

DROSOPHILA

Information Service

52

May 1977

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ANNOUNCEMENTS I

We are very pleased to announce that Prof. Philip Hedrick will assume the editorship of DIS; all communications concerning DIS should be sent to him at the following address:

DROSOPHILA INFORMATION SERVICE
c/o Prof. P. Hedrick
Division of Biological Sciences
University of Kansas
Lawrence, Kansas 66045

Telephone: (913) 864-3763

Prof. I.H. Herskowitz whose current Bibliography of *Drosophila* has been a regular feature of DIS for many years, would like to announce a change in the publication procedures for these bibliographies. Previously they were published in book form at frequent intervals; this will no longer be done. DIS will become the only source of this bibliographical information, with a list in each issue and a subject index to appear about every fifth issue.

DIS 53, the last issue to be published in Eugene, will consist primarily of the computerized consolidated world stock list of *D. melanogaster* being prepared by Prof. Dan Lindsley. The issue will also contain a bibliography, as well as research and technical notes.

MELANOGASTER STOCK LISTS: There are no D. melanogaster stock lists in this issue. The major portion of DIS 53 (to be issued soon) will consist of the computerized world stock list consolidating the lists of the major centers, now being prepared by D.L. Lindsley. Other individual stock lists not incorporated into the consolidated list will also appear in that issue. Drosophila species stock lists are updated in this issue in the usual order.

D. MELANOGASTER - NEW MUTANTS

Report of K. Persson University of Umea, Sweden

14 new Minutes According to Lindsley and Grell (1968) several Minute mutants are not available any longer in official stock lists and thus probably lost.

12 new Minute mutants have been isolated by M M Green after X-ray treatment. Some of the mutants are postulated, by recombination tests, to be alleles to lost Minutes. Some others of Green's mutants were found, in complementation tests, to be alleles to previously described and still available Minutes (Persson 1976b). One of Green's mutants was found to be a double Minute (mutant for M(2)H and M(2)m).

One Minute, M(2)21A-B, isolated by B Rasmuson, is not allelic to any previously characterized Minute. It is described elsewhere as Suppressor of zeste 5, Su(z)5 (Kalisch and Rasmuson 1974; Persson 1976a,b)

Table 1 14 new Minute mutants localized by recombination test (r) or complementation test (c). * = lost Minute.

Mutant	Isolated by	locus	Postulated allele to	Localized by
M(2)21A-B	B. Rasmuson	2-0.0	-	r
M(2)38b ^G	M.M. Green	2-56	M(2)38b (2-57)*	r
M(2)40c ^G	"	2-65	M(2)40c (2-65)*	r
M(2)173 ^G	"	2-92.3	M(2)173	c
M(2)c ^G	"	2-108	M(2)c ^{33A}	c
M(2)H ^G	"	2-53.5	M(2)H ^{S5}	c
M(2)m ^G	"	2-54	M(2)m ^{S6}	c
M(2)z ^{G1}	"	2-12.9	M(2)z	c
M(2)z ^{G2}	"	2-12.9	M(2)z	c
M(3)68j173	"	3-68		r
M(3)68k011	"	3R		r
M(3)i ^{G1}	"	3-29	} M(3)i (2-28.9)* and M(3)x	r
M(3)i ^{G2}	"	3-29		r
M(3)i ^{G3}	"	3-29		r

The mutants are included in the D. melanogaster stocklist of the Department of Genetics, University of Umea, Sweden.

References: Kalisch, W.-E. and B. Rasmuson 1974, Hereditas 78:97-104; Lindsley, D.L. and E.H. Grell 1968, Carn. Inst. Wash. Publ. 627; Persson, K. 1976a, Hereditas 82:57-62; Persson, K. 1976b, Hereditas 82:111-120.

Report of W.D. Kaplan City of Hope Medical Center, Duarte, California

iav: inactive. 1-18.8. Morphologically normal, extremely inactive. Population remains quiet in culture bottles spread out evenly on surface of food; spacing depends upon density. Can walk or fly when literally forced to do so. Settles into inactivity within seconds after bottles have been disturbed. Fertile Rkl.

Report of G. Trippa*, G.A. Danieli**, R. Costa** and R. Scozzari*

*University of Rome and **University of Padova, Italy.

Pgm^G or Pgm^{1.10}: phosphoglucosomutase^{1.10}. A new electrophoretic allele of phosphoglucosomutase (PGM) has been found in a natural population collected near Rome, by using the method of Spencer et al. (Nature 1964, 204:742), modified for single fly homogenates, designated as Pgm^G. This rare allele exhibits a single anodal band of PGM activity and was found in the heterozygote state with the common alleles Pgm^A or Pgm^B. The heterozygotes show no hybrid bands. The new isozyme variant migrates more rapidly than does Pgm^A, but intermediate between Pgm^A and Pgm^C. A new classification of the Pgm alleles is reported in the section Research Notes of the present issue.

Report of J.R.S. Whittle

University of Sussex, Brighton, England.

Chp: Chopped. A mutation with a dominant 'scalloping' effect upon the wing margin and recessive lethality, which appeared following EMS mutagenesis. There are gaps in the distal costa and immediately distal to it in the triple row, in the double row at the distal tip of vein 3 and in the margin hairs proximal to vein 5. Veins 3 and 5 are thickened. This phenotype is similar to that of Beaded but Chp is located in the distal tip of the X-chromosome at approximately 2 cMs. The penetrance of the lethality and of the wing effect is complete.

Mar: Margin. A mutation with a dominant effect on scalloping on the wing margin and recessive lethality, which arose following EMS mutagenesis. Triple row and double row bristles and marginal hairs all have gaps and in extreme cases the wing outline appears ragged. The mutation resembles Beaded^G phenotypically but is not allelic to it and maps left of ro at approximately 72 cMs. The penetrance of the wing effect is incomplete.

Report of S.R. Thompson

Ithaca College, New York

hk⁷³: hyperkinetic-73. Parris, 1973. Discovered in a selection experiment for interaction with Sh⁵. hk⁷³ is a recessive sex-linked mutation which exhibits the characteristic leg shaking of hyperkinetic mutants. hk⁷³ was mapped to 29.2 on the X-chromosome, this position is close to that of the mutants Hk¹ (30.9) and Hk² (30.4). Both Hk¹ and Hk², although originally described as dominant mutants, now behave as typical recessives, so that tests for allelism were performed with both of these mutants with hk⁷³. All heterozygotes exhibited the same degree of leg shaking as homozygotes. Therefore, it is concluded that hk⁷³ is an allele of the hyperkinetic locus of D. melanogaster.

Report of N. Altorfer

University of Brussels, Belgium

ap⁷³ⁿ: apterous⁷³ⁿ. 2-55.4. Spontaneous in F₂'s of the cross y ct oo x w ♂♂. Similar and allelic with ap⁴ and ap^{56f}. Viability low: 50% to 80% of the flies die within 2 to 10 days. The surviving flies are fertile and long lived. Ovaries of the lethal flies remain very small and the fore ventriculus is packed with an exceedingly long peritrophic tube. Bristles and hairs on sides of the thorax are absent.

The wing imaginal disk of third instar larvae is smaller than normal. After metamorphosis, all structures of the wing surface and wing margin are missing. Structures of the ventral wing hinge are present but not those of the dorsal hinge, except for some auxiliary sclerites.

Heterozygotes with ap⁴ and ap^{56f} show a striking complementation effect: wings and halteres are much longer than in any of the parent lines, but with extreme variability in shape and length. Viability of ap^{56f}/ap⁷³ⁿ is nearly normal but ap⁴/ap⁷³ⁿ is partially lethal. Both heterozygotes are fertile.

Report of G. Trippa

Città Universitaria, Istituto di Genetica, Rome, Italy

fs(2)TLM: female sterile(2) 89.7 ± 1 . G. Trippa, A. Loverre and A. Micheli, 1975.

This gene has been recovered associated with an SD factor in a second chromosome isolated from a natural population of Ranna (Sicily, Italy). The external morphology of both female and

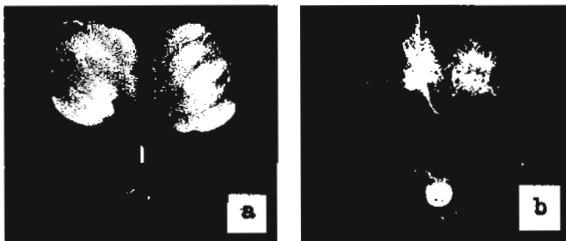


Figure 1. Ovaries of normal (a) and fs(2)TLM homozygous female (b).

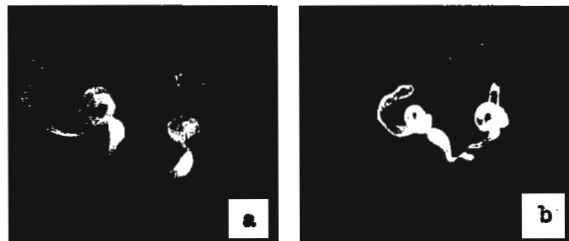


Figure 2. Testes of normal (a) and fs(2)TLM homozygous male (b).

male individuals is normal. Homozygous females are completely sterile and do not lay eggs. Ovaries are underdeveloped (Figure 1) and the number of ovarioles is lower than in normal females. Apparently vitellogenesis is absent. Males are fertile but their testes are ill-shaped and small (Figure 2). Spermiogenesis is normal with spermatozoa production. RK3.

Report of F. Waddle

Fayetteville State University, North Carolina

m^{how}: miniature-heldout wings. Spontaneous (possibly caused by a male recombination inducer). Wings of most flies divergent (up to 45°) even in uncrowded cultures. Otherwise similar to m. RK1.

wy^{spn}: wavy-spoon. Spontaneous (possibly caused by a male recombination inducer). Males similar to wy. The portion of the wing distal to the wave is more deeply dished in females. RK2.

Ly²: Lyra-2. Spontaneous (possibly caused by a male recombination inducer). Similar to Ly except that the eyes tend to be rougher. RK1.

Report of B.W. Geer and J.T. Bowman

Knox College, Galesburg, Illinois and Utah State University, Logan, Utah

ms(1)7: male sterile(1)7. EMS-induced in Canton-S stock. The mutant gene was localized to section 19E of the X-chromosome using Y-chromosomes bearing X-chromosome segments. Males are sterile at 18°, 23° and 28°C. Females are fertile when homozygous for the mutant gene and the viabilities of mutant males and females are good. Sperm of the mutant males are ultrastructurally normal, transferred to females during copulation, and are lost from the seminal receptacle of mated females at a near normal rate as eggs are oviposited. The mutation blocks a late sperm function during the fertilization process.

