutations belong to 8 complementation groups. The salient features of these mutations are as follows:

1) all the mutations were viable and recessive except only one which is semi-dominant
2) wings were held up or extended or looping
3) most of these were flightless or weak in flight
4) all these had shown defects in IFMs, two of these had shown more than 80% degeneration in both DLMs and DVMs, another two of them had shown more than 50% of defect in DLMs, the remaining four had shown about 20% degeneration of IFMs
5) five of these mutations were fertile in homozygous condition
6) all these had shown various levels of penetrance and expressivity.

The combination of genetic analysis of mutations and the molecular characterization of the normal and mutant genes and proteins has permitted powerful correlations of function and structure and initiation of experiments testing mechanisms active in complex developmental processes (Epstein and Bernstein, 1992). Several mutants are already known which affect the development of IFMs. Most of these were X chromosome mutants, namely, *erect wing* (*ewg*), *flap wing* (*flw*), *indented thorax* (*int*), *upheld* (*up*), *shibire*^{ts} (*shi*^{ts}), *vertical wing* (*vtw*). All these mutants showed defects in DLMs and DVMs except *shibire*^{ts} where the DVMs were normal. There are two mutations on chromosome 3, namely, *Actin 88F* which showed defect in both DLMs and DVMs and *stripe* (*sr*) showed defects in only DLMs. *Myosin heavy chain* (*Mhc*) is the only gene known on chromosome 2 which is involved in both DLMs and DVMs formation. Many alleles of this gene have been reported which affect IFMs at various degrees (Lindsley and Zimm, 1992). In the present study, in all the 16 newly isolated mutants, both DLMs and DVMs were affected and showed different levels of penetrance and expressivity. However, DLMs were more severely affected than the DVMs. Of the 16 mutants 4 of them showed defects in DVMs at various levels. Investigations in this direction will be interesting to find out the genes involved for DVMs development. Further characterization, mapping and developmental analysis of these mutations are in progress.

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X-chromosomal linkage of larval secretion fractions in the *Drosophila nasuta* subgroup.

In earlier studies we have analysed the patterns of larval secretion protein fractions in various members of the *Drosophila nasuta* subgroup. These members are taxonomically closely related and exhibit different degrees of reproductive isolation. By employing alternatively 13.4% and 15% SDS-Polyacrylamide separating gels with 5.6% stacking gels, we could show that: (1) Homologous secretion fractions in individual *Drosophila nasuta* subgroup members (Figure 1) differ by their electrophoretic mobility. By this, we grouped them into five (I - V) domains (Ramesh and Kalisch, 1989). (2) Domain I - III fractions are glycosylated (Zajonz *et al.*, 1996b). (3) Domain II and III fractions are X-chromosomal linked. Data are based on the F1 progeny from parents, which are cross fertile subgroup members indicating different electrophoretic mobilities of homologous fractions (Ramesh and Kalisch, 1989). (4) By puff analysis of polytene chromosomes and recombination analysis of F1 and F2 patterns we could assume that synthesis of domain II and III fractions probably is clustered in the huge puff of salivary gland X-chromosome division 10 (Ramesh and Kalisch, 1988a). (5) Chromosomal location of domain I fractions so far was hampered by almost the same electrophoretic mobility in all subgroup members (Figure 1). However, recently we got the first hint for X-chromosomal linkage of domain I fractions from the F1 progeny of *Drosophila nasuta nasuta* females and *Drosophila sulfurigaster sulfurigaster* males by using an elongated 5.6% stacking gel together with our standard 13.4% separating gel (Zajonz *et al.*, 1996a).

In the present study, we have analysed domain I fractions by using gradient gels for pattern analysis of cross fertile subgroup members *Drosophila nasuta nasuta* (wild type Mysore I) and *Drosophila nasuta albomicans* (Okinawa; No. 155112-1751.0, stock list 1990 of Bowling Green) as well as for *Sandhya* (*Drosophila nasuta nasuta*^{Sa}), a dominant

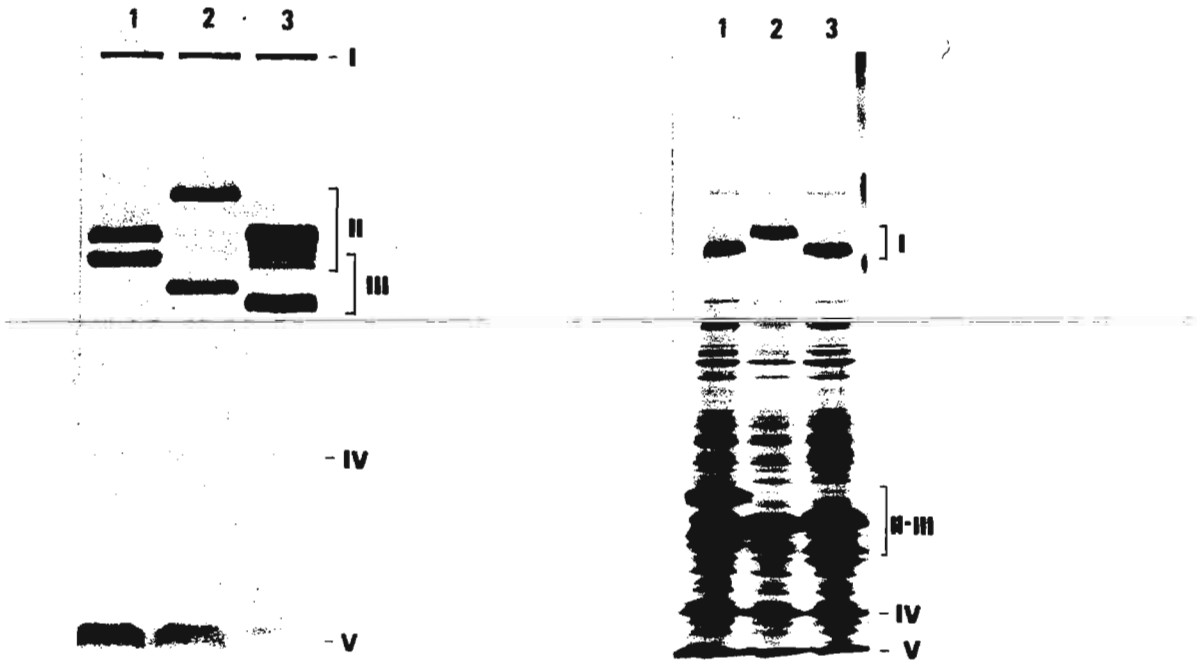


Figure 1 (above, left). Larval secretion fractions in (1) wild type Mysore I mutant *Drosophila n. nasuta*^{Sa}, (2) *Drosophila n. nasuta* (wild type Mysore I), (3) *Drosophila n. albomicans* (Okinawa). 15% SDS-Polyacrylamide separating gel and 5.6% stacking gel (not shown); CBB-staining; two secretion plugs used for each lane; depiction of domain I - V according to Ramesh and Kalisch (1989).

Figure 2 (above, right). Different electrophoretic mobility of domain I fractions in (1) *Drosophila n. nasuta* (wild type Mysore I), (2) wild type Mysore I mutant *Drosophila n. nasuta*^{Sa} (3) *Drosophila n. albomicans* (Okinawa). 4 - 20% SDS-Polyacrylamide gradient gel with 3% stacking gel (not shown); CBB-staining; six salivary glands were used for each lane. Note that domain II and III fractions can not be separated in lane (2) by the technique used.

and spontaneous secretion protein mutant from the Mysore I wild type strain (Kalisch and Ramesh, 1988b). Furthermore, we checked domain I fractions in *Drosophila nasuta kepulauana* (Brunei, Borneo; 15112-1761.1), *Drosophila kohkoa* (Bon Chakkrarat, Thailand; 15112-1771.0), *Drosophila sulfurigaster albostrigata* (Ari Ksatr, Kambodia; 15112-1811.1) and *Drosophila sulfurigaster sulfurigaster* (Kavieng I, New Ireland; 15112-1831.1).

Figure 1 provides a comparative picture of salivary gland secretion protein fractions obtained after electrophoresis on a 15% SDS-Polyacrylamide gel. For more details concerning double band character of domain II and III fractions as well as methodological details see Ramesh and Kalisch, 1988a. Note that homologous domain I, IV, and V fractions in individual strains indicate almost the same electrophoretic mobility.

Figure 2 indicates differences of domain I fraction mobility, using a 4 - 20% SDS-Polyacrylamide gradient gel together with a 3% stacking gel (not depicted). In comparison with Figure 1 the increased number of fractions is due to the use of whole salivary glands instead of only secretion plugs. Domain II and III fractions can hardly be separated in Figure 2 (lane 1 and 3) and can not be separated in (2) by use of the 4 - 20% gradient gel.

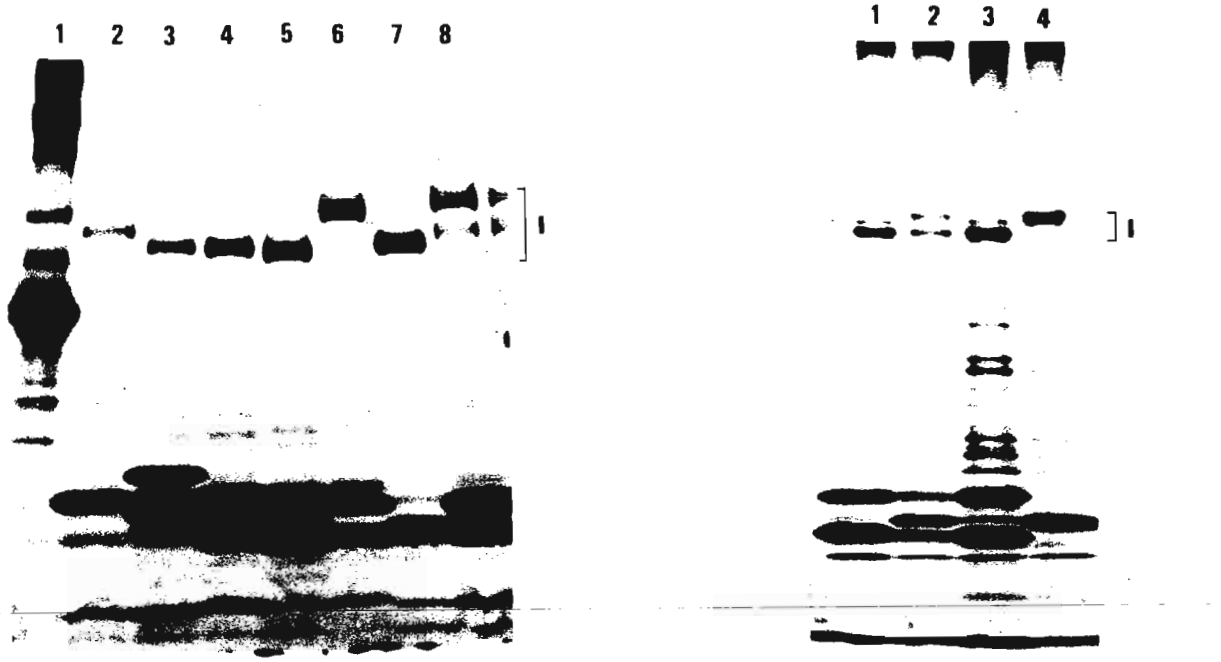


Figure 3 (above, left). Electrophoretic mobility of domain I fractions in different *Drosophila nasuta* subgroup members. (1) Phosphorylase b marker protein, SIGMA, P 8906; (2) *Drosophila n. nasuta*^{Sa} females, 4 secretion plugs; (3) *Drosophila n. nasuta* males, 5 plugs; (4) *Drosophila n. albomicans* females, 5 plugs; (5) *Drosophila n. kepulauana* females, 6 plugs; (6) *Drosophila kohkoa* females, 6 plugs; (7) *Drosophila s. albostrigata* males, 5 plugs; (8) *Drosophila s. sulfurigaster* females, 4 plugs. 4 - 20% SDS-Polyacrylamide gradient gel with 3% stacking gel (not depicted); CBB-staining.

Figure 4 (above, right). X-chromosomal linkage of the domain I fraction. (1) *Drosophila n. nasuta* female larvae, (2) F1 female larvae of the crossing *Drosophila n. nasuta* females x *Drosophila n. nasuta*^{Sa} males, (3) F1 male larvae of the same crossing, (4) *Drosophila n. nasuta*^{Sa} male larvae. Five plugs were used for each lane; 4 - 20% SDS-Polyacrylamide gradient gel with 3% stacking gel (not depicted); silver-staining (Ansorge, 1985).

Surprisingly, domain I fractions in *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans* are almost identical (158 kD) in the 4 - 20% gradient gel of Figure 2, whereas *Sandhya* indicates a value of 178 kD for the homologous fraction. However, matching of kD values in *Drosophila nasuta nasuta* and in *Drosophila nasuta albomicans* are incidental. In an additional study (in preparation) we have found various kD values even for domain I fractions in several wild type strains of *Drosophila nasuta albomicans*.

Additional data of remaining subgroup members in Figure 3 also indicate significantly different electrophoretic mobility of domain I fractions. Based on these results, we assume that subgroup member-specific patterns of domain I

fractions could be used in the same way for taxonomic identification as it has already been done with domain II and III fractions (Ramesh and Kalisch, 1989).

A comparison (Figure 4) between the patterns of P-generation *Drosophila nasuta nasuta* females and *Drosophila nasuta nasuta*^{Sa} males as well as F1 males and females proves that domain I fractions are X-chromosomal linked. We also obtained comparable data for remaining subgroup members (not depicted). However, as already mentioned in Zajonz *et al.* (1996a), the second (and smaller) domain I fraction in *Drosophila sulfurigaster sulfurigaster* [(8) in Figure 3] is autosomally linked (data not depicted).

Note that in Figure 4 we used the very sensitive silver-staining by which: (1) the glycosylated domain I (and II) fractions are stained yellow in the gel and, therefore, indicate a lower contrast; (2) additional fractions become visible on top of each lane (Kalisch and Ramesh, 1997); (3) one additional fraction (brown color in the gel) becomes visible on top of the domain I fraction in *Drosophila nasuta nasuta* [lane (1) and (3)].

So far, we have not found any recombinant pattern (concerning the domain I and II fractions) in thirty F1 males from heterozygous *Drosophila nasuta nasuta/Drosophila nasuta nasuta*^{Sa} mothers. However, additional experimental work is needed (so far hampered by the absence of suitable genetic markers) to prove our assumtion that even the synthesis of the domain I fraction is clustered within the salivary gland X-chromosome division 10 puff.

Nevertheless, differences of domain I - III fractions in individual *Drosophila nasuta* subgroup members as well as individual wild type strains on one side and differences of domain I - III fractions in the *Sandhya* mutant on the other have prompted us to assume that DNA sequencing of the X-chromosomal division 10 puff could give some interesting insights on evolutionary genetic processes, which have occurred within the *Drosophila nasuta* subgroup.

Acknowledgments: The authors like to thank Mrs. Monika Soukupová for conducting the SDS-Polyacrylamide gradient gels and Mrs. Christel Plehn for technical assistance. S.R.R. is thankful to DAAD, Germany, for the award of a scholarship.

References: Ansorge, W., 1985, J. Biochem. Biophys. Methods 11: 13-20; Kalisch, W.-E. and S.R. Ramesh 1997, Dros. Inf. Serv., this issue; Ramesh, S.R. and W.-E. Kalisch 1988a, Biochem. Genetics Vol. 26, Nos. 7/8: 527-541; Ramesh, S.R. and W.-E. Kalisch 1988b, Dros. Inf. Serv. 67: 51-52; Ramesh, S.R. and W.-E. Kalisch 1989, Genetica 78: 63-72; Zajonz, M., S.R. Ramesh, and W.-E. Kalisch 1996a, Dros. Inf. Serv. 77: 47-48; Zajonz, M., S.R. Ramesh, and W.-E. Kalisch 1996b, Dros. Inf. Serv. 77: 76-78.

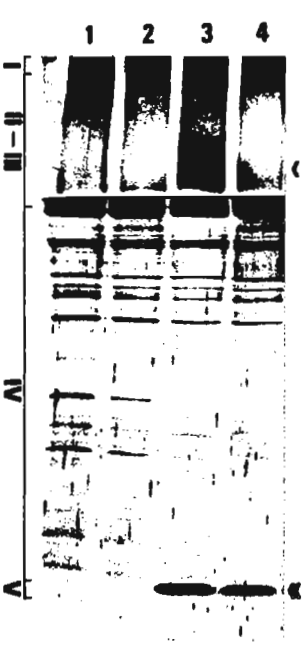
Kalisch, W.-E.,[1] and S.R. Ramesh[2]. [1]Ruhr-Universität Bochum, FR Germany and [2]University of Mysore, India. [1]e-mail: wolf.kalisch@rz.ruhr-uni-bochum.de Salivary gland secretion fractions in *Drosophila hydei.*

In earlier studies (Zajonz *et al.*, 1996a and b), we could show that patterns of secretion fractions in *Drosophila hydei* using SDS-Polyacrylamide gels are: (1) species-specific and completely different from *Drosophila melanogaster*; (2) wild-type-specific by different electrophoretic mobility of homologous fractions; (3) wild-type-specific by the presence of individual fractions; (4) sex-specific by individual fractions in several wild type strains (Figure 1).

Our earlier experiments were conducted by using our standard 13.4% SDS-Polyacrylamide separating gels in combination with elongated 5.6% stacking gels and analysed by silver-staining. In the present paper we have checked *Drosophila hydei* strains by using 4 - 20% SDS-Polyacrylamide gradient separating gels with 3% stacking gels to spread the prominent fractions depicted in Figure 1. We used CBB-staining as well as CBB/silver-staining.

Domains I - V indicate to what extent the 4 - 20% gradient gels in Figures 2 and 3 depict more details in comparison with the 13.4% gel in Figure 1. Domains are based on so far comparative pattern analyses of homologous fractions in different *Drosophila hydei* wild type strains. Otherwise, the domains are arbitrary. Thus, individual domain fractions in *Drosophila hydei* and, for instance, *Drosophila nasuta* (Kalisch and Ramesh, 1997) probably lack any homology concerning functional or biochemical aspects.

Comparison between CBB-staining (Figure 2, lane 1 and 3) and additional silver-staining (lanes 2 and 4) in male and female patterns depict that sex-specific fractions obviously are present in both sexes of the Tübingen wild type, but indicate sex-specific differences concerning the gene expression. Even if one considers that the patterns of individual lanes in CBB/silver-stained SDS-Polyacrylamide gradient gels can be different by: (1) the quantity of proteins extracted from the glands, (2) by staining conditions, and/or (3) by photographic parameters, one can´t ignore sex-specific differences between lanes 2 and 4 by their prominence and by the reproduction of experimental data. The interpretation of sex-specific fractions in *Drosophila hydei* larvae is still missing. But their existance probably could contradict the proposition so far made, that secretion fractions are exclusively used for fixing the pupa to a substratum (Fraenkel and Brookes, 1953; Shirk *et al.*, 1988; Riddiford, 1993).



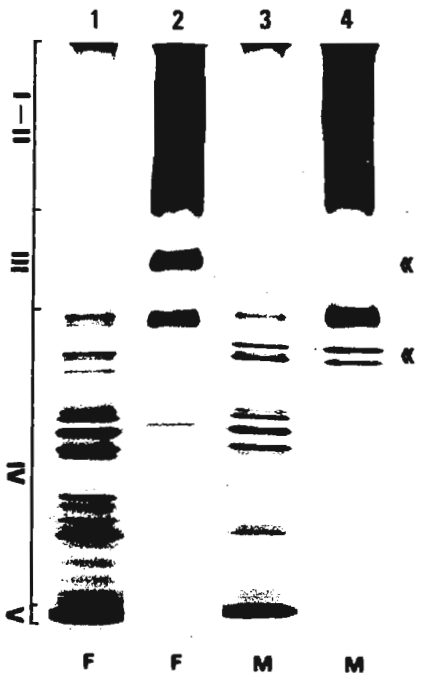


Figure 1 (above, left). Larval secretion fractions from the two salivary gland plugs of an individual *Drosophila hydei* wild type larva: (1) Münster female, (2) Münster male, (3) Zürich female, (4) Zürich male. Note that Zürich shows a strain-specific fraction [«] as well as a sex-specific fraction in the female [<]. 13.4% SDS-Polyacrylamide gel with 5.6% stacking gel; silver-staining. Minor fractions belong, at least in part, to salivary gland cell fractions which remain attached to the plugs in various amounts by the preparation technique used (Ramesh and Kalisch, 1988).

Figure 2 (above, right). Sex-specific salivary gland fractions in *Drosophila hydei* wild type Tübingen larvae comparing CBB-staining (lanes 1 and 3) and additional silver-staining (2 and 4) in 4 - 20% SDS-Polyacrylamide gradient gel with 3% stacking gel (not shown). Five salivary glands were used in each sex; M = males and F = females. « = fractions with different gene expression in females and males. Note the difference of CBB- and silver-staining (Ansorge, 1985) of domain I and II fractions.

Additive silver-staining of Coomasie Brillant Blue R 250 stained patterns in Figure 2 needs additional information: (1) Domain I and II fractions are hardly to depict by the standard CBB-staining used, but get overstained by the silver-staining (by which divisioning into domain I and II, compare Figure 3, is no longer possible). The biochemical details of these different staining behaviours are still unknown. (2) CBB stained fractions in lane 1 and 3 represent secretion fractions and salivary gland cell fractions (most of the smaller domain IV fractions shown). These fractions have not lost their prominent contrast during the additional silver-staining in lane 2 and 4, but have been reduced in photographic contrast not to overexpose domain I - III fractions in the same lanes.

In additional experiments we used PAS-staining (not depicted), but we failed to find any glycosylated secretion fraction in *Drosophila hydei.*

For a more detailed staining of domain I and II fractions, we used plugs instead of whole salivary glands (Figure 3) and conducted a more intensive (2 hour) CBB staining. By this, we found that in contrast to Figure 2 (lanes 1 and 3) at least individual domain I and II fractions of 288 kD and 182 kD could be shown by CBB-staining.

The Münster and the Alicante wild types (which indicate no sex-specific fractions) differ by the electrophoretic mobility of the domain III fraction and by the presence of the 16 kD domain V fraction in the Münster wild type.

From data of Figure 3 and the reciprocal crossing (not depicted) we conclude that domain III fractions are gonosomally linked, whereas the domain V fraction is autosomally linked. Experiments are in progress to localize the gonosomal and autosomal genes by a technique so far not used in *Drosophila* genetics: Recombination analysis of phenotypic markers are combined with recombination analysis of secretion patterns in always one and the same recombinant. This is possible by secretion analysis of single pupae after hatching of the flies; a technique which we have already established earlier (Kalisch and Ramesh, 1988).

Acknowledgments: The authors like to thank Mrs. Monika Soukupová for conducting the SDS-PAGEs and Mrs. Christel Plehn for technical assistance. S.R.R. is thankful to DAAD, Germany, for the award of a scholarship.

References: Ansorge, W., 1985, J. Biochem. Biophys. Methods 11: 13-20; Fraenkel, G., and V.J. Brookes 1953, Biol. Bull. (Woods Hole) 105: 442-449; Kalisch, W.-E., and S.R. Ramesh 1988, Dros. Inf. Serv. 67: 51-52; Kalisch, W.-E., and S.R. Ramesh 1997, Dros. Inf. Serv., this issue; Ramesh, S.R., and W.-E. Kalisch 1988, Biochem. Genetics, Vol.

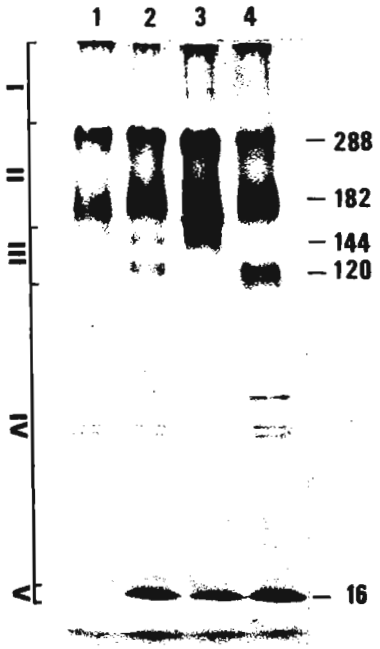


Figure 3. Chromosomal linkage of secretion protein fractions in *Drosophila hydei* larvae. (1) wild type Münster females, (2) F1 females of the crossing: wild type Münster female × wild type Alicante male, (3) F1 males of the same crossing, (4) Alicante males. Five plugs were used for each lane of the 4 - 20% SDS-Polyacrylamide gradient gel covered by a 3% stacking gel (not shown); CBB-staining. kD values are based on phosphorylase b marker protein (P 8906, Sigma). Note that domain III fractions indicate X-chromosomal linkage, whereas domain V fraction indicates autosomal linkage.

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Kalisch, W.-E.,[1] and S.R. Ramesh[2]. [1]Ruhr-Universität Bochum, FR Germany and [2]University of Mysore, India. [1]e-mail: wolf.kalisch@rz.ruhr-uni-bochum.de

Stain-specific characterization of larval secretion fractions.

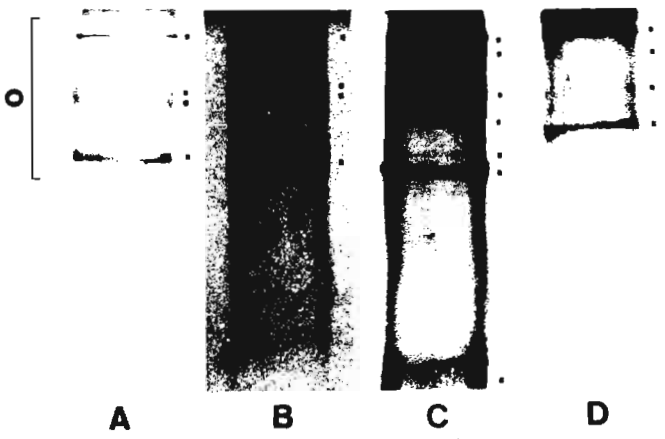
In several studies concerning the biochemical genetics of salivary gland secretion fractions in the *Drosophila nasuta* subgroup we could show that the major fractions could be grouped into five domains (I - V) which are characterized by variations in the electrophoretic mobility of homologous secretion fractions in: different subgroup members, different wild type strains, and mutants (Ramesh and Kalisch, 1988; Kalisch and Ramesh, 1997). In a recent paper we could show that at least two additional fractions (which are named domain 0 fractions in the following) could be localized when the larval secretion plugs of *Drosophila nasuta* subgroup members and other species were electrophoresed on 13.4% SDS-Polyacrylamide gels with elongated 5.6% stacking gels. To visualize these fractions, one has: (1) to fix and eluate the salivary glands with ethanol (instead of a TCA and ethanol/chloroform mixture) and (2) to use silver-staining instead of the common Coomassie Brilliant Blue staining technique (Zajonz *et al.*, 1996).

In the present paper we have checked these domain 0 fractions by using alternatively 4 - 20% and 4 - 12% SDS-Polyacrylamide gradient gels covered by 3% stacking gels. Furthermore, we have used various staining techniques to characterize the differences between the individual fractions.

We checked larval secretion fractions of *Drosophila nasuta nasuta* [wild type Mysore I, No. 15112-1781.0 of the species stock list (1990) from Bowling Green], *Drosophila repleta* (No. 15084-1611.0), *Drosophila rubida* (No. 15115-1901.0), and *Drosophila simulans* (wild type Ethiopia). Staining techniques used are: (CBB) Coomassie Brilliant Blue R 250 (Diezel *et al.*, 1972); Silver (Ansorge, 1985); (PAS) Periodic Acid Schiff (Jay *et al.*, 1990); Alcian Blue 8GX (Krueger and Schwarz, 1987); and Sudan black B (Andrews, 1986).

Figure 1 (A and B) indicates that the domain 0 fractions are not visible by standard CBB-staining in 4 - 20% SDS-Polyacrylamide gradient gels. The reason probably is the use of TCA to fix the tissue in the standard CBB technique. A more detailed description of the *Drosophila nasuta* pattern in a 4 - 20% SDS-Polyacrylamide gradient gel is given in Kalisch and Ramesh (1997).

Figure 1. Comparison of homologous larval secretion fractions in *Drosophila nasuta* using diffent staining-techniques. (A) CBB [5P]; (B) CBB [5G], (C) Silver [3P], (D) PAS/Alcian [16G], (E) Sudan black [15P]. Number of glands [G] or secretion plugs [P] used for individual lanes are included in parenthesis. 4 - 20% SDS-Polyacrylamide gradient gels covered by 3% stacking gels (not shown). Domain I - V sectioning of secretion fractions according to Kalisch and Ramesh, 1997. For domain 0 fractions and different staining contrast of homologous fractions see text.



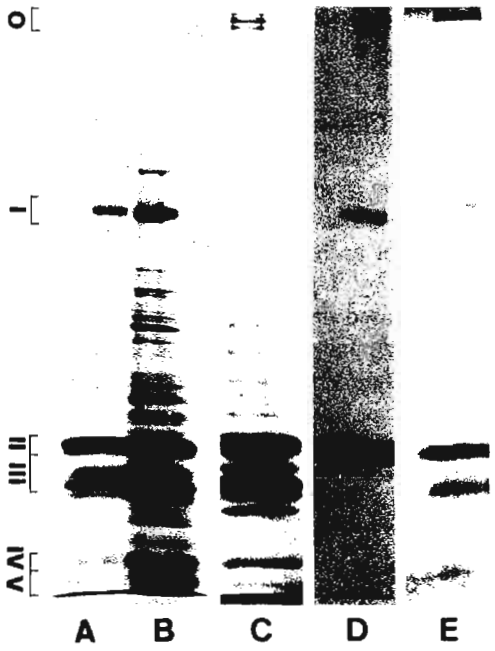


Figure 2. Comparison of domain 0 larval secretion fractions in: (A) *Drosophila nasuta* [1P], (B) *Drosophila repleta* [2P], (C) *Drosophila rubida* [1D], (D) *Drosophila simulans* [2D]. Number of glands [G] or plugs [P] used for individual lanes are included in parenthesis. Silver-staining; 4 - 12% SDS-Polyacrylamide gradient gel covered by a 3% stacking gel (not shown).

By using the more sensitive Silver-staining (Figure 1C), four domain 0 fractions become visible. By the use of phosphorylase b marker proteins (P 8906, SIGMA) we could calculate their Molcular Weight and found a range between 500 - 600kD. Note that we used secretion plugs in (C), but cell fractions attached to the plugs become visible by Silver-staining. However, CBB-staining in (B) and Silver-staining in (C) are not identical, indicating that staining differences in domain 0 fractions are not exceptional for larval secretion fractions.

Biochemical and functional aspects of domain 0 fractions so far are still unknown. The fact that we found quantitatively almost equal amounts of domain 0 fractions in whole salivary glands and in (carefully prepared) glue plugs as well as various patterns in different species (Figure 2) should indicate that: fractions of the cell (housekeeping proteins), nuclear fractions (including fractions of membranes), and/or methodological artifacts (Tasheva and Dessev, 1983) should not be part of the domain 0 fractions.

We used a 4 - 12% SDS-Polyacrylamide gradient gel to spread the domain 0 fractions (Figure 2). We checked *Drosophila nasuta*, *Drosophila repleta*, *Drosophila rubida*, and *Drosophila simulans* in one and the same gel. In several experiments, at least four fractions were found in each species. In *Drosophila rubida* (Figure 2C) we found additional fractions of which it is so far unclear whether or not the smallest one (at the bottom of the lane) belongs to domain 0 or domain I fractions.

The fact that the electrophoretic mobility of most of the domain 0 fractions is similar and can only be spread sufficiently in a 4 - 12% gradient gel obviously does not reflect biochemical similarity. We could show by Alcian-blue-staining (together with PAS-staining in Figure 1C) that only one of the four domain 0 fractions is Alcian-positive. From

the experiments with 4 - 12% gradient gels (data not shown), we know that the third fraction of domain 0 fraction (from top) is the Alcian-positive one.

We used Alcian blue in a salt solution (Scott and Dorling, 1965). By this, our data could be interpreted that the domain 0 fraction contains acid glycosaminoglycans (mucopolysaccharides). Staining of the same domain 0 fraction and the domain I - III fractions by Sudan black (Figure 1D) could be a hint that the Alcian-positive domain 0 fraction is a protein. But lipoproteins are also stained by the Sudan black technique we used.

Contrast of Silver-stained domain I fraction in Figure 1C is very low by the original light-yellow color of this fraction (compare Kalisch and Ramesh, 1997). Domain III-V fractions in Figure 1D are PAS-negative by the staining combination used (domain III) as well as by the completely missing (domain IV and V) glycosylation (Ramesh and Kalisch, 1989).

Data so far collected indicate that domain 0 fractions quantitatively are a minor group of biochemically different larval secretion fractions which are found not only in the *Drosophila nasuta* subgroup, but in others too.

Acknowledgments: The authors like to thank Mrs. Monika Soukupová for conducting the SDS-PAGEs and Mrs. Christel Plehn for technical assistance. S.R.R. is thankful to DAAD, Germany, for the award of a scholarship.

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Derzhavets, Elena, A. Korol, T. Pavlicek, and E. Nevo. Institute of Evolution of Haifa, Mount Carmel, Haifa 31905, Israel, E-mail: Korol@esti.haifa.ac.il. Adaptation to stressful environment and mutation rate: a case study in *Drosophila melanogaster*.

Mutation rate is one of the most fundamental characteristics of the genetic system. Theoretical models predict that under normal constant environmental conditions mutation rate (*mtr*) should evolve toward the lowest possible level (Liberman and Feldman, 1986), although the cost of fidelity may counterbalance this trend leading to some intermediate equilibrium value of *mtr* (Ishii *et al.*, 1989; Kondrashov, 1995). With frequently changing environment, selection may favour higher *mtr*s (Gillespie, 1981; Ishii *et al.*, 1989; Haraguchi and Sasaki, 1996). An appreciable genetic variation in spontaneous mutation rate has been documented for *Drosophila* (see Ashburner, 1989). Likewise, it is well known that an individual subjected to stressful ecological conditions reacts by an increase in *mtr* (reviewed in Woodruff *et al.*, 1983; Ashburner, 1989). However, next to nothing is known whether microevolutionary adaptation to stressfully fluctuating conditions in natural populations indeed results in increased level of mutations.

We conducted an experimental test of this prediction employing freshly collected material from the "Evolution Canyon" at Mount Carmel, Israel (Nevo, 1995). The opposite slopes of the Canyon differ in the level of solar radiation up to three-fold. Consequently, the abiotic conditions on the south-facing slope (SFS) are much more stressful and variable as compared to those on the north-facing slope (NFS). Earlier, we have shown that isofemale lines of *D. melanogaster* and *D. simulans* established from flies of the opposite slopes demonstrate significant differences for a number of adaptively important traits (Derzhavets *et al.*, 1996; Nevo *et al.*, 1997): lines derived from the SFS appeared to manifest higher resistance to heat and drought stress. Likewise, our recent fluctuating asymmetry test of wing measurements on *D. melanogaster* showed a significantly higher between-individual variation on SFS as compared to NFS (see Derzhavets *et al.*, this issue). These data can be considered as an indication of population differentiation on a microscale level, in spite of a rather small distance of 200 m between the stations 2 and 6 on the opposite slopes.

According to the foregoing theoretical models, one could expect a higher mutation rate in the SFS subpopulation as compared to the NFS. This expectation was confirmed in experiments with the fungus *Sordaria fimicola* collected from the Canyon (Lamb *et al.*, 1997). Likewise, we have compared isofemale lines of *D. melanogaster* from the opposite slopes of the Canyon for the rate of male recombination (Derzhavets *et al.*, 1996, and unpublished results). It appeared that SFS-flies manifest significantly higher recombination rate. Although increased male recombination is a component of the hybrid dysgenesis syndrome, the latter cannot be considered as an explanation of our results because the foregoing interslope difference was observed for both directions of crosses between the wild type flies and multiple marker stocks (see also Scobie and Schaffer, 1982). The reviewed data on

Drosophila from the Canyon were obtained on isofemale lines maintained in standard laboratory conditions for about 1-2 years.

Here we report the results on spontaneous mutation rate in males of *D. melanogaster* from the Canyon. The rate of sex-linked recessive lethal mutations was evaluated using the standard *Basc* test (Ashburner, 1989). The stock *Basc*, $sc^{8}sc^{Sl}w^{a}B^{l}$ was obtained from the European Drosophila Stock Center, Umea, Sweden. *D. melanogaster* males were taken from two opposite slopes of the Canyon during June-August 1996.

Freshly caught males were mated *en masse* to homozygous *Basc* females, P1 males and females were kept together for 3 days at 22-25°C, then discarded and the cultures incubated at 22-25°C. F1 offspring were collected for matings between 10th-to-17th days and their F1 daughters then mated individually to 3-4 *Basc* males. Each F2 culture carrying a putative lethal was tested by taking three F2 females and crossing them individually with their F2 brothers. It is noteworthy that the genetic background of the compared males in our test is of natural origin, in contrast to other known studies of mutation rate that are based on the accumulation technique.

Table 1. Frequency of newly arisen six-linked recessive lethals and sterile cultures derived from freshly caught males of *D. melanogaster* from the opposite slopes of Lower Nahal Oren Canyon (Mount Carmel, Israel)

Slope	Chromosomes			Mutation rate[a]		
	lethal	sterile	total	per X	per genome[b]	per locus[c]
NFS	1	0	1410	7.1×10^{-4}	3.0×10^{-3}	8.9×10^{-7}
SFS	6	2	1291	4.6×10^{-3}	2.0×10^{-2}	5.8×10^{-6}

[a] calculated for recessive lethality only; [b] assuming that the proportion of loci that can mutate to recessive lethals is the same in X chromosome and autosomes, and applying the estimate that X chromosome comprises about 23.6% of *D. melanogaster* genome (Ashburner, 1989); [c] applying the estimate that mutation at about 800 loci in the X chromosome can result in recessive lethality (Abrahamson *et al.*, 1980).

The results we obtained are shown in Table 1. Fisher's exact text for 2×2 contingency tables gives a significant ($P = 0.04$ $9< 0.05$) difference between NFS and SFS in the frequency of lethals and no difference ($P = 0.227 > 0.05$) for the frequency of sterility. The same test gives $P = 0.014$ when lethal and sterile cases are combined. Thus, we can conclude that higher mutation rate is characteristic of SFS, although further tests are desirable.

Recently, singificant intra-population difference in the rate of recessive sex-linked lethals was described when the scoring was conducted separately on large- and small-sized males (Ivannikov and Zakharov, 1996). The authors interpret the established dependence of *mtr* on body size as an indicator of stress conditions that might affect the organism's development. This explanation corroborates the known tendency of increased mutation when the organism is subjected to adverse ecological conditions (reviewed in Woodruff *et al.*, 1983; Ashburner, 1989). The question we tried to address is whether population adaptation to adverse conditions brings about a genetically determined increase in *mtr*. Our current data together with indirect evidence based on increased male recombination (Derzhavets *et al.*, 1996) allow us to assume that this is indeed the case. Similar results were obtained on mutation rate in the fungus *Sordaria* from the Canyon (Lamb *et al.*, 1996).

One additional point deserves mentioning here. Experimental tests showed that genetic material selected for increased resistance to a specific ecological stress may manifest, as a correlated response, a reduced level of spontaneous mutations and resistances to other stresses including mutagenic ones (Hoffmann and Parsons, 1989; Meerson *et al.*, 1993). If the interslope differences in *mtr* are a manifestation of such a correlated response, one should expect a lower mutation rate in the stress-resistant material, *i.e.*, that from SFS. Exactly the opposite was found in our test (see Table 1), which fits the theoretical explanation assuming that in a population adapting to stressful and variable conditions an increased mutation rate is evolutionarily profitable and is selected for (Gillespie, 1981; Ishii *et al.*, 1989).

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Watada, Masayoshi,[1] **Sumie Shigeoka**[1]*, **and Yutaka Inoue.**[2] [1]Department of Biology, Faculty of Science, Ehime University, Matsuyama, Ehime 790. [2]Osaka University of Foreign Study, Minoo, Osaka 562, Japan. *Present address: Kameoka-Syuzo and Co., Ltd., Ikazaki, kita-gun, Ehime 795-03. Inversion lethal and female sterile mutants of X chromosome from a natural population of *Drosophila melanogaster* in Japan.

In laboratory experiments, spontaneous mutations of lethal and fertile genes were well studied using X chromosomes of *Drosophila melanogaster* (Ashburner, 1989). Typically, spontaneous sex-linked lethal frequencies in normal stocks of *D. melanogaster* are on the order of 0.08-0.30%, which corresponds to specific locus mutation rates of between 0.1×10^{-5} and 0.3×10^{-5}. The frequency of recessive sterile mutations in *D. melanogaster* is considerably less than that of lethals. Frequencies of recessive male and female sterile mutants are about 10-15% of that of recessive lethals.

On the other hand, lethal and sterile mutations in natural populations of *D. melanogaster* have been studied usually using second and third chromosomes (Mukai, 1978). The frequenciy of recessive lethal and sterile mutants in natural populations is high because of covering by the dominant wild alleles. However, lethal and sterile mutations on the X chromosome have rarely been reported from the natural populations of *D. melanogaster*. These mutants are thought to be easily selected out from the natural populations.

In this paper, we report the result of a preliminary survey of lethal or strile mutations of genes on the X chromosome of a natural population of *D. melanogaster* in Japan.

Materials and Methods: From a natural population of Ozu in Ehime prefecture, 251 inseminated females of *Drosophila melanogaster* were collected using banana bait traps. Females were put into a separate vial. Virgin female progeny of these flies were crossed to FM7a males. Figure 1 shows a mating scheme for screening recessive lethal and sterile mutants of X chromosomes. A lethal mutant was checked by the absence of F3 wild-type males. Male sterility was surveyed by the progenies from the cross between Canton-S females and F3 wild-type males. Female sterility was examined using homozygous females of a wild type from the stock lines. For a lethal mutant of X chromosome, a recombination experiment was performed using a strain marked with *y cv v f*. The salivary chromosome with a lethal gene was also examined using progeny of the cross between lethal heterozygous females and Canton-S males.

Results and Discussion: Out of 251 strains examined, no male sterile mutant of X chromosome was found in this study as expected. However, one lethal and three female sterile mutants in the X chromosomes were found from the Ozu population. The frequencies of the lethal and female sterile mutants in Ozu population are 0.4 and 1.2%, respectively. These frequencies appear to be higher than that of spontaneous mutation of the X chromosomes, although only one strain has been found as a lethal mutant in this study.

The lethal mutant was mapped using a chromosome marked with *y cv v f*. A map position of the lethal mutant was estimated to be near the *v*. However, the recombination values were 6.6% between *y* and *cv*, 9.2% between *cv* and *v*, and 13.2% between *v* and *f*. These recombination values were very low, compared with the standard distances between the marker genes. This result suggests a suppression of recombination by the lethal chromosome. Therefore, the polytene chromo-

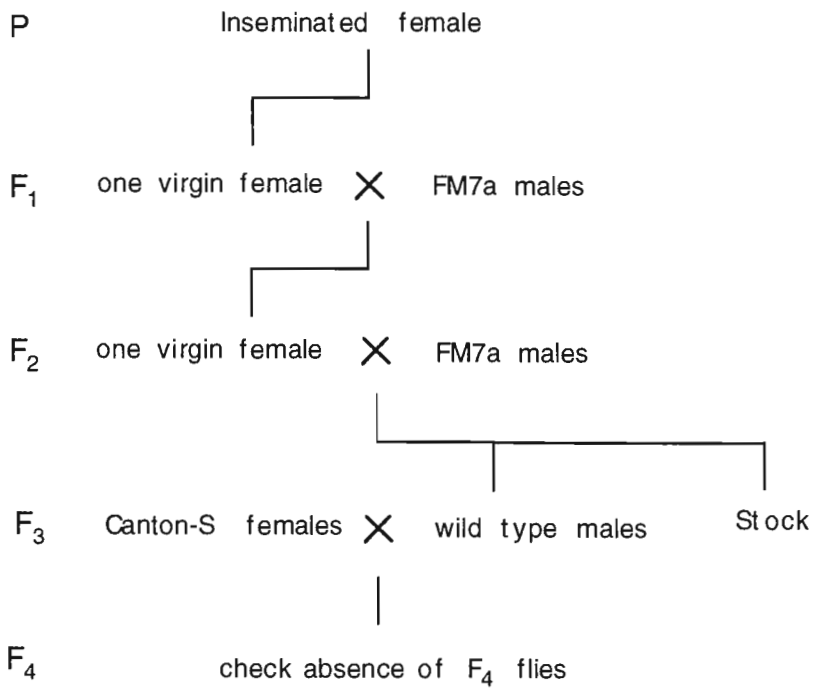

Figure 1. A mating scheme for screening recessive lethal and sterile mutants of X chromosomes.

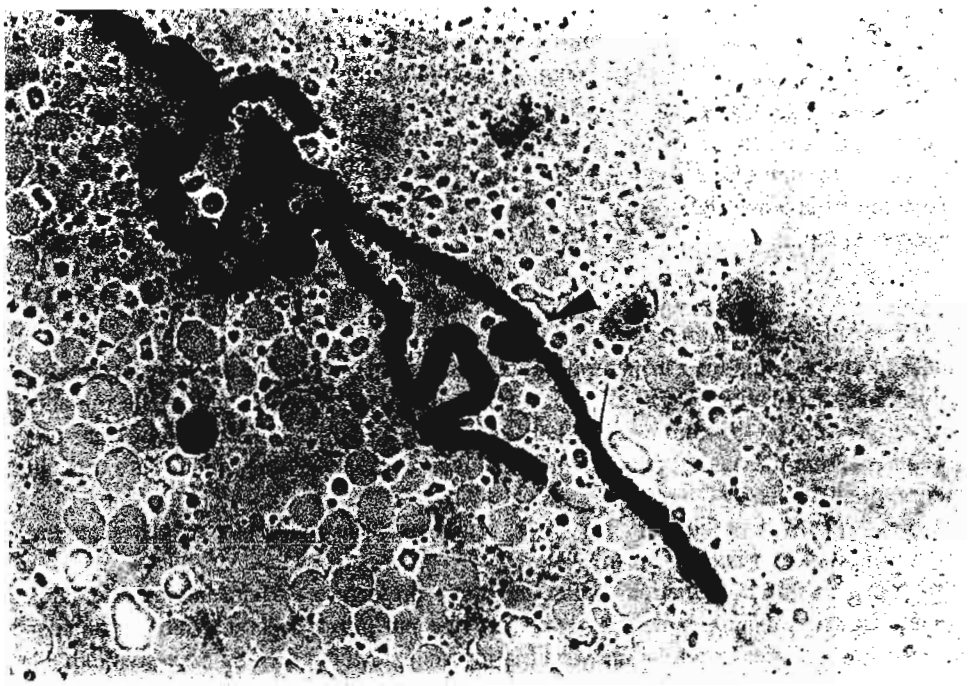

Figure 2. Salivary chromosome of a heterozygote of *In(1)OZ* and Canton-S. An arrow indicates an inverted region.

some of the lethal strain was studied using third instar larvae produced by the cross between lethal heterozygous female and Canton-S males. Observation of salivary chromosome of the lethal showed that this chromosome had an inversion at the breakpoint of 8F and 11A/B (Figure 2). We named this X chromosome inversion with a recessive lethal as *In(1)OZ*.

Many inversions have been described in the previous papers (Lindsley and Zimm, 1992). Among the described inversions, X chromosome inversions of *D. melanogaster* from natural populations were relatively rare compared with those of the second and third chromosomes of *D. melanogaster* (Lemeunier *et al.,* 1986). Ozu population of *D. melanogaster* was well studied with respect to chromosome inversions. Inoue and Watanabe (1979) reported 4 unique endemic inversion from Ozu population among 17 total endemic inversions unique to Japan. Thus, the Ozu population carries more unique endemic inversion than any other populations in Japan. It has been suggested that some extent of dysgenesis may have contributed to the inversion mutations in Ozu population. However, we have, at present, no molecular data that these mutations may have occurred by transposons.

The allelism of three female sterile mutants was examined by complementation tests. The result shows that two mutations are allelic, indicating the transmission of the sterility gene between generations in Ozu population. The frequency of female sterile mutations of X chromosome seems to be higher than that of lethals. Some female sterile mutation on autosomes have male sterility or low viability as homozygotes (Ashburner, 1989; Watanabe and Oshima, 1973). However, Lindsley and Lifschytz (1972) reported that about 8% of the major autosomes from nature carried female steriles, presumably because they are covered by dominant genes. Female sterile genes on X chromosome may be also transmitted in a manner similar to autosome sterile genes if they have no negative effect on the fertility and viability of female heterozygotes and male hemizygotes.

Acknowledgments: We thank Dr. Damon Lisch for critical reading of manuscripts.

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Ivanov, Yu.N., and A.V. Ivannikov. Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk, 630090, Russia. FAX: (3832) 35 65 58. Difference in mutation rates between flies of small and large body sizes in natural *Drosophila melanogaster* populations and regulatory meaning of this phenomenon.

Flies often differ in body size within *D. melanogaster* populations, larger individuals weighing up to 5 times as much as their smaller mates. We have found that these additionally differ in the frequency, at which lethal chromosomes 2 and MR-factors occur in them. Our intention was to answer the question as to whether there is a difference between the size different flies in the frequency at which mutations occur in their gametes.

Measuring the rates of spontaneous mutation in X chromosome of males from four Altai populations, we visually classed the males into small, medium and large. The results of a comparative study on the mutation rates in X chromosome with respect to the size groups are presented in the Table. Of the seven mutations arisen in 4770 gametes, six were confined to the small, one to the medium, and none to the large males. Fisher's precise method, which we have herein applied for evaluating 2×2 tabular data instead of χ^2, reveals significant differences in mutability between the small and large males. Given the zero hypothesis (*i.e.*, that the mutation rates are all equal), the probability of the differences is somewhat low ($P = 0.0051$). On the face of it, the zero hypothesis was rejected for an alternative assumption that mutability is stronger in the gametes of small males than in those of large ones. Relating the medium-size males to the small or large ones, the differences still hold significant ($P = 0.0141$ and 0.0057, respectively).

Mutability should necessarily be elevated in small flies compared with large ones, because the mutation process acts as a factor that regulates the population density of the species. Larvae end up as small flies when the population density is high and as large flies when it is low. Therefore, the dimensions of a fly body are closely associated with population density. If it is high (*i.e.*, the flies are small), there should be factors acting in a manner to restrict the population growth. Really, females lay less eggs because they are small; mortality rates, accounted for by no matter which biocoenotic factors, increase; additionally, we have found that the mortality rates increase due to elevated mutability rates. Decomposing the spontaneous mutation process in the *D. melanogaster* genome into the basic types of mutations (Ivanov, 1991) yields a zygote mortality rate accounted for by dominant lethals in the population as follows: $S = 13.5u - 45.5u^2$, where u is the mutation rates in X chromosome. As can be seen, S is nearly in a direct proportion to mutability u. This value of S is by far not low, as even at normal mutation rates ($u = 0.3\%$) dominant lethals make up as much as 4% of all zygotes. In contrast, at a low population density (*i.e.*, when flies are large) the effect of all these population size-restricting factors is attenuated, which urges the population to grow in abundance.

In connection with the regulatory role of the mutation process, the significance of redundant DNA in the genome and the causes of non-adaptive karyotype structure in many species become clear. The DNA redundancy exists in the form of 1) blockwise and 2) intercalar heterochromatin (between genes), as well as in the form of 3) introns (inside genes). Heterochromatin is represented by genetically inactive (empty) DNA sites from repeated nucleotide sequences or "repeats".

Dominant lethals (DLM) are divided into two types: 1) chromosomal and 2) genic. In *D. melanogaster*, chromosomal DLM make up 95%, and genic ones 5% of all the DLM (Ivanov, 1991). Chromosomal DLM are simple disruptions of chromosome arms with a loss of acentric fragments in the course of subsequent cell division, *i.e.*, terminal deletions. Genic DLM are ordinary recessive lethals with some penetrance in heterozygotes.

Chromosomal DLM are a factor determining the number of chromosome arms in the species karyotype. This followed from the fact that the mean number A of chromosomal DLM in the genome is a decreasing function of the number f of chromosome arms: $A = k(1 - \alpha f)$ where k and α are positive constants. From the formula one can see that as the number f increases, *i.e.*, as karyotype is fragmented into progressively smaller chromosomes, the number A of chromosomal DLM decreases and becomes zero as soon as the equality $\alpha f = 1$ is reached. On the contrary, in order that the number of DLM in the genome increases, it is necessary to diminish the number f, *i.e.*, the genome becomes more vulnerable to chromosomal DLM at a small number of arms. This is connected with the fact that the genic contents of the arms increases and their losses become more dangerous.

The presence of heterochromatin (repeats) in chromosomes makes the genome vulnerable to DLM, since it increases the length of the chromosomal DNA thread as the size of the target hit by mutations. In cytogenetics, it is well known that structural damages are distributed along the chromosome nonuniformly. It is noteworthy it is just heterochromatin regions that are characterized by an increased vulnerability (Bochkov *et al.*, 1984). Thanks to this, the species has the capacity of regulating its numbers by means of changing the mutation rate. Indeed, in order that the mutability can be a regulator of the population size, an effective increase of lethality is necessary through elevation of the mutation rate, which is achieved by means of increase of the genome vulnerability to DLM at the expense of repeats.

The intronic gene structure in eukaryotes has, *inter alia*, the meaning that introns, occupying up to 50% and more of the gene length, elongate thereby, similarly to the intercalar heterochromatin, the DNA thread and heighten the DLM probabiltiy, which is necessary for limitation of the species abundance.

The structure of the genome in *D. melanogaster* in which the numbers are regulated by mutations is in a good accordance with these notions and thereby confirm them.

1) The *D. melanogaster* karyotype consists only of 4 chromosomes, the number of arms being no more than 8. The genetic contents of the arms is very large and their breaks are very effective as DLM whose proportion is about 54% of all spontaneous mutations in the genome (Ivanov, 1991). As a contrast, the grayling (*Thymallus thymallus*) karyotype may be considered where there are 100 - 106 chromosomes and 170 chromosome arms (Severin, 1979). DLM in the grayling do not play practically any role, because the genetic contents of each of so numerous arms is negligibly small, and their breaks and losses are not dangerous and do not represent DLM, which is confirmed by the variation of the chromosome number in the karyotype.

2) Blockwise heterochromatin occupies about a third part of the whole *D. melanogaster* genome. In man (*Homo sapiens*) the fraction of DNA with repeats makes up 36% of the whole DNA, and is therein two times larger than the blockwise heterochromatin (Bochkov *et al.*, 1984). It is possible that in *D. melanogaster* this fraction is also considerably larger than that of blockwise heterochromatin and sometimes exceeds 50%, which is what determines the high DLM level.

3) Hetero- and euchromatin are distributed in chromosomes in a way that the probability of the genome being hit by DLM is maximal. The euchromatin is located in distal, and the heterochromatin in proximal, regions of chromosome arms. That is why in any large chromosome the region whose breaks are lethal occupies practically the whole chromosome, which would not have been the case, if the heterochromatin had been localized distally.

Table 1. Frequency of occurrence of X-linked recessive lethal and visible mutations in the gametes of males with different body size from four Altai populations of *D. melanogaster*, September 1992.

Male group by size	Average male weight of the group over all populations, mg	n	N	*u*, %
Small	0.26 - 0.53	6	1541	0.389
Medium	0.78 - 0.79	1	1054	0.095
Large	0.74 - 0.90	0	2175	0

n is the number of mutations; N is the gamete sample size; *u* is the frequency of occurrence of mutations

In this way, the total length of the chromosomal DNA thread in the *D. melanogaster* genome exceeds by more than two times its unique (translatable) part, the size of arms and localization of blockwise heterochromatin in them being such that the chromosomal DLM probability is maximal.

The modus operandi of mutability is consistent with the basic principle of life organization, of which the crux is not the maximization of an individual's fitness, but allocentrism, *i.e.*, pursuing collective interest of all the species within the ecosystem. Mutability is liable to change adequately to the population density in order to even out fluctuations in the species population size. The agents that promote mutability (as long as this is a necessary requirement for lowering the population size for the sake of ecosystem stability) may be any factors of genetic instability (plasmids, viruses or other mobile genetic elements) which are activated by metabolites, typical of the larvae and flies on the overpopulated substratum. The body of evidence is now great that such agents are primarily MR-factors that are quite frequent in all populations (Hiraizumi, 1971; Thompson and Woodruff, 1978; Green and Shepherd, 1979).

In the case of the tolerant (non-mutant) forms, the selection magnitude changes depending on their frequency, so that selection maintains a stable polymorphism in the population (Luchnikova, 1978), and in the case of mutant forms, the selection rapidly eliminates them, *i.e.*, generally speaking, is a kind of guardian of species permanence (Jenkin, 1867; Agassiz, 1874; Danilevskiy, 1885). The intraspecific competition is an allocentric property of species, since it is exacerbated in overpopulation and limits thereby the species abundance, protecting the ecosystem from degradation. It is now becoming clear that the mutation process is not a factor of transmutation (speciation) and biogenesis, either, but, similarly to selection and competition, exerts regulatory functions in the ecosystem.

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Kim, W., J.M. Kim, and D.J. Shin. Department of Biology, Dankook University, Cheonan-Si, Choong-Nam 330-714, Korea. Molecular analysis for specific *hobo* deletion derivatives in the Korean population of *Drosophila melanogaster*.

On the basis of our results of *in situ* and Southern blot hybridization using *Xho*I restriction enzyme (Kim and Kim, 1996), the Korean population of *D. melanogaster* appeared to have a low copy number of 3.0 kb putative full-size *hobo* elements and a high copy number of internally deleted *hobo* elements. This result is somehow comparable with the earlier reports detected from American and Eurasian populations of *D. melanogaster* (Periquet *et al.*, 1989a, b; Pascual and Periquet, 1991; Boussy and Daniels, 1991). Periquet *et al.* (1989a, 1990) reported the presence of two major classes of *hobo* elements, a 3.0 kb element class and one particular deletion derivative class of elements called the *Th* element, which have accumulated in all naturally-occurring strains throughout the Eurasian continent. They suggested that the presence of *Th* element might be interpreted as potential regulatory elements of the *hobo*-induced hybrid dysgenesis.

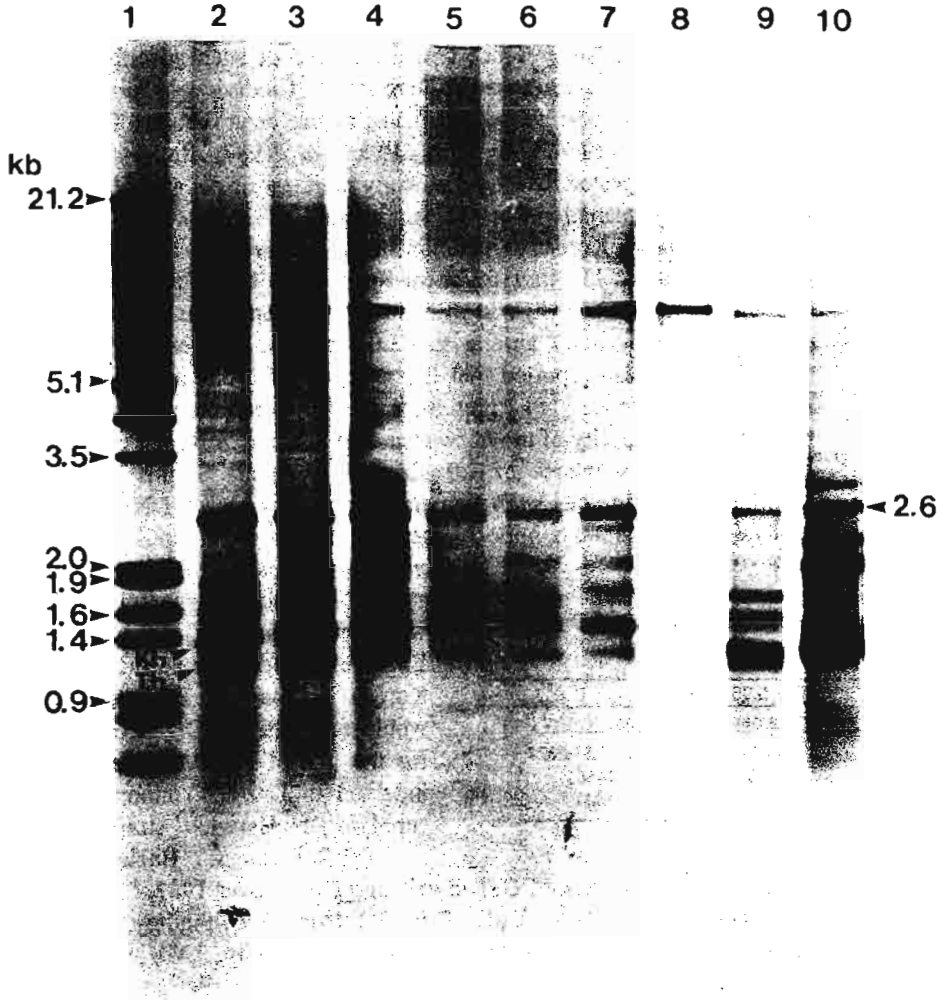

Figure 1. Southern blot analysis for the structure of *hobo* elements from Korean lines of *D. melanogaster*. Genomic DNAs were digested with *Xho*I, and hybridized with the 2.6 kb *Xho*I restriction fragment of the pH108 plasmid as a probe. Lanes are as follows: (1) Dig-labelled DNA marker III, (2) Cheonan 96-33 (H^+), (3) Cheju 96-29 (H^+), (4) Cheonan 96-15 (H^0), (5) Cheju 96-12 (H^0), (6) Cheonan 96-6 (H^-), (7) Cheju 96-9 (H^-), (8) Basc (E), (9) Harwich^Y (E), (10) 23.5*/Cy (H). H^+, H^0, and H^- strains were classified by reference tests of Pascual and Periquet (1991).

Based on the result of Southern blot hybridization, a specific 1.7 kb *hobo* deletion derivative (1.3 kb *Xho*I restriction fragment in Figure 1) is the most preserved in all of the Korean lines tested and is termed *Kh* element. The 1.5 kb *Th* element, giving a 1.1 kb fragment and 3.0 kb full-size *hobo* element (2.6 kb fragment) are also observed in these lines (Figure 1). The entire 1.7 kb sequence of four *Kh* elements derived from Korean lines have been obtained by polymerase chain reaction (PCR) and DNA sequencing. PCR amplification of *Kh* element sequence was performed on the genomic DNA using the following two primer sequences in pH108 (Streck *et al.*, 1986): #1, 5'-CAGAGAACTGCAAGGGT GGC-3' (1-21), and #2947,

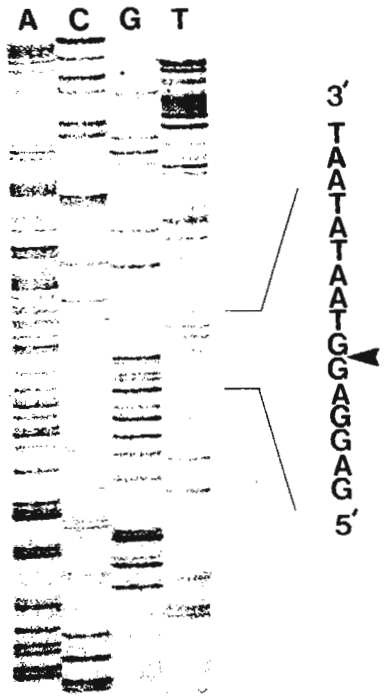

Figure 2. Sequencing gel autoradiograph of a segment of the 1.7 kb *Kh* element from a Korean line. The arrow shown is that of the breakpoint (938/2192) of the *Kh* element from an autonomous 2959 bp HFL1 *hobo* element.

5'-GCCCGCCACTCGCACTCTAC-3' (2947-2928) by the method of Kim and Kidwell (1994). The 1.7 kb fragments of individual *Kh* elements were cloned into pCR™II vector plasmids, and subsequently sequenced by the dideoxy-chain-termination method (Sanger *et al.*, 1977) using the Sequenase kit (U.S. Biochemical, Cleveland) according to the supplier's protocol. We also determined the sequences of *Th*1 and *Th*2 elements isolated from these lines, to compare the breakpoint to *Kh* element.

The sequence of all *Kh* elements tested in these populations suggested that they might have been derived from the autonomous *hobo* element HFL1 (Calvi *et al.*, 1991) by a 1253 bp internal deletion between positions 939 and 2191 (Figure 2). The sequences of *Th*1 and *Th*2 elements appeared to be identical to that of the HLF1 with the exception of internal deletions of 1442 bp and 1455 bp removing nucleotides 940-2381 and 923-2377, respectively (Table 1). Therefore, all of these *hobo* deletion derivatives seem to be derived from the HLF1 *hobo* element, not from pH108.

The massive presence and the spread of such specific deletion derivatives might be due to a selective favor of individuals carrying high copy numbers of these deleted elements, as has been reported for the *KP* element in the P-M hybrid system (Black *et al.*, 1987; Rasmusson *et al.*, 1993). It is suggested that the high copy numbers of *Kh* and *Th* elements provides an explanation for the suppression of *hobo*-mediated hybrid dysgenesis in the Korean population of *D. melanogaster*. However, the presence of a 2.6 kb *Xho*I fragment by itself in this study cannot be a sufficient prediction of hybrid dysgenesis or autonomous because of the sequence heterogeneity among the 3.0 kb *hobo* element. Bazin and Higuet (1996) also reported that the structure of the S region where an amino acid sequence (TPE) presents a repetition polymorphism could be specific to the activity of the *hobo* element. Further work will be required to identify the sequence of 3.0 kb *hobo* elements in this population whether an autonomous *hobo* element is present or not. A DNA sequence analysis of the 3.0 kb *hobo* element in the Korean population of *D. melanogaster* is in progress.

Table 1. Comparison of sequence differences between deletion derivative *Kh* and *Th* element derived from Korean lines of *D. melanogaster*.

	HFL1 (2959 bp)		
	Kh element (bp)	*Th* (1) element (bp)	*Th* (2) element (bp)
Internal deletion site	939-2191	940-2381	923-2377
Deletion size	1253	1442	1455
Size in genome	1706	1517	1504

Acknowledgments: This work was funded by a Genetic Engineering Research Project from the Korean Ministry of Education. We would like to thank Dr. M.G. Kidwell for the gift of pH108. We are also indebted to Dr. G. Yannopoulos for supplying the Harwich[Y] strain.

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Shastri, Mythreyi D., and Jack R. Girton. Department of Zoology and Genetics, Iowa State University, Ames, Iowa. Prolonged exposure to carbon dioxide does not affect the electroretinograms permanently.

In this study, we examined the effects of prolonged exposure to carbon dioxide on the electrical activity in the visual system of *Drosophila.* Electroretinograms (ERG) were measured in *Drosophila* after exposure to carbon dioxide followed by a lengthy time period for recovering from anaesthesia. Previous studies on the effect of anaesthetics on electroretinograms have focused primarily on ether. The studies noted that ether and carbon dioxide cause temporary alterations in ERGs during exposure (Stark, 1972). We show statistical evidence that the amplitude and the shape of the electroretinogram is not altered permanently by prolonged exposure to carbon dioxide.

The electroretinograms in this study have been characterized in terms of amplitude and shape. The normal ERG has quick responses to the beginning and ending of the light stimulus. These are the "on" and "off" transients due to electrical activity in the synaptic junctions with the L1 and L2 cells of the lamina. The sustained corneal negative is due to the electrical activity in the retina (Hotta and Benzer, 1969). The amplitude or height of the ERG is the distance between the tips of the "on" and "off" transients. The amplitude of the ERG is measured as mV/Division. In the figure, one division is seen as one large square with twenty five small squares.

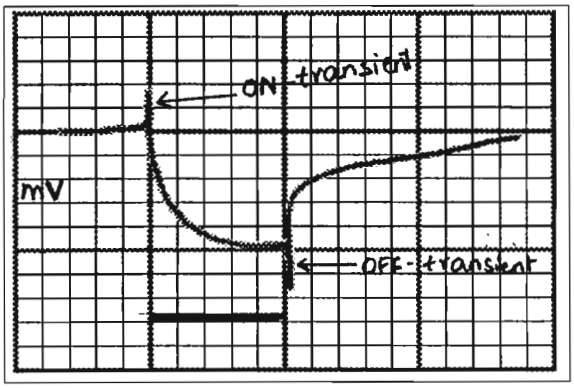

Figure 1.

The ERGs were measured in *y w sn* flies. Flies with the *white* mutation are used instead of wild type flies to measure ERGs because the white eyes do not 'light adapt' as quickly as the red eyes. ERGs were also measured from w^a fa^g and w^a fa^g*;Bpt* flies. w^a *fa* flies have apricot coloured eyes and rough facets. In w^a fa^g*;Bpt* flies, the rough eyes have a black patch on the retina. This is due to death and degeneration in the retina and the underlying optic lobes (Duus *et al.*, 1992).

Flies were prepared for measuring ERG, in batches. Flies in each batch were prepared for the experiment by anesthetizing with CO_2 and gluing to a coverslip with nail polish. Each batch contained flies exposed to 3 min, 5 min, and 8 min of carbon dioxide. The coverslip was laid on a block of agar, prepared with 0.9% NaCl, and the flies were connected to the block with small strips of agar. A silver reference electrode was inserted into the block of agar, and the signals from the bodies of the flies (background noise) were grounded. A fiber optic illuminator provided a white light stimulus that was focussed onto the eyes of the fly by using a mirror and a convex lens. Wavelength filters of 470 nm and 568 nm were used to change the wavelength of the stimulus. The light stimulus always lasted for one second and is shown as a black bar in the figure.The average amplitude of the ERG was measured at two wavelengths, 470 nm and at 568 nm. Electroretinograms were measured from each fly by inserting a recording electrode filled with 0.9% NaCl into the eye. Signals from the recording electrode were amplified 10x by a preamplifier, Dagan corporation (Minneapolis, MN) and then displayed on the screen of an oscilloscope. The recording electrodes were made by using glass capillaries with filament, No. TW100F-3, World Precision Instruments, (Sarasota, FL).

Statistical analyses were done on the amplitudes of the ERGs recorded from *y w sn* flies. Seven flies, exposed to 3 min of carbon dioxide show an average amplitude of 8.21 mV (470 nm) and 8.14 mV (568 nm). Five flies, when exposed for 5 min have average amplitudes of 6.1 mV (470 nm) and 5.9 mV (568 nm). Even after exposure to carbon dioxide for 8 min, ten flies show amplitudes of 5.8 mV (470 nm) and 5.6 mV (568 nm). The differences between these means is not significant ($P > 0.05$). The electroretinograms always showed the on and off transients (Figure 1). Electroretinograms measured in the flies with other genotypes were not affected by prolonged exposure to carbon dioxide.

In our study, we have conclusively shown that prolonged exposure to carbon dioxide does not alter the amplitude and shape of the ERGs permanently. Previous studies have measured the effects of anaesthetics on electroretinograms. Prolonged exposure to ether for more than 3 min permanently blocked the transients and sometimes, even lowered the corneal negative. Measurement of ERGs during short exposure to carbon dioxide showed reversible changes. The off-transient disappeared in less than 5 sec and the on-transient disappeared in less than 10 sec (Stark, 1972). These studies, taken together, indicate that the metabolic and synaptic effects of these two anaesthetics on

electroretinograms are very different. We suggest that carbon dioxide should be the anaesthetic of choice when measuring electroretinograms.

Acknowledgments: We thank Dr. DeMao Chen for teaching us how to measure electroretinograms at Dr. William Stark's laboratory, St. Louis University. We thank Dr. Jorgen Johansen for providing advice, equipment and laboratory space at Iowa State University.

References: Duus, K. M., W. J. Welshons, and J. R. Girton 1992, Dev. Biol. 151:34-47; Hotta, Y., and B. Benzer 1969, Nature 222:354-356; Stark, W. S. 1972, Dros. Inf. Serv. 48:82.

Goode, S. Department of Genetics, Harvard Medical School. Additional gain of function phenotypes associated with the *Ocellarless* gene of *Drosophila melanogaster*.

Ocellarless (*Oce*, 1-5.7)/+ females are missing 60-80% of ocellar and 90-95% of postvertical head bristles and sometimes show incised margins on the wings (Lindsley and Zimm, 1992). We report additional phenotypes of *Oce*/+ females. We find that ocelli of *Oce*/+ females are usually moved closer together, or fused, and that additional head bristles are often missing or absent (Figure 1, A-C). The wings of *Oce*/+ flies typically have a gap in the fifth longitudinal wing vein and less frequently in the posterior cross vein (Figure 2, A-C). *Oce*/+ phenotypes do not result from haplo-insufficiency, since females heterozygous for *Df(1)HC244*, which removes DNA spanning the 3E to 4F region (approximate meiotic map positions 1-5 to 1-11.5), are completely wild type.

Both *Oce* wing vein gap and ectopic bristle phenotypes resemble phenotypes associated with loss and gain of function mutations of Notch receptor and the *Drosophila* EGF receptor (DER; Clifford and Schüpbach, 1989; Diaz-Benjumea and Hafen, 1994; Schellenbarger and Mohler, 1978; unpublished observations). *brainiac* (*brn*) maps within 0.2 map units of *Oce*, at position 5.9, and *brn* mutant animals display phenotypes common to both the Notch and EGF receptor signaling pathways (Goode *et al.*, 1992, 1996). We ruled out the possibility that *Oce* mutations are gain of function *brn* alleles. $Df(1)rb^{33}$, which was synthesized on an *Oce* chromosome (Banga *et al.*, 1986), fails to complement *brn* mutations, but still retains dominant *Oce* phenotypes.

Oce phenotypes are completely penetrant in *Oce*/*w v* l^{14ts} or *Oce*/*FM3* females reared at 29°C (n > 1400), making a simple F_1 reversion screen for rearrangements in the *Oce* gene easy. These rearrangements should be useful for isolation of *Oce* DNA sequences, since a genomic walk spanning the 3F-4A region has been completed (Goode *et al.*, 1996). Elucidation of the *Oce* molecular structure and function may add to our knowledge of Notch and/or DER signaling pathways.

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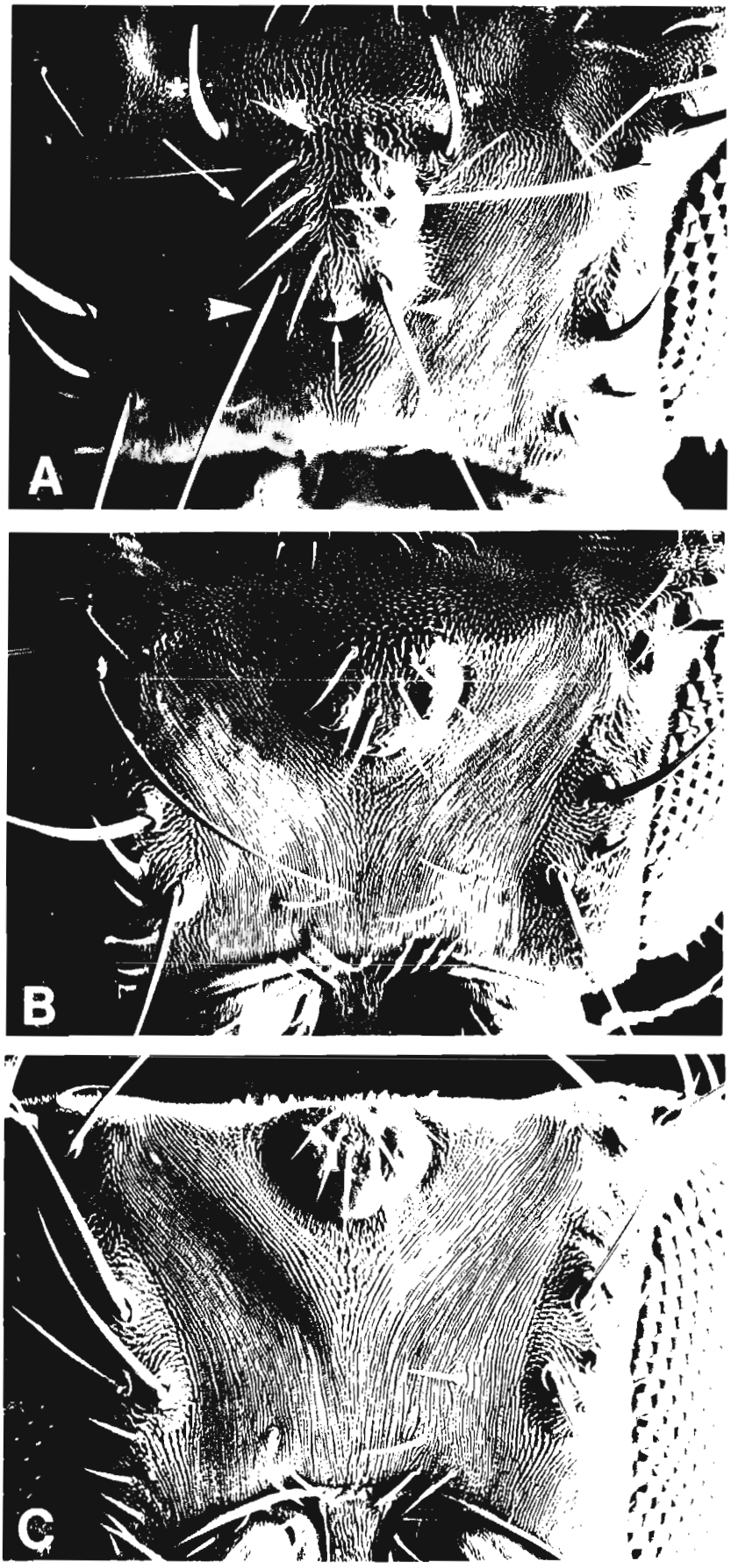
Figure 1 (next page). *Oce* head phenotypes. Scanning electron micrographs of the dorsal side of wild type (A), and *Oce* (B,C) adult heads. Arrows point to the ocellar bristles and stars demarcate the postvertical bristles of wild type flies (A). These bristles are frequently missing in *Oce*/+ flies (B, C; Lindsley and Zimm, 1992). Further, the ocelli (arrows, A) are either moved closer together (B), or fused (C). Other head bristles are often missing or misplaced in *Oce*/+ flies. The "wild type" fly in (A) has an extra microchaete (x).


A
B
C

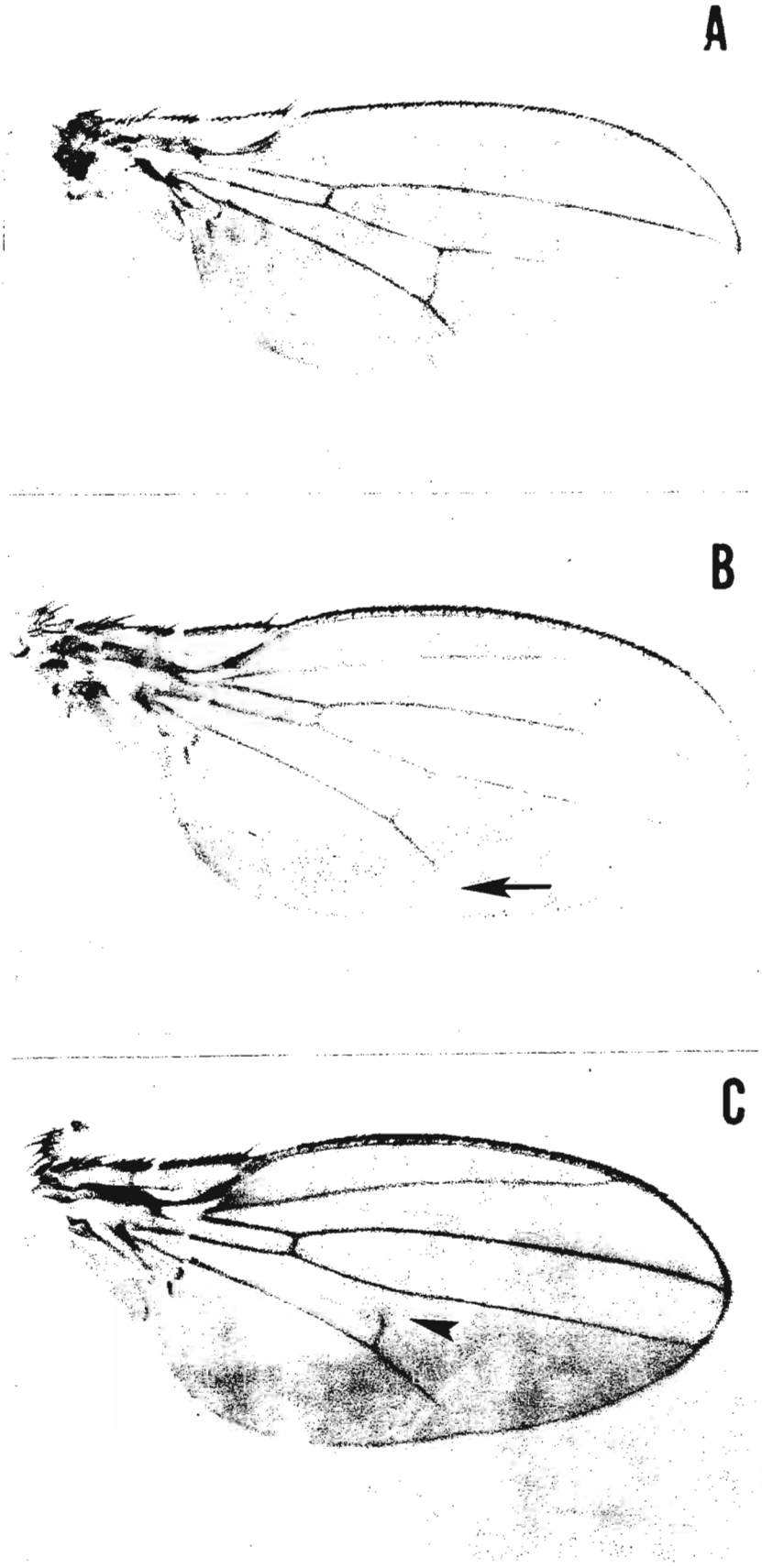


Figure 2. *Oce* wing phenotypes. Hoyer's mounts of wild type (A) and *Oce* mutant wings (B, C). *Oce* mutants frequently have a gap in the fifth longitudinal wing vein (B, arrow), and less frequently, in the posterior cross vein (C, arrow).

Di Pasquale, Anna, Fara Misuraca, and Valentina Tesoro. Dipartimento Di Biologia Cellulare e Dello Sviluppo 'A. Monroy" - Sezione di Genetica, Università di Palermo, Parco D'orleans II, 90128 Palermo, Italy. Tel. 091-424459; Fax 091-420361. Immune response in the *tu-pb* melanotic tumor strain of *Drosophila melanogaster*: preliminary data.

It is well known that *Drosophila*, like other insects, resist bacterial infections through the induction of both cellular (Lackie, 1988) and humoral (Faye and Hultmark, 1993) responses; the cellular response involves the mobilization of hemocytes, which phagocytose or encapsulate microorganisms, whereas the humoral response utilizes antibacterial peptides, which effectively lyse bacteria or are bacteriostatic. Antibacterial peptides are synthesized in the fat bodies and secreted into the circulating hemolymph. Another component of the insect immunity is the activation of phenoloxidase and the clotting of the hemolymph. Phenoloxidase catalyzes the synthesis of melanin, resulting in the encapsulation of invading cells (Hultmark, 1993).

Several genes encoding anti-bacterial peptides have been cloned and characterized in *Drosophila melanogaster*. The induction of antibacterial peptides is controlled at the transcriptional regulation level (Faye and Hultmark, 1993).

The immune response has been shown to be activated not only in adults but also in pupae and third instar larvae. Recently it has been reported (Lemaitre *et al.*, 1995) that some genes controlling embryonic development [*dorsal (dl), Toll,* and *cactus*] are expressed in larval and adult fat bodies, where their RNA expression is enhanced upon injury. Mutants of these genes result in a melanotic tumour phenotype which is considered to be a spontaneous immune-like response. The Dorsal protein (Dl), normally localized in the cytoplasm of the fat body, is rapidly imported in the nucleus upon bacterial challenge. This nuclear uptake of Dl occurs spontaneously in mutants exhibiting melanotic tumours.

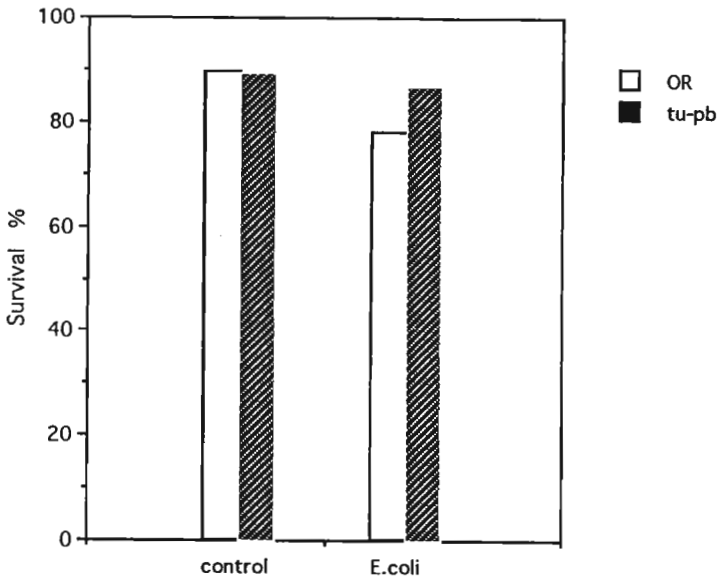


Figure 1. Survival of wild-type and mutant adults to bacterial infection.

In order to elucidate a possible relation between melanotic tumour manifestation and the immune response, we have started to investigate the response to the bacterial challenge in the melanotic tumour *tu-pb* strain of *D. melanogaster*.

Tu-pb is an atypical melanotic tumour mutant in which tumour manifestation is restricted to the head of the adult; penetrance is also incomplete and different in the sexes. Genetic analysis indicated that the *tu-pb* phenotype depends on at least two different loci, one recessive on the 3rd chromosome, the other, apparently semidominant, on the 2nd chromosome: only genotypes including both the two large *tu-pb* autosomes elicit tumour manifestation. *Tu-pb* larvae lack the precocious transformation of plasmocytes into lamellocytes which is typical of all melanotic tumour mutants, but retain most of the crystal cells in the lymph glands (Di Pasquale Paladino and Cavolina, 1983, 1984; Di Pasquale Paladino *et al.*, 1988).

To determine if *tu-pb* individuals have a different degree of susceptibility to bacterial infection, we analyzed the survival rates of Oregon-R wild-type flies and *tu-pb* mutants after bacterial challenge.

Injury experiment (control) were performed by pricking third instar larvae or adults with a thin needle; for bacterial challenge, the needle was previously dipped into a concentrated bacterial culture of *E. coli*. Preliminary results give evidence that the *tu-pb* mutant exhibits slightly elevated survival rates as compared with the wild-type strain, after bacterial infection (Figure 1). Moreover, treatment of larvae sets in a melanization reaction which appears to persist in the adults and which can be induced by infection and even by a simple injury. *Tu-pb* flies exhibit a significantly higher level of this melanization reaction (Figure 2). To investigate about a possible correlation between resistance to infection,

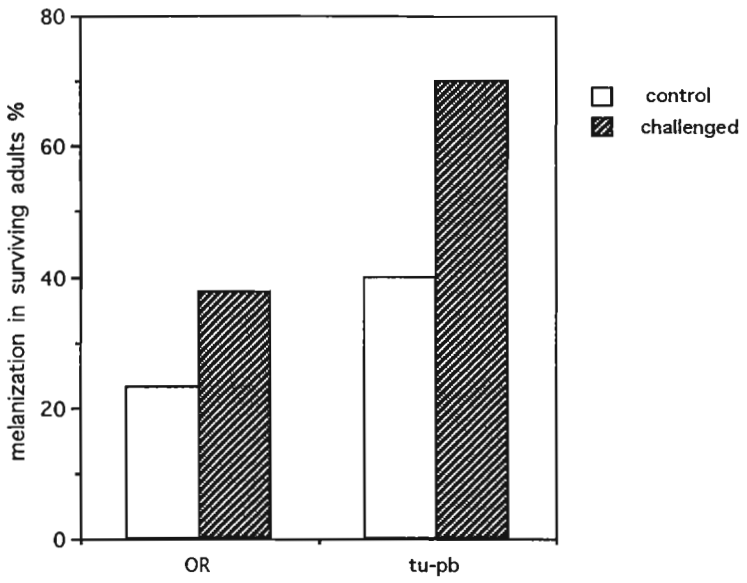


Figure 2. Percentage of melanization in Oregon-R and*tu-pb* adults survived to injury (control) or bacterial challenge as third instar larvae.