Report of E. Gateff

Biologisches Institut I (Zoologie), Freiburg, West Germany

1(1) bwn: lethal (1) benign wing imaginal disc neoplasm. EMS-induced in a wild-type Oregon-R stock. Located at 34.4 ± 0.6 (G. Jürgens). Lethal at the time of puparium formation. The mature mutant larvae appear slightly bloated. The third larval instar is extended up to four days. The wing imaginal discs are clumped, enlarged incapable of differentiation and represent transplantable, benign neoplasms. The remaining imaginal discs develop initially normally, but in older mutant larvae show a highly abnormal folding pattern and mode of growth. Imaginal disc cells invade the epithelia of adjacent imaginal discs. The metamorphosed testpieces of such mixed imaginal disc epithelia show mixed patterns consisting of structures belonging to different imaginal discs. Thus, the mutant imaginal disc cells seem to have lost the capacity to differentiate between cells of their disc of origin and those belonging to another imaginal disc. The imaginal disc cells invade also the central nervous system, where they destroy large regions of the neuropile and the cortex. Finally, imaginal discs from very old mutant larvae lose their capacity to differentiate into any cuticular pattern. In addition to the imaginal disc abnormalities the large polytene, ecdyson-secreting cells in the ring glands are degenerated. Thus, the mutation 1(1) bwn transforms the wing imaginal disc into a lethal, transplantable and benign neoplasm. In the remaining imaginal discs it causes defects in the mechanism by which imaginal disc cells distinguish between cells of their own and cells of a foreign imaginal disc.

1(2) gd-1: lethal (2) giant disc-1. EMS-induced in a second chromosome carrying the markers a, px, and or. Located at 10.4 ± 2.5 (G. Jürgens). 1(2)gd-1 complements with 1(2)gd. Lethal shortly after pupation. The mutant imaginal discs are 2 to 4 times larger than those of their wild-type counterparts. They exhibit abnormal shapes and folding patterns. Occasionally, however, perfectly duplicated leg and antennal imaginal discs can be observed. The imaginal discs possess incomplete differentiation capacities. Most metamorphosed testpieces show exclusively hair patterns. Only rarely are single bristles found in the metamorphosed testpieces. Testpieces consist very often of only transparent cuticle without hairs. The 1(2)gd-1 mutation affects apparently the mechanism which controls the size of the imaginal discs and the proportions of the different regions within them. It may thus affect the differentiation of compartment borders. References: Bryant, P. 1969, DIS 44:47.

1(2) mbn: lethal (2) malignant blood neoplasm. EMS-induced in a second chromosome marked with a, px, and or. Not located. Lethal at the time of puparium-formation. The number of free blood cells in the hemolymph of a mature mutant larva is 35-40 times increased when compared to the blood cell count in the hemolymph of a mature wild-type larva. Most free blood cells resemble plasmatocytes. Podocytes are rarely observed. Crystal cells are present in reduced numbers. A small percent of blood cells exhibit sickle-like shape. No encapsulation or melanization of larval tissues takes place. However, larval tissues, such as the imaginal discs, the fat body and the muscles become destroyed by invasion and phagocytosis. The drastic increase of free blood cells in the mutant hemolymph is due to the continuous and uncontrolled production of immature phagocytic blood cells within the hematopoietic organs - the lymph glands which are themselves up to 3000 times enlarged. Thus, the 1(2)mbn mutation causes malignant transformation of the progenitors of the phagocytic blood cell types in the lymph glands which accumulate in the hemolymph and destroy large portions of the larval tissues.

1(3) gl: lethal (3) giant larvae. EMS-induced in a stock marked with a, px and or. Not located. The mutation shows incomplete penetrance. Lethal at the time of puparium formation. The mature, mutant larvae are enlarged and bloated. As a result of the reduction of the fat body they appear transparent. The third larval instar can be prolonged up to 1 week. As in 1(2)gl⁴ (Gateff, 1971; Gateff and Schneiderman, 1969, 1974), the 1(3)gl imaginal discs represent transplantable, benign and lethal neoplasms and the brain develops a malignant neuroblastoma.

References: Gateff, E. 1971, Dissertation, Univ. of Calif., Irvine; Gateff, E. and H.A. Schneiderman 1969, Natl. Cancer Inst. Monogr. 31:365-397; _____ 1974, Wilhelm Roux' Archiv 176:23-65.

1(3)mbn: lethal (3) malignant blood neoplasm. EMS-induced in a stock in which the second chromosome carried the markers a, px and or. Not located. Lethal at the time of puparium-formation. Mature mutant larvae contain in their hemolymph 150 times more free blood cells than mature wild-type larvae. As a result of this, mutant larvae appear bloated. The predominant blood cell type is an immature round cell which resembles plasmatocytes. A small percent of the cells show a sickle-like shape. Podocytes are virtually absent and crystal cells are much reduced in numbers. The increased blood cell count in the 1(3)mbn hemolymph is caused by the steady production of immature plasmatocytes in the hematopoietic organs, the lymph glands. The mutant blood cells possess the capacity to encapsulate and melanize the larval tissues. Furthermore, mutant blood cells invade and destroy larval tissues, such as the imaginal discs, the fat body and the muscles. Thus, the mutation 1(3)mbn renders the progenitors of the plasmatocytes malignant.

LINKAGE DATA

Report of F.R. Waddle and I.I. Oster

Fayetteville State University, North Carolina; Bowling Green State University, Ohio.

Su(SD)X: Suppressor of segregation-distorter: X-Linked. Nine X chromosomes capable of suppressing SD have been mapped with the use of the markers y, m and f^{36a} and an SD-bearing chromosome that has a high average K value ($\approx .98$) but lacks St(SD). The suppressors of all tested chromosomes completely suppressed the expression of SD. There were no indications that any of the nine X-chromosomes carried suppressors outside the m - f region. For one chromosome, it was possible to determine that the suppressor is located to the left of sd. The nine tests gave a total of 289 recombinants between m and f^{36a} that could be recognized (one test chromosome carried Bx^J which interfered with the expressing of m). Of these, 170 involved recombination between m and the suppressor, while the remaining 119 involved recombination between the suppressor and f^{36a}. The suppressor is thus located at 58.8% of the m to f distance or at map position 48.2. This position is within the region determined by Kataoka and very nearly the same distance from Bar as determined by Sandler.

References: Kataoka, Y. 1967, Japan J. Genetics 42:327-37; Sandler, L. 1962. The American Naturalist. XCVI:161-5.

DROSOPHILA SPECIES STOCKS

LEEDS, ENGLAND: UNIVERSITY OF LEEDS Department of Pure and Applied Zoology

busckii	funebri	obscura	subobscura
cameraria	hydei	phalerata	testacea
confusa	immigrans	simulans	transversa
deflexa	melanogaster		

AHMEDABAD, INDIA: GUJARAT UNIVERSITY School of Sciences, Department of Zoology

<u>simulans</u>	<u>Other species</u>	hydei	pseudoobscura
1 y w	ananassae	kikkawai	seguyi
2 v	bipectinata	malerkotliana	simulans
		nasuta	

MILAN, ITALY: UNIVERSITA DI MILANO
Istituto di Genetica

simulans
Wild stocks

Chromosome 3

3 st

Stocks selected for
tumor manifestation

1 Aspra
 2 Giannutri

4 tu B1
 5 tu Aspra

TÜBINGEN, GERMANY: INSTITUT FÜR BIOLOGIE II
Lehrstuhl für Populationsgenetik

subobscura
Wild stocks

Chromosome A

cn

ma

Enzyme markers

Belgrad
 Lipari
 Ponza 2/3
 Norwegen 74
 Tübingen 75
 Tunis

pm ct sn

y

Bx

Oc

pm, Bx

pm, Bx, In (A9)

Chromosome O

cu

ch, cu

Ba/l8

Va/+

Va/Ba²¹⁰

α -Gpdh F (Chrom. U)

Mdh F (Chrom. U)

Me F (Chrom. O)

Pgm F (Chrom. I)

Idh S (Chrom. I)

Other species

Homozygous for
gene arrangements

Chromosome E

dp¹

vg, pp

Chromosome U

nt, fd^{Mi}

ltr

pseudoobscura

obscura

ambigua

subsilvestris

bifasciata

busckii

immigrans

kuntzei

hydei

Küsnacht
 Tunis 1
 Tunis 2
 Zürich U1+2

Chromosome I

int, ey, wt

BARCELONA, SPAIN: UNIVERSITY OF BARCELONA
Faculty of Biology, Department of Genetics

ambigua - Spain
 bifasciata - Pavia, Italy
 busckii - Spain
 buzzati - Spain
 emarginata
 funebris - Spain
 guanche - Canary Islands, Spain
 hydei - Spain
 immigrans - Spain

kuntzei - Spain
 lebaonensis - Spain
 littoralis - Spain
 mercatorum - Spain
 nigrosarsa - Spain
 obscura - Spain
 phalerata - Spain
 pseudoobscura - Texas
 repleta - Spain

simulans - Spain
 subobscura - Spain
 testacea - Spain
 transversa - Spain
 virilis - Spain
 Parascaptomiza disticha - Spain
 Zaprionus vittiger - Canary
 Islands, Spain

OULU, FINLAND: UNIVERSITY OF OULU
Department of Genetics

busckii
 simulans
 bifasciata
 obscura
 subobscura

guanche
 subartica
 unimaculata
 funebris

virilis
 littoralis
 lummei
 ovivororum
 ezoana

limbata
 phalerata
 transversa
 testacea

DÜSSELDORF, GERMANY: UNIVERSITÄT DÜSSELDORF
Institut für Allgemeine Biologie

<u>hydei</u>	18 w ^{m3}	<u>Multichromosomal</u>
1 wild	19 w Anp	
	20 v sc sn y m ch bb	29 bb;p;vg (1;2;5)
	21 w lt/Y & +/Y	30 st;sca;jv (2;3;5)
<u>Chromosome 1</u>	22 ch y m/Y & w lt/Y	31 st;vg;ng;se;Hex (2;3;4;5;6)
2 Ba	23 v f/Y & w lt/Y	
3 ch ^{to-1} y ^{tu-1} m ^{tu-1}	24 w lt/Y & or/Y	many stocks with T(X,Y)
4 cho ²	25 to ²	
5 f ²	<u>Chromosome 2</u>	several stocks with mutant morphology of Y chromosomal lampbrush loops
6 g y m	26 edu	
7 m ^{tu-2}		2 stocks with T(X,A) [=T(X,?3)]
8 N/w lt	<u>Chromosome 3</u>	<u>Other species</u>
9 pn		bifurca
10 v ¹ [(Y,A)]	27 cn	eoheydei
11 v ³ (homozygous lethal)		fulvimacula
12 v f	<u>Chromosome 5</u>	immigrans
13 w		neohydei
14 w ^a	28 red eye, brown thorax	simulans
15 w lt		virilis +
16 wml		virilis w
17 w ^{m2}		

BELLATERRA, SPAIN: AUTONOMOUS UNIVERSITY OF BARCELONA
Faculty of Science, Department of Genetics

simulans subobscura immigrans busckii

HAREN, NETHERLANDS: UNIVERSITY OF GRONINGEN
Genetisch Instituut

<u>hydei</u>	<u>simulans</u>	<u>Other species</u>	<u>obscura</u>
wild	wild	burlai	subobscura
inversion stock	mutant: v	funnebris	virilis
	mutant: w	immigrans	yakuba

VARANASI, INDIA: BANARAS HINDU UNIVERSITY
Department of Zoology

<u>ananassae</u>	b se	px	<u>Other species</u>
<u>Chromosome 1</u>	cu b	pc	ananassae (7 strains)
	b		biplectinata (Calcutta)
y	cu	<u>Unlocated mutants</u>	malerkotliana
vs	se		nasuta
	ss ^a	dct	kikkawai
<u>Chromosome 2</u>		sp	latifshahi
cu b se	<u>Chromosome 3</u>	ci	seguyi
cu se	px pc	arch	ficuspila
			andamanensis

VIENNA, AUSTRIA: UNIVERSITY OF VIENNA
Institut für Allgemeine Biologie

1. mercatorum - delete
2. subobscura - the markers of all chromosomes have been abolished. We plan to build up a new collection and will send the new list, as soon as available.