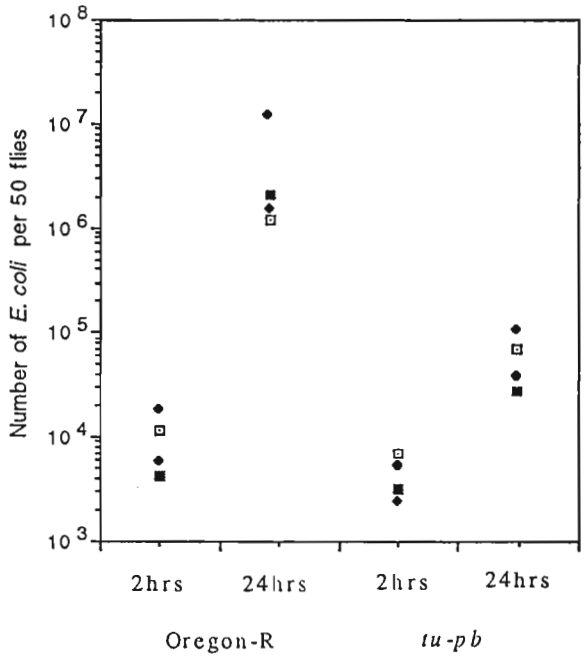


Figure 3. Bacterial growth in challenged Oregon-R and *tu-pb* adults infected with *E.coli* ampicillin resistent strain. Flies were homogenized either two or 24 h after infection, and lysates were cultured on plates containing ampicillin.

melanization and bacterial growth, bacterial proliferation assays were performed on challenged insects. The results obtained indicate that *tu-pb* mutants are able to inhibit bacterial growth more efficiently than wild type (Figure 3).

These preliminary observations could suggest that the *tu-pb* mutation probably enhances other immune mechanisms as proteolytic cascades and cellular reactions, essential for the host defense.

References: Di Pasquale Paladino, A., and P. Cavolina 1983, Dros. Inf. Serv. 59: 31; Di Pasquale Paladino, A., and P. Cavolina 1984, Dros. Inf. Serv. 60: 84; Di Pasquale Paladino, A., P. Cavolina, G. Romano, and R. Ribaudo 1988, Experientia 44: 777-779; Faye, I., and D. Hultmark 1993, In *Parasites and Pathogens of Insect*, vol. 2, Academic Press, pp. 25-53; Hultmark, D., 1993, TIG 9: 178-183; Lackie, A.M., 1988, Adv. Insect. Physiol. 21: 85-178; Lemaitre, B., M. Meister, S. Govind, P. Georgel, R. Steward, J.M. Reichart, and J.A. Hoffmann 1995, EMBO J. 14: 536-545; Sparrow, J.C.,1978, *The Genetics and Biology of* Drosophila, 2b (ed., M. Ashburner and T.R.F. Wright), pp. 277-313, Academic Press, N.Y.

Catchpole, Roger. Department of Biology, University of Leeds, Leeds, LS2 9JT. Email: r.d.j.catchpole@leeds.ac.uk The effect of maturity on courtship behaviour in two species of *Drosophila.*

Any examination of the literature associated with the measurement of sexual isolation, as well as the general courtship behaviour of *Drosophila,* shows that individuals of a range of ages are commonly used in different studies on the same species (*e.g.,* Stalker, 1942; Spieth, 1951). Both the receptivity of the female (Manning, 1959) and the willingness of the male to initiate courtship (Spieth, 1974) has been shown to be age dependent. In view of this, it is important to determine when maturity has been reached before any study of courtship behaviour is undertaken. Differences in temperature, diet and genotype will all lead to different rates of development that make any definitive estimate of when a particular species reaches maturity problematic. In spite of this fact many studies show no evidence of any direct measurement (*e.g.,* Spieth, 1951; Bastock and Manning, 1955; Bastock, 1956; Cowling and Burnet, 1981; Cobb *et al.,* 1986; Crossley *et al.,* 1995).

The differences that can be present, both within and between species, are well illustrated in the following data that were collected as part of a larger study on the effects of founder events and selection on isolation within two species; *D. melanogaster* and *D. virilis*. As a large number of assortative mating tests had to be carried out, it was important to determine the age at which each species became sexually mature and therefore receptive to courtship. Newly emerged individuals of each species were collected at six hour intervals over a number of days and stored individually in glass vials on standard food medium (Shorrocks, 1971). These virgins were then used in single pair mating tests to determine the maturity threshold for each species. The latency to key stages in the mating sequence were recorded in order to determine the relationship between age and sexual maturity in both males and females. The measurements consisted of latency to tarsal contact where the male first makes deliberate physical contact with the female, latency to the first wing vibration where the male first begins actively to court the female and latency to copulation where the male mounts the female and intromission occurs. All observations were carried out at a constant temperature ($21 \pm 2^{\circ}C$) and in uniform lighting conditions. Five pairs were observed for each time interval.

Analysis of the effect of age on the courtship behaviour of *D. melanogaster* showed that there was no significant relationship for any of the key behaviours that were measured between 2-15 days, as may be seen from Table 1 and Figures 1-3. Only data for adults aged between 2-15 days were used, as sample sizes were not constant after 15 days and

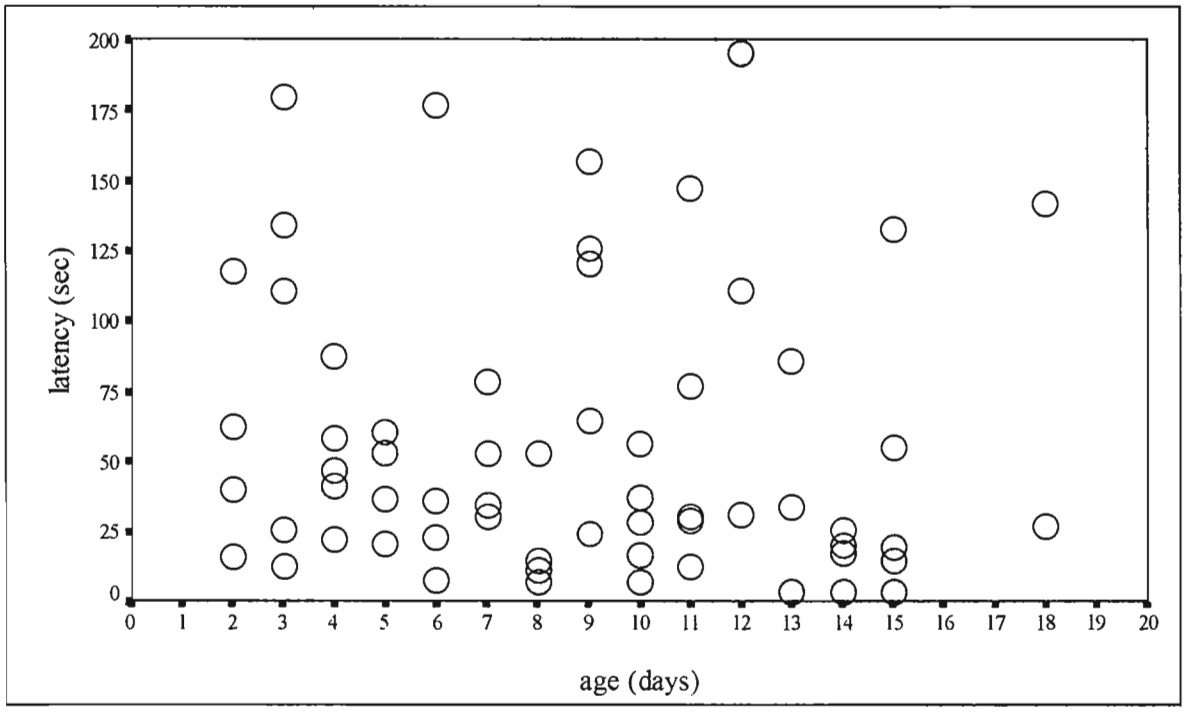


Figure 1. Age and latency to tarsal contact. Graph shows latency to tarsal contact in virgin males collected from a single population.

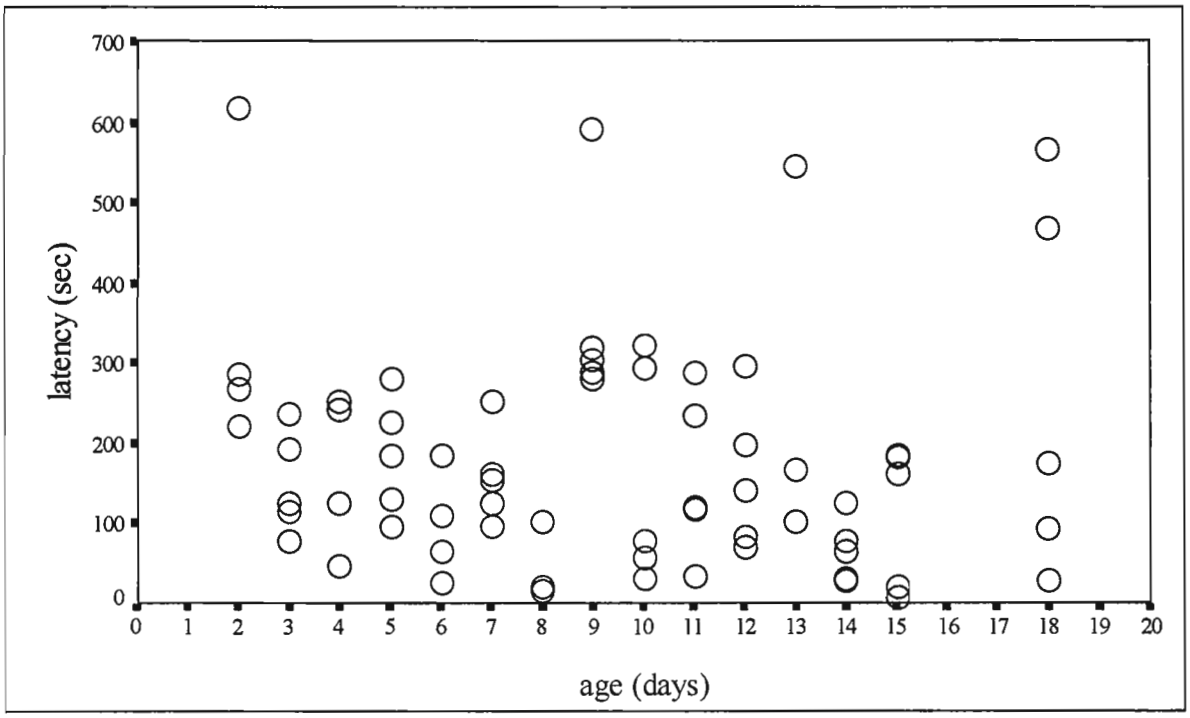


Figure 2. Age and latency to wing vibration. Graph shows latency to wing vibration in virgin males collected from a single population.

Table 1. Maturation and courtship behaviour. This table shows the results for a model I regression between age (2-15 days) and latency to each key behaviour.

Behaviour	b	S.E.	t	p
tarsal contact	0.078	0.168	0.465	0.645
wing vibration	0.085	0.164	0.520	0.605
copulation	0.190	0.194	0.979	0.332

Table 2. Maturation and courtship behaviour. This table shows the results for a least squares regression which was applied to a range of different ages between 12-17 days.

Behaviour	b	S.E.	t	p
tarsal contact	- 1.202	0.780	- 1.540	0.139
wing vibration	- 1.733	1.059	- 1.636	0.117
copulation	- 1.090	1.222	-0.892	0.382

were non-existent before 2 days. This avoided any bias that might result from the restricted sampling of the widely dispersed data. It was clear that sexual maturity developed within two days of emergence in both males and females and did not show any decline over the period that was examined. The results were generally consistent with other studies which have shown the rapid development of sexual maturity in this species over a range of conditions (Manning, 1959; Long *et al.*, 1980; Spieth, 1952, 1958). The period over which active mating occurred was, however, longer than the 12 days reported by Spiess (1970).

Analysis of the effect of age on the courtship behaviour of *D. virilis* showed that there was no significant relationship for any of the key behaviours that were measured between 12-17 days, as may be seen from Table 2 and Figures 4-6. There was, however, a long phase of juvenile development during which time no courtship or mating occurred, see Figures 5-6. There appeared to be a clear and parallel maturation period in both males and females up until nine days after emergence. This pattern was not apparent in the data for tarsal contact, although the variation between individual observations was higher during the initial period, see Figure 5. The single points at 900 seconds in Figures 5 and 6 represent five measurements in which the associated behaviour did not occur. They were scored at 900 as this was

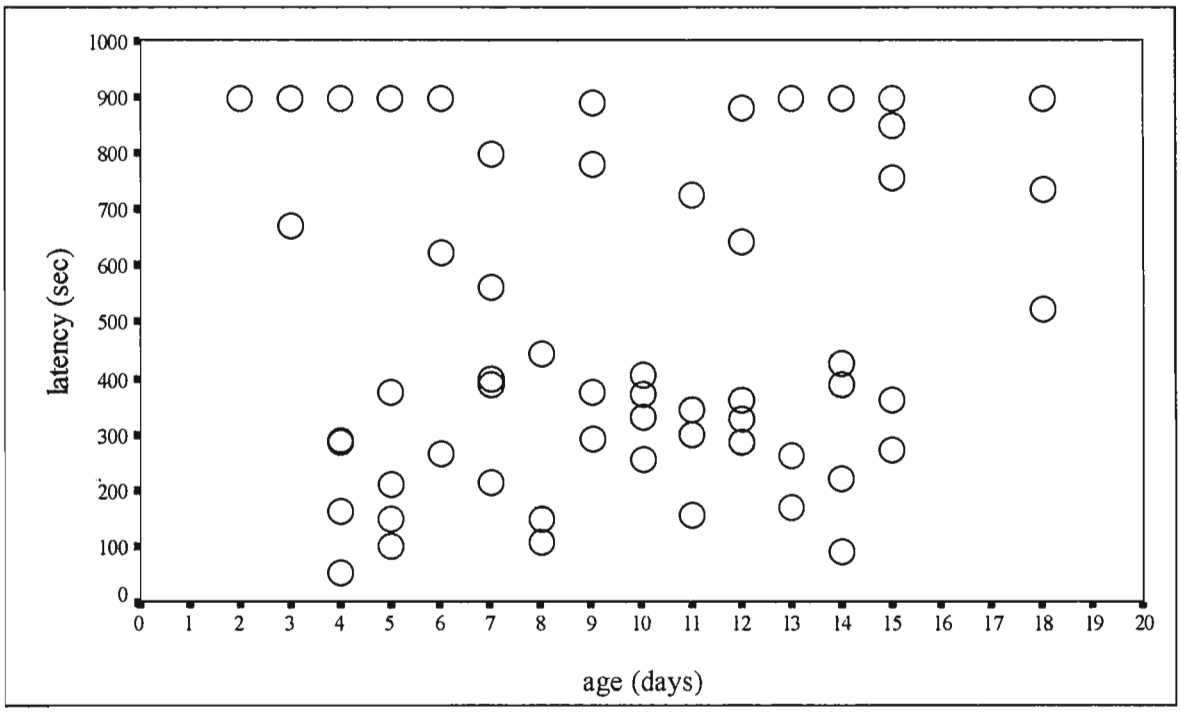


Figure 3. Age and latency to copulation. Graph shows latency to wing vibration in virgin males collected from a single population.

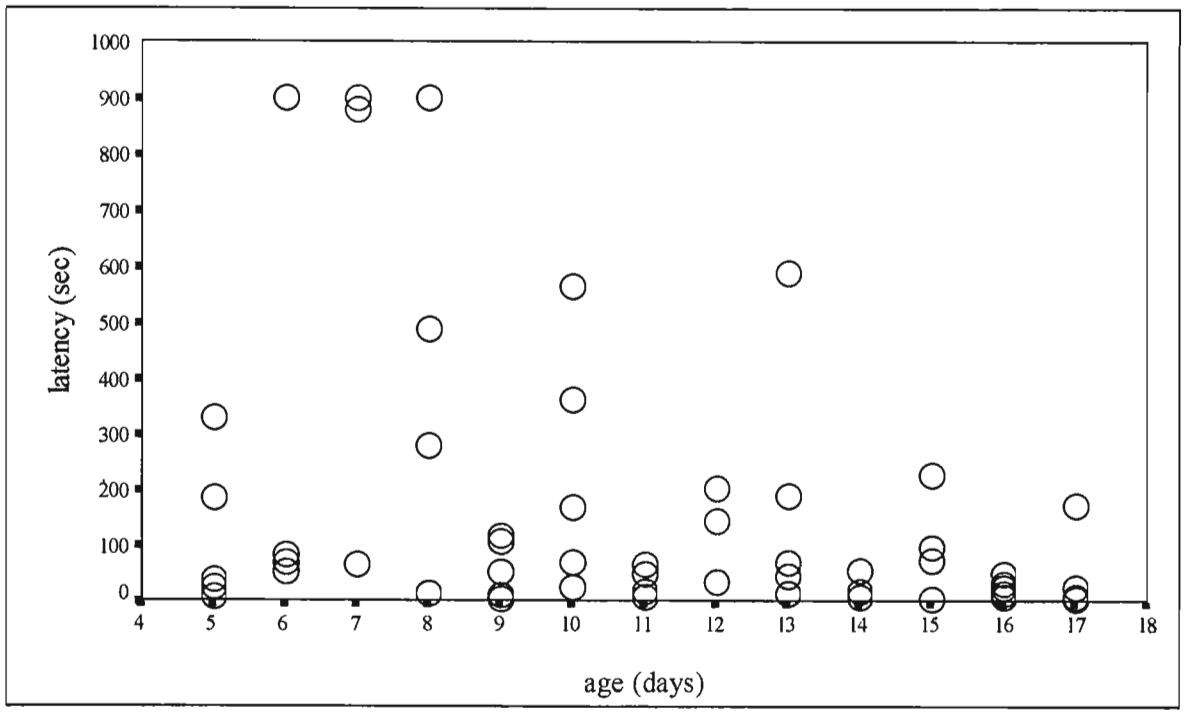


Figure 4. Age and latency to tarsal contact. Graph shows latency to tarsal contact in virgin males collected from a single population.

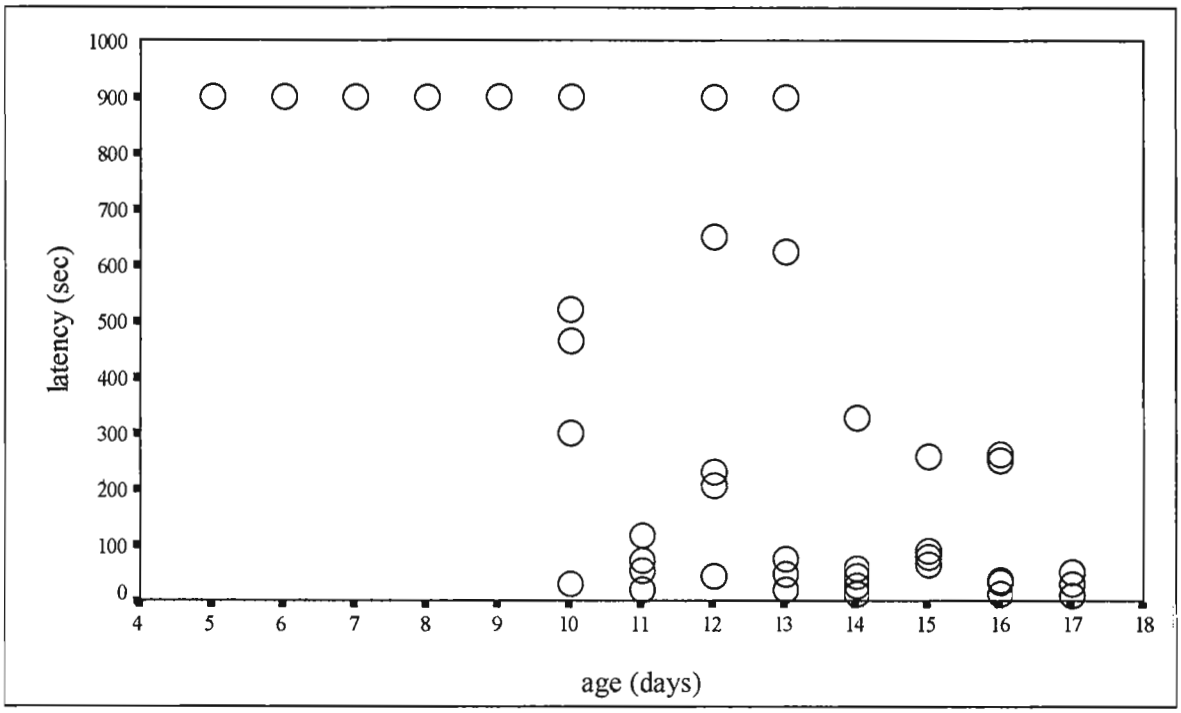


Figure 5. Age and latency to wing vibration. Graph shows latency to wing vibration in virgin males collected from a single population.

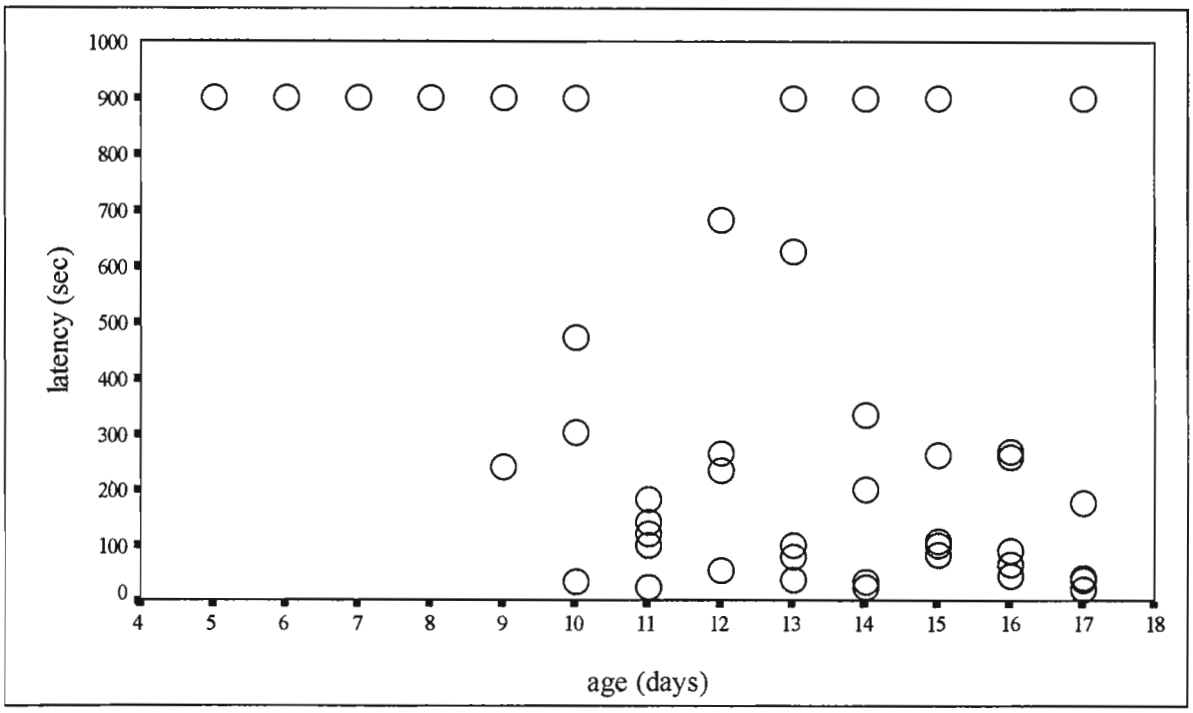


Figure 6. Age and latency to copulation. Graph shows latency to copulation in virgin females collected from a single population.

the end of the observation period; none of the behaviours of either species actually occurred at this time; therefore, all such values indicate a failure to observe any change. The results were not consistent with the work of Stalker (1942) who observed that substantial mating occurred for this species after only 4 days. Other workers have been more conservative and not used individuals younger than 10 days (Spieth, 1951) or even 14 days (Hoikkala and Lumme, 1984). Generally there appears to be a need to quantify the rate at which species mature under different conditions rather than assume that an arbitrary choice will be acceptable in courtship studies. Clearly the rates of development can vary greatly even within one species as this comparison has shown.

References: Bastock, M., 1956, Evolution 10: 412-429; Bastock, M. and A. Manning 1955, Behaviour 8: 86-111; Cobb, M., B. Burnet, and K. Connolly 1986, Behaviour 97: 182-212; Cowling, D.E. and B. Burnet 1981, Animal Behaviour 29: 924-935; Crossley, S.A., H.C. Bennet-Clark, and H.T. Evert 1995, Animal Behaviour 50: 827-839; Hoikkala, A. and J. Lumme 1984, Behavior Genetics 14: 257-268; Long, C.E., T.A. Markow, and P. Yaeger 1980, Behavior Genetics 10: 163-170; Manning, A., 1959, Behaviour 15: 123-145; Shorrocks, B., 1971, Dros. Inf. Serv. 46: 149; Spiess, E.B., 1970, Mating propensity and its genetic basis in *Drosophila*. In: *Essays in Evolution and Genetics in Honour of Theodosius Dobzhansky* (ed. M.K. Hecht and W.C. Steere), pp. 315-379. Wiley; Spieth, H.T., 1951, Behaviour 3: 105-145; Spieth, H.T., 1952, Bulletin of the American Museum of Natural History 99: 395-474; Spieth, H.T., 1958, Behaviour and isolating mechanisms. In: *Behavior and Evolution* (ed. A. Roe and G.G. Simpson), pp. 363-389. Yale University Press; Spieth, H.T., 1974, Annual Review of Entomology 19: 385-405; Stalker, H.D., 1942, Genetics 27: 238-257.

Choo, J.K., and C.H. Ahn. Department of Biology, Chung-Ang University, Seoul 156-756, Korea.
Identification of genotype and its relationship with map gene pattern in a population of Korean *Drosophila melanogaster*.

In *Drosophila melanogaster*, the structural gene of alpha-amylase (E.C. 3.2.1.1; α-1,4-glucan glucanohydrolase) encoding a monomeric enzyme (54,500D) is controlled by allelic, codominant and duplicated genes located near site 78 of the second chromosome. In natural populations, eight variants of the amylase genotype have been reported (Lindsley and Zimm, 1992), and two regulatory factors in *Amy* gene expression have been well identified. Of them, *mapP*, a regulatory gene, affects the tissue- and age-specific expression of the *Amy* gene in the posterior region of the adult midgut (Klarenberg *et al.*, 1986). The other factor known as the dietary glucose repression depresses the level of *Amy* activity and its product in each developmental stage (Benkel and Hickey, 1987). In our study, the genotype and frequency of the *Amy* variants of *D. melanogaster* collected from a natural population were analyzed and the expression and genetic regulation of alpha-amylase were investigated at the tissue level.

Materials and Methods: The flies used in our study were collected at Cheon-An city near Seoul, Korea by sweeping net. To determine genotype and frequency of each variant, polyacrylamide (7.5%) gel electrophoresis was performed. After electrophoresis, activity staining of alpha-amylase needed incubation with separating gel in 2% starch and I_2-KI solution. The protein content was measured by the method of Bradford (Bollag and Edelstein, 1991). The specific activity of alpha-amylase of each *Amy* genotype was determined by the method of starch-iodine and DNSA (Doane, 1969). Pattern analysis of amylase activity in midgut (map) was carried out by the method of Abraham and Doane (1978) and each pattern was determined by the method of Doane (1980).

Table 1. Protein content and specific activity of amylase from each *Amy* genotypes in a natural population *of D. melanogaster.*

Genotype	No. of Line	Frequency (%)	Protein content[1] (μg)	Specific activity (unit/min)
Amy^{1}	147	75.00	12.9453	2.0179
$Amy^{1\cdot3}$	33	16.84	13.7170	1.8629
$Amy^{1\cdot2}$	7	3.57	13.8156	1.8286
$Amy^{1\cdot2\cdot3}$	4	2.04	13.1786	2.0662
$Amy^{1\cdot3\cdot6}$	4	2.04	13.5442	1.3989
$Amy^{1\cdot6}$	1	0.51	12.8061	1.2432
Total	196	100.00	13.1226[2]	1.9695[3]

[1]Protein content of crude extract; [2]Average of protein contents of six genotypes; [3]Average of specific activities of six genotypes

Results and Discussion: Frequency, protein content and specific activity of each *Amy* genotype are shown in Table 1. It was revealed that the population analyzed in this study consisted of six *Amy* genotypes designated Amy^{1}, $Amy^{1\cdot2}$, $Amy^{1\cdot3}$,

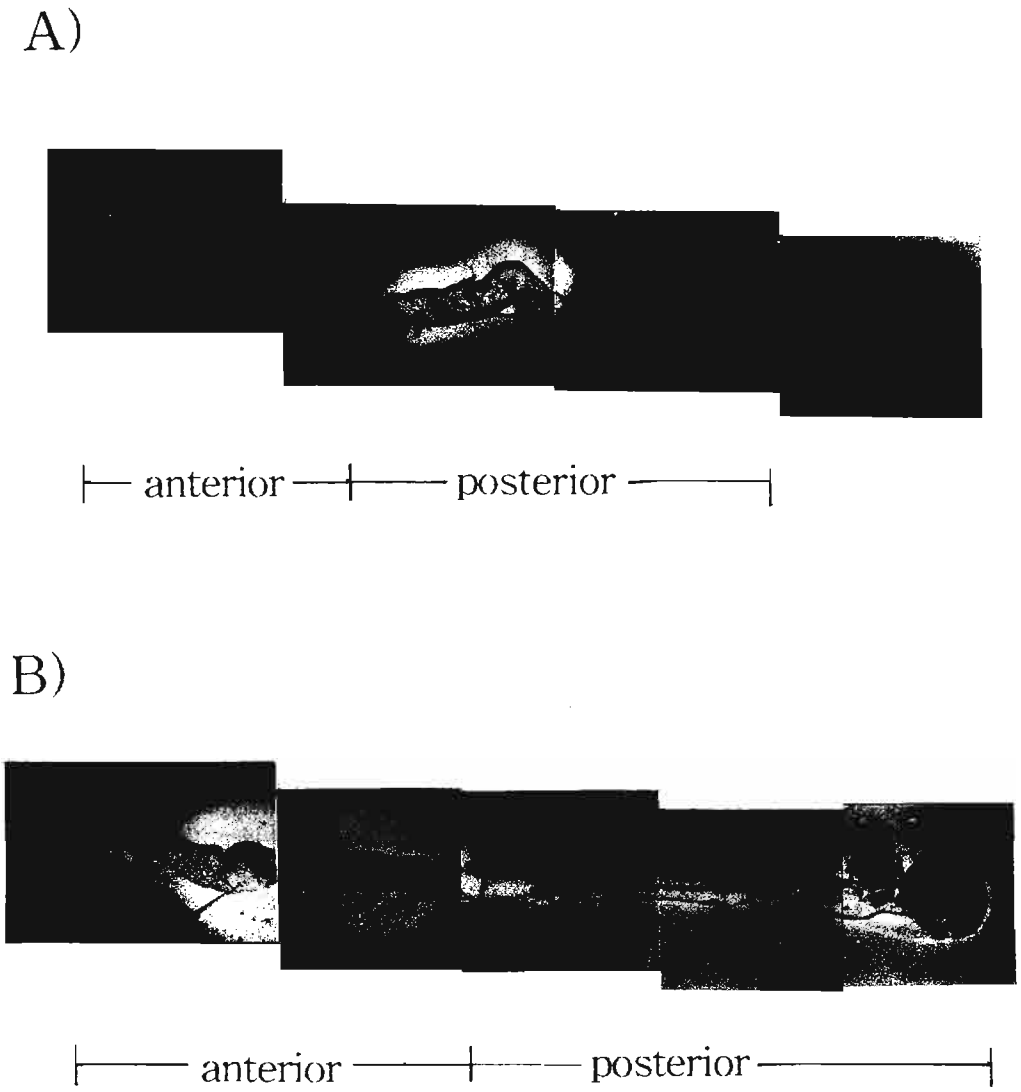


Figure 1: Alpha-amylase expression in the midgut of 120 hr third instar larvae reared on the standard medium. The colorless area indicates the portion containing amylase activity. A) mapA^{000}P^{12}, B) mapA^{123}P^{00}.

Amy$^{1\cdot3\cdot6}$, *Amy*$^{1\cdot2\cdot3}$, and *Amy*$^{1\cdot6}$. The frequencies of *Amy*^{1} and *Amy*$^{1\cdot3}$ appeared to be 75.00% and 16.84%, respectively, indicating that these two genotypes would be the common and ancestral form in this natural population. The protein concentration of each genotype appeared not to be significantly different and the average of six genotypes was 13.1226μg. The average of the specific activity of amylase was 1.9695 unit/min and *Amy*$^{1\cdot3\cdot6}$ and *Amy*$^{1\cdot6}$ showed relatively low activity.

Figure 1 represents the histological distribution of amylase activity, indicating differential distribution within a genotype (Figure 1, A and B). Also, map pattern of the adult indicated higher activity at the posterior region than at the anterior region (83.5%; 175/209 midgut). This result was shown similarly in Klarenberg *et al.* (1986) which observed *trans*-regulation of amylase activity in larval and adult midgut.

To elucidate the genetic independence of amylase genotype in a natural population, the method of amylase-substrate PAGE was employed. The electrophoretic pattern of amylase appeared to be consistent with genotype and map pattern, suggesting that amylase and map genes are genetically independent.

In this paper, it would be emphasized that the amylase gene showed polymorphism in a natural population and the amylase activity and its gene expression were independent from map gene associated with tissue-specific expression of amylase.

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Derzhavets, Elena, A. Korol, and E. Nevo. Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31905, Israel, e-mail: korol@esti.haifa.ac.il. Differences in fluctuating asymmetry in *Drosophila melanogaster* caused by microclimatic contrasts.

Temperature and humidity are among the most important factors affecting insect adaptation and evolution. We have studied the effect of contrasting differences for these factors at a microsite, "Evolution Canyon", Lower Nahal Oren Canyon, Mount Carmel, Israel, on the variation for fluctuating asymmetry of wing scores in *Drosophila melanogaster*. The opposite slopes of the Canyon contrast sharply due to difference in solar radiation (Nevo, 1995). Our previous studies with *D. melanogaster* isofemale lines derived from the Canyon revealed significant interslope differences in a number of adaptively important traits including oviposition temperature preferences, viability and longevity changes caused by short-term and lifetime temperature treatments, and resistance to drought stress at different temperatures (Nevo *et al.*, 1997). A part of these tests was also conducted for the sibling species *D. simulans*, displaying parallel results to those of *melanogaster*. Likewise, we found considerable differences in the characteristics of the "transmission system" in *D. melanogaster*: higher rates of male recombination (Derzhavets *et al.*, 1996a) and sex-linked lethal mutations (unpublished results) in lines of ecologically stressful south-facing slope as compared to those of the less stressful north-facing slope, and different patterns of potential for P-M hybrid disgenesis (Derzhavets *et al.*, 1996b).

Deviation from bilateral symmetry was considered by many authors as an indicator of stress, either external or genetic (e.g. Palmer and Strobeck, 1986; Jones, 1987; Parsons, 1992; Markow, 1995). It was demonstrated that fluctuating asymmetry correlates positively with the level of inbreeding, genetic disbalance, general or specific ecological stresses, although in some cases no clear evidence was obtained (Alibert *et al.*, 1994; Fowler and Whitlock, 1994; McKenzie and Yen, 1995; Freebairn *et al.*, 1996). In a long-term study on the Australian sheep blowfly, *Lucilia cuprina*, it was demonstrated that asymmetry scores may serve as relevant and sensitive indicators of population gene pool adaptation to new environmental challenges (McKenzie and Yen, 1995). Thus, it is of interest to employ the asymmetry test in analysis of microsite population adaptation caused by microclimatic geographic differentiation.

Material and Methods: Wild type inseminated females were collected in June-July 1994 from the two opposite slopes of "Evolution Canyon": ecologically stressful south-facing slope (SFS) and the less stressful north-facing slope (NFS). The resulting isofemale lines were kept under standard laboratory conditions. Flies to be measured were reared at 25°C under controlled low-density conditions. This was achieved by placing 10 pairs of flies in a vial for 24 hours. From the progeny emerging from each of the vials during the first four days, five males and five females were taken at random in order to measure wing parameters. Thus, a total of 10 flies from each of the isofemale lines (five lines from SFS and six from NFS) were examined. Wings were prepared for measurement by laying them on two-sided sticky tape and covering them with a coverslip. The left and right wings from each fly were dissected and mounted on the slide. The measured complex of wing parameters involved the wing length along longitudinal vein and the wing width from the extreme of the fifth vein to the coastal border (see Figure), and several derivative traits. These measurements were conducted using an interactive image analyzing system, WScanArray 3 Image Analyzer (Galai Production Ltd, Israel). In order to reduce the uncontrolled variation caused by manual clicking of the cursor on boundary points on the wing, all wings were scored five times and then the initial scores averaged to obtain mean trait scores per wing. All measurements were taken by the same person. For each fly, trait scores of the two wings were used to derive the 'directional asymmetry' $DA = right\text{-}left$, 'fluctuating asymmetry' $FA = \mathrm{abs}(DA)$, 'relative directional asymmetry' $RDA = 100 \cdot DA/M$, where $M = (right + left)/2$ and 'relative fluctuating asymmetry' $RFA = \mathrm{abs}(RDA)$ indices. Clearly, the indices within the pairs {*DA* and *RDA*} and {*FA* and *RFA*} have the same sense, but the preference of the 'relative' indices is in the possibility to make comparisons between different traits. However, the results for these two types of indices may differ if asymmetry is correlated with the initial scores. Thus, all our calculations were conducted for both types. Very close results were obtained. Therefore, we present the results only for the 'relative' indices.

Results and Discussion: One would consider the dissection of the total between-individual phenotypic variation of a trait into genetic (between-line) and non-genetic (within-line) components as a natural way of data analysis and presentation for the considered situation. However, analysis of allozymic variation shows that in spite of about two-

Table 1. Variation of wing mean trait scores and asymmetry indices

Trait	sex	SFS			NFS		
		mean	σ^2_P	σ^2_L	mean	σ^2_P	σ^2_L
		trait mean scores *M*=(*right*+*left*)/2					
L1	f	31.84±0.31	2.40	1.10	30.98±0.35	3.69	2.82
	m	28.01±0.29	2.16	1.56	27.40±0.28	2.28	0.64
L2	f	31.65±0.31***	2.43	1.06	30.04±0.29	2.47	2.19
	m	28.39±0.25*	1.59	0.79	27.61±0.29	2.53	0.46
L3	f	14.44±0.15	0.59	0.25	14.29±0.13	0.47	0.34
	m	13.28±0.17	0.68	0.52	13.22±0.15	0.66	0.26
L4	f	16.56±0.17	0.69	0.34	16.39±0.15	0.65	0.58
	m	15.06±0.17	0.71	0.56	14.94±0.13	0.48	0.17
R1	f	49.86±0.27	1.82	1.28	49.25±0.19	1.10	0.38
	m	50.35±0.24	1.42	1.23	50.19±0.21	1.35	0.67
R2	f	46.59±0.25	1.61**	1.37	46.59±0.14	0.59	0.34
	m	46.85±0.24	1.49	1.35*	46.95±0.16	0.76	0.17
R3	f	48.84±0.29***	2.07	1.02	50.34±0.32	2.96	2.07
	m	50.26±0.38	3.65	3.65	51.22±0.30	2.66	1.54
		relative directional asymmetry *RDA*=(*right-left*)/*M*·100%					
L1	f	-0.79±0.94	22.09	5.11	-0.67±0.94	26.42	5.38
	m	0.11±0.78	15.05	0.87	-0.03±0.72	15.68	1.32
L2	f	-0.59±0.44	4.97	0.64	-0.04±0.49	7.29	2.19
	m	-1.32±0.47++	5.57	0.71	-0.07±0.42	5.20	0.90
L3	f	0.61±0.93	21.72**	1.82	-0.81±0.50	7.62	2.92
	m	-1.16±0.92	21.07*	1.28	-0.33±0.54	8.64	1.00
L4	f	-1.18±0.85	17.98	3.76	-1.42±0.54++	8.88	3.31
	m	-0.40±0.76	14.44	0.79	-0.87±0.64	12.46	1.80
R1	f	0.10±0.52	6.81	1.74	0.29±0.44	5.66	0.56
	m	-0.72±0.41	4.29	0.28	-0.01±0.35	3.65	0.55
R2	f	0.95±0.51	6.40	1.25	0.32±0.36	3.92	1.51
	m	-0.42±0.58	8.41	0.58	0.28±0.38	4.37	0.45
R3	f	0.35±0.83	17.56	1.10	-0.80±0.77	17.98	7.56
	m	-0.14±0.85	17.89	1.80	-0.55±0.57	9.73	0.46
		fluctuating asymmetry *RFA*=abs(*RDA*)					
L1	f	3.44±0.64	10.37	3.24	3.69±0.65	12.82	3.53
	m	2.99±0.48	5.76	1.10	3.14±0.43	5.48	0.46
L2	f	1.47±0.35	3.10	0.29	1.99±0.33	3.20	0.76
	m	2.12±0.33	2.69	0.72	1.78±0.26	1.96	0.50
L3	f	3.46±0.62	9.67**	3.28*	2.17±0.34	3.46	0.38
	m	3.81±0.54*	7.34**	1.49	2.45±0.29	2.53	1.30
L4	f	3.47±0.53	6.92	2.05	2.47±0.39	4.67	2.37
	m	2.74±0.52	6.81	2.50	2.72±0.43	5.57	1.00
R1	f	1.96±0.34	2.82	0.77	1.47±0.34	3.53	1.02
	m	1.68±0.28	1.90	0.64	1.55±0.20	1.17	0.28
R2	f	1.97±0.36	3.31	0.88	1.48±0.24	1.77	0.53
	m	2.19±0.38	3.57*	1.00	1.66±0.23	1.61	0.64
R3	f	3.34 0.49	6.05	2.86	3.23 0.51	7.78	4.12
	m	3.43 0.48	5.66	0.85	2.56 0.33	3.28	1.44

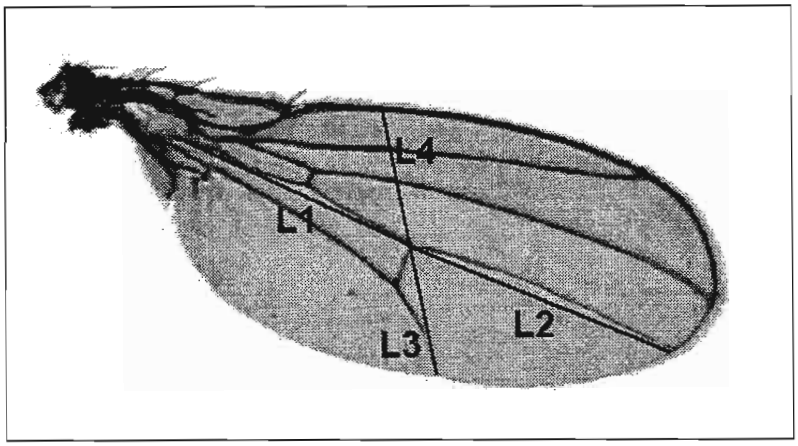


Figure 1. Initial wing traits (L1-L4) measured on the interactive image analyzing system.

year rearing of the isofemale lines as small closed populations (40-100 flies per line per generation), they still manifest rather high genetic variation (unpublished data). In such a case, the 'phenotypic level' is of primary importance when conducting interslope comparisons.

The presented data (Table 1) show significant difference for some wing 'size' and 'shape' mean trait values (L2 and R3) and trait variance (R2). In the latter case, higher variation is characteristic of SFS. The same pattern is manifested by the asymmetry indices: in any case of significant interslope difference in the level of variation, both the directional (*RDA*) and fluctuating (*RFA*) asymmetries are more variable in the material derived from SFS. It is noteworthy, that no significant deviation of *RDA* from zero is observed for the trait L3, where the highest interslope difference for σ^2_{FA} is established. Thus, significantly higher variance of *FA* and for L3 scores in SFS flies reflects higher variation in fluctuating asymmetry, parallel in males and females. Earlier (Nevo *et al.*, 1997), we found a higher intraslope variation of adaptively important traits in isofemale lines derived from the SFS as compared to NFS. This effect appeared to be a result of higher between-station variation on SFS. Our present data (see Table 1) demonstrate that higher variation on SFS is also manifested at the within-station level.