ATHENS, GEORGIA: UNIVERSITY OF GEORGIA
Department of Zoology

<u>pseudoobscura</u>	47	cd	<u>Chromosome 5</u>
<u>Chromosome 1</u>			
3 ct se ll sp tt		<u>Chromosome 3</u>	80 or L (SC)/or + (ST);spa
5 v	73	pr(ST)	<u>Multichromosomal</u>
6 Pt	74	pr(AR)	
7 w	76,85	or px	42 ss ^a ;or
8,9 y sn v co sh	77	or Bl pr spr (ST)/	83 gl;or pr(ST)
10 co sh		lethal (CU)	80 or L(SC)/or + (ST);spa
11 sh	78	or Bl pr cv (AR)/	128 sh;or(ST)
12 se		lethal (CU)	
<u>Chromosome 2</u>	79	or Bl Sc pr (ST)/lethal	<u>Chromosome 3 Inversions from</u>
36 gl ²	80	or L (SC)/or + (ST);spa	<u>Mather, California, 1959</u>
37,43 upt gl	86	or pr (ST)	(At least 5 strains of each
39,129 Ba	90,92	or	inversion type available)
40 upt bx Ba gl (INV)/		<u>Chromosome 4</u>	148-157 AR
Δ ubx gl ²	118	in hk j	188-208 PP
42 ss ^a ;or	119,120	in hk j Cy (INV)/	246-252 CH
44 ca		lethal	261-270 TL
45 gl			292-300 ST

HONOLULU, HAWAII: UNIVERSITY OF HAWAII
Department of Genetics

<u>mercatorum</u>	m	RSB-7-Im (sl)
<u>Bisexual wild stocks</u>	slY-1 w;vl	RSB-7-Im (w)
Man-11 (Manizales, Colombia)	sl;v;pm;vl	RSS-18-Im (sl)
Oatu	br	S-1-Im (sl)
Rochester		slY-1 w-Im
Reg-1 (Registro, São Paulo, Brasil)	<u>Parthenogenetic stocks</u>	sl;v;pm;vl-Im
Salvador	K23-O-Im	29cI droopy-Im
<u>Bisexual mutant stocks</u>	K28-)-Im	
sl w	O-3-Im	<u>Other Species</u>
	Rs-3-Im (sl)	paranaensis

THESSALONIKI, GREECE: UNIVERSITY OF THESSALONIKI
Laboratory of General Biology

ananassae	biplectinata	kikkawai	serrata	tsakasi
auraria	funbris	rajasetati	takahashi	yakuda

TÜBINGEN, GERMANY: MAX-PLANCK-INSTITUT FÜR BIOLOGIE
Abteilung Beerman

<u>hydei</u>	<u>simulans</u>	<u>Other species</u>
tob ^P	wild	miranda
	y w	pseudoobscura
		virilis

NEW MUTANTS

subobscura

Report of M.L. Rivera
 University of Barcelona, Spain

All of these mutants were found in natural populations.

lancet-wing. Wings pointed, seldom slightly scalloped. It is not allelomorph of pointed (Gordon 1936), neither of pointed₄₄ (Gordon et al. 1939). Classification sometimes difficult. Expression variable depending on the rest of the genotype. Complete penetration. Recessive. Chromosome E.

arch. Wings curved downwards over the abdomen. The curvature is along both axes. Wings slightly longer than wild type. Good classification. Expression variable. Complete penetration. Recessive. Seldom wings are just slightly wavy. Chromosome U.

bordeaux. Eyes of a Bordeaux wine bright color. Viability normal. Good classification. Recessive. Chromosome J.

curlx. Wings curled upwards along all their length and slightly divergent. Good viability. Easy classification. Complete penetration. Recessive. Chromosome O.

wave. Wings waved along the transversal axis, slightly curved in general, upwards in the margin. Good viability. Easy classification. Recessive. Chromosome O.

arista-tarsus. Arista of the antennae thickened, sometimes of jointed appearance. Good classification. Expressivity slightly variable. Complete penetration. Recessive. Chromosome O.

malt-brown. Eyes of a brown and not bright color, slightly yellowish in the rims when young. Classification difficult. Expressivity variable. Complete penetration. Recessive. Chromosome E.

blistered-plexus. The 2nd and 3rd longitudinal veins are very near one another. The 2nd longitudinal vein, before reaching the margin, divides in two. There is an extra vein zone limited by the division in the part of marginal and submarginal cell. Between the 3rd and 4th longitudinal veins there is more space than usual. The 5th longitudinal vein shows another extra vein zone. Very often the wings show bubbles or may be spoiled. Good viability. Easy classification. Recessive. Chromosome U.

sparse-margin. Hairs more spaced all around the wing margin. The 4th vein does not reach the margin in most individuals. Sometimes it does not reach either the 2nd or the 5th. In rare individuals it appears as a delta formation in the wing. Sometimes the distal edge of the wing has a convex bay. Veins not very conspicuous. Some individuals have the anterior and posterior crossveins set backwards. Expression very variable depending mainly on the rest of the background. Recessive. Chromosome E.

tibia-tarsus. Tibia and tarsus of the three pairs of legs thicker and shorter. They are darker, too. Viability inferior to the normal. Males show better expressivity. Complete penetration. Recessive. Chromosome J.

ciruela. Eyes of a bright plum color. Testes not orange when young. Allelomorphism not tested for copper (Spurway 1945). Good classification. Recessive. Chromosome E (determined by Rosa Miró).

tarsi. The first tarsal joint, especially in posterior legs, shorter and curved. Viability inferior to wild type. Expressivity variable. Incomplete penetration. Recessive. Chromosome U.

dorso-scute. Dorsocentral and scutellar bristles shorter and slightly thinner. Sometimes some of them are of normal length and consistency. Fair viability. Complete penetration. Recessive. Chromosome J.

dark-leg. Tibia and tarsus of the three pairs of legs thicker and shorter. They are darker, too. Viability inferior to the normal. Expressivity very variable. Males show better expressivity. Complete penetration. Recessive. Chromosome J.

short-leg. Tibia shorter and arched. Tarsi short and deformed mainly in the third pair of legs. In a few males tarsi combs appear as fused and very small, so that they look like only one. Viability inferior to the normal. Classification sometimes a bit difficult. Expressivity variable. Complete penetration. Recessive. Chromosome E.

Report of W. Pinsker
Universität Tübingen, Germany

vg: vestigial. Spontaneous mutant in a laboratory stock. Wings extremely reduced and held at right angles to body. Halteres also reduced. Wing size varies according to the genetic background. Postscutellar bristles erect and shorter. Localized on chromosome E. Viability about 50% of normal. Completely recessive.

gibberosa

Report of C. Koelling and R.L. Seecof
City of Hope Medical Center, Duarte, California

y: yellow. Spontaneous sex-linked recessive (single male) cuticle yellow, bristles of adult and mouthparts of larva brown, easily distinguished from black counterparts of normal. Rkl.

QUOTABILITY OF NOTES

Note: It is not the policy of DIS to enter permissions to quote in this section for material appearing in the same issue.

Ashburner, M. & T. Littlewood 51:145
Band, H.T. 46:106, 119, 120, 145
47:86, 87, 88
Deltombe-Lietaert, M.-C., M. Libion-Mannaert
& A. Elens 51:132
Elens, A. 51:107

Holm, D.G., M. Baldwin, P. Duck & A. Chovnick
44:112
Libion-Mannaert, M., M.-C. Deltombe-Lietaert,
J. Delcour & A. Elens 51:28
Oksala, T.A. 51:54
Van den Haute, J. & A. Elens 51:126

For previous listings see DIS 38, 42, 43, 44, 45, 47, 48, 49, 50 and 51.

pseudoobscura

Report of W.W. Anderson and R.A. Norman
University of Georgia, Athens, Georgia

Brief descriptions and map positions of currently-available mutants of *D. pseudoobscura*. Descriptions of *D. pseudoobscura* mutants are scattered over a literature now thirty or forty years old. To accompany the list of *D. pseudoobscura* stocks in this issue of DIS, we summarize below the descriptions of mutants currently available and also give their map positions. The linkage data are from Sturtevant and Tan (Jour. Genet. 34:415-432, 1937) and Sturtevant and Novitski (Genetics 26:517-541), with one or two additions. A few subsequent studies (e.g., Dobzhansky and Epling, 1948, Proc. Natl. Acad. Sci. U.S. 34:137-141; and Levine and Levine, 1955, Genetics 40:399-405) show that corrections may be necessary, but additional data are available for so few loci that changes in the maps do not seem warranted at this time. We are grateful to Prof. Eliot Spiess and Mr. Boris Spassky for their help in describing several mutants.

CHROMOSOME I

co	compressed	1-105.7	Variable reduction in size of eyes; the eyes of an individual may differ in expression; frequently a mid-dorsal depression on thorax, which is more extreme anteriorly.
ct	cut	1-21.9	Edges of wings nicked.
ll	lanceolate	1-159.7	Wings narrowed at tips and slightly divergent.
Pt	pointed	1-0.0	Wings severely reduced. Viability of homozygotes good.
se	sepia	1-135.7	Dark brown eye color - darker with age.
sh	short	1-201.9	Wing veins do not run full length of wing.
sn	singed	1-66.5	Bristles twisted and shortened.
sp	snap	1-176.7	Wing vein L2 interrupted.
tt	tilt	1-204.2	Upward tilt to end of wings; 3rd wing vein short; L2 and L4 sometimes short.
v	vermillion	1-69.1	Eye color bright scarlet
w	white	1-65.3	White eyes.
y	yellow	1-59.3	Body color yellow; bristles brown with yellow tips.