Our results corroborate the conclusion resulted from an experimental study conducted on populations of *D. melanogaster*, derived from laboratory strain kept for 4 years (Cavicchi *et al.*, 1985). The authors found that population adaptation to new temperatures affects wing traits related to our scores L2 and, especially, L3. The observed changes were interpreted in terms of independent units of selection represented by two wing compartments, with determinants of cell size and cell number being the target of selection.

An interesting point is that for all seven traits examined significant fluctuating asymmetry is observed for both slopes for both sexes. Nevertheless, in only one case significant interslope difference was found (for L3 in males, though non-significant difference of the same sign is noted). Our previous studies showed that the lines derived from the opposite slopes are different with respect to their reactions to stressful environments (Nevo *et al.*, 1997; Derzhavets *et al.*, 1996). Nevertheless, if fluctuating asymmetry of the foregoing wing scores can indeed serve as an indicator of stress, it follows from the foregoing results that the conditions of rearing of the scored material were, on the average, nearly equally stressful for the two groups of lines. This allows for further tests to compare the reaction norms of *FA* in the two groups on stress factors characteristic to the natural habitat of "Evolution Canyon".

References: Alibert, P., S. Renaud, B. Dod, F. Bonhomme, and J.-C. Auffray 1994, Proc. R. Soc. Lond. B 258: 53-59; Cavicchi, S., D. Guerra, G. Giorgi, and C. Pezzoli 1985, Genetics 109: 665-689; Derzhavets, E.M., E. Nevo, and A.B. Korol 1996b, Dros. Inf. Serv. 77: 124-126; Derzhavets, E.M., A.B. Korol, and E. Nevo 1996a, Dros. Inf. Serv. 77: 92-94; Fowler, K., and M.C. Whitlock 1994, Heredity 73: 373-376; Freebairn, K., J.L. Yen, and J.A. McKenzie 1996, Genetics 144: 229-239; Jones, J.S., 1987, Nature 325: 298-299; Markow, T.A., 1995, Annual Review Entomology 40: 105-120; McKenzie, J.A., and J.L. Yen 1995, Heredity 75: 181-187; Nevo, E., 1995, Proc. R. Soc. Lond. B 262: 149-155; Nevo, E., E. Rashkovetsky, T. Pavlicek, and A. Korol 1997, Heredity, in press; Palmer, A.R., 1994, in: Markow, T.A. (Ed.) *Developmental Instability: Its Origins and Evolutionary Implicaqtions*, Kluwer Acad. Publ., Netherlands, pp. 335-364; Palmer, A.R., and C. Strobeck 1986, Annual Reviews of Ecology and Systematics 17: 391-421; Parson, P.A., 1992. Heredity 68: 361-364.

Table Footnote: The 'size' traits L1-L4 are as shown in the Figure (the measurements are given in some arbitrary units formed by the Image analyzing system), the 'shape' traits are denoted as follows: R1 = 100·L2/(L1 + L2), R2 = 100·L3/(L3 + L4), and R3 = 100·(L3 + L4)/(L1 + L2). The interslope differences are significant at $^{*}(P < 0.05)$, $^{**}(P < 0.01)$, and *** $(P < 0.001)$, respectively; $^{++}$ significant $(P < 0.01)$ deviation of *RDA* from zero; note that for all traits deviations of *FA* are highly significant for both slopes and both sexes.

Berrigan, David. Department of Zoology, Box 351800, University of Washington, Seattle Washington 98195 USA. Pulsed emissions of CO_2 during mating in *Drosophila melanogaster*.

The biochemistry, genetics, and behavior associated with courtship and mating have been the focus of much work (recent reviews in Wolfner, 1997; Pitnick, 1996; Hall, 1994). The evolution of reproductive behavior is likely to be influenced by the costs of such behavior. These costs could occur as energetic expenditures during courtship and mating or as longer term life history consequences of mating (*e.g.* Partridge and Farquhar, 1981).

In order to quantify the energetics of courtship and mating, I recently recorded CO_2 production in a number of pairs of mating *D. melanogaster*. To measure metabolic rates, I used a Licor-6262 CO_2 (Lincoln, Nebraska, USA) analyzer together with flow controller and a Sable Systems (Las Vegas Nevada, USA) data acquisition package. Similar methods for individual insects are discussed in detail in Lighton (1991) and Berrigan and Partridge (1997).

A typical recording is illustrated in Figure 1. This recording was made with a flow rate of 40 ml min^{-1} in a tubular glass chamber 4 cm long and 2 cm in diameter. These flies began copulating about 12 minutes after the start of the figure and finish at about 36 minutes. I deleted 15 minutes of the recording prior to the section shown and ten additional minutes of recording at the end. Note that when the flies are motionless for a few minutes, then CO_2 emission stabilizes at rates approximately equal to the level in between the pulses during mating. The apparent increase in metabolic rate prior to copulation (from about 6 to 12 minutes) could reflect costs of courtship; however, it is not apparent in all recordings and more detailed observations are required to determine if the resolution if this system is sufficient to estimate the costs of courtship. Videotaping the flies during courtship and copulation could help correlate measured metabolic rates with specific behaviors.

Two aspects of these recordings are worth mentioning. First, metabolic rates during mating are fairly similar to those during routine activity. Thus, the energetic costs of courting and copulation are not very high in *D. melanogaster* when compared to the costs of pedestrian locomotion. This is obvious upon inspection of the behaviors that occur during courtship and copulation but worth verifying because of the theoretical importance of costs of reproduction. Second, one or both of the copulating flies release pulses of CO_2 during copulation. These pulses appear to differ from the CO_2 pulses associated with discontinuous ventilation in insects (Lighton, 1994). The level of CO_2 emission does not decline to zero between pulses; therefore, it seems unlikely that the spiracles of both flies are closed.

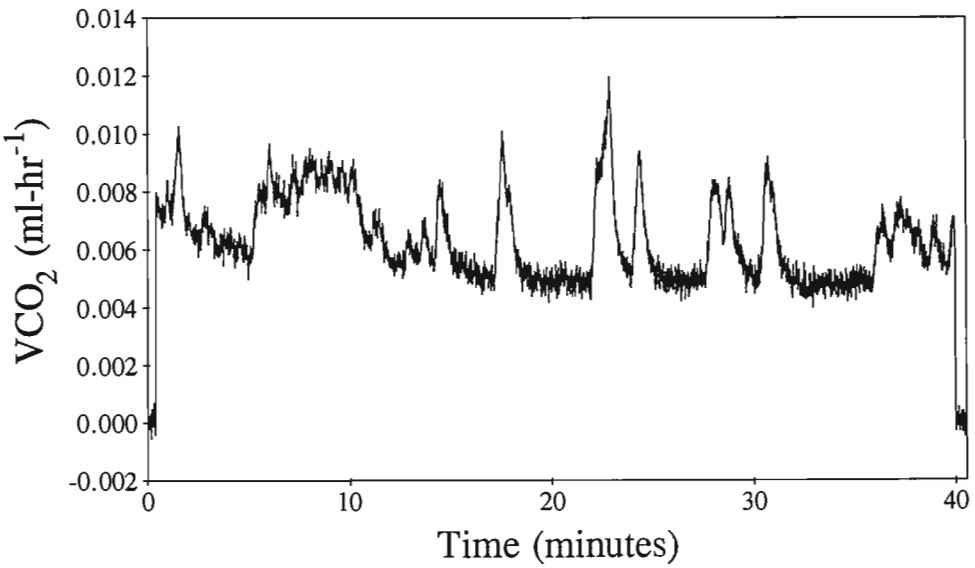


Figure 1. Metabolic rate of a pair of *Drosophila* before, during and after mating. The near zero readings at the beginning and end of the recording were obtained from an empty cuvette. Average CO_2 production was 0.00745 ml hr^{-1} from 0-12 minutes, 0.0058 ml hr^{-1} from 12 to 36 minutes and 0.0065 ml hr^{-1} after 36 minutes. Flow rate of CO_2 free air through the cuvette in this recording was 40 ml min^{-1}. Similar recordings have been obtained on a number of copulating flies, the magnitude and number of pulses of CO_2 emitted varies among pairs.

My hypothesis is that these pulses occur when muscular activity by the male associated with the transfer of sperm and/or seminal fluid results in the release of a pulse of CO_2. If this hypothesis is correct, then it would be interesting to document the correlation between the number and magnitude of the pulses and the number of sperm and amount of seminal fluid transferred. Recordings of CO_2 pulses could be a non-invasive method for assessing male reproductive performance.

References: Berrigan, D., and L. Partridge 1997, In press, Comparative Biochemistry and Physiology; Hall, J. C., 1994, Science 264:1702-1714; Lighton, J. R. B., 1991, pp. 201-208, in P.A. Payne, Ed., Concise Encyclopedia on Biological and Biomedical Measurement Systems. Pergamon Press, Oxford; Partridge, L., and M. Farquhar 1981, Nature 294:580-582; Lighton, J. R. B., 1994, Physiol. Zool. 67:142-162; Pitnick, S., 1996, Amer. Nat. 148:57-80; Wolfner, M. F., 1997, Insect Biochem. Molec. Biol. 27:179-192.

Zhimulev, Igor F., and Natalia I. Mal'ceva. Laboratory of Molecular Cytogenetics, Institute of Cytology and Genetics, Novosibirsk 630090, Russian Federation. Action of ecdysterone on salivary gland and nurse cell polytene chromosomes of *Drosophila melanogaster otu* mutant *in vitro*.

The main purpose of this work was to investigate the reaction of polytene chromosomes from ovarian nurse cells to ecdysterone. Normally the nuclei of nurse cells (ncs) have a reticular structure because they contain dispersed chromosomes that are of little cytological value. However, in the present study we used flies of the $otu^7/y\ w\ sn^3\ otu^{11}$ genotype, since their nurse cell nuclei contain polytene chromosomes with clear-cut banding patterns. To obtain NC polytene chromosomes of best cytological quality, this fly stock was kept at 16°C (Mal'ceva *et al.*, 1995, 1997). Both salivary glands (sgs) of larvae at Puff Stages 1-3 and ovaries of 5 day old adult flies were dissected in Ephrussi-Beadle's solution and incubated in Robb's medium according to the protocol given below (Figure 1). Organs were incubated *in vitro* using 3×10^{-7}M of ecdysterone (20-OH ecdysone, Serva), according to Ashburner's technique (Ashburner, 1972).

Table 1. Changes of puffing patterns of salivary gland and nurse cell chromosomes *in vitro*.

Cell type	Experiment	Puffing pattern
Salivary gland cells	6-24h incubation without ecdysterone, 16°C	Inactivation of all ecdysterone puffs. Induction of "incubation" puffs: 47D, 50C, 60C and 93E
Ovarian nurse cells	6-24h incubation without ecdysterone, 16°C	Induction of "incubation" puffs: 47D and 50C
Salivary gland cells	2-6h incubation with ecdysterone, 25°C	Development of ecdysterone puffs to PS7
Salivary gland cells	3-24h incubation with ecdysterone, 16°C	Changes of puffing patterns till PS10-11 after 16h incubation
Ovarian nurse cells	3-24h incubation with ecdysterone, 16°C	No reaction of polytene chromosome loci to ecdysterone
Salivary gland cells	12h preincubation without ecdysterone and 8h incubation with ecdysterone, 16°C	Induction of late larval puffs (63E, 66B, 78C), induction of late prepupal puff 93F9-10
Ovarian nurse cells	12h preincubation without ecdysterone and 8h incubation with ecdysterone, 16°C	No reaction of polytene chromosome loci to ecdysterone
Salivary gland cells, ovarian nurse cells	6h preincubation without ecdysterone and 6h incubation with ecdysterone, 16°C	Induction of early late ecdysterone puffs, "incubation" puffs and puff 46F in salivary gland polytene chromosomes. No induction of puffs in nurse cell polytene chromosomes
Salivary gland cells, ovarian nurse cells	6h preincubation without ecdysterone, 6h incubation with ecdysterone and 6h preincubation with ecdysterone, 16°C	Occasionally there are "incubation" puffs and puff at 93F9-10. No ecdysterone puffs in salivary gland cells. No puffs in nurse cells

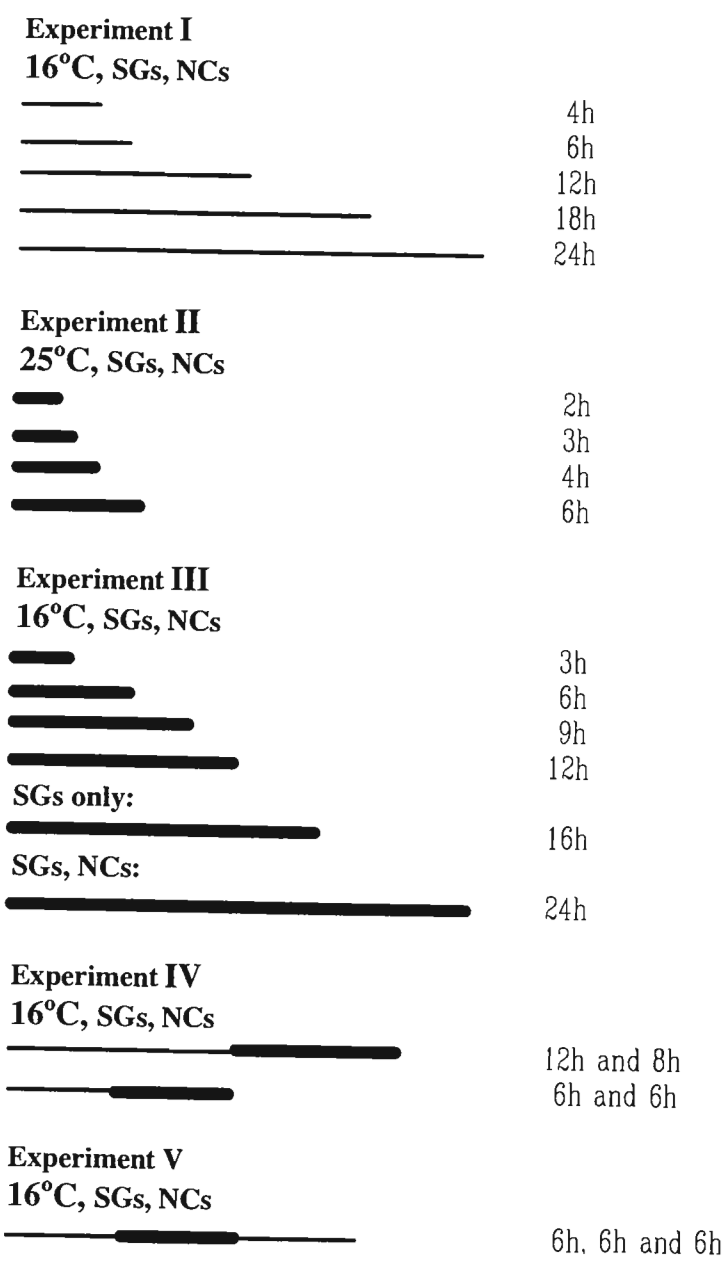


Figure 1. Scheme of incubations of salivary glands (sgs) and ovaries with nurse cells (ncs) at different temperatures. Periods (hours) of incubations with ecdysterone (thick lines) and without (thin lines) are indicated by figures.

In the first experiment, sgs were incubated in Robb's medium without hormone (experiment I of Figure 1). During the 4-24 hour incubation period obvious changes in SG chromosome puff patterns occurred. After the first 6 hour period, all the ecdysterone-stimulated puffs (2B3-5, 46F, 74EF, 75B and 85F) had disappeared. Even after 24 hours incubation *in vitro* the SG chromosomes showed very clear banding patterns and general polytene structure (Figure 2, Table 1). We know that "incubation" puffs sometimes appear on polytene chromosomes when sgs are cultured *in vitro* and that the pattern of puffs observed depends on medium used (Ashburner, 1972; Biyasheva *et al.*, 1985). In our experiments, several new puffs, namely 47D, 50C, 60C and 93E appeared during the 24 hour incubation (Figure 3). The 47D and 50C puffs also appeared in Grace's medium (Ashburner, 1972), but the 60C and 93E puffs are probably specific for Robb's medium.

Except for the development of puffs in the 47D and 50C regions, no significant changes were found in morphology of the NC polytene chromosomes during this incubation period (Table 1).

When sgs were incubated from 2 to 6 hours with ecdysterone at 25°C (experiment II, Figure 1), the development of puffing patterns stopped at PS7, as often happens with SG polytene chromosomes incubated *in vitro*. To overcome this disadvantage, we cultured sgs at a much lower temperature (16°C) for 16 hours and found that the puffing pattern had reached its final stage of development (Figure 4b). This final stage cannot be passed when SG are incubated with ecdysterone (Richards, 1976a,b). No induction of ecdysterone puffs occurred in NC polytene chromosomes during the entire incubation period (Figure 4c).

We thought that perhaps the development of ecdysterone puffs in nurse cells of *otu* mutants might be blocked by some unknown inhibitors. Therefore we preincubated sgs and ovaries in pure medium for 12 hours and then added hormone for 8 hours. In this case, in SG chromosomes, puffs appeared which are specific for late larvae (63E, 66B, 78C). Similar results were obtained after 6h preincubation without hormone and 6h incubation with ecdysterone (Figures 5,6). For unknown reasons, puff 93F9-10 appeared, which normally developed in very late prepupae (Ashburner and Berendes, 1978). No reaction of NC chromosomes to the hormone was found in these experiments.

Changes of ecdysterone concentration are very important during development of ecdysterone puffs in polytene chromosomes of sgs. The high ecdysterone titre followed by its absence can induce a new wave of puffs (Richards,

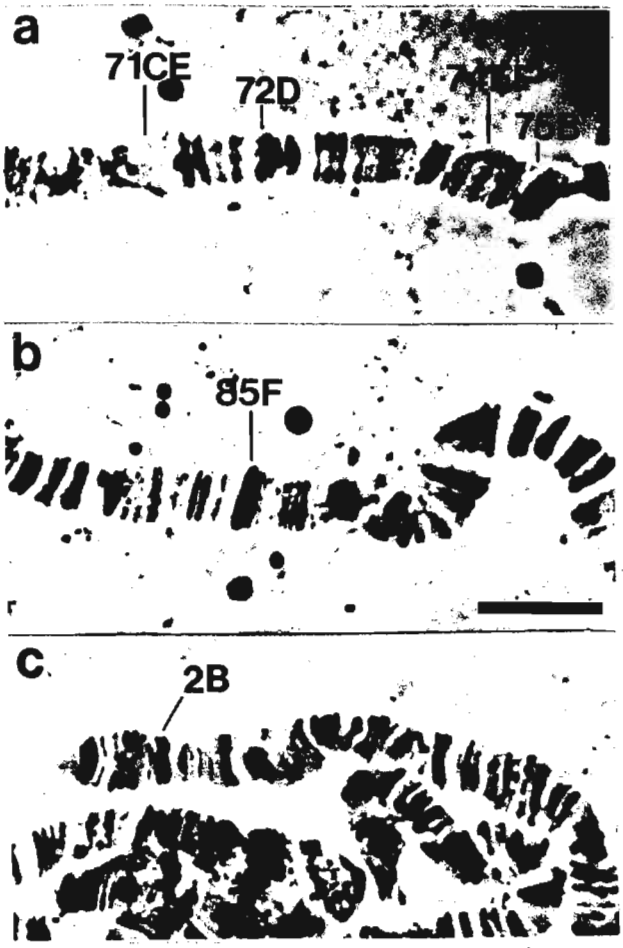


Figure 2 (above). Inactivation of ecdysterone puffs after 24 hour incubation of the salivary glands in Robb's medium without ecdysterone. Bar represents 10mm.

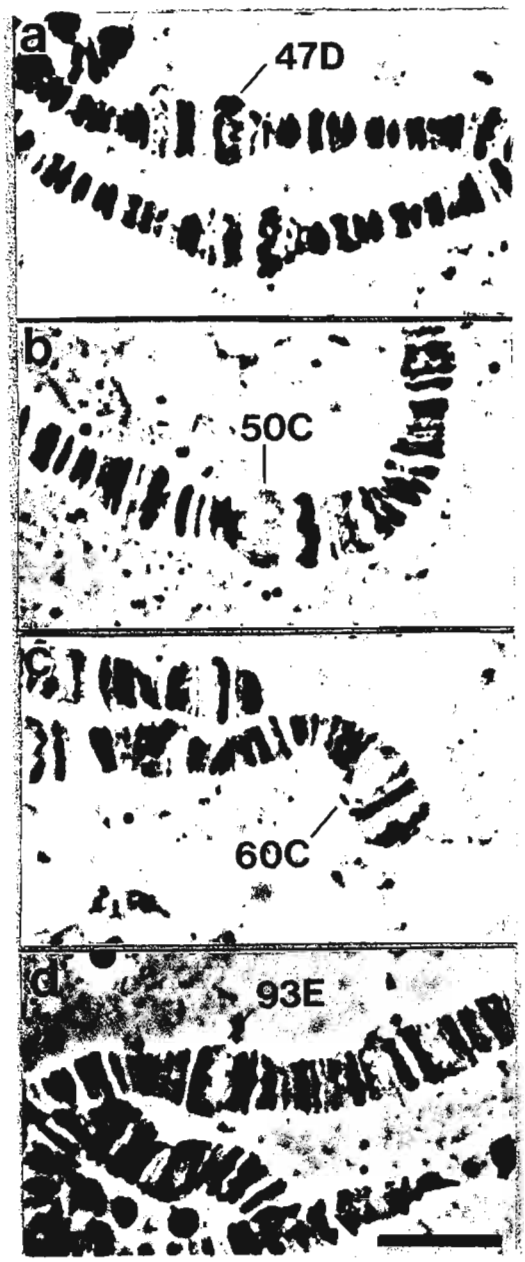


Figure 3 (at the right). Puffs induced during 24 hour cultivation of the salivary glands in Robb's medium without ecdysterone. Bar represents 10mm.

1976a,b). Therefore, we expected that such changes could be useful for puff induction in NC chromosomes. For this reason we performed experiment V (see Figure 1 and Table 1). Again no induced puffs were found in NC chromosomes.

It could be that long incubations *in vitro* will lead to the induction of heat shock puffs, which in turn could block ecdysterone induction. However, in our experiment (Figure 7) we saw only the formation of tiny heat-shock puffs on NC polytene chromosomes mainly in 63B, 67B, 87A and 93D regions. Heino also reports that it is difficult to induce heat-shock response in ncs (Heino *et al.*, 1995).

Our data show that the NC polytene chromosomes do not form puffs in response to ecdysterone, at least under the experimental conditions described above.

Acknowledgments: Authors are indebted to Drs. G. Richards, E.S. Belyaeva and R.C. King for valuable advice, to Dr. J. Fristrom for his gift of Robb's medium, and to I.P. Selivanova for her technical assistance.

References: Mal'ceva, N.I., H. Gyurkovics, and I.F. Zhimulev 1995, Chrom. Research 3: 191-200; Mal'ceva, N.I., E.S. Belyaeva, R.C. King, and I.F. Zhimulev 1997, Dev. Genet. 20: 163-174; Ashburner, M., 1972, Chromosoma 38: 255-281; Biyasheva, Z.M., E.S. Belyaeva, and I.F. Zhimulev 1985, Chromosoma 92: 351-356; Richards, G., 1976a, Develop. Biol. 54: 256-263; Richards, G., 1976b, Develop. Biol. 54: 264-275; Ashburner, M., and H.D. Berendes 1978,

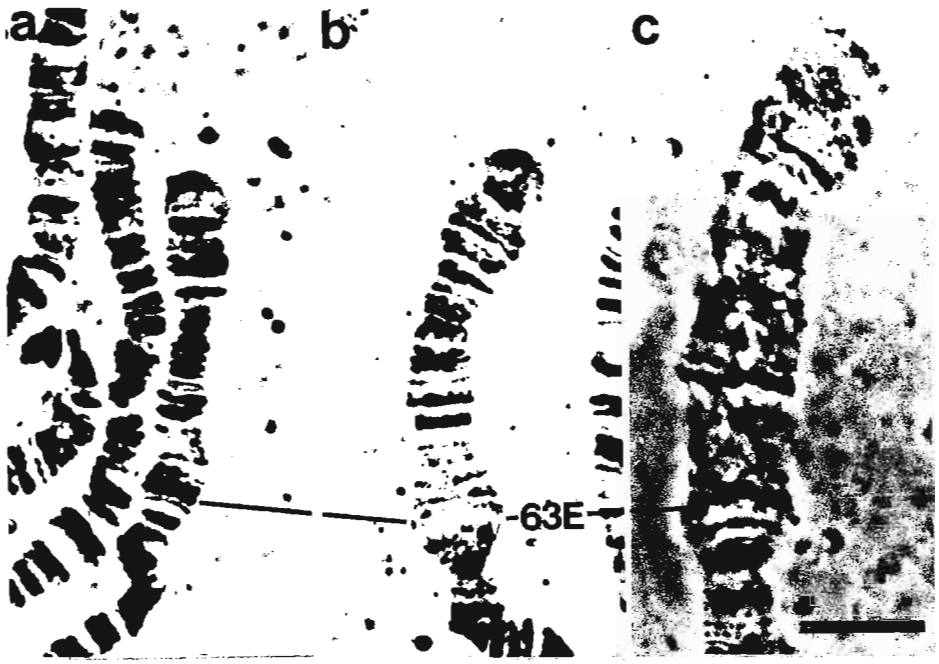


Figure 4. Morphology of the SG 3L polytene chromosome after 24 hour incubation without ecdysterone (a), 16h incubation with ecdysterone (b), and after 24 hour incubation of NC with ecdysterone (c). Bar represents 10mm.

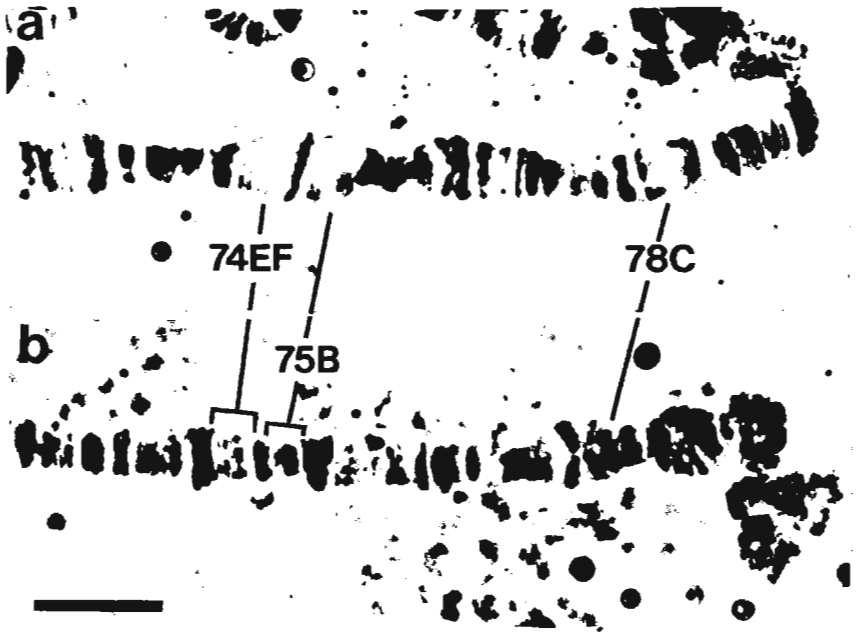


Figure 5. Puffs in 74EF - 75B and 78C regions of SG (a) and NC (b) polytene chromosomes, after 6 hour incubation at 16°C without ecdysterone and then 6 hour incubation with hormone. Bar represents 10mm.

In: *The Genetics and Biology of* Drosophila, (ed., M. Ashburner and T.R.F. Wright), 2b: 316-395. Academic Press, London; Zhimulev, I.F., 1994, Chromomeric Organization of Polytene Chromosomes. 565 p. Nauka, Novosibirsk; Heino, T.I., V.P. Lahti, M. Tirronen, and C. Roos 1995, Chromosoma 104: 44-55.

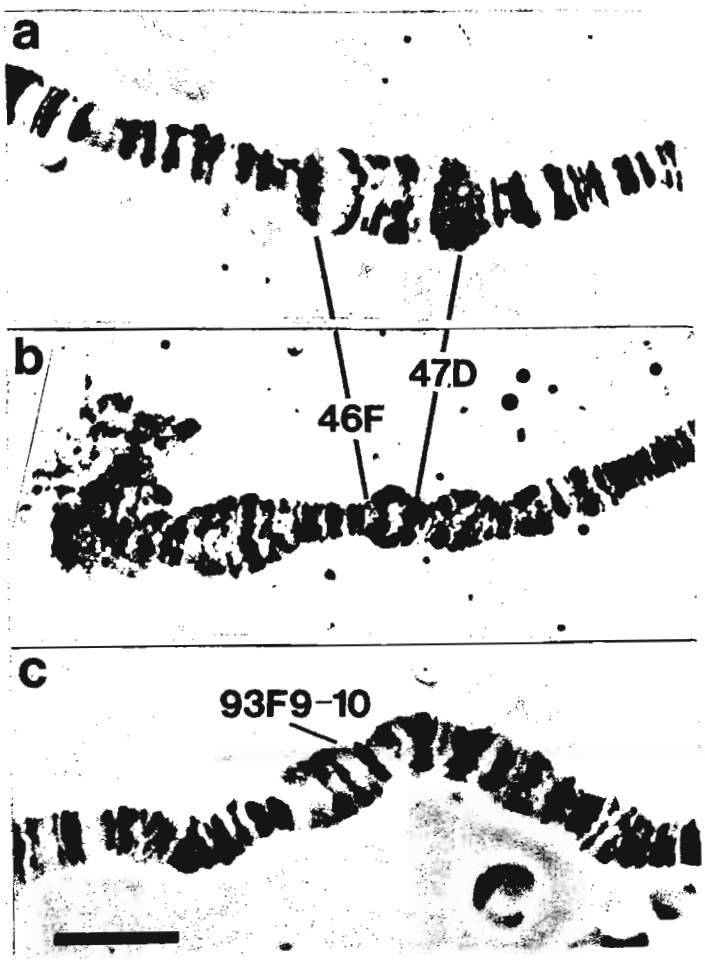
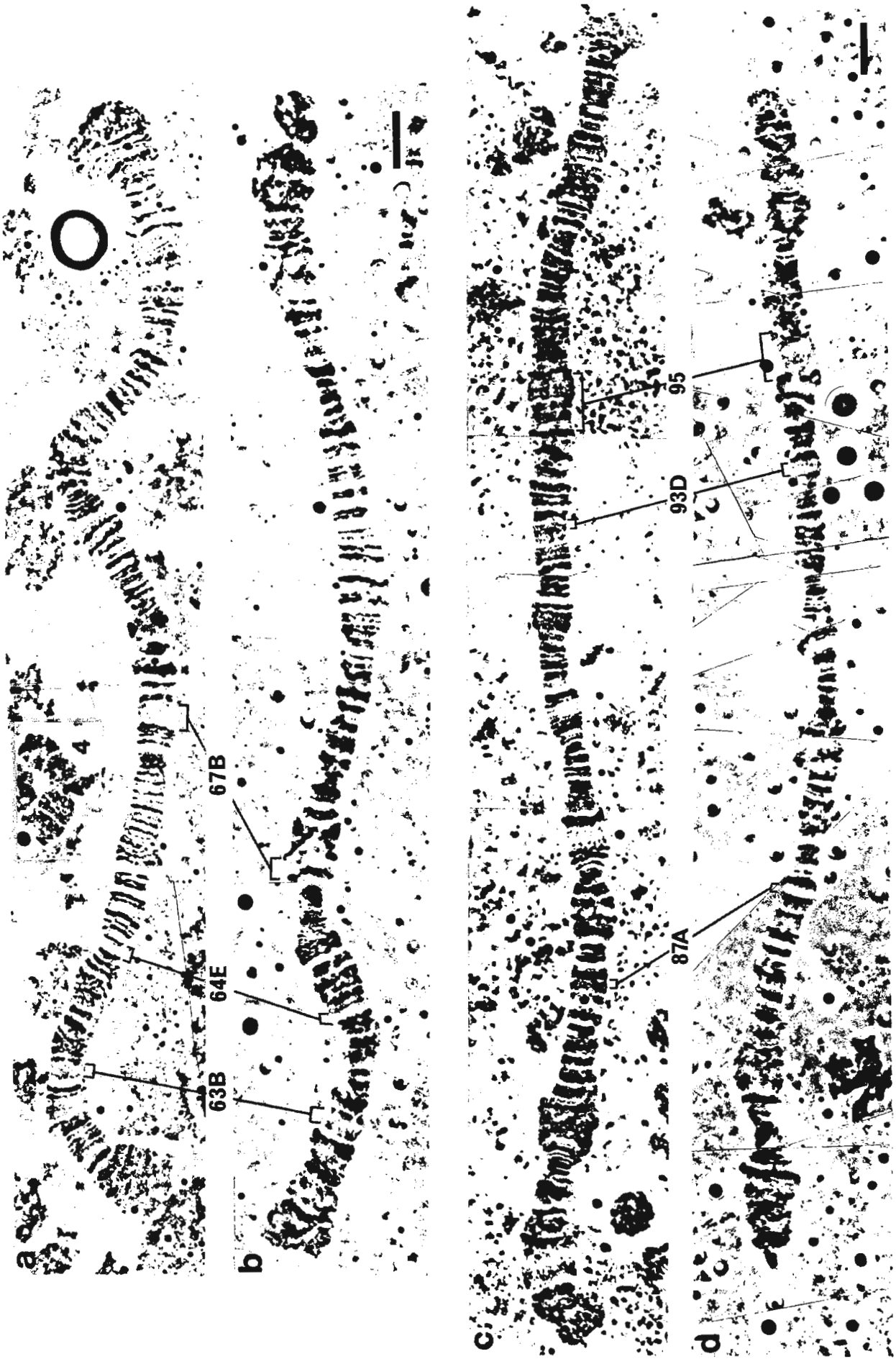


Figure 6. Induction of puffs in 46F and 47D regions in SG (a) and NC (b) after 6 hour incubation in Robb's medium without ecdysterone and 6 hour incubation with hormone. (c) - Puff in 93F9-10 region which appeared after subsequent incubations of SG for 6 hour in Robb's medium without ecdysterone, 6 hour with ecdysterone and 6 hour without ecdysterone. Bar represents 10mm.

Figure 7 (next page). 3L and 3R NC polytene chromosomes in control (a and c) and after 30 min at 37°C (b and d). Bar represents 10mm.


a
63B
64E
67B
b
c
87A
93D
95
d

Rashkovetsky, Eugenia, A. Korol, T. Pavlicek, and E. Nevo. Institute of Evolution of Haifa, Mount Carmel, Haifa 31905, Israel, e-mail: korol@esti.haifa.ac.il Mate-choice in *D. melanogaster*: incipient premating sexual isolation in ecologically contrasting slopes at "Evolution Canyon".

A remarkable microscale differentiation has been established in a *D. melanogaster* natural population at "Evolution Canyon", Lower Nahal Oren, Mount Carmel (Israel) (Nevo, 1997a,b; Nevo *et al.*, 1997; Derzhavets *et al.*, 1996a,b; 1997). The studies were conducted on isofemale lines set up from flies of the opposite slopes of the Canyon and maintained in normal laboratory conditions for 1-2 years. The measured traits included viability and longevity changes caused by short-term and lifetime temperature treatments, changes in fly weight due to desiccation/starvation treatments at different temperatures, fluctuating asymmetry test of wing scores, and rates of mutation and recombination. The obtained evidence leads to the conclusion that strong microclimatic natural selection can override migration and generate a complex slope-specific adaptive syndrome contributing to fitness at a microsite.

The foregoing tests also showed (both for *D. melanogaster* and *D. simulans*) that adaptation to contrasting ecological conditions of the opposite slopes has resulted in strong genetic divergence for habitat choice (Nevo *et al.*, 1997). The direction of the revealed interslope difference in the oviposition preferred temperatures is exactly the one expected in accordance with habitat selection and habitat choice models (Hedrick, 1990). Theoretical models predict that selection in systems with spatially heterogeneous selection can bring about a genetically determined reduction in the migration rate (Wiener and Feldman, 1993). In view of that, our results established a promising natural model to bridge theory and evidence in an in-depth analysis of behavioral adaptation under heterogeneous stressful conditions (Parsons, 1993) including the interactions between selection, migration, and habitat choice. This model is one of several model organisms currently studied at the "Evolution Canyon" research program.

Inter-population geographical isolation (allopatry) may promote the evolution of sexual isolation as a first step of speciation (Dobzhansky and Pavlovsky, 1957; Mayr, 1963). Incipient sexual isolation among geographic races of the same species has been reported in a number of studies (*e.g.,* Wu *et al.*, 1995; Noor, 1995; Wade *et al.*, 1995). Habitat choice caused by strong microsite ecological-genetic differences can also develop a tendency toward within-population reproductive isolation (sympatry). However, the model of sympatric speciation is by far more controversial than the allopatric model and suffers from scarce evidence in natural populations. Here we report the results of some pilot tests on sexual behavior conducted on *D. melanogaster* genotypes derived from subpopulations of "Evolution Canyon" that display sharp adaptive divergence over a short distance, of 200 m, presumably resulting from the contrasting interslope ecological differences.

The experiments were conducted on two specially designed testing groups (one per slope) that have been constructed by crossing 25 isofemale lines from each slope. The lines were derived from inseminated females caught at midslope stations of the opposite slopes of the Canyon and maintained in normal laboratory conditions for about 2 years (see Nevo *et al.*, 1997). To establish the testing groups, 10 males and 10 virgin females of each line were crossed and then maintained under random mating for 12 generations. We conducted three tests, aimed to examine (*i*) mate choice, (*ii*) dynamics of mating pair formation, and (*iii*) mating speed and copulation duration.

Mate-choice: The first test aimed to measure biases in choices of males 'exposed' to females of their own and opposite slopes. The experiments were conducted in a special apparatus made of 2 mm plexiglass. The apparatus included 3 compartments. The main chamber (11×11×11 cm) and offshoots (3×3×10 cm) were separated by thin nylon nets, permitting flies to see and smell each other, but not to cross the nets. Virgin flies used for the test were aged for 7 days after eclosion. Fifty tested males were introduced without etherization into the main chamber of the apparatus; simultaneously, 25 females of the testing group of the more stressful warm and xeric south-facing slope (SFS) were introduced into one offshoot and 25 females of the testing group of the cool and mesic north-facing slope (NFS) into the other one. Direct observations of males choice were made over a period of 60 minutes. The number of males interested in females from one or another offshoots was recorded (Table 1).

Males of both testing groups discriminate against females of the groups of the opposite slopes. Out of 50 males from the NFS-group 70.4% were oriented to females from their own slope and only 46.4% to SFS-females. Similarly, 49.6% of males from SFS preferred females of their own origin and only 26.8% - females from NFS (note, that the sums of these frequency indices in some combinations are more than 100%, because males were often interested not only in females of one sample, but also in a second-batch female). The differences between the number of homo- and heteroslope choices (D = homo-hetero) were highly significant and rather similar for males of both slopes: for SFS males $D = 11.4$, Student's test $t = 6.69$, df = 8, $P < 0.001$); for NFS males $D = 12.0$, $t = 5.59$, df = 8, $P < 0.001$). Thus, with respect to sexual activity, males of both slopes demonstrated a bias toward females of their own origin..

According to the data in Table 1, males of the SFS-group evolved a lower level of sexual activity than NFS-males. This difference is manifested when comparisons d = NFS-SFS are made for both homo- and heteroslope choices. The interslope difference (d) of the mean scores was: for homoslope choice $d = 10.4$, $t = 4.61$, df = 8, $P < 0.01$; for heteroslope choices $d = 9.8$, t = 6.31, df = 8, $P < 0.001$. These results fit the generalization of a reduced metabolic rate and energy-saving behavior in populations adapted to stressful environments (Parsons, 1993).

The dynamics of mating pairs formation: In order to evaluate the dynamics of mating pairs formation, females and males of each testing group were mated in all possible combinations (referred to as homo- and hetero-crosses) in a mating chamber. For each cross, the mating experiment was conducted in three independent blocks, each with 25 virgin females and 25 males. Number of couples was recorded at 5-min intervals for an hour. The greatest number of mating couples was recorded in the homo-cross (SFS×SFS) after 15-20 minutes from the beginning of the experiment; here matings were virtually finished during the first 30 min. The second homo-cross (NFS×NFS) manifested a slower dynamics of pair formation. The greatest number of mating pairs was recorded after 25-30 min and the maximum number of couples per registration was lower. Nevertheless, these differences did not reach the border of significance, hence further tests are needed.

Mating speed and duration of copulation: In this test, 25 individual pairs were observed for each of the four possible crossing combinations (SFS×SFS, NFS×NFS, SFS×NFS and NFS×SFS). The foregoing two parameters were registered during the first 120 min from the beginning of the test. The pairs that did not mate during this period were recorded as 'non-mated' and were not taken into account. In order to get 25 mated couples, the test was continued with additional pairs. The obtained estimates indicated a significantly higher mating speed in intraslope matings than that in interslope ones, *i.e.* a positive assortative mating, especially strongly manifested by NSF females (Table 2).

Table 1. Male mate choice: mean number of males registered on the nets of the offshoots with females of their own and opposite slopes.

	Females	
Males	SFS	NFS
SFS	24.8 ± 1.53	13.4 ± 0.75
NFS	23.2 ± 1.36	35.2 ± 1.66

Table 2. Mating speed and copulation duration (in min).

		Males	
Female	trait	SFS	NFS
SFS	mating speed	11.8 ± 0.86	14.3 ± 0.81
NFS		20.0 ± 0.78	8.6 ± 0.92
SFS	duration of	19.6 ± 0.51	21.3 ± 0.49
NFS	copulation	18.1 ± 0.58	23.6 ± 0.57

In both types of crosses (homo- and hetero-), males attempted to court immediately, showing no significant differences in their courtship. Mating success and, therefore, mating speed are influenced, to a large extent, by the level of females' receptivity. The results suggest that with respect to homogeneous mating, females of the SFS-subpopulation have evolved a decreased receptivity to their own males as compared to NFS-females (11.8 versus 8.6 mean scores, t = 2.54, df = 48, $P < 0.05$). This fits the generalization of a reduced metabolic rate in natural populations adapted to stressful environments (Parsons, 1993). The foregoing data on heterogeneous crosses imply that interslope population differentiation brought about a highly significant mating selectivity of NFS-females in favor of males of their own origin (compare the mean scores 20.0 and 8.6 for hetero- and homo-crosses, $t = 9.45$, $P < 0.001$). The same, but less pronounced, trend is exhibited by SFS-females ($t = 2.12$, $P < 0.05$). In accordance with the results on mating speed, NFS flies manifest also significantly longer copulation in homogeneous crosses than do SFS flies ($t = 5.23$, $P < 0.001$). Likewise, a highly significant reduction in copulation duration was observed in the heterogeneous cross NFS (females) × SFS (males) as compared to the homogeneous one, NFS (females) × NFS (males) ($t = 9.44$, $P < 0.001$). However, an opposite although less significant trend was observed in the heterogeneous cross SFS (females) × NFS (males) ($t = 2.40$, $P < 0.05$). With the exception of the last difference, all results of the third experiment correspond to the assumption of non-random mating behavior of the tested populations with a tendency to positive assortative mating.

The foregoing results allow us to suggest that population differentiation at "Evolution Canyon" caused by strong interslope ecological contrasts has resulted in an incipient premating sexual isolation system, in spite of the extremely small distance between the slopes. Further tests are needed for an in-depth study of this evolving sympatric isolation system.