CHROMOSOME II

Ba	Bare	2-62.1	Bristles on thorax and head absent or very short. Homozygous viable in some strains; lethal in others (such as the Bare/Delta "analyzer stock").
bv	brevis	2-?	Bristles uniformly short and stubby.
bx	bithorax	2-52.0	Balancers enlarged and winglike.
ca	claret	2-60.2	Eye color ruby.
cd	cardinal	2-?	Eye color red (yellowish vermillion).
Δ	Delta	2-8.4?	Veins of wings broaden into deltas at junction with margin; surface of eyes rough. Homozygous lethal.
ga	garnet	2-?	Eye color deep purplish ruby.
gl	glass	2-83.3	Eyes have glassy texture; they are also slightly reduced in size and yellowish.
pcv	posterior crossveinless	2-?	Crossveins absent or just partially present.
ss ^a	spineless		
	aristapedia	2-?	Antennae enlarged and tarsuslike.
upt	upturn	2-0.0	Wings curled upward.
ubx	ultrabithorax	2-?	Extreme bithorax.

CHROMOSOME III

amy	amylase	3-33.0	Electrophoretic variants of the enzyme amylase.
Bl	Blade	3-77	Wings blade-shaped; leading edge is always either straight or concave, never convex; in a few strains half of the wing is cut off. Homozygous lethal.

cv	crossveinless	3-68.0	Crossveins absent or partially present.
L	Lobe	3-?	Eyes small with nick in anterior edge. Homozygous lethal.
or	orange	3-0.	Eyes bright orange.
pr	purple	3-49.9	Eyes brown with age.
px	plexus	3-22.3	Wings have network of extra veins.
rg	rugose	3-?	Extreme rough eyes.
r	rough	3-?	Rough eye texture.
Sc	Scute	3-28.3	Reduction in or loss of at least one scutellar or dorso-central bristle.
spr	spread	3-?	Wings outstretched perpendicular to body.

CHROMOSOME IV

Cy	Curly	4-67.2	Wings turned down or up; wing veins, particularly 2nd and 3rd longitudinal veins, irregular as if drawn with a shaky hand. Homozygous lethal.
hk	hooked	4-40.2	Bristles hooked at tip or blunted.
inc	incomplete	4-0.0	Fourth and fifth wing veins shortened.
j	jaunty	4-17.1	Slight upturning at tip of wing.

CHROMOSOME V

spa	sparkle	5-?	Eyes rough in varying degrees and somewhat bulging.
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RESEARCH NOTES

Starmer, W.T. and W.B. Heed. University of Arizona, Tucson. The infection of *Drosophila* cultures by species of the genus *Prototheca*.

During the course of our investigation of yeasts associated with natural populations of *Drosophila* we encountered three species of *Prototheca* (a unicellular algae devoid of chlorophyll) in the necrotic tissues of cacti. Several species of *Drosophila* in the Sonoran

Desert utilize rotting cacti for breeding and feeding. The three species of *Prototheca* accounted for 2.3% of 132 yeast isolates from cacti. A concomitant survey of yeasts in the crops of *Drosophila* adults (187 isolates) did not yield *Prototheca* species. We recovered *Prototheca zopfii* Krüger from organpipe cactus, *Prototheca filamenta* Arnold and Ahearn from senita cactus (8×10^5 cells/cc of cactus tissue), and an unknown species of *Prototheca* from cochal cactus (9.5×10^5 cells/cc of cactus tissue). Subsequently, an infection of *Prototheca* spread throughout the *Drosophila* cultures maintained in our laboratory. It is not known if *Prototheca* is pathogenic to *Drosophila* but the infected cultures became overgrown with the organism, covering the surface of the medium and surface of many of the flies, resulting in the problematic maintenance of many cultures. Cultures of *D. mojavensis* and *D. pseudoobscura* were affected most severely.

Species of *Prototheca* have also been isolated from slime fluxes of trees (Phaff, Yoneyama and Do Carmo-Sousa, 1964 and Phaff, Miller and Mrak, 1966) on which *Drosophila* breed and feed. The species produce thick-walled cells which divide internally by irregular cleavage forming 2-15 aplanospores (Arnold and Ahearn, 1972). Yeasts, on the other hand typically reproduce by budding. *Prototheca* species are usually many times larger than yeasts and probably cannot be ingested by adults because of the limitation imposed by the feeding apparatus.

We have attempted to eradicate the infection from the cultures by treatment with cycloheximide (.1% w/v actidione) with limited success. We are open to suggestions concerning its eradication.

Reference: Arnold, P., D.G. Ahearn 1972, Mycol. 64:265-275; Phaff, H.J., M. Yoneyama and Lidia Do Carmo-Sousa 1964, Riv. Patol. Veg. 4:485-497; Phaff, H.J., M.W. Miller and E. M. Mrak 1966, In: The Life of Yeasts, Harvard Univ. Press p. 110.

Oishi, K., A. Tanaka and A. Fukunaga.
Kobe University, Japan. A novel sex-transforming gene in *D. melanogaster*.

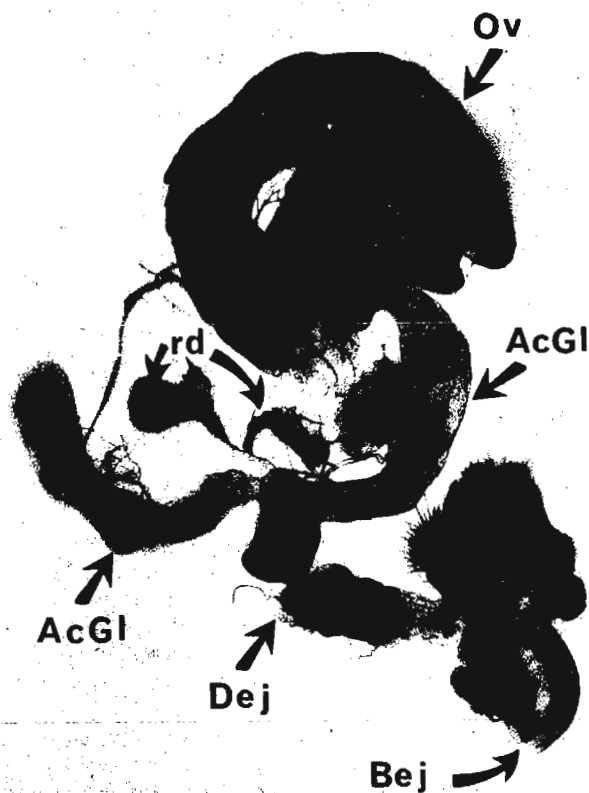
references in Miyamoto and Oishi 1975, *Genetics* 75:55; sex-ratio organism previously called SR-spirochetes but now recognized as Mycoplasma-related specifically with a newly established genus *Spiroplasma*, references in Williamson and Whitcomb 1975, *Science* 188:1018), and the

other sex specific-lethal genes.

One of the sex-specific lethal genes we have been studying is the gene maleless (*mle*: 2-55, Fukunaga, Tanaka, and Oishi 1975 *Genetics* 81:135) which is characterized by its male-specific lethality and is unique among sex-specific lethals so far known in its late lethal stage (pupal when mothers are heterozygous for *mle* and late-larval when mothers are homozygous, Tanaka, Fukunaga, and Oishi, in preparation).

Recently we have noticed that in one line of *mle* stock maintained by crossing

Figure 1. Internal genitalia of transformed "male". AcGl: accessory gland. Bej: ejaculatory bulb. Dej: ejaculatory duct. Ov: ovary. rd: rudimentary testis and/or seminal visicle.



S^{M1}(Cy)/*mle* females and males, a few non-Cy males emerge. These males are apparently larger in size than their Cy sib males but are nearly normal in external morphology characteristic to males such as the presence of sex-comb, coloration and the structure of abdominal tergites, and the external genitalia. Internal genitalia are also normal except for the testes which are absent or only rudimentary buds. Most of these "males" have one or two structures which closely resemble the rudimentary ovary, and in about 5-10% of these males, surprisingly,

a mature-looking ovary is found to be present (Figure 1). The ovary appears as though it is floating around without being associated with any definite structure.

Brown and King (1961, *Genetics* 46:143) observed oogenesis-like processes in the rudimentary "testis" of XX;*tra/tra* transformed males, but never a mature egg. Watanabe (1975, Japan. J. Genet. 50:269) described a second chromosome transformer gene, *tra-2*. The testis of XX;*tra-2/tra-2* transformed males was a reduced bud-like structure, and no ovarian structure was observed.

A stock is now established in which non-Cy flies are all males apparently because non-Cy females are transformed into "males" (non-Cy XY males are lethal due to the *mle* gene). Further studies are under way.

Supported in part by a grant-in-aid from the Ministry of Education, Japan, No. 054177, 1975.

Kerkis, A.Ju., I.F. Zhimulev and E.S. Belyaeva. Institute of Cytology and Genetics, Novosibirsk 630090, USSR. EM autoradiographic study of ^3H -uridine incorporation into *D. melanogaster* salivary gland chromosomes.

RNA synthesis in 100A-F region (3R chromosome) and in some non-identified regions of *D. melanogaster* salivary chromosomes has been examined with EM autoradiography.

Third instar larvae compound for *gt* alleles (*gt*^{E-6}/*gt*^{l3z}) (1) were used. The salivary glands were dissected in Ephrussi-Beadle saline, transferred into the same medium con-

taining ^3H -uridine (1m Ci/ml, specific activity 49 Ci/mM, Amersham) and incubated for 5 min. The glands were then fixed in the cold methanol: propionic acid (3:1) for 10-15 min and squashed in 45% acetic acid in Petri dishes coated with carbon. Just after squashing slides frozen in liquid nitrogen, coverslips were removed, preparations were postfixed in 2% osmium tetroxide and embedded in Araldite. The sections ranging in thickness from 500 angstroms to 800 angstroms were mounted on Formvar-Carbon coated 200 mesh Nickel grids. A monolayer of Ilford L4 emulsion was applied to the grids. After 2-4 months of autoradiographic exposure, the preparations were developed (3 min) using Kodak D19 developer. Preparations were examined under a JEM-7 electron microscope at 50 and 80 kv and original magnification was from X4000 to X15000. This technique has been described in detail previously (2).

Figure 1 illustrates ^3H -uridine incorporation into unknown chromosome regions whose morphology is similar to that of interbands (Figures 1a,b,c,d,f,h, arrows) and puffs (42 region in Figures 1e,g and in Figure 1h, double arrow). Centromere region of 2R chromosome is also clearly labelled (Figures 1e,g, 40-41B region). (See also Lakhotia and Jacob (3)). It may be noted that silver grains are almost completely absent over the compact bands.