References: Derzhavets, E., A. Korol, and E. Nevo, 1996a, Dros. Inf. Serv. 77:92-94; Derzhavets, E., E. Nevo, and A. Korol, 1996b, Dros. Inf. Serv. 77:124-126; Derzhavets, E., A. Korol, and E. Nevo, 1997, Drosophila Information Service, this issue; Dobzhansky, Th., and O. Pavlovsky, 1957, Nature 23:289-292; Hedrick, P.W., 1990, Heredity 65:145-149; Mayr, E., 1963, *Animal Species, and Evolution*, Harvard Univ. Press, Cambridge (Mass.); Nevo, E., 1995, Proc. R. Soc. Lond. B 262:149-155; Nevo, E., 1997a, Theor. Pop. Biol. (in press); Nevo, E., 1997b, Annu. Plant Genet. Newsletters (in press); Nevo, E., E. Rashkovetsky, T. Pavlicek, and A. Korol, 1997, Heredity, in press; Noor, M.A., 1995, Nature 375:674-675; Parsons, P.A., 1993, Biol. Rev. 68:313-333; Wade, M.J., N.W. Chang, and M. McNaughton, 1995,

Heredity 75:453-459; Wiener, P., and M.W. Feldman, 1993, Evol. Ecology 7:251-269; Wu, C.-I., H. Hollocher, D.J. Begun, C.F. Aquadro, Y. Xu, and M.-L. Wu, 1995, Proc. Nat. Acad. Sci. USA 92:2519-2593.

Gaining Access to the BIOSCI/bionet.DROSOPHILA Newsgroup

This information was provided by Dave Kristofferson, BIOSCI/bionet Manager.

The BIOSCI/bionet DROSOPHILA newsgroup can be accessed on USENET by reading bionet.drosophila using your local USENET newsreader or on the World Wide Web at

http://www.bio.net/hypermail/DROS/.

The Web address includes access to the complete newsgroup archive going back to the group's beginning in July 1993. If your browser is configured to send e-mail, you can also post to the newsgroup via this Web site.

For readers who prefer to use e-mail exclusively, there are two sign-up procedures based on your location.

For users in the Americas and Pacific Rim countries, address a message to biosci-server@net.bio.net and include the line

subscribe dros

(NOT "subscribe drosophila" as printed in DIS 77)

in the *body* of the message. Anything put on the Subject: line will be ignored. To cancel your subscription repeat the same procedure using the

unsubscribe dros

command. Your address on the From: line of your mail message must match the address on the list for this to work. Please address any problems to biosci-help@net.bio.net for technical assistance.

For users in Europe, Africa and Central Asia, address a message to mxt@dl.ac.uk and include the line

sub bionet-news.bionet.drosophila

in the body (not the Subject:) of the message. To unsubscribe send in the command

unsub bionet-news.bionet.drosophila

Technique Notes

Lindsley, D.L. and L.M. Wickline. Department of Biology, University of California, San Diego, La Jolla, CA 92093. A *Y* chromosome duplicated for salivary regions 14A, B, F, and 15, and chromosome 4, and carrying, among other normal alleles, y^+ and f^+.

In investigations of lethals in regions 14 and 15 of the *X* chromosome, lethal-bearing males are kept in combination with $Dp(1;4)r^+$ or derivatives thereof. In order to follow this duplication, which carries f^+, in crosses, all other alleles of *f* must be mutant. As soon as there is a second f^+ element in the cross, one loses track of the duplication, and its presence in flies must be determined by individual test crosses. This problem plagued us in a mapping exercise involving lethal mutations in regions 14A and B; accordingly, we decided to append a derivative of *Dp(1;4)r+* to the *Y* chromosome so that the regular segregation of the Y could allow us to follow the duplicated segment of the *X*. The derivative used was a *Df(1)81h24b,* a deletion of *para* generated by D. Falk; $Dp(1;4)r^+$ extends from 14A2 through 16A7; *Df(1)81h24b* deletes material between 14B9 and 14E.

Females carrying $C(1)DX, y\ w\ f/B^S Y y^+/Dp(1;4)r^+$ were irradiated with 4000 rads of X rays and crossed to *y cv v f* males. Progeny which had lost one of the terminal Y markers and had retained f^+ were selected and crossed to *C(1)DX, y w f/Y* sisters. Of 70*cv v* sons (i.e., which had lost B^S), 12 were fertile (the remainder had presumably lost more of *YL* than its terminal euchromatic marker); of these only one proved to have f^+ appended to a fertile *Y*; in the remainder of cases, f^+ continued to segregate at random with respect to y^+ on the *Y*. Of 24 *y cv v* B^S males recovered, five were fertile and in every case f^+ segregated at random from B^S.

The new order of the duplicated *Y* is as follows:
1A|14A2—14B9|14E—16A7|102F2—101A|YL.YS
We designate this derivative *Dp(1;4;Y)81h24b.*

Kotliarevski, Deema Israel. Laboratory of Optimal Aerosol Application of Pesticides. Institute of Chemical Kinetics and Combustion. 630090 Novosibirsk. Russia. e-mail: naber@kinetics.nsk.su. The simplest low-cost medium for rearing *Drosophila melanogaster*.

The author has developed a very simple and cheap rearing medium for the fruit-fly, *Drosophila melanogaster*.

The medium consists of 30% oat flour and 70% water. The flour is put in the rearing tubes, then water is added. The tubes are closed with cotton plugs and are placed in the boiling water-bath for 45 minutes. After cooking these tubes are dried a little in a drier (80-100°C for 20-30 minutes), then they are cooled in the drier to room temperature. This medium was compared with a common medium consisting of agar, yeast, sugar and semolina. Three species of *Drosophila* were taken in the experiment including wild-type and four mutant lines of *Drosophila melanogaster* (kindly given by Dr. B.F. Tchadov from the Institute of Cytology and Genetics, Novosibirsk). The mutant lines were reared on the medium for at least ten generations, and no deviations from the norm (as compared with the common medium mentioned above, which served as a control) in dimensions, development and fecundity were observed. The proposed medium is at least ten times less expensive than the common medium and is free of expensive agar, sugar and yeast (and it is not inoculated with yeast). It is dense enough for flies. The residues of the medium are removed (during washing tubes) not so easily as in the case of agar-containing media, but the problem is solved by mechanical washers. Also the medium is proposed composed of 5% oat flour and 0.8-1% agar.

Wickline, L.M., and D.L. Lindsley. Department of Biology, University of California, San Diego; La Jolla, CA 92093. Construction of a *sog* ^+Y.

We have generated a male-fertile *Y* chromosome, marked with y^+ and B^S, which carries a normal allele of *sog*: short gastrulation (at 13D/E). This duplication sog^+Y was made by irradiating a recombinant between the proximal element of *T(1;Y)B32* and *C(1;Y)XYL*YS129-16* (see Figure 1). A stock was generated in which males carried this recombinant compound chromosome. Such males were irradiated with 4000 rads and crossed to various free-*X*-bearing females. In

males carrying a *T(1;Y)* and no free *Y*, the two elements of the translocation segregate from one another regularly (Nicoletti and Lindsley, 1960). So, from this cross, the only viable progeny were those in which the majority of the X euchromatin had been deleted from the translocated element. Of these surviving progeny, B^S males were selected. These males were then tested for fertility and for the ability of the duplication to cover *sog*.

Of 219 B^S males recovered, only one was both fertile and able to cover *sog*. This duplication does not cover *sd*, *exd* or *baz*.

This $Dp(1;Y)B^S sog^+ y^+$ has been used to cover the recessive lethality of *sog* so that complementation tests between alleles could be completed, as well as to simplify various crossing schemes involving *sog*.

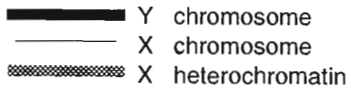


Figure 1a

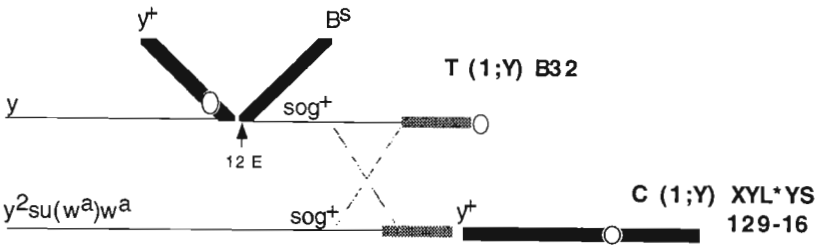
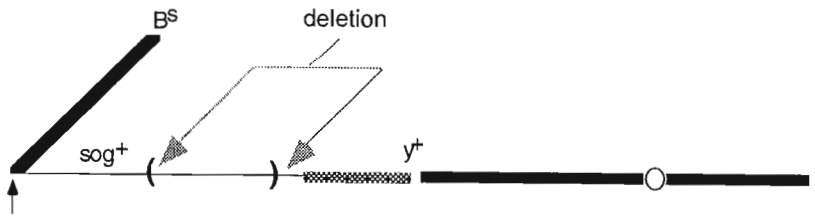


Figure 1b



References: Brosseau, G.E., B. Nicoletti, E.H. Grell, and D.L. Lindsley 1961, Genetics 46: 339-346; Nicoletti, B., and D.L. Lindsley 1960, Genetics 45: 1705-1722.

Eisses, K.Th., and M. Santos. Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, 08193 Bellaterra (Barcelona), Spain. E-mail: IBGF2 or IBGF1@cc.uab.es. Easy and reliable distinction between females of *Drosophila melanogaster* and *Drosophila simulans* from a Spanish population based on abdominal pigmentation patterns.

Since the discovery of Sturtevant (1919) that *Drosophila melanogaster* has a closely resembling sibling species *D. simulans*, both species turned out to be cosmopolitan and coexistent (Lachaise *et al.*, 1988). The two species are mainly distinguished by checking the male offspring of isofemale lines because of different genital arches (Coyne, 1983; Shorrocks, 1972). Based on measurements of eye sizes of *D. melanogaster* and *D. simulans* it is possible to make a distinction between the females (Burla, 1951; Gallo, 1973; McNamee and Dytham, 1993) but it is a painstaking job when large numbers of flies have to be examined. A high number (up to 45%) of misqualifications of *D. melanogaster* have been reported, based on different eye size definitions (McNamee and Dytham, 1993 and references therein). Based on a paper by Gallo (1973) we decided to examine whether morphological distinction through differences in abdominal pigmentation patterns was applicable in our population of *D. melanogaster* and *D. simulans*.

The apparent *D. melanogaster* females trapped in a semi-abandoned *Opuntia ficus-indica* plantation in Carboneras (Almería, Spain; 37°00'51"N; 1°53'33"W) were separated by eye size only from *D. simulans*, whereas the flies trapped in other locations nearby were separated at species and sex level by eye size and genital arches. Simultaneously we checked whether or not *D. simulans* female eye size correlated with a distinguishable pigmentation pattern. All flies deemed *D. melanogaster* were subjected to electrophoresis on hydrolyzed potato starch (Poulik, 1959) and stained for alcohol dehydrogenase (ADH; EC 1.1.1.1.), which is a diagnostic enzyme between *D. melanogaster* and *D. simulans* (Eisses *et al.*, 1979). The number of misqualifications of *D. melanogaster* was calculated (Table 1 A). Almost 21% of the female flies turned out to be *D. simulans* (Table 1 A 1). For females and males together a general misqualification of 11% was obtained (Table 1 A 2).

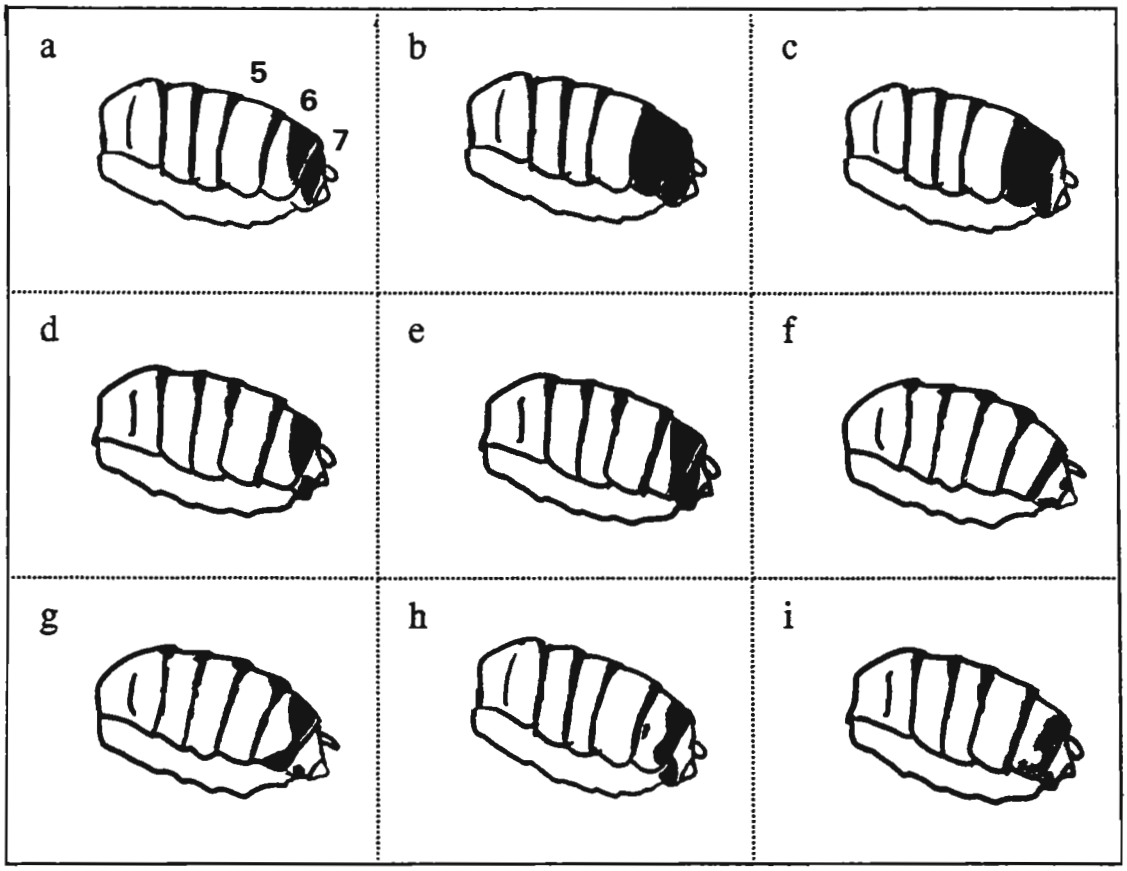


Figure 1. Pigmentation patterns of the 6th and 7th tergite of *D. simulans* (a) and *D. melanogaster* (b - i). Within *D. melanogaster* variation is present in the natural population and in homozygous or isogenic laboratory strains Groningen-SSN (b) and Groningen-FFF (d). None of the *D. melanogaster* strains was monomorphic.

Table 1. Number of misqualifications of *D. melanogaster* based on electrophoresis of flies trapped in banana baits in a semi-abandoned *O. ficus-indica* plantation (A-1) and other locations close to Carboneras (A-2), and of flies emerging from *O. ficus-indica* fruits collected at the plantation (B).

	Method of distinction	Total number of flies	% *D. simulans*
A1	Eye size	226 females	20.8
A2	Eye size/Genital arch	437 females/males	11.0
B	Pigmentation of 6th tergite and eye size	1078 females	1.68 ± 0.26*
	Genital arch	1092 males	0.64 ± 0.03*

* Empirical Standard Deviation

The large number of flies emerging from *Opuntia ficus-indica* fruits (prickly pears) collected in the Carboneras plantation were separated primarily by examining the pigmentation pattern of the sixth tergite and in cases of doubt the eye size was examined as well. Approximately equal numbers of female and male flies have been checked by each of us. After electrophoresis the

average percentage of misqualifications of the females was calculated to be 1.68% ± 0.26. This experimental error is in the same order as misqualifying male flies (Table 1 B), and mainly due to large sample sizes and time pressure.

The most important difference between *D. simulans* and *D. melanogaster* is the black pigmentation which runs to the ventral margin in *D. melanogaster* in various patterns (Figure 1 b,c,e-i), whereas the pigmentation border line in *D. simulans* makes an angle with the tergite margin. It forms a continuous line with the pigmentation border line in the seventh tergite (Figure 1 a). The abdominal pigmentation pattern with respect to the sixth tergite was found to be monomorphic in *D. simulans* in our population, and in at least two other world-wide different populations (Brasil: Gallo, 1973; USA: Thompson *et al.*, 1979) in contrast to *D. melanogaster* (David *et al.*, 1990; Robertson *et al.*, 1977; this paper). The seventh tergite in *D. simulans* was almost completely pigmented but for a small area adjacent to the sixth tergite and its ventral margin (Figure 1 a). However, like in *D. melanogaster*, some variation existed in the pigmentation pattern of the seventh tergite of *D. simulans* with only small spots like those of *D. melanogaster* in Figure 1 g. Robertson *et al.* (1977) described the locus *fap* (female abdomen pattern) to be residing on the extreme tip of the 3L chromosome, with some effects related to chromosome four. The *D. melanogaster* Groningen-FFF strain, used as a reference in electrophoresis, showed a pigmentation pattern in the sixth tergite like *D. simulans* (Figure 1 d), but in contrast the seventh tergite was pigmented differently. This strain was homozygous for Adh^F and $\alpha Gpdh^F$ on the second chromosome and Odh^F on the third chromosome. This might be the reason why the Groningen-FFF strain with some homozygosity on the third chromosome was different from the other *D. melanogaster* strains.

Acknowledgments: Montse Peiró is thanked for doing part of the electrophoreses and Albert Kamping (University of Groningen) for providing the Groningen reference strains of *Drosophila melanogaster*. This work was funded by Contract No. CHRX-CT92-0041 from the Commission of the European Communities.

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Szakmary, A. Laboratory of Molecular Genetics, NIEHS, NIH, P.O.Box 12233 Research Triangle Park NC 27709. A short guide to scoring mosaic cell clones in the *Drosophila* eye.

Somatic Mutation and Recombination tests are quick, simple and easy to perform. Because of their versatility more and more researchers in different fields use them for different purposes. Several different systems have been developed. Some use bristles and color on the body (*e.g.*, markers *y* and *sn*) or trichomes on the wings (markers *mwh* and *flr*). Mosaicism in the eye allows a lot of variation due to the large number of possible genetic markers. Still the best way to learn to identify mosaic clones correctly is to visit a laboratory where it is done routinely. This short guide is intended to help when eye mosaicism should be used and a visit is not possible. Photographic examples will be made at the image section of FlyBase.

We use a regular dissecting microscope (binocular) at a magnification of 35-75×. The lights are common light boxes with swan neck point lights. Position the lights on the same side giving you light and shade but minimum reflection. Adjust brightness to whatever feels comfortable over a long time period.

Scoring mosaic clones is certainly subjective; therefore, consistency is more important than absolute accuracy. One rule of thumb is, if you are in doubt whether you have a mosaic clone exclude it. When scoring do record spot sizes, *i.e.*, number of mutant ommatidia in a clone. This can give you additional information, *e.g.*, age of larvae at time of treatment, and serve as an additional control, because the distribution over size classes (2, 3-4, 5-8, 9-16, 17-32 etc.) should fit a Poisson-distribution (often only one-sided). However, factors such as metabolic activation or stability of the mutagen can distort it.

The flies are scored initially in a solution of 90% EtOH, 9% water, 1% Tween20. Once the flies are dead, this solution can be replaced by 2% aqueous Tween20. I prefer dark, matte tiles because they are easier on the eyes. Focus on the highest points of the eyes and bring other parts into focus by lifting the plate on one side. Position and move the flies with a thin but stiff brush. Turning the flies from light into the shadow and back will reveal mosaic clones that would not be visible in direct bright light. The shape of ommatidia and spots is important to distinguish them from artifacts like chemical burns or developmental disturbances. Because ommatidia are hexagonal in shape small spots have typical shapes of multiple hexagons. The color of spots depends not only on genotype but also on the size of the spots in the case of the white gene as marker in a wild-type background only large mosaic clones appear white. The clones are actually

pigmentless and transparent. Small clones, therefore, appear dark like a tunnel entrance. Other kinds of spots can be clones of other genotypes like roughest or chocolate or artifacts as mentioned before and should be excluded. Insect eyes do contain hairs that in the solution sometime stick together and appear like small spots, changing the angle will help to detect this. Two mosaic clones should be scored as single event if they are separated by 4 normal ommatidia or less and as two independent events if separated by more. Spots larger than 64 ommatidia are rare and can be categorized rather than their size accurately determined (as A = 64-128, B = 128-256, C = half eye, or D = full eye). They should be excluded from mean spot size calculations. Over time more dust and other debris accumulates on the eyes which can be mistaken for a mosaic clone. Brushing over the spots, therefore, becomes important. The age of the flies scored can be important too. 3-4 day old flies are usually best.

Mosaic clone frequencies are generally given as mosaic clones per 100 eyes scored. Other frequencies, *e.g.*, mosaic clones per eye or eyes with clones per eyes scored, are generally less useful. Ideal sample sizes depend very much on the purpose of the experiments and the mosaic clone frequency. To classify a chemical as non-mutagenic, a minimum of 2000 scored eyes will be necessary, while clone frequencies clearly above control level can be significant with much less. I often score till reaching 100 mosaic clones in experiments comparing different genotypes with a standard mutagen. Historic controls are useful to observe stocks over a longer period of time but cannot replace concurrent controls (equal in size to treated groups). However, clone frequencies that do not exceed the highest values in the historic controls should not be considered positive no matter how low the concurrent control frequency is (for statistics see Frei and Würgler, 1995). The variations between repeats can be fairly large compared to other types of experiments due to slight variations in age of cultures at time of treatment or feeding behavior.

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Crisp, Jonathan, and John Merriam. Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA 90095-1606. Efficiency of an F1 selection screen in a pilot two-component mutagenesis involving *Drosophila melanogaster* misexpression phenotypes .

Introduction: One of the main goals of genetic research concerns the identification of genes and their role in development. The most common method for determining the significance of a gene involves the loss of function approach in which the result of gene inactivation (deletion, mutation, etc.) is observed. In contrast, the gain of function approach utilizes misexpression of a genetic region or putative gene in order to recognize new genes. The idea used here is to hop around a P element with the promoter and Gal4-UAS of pUAST (Brand and Perrimon, 1993) and expose new insert sites to Gal4 regulation. In this way, a Gal4-dependent phenotype will result if the P element lies close enough to an endogenous gene and if the endogenous gene protein has an effect on the cells expressing Gal4.

For this purpose a P element construct was made with the promoter and UAS of pUAST located at the 5' end of the construct oriented to transcribe leftwards away from the P element (Merriam, Harrington, Merrill, Phillips, Warden, Martin-Blanco, Nygren and Poole, in prep.). This new construct carries a genomic y[+] sequence as its marker; it was transformed and an X linked strain (1-37) selected for further studies. This strain, 6.11 #4, is available from the Bloomington stock center.

Initial experiments with this (responder) strain recovered 730 exceptional y[+] sons of independent origin from 2540 fathers cultured separately. When crossed to females from a strain carrying the sca-Gal4 driver (y w; {w[+], sca::Gal4}/CyO virgins), 102 (13.8%) of the cultures showed a Gal4-dependent phenotype in the y+ w+ progeny.

In this paper a pilot two-component mutagenesis involving misexpression phenotypes was performed in which the steps traditionally used to produce the F1 generation were combined with a F2 screen. This screen has been termed an F1 screen; it enabled selection of phenotypes to be applied directly to the F1 generation instead of the F2 generation. The questions asked here are whether the F1 screen will find the same number of "keeper" inserts as the F2 screen and how much savings in time are realized.

Materials and Methods: The pilot two-component mutagenesis performed in the F2 screen involved females with the responder P element located on the X chromosome (Figure 1), which have grey bodies due to the y+ gene insert on the P element and white eyes (yw[UAS, y+]/yw[UAS, y+]) (ref Merriam, *et al.*, unpublished). The males have wild type red eyes and kinked bristles linked to the 2-3 transposase gene (Ki 2-3/Ki 2-3). Once this cross is performed, the offspring will have both the responder P element and transposase, creating a situation in which the P element can mobilize ("hop") to new, random locations. The males of this offspring (yw[UAS, y+]/Y; Ki 2-3/+) are crossed to tester females with white eyes and yellow bodies (yw). Any male offspring from this cross with grey bodies are indicative of the P element "hopping" from the X chromosome to an autosome, since the original y+ insert was on the X chromosome.

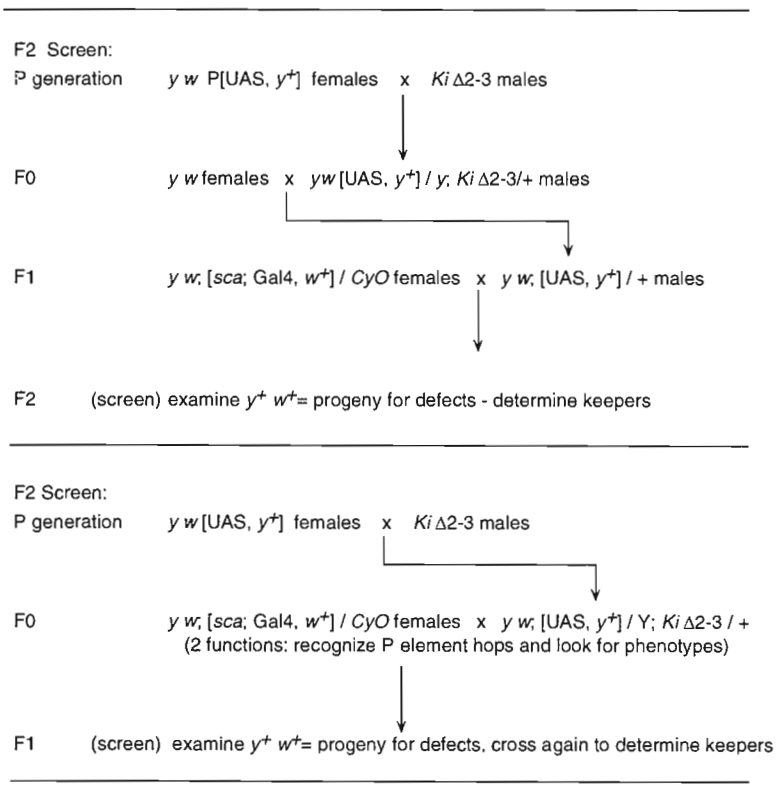

Figure 1. Comparison of F2 and F1 screens.

These grey bodied males are then crossed to females heterozygous for curly wings and a chosen driver element such as scabrous, along with the GAL4 gene (yw; [sca: GAL4, w+]/CyO) in order to recognize "keeper" phenotypes. A keeper phenotype is one in which the misexpression of the region adjacent to the P element insertion has led to an observable phenotype. A heterozygous driver with GAL4 allows lethal jumps to be preserved, for if the responder integrated next to a gene that was lethal when misexpressed, this would not produce any offspring while the dark bodied curly flies that did not receive the GAL4 gene would be viable. Due to the temperature sensitivity of GAL4, all crosses for both F1 and F2 screens were incubated at 25°C.

In the F1 screen (Figure 1), instead of crossing the F0 males to a yw female in order to isolate the P element jump and eliminate the Ki phenotype, the males were crossed to the driver element in a yw background. However, only the non-Ki males observed to contain a phenotype different from wild type were used for the next step. The rules used to screen the F1 progeny involved careful inspection of the scutum and scutellar bristles. Any deviation from the observed wild type pattern was noted as a phenotype that was different from wild type, and this made up one of the three classes of y+ flies representing a "hop" that were kept. The other two distinguishing features of flies that were selected for in the F1 generation included the y+w Cy progeny that did not have any y+w+ brothers, indicating a possible lethal phenotype, and the y+w Cy brothers of those y+w+ progeny displaying a phenotype. No Ki progeny were selected because the Ki gene affected scutum and scutellar bristle appearance, creating false phenotypes. Thus, the F1 screen combined the recognition of P element hops and the phenotype identification into one step.

The next step in the F1 screen involved test crossing selected males to a new driver (sd-Gal4) females and examining the F2 progeny for keeper phenotypes. Ths savings represented is that only a few F1 males are so screened, compared with the "F2 screen." The rules used to select keepers included a detailed inspection of the scutum and scutellar bristles along with the wings, since the sd driver is involved in the development of these areas. If four or more F2 progeny exhibited the same phenotype at the time of the initial screen, the jump was called a keeper. The number of progeny exhibiting a specific phenotype is given in Table 2. Keepers were determined in the same manner in the F2 screen.

Once the keepers were identified on the basis of their phenotype in combination with the sd driver, analysis of the progeny revealed the chromosome location of the y+ insert. If the y+ insert were on the second chromosome, the two progeny would either be y+w+ or w+ Cy, with no y+ Cy. The progeny with the insert on the third chromosome would show all possible offspring,and thus the insert location was determined for the eight keepers (Table 2).

Results and Discussion: Refer to Table 1 and Table 2 for the data of the two screens. When evaluating the effectiveness of the F1 selection imposed screen in comparison to the F2 selection imposed screen, the number of keeper phenotypes must be considered along with the effort required to generate these keepers. Selection at the next round generated 8 keepers out of 45 F1 crosses, while the F2 screen generated 102 keepers out of 730 F2 crosses, having the respective ratios of 18% to 14% keepers/successful hop crosses (Table 2). It should be noted that there were no lethal mutations observed in the F1 screen, while 24 lethal mutations were observed in the F2 screen.

Table 1.

	F1 Screen	F2 Screen
Number of initial fathers	120	2540
Number of F1 crosses	45	730
Number of F2 crosses	8	102
Ratio of keepers / F1 crosses	0.18	0.14

Table 2.

Initial Phenotype of F1(*sca* driver)	F2 Keeper Phenotype (*sd* driver)	Chromosome location
\$5, missing scutellar	small crumpled wings	2 (homozygous viable)
\$6, missing scutellars	small crumpled wings	2 (homozygous viable)
\$24, smaller wings	jagged wing edges	3 (homozygous viable)
\$26, *w Cy* heterozygote	crumpled wings	2 (homozygous viable)
\$27c, *w Cy* heterozygote	crumpled wing edges	2 (homozygous viable)
\$38, missing scutellar	wings 2/3 size	3 (homozygous viable)
\$77, *w Cy* heterozygote	small crumpled wings	2 (homozygous viable)
\$115, wrinkled wing	wrinkled wing	2 (homozygous lethal)

Table 3. Estimated time in minutes required to generate a keeper.

Mobilization cross (F0) (1 min per cross)
F1 screen total 120 +120 (transfer)= 240 mins.
F2 screen total 2540 mins.
F1 score (4 min)
F1 screen total 480 mins.
F2 screen total 10288 mins.
F1 cross (2 min)
F1 screen total 64 x 2 = 128 mins.
F2 screen total 1460 mins.
F2 score (7 min)
F1 screen total 224 mins.
F2 screen total 5110 mins.
Totals
F1 screen 1072 mins.
F2 screen 19398 mins.
Minutes per keeper
F1 screen 134
F2 screen 190

The effort required to generate each keeper was calculated using time estimates for each step in the respective screens, with the data shown in Table 3. One fact that must be mentioned is that each parent cross of the F1 screen was transferred to a second vial five days after the initial cross, to generate more progeny. This was not done for the F2 screen. Additionally, the F1 phenotypes selected to cross with the scabrous driver were also transferred five days after the cross, while the F2 screen was kept in a single vial. When this is broken down into the effort required to generate each keeper, the F1 screen is approximately one hour faster per keeper than the F2 screen.

In the F2 screen, once a y+ male was identified it was assumed that each y+ progeny in the cluster of F1 progeny was a result of the same "hop", and therefore only one was crossed to generate F2 progeny. In the F1 screen, it was assumed that there could be more that one "hop" per cluster and thus every fly with a distinct phenotype was crossed to generate F2 progeny. However, one of the keepers (\$26) from the F1 screen gave interesting results. This was the result of the cross of a brother of a Ki male, a cross that should not have been made according to the rules of F1 phenotype selection. The fact that this cross generated a keeper phenotype provided evidence that more than one "hop" could occur in the same cluster, since the crossed brothers of \$26 did not exhibit a keeper phenotype. If the results were corrected for \$26, the efficiency percentage would drop from 6.7% to 5.8%, while the minutes per keeper value increases from 134 to 151.

Conclusions: In a direct comparison of the two types of screens involved in this experiment, the F1 screen has advantages over the classic F2 screen. The proportion of keeper phenotypes obtained was greater than the F2 screen, indicating that the F1 screen did not suffer from a decrease in positive results. Considering effort, the time difference between the F1 and F2 screens was found to be large, lending additional support to the efficiency of the F1 screen. Once the keepers were identified, the next step would be to perform crosses that generate a homozygous stock of each individual keeper. This stock can then be crossed to other drivers and analyzed by molecular genetic techniques in an effort to identify new genes through gain of function phenotypes.

Castrezana, Sergio. Department of Zoology, Arizona State University, Tempe, AZ 85287. A new recipe for rearing cactophilic *Drosophila*.

The cactophilic species *Drosophila mettleri* and especially *D. nigrospiracula* have difficulty adapting to laboratory culture. Both species are associated with necrotic Saguaro (*Carnagiea gigantea*) or Cardon (*Pachycereus pringlei*) in nature, with *D. nigrospiracula* breeding in the cactus tissue and *D. mettleri* in soil soaked with fermented cactus juice (Heed, 1978).

In developing an improved method to culture these species in the laboratory, I first made a food from commercial instant mashed potatoes (Betty Crocker potato flakes) with a piece of sterilized Saguaro cactus added to the surface. Although the number of offspring was higher than with standard cornmeal or banana recipes, sometimes field-caught flies carried mold that efficiently use the food, directly affecting the development of the flies. Thus, cultures were

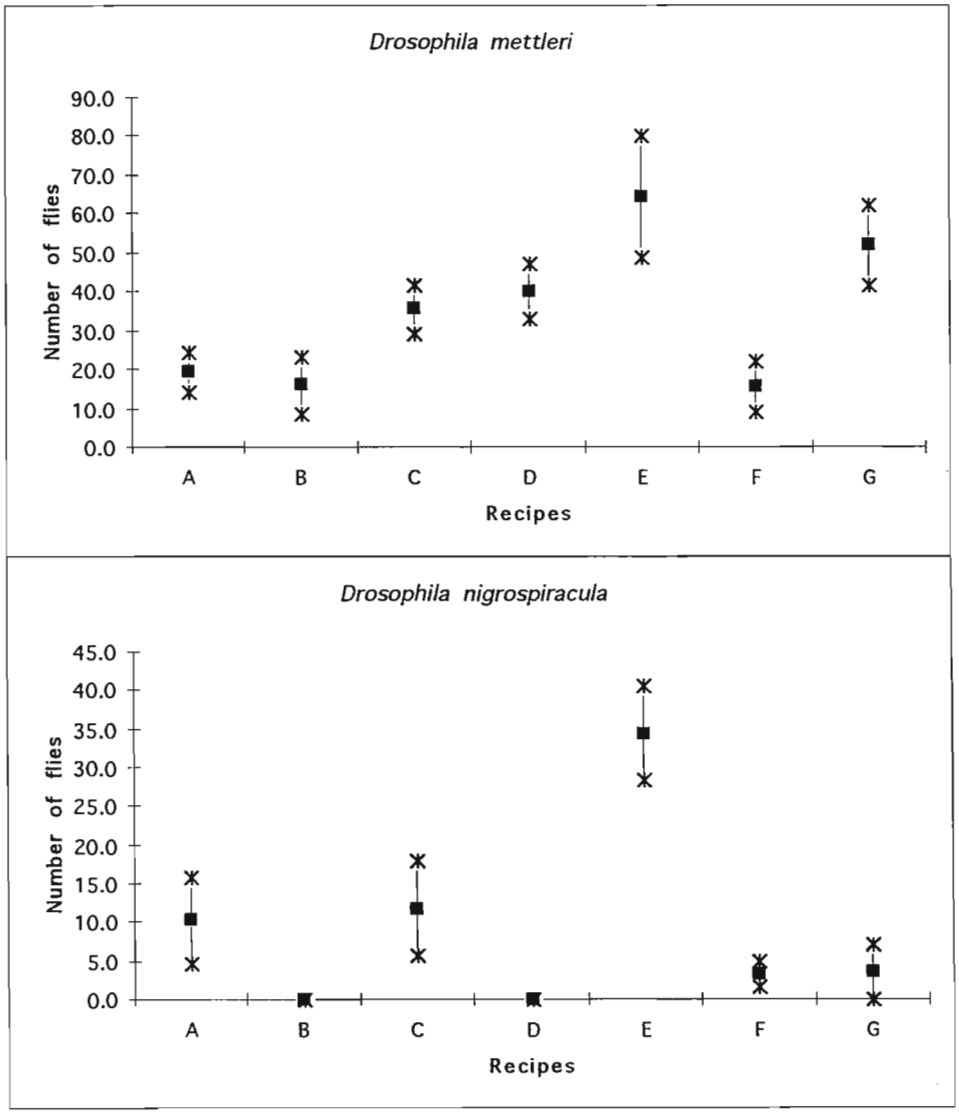

Figure 1. Mean ± SE number of flies emerging from each recipe.

inconsistent. Antibiotics, such as Penstrep, typically used in *Drosophila* media, controlled the mold, but the flies refused to oviposit on the food.

Seven modifications of potato medium were tested in order to find one that would control mold while stimulating oviposition and allowing development. Table 1 shows the seven recipes that were tested; for each test I used four vials of food; in each vial, one mated pair. When the Saguaro is present in the recipes, it is blended with the water. The liquid, either water or water/Saguaro blend, is cooked until it reaches 85°C. It is then cooled to 60°C, and the propionic acid is added. The potato blended flakes are then added and blended.

For both species, recipe E is clearly the best, although the difference is most dramatic for *D. nigrospiracula*.

Table 1. Ingredients and their proportions in seven recipes.

Recipe	Potato grams	Water mililiters	Saguaro tissue grams	Propionic acid mililiters	Antibiotic mililiters
A	15	80	-	0.5	-
B	15	75	-	0.5	0.06
C	15	75	2	0.5	-
D	15	75	2	0.5	0.06
E	15	80	5	0.5	-
F	15	80	5	-	0.06
G	15	80	5	0.5	0.06

Catchpole, Roger. Department of Biology, University of Leeds, Leeds, LS2 9JT. Email: r.d.j.catchpole@leeds.ac.uk The effect of substrate hardness on the oviposition behaviour in two species of *Drosophila*.

Drosophila feed on microorganisms that grow on a range of different substrates (Sang, 1950; Begon, 1982). The type of substrate is commonly used to identify the 'guild' to which a particular species might belong, *e.g.* fungal, fruit and sap. Rearing *Drosophila* under laboratory conditions can be done without the use of these substrates if the basic nutritional requirements of both the adult and larval life stages are met. Several authors have examined the exact nutritional requirements for growth in artificial culture (Begg and Robertson, 1950; Royes and Robertson, 1964; Sang 1978), although in practice a more pragmatic approach is usually taken (*e.g.* Ashburner and Thompson, 1978; Shorrocks, 1971). Most general food media usually contain varying proportions of sugar, agar, yeast, cereal and a mould inhibitor. Of particular interest to the current study are the effects that changes in the concentration of agar have on the oviposition behaviour of two species of *Drosophila*; *D. melanogaster* and *D. virilis*. Agar is an important element in a recipe because it provides a stable substrate in which the various ingredients can be evenly fixed and in which the larvae can burrow and feed. It also provides the adult with a stable oviposition substrate into which eggs can be inserted without sinking. This is important as the egg filaments must remain above the substrate surface to avoid anoxia.

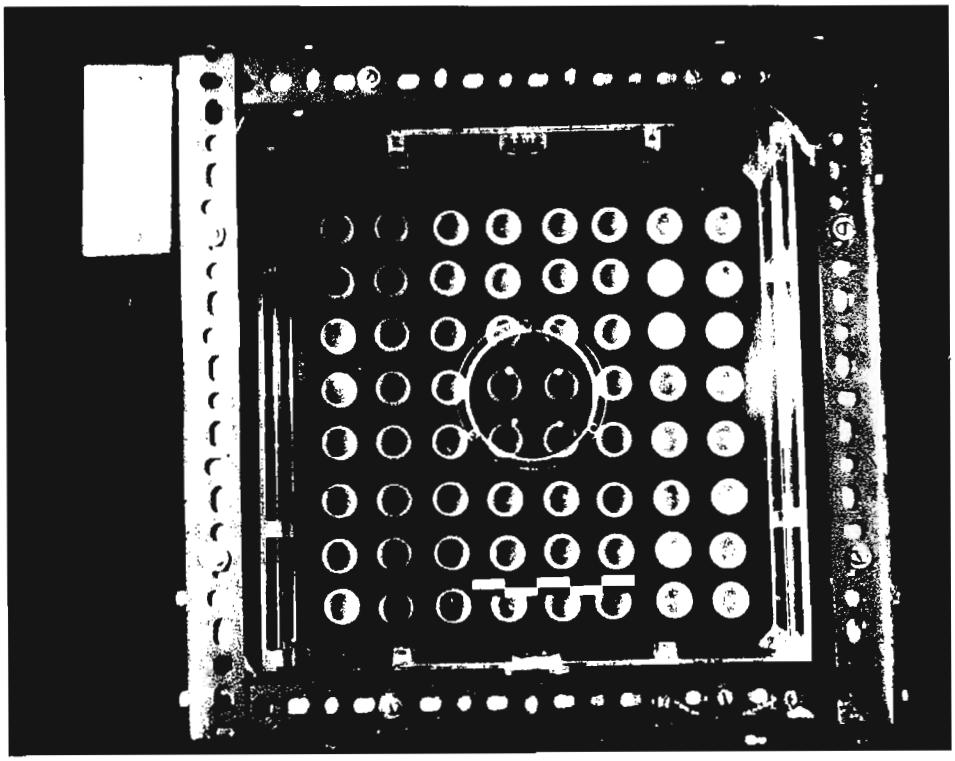

Figure 1. Perspex observation chamber with 200 mm scale bar and lighting unit.

In order to test the effect of different agar concentrations, a small amount of standard food medium (Shorrocks 1971) was placed in an equidistant array of nylon test tube caps, each of which contained 5ml of food. Six replicate patches of four different concentrations of agar (0.01g/ml, 0.02g/ml, 0.03g/ml and 0.04g/ml) were randomly arranged in a 4 × 6 array in the centre of the chamber as shown in Figure 1. Forty gravid females and 3 mature males were selected from stock populations of each species, sorted and placed in the observation chamber. When *D. melanogaster* was examined, individuals were only kept in the chamber for 24 hrs, whereas *D. virilis* needed a further 24 hrs before any appreciable oviposition had occurred. Each chamber was uniformly lit and kept in a constant temperature room for the duration of the experiment at a temperature of 20 ± 1°C. The light source was connected to a time switch which was set on a 12 hr light/dark cycle in order to avoid any interference with the flies' circadian oviposition rhythm. After exposure, the patches were removed and examined under a low power binocular microscope for the presence of eggs. This procedure was repeated three times to give three replicated experiments.

The results in Figure 2 clearly show that there were considerable differences in oviposition preference for food substrates of different hardness even when the concentration of agar changed by as little as 0.01g/ml. The greater number of eggs present on patches containing the lowest concentration of agar is of considerable interest as it may be indicating an active preference of oviposition site. An alternative interpretation could be that the females simply fed more often on

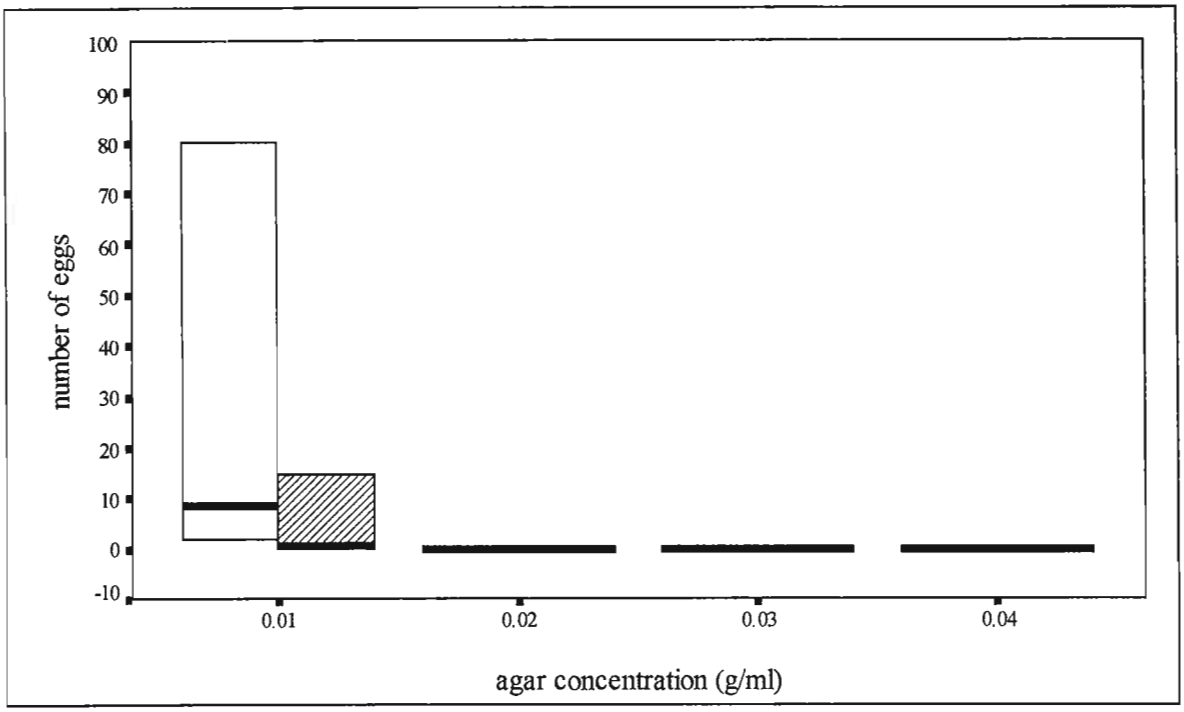

Figure 2. Oviposition preference in four different concentrations of agar. Graph shows median values and the interquartile range. Hatched bar shows data for *D. virilis* and the clear bar shows data for *D. melanogaster*.

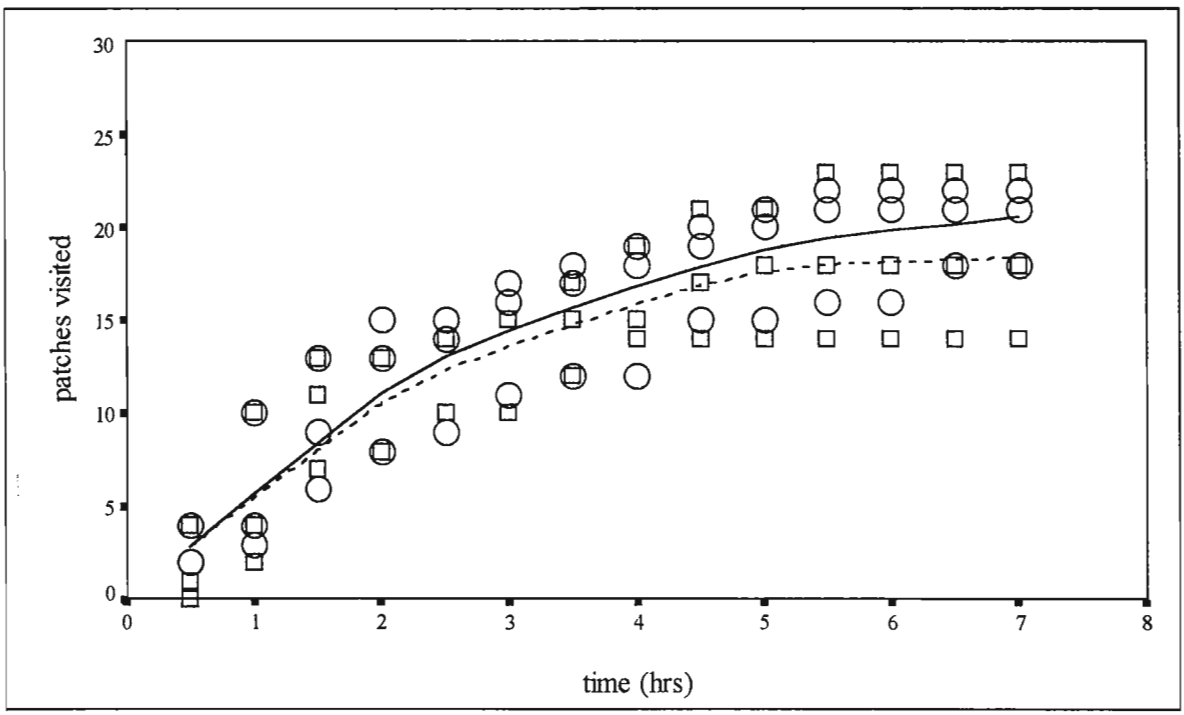

Figure 3. Cumulative number of patches visited during observation period. Dotted line and squares show *D. virilis* data while the solid line and circles show the *D. melanogaster* data.

softer patches and that the presence of eggs was coincidental rather than deliberate. This is not supported from data on wild populations which suggest that there is an effective separation between oviposition and breeding sites in at least some species (Kearney, 1979). Another explanation could have been that only the softest patches were 'discovered' during the oviposition period. This was not supported by the data shown in Figure 3, however, which were obtained from direct observation during the first eight hours of the experiment. The graph clearly shows that during this period, both species discovered most of the patches that were present. Interestingly no eggs were laid on the standard concentration of 0.02g/ml which was used to rear the stocks during the preceding year. This suggests that even though the stocks had been in the laboratory for some time, variation was still present for oviposition preferences. Whether this was linked to higher survival during the preadult stages within the softer substrate was unknown. There appears to be a need for further research on the response of wild caught stocks to general food substrates as this data suggests that there may be some room for improvement, especially from the perspective of the individual *Drosophila*. In summary, patch hardness appeared to have a profound affect on the choice of oviposition site. Both species chose a patch that was 'softer' than the standard substrate on which they were reared. This may be closely linked to fitness within wild populations or could be an artefact of adult feeding behaviour.

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Catchpole, Roger, and Bryan Shorrocks. Department of Biology, University of Leeds, Leeds, LS2 9JT. Email: r.d.j.catchpole@leeds.ac.uk An alternative method for population estimation in large scale laboratory populations of *Drosophila*.

When large laboratory populations of *Drosophila* are being studied, more traditional estimates of population size such as mark-release-recapture may not be practical, because of the sampling effort required. This is especially the case if a large number of replicates are being studied. As an alternative, the number of individuals present on a standard area can be counted to give an estimate of the total population size. This note describes a method for determining population density through the use of curvilinear functions fitted to observations that were taken at known population densities for two species of *Drosophila*: *D. melanogaster* and *D. virilis*.

Drosophila populations are commonly kept in a number of different containers in laboratories which range from half pint milk bottles, that support several hundred individuals, to large scale population microcosms that are capable of supporting many thousands of individuals. Generally, larger populations are usually kept in purpose built 'cages' such as the one shown in Figure 1. When such containers are used it is easily possible to define an area in which the number of individuals can be counted at regular intervals. The container shown in Figure 1 has two transparent inspection windows which are ideally suited to this activity.

Before any estimates of population size could be produced it was necessary to calibrate the windows using known densities of flies. Adults were removed from established cage populations of each species and placed in empty cages at densities of 10, 30, 50, 300, 600 and 1200 individuals. A sex ratio of 50:50 was maintained in order to simulate the age structure that would be present under 'normal' sampling conditions. After sorting, the flies were allowed to recover from anaesthetisation for 24 hrs before any observations were made. The numbers of individuals on the windows were subsequently recorded at 15 minute intervals, over an 8 hour period. Immediately after each observation the cages were tapped to dislodge any individuals that had settled to ensure independent counts. The cages were placed in an incubator at 20±1°C and illuminated throughout the observation period.

Initially a number of different functions were fitted to the data for each species. These were derived from the straight line, quadratic, cubic, power, exponential and logistic equations. In each species the models that provided the three best fits were the straight line ($y = b_0 + b_1x$), quadratic ($y = b_0 + b_1x + b_2x^2$) and cubic ($y = b_0 + b_1x + b_2x^2 + b_3x^3$). The adequacy of the fit was determined by comparing the adjusted coefficients of determination (R^2_{adj}) for each model which were calculated in the following manner:

$$R^2_{adj} = 1 - \frac{(n-1)(1-r^2)}{n-p}$$

where n = number of observations, p = number of parameters and r = correlation coefficient (SAS, 1990). Although both R^2 and R^2_{adj} are commonly used to describe the goodness of fit of particular models, R^2_{adj} was chosen in this case as it generally provides a better estimate of a models adequacy by adjusting for the number of parameters that are present (SAS, 1990). Using R^2 as a selection criterion can be misleading because as the number of parameters increases, so the fit of the model will improve. Unless a perfect straight line is present, a simple model will always have a lower value in comparison to a higher order model such as a quadratic or cubic. The values for the models with the three highest R^2_{adj} coefficients are shown in the legend of Figures 2 and 3. The best linear estimate of population density was chosen by applying a stepwise multiple regression to successive powers of the independent variable. This procedure sequentially adds and removes additional variables to determine whether changes in the model significantly reduce the residual variance that is present (Sokal and Rohlf, 1995). The significance level for retaining a variable was adjusted to 0.017 from an initial probability of 0.05 using the following formula where p = number of parameters and a = probability level (Sokal and Rohlf, 1995):

Table 1. Model determination for *D. melanogaster*. Stepwise multiple regression of successive polynomial terms.

variables in the equation			
variable	b	t	p
b_1 x (straight line)	0.161	74.017	<0.001
b_0 (constant)	0.492	12.209	<0.001
variables not in the equation			
b_2x^2 (quadratic)	n/a	1.905	0.057
b_3x^3 (cubic)	n/a	1.551	0.122

Table 2. Model determination for *D. virilis*. Stepwise multiple regression of successive polynomial terms.

variables in the equation			
variable	b	t	p
b_2x^2 (quadratic)	0.006	52.030	<0.001
b_0 (constant)	1.171	18.904	<0.001
variables not in the equation			
b_1 x (straight line)	n/a	-0.007	0.879
b_3x^3 (cubic)	n/a	-0.114	0.019

$$1-(1-\alpha)^{\frac{1}{p}}$$

The results for this analysis are shown in Tables 1 and 2 which indicate that the best fit for *D. melanogaster* was the straight line while the quadratic provided a better fit for *D. virilis*. The models gave the following transformed values for the different parameters:

D. melanogaster (straight line fit $y = b_0 + b_1x$)
$y = 0.492 + 0.161\,x$

D. virilis (quadratic fit $y = b_0 + b_1x + b_2x^2$)
$y = 1.189 + -0.003\,x + 0.006\,x^2$

In order to estimate the number of individuals that were present, the equations for each model had to be re-arranged and solved for x. Simply swapping the variables in the analysis would have violated one of the basic assumptions of model I regression; that the independent variable is measured without error and is a 'fixed' variable (Sokal and Rohlf, 1995). In consequence the equations were re-arranged in the following way so that the actual numbers that were present in the cages could be estimated from the observed values:

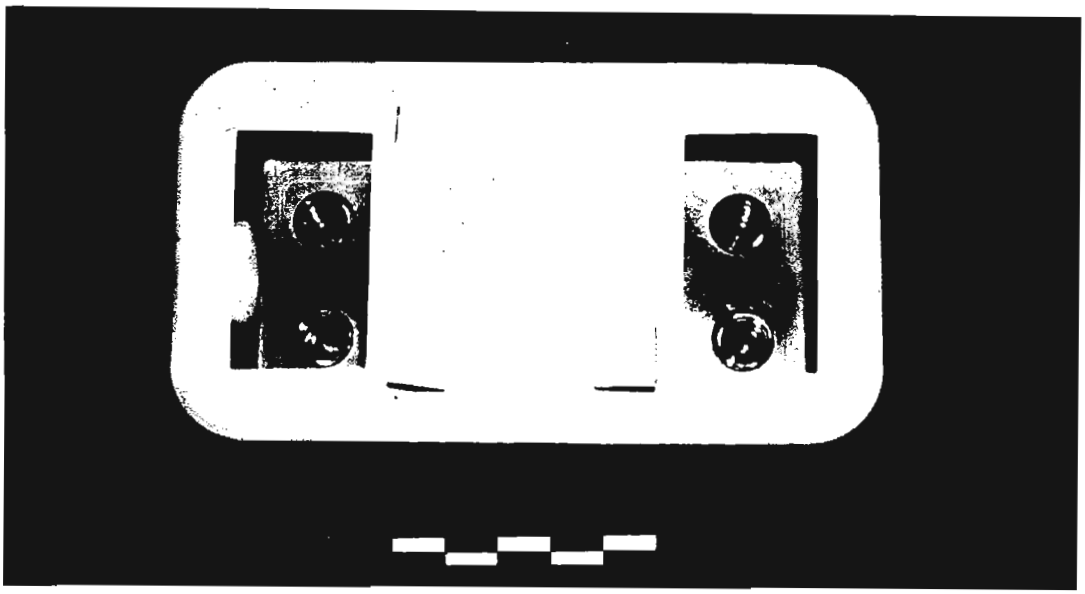


Figure 1. Polythene population cage with 200 mm scale bar.

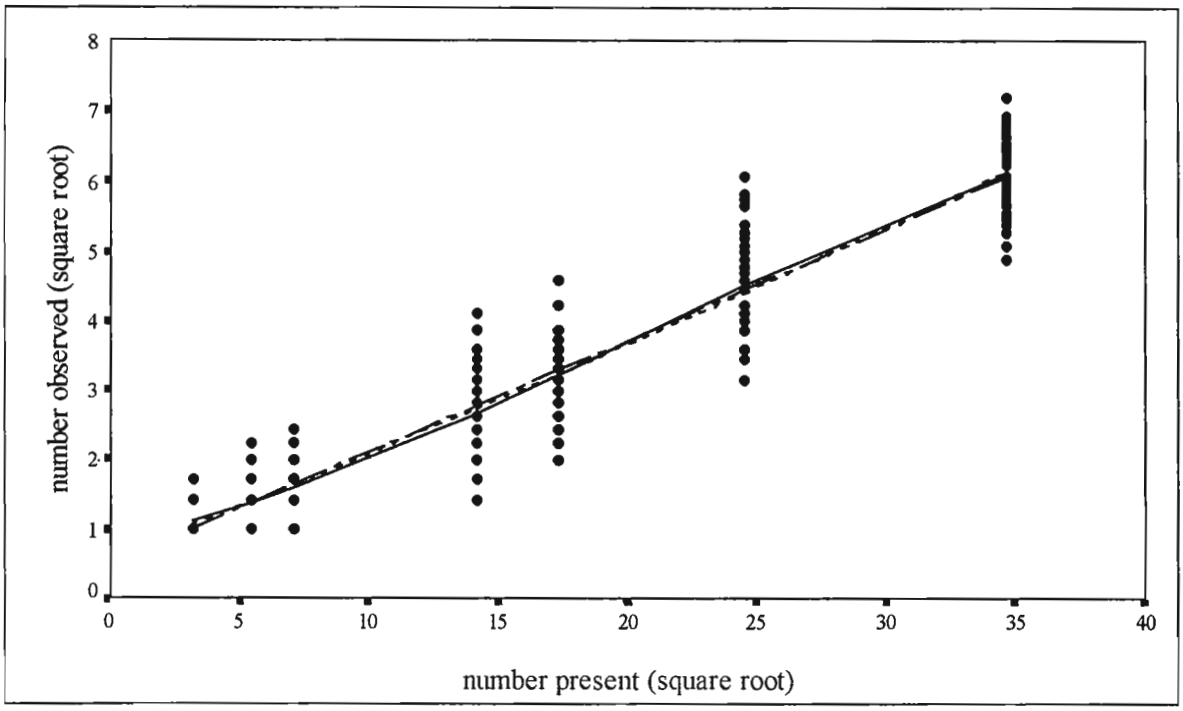


Figure 2. Curve estimation for *D. melanogaster* populations. Solid line represents the cubic fit ($R^2 = 0.931$), dotted line the quadratic fit ($R^2 = 0.929$) and the long dotted line the linear fit ($R^2 = 0.929$).

straight line ($y = b_0 + b_1x$) $$x = \frac{y - b_1}{b_0}$$

quadratic ($y = b_0 + b_1x + b_2x^2$) $$x = \frac{-b_1 \pm \sqrt{b_1^2 - 4(b_0 - y)b_2}}{2(b_0 - y)}$$

There were very few qualitative differences between the three models that were fitted to the data for *D. melanogaster*. The analysis clearly indicated that the straight line was the most suitable model and that the addition of extra terms had no effect on the amount of residual variance that was present. The data for *D. virilis*, however, was more complex and a higher order model was more suitable. Although the cubic model was also significant, the quadratic model had the highest level of significance and, qualitatively, also gave the best fit, see Table 2 for details. The data for *D. virilis* showed that at low densities the window counts failed to consistently detect flies up to a density of about 200 individuals. This meant that for some of the counts, no individuals were observed when they were actually present in the cage. Bearing this in mind, the straight line gave a qualitatively better estimate for populations at lower densities but was less adequate at moderate densities, see Figure 3 for details.

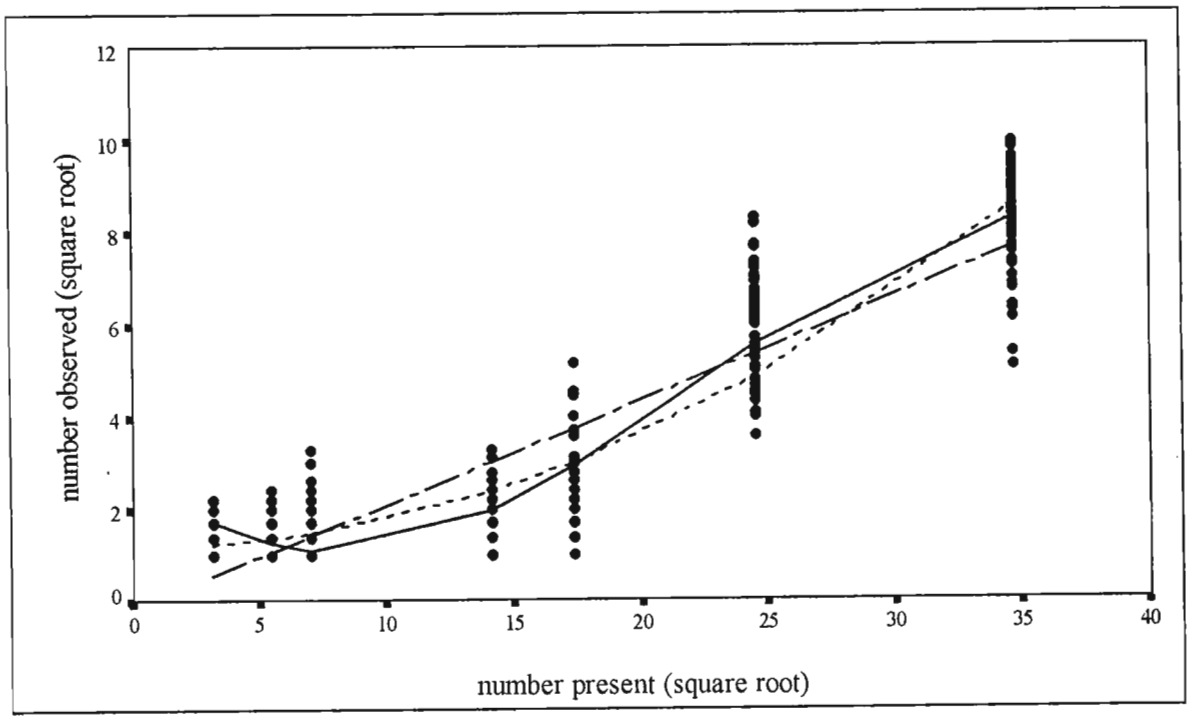


Figure 3. Curve estimation for *D. virilis* populations Solid line represents the cubic fit ($R^2 = 0.889$), dotted line the quadratic fit ($R^2 = 0.866$) and the long dotted line the linear fit ($R^2 = 0.813$).

After the most suitable models were identified, they were then used to estimate changes in population density for 40 replicate populations during the course of a long term study. Sampling all these populations took approximately one hour each week in contrast to the considerably greater length of time that would have been required even for the most basic mark-release-recapture estimate. The method provides a quick and economic way of measuring changes in population density within large cage environments.

References: SAS, 1990, *SAS/STAT User's Guide: Volume 2 (Version 6, Fourth Edition)*. SAS Institute Inc.; Sokal, R.R., and F.J. Rohlf 1995, *Biometry*. W.H. Freeman.

Catchpole, Roger. Department of Biology, University of Leeds, Leeds, LS2 9JT. Email: r.d.j.catchpole@leeds.ac.uk The effect of substrate hardness on penetration resistance.

This study provides an outline of the methodology for the measurement of penetration resistance in *Drosophila* oviposition substrates. Although the method was applied to standard laboratory medium (Shorrocks, 1971), it can potentially be applied to any substrate that is commonly found in the field. The preferential utilisation of different substrates by *Drosophila* has been noted for some time (Begon, 1982). Clearly oviposition sites will differ in hardness not only because of intrinsic differences in the substrate type but also because of environmental factors such as moisture content. It may, therefore, be of some interest to relate differences in hardness between different substrates to the oviposition behaviour of particular species. In another paper in this volume, Catchpole (1997) showed clear and persistent choices between different concentrations of agar even when these differences were extremely small. In order to determine whether these differences produced a physical effect, the resistance to penetration was measured using a force transducer in an attempt to simulate the insertion of an ovipositor in the substrate surface. This method has potentially wider applications in co-evolutionary studies where the ovipositor sizes of a number of different species could be related to the penetration resistance of their preferred oviposition substrates.

Table 1. Penetration resistance in four different concentrations of agar. One way ranked ANOVA with a Bonferroni *post-hoc* comparison. Values that are underlined are not significantly different from each other when p = 0.05.

Source	df	SS	MS	F	p
between	3	119606	39868	209.03	<0.001
within	116	22125	190		
total	119	141731			

0.01g/ml 0.02g/ml 0.03g/ml, 0.04g/ml

Resistance was measured by mounting an entomological pin on a force transducer as previously mentioned. The force transducer converted the mechanical force to an electrical signal which was then measured using an oscilloscope. Attempts to mount an ovipositor failed as there was no reliable way of attaching the structure to the transducer arm.

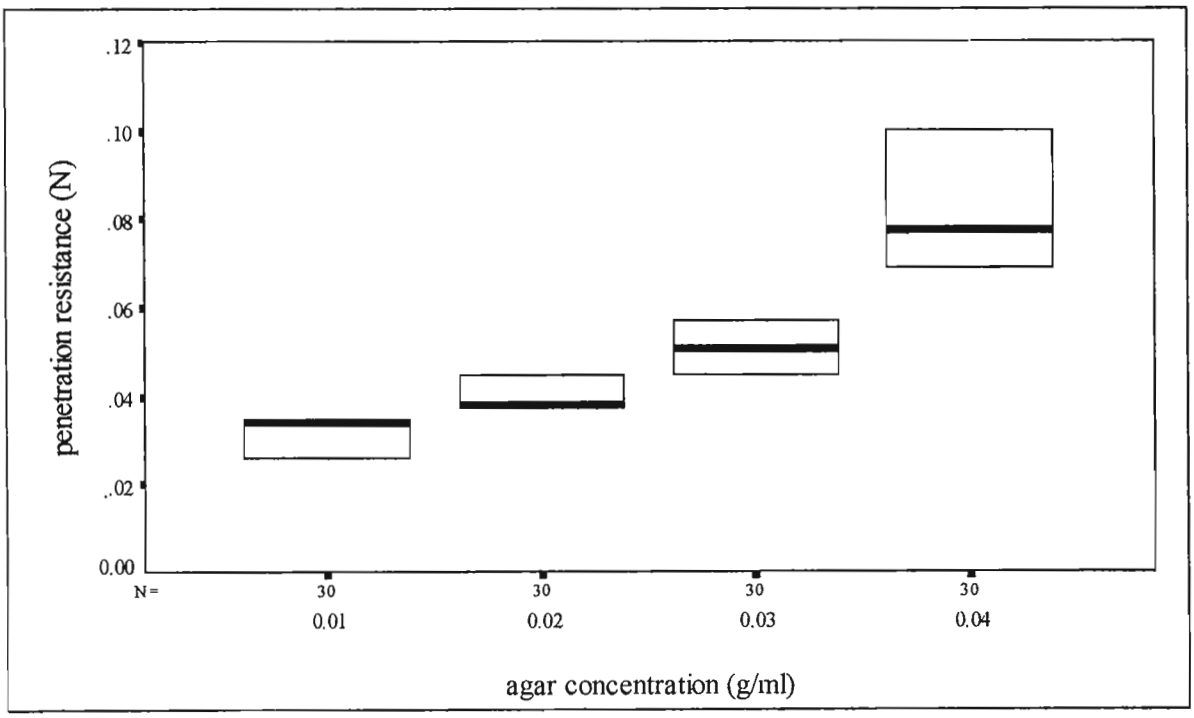


Figure 1. Penetration resistance in four different concentrations of agar. Graph shows median values and the interquartile range.

Although a pin of similar dimensions and shape to an ovipositor was used, the measurements were not intended to be a wholly realistic simulation of ovipositor insertion but simply a relative assessment of the forces involved. The electrical signal was recorded on an oscilloscope (Gould DSO 1602) and the resulting traces were measured and converted into Newtons (N) for analysis. Measurements were made on 30 separate samples of food at four different agar concentrations of 0.01g/ml, 0.02g/ml, 0.03g/ml and 0.04g/ml. This was done by dropping the mounted pin from a standard height of 3mm into each sample. The samples consisted of a standard amount of food held in a small nylon test tube cap. Separate caps were used for each measurement to avoid any influence from previous inser-tions.

Even though the differences in agar concentration were small, these were sufficient to produce clear physical differences, as can be seen from Table 1 and Figure 1. Results that were not significantly different from each other were underlined in Table 1. The larger variation in measurement at the highest concentration, shown in Figure 1, may have been due to differences in food preparation. All other concentrations were derived from a single batch while the highest concentration contained some samples from a second batch of food. In species with small ovipositors these physical differences could lead to a substantial increase in energetic costs either through the physical effort required to insert the ovipositor plates into the harder substrate or through the location of more suitable sites. Smaller species will also pay an additional penalty at the larval stage because of the increased cost of burrowing within harder substrates. In summary, clear physical differences were present between food media containing only small alterations in agar content. These differences were easily measured using a method that could be applied to a much wider range of substrates.

References: Begon, M., 1982, Yeasts and *Drosophila*. In: *The Genetics and Biology of Drosophila* 3b (ed. M. Ashburner, H.L. Carson, and J.N. Thompson, jr.) 345-384, Academic Press; Shorrocks, B., 1971, Dros. Inf. Serv. 46: 149.

Request for Assistance

Robert Farkas. Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlarska 3, 833 06 Bratislava, Kramare, Slovakia. Tel. (+421 7) 373-800, ext. 244; fax (+421 7) 374-247; email ueenfark@savba.savba.sk or farkas@uee.savba.sk

I am interested in obtaining the following wild type and mutant strains. I appreciate your help in locating any of them. Thank you.

Gruta
Falsterbo
Formosa
Stromsvreten 10
turnipe (any allele)

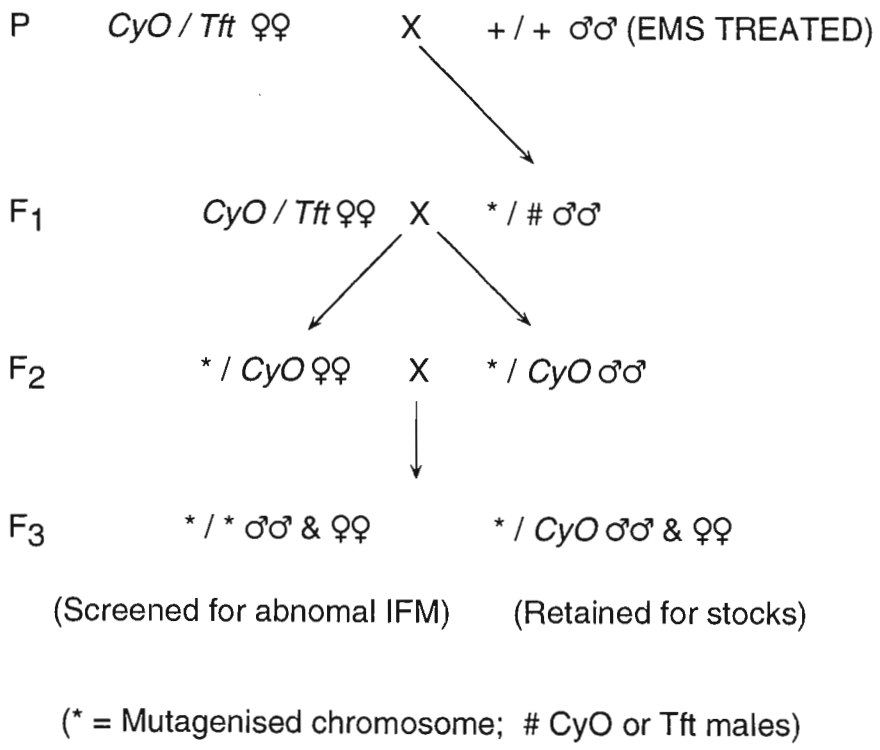
Mutation Notes - Drosophila melanogaster

Report of Upendra Nongthomba and N.B. Ramachandra. Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore, India.

Isolation of an allele of yellow body mutation in *Drosophila melanogaster* by an unusual pattern of inheritance.

Chemical mutagenesis is commonly used in *Drosophila* genetics to induce point mutations and chromosomal aberrations (Ashburner, 1989). One of the main areas of our research activity is to induce mutations in general and then to select viable recessive mutations confined to second chromosome which affects the Indirect Flight Muscle (IFM) development in *Drosophila melanogaster* by using ethyl methanesulphonate (EMS). The protocol used for induction and detection of mutations on the second chromosome is as follows:

In this proto-col, 3500 lines of the flies were set up to screen for mutations. All the males and females of the F2 progeny were analyzed. Surprisingly, in one line, out of 25 males and 30 females, only one male had a yellow body phenotype along with the *CyO/Tft* markers. This was not an expected result. Perusal of the literature, since 1916, when Morgan and Bridges reported for the first time the yellow body mutant, many alleles of yellow body mutant, localized on X-chromosome have been reported (Lindsley, 1992). In the present study, the mutagenized X-chromosome is not retained in the males of F1 progeny. Then, the question is, how come a yellow body mutant appeared in the F2 generation? The possibilities are it might have arisen by spontaneous mutation or by some means yellow body colour gene was inserted to autosomes and then activated or by any other unknown mechanisms.



To understand the inheritance pattern and to preserve the mutant chromosome, this yellow body mutant was crossed to three females of a strain of *D. melanogaster* having normal X-chromosome with *CyO/Tft* markers on chromosome 2. This was done because the virgin females of the above strain were readily available since these are being used for routine experiments. From this cross, one can usually expect that all the F1 individuals should possess wild type body colour with markers, if the yellow body colour is recessively inherited. Interestingly, the phenotype of the observed progenies were different from the expected results. In the F1 generation, out of 31 flies scored, 23 (11 males and 12 females) had the yellow body phenotype and 8 (4 males and 4 females) had wild type phenotype. These yellow body mutant flies along with the markers bred true and are being maintained as stocks. This indicates that the yellow body colour gene is not behaving as recessive or dominant genes, but it behaves as unusual dominant-like gene and it appears in the F1 progenies in 3:1 ratio. The puzzling question now is how a single copy of the X-chromosome present in the yellow body male could give two copies of the yellow body gene or X-chromosome to generate yellow body females? This is possible only if the female parents have at least one copy of yellow body mutant chromosome or by contamination. However, our data on the ratio and progenies scored showed no such indications. Moreover, the strain

having normal X-chromosome with *CyO/Tft* markers on second chromosome is being used for routine experiments and is a pure line.

Interestingly, subsequent crosses to Canton-S strain and yellow allele 1 of *D. melanogaster* (obtained from our Drosophila Stock Centre; Mysore) have shown the sex-linked pattern of inheritance. Therefore, this is an unusual pattern of inheritance observed only for one generation.

The phenotypic characteristics of the yellow body mutant isolated in the present study are as follows: Adult body colour is lighter than yellow allele 1 type. Hairs and bristles are brown with yellow tips. Wing veins and hairs are yellow. Larval setae is yellow to brown. Larval mouth parts are golden brown and mouth hooks are dark brown. The viability of this mutant is excellent. Thus, the yellow body mutant isolated in the present study is recessive sex-linked and allelic to yellow 1 type and we have named this mutant as y^{RU} allele.

Acknowledgments: We are grateful to Prof. H.A. Ranganath, Principal Investigator, Drosophila Stock Centre, and chairman of our department; as well as to Prof. K. VijayRaghavan, TIFR, Bangalore for their constant encouragement and providing facilities. The financial assistance from the Department of Science and Technology, New Delhi (Grant No. SP\SO\D - 72/93 Dated 14.11.1994), is gratefully acknowledged.

References: Ashburner, M., 1989, In: Drosophila. *A Laboratory Handbook.* CHS Press, London; Lindsley, D.L., and G.G. Zimm 1992, In: *The Genome of* Drosophila melanogaster. Academic Press Inc., USA.

Report of Pascal Heitzler. Laboratoire de Genetique et de Biologie Moleculaire et Cellulaire, BP163, 67404 Illkirch Cedex, France.

New FM7 versions from Strasbourg:

- FM7d: FM7, y[31d] sc[8] B, fertile.
- FM7e: FM7, y[31d] sc[8] oc ptg B, female sterile.
- FM7e P[ftz-lac,ry+]: FM7, y[31d] sc[8] P[ftz-lac,ry+] oc ptg B, "ftz blue FM7e".
- FM7f: FM7, y[93j] sc[8] oc ptg B, female sterile, y[-] marker.
- FM7f P[ftz-lac,ry+]: FM7, y[93j] sc[8] P[ftz-lac,ry+] oc ptg B, "ftz blue FM7f".
- FM7g: FM7, y[31d] sc[8] w[a] oc ptg v[Of] B, female sterile.
- FM7g fa[n]: FM7, y[31d] sc[8] w[a] fa[n] oc ptg v[Of] B.
- FM7g ct[ns]: FM7, y[31d] sc[8] w[a] ct[ns] oc ptg v[Of] B.
- FM7h: FM7, y[31d] sc[8] w oc ptg B, female sterile.
- FM7h N[PlacW]: FM7, y[31d] sc[8] w N[PlacW] oc ptg B, lethal, "N blue FM7h".
- FM7i: FM7, y[93j] sc[8] w oc ptg B, female sterile, y[-] marker.
- FM7j: FM7, y[93j] sc[8] w, very good fertility, y[-] marker.

Comments: These chromosomes represent new useful versions of the effective FM7 balancer performed at Strasbourg. The previous sn[X2] female sterile marker from FM7c has been replaced advantageously by the female sterile oc[1] inversion because sn males often stick on the food medium. The different markers used were introduced from In(1)dl-49 into FM7 through the medium of the In(1)sc[8] In(1)dl-49 chromosome. The amorphic y[93j] allele was EMS-induced on FM7, y[31d]. The B[+] FM7j version was obtained after unequal crossing over within the tandem duplication of B. The P[ftz-lac,ry+] insertion on a FM7 chromosome was obtained by Hiromi and recombined here on a FM7e version. N[PlacW] is a PlacW enhancer trap induced N haplo-insufficient allele obtained in the Jan's lab on In(1)dl-49, w; it was introduced in the FM7h version. I remember that occasional spontaneous compound-X chromosomes occur with balancers of the X and a normal X chromosome.

Report of R.D. Bien-Willner, W.W. Doane, and D.W. Scheel. Department of Biology, Arizona State University, Tempe, AZ 85287-1501.

The "nw^{P2ry+}" mutation of *Drosophila melanogaster* proves to be an allele of *tapered.*

We earlier described a recessive, *P*-induced mutation in *D. melanogaster* (Scheel and Doane, 1994; Bien-Willner *et al.*, 1996) that, in homozygotes, produces "narrow-like" wings with pointed tips, reduces viability, causes complete behavioral male sterility, and decreases the fertility of females. This mutation was assigned to chromosome *2R* through genetic analysis and tentatively placed in region 54E by *in situ* hybridization of a biotinylated *P* element probe to

polytene chromosomes. Based on its overall phenotype, tentative location, and a weak interaction between it and the nw^2 allele in regard to wing shape, it was named $nw^{PZ[ry+]}$ *FlyBase* (1997) lists it as nw^{PZry+}.

Despite its proposed classification as a *nw* allele, uncertainty remained about the exact chromosomal location of the *PZ* element that had induced the so-called nw^{PZry+} mutation. This was due to a complex chromosomal aberration in the region of its insertion that made the insertion site difficult to interpret. In addition, the weak narrow-like wing phenotype of presumptive nw^{PZry+} / nw^2 flies often overlapped wild-type, raising doubts about the presence of the nw^2 allele in our test stocks. These stocks, which produce few homozygotes at either 18° or 25°C, had been obtained from the Bloomington, Mid-America, and Umeå Drosophila Stock Centers. In our hands, none of them yield homozygotes with a wing phenotype like that originally described for nw^2; rather, their wings appear wild-type. (Unfortunately, our laboratory had lost the nw^2 strain used by Doane and Clark [1984], which did produce homozygotes with the wing phenotype characteristic of this mutant.)

The only other gene on chromosome *2R* with a mutant phenotype similar to our *PZ*-induced mutation is *tapered* (*ta*; 2-56.6) which, prior to this report, had a single mutant allele called ta^1 (Lindsley and Zimm, 1992; *FlyBase*, 1997). Although this locus lies about 23 cM centromere-proximal to *nw*, the description of the ta^1 phenotype matched that of "nw^{PZry+}" almost perfectly. We therefore obtained several ta^1 stocks from the Mid-America Stock Center and tested the chromosome carrying ta^1 from each one over our "nw^{PZry+}"-bearing chromosome for potential allelic interaction. Contrary to expectation, the heterozygotes expressed the typical ta^1 mutant phenotype, suggesting the two mutations are alleles.

We now have conclusive evidence that "nw^{PZry+}" is actually a recessive mutant allele of the *tapered* gene (Doane, Bien-Willner and Scheel, in preparation) and, therefore, have renamed it ta^2. Supporting evidence includes: (1) a third mutant allele, ta^3, which was induced by excision of the *PZ* element from ta^2, behaves as a recessive lethal that is able to uncover the *tapered* mutant phenotype when tested in *trans* over either ta^1 or ta^2, (2) data from two different 3-point crossover analyses place the above three *ta* mutant alleles at the same locus on the genetic map, and the site of this locus is consistent with the published site of the *ta* gene, (3) three different deficiencies in the second chromosome, namely *Df(2R)B5*, *Df(2R)X3*, and *Df(2R)eve* (Bloomington Stock Center), uncover the mutant phenotypes of the above *ta* alleles, while four others, *Df(2R)cn76k3*, *Df(2R)cn88f34a*, *Df(2R)spleN3* (Michael Ashburner's laboratory, University of Cambridge) and *Df(2R)M41A4* (Mid-America Stock Center) do not. Thus, in addition to identifying two new *ta* mutations, we have defined the cytogenetic location of the *ta* gene, which lies within region 46C3-C4; 46C9-C11 of the polytene chromosome map for *2R*. Furthermore, we have collected preliminary data indicative of genetic interactions between the dominant nw^D allele of the *narrow* gene and the ta^1 and ta^2 alleles of *tapered*, suggesting that these two linked genes may be part of the same developmental pathway(s).

Acknowledgment: We thank Joyce Ann Bradley for media preparation and the curators of Drosophila Stock Centers, including John Roote of the Ashburner laboratory, for providing the *D. melanogaster* strains used here. Partial support for this research was provided by a grant from the Howard Hughes Medical Institute through the Undergraduate Biological Sciences Educational Program.

References: Bien-Willner, R.D., D.W. Scheel, and W.W. Doane 1996, 37th Ann. *Drosophila* Res. Conf., San Diego, *Program & Abstracts*, p. 140; Doane, W.W., and A.G. Clark 1984, Dros. Inf. Serv. 60:234; *FlyBase*, 1997, A *Drosophila* Genetic Database. Available from the ftp.bio.indiana.edu network server and Gopher site; Lindsley, D.L., and G.G. Zimm 1992, In: *The Genome of* Drosophila melanogaster, Academic Press, NY, p. 701; Scheel, D.W., and W.W. Doane 1994, Dros. Inf. Serv. 75:34-35.

Report of Mary Roberts and F. Rob Jackson. Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111.

A new *miniature-dusky* allele.

We report the isolation of a new spontaneous allele of the *miniature-dusky* (*m-dy*) gene complex. The new mutation was induced in a single male which carried an X chromosome marked with an existing *dy* allele (dy^{And}). It was identified among the progeny of a genetic cross designed to mobilize a *dy* transgene; (*y w* dy^{And}/*y w* dy^{And}; +/+; +/+ ♀ × +/Y; *P*{*dy12*}/+; *Sb P*{Δ2-3}/+ ♂); however, the mutant allele did not arise in a dysgenic individual. Genetic analysis indicated that the new mutation: (1) was recessive, (2) mapped to the X chromosome, and (3) failed to complement both *m* and *dy* alleles. We refer to this new *m-dy* allele as m^{MR}. The m^{MR} mutation causes reduced wing size similar to other *m-dy* alleles, but the reduction in wing surface area is more extreme than that observed in any other *m-dy* mutant with the exception of m^D, a dominant allele of the *m-dy* complex. Indeed, the phenotype associated with m^{MR} is similar to that previously described for *m dy* double mutants, consistent with the presence of the dy^{And} allele on the parental

chromosome. Southern blot analysis using DNA probes spanning the *m-dy* complex indicates that the m^{MR} mutation is a 19-25kb chromosomal deletion in the *m-dy* interval. Our unpublished molecular analysis of this region indicates the existence of separable *m* and *dy* transcription units, and we postulate that m^{MR} removes part or all of both transcription units.

Mutation Notes - Other Species

Report of E. Solé. Dept. Genètica, Facultat de Biologia, Universitat de Barcelona, Spain.
Spontaneous *yellow* mutation in the *ch cu* strain of *Drosophila subobscura.*

Two *yellow* male flies spontaneously arose in a homokaryotypic stock of *D. subobscura* kept in the laboratory for a long time. This stock bears the recessive mutations ch (*cherry*, bright red eyes) and *cu* (*curled*, wings curled concave upwards), both located on chromosome O. Another *yellow* male fly arose after some generations in a cross between a wild male and five *ch cu* females. Only the right half of this mutant individual was *yellow*; his half left was wild type. It was fertile and no mutant flies appeared either in the F1 or F2 of a cross with *ch cu* females.

The *yellow* mutation is recessive, located in the A (sexual) chromosome and has been previously described in *D. subobscura* (Krimbas, 1993; Mestres, 1996).

References: Krimbas, C.B., 1993, Drosophila subobscura: *Biology, Genetics, and Inversion Polymorphism.* Verlag Dr. Kovac, Hamburg; Mestres, F., 1996, Dros. Inf. Serv. 77: 148.

Report of Albert Kamping and Wilke van Delden. Department of Genetics, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.
A rare *α-Gpdh* allele in *Drosophila simulans.*

In contrast to many other allozyme loci, *α-Gpdh* is remarkably invariable in *Drosophila.* The *α-Gpdh* locus is monomorphic for electrophoretic variation in almost all *Drosophila* species. Some species show alleles at very low frequencies and in only two out of almost 200 species that have been analyzed, the *α-Gpdh* locus is classified as polymorphic (*D. melanogaster* and *D. subarctica*). The low level of variation is ascribed to the important functions in energy metabolism of the enzyme. New mutants at this locus are assumed to be deleterious, and only under conditions without biochemical or physiological constraints new mutants may be maintained. Allele substitutions have taken place in the evolution of *α-Gpdh* in *Drosophila*, because different species carry different alleles. Alleles with identical electrophoretic mobility are restricted to certain species or species groups. The distribution and uniformity in alleles within and between species cannot be explained without the action of natural selection, where metabolic function of the enzyme and ecological niches of the species are assumed to be main factors in the evolutionary process of *α-Gpdh.*

Table 1. Changes of the rare $\alpha Gpdh^{S}$ allele frequency in laboratory *D. simulans* populations, started with different initial frequencies at 20°C and 29.5°C and raised under uncrowded conditions.