For the careful study of ^3H -uridine incorporation into the distal end of 3R chromosome, 100A-F was chosen. EM photographs of 32 sections have been analyzed and the map was derived (Figure 2a). The band number was found to be much lower than in Bridges' revised map (4) and corresponds more to the first map (5). All the bands, 100A1-2, 100A5-6, 100B1-2, 100B4-5, 100C1-2 and 100C4-5, considered as "double bands" (4), are seen on the electron microscope photographs as compact and homogeneous single bands (Figure 3). Thus our observations give further support to the opinion that the great number of "doublets" might be a fixation artifact (6-10).

We cannot detect thin bands 100A3, 100A4, 100A7 or 100B3 and therefore suppose that chromosome region between broad compact bands 100B1-2 and 100B4-5 may be considered as a real interband (Figures 3b,c) along with regions between 100C1-2 and 100C3, 100C3 and 100C4-5, which are represented as interbands in Bridges' map (4). Regions between bands 100A1-2 and 100A5-6, 100A5-6 and 100B1-2 can be supposed to be interbands also. The telomere, 100F1-5 region, on all the sections, looks like reticular, partly decondensed material (Figure 3a). No individual bands were resolved.

Figure 4 shows some examples of labelled 100A-F region at chosen exposure time. To estimate the level of labelling in each region, all the silver grains were counted and plotted on the photographic map of the 3R chromosome. Thirteen EM autoradiographs were analyzed and the results are presented in Figure 4a. It is seen that interbands between 100B1-2 and 100B4-5, between 100C3 and 100C4-5, as well as decondensed bands 100A5-6, 100C3 and 100F1-5 are well labelled. On the other hand, compact bands 100A1-2, 100B1-2 and 100B4-5 are almost completely unlabelled. Figure 4a demonstrates that bands 100B1-2 and 100B4-5 are labelled weaker than an interband between them. It means that the grains over this interband are not caused by scattering of beta-particles from these bands. The background on these slides is practically absent. Therefore interbands may represent small regions of DNA transcribed intensively. Taking into account that interband contains about 5-10% of band DNA it is possible to suggest that the intensity of RNA synthesis per equivalent length of DNA molecule in interband and puff is similar. This question was discussed in detail in another paper (11).

Acknowledgements. We are thankful to Prof. Dr. I.I. Kiknadze for initiation of this study, to Dr. N.B. Khristoljubova for help and advice. We would like to thank Dr. T.C. Kaufman for kindly supplying the *gt* stocks.

References: (1) Kaufman, T.C. 1972, *Genetics* 71:s 28-29; (2) Kerkis, A.Ju., I.F. Zhimulev, E.S. Belyaeva 1975, *Tsitologia* (USSR), in press; (3) Lakhotia, S.C. and J. Jacob 1974, *Exp. Cell Res.* 86:253-263; (4) Bridges, P.N. 1941, *J. Hered.* 32:64-65; (5) Bridges, C.B. 1935, *J. Hered.* 26:60-64; (6) Beermann, W. 1962, *Riesenchromosomen. Protoplasmatologia* Bd. IVD, Wien, Springer; (7) Berendes, H.D. 1968, *DIS* 43:115; (8) Berendes, H.D. 1970, *Chromosoma* 29:118-130; (9) Breugel, F.M.A. van, A. Ray and H. Gloor 1968, *Genetica* 39:165-192; (10) Sorsa, M. 1969, *Ann. Acad. Sci. Fen. AIV*, 151; (11) Zhimulev, I.F. and E.S. Belyaeva 1975, *Theor. Appl. Genet.* 45:335-340.

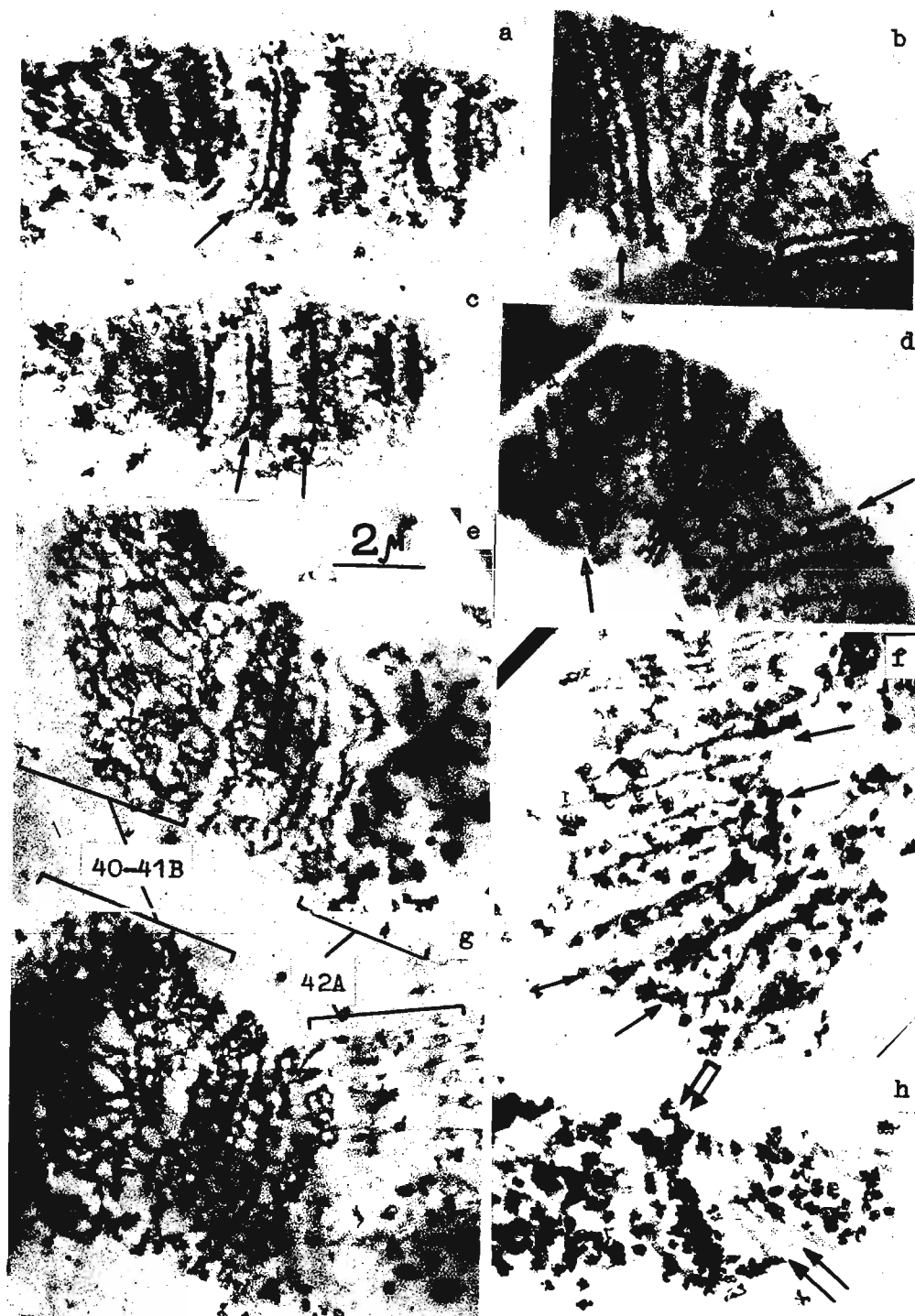


Figure 1. Labelling patterns of unknown chromosome regions. a and c, b and d, e and g are pairs of serial sections. Two microns scale is valid for all photographs.

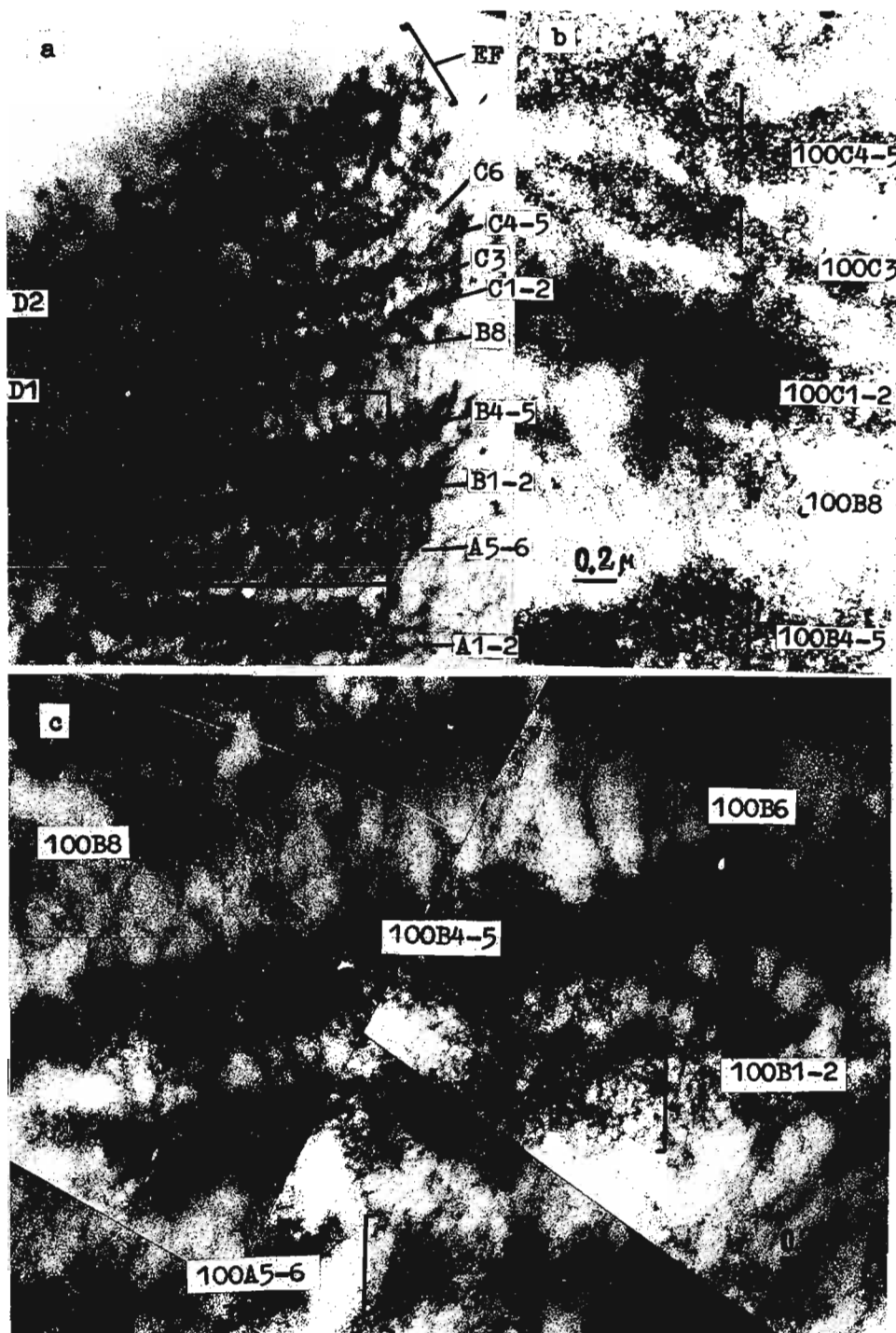


Figure 3. EM photographs of 100A-F region:

- a) low magnification (bands designated according to Bridges' revised map (4))
- b) higher magnification of 100B-C region
- c) region 100AB outlined in 3a

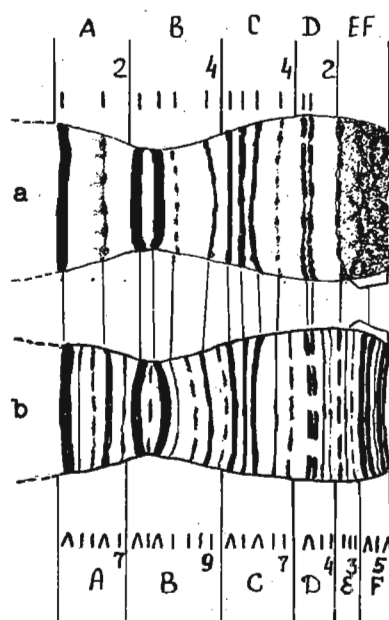


Figure 2. Comparison of band number.

- a) EM map of 100 AF region
b) banding pattern according to Bridges (4)

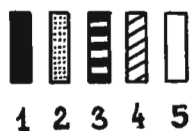
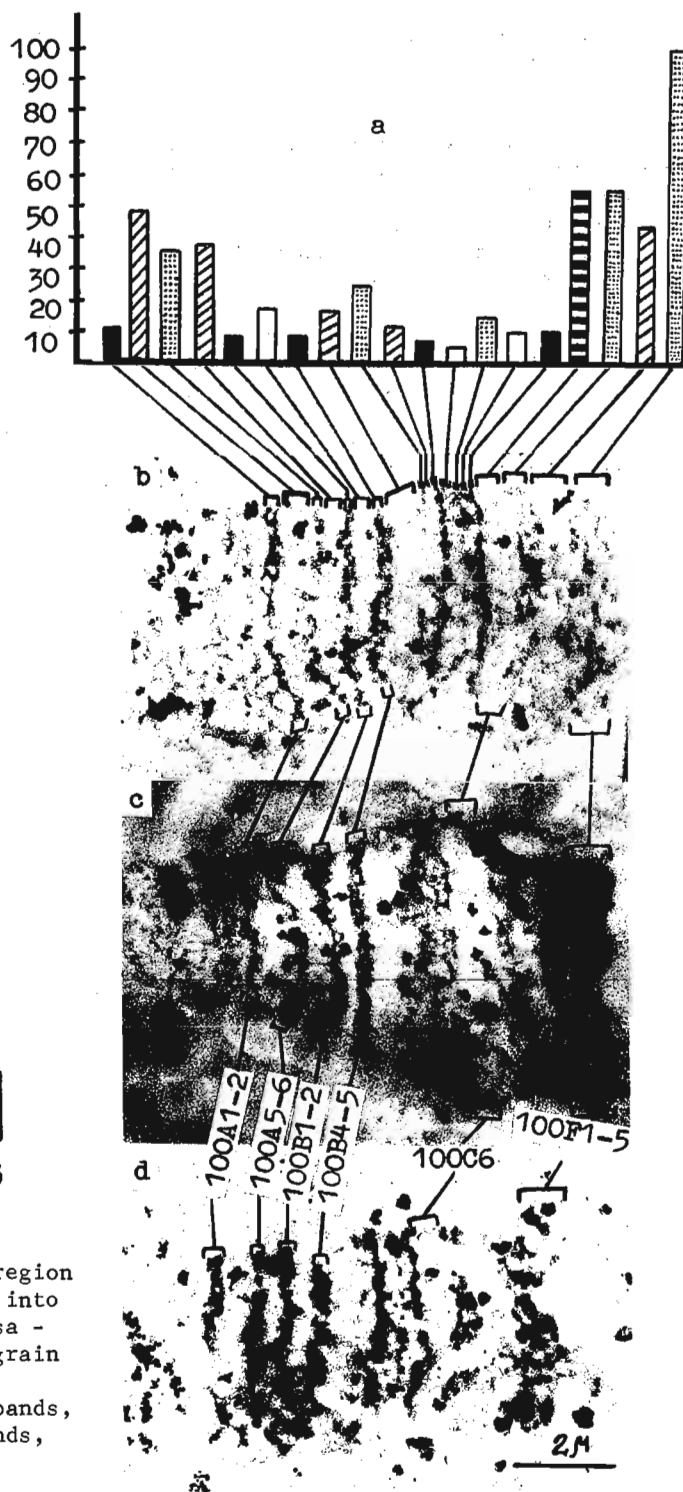


Figure 4. Labelling patterns of 100A-F region

- a) intensity of ^3H -uridine incorporation into individual chromosome regions: Abscissa - chromosome regions; Ordinate - total grain number over them for 13 sections.
1 - condensed bands, 2 - decondensed bands,
3 - small puffs, 4 - supposed interbands,
5 - real interbands

b,c,d) labelled 100A-F regions



Beppu, K. and E. Momma. Hokkaido University, Sapporo, Japan.
Microdistribution of drosophilid flies in the vicinity of the stream.

The series of 'Drosophila survey of Hokkaido' (cf. references) has concerned microdistribution of *Drosophila*. The robusta species group and *D. ezoana* were collected in abundance in the vicinity of the stream (Kaneko and Tokumitsu 1969, Takada 1971, and Toda 1973). In this survey,

six traps were set up at different distances from the stream (0, 5, 10, 15, 25 and 50 m) at each of four localities near Sapporo City - Jozankei, Moiwa, Soranuma and Nopporo.

Numbers of flies collected are shown in Table 1. *D. ezoana*, *D. lacertosa*, *D. okadai*, and *D. neokadai* are very restricted in their habitats within the vicinity of the stream. However the other species of robusta group did not show such a restriction. From these facts, the habitat preferences of these species seem to be peculiarly specific within the two types.

Table 1. Collected number of flies at each trap spot.
(Collections were carried out from May to October at Nopporo and from July to October at Jozankei, Moiwa and Soranuma).

Locality	Distance from water's edge of the stream					
	0	5	10	15	25	50 m
<u><i>D. lacertosa</i></u>						
Jozankei	21	25	3	3	2	-
Moiwa	36	32	7	1	1	2
Soranuma	47	3	3	3	-	-
Nopporo	443	162	117	80	18	8
<u><i>D. okadai</i></u>						
Jozankei	48	101	10	1	2	1
Moiwa	2	-	1	-	-	-
Soranuma	115	9	6	1	2	-
Nopporo	4	-	1	-	-	-
<u><i>D. neokadai</i></u>						
Jozankei	34	18	1	3	-	-
Moiwa		No specimens were collected				
Soranuma	135	6	2	1	-	-
Nopporo	16	5	1	-	-	-
<u><i>D. moriwakii</i></u>						
Jozankei	3	2	28	10	19	8
Moiwa	86	111	114	37	26	30
Soranuma	373	277	212	388	165	97
Nopporo	54	26	82	51	39	57
<u><i>D. sordidula</i></u>						
Jozankei	1	-	-	1	1	-
Moiwa	1	1	-	-	-	-
Soranuma	26	9	13	29	19	12
Nopporo.	1	-	1	-	-	-
<u><i>D. pseudosordidula</i></u>						
Jozankei	3	-	-	2	1	-
Moiwa	2	1	-	-	-	-
Soranuma	4	1	11	7	12	2
Nopporo	10	2	8	-	2	3
<u><i>D. ezoana</i></u>						
Jozankei	158	61	2	-	-	-
Moiwa		No specimens were collected				
Soranuma	2	-	-	-	-	-
Nopporo		No specimens were collected				

References: Kaneko, A. and T. Tokumitsu 1969, J. Fac. Sci. Hokkaido Univ. VI. Zool. 17: 244-256; Takada, H. 1971, J. Fac. Gener. Edu. Sapporo Univ. 2:15-30 (In Japanese with English summary); Toda, M.J. 1973, J. Fac. Sci. Hokkaido Univ. VI. Zool. 18:532-550.

Parsons, P.A. La Trobe University, Bundoora, Victoria, Australia. Temperature and humidity preferences of endemic southeast Australian *Drosophila* species in the wild.

Endemic Australian species, mainly *D. (Scaptodrosophila) inornata* Malloch, can be collected by sweeping off foliage into a net and not conventional baiting with bananas or other baits. It is therefore possible to obtain a fairly precise idea of the temperature/humidity relationships of adults in the field. The flies

have so far been found in permanently moist habitats such as tree fern gullies which minimize the normal temperature/desiccation extremes of the Mediterranean climate of southeastern Australia (Parsons et al., 1975).

Based on collections in over 17 localities in southeastern Australia from west of Melbourne to Sydney, microhabitats occupied by adults of endemic *Drosophila* species can be characterized as follows:

Temperature °C	Relative humidity (%)	Location of flies on foliage
< 12	90 - 100	usually none
12 - 15	90 - 100	widely scattered in foliage - may be considerable distances from permanent water
15 and up	90 - 100	1-4m above permanent moisture
15 - 21	≤ 80	1-2m above permanent moisture
≥ 21	> 80	very close to permanent moisture (< 1/2m)

Below 12°C flies are inactive but can occasionally be aspirated off the foliage where they are motionless (Grossfield & Parsons, 1975), and from 12°C to 15°C flies are few and widely scattered. Therefore both in Australian cosmopolitan (*D. melanogaster* and *D. simulans*, McKenzie, 1975) and endemic species, 12°C appears to be a lower boundary temperature for population continuity. Above 15°C as the temperature/desiccation stress increases, flies approach water progressively more closely.

For 15°C and up, flies were mainly collected on tree fern fronds in the 15 - 20°C range (mean 17.7 ± 2.0°C), while the ambient temperatures adjacent to the fern gully refuges were 16 - 27°C (mean 20.0 ± 3.4°C); that is, the temperature where flies were collected is less variable than the general weather conditions as expected if flies select optimal microhabitats. Minimum temperatures in the habitats during summer were from permanent running water (13 - 18°C, mean 14.5 ± 2.3°C). In one habitat the water was 18°C, ambient temperature 27°C and flies were from fern fronds almost at 20°C. Therefore flies adjust to microhabitats based upon temperature/humidity relationships optimal to their adaptive requirements. Such behavioral responses are expected since small insects have an extremely high ratio of surface area to volume, so that the amount of water lost by evaporation is large compared with the amount stored.