Temperature	Initial frequency	Generations 1	5	10	15
20°C	.25	.24	.25	.16	.14
	.50	.53	.52	.37	.31
	.75	.74	.69	.59	.59
29.5°C	.25	.28	.29	.21	.19
	.50	.51	.49	.41	.36
	.75	.72	.69	.65	.68

D. melanogaster is one of the exceptions concerning the level of variation at the *α-Gpdh* locus. Almost every wild population of *D. melanogaster* is polymorphic for two common alleles, Slow (*S*) and Fast (*F*). The sibling species *D. simulans* is monomorphic and carries an allele with identical electrophoretic mobility as the *D. melanogaster F*-allele. In consecutive years we observed an additional *α-Gpdh* variant in a wild population of *D. simulans* in The Netherlands. Electrophoretic mobility of this allele is comparable with the *S* allele of *D. melanogaster*, and its frequency reaches the level of polymorphism. Four out of 21 captured *D. simulans* females produced progeny (no hybrids) carrying the *S* allele in a frequency not significantly different from .25. We derived homozygous *S* and *F* strains, and laboratory populations with different

initial frequencies were started at 20°C and at 29.5°C under uncrowded conditions, to test whether the rare *S* allele would persist in the populations. The F2 generations were analyzed, and the observed numbers of genotypes were tested for deviations from the expected ratio and allele frequencies were followed for 15 generations, with 100 individuals per sample. We also tested the F2 generation under more stressful developmental conditions.

In Table 1 the frequencies of the rare α-*Gpdh*^{S} allele in *D. simulans* populations started with different frequencies at 20°C and 29.5°C under optimal developmental conditions are presented. In generation one (= F2) no deviations from the expected 1:1 or 1:2:1 genotypic ratios were observed. A slight decrease in α-*Gpdh*^{S} frequency is observed after 10 and 15 generations at both temperatures. At 29.5°C a tendency for heterozygote advantage was observed at all three starting frequencies in generation one, and the combined data showed a significant excess of heterozygotes ($P < 0.05$). The tendency of heterozygote advantage was also observed in later generations at 29.5°C. This is possibly the reason for the lower decrease in α-*Gpdh*^{S} frequency at that temperature. Under stressful developmental conditions, *i.e.,* high larval crowding, a highly significant deviation from the expected numbers of genotypes was observed ($X^2_2 = 20.27$, $P < 0.001$). Under these conditions, individuals homozygous for the rare *S* allele have a significant fitness disadvantage compared to homozygous *FF* and heterozygote individuals. Fitness values derived from F2 ratios of the three genotypes were .65, 1.00, and .86 for *SS* homozygotes, heterozygotes, and *FF* homozygotes, respectively.

We observed clear differences in α-*Gpdh* genotype frequencies between optimal and stressful conditions. The fitness differences among α-*Gpdh* genotypes under stressful conditions may be caused by functional restrictions of the enzyme product of the rare allele. Only under conditions without these restrictions, the rare allele may be maintained in the population or species.

References: Coyne, J.A., W.F. Eanes, J.A.M. Ramshaw, and R.K. Koehn 1979, Syst. Zool. 28: 164-175; Johnson, F.M., and H.E. Schaffer 1973, Biochem. Genet. 10: 149-163; Lakovaara, S., A. Saura, and P. Lankinen 1977, Evolution 31: 319-330; Lakovaara, S., and L. Keränen 1980, Hereditas 92: 251-258; O'Brien, S.J., and R.J. MacIntyre 1972a, Genetics 71: 127-138; O'Brien, S.J., and R.J. MacIntyre 1972b, Biochem. Genet. 7: 141-161.

Report of F. Mestres and M. Pascual. Dept. Genètica. Universitat de Barcelona. Barcelona (Spain).
bm (*bombolles*) a wing mutation of *D. subobscura.*

When analyzing a sample of *D. subobscura* flies from Observatori Fabra (Barcelona) many flies with abnormal wings were detected. Young flies presented big bubbles in the wings due to lymph accumulations between the two cell layers of this body structure. Usually bubbles tear after a few days releasing the lymph. As a consequence the wings get crumpled in the places where lymph bubbles were present and the wing tips curl upwards. Many individuals also showed bubbles inside their abdomens, being bulky and presenting alterations in the tegument of their abdominal segments.

The trait is autosomal and recessive. Its penetrance is incomplete and its expressivity is variable.

All these characteristics are similar to other mutations described in *D. subobscura* as *bubble*, *bladder* and *blister-curly* (Krimbas, 1993).

References: Krimbas, C.B., 1993, *Drosophlia subobscura*: Biology, Genetics and Inversion polymorphism. Verlag Dr. Kovac.

Teaching Notes

Woodruff, R.C.,[1] **and J.N. Thompson, jr.**[2]
[1]Department of Biological Sciences, Bowling Green State University, Bowling Green, OH, and [2]Department of Zoology, University of Oklahoma, Norman, OK. A teaching exercise combining Mendelian genetics and gene therapy concepts in *Drosophila*.

The following genetic laboratory exercise with *D. melanogaster* should give students an increased understanding of Mendelian genetics, including segregation, independent assortment, and sex linkage. In addition, it could be tied into an introduction to the use of the model system *Drosophila* in exploring the applications of gene therapy.

The objective of the one-generation cross is to identify the chromosomal location of a wild-type white gene, w^+, that has been transformed as part of a P DNA element, P[w$^+$], into an X chromosome containing a defective white gene, w^{1118}. The attainment of this objective will confirm that these red-eyed (w^{1118} P[w$^+$]) flies have their defective white gene corrected by gene therapy (see Engels, 1996, for a review of the P element and Spradling, 1986, for a review of transformation in *Drosophila*). In addition, students using Mendelian genetic crosses can identify the chromosomal location of the P-element transposase source, P[Δ2-3](99B), that causes the somatic movement of the inserted P[w^+] element. From this exercise, and from discussions, the students can also learn about the genetics and regulation of the P DNA element and how this transposon is used as a gene-transfer vector and as a marker to localize, clone, and sequence genes.

In this cross, w^{1118} P[w^+]038 females, which have red eyes, are mated with *CyO/Sp;* ry^{506} *Sb* P[ry$^+$ Δ2-3](99B)/TM6, *Ubx* males, which contain the P-element transposase source (Δ2-3) on the third chromosome. The dominant markers in these males (*CyO* = Curly wings, 2nd; *Sp* = Sternopleural bristles, 2nd; *Sb* = Stubble bristles, 3rd; and *Ubx* = Ultrabithorax, 3rd) are used to balance the second and third chromosomes, *i.e.*, each of the markers is always in the heterozygous state, since they are homozygous lethals. See FlyBase (http://Morgan.Harvard.edu) for a complete description of mutant markers and P insertions. Hence, the complete cross, with all genotypes, is:

FEMALES					MALES				
w^{1118} P[w^+]038 / w^{1118} P[w^+]038	+ / +	+ / +	+ / +	X	+ / Y	*CyO* / *Sp*	*ry*^{506} *Sb* P[ry$^+$ Δ2-3](99B) / TM6,Ubx	+ / +

However, a more simplified cross that does not show the location of P[w^+] and Δ2-3 could be presented to students. In this cross, the phenotypically uninformative *ry*, *ry*$^+$, *Sp*, and *Ubx* genes and the multiple-inversion TM6 chromosome should be shown as wild types (+).

FEMALES					MALES			
w / *w*	+ / +	+ / +	+ / +	X	+ / Y	*CyO* / +	*Sb* / +	+ / +

Students should be told that the P-element transposase source (Δ2-3) in males could be on the X, Y, second (*CyO* or *CyO*$^+$), third (*Sb* or *Sb*$^+$), or fourth chromosomes (the latter in a homozygous state), and that the P[w$^+$] insert is in a homozygous state on either the X or an autosome (2nd, 3rd, or 4th) in the females.

From the results of this cross, ask students to determine the chromosomal location of the Δ2-3 P transposase source in the male parents and the location of the P[w$^+$] insert in the female parents. Students could record the F1 results in a table like the one shown below.

The F1 females will have red eyes, because they are $w^{1118}/+$ (w^{1118} is a recessive mutation), whereas, one-half of the F1 males will have eyes with red and white mosaic spots. These mosaic eyes are caused by white spots in which the P[w$^+$] element has excised during fly development, w^{1118} cells, on a background of cells that are red, *i.e.*,

Record Number of F1 Flies with Mosaic Eye Spots

Sex	*Cy* (Curly wings)	*Sb* (Stubble bristles)	*Cy Sb* (Curly & Stubble)	Wild type
Males:				
Females:				

What chromosome contained the P[w$^+$] element? _____
Why?

What chromosome contained the Δ2-3 P transposase? _____
Why?

w^{1118} P[w$^+$]. Note that for cells to be white the P[w$^+$] element has to excise and then not insert into a new chromosomal position, or the P[w$^+$] element has to lose part of the white DNA during a transposition event; such imprecise P-element excisions do occur. The size of white spots with be larger the earlier the P[w$^+$] excisions occur during eye development.

Based on sex linkage and Mendelian genetics, the students should be able to determine that the F1 results could only be possible if the P[w$^+$] element was part of the w^{1118} containing X chromosome in parental females. In addition, the F1 results should allow students to determine the chromosomal location of the Δ2-3 transposase source; only males that have short bristles (either *Sb* or *Cy Sb* flies) will have mosaic eyes. Hence, Δ2-3 must be inserted into the *Sb* containing third chromosome of the parental males.

In introducing this exercise to students, one could review Mendelian genetics, *Drosophila* cytogenetics, sex linkage, and gene symbolism. In addition, gene therapy could be reviewed, including how gene replacement was first performed in *Drosophila* (Spradling and Rubin, 1982; Rubin and Spradling, 1982). This could then lead to a general discussion of transposable DNA elements, how elements such as Alu and *mariner* have been observed to cause gene and chromosomal mutations in humans (Cooper and Krawczak, 1993), and the current status of gene therapy in humans.

The two stocks used in this exercise can be obtained from the National Science Foundation funded Mid-America *Drosophila melanogaster* Stock Center as stocks number 3057 {w^{1118} P[ry$^+$ Δ2-3](99B)} and 3159 {*CyO/Sp*; ry^{506} *Sb* P[ry$^+$ Δ2-3]/ TM6, *Ubx*}. Send requests to Mid-America Drosophila Stock Center, Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio, 43403 or Dmelano@bgnet.bgsu.edu.

References: Cooper, D.N., and M. Krawczak 1993, *Human Gene Mutation*, Bio Scientific Publishers, Oxford; Engels, W.R., 1996, P elements in *Drosophila*. wrengels@facstaff.wisc.edu; Rubin, G.M., and A.C. Spradling 1982, Science 218: 348-353; Spradling, A.C., 1986, P element-mediated transformation. In: Drosophila - *A Practical Approach* (Roberts, D.B., ed.), pp 175-197, IRL Press, Oxford; Spradling, A.C., and G.M. Rubin 1982, Science 218:341-347.

Stock List

The Moscow Regional *Drosophila melanogaster* Stock Center

Dubna, Russia

Version 5-97

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In the following list, the superscripts are denoted by square braskets.

Wild Stocks

001	Algeria
002	Berlin-wild
003	Canton-S
004	Crimea
005	D-32
006	D-18
007	D-32 (from IBD)
008	Formosa
009	Gruta, Argentina

010 lnozemtzeva
011 LA
012 Magarach
013 Oregon-R (South Africa)
014 Oregon-R (Umea, Sweden)
015 Pacific-2
016 P-86
017 Silhonette-8 (+S)
018 iso+S-5(F236)
020 Stellenbosch
021 Ultuna

Chromosome 1 Stocks

1001 bo
1002 br
1003 cho[2]
1004 cv ct v f car
1005 Dp(1)dor Y3T,y[+]/C(1)DX, y f
1006 Dp(1;f)R/l(1)5-39-1 w[e59]
1007 Dp(1;1)w[a], (w[a]/w[a])/C(1)DX, y f
1008 Df(1)S39/FM6, l(1)69a/Dp(1;Y) y[2]67g
1009 g[2]
1010 g[76b]/C(1)DX, y f
1011 g[2] mei-41[A1]f/C(1)DX, y f
1012 l(1)1Aa[7]y[2]w[i]ct f/FM7
1013 l(1)1Aa[17]/FM7
1014 l(1)1Ac[1]y[J1]sc[J1]v f mal su(f)/Df(1)arth In(1)sc[S1L]sc[8R]In(1)dl-49, arth y ac w[a] v[Of] f/y[+] Y
1015 l(1)1Ad[5] y w/FM7
1016 l(1)1Ag y cho sn/FM6, l(1)69a/y[2] Y[611]
1017 l(1)1Bb[20]/FM7
1018 l(1)1Bc[4] w[i] ct f/FM7
1019 y l(1)1Be w[a]/FM3/y[2] Y67g
1020 y[2]l(1)1Bf w[i] ct f/FM6, l(1)69a/y[+] sc Y
1021 l(1)2Cb[3]/FM7
1022 l(1)2Fe/FM6, l(1)69a/B[S] w[+] y[+] Y
1023 l(1)3Ac[104]/FM7
1023.1 l(1)3Ac[104]/FM6, l(1)69a/B[s] w[+] y[+] Y
1024 l(1)3Ba[31]/C(1)DX y f/w[+] y[+] Y
1026 l(1)3E/FM7
1027 l(1)7Dn/FM4, l(1)?ts
1028.1 l(1)Pgd[35] pn/FM4
1028.2 l(1)Pgd[35] pn/C(1)DX,yf/Dp(1;Y)dor3T, y[+] Y
1029.1 l(1)Pgd[39] pn/FM4
1029.2 l(1)Pgd[39] pn/C(1)DX, y w f/w[+] Y
1030.1 l(1)Pgd[71]/FM4
1031 l(1)Pgd[109]/FM4
1032.1 l(1)Pgd[111] pn/FM4
1032.2 l(1)Pgd[111] pn/C(1)DX, y f/w[+] Y
1034 mei-9[AT1]/C(1)DX, y f
1035 mei-9[L1]

1036 mus102[A1] cv v f car/C(1)DX, y f
1037 pn
1038 pn[MS2]l[DES]/FM6/Pgd[-] pn[-] w[+] Y
1039 r[39k] f B/FM6
1040 Basc/rud
1041 N[88c52]/FM7
1042 sn[MR-2]
1043 svr su(w[a])w[a]
1044 Tp(1;2)w-ec,cm ct[6] sn[3]/C(1)DX, y w f
1045 v (LA;LA)
1046 v[87l1S]
1047 w[1]
1048 w[-59K13], Df(1)w Dp(1;3) w[vco]
1050 w[a]
1052 w[a] su(f)
1053 w[Bwx]
1054 w[bl]
1055 w[ch] spl
1056 w[co] sn[2]
1057 w[col]
1058 w[e]
1059 w[h]
1060 w[m4], In(1)3C2-3;20B
1061 w[m51b19], In(I)3C2-3;20D
1063 w[m88c60]/FM7
1064 w[m88c72]/FM7
1065 w[m91l12]/FM7
1067 w[sat]
1068 w[sp]
1069 w[sp2]
1070 w[spA]=w[sp76d]
1071 w mei-41
1072 w mus 101[A1]/C(1)DX, y f
1073 w mus 101[D1] f/C(1)DX, y f/B[S] Y
1074 X[c2], y v/sc[8] Y y[+]
1075 y[1]
1076 y[2]
1077 y[2s]
1078 y[34c]
1079 y[td]
1080 y ac sc pn w[e59]/C(1)DX, y f
1081 y ac z Dp(1;1)w[60h30]/C(1)DX, y w f
1082 XY, y B/C(1)DX, y f/Y
1083 y cv ct v f car
1084 y cv l(1)ts[403]/FM6, v
1085 y ec ct v f/C(1)DX, y f
1086 y mei-9[L1] cv/B[S] Y y[+]
1087 y mei-9[L1] cv mei-41[D5]/C(1)DX, y f
1088 y mus 102[A1] g[2] mei-41[A1] f/C(1)DX, y f
1089 y sc z w[a] ec/C(1)DX, y f
1090 y sc[s1] sc[8] w[a] ("Muller-5")
1090.1 y sc[s1] sc[8] w[a]; T(1;2)y[+89e8]/C(1)DX, y f
1090.2 y sc[S1] sc[8] w[a]; T(1;3?)y[+91l22]/C(1)DX, y w f
1091 y sn[X2] v[Of] B[M1] mal[6],
In(1)dl-49, In(1)B[M1]Df(1)mal[6]/C(1)DX, y f/y[+] Y

mal[+]
1092 y sn[X2] v[Of] B[M1] mal[6],
ln(1)dl-49, In(1)B[M1]Df(1)mal[6]/C(1)RM, y v f mal[2]/
y[+] Y mal[+]
1094 y w
1095 y w[258-45]/FM4
1096 y w[a]/w[+] Y
1097 y w[a] mei-9[a]/C(1)DX, y f/y[+] Y
1098 y[2] ct f mal[5], Df(1)mal[5]/C(1)RM, y v f mal[2]/y[+]
Y mal[106]
1099 y[2] sc car Dp(1)sc[v1], y[+]
1100 y[2] sc w[-] spl/y[+] Y
1101 y[2] sc w[-] spl
1102 y[2] sc w[i] w[ch]/C(1)DX, y f
1103 y[2] su(w[a]) w[a]
1104 y[2] w[a]/C(1)DX, y f
1105 y[2] w[bf] spl ec ct/C(1)DX, y f
1106 y[2] w[bf] spl sn[3]
1107 y[2] w[spA]
1108 z
1109 z Tr(w[+])/C(1)DX, y f

Spontaneous and Radiation-Induced w and y Mutations

w[s002] w[84e]/C(1)DX, y f
w[s003] w[84f]
w[s004] w[87h]
w[s005] w[88c45]
w[s006] w[88d46]
w[s007] w[91l14]
w[s008] w[92l]/C(1)DX, y f
w[s009] w[94l2]/C(1)DX, y f
w1 w[22cH1]
w3 w[66g]
w4 w[67a]
w5 w[67b]
w6 w[67g]/C(1)DX, y f
w7 w[71k]
w8 w[72a29]/C(1)DX, y f
w9 w[72a189]
w10 w[72b], In(1)3C2;8F
w11 w[72d75]/FM7
w12 w[74b], y sc z w[74b]/C(1)DX, y f
w13 w[74b29]/C(1)DX, y f
w14 w[74b166],y[31d] w[74b166]+In(1)FM4
w15 w[74c], T(1;3)3C2; 65B/FM4
w17 w[74c157], y sc z w[74c157/C(1)DX, y f
w18 w[74d10]/C(1)DX, y f
w19 w[74d50], y sc z w[74d50]/C(1)DX, y f
w20 w[74d145], y sc z w[74d145]/C(1)DX, y f
w21 w[74f]
w22 w[74j], y sc z w[74j]/C(1)DX, y f
w23 w[75a], y sc z w[75a]/C(1)DX, y f

w24 w[76a], y sc z w[76a]
w25 w[76b2], y sc z w[76b2]/C(1)DX, y f
w26 w[76b37]
w27 w[76b55], y sc z w[76b55]
w28 w[76b98], y sc z w[76b98]/C(1)DX, y f
w29 w[76b129]
w30 w[76j]
w31 w[76j2], y sc z w[76j2]/C(1)DX, y f
w32 w[76j3]
w33 w[76j10], y sc z w[76j10]
w34 w[76l1]/FM4
w35 w[76l2]
w37 w[77a63]/C(1)DX, y f
w38 w[77a112], y sc z w[77a112]
w39 w[77d], In(1)3C2; 20C/FM4
w40 w[78e]
w41 w[78h], T(1;3)3C2;91E/C(1)DX, y f
w42 w[79b3], In(1)3C2;16D/C(1)DX, y f
w43 w[79B4]
w44 w[79b5], In(1)3C2;2B17/FM4
w45 w[79b6], y sc z w[79b6]/C(1)DX, y f
w46 w[79b7], In(1)3C2;7E/FM4
w47 w[79b8], y sc z w[79b8]
w48 w[79b11]
w51 w[79d5], In(1)3C2;4B
w52 w[79d6]/FM4
w53 w[79f]
w54 w[79g], In(1)3C2;4A/FM4
w56 w[79h3]
w57 w[79h4]
w58 w[79h5]
w59 w[81a], In(1)2F;3C2;b
w60 w[81c40]
w61 w[81c44]
w62 w[81f3], In(1)3C2;18E/FM4
w63 w[81k2]
w64 w[81k3]
w65 w[81k4]
w66 w[81k5]
w67 w[81k6]
w68 w[81k7]
w69 w[81k8]/C(1)DX, y f
w70 w[81k10]
w71 w[81k11], T(1;3)3C2;87F/FM4
w72 w[81k12]
w73 w[82c]
w74 w[83b21]/FM4
w75 w[83b32]/C(1)DX, y f
w76 w[83b38]
w77 w[83b49]
w78 w[83c19], In(1)3C2;20B/C(1)DX, y f
w79 w[83c20]
w80 w[83d13], In(1)3B;3C10/FM4
w81 w[83f10]
w82 w[83f29]

w83 w[83f40]/FM4
w84 w[83f42]/C(1)DX, y f
w85 w[83f49]/FM4
w86 w[83f58]/FM4
w87 w[83k]/C(1)DX, y f
w88 w[84f]
w89 w[84g]
w90 w[85d1], y[31d] w[85d1]
w91 w[85d2], Df(1)2D3;3C3/FM4
w92 w[85e1], T(1;3)3C2;65E5//C(1)DX, y f
w93 w[85f2]/C(1)DX, y f
w94 w[87e36]/C(1)DX, y f
w95 w[87f39]/C(1)DX, y f
w96 w[87g14]
w97 w[87g67]
w98 w[87g75]/C(1)DX, y f
w99 w[87h40]
w100 w[87h83]/C(1)DX, y f
w101 w[88b10]
w102 w[88b25]/FM7
w103 w[88b64], In(1)3C2;4F14/FM7
w104 w[88c34a], T(1;2)3C2;24F8
w105 w[88c34b], T(1;3)3C2;92C2/FM4/y[+] w[+] Y
w106 w[88c78], Df(1)3C2;3C9/FM7
w107 w[88c82]/FM7
w108 w[88c83], Df(1)3C1;3C10/FM7
w109 w[88c87], In(1)3C2;19E
w110 w[88d32]/C(1)DX, y f
w111 w[88d36]/FM7
w112 w[88d39], In(1)3C2;19E+T(1;2)3C1;58E/FM7
w113 w[88d54]/FM7
w114 w[88d80], T(1;3)3C2;81E/FM7
w115 w[88d107], T(1;3)3C2;63E6/C(1)DX, y f
w116 w[88e73], In(1)3C2;4A2/FM7
w117 w[88f18]
w119 w[88f67]/C(1)DX, y f
w120 w[88f83], In(1)3C2;20B/FM7
w121 w[88g5]
w122 w[88g20]/FM7
w123 w[88g23]
w124 w[88g52], Df(1)3C2;3C12/FM7
w125 w[88g97]/C(1)DX, y f
w126 w[w88h75]/FM7
w127 w[m89d20], Df(1)3C1;3C7/FM7
w128 w[89d62]+Df(1)3A4;3B1/FM7
w129 w[89d64]+T(1;3)7A;77B/FM7
w130 w[89d97]
w131 w[89d],Df(1)3B4;3C10/FM7
w132 w[89e12], In(1)3C2;17D
w133 w[89e16], Df(1)2E1;3C3/FM7
w134 w[89e24], Df(1)2E2;3C3/FM7
w135 w[89e32a], Df(1)3A3;3C3/FM7
w136 w[89e32b]
w137 w[89e40], Df(1)3B3;3C3/FM7
w138 w[89e52], In(1)3C2;19E4/C(1)DX, y f

w139 w[89e68]
w140 w[89e72], Df(1)3A3;3C3/FM7
w141 w[89e80]+In(1)5B;19D/FM7
w142 w[89e96a]
w143 w[89e96b], Df(1)3C1;3E7/FM7
w144 w[91l1a]
w145 w[91l1b]+T(1;2)3C5;35F;30C4;20A;3C6;36A/FM4
w146 w[91l5]/FM7
w147 w[91l8], Df(1)2C8;3C3/FM4
w148 w[co91l12]+In(1)2A1;17F+In(1)2D4;12F7/FM7
w149 w[91l14]/FM4
w150 w[91l18], Df(1)3A4;3C3/FM4
w151 w[91l21], In(1)2A2;3C2/FM7
w152 w[91l22]
w153 w[92g13]
y[s000] y[74e3]
y[s001] y[84dS]/C(1)DX, y f
y[s002] y[84e61]
y[s003] y[88c41], Df(1)arth y[88c41] ac/FM7
y[s004] y[88c50]
y[s005] y[88f24]
y[s006] y[88g16]
y[s007] y[89l];br[12]/CyO
y[s008] y[90e15] w[81k6]
y[s009] y[91h1]/C(1)DX, y f
y[s010] y[91h2]
y[s011] y[91i]
y[s012] y[94j]/C(1)DX, y f
y[s013] y[95h]/C(1)DX, y f
y1 y[66c]
y2 y[71k2]
y3 y[71l1]
y4 y[71l2]
y5 y[72a]
y6 y[72d1]
y7 y[72d2]
y8 y[72d4]
y9 y[74b46]
y10 y[74b51], In(1)1B1;20D
y11 y[74b121]
y12 y[74b151]
y13 y[74c166], In(1)1B1;9D
y14 y[74d1]
y15 y[74d2]
y16 y[74d30]
y17 [74d40]
y18 y[74e], In(1)1B1;20D
y19 y[74e2]
y20 y[74k]
y21 y[-75a22-1(2)] w mus-10/C(1)DX, y f/ Dp y[2] Y 67g[19.1]
y22 y[75l1]
y23 y[75l2]
y24 y[75l3]
y25 y[76b37], T(1;2)1B1; 60F4/C(1)DX, y f
y26 y[76b94], In(1)1B1;16C

y27 y[76i]
y28 y[76j]
y29 y[77a]
y30 y[77d]/C(1)DX, y f
y31 y[77f]/C(1)DX, y f
y32 y[78a]/C(1)DX, y f
y33 y[78d], Df(1)1A5;1B2/FM4
y34 y[78j], T(1;3)1B1;81F/C(1)DX, y f
y35 y[79b18]
y36 y[79d-sd]
y37 y[79d], T(1;2)1B1;23E/FM4
y38 y[79d1]/FM4
y39 y[79d2]
y40 y[79d3]
y41 y[79g]
y42 y[79h2], Df(1)1B1;1B5/FM4
y43 y[81c1]
y44 y[81c2]
y45 y[81c3]
y46 y[82c1]/C(1)DX, y f
y47 y[82c2]
y48 y[82c3]
y49 y[83c]/FM4
y51 y[83f12]
y52 y[83f26]
y53 y[83f58]
y54 y[84f]/C(1)DX, y f
y55 y[85b]
y56 y[85c1]/FM4
y57 y[85c2]/FM4
y58 y[85f2], In(1)1B1;11E3/FM7
y59 y[85f3], T(1;2)1B1;28B/FM4
y60 y[87e45]
y61 y[87e85], In(1)1B1;3A5/FM7
y62 y[87f14]
y63 y[87f72]
y64 y[87g49]+In(1)7C;20D
y65 y[87g74]
y66 y[87g92]
y67 y[87h50]/C(1)DX, y f
y68 y[88c60]
y69 y[88c63], In(1)1A8;18D2/FM4
y70 y[88c93]
y71 y[88c104], In(1)1B1;1E5
y72 y[88d48a]
y73 y[88d48b], In(1)1B1;1B8
y74 y[88d100]
y75 y[88d102]/FM7
y76 y[88e20]
y77 y[88e24], Df(1)1A3;1B5/FM7
y78 y[88e66]
y79 y[88e75]
y80 y[88f17]/C(1)DX, y f
y81 y[88f86], In(1)1A8;19E8/FM7
y82 y[88g56]

y83	y[88g98]
y85	y[88h41]/FM7
y86	y[89d36], In(1)1B1;2B17+In(1)2C1;7C19
y87	y[89d68], In(1)1A8;20A
y88	y[89d72]
y89	y[89e20]
y90	y[89e56]
y91	y[89e60]
y92	y[89e100]
y93	y[91l15], In(1)1B1;4A5/FM7
y94	y[91l18]
y95	y[91l20]
y96	y[92g24]

Chromosome 2 Stocks

2000	b cn vg
2001	b cn vg[B]/ b In(2R) bw[VDe1]
2002	b In(2) bw[VDe1]/b lt l cn mi sp
2003	b j
2004	In(2LR)Pm, b bw[V1]/In(2LR)Cy, net dp[txI] Cy b pr Bl lt[3] cn[2] L[4] sp[2]
2005	b so[2] (cn?)
2006	b vg
2008	bw
2009	bw[D]
2010	cn bw
2011	cn bw mus 205[A1]
2012	cn
2013	j
2014	z w[11E4]/y[+] Y; l(2)br38[TE94]=l(2)34Da/CyO
2015	l(2)br20[SF2]=l(2)34Db Adh[nf3] rd[s] pr cn/CyO
2016	b l(2)brS[SF22]=l(2)34Dc Adh[n4]/Cy0
2017	l(2)br32[S37]=l(2)34Dd/Cy[2004]
2018	l(2)br16[SF1]=l(2)34Dc Adh[nf3] rd[s] pr cn/CyO
2019	l(2)br39[BG1]=l(2)34Df/CyO
2020	b l(2)br17[SF10]=l(2)34Dg Adh[n4]/Cy Bl
2021	l(2)br24[SF15]=l(2)34Ea Adh[n4]/CyO
2022	l(2)br31[CH61]=l(2)34Eb Adh[D] pr cn/CyO Adh[nB]
2023	l(2)br30[HG15]=l(2)34Fa Adh[n1] rd[S] pr cn l(2)CA3 l(2)CA8/CyO
2024	b l(2)br1=wb[SF25]=l(2)34Fb Adh[n2] pr cn/CyO, Cy dp[lv1] pr cn[2]
2025	b l(2)br8[SF9]=l(2)34Fc Adh[n4]/CyO
2026	b l(2)br15[CR5]=l(2)34Fd Adh[n2] pr cn l(2)CA3/CyO
2027	ms(2)br40[1715]=l(2)34Fe/CyO
2028	l(2)br12[HG8]=l(2)35Aa Adh[n1] cn bw/CyO
2029	b l(2)br22[HG46]=l(2)35Ba pr/CyO
2030	b l(2)br3[AR2]=l(2)35Bb pr/Cy Bl
2031	Adh[n1] l(2)br4[HG1]=l(2)35Bc rd[S] pr cn l(2)CA2/CyO
2032	b l(2)br9[AR4]=l(2)35Bd pr cn/Pm b
2033	b l(2)br10[AR3]=l(2)35Be pr/SM5
2034	Adh[n7] l(2)br2[HG27]=l(2)35Bf cn vg/CyO
2035	Adh[n11] l(2)br26[HG21]=l(2)35Bg cn vg/CyO

2036 Adh[n10] l(2)br7[HG36]=l(2)35Bh cn vg/CyO
2037 Adh[n7] l(2)br27[HG29]=l(2)35Ca cn (vg)/CyO
2038 Adh[n10] l(2)br33[HG38]=l(2)35Cb cn vg/CyO
2039 Df(2L)rd[9], Sco l(2)br50[AM7]=l(2)35Cc/CyO
2040 Adh[n10] l(2)br34[HG39]=l(2)35Cd cn vg/CyO
2041 b l(2)br43[VS2]=l(2)35Ce pr cn bw/SM5
2042 l(2)br35[B7]=l(2)35Da cn bw sp/CyO
2043 Adh[n7] l(2)br28[HG31]=l(2)35Db cn (vg)/CyO
2044 Adh[n7] l(2)br36[HG34]=l(2)35Dc cn (vg) l(2)CA5/CyO
2045 b l(2)br37[P28]=l(2)35Dd pr cn bw/CyO
2046 In(2L)NS, l(2)br46[AM2]=l(2)35De/J Bl[+]
2047 b l(2)br44[P15]=l(2)35Df pr cn bw/CyO
2048 b l(2)br45[P29]=l(2)35Dg pr cn bw/CyO
2049 In(2L)dpp[36]Sp Bl, Dp(2;2)DTD48, ho[2] l(2)br47[AM3]= l(2)35Ea/Gla vg[st]
2050 In(2L)C163, l(2)br48[AM4]=l(2)35Eb, l(2)CA7/CyO
2051 T(2;3)DTD14, dpp[4] l(2)br49[AM5]=l(2)35Ec/CyO
2052 MR[h12]/Cy
2053 nub b pr
2054 or[45a] sp[2]
2055 phr
2056 pu
2057 rk cn bw
2058 sca
2059 sca l(2)C/SM5
2060 so
2061 vg
2062 vg[np]
2063 y[D]/SM5
2064 Gla vg[st]/CyO

Spontaneous and Radiation-Induced *b*, *cn* and *vg* Mutations

b[s001] b[87g3]
b[s002] b[87h6]/Cy[2004]
b[s003] b[88c30]
b[s004] b[88d21]
b[s005] b[88g16]
b[s006] b[88g29]
b[s007.1] b[91l10a]
b[s007.2] b[91l10a] vg
b[s008] b[91l10b]
b[s009] b[91l11]
b[s010] b[94l13]
b1 b[66a]
b2 b[71K1], In(2LR)34D4;43C2/SM5
b3 b[71k2]
b4 b[74b2]
b5 b[74b4]
b6 b[74b5]
b7 b[74c2]
b8 b[74c4]/Cy[2004]
b10 b[74c6], Df(2L)34D4-8/Cy[2004]

b11 b[74d4]
b12 b[74d6]
b13 b[75a]
b14 b[76b1]
b16 b[76e1]
b17 b[76e2]
b18 b[76j1]
b19 b[76j2]
b20 b[76j3]
b21 b[76k1]
b22 b[76k2]
b23 b[77a1]
b24 b[77a2]
b25 b[77a3]
b26 b[77a4]
b27 b[77a5]
b28 b[77c], Df(2L)34D4;34F/Cy[2004]
b29 b[77j]
b30 b[78a]
b31 b[78f1]
b32 b[78f2]
b33 b[78g]
b34 b[78j], Df(2L)34D4;35A/SM5
b35 b[78k1]
b36 b[78k2]
b37 b[78k3]
b38 b[78k5]
b39 b[79a1]
b40 b[79a3]/Cy[2004]
b41 b[79a4]
b42 b[79b1]
b43 b[79b3], Df(2L)34D4;35A/Cy[2004]
b44 b[79b4], Df(2L)34D4;34E/SM5
b45 b[79d2]
b46 b[79d5], In(2L)34D4;35B10/SM5
b47 b[79d6], T(2;3)34A;80C;79C;34D6/Cy[2004]
b48 b[79d8]
b49 b[79d10]
b50 b[79d11]
b51 b[79d13]
b52 b[79f2]
b53 b[79g2]
b55 b[79h2]
b56 b[79h3]
b57 b[80k], Df(2L)34D4;35B10/CyO
b58 b[80l], Df(2L)34D4;34E3/Cy[2004]
b59 b[81a], In(2LR)34D4;41D/SM5
b60 b[81a2]
b61 b[81c]
b62 b[81c2]
b63 b[81c17]
b64 b[81f2], Df(2L)34D4;34F2/SM5
b65 b[81f3], In(2L)34D4;35B10
b66 b[81k]
b67 b[81l7], In(2L)34D4;40F/SM5

b68 b[81l40]/SM5
b69 b[81l42], Df(2L)34D4;34D8/SM5
b72 b[82c16]
b73 b[82c44], In(2L)34D4;40F/SM5
b74 b[82c54] (vg)/SM5
b75 b[83b11] (vg)/SM5
b76 b[83b22], In(2L)34D4;35B8 (vg)/SM5
b77 b[83b40]
b78 b[83c20]
b79 b[83c25]
b80 b[83c26] (vg)
b81 b[83c35a]/SM5
b82 b[83c35b]
b83 b[83c36]
b84 b[83c47]
b85 b[83d29a], Df(2L)34D4;35E2/SM5
b86 b[83d29b]
b87 b[83d35]
b88 b[83d36]
b89 b[83f17]
b90 b[83f18]
b92 b[83f52]
b93 b[83fXD]
b94 b[83l1]/SM5
b95 b[83l2], Df(2L)34D4;34E2/SM5
b96 b[83l3]
b97 b[84g]
b99 b[84h70] Bl/Pm b
b100 b[85b1], Df(2L)34D4;34E2/SM5
b101 b[85b2]/SM5
b102 b[85b3]
b103 b[85b4]/Cy[2004]
b104 b[85c1], Df(2L)34D4;34F2/CyO
b105 b[85c2], T(2;3)34C7;34E2;95C4/Cy[2004]
b106 b[85f1], Df(2L)34D4;34E5/SM5
b107 b[85f2], Df(2L)34D1;34E5/SM5
b108 b[87e25], Df(2L)34C1;35B10/CyO
b109 b[87e58]
b110 b[87e128]/Cy[2004]
b111 b[87e152], In(2LR)34D4;41A/Pm b
b112 b[87f2]/Cy[2004]
b113 b[87f115]/Cy[2004]
b114 b[87f124]
b115 b[87f144]
b116 b[87g23], Df(2L)34D4;34F2/Pm b
b117 b[87g62/Cy[2004]
b118 b[87g65]
b119 b[87h22]
b120 b[87h38]/Pm b
b121 b[88b12]
b122 b[88b15], Df(2L)34C7;35E2/CyO
b123 b[88b42]/Cy[2004]
b124 b[88b62]
b125 b[88c4]
b126 b[88c8]

b127 b[88c24]/Cy[2004]
b128 b[88c25], Df(2L)34C1;35B4/CyO
b129 b[88c58], Df(2L)34D4;35C2/CyO
b130 b[88c73]/Pm b
b131 b[88c75], Df(2L)34C5;35E1/CyO
b132 b[88c107]
b133 b[88c112]
b134 b[88d12]
b135 b[88d28a]
b136 b[88d28b] + In(2L)35C;40A/Cy[2004]
b137 b[88d39]
b138 b[88d50]
b139 b[88d70]
b140 b[88e16], In(2L)34D4;36C10/CyO
b141 b[88e32], Tp(2)34D6;51D11;50C14+Tr(2)34D4;40C +
Tr(2)40D;50C12/Cy[2004]
b142 b[88e45], In(2L)34D4;40E/Cy[2004]
b143 b[88e55]
b144 b[88e71]
b145 b[88e88], T(2;3)34D4;72A1+T(2;3)34D2;80D+
+T(2;3)40D;71F4/Pm b
b146 b[88f5]+In(2R)52D12;60B2/Cy[2004]
b147 b[88f32], Df(2L)34D4;34E3/Cy[2004]
b148 b[88f40], Df(2L)34D4;34F4/Cy[2004]
b149 b[88f43], Df(2L)34D4;34E5/Cy[2004]
b150 b[88f69]/Cy[2004]
b151 b[88g4], Tp(2)34D3;56F16;48E1+T(2;3)34D4;62F5;48E1;63A1/Gla
b152 b[88g15]
b153 b[88g22], T(2;Y)34D4;Y + T(2;3)34D5;87F8 + In(2R)41A;52A14 +
Tp(3)69D2;92A2;93A6/Cy[2004]
b154 b[88g26], Df(2L)34D3;35A2 + T(2;4)33B2;101E/CyO
b155 b[88g41]
b156 b[88g68], Df(2L)34D3;35B2 + T(2;3)34D2;76A6/CyO
b157 b[88g83]/Cy[2004]
b158 b[88g96], Df(2L)34D3;34F2/Pm b
b159 b[88g97] + Df(2R)42A14;42B4/CyO
b160 b[88g98]/Cy[2004]
b161 b[88h36]
b162 b[88h49], Df(2L)34C7;35A4/CyO
b163 b[89d32]/Cy[2004]
b164 b[89d48]
b165 b[89d88]
b166 b[89e8], Df(2L)34D4;34E6/Cy[2004]
b167 b[89e12], T(2;3)34D4;79C3/Cy[2004]
b168 b[89e20]/Cy[2004]
b169 b[89e60]
b170 b[89e64a], In(2LR)34D4;45F6/Cy[2004]
b171 b[89e64b], Df(2L)34C7;34E2 + T(2;3)47C4;69C5/CyO
b172 b[89e64c] + T(2;3)55E10;98C3/Cy[2004]
b173 b[89e68], Df(2L)34D4;35A2 + T(2R;Y)60F4/Pm b
b174 b[89e72], Df(2L)34D4;34F4/CyO
b175 b[89e80a], Df(2L)34C3;35B10/CyO
b176 b[89e80b], Df(2L)34D4;35B10/Cy[2004]
b177 b[89e88a], Df(2L)34D1;35D7/CyO
b178 b[89e88b], Df(2L)34C4;35B7/CyO

b179 b[89e96], Df(2L)34D4;34F4/CyO
b180 b[89e100], In(2L)34D4;40E/Pm b
b181 b[91l4]/CyO
b182 b[91l5], Df(2L)34D3;34F2/Cy[2004]
b183 b[91l7], T(2;3)34D4;61D/Pm b
b184 b[91l9], In(2L)34D4;40F/CyO
b185 b[91l13]
b186 b[91l14] + In(2R)41A;60A2 + T(2;3)60A4;65F/Cy[2004]
b187 b[91l16]
b188 b[91l24]/Cy[2004]
b189 b[92g18]/Cy[2004]
cn[s001] cn[88c23]
cn[s002] cn[91l5]
cn[s003] cn[94l9]
cn1 cn[67d]
cn2 cn[74b1]/Cy[2004]
cn3 cn[74b2] vg/Cy[2004]
cn4 cn[74b3]/Cy[2004]
cn5 cn[74b4]
cn6 cn[74c3], Df(2R)43D5;43F7/Cy[2004]
cn7 cn[74c5]
cn8 cn[74c6], Df(2R)43D5;43F7/Cy[2004]
cn9 cn[74c7], T(2;3)43E15;98A12
cn10 cn[74c8]
cn11 cn[74c9]
cn13 cn[74d1]
cn14 cn[74d4]
cn15 cn[74d5], In(2LR)24C4;43E15
cn16 cn[74d7]
cn17 cn[74d8]/Cy[2004]
cn18 cn[76b]
cn19 cn[76e2]
cn20 cn[76i]/SM5
cn21 cn[76k1]/CyO
cn22 cn[76k2]
cn23 cn[76k3], Df(2R)43B1;44B7/SM5
cn24 cn[77a2]
cn25 cn[77a3]
cn27 cn[77a6], Df(2R)43E1;43F6/SM5
cn28 cn[77a9]
cn29 cn[77a10]
cn30 cn[77c2]
cn31 cn[78a]
cn32 cn[78b1]
cn33 cn[78f] vg
cn34 cn[78g]
cn35 cn[78j2]
cn36 cn[78k1]/CyO
cn37 cn[78k5], Df(2R)43E5;44B2/Cy[2004]
cn38 cn[78l]
cn39 cn[79b5]
cn40 cn[79b7]
cn41 cn[79b8], In(2R)43E15;56D2/CyO
cn42 cn[79b9], Df(2R)43E7;43E18/SM5
cn43 cn[79b10] vg/CyO

cn44 cn[79b11]+Df(2L)22A3;22D/Pm b
cn45 cn[79b13], Df(2R)43E2;43E18/SM5
cn46 cn[79b15]
cn47 cn[79b18], Df(2R)43D1;43E15/Gla vg[st]
cn48 cn[79c]/CyO
cn49 cn[79d1]
cn50 cn[79d2]/CyO
cn51 cn[79d6]
cn52 cn[79d9], Tp(2R)41A;43F1;60A;41B;43E15;60B/Pm b
cn53 cn[79d12]/Pm b
cn54 cn[79d15] vg
cn55 cn[79d18]
cn56 cn[79h1]
cn57 cn[79h2]/SM5
cn58 cn[79h3]
cn59 cn[79h5]/SM5
cn60 cn[79h7]/SM5
cn62 cn[81a1]
cn63 cn[81a2]+In(2L)22D;36C/SM5
cn64 cn[81c21a] vg/CyO
cn65 cn[81c32] vg
cn66 cn[81c51]
cn67 cn[81f1], In(2R)41B;43E16
cn68 cn[81f3]/SM5
cn69 cn[81f5]+Df(2R)42A5;42B1+T(2L;Y)37D4/Cy[2004]
cn70 cn[81k1]
cn71 cn[81k2]
cn72 cn[81k3], Df(2R)43D1;43F8/SM5
cn73 cn[81k4], Df(2R)43D4;43E18/SM5
cn74 cn[81k5]
cn75 cn[81l], In(2R)43E1;43E18/CyO
cn76 cn[81l1], Df(2R)43E5;43F8/SM5
cn77 cn[81l2], Df(2R)43E4;43E18/SM5
cn78 cn[81l4]/Cy[2004]
cn79 cn[82c1]
cn80 cn[82c33] + In(2LR)28B;45B/SM5
cn81 cn[82c41] vg
cn82 cn[82c63] vg
cn83 cn[83b27] + In(2L)22C;36C/SM5
cn84 cn[83b31]
cn85 cn[83b33], Df(2R)43D1;44B2/SM5
cn86 cn[83c17] + In(2R)41A;42E/Cy[2004]
cn87 cn[83c23], Df(2R)43D1;44B6/SM5
cn88 cn[83c58]
cn89 cn[83d15]
cn90 cn[83d21], Df(2R)43D4;43F8/SM5
cn91 cn[83f16]
cn92 cn[83f17]
cn93 cn[83f50]
cn94 cn[83f51]
cn95 cn[83l], Df(2R)43C3;43E18/SM5
cn96 cn[84f37]
cn97 cn[84f76]
cn98 cn[84g]
cn99 cn[84h], Df(2R)43C1;44A7/SM5