Therefore for the Australian endemics there are boundary conditions imposed by temperature and humidity as for the cosmopolitans, although the upper boundaries for the cosmopolitans are likely to be less stringent (Parsons, 1973). The upper boundary conditions for the Hawaiian endemics are in qualitative agreement with the Australian endemics (Carson et al., 1970). Until the breeding biology of the Australian endemics can be established, it will not be possible to relate boundary conditions to resource utilization as is so for the cosmopolitans.

I am grateful to Dr. Ian Bock for help in species identifications, and the Australian Research Grants Committee for financial support.

References: Carson, H.L., D.E. Hardy, H.T. Spieth and W.S. Stone 1970, Essays in Evolution and Genetics in Honor of Th. Dobzhansky (Eds. M.K. Hecht and W.C. Steere), Appleton-Century-Crofts, p. 437; Grossfield, J. and P.A. Parsons 1975, Proc. Roy. Soc. Vict., 87:235; McKenzie, J.A. 1975, Aust. J. Zool. 23:237; Parsons, P.A. 1973, Behavioural and Ecological Genetics: A Study in *Drosophila*, Clarendon Press, Oxford; Parsons, P.A., I.R. Bock, D.A. Hay and J. Grossfield (in preparation).

Kortselius, M.J.H. and E. Vogel. State University of Leiden, The Netherlands. A comparison of feeding and abdominal injection in mutagenicity studies with adult *Drosophila* males.

In mutagenicity screening programs with *Drosophila melanogaster*, the chemicals are usually applied to adult flies by either feeding or abdominal injection. There is experimental evidence indicating that the route of administration influences the mutagenic effectiveness of the chemical, depending on the physico-

chemical properties of the compound. Vogel & Lüers (1974) found 2,4,6-triCl-PDMT, a triazene with a very low water solubility, strongly mutagenic when fed, but not mutagenic at all when injected. This raises the question of whether there exist mutagens which, conversely, register as negative when fed. For such a comparative study we selected beta-propiolactone (BPL) because of its known instability in aqueous solution; at 25°C the half-life in aqueous solution is approximately 3½ h. X-linked recessive lethal frequencies were determined by means of the Basc test.

Adult feeding experiments were carried out according to the technique described by Vogel & Lüers (1974). 2-day-old Berlin K males were fed with 5% sucrose solution containing BPL or with 5% sucrose controls for three days. Freshly prepared solutions were replaced 6 times in experiment 1 and 3 times in experiments 2 and 3. In order to ensure immediate uptake of the mutagen, the flies had been starved for 9 h before exposure to the freshly prepared solutions. Immediately after treatment the males were mated individually to three 3-day-old Basc females. Different germ cell stages were sampled in three subsequent broods of three days duration each.

Abdominal injection of 1-day-old Berlin K males with freshly prepared 0.7% NaCl solutions containing BPL was carried out by hand. In this way it was possible to apply 0.2 µl (±0.05) of the test solution into the flies within 10 minutes after preparation of the solution. 24 h after injection the males were crossed with Basc females as mentioned above.

Under our experimental conditions, BPL is clearly mutagenic to *Drosophila* both after oral application (Table 1) and injection (Table 2).

After oral application the recessive lethal frequencies are relatively low (1.2%), even after exposure to such a high concentration as 25 mM of BPL. The mutation frequencies seem to be independent of the concentration of BPL the flies were exposed to. From these results one might conclude that BPL is only a weak mutagen to *Drosophila*.

The results after injection show quite a different picture (Table 2). When injected, BPL is a very potent mutagen to *Drosophila*, inducing up to 15% recessive lethals. Exposure to a 10

Table 1. X-linked recessive lethal frequencies after application of beta-propiolactone by adult feeding for three days.

	concentration in mM	days after treatment	X-chromosomes tested	n lethals	recessive lethal frequency in %
expt. 1	0	1 - 3	361	0	-
		4 - 6	311	0	-
		7 - 9	83	0	-
		1 - 9	755	0	-
	10	1 - 3	701	10	1.43 ± 0.45
		4 - 6	295	2	0.68 ± 0.48
		7 - 9	54	0	-
		1 - 9	1050	12	1.24 ± 0.34
expt. 2	0	1 - 3	1005	0	-
	10	1 - 3	991	10	1.01 ± 0.32
expt. 3	0	1 - 3	604	1	0.17 ± 0.17
		4 - 6	218	0	-
		7 - 9	121	0	-
		1 - 9	943	1	0.11 ± 0.11
	25	1 - 3	984	10	1.02 ± 0.32
		4 - 6	449	9	2.00 ± 0.66
		7 - 9	145	0	-
		1 - 9	1578	19	1.20 ± 0.27

Table 2. X-linked recessive lethal frequencies after application of beta-propiolactone by abdominal injection of 0.2 μ l.

	concentration in mM	days after treatment	X-chromosomes tested	n lethals	recessive lethal frequency in %
expt. 4	0	2 - 4	800	1	0.13 \pm 0.12
		5 - 7	578	2	0.35 \pm 0.24
		2 - 7	1378	3	0.22 \pm 0.13
	10	2 - 4	799	25	3.13 \pm 0.62
		5 - 7	495	10	2.02 \pm 0.63
		2 - 7	1294	35	2.70 \pm 0.45
	25	2 - 4	726	101	13.9 \pm 1.3
		5 - 7	346	36	10.4 \pm 1.6
		2 - 7	1072	137	12.8 \pm 1.0
expt. 5	0	2 - 4	563	2	0.36 \pm 0.25
		5 - 7	325	0	-
		8 - 10	168	0	-
		2 - 10	1056	2	0.19 \pm 0.13
	25	2 - 4	940	154	16.4 \pm 1.2
		5 - 7	257	30	11.7 \pm 2.0
		8 - 10	25	2	8.0 \pm 5.4
		2 - 10	1222	186	15.2 \pm 1.0

mM solution of BPL was 2 times more effective when injected, as compared with feeding. Injection of 25 mM BPL was even 10 times more effective than was feeding. In addition, the mutation induction after injection is clearly concentration-dependent (expt. 4).

The quantitative differences in BPL-induced mutation frequencies at equimolar concentrations are explained as resulting from the instability of the compound in aqueous solution.

1-(2-hydroxyethyl)-3-(2-chloroethyl)-3-nitrosourea (CNU-ethanol) was used as a reference mutagen. Berlin K males were exposed to 5 mM CNU-ethanol either by feeding for two days or by abdominal injection of 0.2 μ l, using a microapplicator set similar to that described by Mollet & Würgler (1974).

Table 3. X-linked recessive lethal frequencies after application of 5 mM CNU-ethanol either by adult feeding for two days or by abdominal injection of 0.2 μ l.

	days after treatment	X-chromosomes tested	n lethals	recessive lethal frequency in %
adult feeding	1 - 3	493	47	9.5 \pm 1.3
injection	1 - 3	362	30	8.3 \pm 1.4

The mutation frequencies in the CNU-ethanol experiments show no remarkable differences when fed or injected (Table 3). This is in line with findings with other reference mutagens, e.g. Trenimon (Mollet & Büchi, 1974; Vogel & Lüers, 1974).

Vogel & Lüers (1974) directed attention to the fact that in *Drosophila* certain mutagens are only detectable after oral application. On the other hand, our investigations demonstrate that mutagens might be missed or considered to be only weakly active if adult feeding were the only treatment procedure. From these observations it is obvious that for mutagenicity screening in *Drosophila* both application techniques should be taken into consideration, as has been recommended in Vogel & Sobels (1976).

References: Mollet, P. & F.E. Würgler 1974, DIS 50:202; Mollet, P. & R. Büchi 1974, DIS 51:96-97; Vogel, E. & H. Lüers 1974, DIS 51:113-114; Vogel, E. & F.H. Sobels 1976, in "Chemical mutagens" (A. Hollaender, ed.) vol IV:94-127 (Plenum Press, New York-London).

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Bewley, G.C. and J.C. Lucchesi. North Carolina State University, Raleigh, and The University of North Carolina, Chapel Hill. Evidence for a factor in the conversion of α -glycerophosphate dehydrogenase isozymes in *D. melanogaster*.

We have been exploiting the gene-enzyme system L-glycerol-3-phosphate dehydrogenase (α -GPDH, E.C. 1.1.1.8) in *Drosophila melanogaster* for a study on the epigenetic modulation of gene expression during development. The isozymes of α -GPDH have been demonstrated to be both tissue and developmentally specific (Wright and Shaw, 1969; Rechsteiner, 1970) while apparently the

product of a single structural gene (Wright and Shaw, 1969; Bewley, et al., 1974). In addition, each isozyme differs significantly in kinetic parameters and in particular with respect to their thermal stabilities: GPDH-1 (adult form) is stable at 50°C but decays at 57°C while GPDH-3 may arise by specific post-translational modification of a single polypeptide (Wright and Shaw, 1969; Bewley, et al., 1974) although no direct evidence in support of this model is currently available. In this communication we report the discovery and preliminary characterization of a factor found in larval extracts which converts thermal stable α -GPDH activity (GPDH-1) to thermal labile activity (GPDH-3) at 50°C.

Null alleles for α -GPDH were provided by Dr. S.J. O'Brien and homozygous stocks for any one allele were constructed according to the free crossing over scheme of O'Brien and Shimada (1974). The conversion of α -GPDH isozymes was studied by incubating partially purified adult enzyme (PPA) or partially purified larval enzyme (PPL) with crude adult or larval extracts from the homozygous null allelic stocks. In all experiments the reactions were incubated at 37°C for 2-4 hours and controls consisted of partially purified enzyme incubated with equal volumes of buffer or BSA (2mg/ml). Upon completion of the incubation, each solution was assayed for activity, thermally denatured at 50°C for 30 min., and the remaining activity determined. In some instances the null extracts were boiled for 5 min., heat treated at 60°C for 30 min., or pre-incubated with RNase (2 mg/ml) for 3 hours prior to incubation with partially purified enzyme.