cn100 cn[84h29], Df(2R)43C1;44B9 + T(2R;Y)56F16/Cy[2004]
cn101 cn[84h80], Df(2R)43E5;43F7/SM5
cn102 cn[85b1]
cn103 cn[85b2] vg 1 ?/Cy[2004]
cn104 cn[85b3]
cn105 cn[85c1], Df(2R)43D3;43E16/SM5
cn106 cn[85c2], Df(2R)43D5;43F2/SM5
cn107 cn[85d3], Df(2R)43E5;43E16/SM5
cn108 cn[85e1], Df(2R)42E4;43E16/SM5
cn109 cn[85e2]
cn110 cn[85e3], Df(2R)43D4;43E16/SM5
cn111 cn[85f2], Df(2R)43C7;43E16/CyO
cn112 cn[85f4], Df(2R)43D4;43E16/SM5
cn113 cn[87e62]
cn114 cn[87e80] vg/Cy[2004]
cn115 cn[87e94], Df(2R)43D7;43E18/Cy[2004]
cn116 cn[87e130]
cn117 cn[87e150]
cn118 cn[87e158]
cn119 cn[87f65], Df(2R)42C1;43F8/Gla vg[st]
cn120 cn[87f99]
cn121 cn[87f147]/CyO
cn122 cn[87f149]/Cy[2004]
cn123 cn[87f165]
cn124 cn[87g12]
cn125 cn[87g18], Df(2R)43D7;44B7/Pm b
cn126 cn[87g54]
cn127 cn[87h32]/Cy[2004]
cn128 cn[88b37]
cn129 cn[88b44] + Df(2R)42A;42E/CyO
cn130 cn[88c3]
cn131 cn[88c25], In(2R)43E14;49B11/Gla vg[st]
cn132 cn[88c36], Df(2R)42B5;44B4/Gla vg[st]
cn133 cn[88c81]
cn134 cn[88c83] + In(2R)50B;56B/Pm b
cn135 cn[88c84]
cn136 cn[88c90], Df(2R)43C3;43F8/Cy[2004]
cn137 cn[88c99], In(2R)41A;43E15
cn138 cn[88c102]/Cy[329]
cn139 cn[88d6], Df(2R)43C4;44B8/CyO
cn140 cn[88d7]/Cy[2004]
cn141 cn[88d29]/Cy[2004]
cn142 cn[88d33] vg
cn143 cn[88d64], Df(2R)43E2;43F4/Cy[2004]
cn144 cn[88d78]
cn145 cn[88e7]
cn146 cn[88e30], In(2LR)40E;43E17/Cy[2004]
cn147 cn[88e43], Df(2R)43A1;44B3 + T(2;3)43A1;93B11/CyO
cn148 cn[88e59]
cn149 cn[88e69], Df(2R)42B4;43E17/Gla vg[st]
cn150 cn[88f5], Df(2R)43D7;43E18/Cy[2004]
cn151 cn[88f29], T(2;3)43E15;70F/CyO
cn152 cn[88f34a], Df(2R)43B1;44B9/CyO
cn153 cn[88f34b], Df(2R)43E10;44A2/Cy[2004]
cn154 cn[88f74]

cn155 cn[88f84], In(2R)41A;43E17/Cy[2004]
cn156 cn[88f87], Df(2R)43D1;44A7/Cy[2004]
cn157 cn[88f93], Df(2R)42D1;44B4, vg/CyO
cn159 cn[88g42b] vg
cn160 cn[88g43], Df(2R)43E5;43E18/Cy[2004]
cn161 cn[88g53] + In(2L)23B1;35D4/Gla vg[st]
cn162 cn[88g69]/CyO
cn163 cn[88g74]
cn164 cn[88g76], Df(2R)43E1;43E18/Gla vg[st]
cn165 cn[88g77]
cn166 cn[88g88], Df(2R)43D1;43E18/Cy[2004]
cn167 cn[88g91], In(2R)41D4;43E18/Cy[2004]
cn168 cn[88h2], Df(2R)43E1;43F7/Cy[2004]
cn169 cn[89d4], Df(2R)42B4;44B7/CyO
cn170 cn[89d64], Df(2R)43C1;44B9/Cy[2004]
cn171 cn[89d88] vg
cn172 cn[89d99], Df(2R)42B4;43F7/CyO
cn173 cn[89e8], Df(2R)42F1;43E18/CyO
cn174 cn[89e12], Df(2R)43E5;44B8/CyO
cn175 cn[89e15]
cn176 cn[89e16], Df(2R)43E1;43E18/Cy[2004]
cn177 cn[89e20], Df(2R)43C1;44B7/CyO
cn178 cn[89e44], Df(2R)42E6;44B7/CyO
cn179 cn[89e48], T(2;3)43E15;62C3/Cy[2004]
cn180 cn[89e52], Df(2R)42F1;44B7/CyO
cn181 cn[89e72a] vg/Cy[2004]
cn182 cn[89e72b] vg/Cy[2004]
cn183 cn[89e76a], Df(2R)43E10;44B7/Cy[2004]
cn184 cn[89e76b] + T(2;3)41A;64A5/Cy[2004]
cn185 cn[89e84], Df(2R)43A1;43E16/Cy[2004]
cn186 cn[89e104], Df(2R)43C8;44B7/Cy[2004]
cn187 cn[91l4a], Df(2R)43D1;44B6/Pm b
cn188 cn[91l4b], Df(2R)43C5;43F6/Cy[2004]
cn189 cn[91l8], Df(2R)42E4;44C5/Gla vg[st]
cn190 cn[91l13a], Df(2R)42E;43E16/Cy[2004]
cn191 cn[91l13b], In(2LR)34E6;43E15/Cy[2004]
cn192 cn[91l14]
cn193 cn[91l28], In(2R)41C;43E15/Cy[2004]
cn194 cn[92g4]/Cy329
cn195 cn[92g12a], Df(2R)43C1;43F8/Pm b
cn196 cn[92g12b], Df(2R)43C1;43F8/Pm b
vg[s001] vg[ni88d44]/Cy[2004]
vg[s002] vg[88d38]
vg[s003] vg[88e18]
vg[s004] vg[88e26]/Cy[2004]
vg[s005.1] vg[88g10]
vg[s005.2] vg[88g10SR]
vg[s006] vg[88g32]
vg[s007] vg[88h7]
vg[s008] vg[89c1]
vg[s009] vg[91i13]
vg[s010] vg[91l2]
vg[s011] vg[91l9]
vg[s012] vg[91l22]/Cy[2004]
vg[s013] vg[92g18]

vg1 vg[67d2]/SM5
vg2 vg[72a1], In(2R)44C3;49D4/Pm b
vg3 vg[74b1], In(2LR)37F2;49D4/Cy[2004]
vg4 vg[74c1]/CyO
vg5 vg[74c4], In(2LR)22A5;49D4/CyO
vg6 vg[74c5]
vg7 vg[74c6]/Cy[2004]
vg8 vg[74c7]/Cy[2004]
vg9 vg[76d1]/Cy[2004]
vg10 vg[76d2], Dp(2R)58B;58D +T(2R;Y)58B/vg[83b27]
vg11 vg[76i1], T(2;3)49D4;84E3/Cy[2004]
vg12 vg[76i2]
vg13 vg[76j1], Tp(2R)49D2;60B;60A;51A;59F2;50F;49E;60C/Pm b
vg14 vg[77a4]/Cy[2004]
vg15 vg[77d1], In(2LR)25C;49D4/Cy[2004]
vg16 vg[78a1], In(2R)41D;49D4/SM5
vg17 vg[78a2], In(2R)49D4;56E/Cy[2004]
vg18 vg[78b1]/Cy[2004]
vg19 vg[78b2]
vg20 vg[78b3]
vg21 vg[78b4], T(2;3)49D4;80C/Pm b
vg22 vg[78f2]/Cy[2004]
vg23 vg[78j1], Df(2R)49D4;50C4/CyO
vg24 vg[78j3], In(2R)41D;49D4/SM5
vg25 vg[78k3], In(2R)49D4;59D4/Cy[2004]
vg26 vg[79a], Df(2R)49C3;49E6 + In(2LR)34B2;49C2/Pm b
vg27 vg[79b1]
vg28 vg[79b3], Df(2R)49C3;49E1 + T(2;3)49C2;94A3/SM5
vg29 vg[79b4], In(2R)41C;49D4/Cy[2004]
vg30 vg[79d2], Df(2R)49D1;49D7/SM5
vg31 vg[79d3], Tp(2R)41A;49D4/SM5
vg32 vg[79d4], In(2R)41E;49D4/Cy[2004]
vg33 vg[79d5]
vg34 vg[79d6]/Gla vg[st]
vg35 vg[79d7], In(2R)41D;49D4/Cy[2004]
vg36 vg[79d8], Df(2R)49D1;49D7/SM5
vg37 vg[79f1]/Gla vg[st]
vg38 vg[79h1]/Cy[2004]
vg39 vg[79h4], In(2LR)24C;49D4/Pm b
vg40 vg[79h5], In(2R)49D4;50A2/Cy[2004]
vg41 vg[79h6], In(2R)41E;49D4 + In(2R)42B2;57F/Pm b
vg42 vg[80l1], Df(2R)49D2;49E7/Cy[2004]
vg43 vg[80l2]/CyO
vg44 vg[81a], T(2;3)49D4;64B2/SM5
vg45 vg[st81a]/SM5
vg46 vg[81b1], In(2R)48C4;49D4/SM5
vg47 vg[81c13]
vg48 vg[81c28], In(2R)41D;49D4/CyO
vg49 vg[81c41d], In(2R)49B12;49D4/Cy[2004]
vg50 vg[81k1]/Cy[2004]
vg51 vg[81l18], In(2LR)36C4;49D4/SM5
vg52 vg[81l24]
vg53 vg[82c13]
vg54 vg[82c14], In(2LR)36D;49D4/Pm b
vg55 vg[82c61], In(2LR)24E2;49D4/SM5

vg56 vg[83b], Df(2R)49B3;49E7/Cy[2004]
vg57 vg[83b24]/Cy[2004]
vg58 vg[83b27]
vg59 vg[83b39], In(2R)49D4;51D2/Cy[2004]
vg60 vg[83c], T(2;3)49D4;65F6/Cy[2004]
vg61 vg[83c3], In(2R)41C;49D4/Cy[2004]
vg62 vg[83c5]/Cy[2004]
vg63 vg[83c7]/Cy[2004]
vg64 vg[83c24]/Cy[2004]
vg66 vg[83c42]/Cy[2004]
vg67 vg[83c43], In(2R)43C3;49D4/Cy[2004]
vg68 vg[83c45]/CyO
vg69 vg[83d]/Cy[2004]
vg70 vg[83d4], In(2R)48F3;49D4/Cy[2004]
vg71 vg[83f15], Df(2R)49D1;49E7/SM5
vg72 vg[83f36], Df(2R)49D1;49E7/CyO
vg73 vg[83f38], In(2R)49D4;59D/SM5
vg74 vg[83fxD], In(2R)48F2;49D4/Cy[2004]
vg75 vg[83l1]/SM5
vg76 vg[83l2]/SM5
vg77 vg[83l3a], Df(2R)49D3;49D7/SM5
vg78 vg[83l3b], In(2R)41E;49D4/SM5
vg79 vg[84f]/SM5
vg80 vg[84f51], In(2R)44F2;49D4/Cy[2004]
vg81 vg[84f65], In(2R)49C4;49E7/SM5
vg82 vg[84h], In(2R)41B;49D4/Pm b
vg83 vg[84h49], Df(2R)49B5;49F10/Cy[2004]
vg84 vg[84hIX], In(2R)48E6;49D4/Pm b
vg85 vg[85b]
vg86 vg[85c], In(2R)41B;49E6/SM5
vg87 vg[85d1]/Cy[2004]
vg88 vg[85d2], T(2;3)49D4;84F4/SM5
vg89 vg[85e2], Tp(2R)41B;49D5;55F/Cy[2004]
vg90 vg[85e3]/Cy[2004]
vg91 vg[85e4] + In(2R)50C11;56C9/Cy[2004]
vg92 vg[85f1], Df(2R)49C1;49E7/Cy[2004]
vg93 vg[85f2], Df(2R)49C1;49E7/Cy[2004]
vg94 vg[85f3]
vg95 vg[87e12], Df(2R)49C1;49E7/Cy[2004]
vg97 vg[87e39]
vg98 vg[87e46]/Cy[2004]
vg99 vg[87e80]/Cy[2004]
vg100 vg[87e90], Df(2R)49D1;49E7
vg101 vg[87e95]/Cy[2004]
vg102 vg[87e131]
vg103 vg[87e140]/Cy[2004]
vg104 vg[87e148], Df(2R)49D4;50C6/CyO
vg105 vg[87f50], Df(2R)49D4;49F14/CyO
vg106 vg[87f96]/Gla
vg107 vg[87f98], Df(2R)49C1;50A12/CyO
vg108 vg[87f147]/CyO
vg109 vg[87f155]
vg110 vg[87g20], In(2R)41C;49D4/Cy[2004]
vg111 vg[87g22], In(2R)41D;49D4/CyO
vg112 vg[87g24]

vg113 vg[87g27], Df(2R)49D1;49D7/CyO
vg114 vg[87g43], In(2R)49D4;60B12/Cy[2004]
vg115 vg[87g50]/Cy[2004]
vg116 vg[87g77]/Cy[2004]
vg117 vg[87h1], T(2;3)49D4;81F/CyO
vg119 vg[87h31] + T(2;Y)41F + In(3R)81D;82E/Cy[2004]
vg120 vg[87h42], In(2R)49D4;56F9 + T(2;Y)56F9/Cy[2004]
vg122 vg[87h49], Df(2R)49C1;49E7/Cy[2004]
vg123 vg[87h55], In(2R)41D;49D4 + T(2;3)41D;90E1/CyO
vg124 vg[88b10]
vg125 vg[88b15], In(2LR)40E;49D4/Cy[2004]
vg126 vg[88b32]
vg127 vg[88b59]/Cy[2004]
vg128 vg[88c1], In(2LR)36E;49D4/Gla vg[st]
vg129 vg[88c3]/CyO
vg130 vg[88c25], T(2;3)49D4;70C4/CyO
vg131 vg[88c28], Df(2R)49D4;49F6/Cy[2004]
vg132 vg[88c30]/Cy[2004]
vg133 vg[88c42], T(2;3)49D4;81E/CyO
vg134 vg[88c45]
vg135 vg[88c62] + Ins(2LR)27C;41A;52D/CyO
vg136 vg[88c64]/Cy[2004]
vg137 vg[88c72], In(2LR)30B;41A;49D4/CyO
vg138 vg[88c87a], Ins(2R)41A;49D4;59A/Cy[2004]
vg139 vg[88c87b]/Cy[2004]
vg140 vg[88c94], In(2LR)36C10;49D4/Gla vg[st]
vg141 vg[88c96]/Cy[2004]
vg142 vg[88c102]/Cy[2004]
vg143 vg[88d4], In(2R)41C;49D4/Pm b
vg144 vg[88d20], In(2R)49A6;49D4/Gla vg[st]
vg145 vg[88d24], T(2;3)49D4;49F14;70C/Gla vg[st]
vg146 vg[88d43], In(2L)22A;34A + In(2R)41D;49D4/Cy[2004]
vg147 vg[88d101]/Cy[2004]
vg148 vg[88e28], In(2R)49D4;60F1/Cy[2004]
vg149 vg[88e55], In(2LR)22A8;49D4 + In(2LR)37A6;55C4/Cy[2004]
vg150 vg[88e76], In(2LR)34A10;49D4/Cy[2004]
vg151 vg[88e94] + In(2LR)35B10;41D/CyO
vg152 vg[88f18], In(2R)49D4;54C4/Cy[2004]
vg153 vg[88f21] + T(2;3)60F3;86D2/Cy[2004]
vg154 vg[88f31], T(2;3)49D4;80C2/Cy[2004]
vg155 vg[88f33]/Cy[2004]
vg156 vg[88f58], T(2;3)49D4;80D5;76B1/CyO
vg157 vg[88f66]/Cy[2004]
vg158 vg[88f80], Df(2R)49D4;50A14/Gla vg[st]
vg159 vg[88g5], Df(2R)49A12;49D7 + In(2R)49A12;51E10/Cy[2004]
vg160 vg[88g26] + Ins(3LR)68C10;80C;87E11/Cy[2004]
vg161 vg[88g33], Ins(2LR)39D5;49D4;59F10/Cy[2004]
vg162 vg[88g38], Df(2R)49B1;49E7/CyO
vg163 vg[88g40]/Cy[2004]
vg164 vg[88g53], T(1;2)19F;49D4/Gla vg[st]
vg165 vg[88g80], In(2LR)25E6;49D4/Cy[2004]
vg166 vg[88g104] + T(1;2)19D;41C/CyO
vg167 vg[88g108]/Cy[2004]
vg168 vg[88h11] + T(2;3)39F;62F/Cy[2004]
vg169 vg[88h64], Df(2R)49D1;49E7/Cy[2004]

vg170 vg[88h72]/Cy[2004]
vg171 vg[88h78]/Cy[2004]
vg172 vg[89d1], T(2;3)49D4;83A6/Cy[2004]
vg173 vg[89d40], Tp(2R)41A;54B;49E;56E;54C;41A;49D4;56F/Cy[2004]
vg174 vg[89d68]
vg175 vg[89e4], In(2R)49D4;52A11/Cy[2004]
vg176 vg[89e16], Df(2R)49C1;49D7/CyO
vg177 vg[89e19] + T(1;2)13E;48D2/CyO
vg178 vg[89e20]/Cy[2004]
vg179 vg[89e23]
vg180 vg[89e24], Df(2R)49D1;49F16/Cy[2004]
vg181 vg[89e47]/Cy[2004]
vg182 vg[89e52b]/Cy[2004]
vg183 vg[89e60], In(2R)49D3;50A14/Cy[2004]
vg184 vg[89e64a] + In(2LR)39A;48E10 + T(2;4)48E10;102F/Cy[2004]
vg185 vg[89e64b]/Gla vg[st]
vg186 vg[89e68] +In(2L)35D;40D/Cy[2004]
vg187 vg[89e76a], In(2R)49C1;49F5/Cy[2004]
vg188 vg[89e76b], In(2R)49A4;49D4/Cy[2004]
vg189 vg[89e84a], Df(2R)49D4;49F6/CyO
vg190 vg[89e84b]/Cy[2004]
vg191 vg[89e84c], Df(2R)49B8;49E7/Cy[2004]
vg192 vg[89e87]/Cy[2004]
vg193 vg[89e88] + Df(2R)52D;53E/Cy[2004]
vg194 vg[89e104b]/Cy[2004]
vg195 vg[91l5]/Cy[2004]
vg196 vg[91l15] + T(2;3)51C;89D/Cy[2004]
vg197 vg[91l19]/Cy[2004]
vg198 vg[92g23], Df(2R)49D3;49E7/Cy[2004]

Chromosome 3 Stocks

3001 e[11]
3002 e[88f24]
3003 mus(3)312[D1]/TM3, y[+] ri p[p] sep bx[34e] e[s] Sb Ser
3004 mus(3)312[D1]/In(3LR)Ubx[130], Ubx[130] ri e[s] ca
3005 mus(3)312/TM3, y[+] ri p[p] sep bx[34e] e[s] Sb Ser; B[S] Y y[31d11]
3006 red
3007 Sod[n1] red/TM3
3008 cSOD[n108] red/TM3
3009 st C(3)G ca/TM3
3010 st C(3)G ca/ri Ubx[130] e[s] ca
3011 st mus(3)301[D1]/st mus(3)301[D1]
3012 st mus(3)302[D2]/TM2, Ubx[130] se e[s]
3013 st mus(3)302[D2]/In(2LR)Ubx[130], ri Ubx[130] e[s] ca
3014 st mus(3)302[D2]/TM3
3015 st mus(3)304[D1]/TM3
3016 st mus(3)304[D1]/In(2LR)Ubx[130], ri Ubx[130] e[s] ca
3017 st mus(3)305[D1]/TM3
3018 st mus(3)305[D1]/In(2LR)Ubx[130], ri Ubx[130] e[s] ca
3019 st mus(3)310[D1]/TM3

Chromosome 4 Stocks

4001 spa - Ore (iso-IV)

Chromosome 1-2 Stocks

4010 su(b)[18]/C(1)DX, y f/Y; b
4011 su(b)[31]/FM4; b
4012 In(1)sc[S1L]sc[8R] + dl-49, y sc[S1] sc[8] w[a]; b cn vg
4013 y[2] w[a]; In(2LR)Pm, b bw[V1]/In(2LR)Cy, net dp[tx1] Cy b pr Bl lt[3] cn[2] L[4] sp[2]

Chromosome 1-3 Stocks

4014 fs(1)K10 w/ClB; mwh se e
4015 w[e]; e[87f]
4016 y w[co]/y[+] Y; flr[3] se/TM2, Ubx[130] se e[s]

Chromosome 2-3 Stocks

4017 cn; e[11]
4018 cn bw; e[11]
4019 Cy/Pm; D/Sb

Chromosome 1-2-3 Stocks

4020 sc z +[is]; Cy; Ubx[130]/Xa
4021 y sc[S1] dl49 sc[8]; bw; st p[p]
4022 y sc[S1] dl49 sc[8] w[a]; bw; st p[p]
4023 y sn[w]; bw; st
4024 y w/y[+] Y; bw; st mus(3)302[D1]
4025 y[2] sc w[a] w[ch] fa; Cy; Ubx/Xa

Conference Programs

In an effort to provide as diverse a source of information on *Drosophila* genetic research as possible, Drosophila Information Service will print programs for research conferences whenever space allows. The editor invites conference organizers or participants to submit copies of meeting programs and a brief description of the theme, location, and time the conference was held. DIS will endeavor to publish the names of authors, the affiliation of the senior author or corresponding presenter, and the title of the talks or posters. This can then be used by readers of DIS to locate individuals pursuing problems of common interest, locate possible postdoctoral researchers, and find sources of materials or information that may not yet be published in other journals. Even if space does not permit publishing all proceedings, we hope that a listing of regional and national conferences will be useful.

38th Annual *Drosophila* Research Conference

16 to 20 April 1997, Chicago, Illinois, U.S.A.

The 38th Annual *Drosophila* Research Conference was held at the Sheraton Chicago Hotel and Towers, Chicago, Illinois, and the 1997 Program Chairs were William R. Engels, Barry S. Ganetzky, F. Michael Hoffmann, and Allen S. Laughon from the University of Wisconsin. The conference was sponsored by the Genetics Society of America, 9650 Rockville Pike, Bethesda, Maryland 20814-3998 [telephone, 301-571-1825; fax, 301-530-7079; e-mail, society@genetics.faseb.org; web site, http://www.faseb.org/genetics]. Large numbers of slide presentations and posters were presented. The plenary session lectures are listed here, in the order they appeared in the program.

Crow, James F. (University of Wisconsin, Madison, WI). Historical perspective.

Sved, John (Sydney University, Australia). Recombination and chromosomal changes induced by P-element derivatives.

O'Brochta, David (University of Maryland Biotech. Inst., College Park, MD). Transforming insects other than *Drosophila* -- The P paradigm and beyond.

Xu, Tian (Yale University School of Medicine, New Haven, CT). *Drosophila* tumor suppressors and their mammalian homologs.

Banerjee, Utpal (University of California, Los Angeles, CA). Predisposition and signalling during cell fate determination in the eye.

Blair, Seth (University of Wisconsin, Madison, WI). Lineage, boundaries and signalling in the developing wing.

Kuroda, Mitzi (Baylor College of Medicine, Houston, TX). Dosage compensation, sex, and the single chromosome.

Laurie, Cathy (Duke University, Durham, NC). Genetic basis of a morphological shape difference in the male genitalia of D. simulans and D. mauritiana.

Edgar, Bruce (Fred Hutchinson Cancer Research Center, Seattle, WA). Growth and cell cycle control in the developing larva.

Bellen, Hugo (Baylor College of Medicine, Houston, TX). Functional diversity of cellular junctions.

Peifer, Mark (University of North Carolina, Chapel Hill, NC). Cell adhesion and signal transduction: The armadillo connection

Wu, C.-Ting (Harvard Medical School, Boston, MA). When homologues meet: Using transvection to study gene expression.

Nusse, Roel (Stanford University, Stanford, CA). Reception and transduction of the wingless signal.

Andrew, Deborah (Johns Hopkins University School of Medicine, Baltimore, MD). Regulation and formation of the larval salivary gland.

Levis, Robert (Fred Hutchinson Cancer Research Center, Seattle, WA). The exceptional telomeres of *Drosophila*.

WORKSHOPS:

Aging
Chair: Steve Helfand (University of Connecticut Health Center, Farmington, CT)

RNA Processing:
Chair: Helen Salz (Case Western Reserve University, Cleveland, OH)

Immunity:
Chair: Deborah Kimbrell (University of Houston, Houston, TX)

Embryonic Development in Non-Drosophilids
Chairs: Susan J. Brown and Rob Denell (Kansas State University, Manhattan, KS)

DNA Repair and Mutagenesis
Chair: Christopher Osgood (Old Dominion University, Norfolk, VA)
Moderator: Jan Eeken (University of Leiden, The Netherlands)

Electrophysiological techniques and analysis, or What DO all those squiggles mean?
Chair: Linda M. Hall (SUNY at Buffalo, Buffalo, NY)

American Society of Naturalists

Society of Systematic Biologists

Society for the Study of Evolution

14 to 18 June 1997, University of Colorado, Boulder

The joint meeting of these three societies was held at the University of Colorado. The host was Jeff Mitton and the Program Organizer was Brian Kreise. A total of 875 talks and poster presentations are listed in the Program, and the following represent those we could identify as concerning research related to *Drosophila*. This list was prepared by Stanton B. Gray, Department of Zoology, University of Oklahoma.

Akashi, H. (University of California, Davis) Natural Selection and the Population Genetics of "Silent" DNA Mutations in *Drosophila*.

Bauer, V.L., and C.F. Aquadro (Cornell University) Molecular Evolution Is Not Male-Driven in *Drosophila melanogaster* and *D. simulans*.

Berrigan, D., and M. Blows (University of Washington) Tests of Evolutionary Hypotheses Using Hybrids Between *Drosophila serrata* and *D. birchii*.

Bettencourt, B.R., M.E. Feder, and S. Cavicchi (University of Chicago) Expression of the 70-kD Heat Shock Protein in *Drosophila* Populations: Laboratory Evolution at Different Temperatures.

Boake, C.R.B., and D.K. Andreadis (University of Tennessee) Head Width, a Sexually Selected Trait in *Drosophila heteroneura*, Is Not Used by Females in Species Recognition.

Boussy, I.A., and M. Itoh (Loyola University) The Hobo Transposable Element Invaded *Drosophila melanogaster* Twice.

Breitmeyer, C. (Arizona State University) Comparative Population Genetics of Endemic Sonoran Desert *Drosophila*.

Chabora, P.C. (Queen's College) Species Range Expansion and *Drosophila*-Parasitoid Interaction.

Chippindale, A., and B. Rice (University of California, Santa Cruz) Measuring Heritable Variation in Net Fitness: A New Technique Using Cytogenic Cloning of *Drosophila*.

Clark, A. (Penn State University) Apparent Selection Inferred From Pleiotropic Effects of P-Element Insertions.

Craig, C.L., and R.S. Weber (Harvard University) Selective Regimes Affecting Replacement Substitutions in *D. melanogaster* and *E. coli* Are Revealed Through the Bioenergetic Costs of Amino Acid Synthesis.

DeAngelis, M. (University of Tennessee) Sperm Transfer and Copulation Duration in *Drosophila silvestris* and *Drosophila heteroneura*.

DeFilippis, V.R., and F.J. Ayala (University of California, Irvine) An Incipient Speciation Event in the *Drosophila willistoni* Group.

Dos Santos, P., and S.R. Matioli (Universidade de Sao Paulo, Brazil) Haldane's Rule in the Fruit Fly *Anastrepha*: Why So Different From *Drosophila*?

Eanes, W., and M. Zurovcova (State University of New York, Stony Brook) Molecular Population Genetics of the Y Chromosome in *Drosophila melanogaster* and *D. simulans*.

Etges, W.J., W.R. Johnson, G. Huckins, G.A. Duncan, and W.B. Heed (University of Arkansas) Inversion Phylogenies and Parallel Population Structures in Cactophillid *Drosophila mojavensis* and *D. pachea*.

Feder, M.E. (University of Chicago) Evolutionary Physiology of Heat Shock Proteins and the Stress Response in *Drosophila*: How Directed Mutagenesis and Natural Variation Can Contribute to Understanding Adaptation.

Fry, J.D., and T.F.C. Mackay (North Carolina State University) Preponderance of Mildly Deleterious Mutations Affecting Male Fertility in Long-Term Mutation Accumulation Lines of *Drosophila melanogaster*.

Fry, J.D., S. Nuzhdin, E. Pasyukova, and T.F.C. Mackay (North Carolina State University) Mapping of Genetic Factors Causing Genotype-By-Environment Interaction for Fitness in *Drosophila melanogaster*.

Gibbs, A., and L. Matzkin (University of California, Irvine) Evolution of Water Balance in the Genus *Drosophila*.

Gilchrist, G.W., and R.B. Huey (University of Washington) Experimental Evolution of Temperature Sensitivity in *Drosophila*: Fitness Estimates on Selected Lines.

Guthrie, E., and P. Service (Northern Arizona University) Estimates of Heritability of Male Effects on Female Oviposition in Two Populations of *Drosophila melanogaster*.

Haller, B.S., and R.C. Woodruff (Bowling Green State University, Ohio) Regulation of Transposable DNA Elements in Heterochromatin: The P Element of *Drosophila melanogaster*.

Hollocher, H., and J. Hatcher (Princeton University) Change in Pigmentation Pattern As It Relates To Speciation in Caribbean *Drosophila*.

Huai, H., and R.C. Woodruff (Bowling Green State University, Ohio) Rapid Evolution and Genetic Instability Coupled Via Clustered Mutations.

Itoh, M., I.A. Boussy, and R.C. Woodruff (Kyoto Institute of Technology, Japan) Decline in a Cline: P Elements in Eastern Australian *Drosophila melanogaster* Populations.

Jaenike, J., and S. Perlman (University of Rochester) Evolution of Host Range in Nematodes Parasitizing *Drosophila*.

Johnson, N.A. (University of Texas, Arlington) Variation in Increased Male Mortality Due to Mating with Males of Different Strains of *Drosophila melanogaster*.

Jones, C., and H.A. Orr (University of Rochester) Genetics of Adaptation: The Genetics of *Drosophila sechellia*'s Resistance to a Naturally Occurring Toxin.

Joshi, A. and L. Mueller (University of California, Irvine). The Evolution Life History and Population Stability in Laboratory Populations of *Drosophila*.

Kann, L.M., and D.M. Rand (Brown University) A Strand Bias to Non-Neutral Mitochondrial DNA Evolution: Evidence from the CytB and ND5 Genes in *Drosophila*.

Kim, J., and J. Kerr (Yale University) Heterochrony in the Molecular Development Cascade of *Drosophila*.

Kliman, R.M., and A. Eyre-Walker (Radford University) Evidence for Recent Selection on Codon Usage in *Drosophila*.

Kondrashov, A.S. (Cornell University) Decline of Fitness in Two Panmictic Populations of *Drosophila melanogaster* Maintained under Relaxed Natural Selection.

Koepfer, H.R., P.C. Chabora, and A. Kermarrec (Queens College, USA) Species Range Expansion and *Drosophila*-Parasitoid Interaction

Krebs, R.A. (University of Chicago) Evolutionary Constraints on Thermotolerance Across the Life Cycle of *Drosophila melanogaster*.

McAllister, B. (University of Chicago) Sequence Variation Within and Between the Neo-X and Neo-Y Chromosomes of *Drosophila americana*, Implications for Sex Chromosome Evolution.

McKean, K.A. (University of California, Riverside) Effects of Sex Ratio Manipulation of Female Fitness in *Drosophila melanogaster*.

Michieli, C.A., K. McGill, and P.M. Service (Northern Arizona University) Evolution of Senescence in Laboratory Populations of *Drosophila melanogaster*.

Mueller, L. (University of California, Irvine) The Evolution Life History and Population Stability in Laboratory Populations of *Drosophila*.

Noor, M.A.F., and C.F. Aquadro (Cornell University) Courtship Song and Sexual Isolation in *Drosophila pseudoobscura* and *D. persimilis*.

Nunney, L.P. (University of California, Riverside) The Effect of Temperature on Fecundity in Female *Drosophila melanogaster*: Evidence for Adaptive Plasticity.

Parsch, J., S. tanda, and W. Stephan (University of Maryland) Site-Directed Mutations Reveal Long-Range Compensatory Interactions in the Adh Gene of *Drosophila melanogaster*.

Pierce, V., and A. Gibbs (University of California, Irvine) Physiological Mechanisms of Evolved Urea Resistance in *D. melanogaster*.

Pletcher, S.D., and J.W. Curtsinger (University of Minnesota) Age-Specific Properties of Spontaneous Mutations Affecting Mortality in *Drosophila melanogaster*.

Polak, M. (Syracuse University) A Micro-Evolutionary Study of the *Drosophila-Macrocheles* System: Fitness Consequences of Ectoparasitism and Heretibility of Resistance.

Price, D. (University of Hawaii) Female Preference and Male Age in *Drosophila*.

Promislow, D., and L. Pearse (University of Georgia) Fitness Consequences of Sexual Selection in *Drosophila*: Artificial Selection Experiments.

Roff, D., G. Stirling, and D. Fairbairn (McGill University, Canada) The Evolution of Threshold Traits: A Quantitative Genetic Analysis of the Physiological and Life History Correlates of Wing Dimorphism.

Russell, A.L., and R.C. Woodruff (Bowling Green State University, Ohio) Genetics and Evolution of the Mariner DNA Element in *Drosophila simulans*.

Schug, M.D., and C.F. Aquadro (Cornell University) Determinants of Microsatellite Evolution in *Drosophila melanogaster*.

Shabalina, S.A., L.Y. Yampolsky, and A.S. Kondrashov (Cornell University) Decline of Fitness in Two Panmictic Populations of *Drosophila melanogaster* Maintained Under Relaxed Natural Selection.

Silva, J.C., J. Clark, P.M. O'Grady, and M.G. Kidwell (University of Arizona) Molecular Evolution of P Elements: Snail's Pace for a Jumping Gene?

Smith, R., and N.A. Johnson (University of Texas, Arlington) Variation in Increased Male Mortality Due to Mating with Males of Different Strains of *Drosophila melanogaster*.

Stirling, G., and D. Roff (McGill University, Canada) Partitioning a Fitness Tradeoff: Genetic and Physiological Components.

Thompson, J.N., jr., and R.C. Woodruff (University of Oklahoma) Mutation Rate: A Simple Concept Has Become Complex.

Verrelli, B.C., and W.F. Eanes (State University of New York, Stony Brook) Polymorphism and Divergence at the Pgm Locus of *Drosophila*.

Wisotzkey, R.G., C.E. Dohrmann, S. Liu, and L.A. Raftery (Massachusetts General Hospital and Harvard Medical School) Conservation of Both Molecular and Developmental Aspects of TGF-Beta Signaling Between *Drosophila* and Vertebrates.

Zeh, D.W., and J.A. Zeh (University of Houston) Reproductive Mode and Speciation: Why We Need Alternatives to the *Drosophila* Model System.

Updated Entries to the Directory of Drosophila Researchers

The following laboratories have submitted updates of research personnel and areas of interest.

India

Department of Zoology, Kumaun University, Nainital 263002, India.

Singh, Dr. B.K.	Reader
Fartyal, Mr. Rajendra Singh	Junior Research Fellow

Switzerland

Department of Cell Biology, University of Basel, Biozentrum, Klingelbergstrasse 70 CH-4056 Basel, Switzerland, Phone: 061/267-3111, Fax: 061/267-2078, e-mail: kloter@ubaclu.unibas.ch

Research Group Gehring:

Baumgartner, Paul	Technician	DNA sequencing
Bello, Bruno	Postdoctoral fellow	developmental genetics, gene regulation
Berry, Meera	Postdoctoral fellow	gene regulation, transcription factors
Edelmann, Lambert	Postdoctoral fellow	gene regulation, developmental genetics
Flister, Susanne	Acad. techn. asst.	developmental genetics, homeotic target genes
Gehring, Walter J.	Professor Dept. Chairman	developmental genetics
Girard, Franck	Postdoctoral fellow	developmental genetics, gene structure and regulation
Glardon, Sacha	Graduate student	developmental genetics, molecular evolution
Janssens, Hilde	Graduate student	developmental genetics, gene regulation
Kloter, Urs	Curator of stocks and Drosophila cell-cultures	
Kurata, Shoichiro	Postdoctoral fellow	developmental genetics, gene regulation
Mirault, Lydia	Postdoctoral fellow	developmental genetics, gene regulation
Punzo, Claudio	Graduate student	developmental genetics, molecular biology
Resendez-Perez, Diana	Postdoctoral fellow	molecular biology, homeodomain specificity
Seimiya, Makiko	Postdoctoral fellow	developmental genetics, homeotic target genes

Department of Cell Biology, University of Basel, Biozentrum, Klingelbergstrasse 70 CH-4056 Basel, Switzerland, Phone: 061/267-3111, Fax: 061/267-2078, e-mail: affolter@ubaclu.unibas.ch

Research Group Affolter:

Affolter, Markus	Group Leader	developmental genetics, gene regulation
Groppe, Jay	Postdoctoral fellow	biochemistry, protein purification
Grieder, Nicole	Graduate student	developmental genetics, gene regulation
Kiefer, Fabrice	Diploma student	developmental genetics
Nussbaumer, Ute	Technician and Curator of stocks	protein purification, developmental genetics
Marty, Thomas	Graduate student	developmental genetics
Vincent, Stéphane	Graduate student	developmental genetics

New Books

Drosophila Cells in Culture

by Guy Echalier (Groupe de Génétique Cellulaire et Moleculaire, Unité Associée 1135, Centre National de la Recherche Scientifique, Université Pierre et Marie Curie, Paris, France.

Academic Press, San Diego
Publication date: February 1997 (ISBN 0-12-229460-2; $135.00, hard cover, 702 pp)

This is an extensive summary of all the technical aspects of *Drosophila* cell culture. It includes critical discussions of the techniques that covers relevant experimental work as well as details about the techniques themselves. The bibliography of more than 1500 entries focuses on *in vitro* cultures of *Drosophila* cells and tissues, with each entry coded to indicate the principle topic covered in the reference (for example, B, cell biology [growth factors, oncogenes, adhesion molecules]; C, culture media and methods; Cd, imaginal discs; Cp, cell primary cultures; Cl, cell lines; Ct, tissue and organ cultures; Cy, cell cycle, karyology; E, ecdysone and other hormones; H, heat-shock; M, molecular biology; R, retrotransposons; r, review or handbook; T, transfection; and V, viruses or other infectious agents).

The main sections of the book are: 1, Composition of the body fluid of *Drosophila* and the design of culture media for *Drosophila* cells; 2, Primary cell cultures of *Drosophila* cells; 3, *Drosophila* continuous cell lines; 4, Karyotype and cell cycle; 5, Biology and biochemisry of cultured cell lines: nucleic acids; 6, Biology and biochemistry of cultured cell lines: proteins; 7, Experimental models of gene regulation: heat-shock response of *Drosophila* cells; 8, Experimental model of gene regulation: cell responses to hormones; 9, Gene transfer into cultured *Drosophila* cells; 10, Transposons; 11, *Drosophila* viruses and other infections of cultured cells. In addition to the extensive bibliography of *Drosophila* literature described above, each chapter has a general bibliography of supporting references.

Drosophila Cells in Culture by Guy Echalier is an excellent resource for anyone engaged in *Drosophila* cell or tissue culture research.

Guide to Authors

Drosophila Information Service prints short research and technique articles, teaching notes, descriptions of new mutations, stock lists, directory information, and other material of general interest to *Drosophila* researchers. The current publication schedule for regular issues is annually in late summer/early fall. To meet this target date, the deadline for submission of materials is typically 1 May. Later submissions can occasionally be accommodated by contacting the editor by email or telephone. Special issues will also be prepared on an irregular schedule.

Manuscripts, orders, and inquiries concerning the regular annual DIS issue should be sent to James Thompson, Department of Zoology, University of Oklahoma, Norman, OK 73019. Telephone (405)-325-4821; email jthompson@ou.edu; FAX (405)-325-7560.

Submission: Submissions are accepted at any time, but the deadline for the annual issue will be about 1 May or until the issue is full. To help minimize editorial costs, proofs will not be sent to authors unless there is some question that needs to be clarified or they are specifically requested by the authors at the time of submission. The editor reserves the right to make minor grammatical, spelling, and stylistic changes if necessary to conform to DIS format and presentation clarity.

Manuscripts should be submitted in duplicate. If possible, a 3.5" or 5.25" diskette should also be sent with the manuscript in Microsoft Word, ASCII, WordPerfect or other common word-processing format. This improves the speed and accuracy of preparing manuscripts and is always greatly appreciated. Please avoid inserting extensive page set up commands, because these must be modified to fit the page set up used by the editors.

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Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

Stock Lists, Specialized Bibliographies, and Long Technical Articles: Long or complex material can generally not be accepted unless it is submitted on diskette, with a printed copy for editorial guidance. We encourage submission of lists and other documentary material to complement presentations in other journals that might have more restrictive space limits or costs. Special justification will, however, be needed for material like bibliographic lists that are now often readily available by other means. Inquiries about formats for this kind of submission are welcomed.

Figures and Tables: Both line drawings and half-tone illustrations will be accepted, but half-tones should be provided in black and white. We are currently unable to publish figures in color. All tables are retyped by us to fit a uniform style, and it is critical that all numbers and symbols be clearly arranged and legible.

Call for Papers

Submissions to Drosophila Information Service are welcome at any time. Typically, we would like to have submissions no later than 1 May to insure their inclusion in the regular annual issue. Submissions in Microsoft Word, which is now the program we use for our page setup, are especially helpful. Submissions by email are also possible, but if they are sent as attached files, we have greatest success using MS Word or ASCII format. In all instances, especially where tables are concerned, it is useful to have a paper copy to facilitate formatting. The Guide to Authors is printed elsewhere in this volume.

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Several years ago, formal standing orders were discontinued due to the need to obtain prepayment for issues. "Standing Orders" are now handled through a mailing list of active subscribers. All individuals on the active subscriber list will receive notices for forthcoming regular and special issues and a Standing Order Invoice to facilitate prepayment. If you would like to be added to the Drosophila Information Mailing List, please write to the editor, Jim Thompson, Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019.

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DROSOPHILA INFORMATION SERVICE --SPECIAL ISSUES

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Publication Date: April 1997

Published by the FlyBase Consortium

FlyBase announces the publication of two new volumes of DIS:

DIS 78 is 554 pages and focuses on graphical map displays of two types:

GENE-ORDER MAPS for *Drosophila melanogaster*, in which the positions of genes are listed according to their polytene chromosome location and relative to the genes which determine their leftward and rightward limits.

TRANSPOSON & VECTOR MAPS, which include graphical and tabular information on the structure, derivation, and uses of important plasmid vectors and transposons.

DIS 79 is 400 pages and includes three different tabulations:

GENETIC NOMENCLATURE GUIDELINES for *Drosophila melanogaster*, compiled by FlyBase and employed in all FlyBase datasets.

SUBCELLULAR & ANATOMICAL NOMENCLATURE, organized hierarchically. These terms are being utilized as a controlled vocabulary in phenotypic and gene expression data captured by FlyBase, and will form the basis of anatomical search tools.

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