The results presented in Table 1 illustrate a putative conversion event when partially purified adult enzyme is incubated with an aliquot of crude larval supernatant fluid from the *Gpdh* null lines. When the α -GPDH activity for PPA + N-0 larval mixtures is compared with both

Table 1. Incubation of PPA and PPL preparations with "null" allelic extracts^a

Incubation Medium	Initial Activity	2 Hour Incubation	Enzyme Activities ^b Thermal Denaturation	4 Hour Incubation	Thermal Denaturation	N
PPA:						
Buffer	1.97 ± .02	2.00 ± .02	1.62 ± .02	2.01 ± .01	1.60 ± .03	3
BSA	1.92 ± .04	1.96 ± .03	1.72 ± .04	2.01 ± .04	1.79 ± .04	3
N-0 (larval)	2.05 ± .04	1.93 ± .04	1.09 ± .03	1.83 ± .03	0.808 ± .05	5
N-0 (larval) Boiled	1.97 ± .01	1.98 ± .04	1.49 ± .02	2.01 ± .04	1.56 ± .06	3
N-0 (larval) Heated 60°C	2.03 ± .05	2.02 ± .01	1.56 ± .03	2.06 ± .01	1.59 ± .02	4
N-0 (adult)	2.12 ± .03	2.12 ± .02	1.67 ± .03	2.07 ± .01	1.57 ± .03	4
PPL:						
BSA	0.856 ± .004	0.808 ± .004	0.000			4
N-0 (adult)	0.860 ± .008	0.800 ± .004	0.000			4

^aAliquots of PPA (GPDH-1) or PPL (GPDH-3) were mixed with equal volumes of each incubation medium and incubated at 37°C. After each incubation time, an aliquot of each solution was denatured at 50°C for 30 min. and the remaining activity determined. N-0 represents an EMS induced null allele for α -GPDH whose complete designation is *Gpdh*^{N-0}.

^bEnzyme activities are expressed as units per ml ± the standard error. N is equal to the number determination.

buffer and BSA control mixtures, there is a marked difference in that the N-0 sample lost 44% of the heat stable activity in 2 hours while the buffer and BSA controls lost only 18% and 12% respectively. When the same comparisons were made at 4 hours of incubation the N-0 sample had lost 57% while the control samples remained relatively unchanged. The kinetics of the conversion process provides evidence for the concentration dependency of larval extracts as a function of the rate of conversion of PPA preparations (Figure 1A), and that the process follows a time dependent course (Figure 1B). Table 1 provides evidence that the conversion

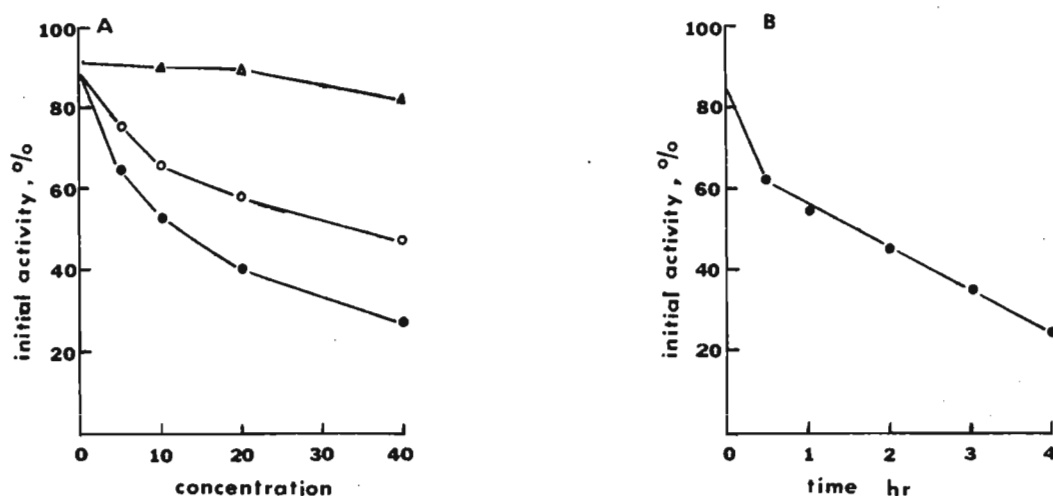


Figure 1. The kinetics of α -GPDH isozyme conversion: (A) The conversion of GPDH-1 to GPDH-3 by the incubation at 37°C of PPA preparations with different concentrations of adult and larval α -GPDH "null" allelic extracts. () adult N-0 extracts (mg live wt./ml) incubated for 2 hours; (o) larval N-0 extracts (larval/ml) incubated for 2 hours; (o) larval N-0 extracts (larvae/ml) incubated for 4 hours. Each point is the mean of three determinations.

(B) The conversion of GPDH-1 to GPDH-3 as a function of time. PPA preparations were incubated at 37°C with N-0 larval extracts at a concentration of 20 larvae per ml. Each point is the mean of two determinations.

factor is thermal labile at 60°C and RNase resistant which may suggest that the factor is protein in nature. Table 1 and Figure 1A also illustrate that the conversion factor is not readily apparent in crude adult extracts (i.e. either it is not present or it is present in reduced levels) and that the conversion process does not result from a simple random reassociation of subunits since it is unidirectional (GPDH-1 \rightarrow GPDH-3) and occurs with a partially purified GPDH-1 preparation upon the addition of a crude larval extract which does not contain active GPDH-3 subunits (i.e. from larvae bearing the N-0 null allele). Experiments where mixtures containing equal volumes of PPA and PPL preparations were incubated at 37°C or under conditions of freeze-thaw in high ionic strength buffer did not result in subunit exchange as evidenced by the failure of GPDH-2 to appear on gels.

In this study an effect on α -GPDH isozymes by endogenous inhibitors or activators is unlikely. In addition the apparent molecular weight for GPDH-1 and GPDH-3, as determined by gel filtration, is 66,000 daltons (Bewley, et al., 1974) which excludes the possibility that these isozymes constitute a polymeric series. The molecular nature of the conversion phenomenon is not known at this time, however it is believed that these results add support to a model of post-translational modification in the differentiation of α -GPDH isozymes.

References: Bewley, G.C., J.M. Rawls, and J.C. Lucchesi 1974, *J. Insect Physiol.* 20: 153-165; O'Brien, S.J. and Y. Shimada 1974, *J. Cell Biol.* 63:864-882; Rechsteiner, M.C. 1970, *J. Insect Physiol.* 16:1179-1192; Wright, D.A. and C.R. Shaw 1969, *Biochem. Genetics* 3:343-353.

Godbole, N.N. and V.G. Vaidya. University of Poona, India*. A new species of genus *Leucophenga* (Drosophilidae) from India: *Leucophenga* (*Leucophenga*) *neoangusta* sp. nov.

Four species of *Leucophenga* have been reported so far from India. A new species belonging to this genus is described here.

DESCRIPTION OF MALE IMAGO: General features and head: Body about 2.7 mm in length. Eyes red, bare. Antenna brown, third segment dark brown with few prominent bristles. Second oral rays including the terminal fork. Palpus minute. First orbital slightly shorter than the third. Post-vertical small. Occiput black.

Thorax: Mesonotum orange, with a dark brown squarish area behind dorsocentrals. Thin dark brown lines along the dorsocentral lines. Scutellum dark brown. Humerals 2, lower longer. Acrostichal hairs in 10 irreg-

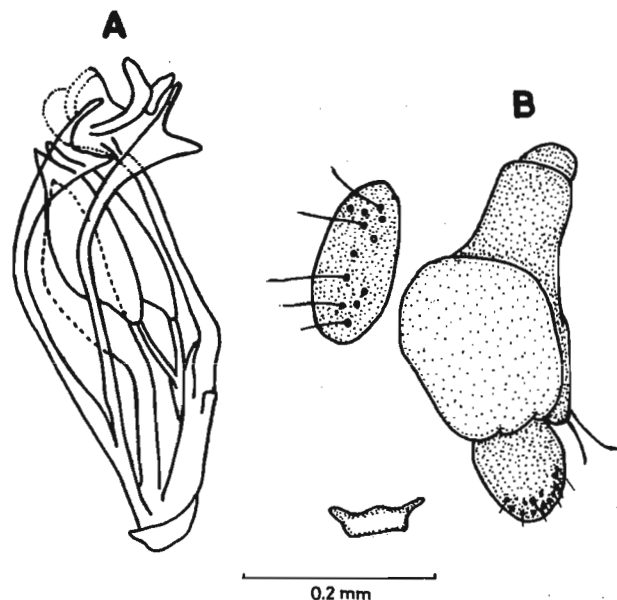


Figure: *Leucophenga neoangusta* sp. nov.
A. Phallic organs; B. Peripheral phallic organs.

ular rows. Cross distance between dorsocentral about four times the length distance. Prescutellars well developed, slightly longer than the anterior dorsocentrals. Anterior scutellars divergent. Posterior scutellars crossing each other. Sterno-index about 0.7.

Legs: Yellowish. Middle and hind knees black. Prespicals on all three tibiae and apicals on second. Two longitudinal rows of cuneiform bristles enclosing two rows of stout

bristles on second tarsus.

Wings: About 2.3 mm in length. Anterior part of wing fuscous. Third costal section with about 8 thornlike warts. Costal index about 2.0. 4th vein index about 2.4. 4C-index about 1.5. 5X-index about 1.2. C1 bristles 2. C3 bristles on basal 2/3.

Abdomen: 1T yellow, remaining tergites mostly black. 2T medially yellow. 3T paler, with median black spot.

Peripheral phallic organs: Genital arch dark brown, broad, pubescent and truncate below. Heel prominent with 3 long bristles. Clasper oval, pubescent and with few stout bristles on the lower half. Anal plate separate from the genital arch, elongate and with about 10 long bristles and numerous small evenly distributed bristles. Decasternum roughly rectangular.

Phallic organs: Aedeagus yellow, apically pointed, pubescent. Anterior paramere oblong. Posterior paramere slender. Ventral fragma narrow and pointed proximally. Novasternum slender, apically bifid.

MATERIAL: Holotype: Male: Poona (India) July 1972 (Vaidya). Deposited with Department of Zoology, University of Poona, Poona 411 007, India.

Paratype: 3 males, 2 females. 1 male deposited with Professor T. Okada, Department of Biology, Tokyo Metropolitan University, Tokyo, Japan.

HABITAT: The flies were collected on vegetable garden plots by sweeping with net. It was not possible to rear them in the laboratory.

RELATIONSHIP: This species is closely related to *Leucophenga* (L.) *angusta*. However, it differs from the latter in having black quadrate area at the caudal part of mesonotum and in having a few stout bristles on the ventral half of clasper.

ACKNOWLEDGEMENT: The authors are grateful to Professor Okada for checking the description and for confirming the identification of the species.

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Thompson, S.R., J. Stein and S. Cruice.
Ithaca College, Ithaca, New York. Glutamic-oxaloacetic transaminase (GOT) activity in pupae and adult *Drosophila melanogaster*.

Enzyme assays were done on whole-fly extracts of various developmental stages where the enzyme source was a crude extract prepared in the following fashion: (1) 20 organisms were weighed and then homogenized in five ml of distilled-demineralized water in a glass-teflon tissue

grinder. (2) The homogenates were sonicated for two minutes in 30-second bursts at peak intensity in a Kontes Ultrasonic Cell Disrupter. (3) The sonicated homogenates were centrifuged at 27,000 x g for five minutes. (4) The supernatant was cleared with not more than five mg of Norite A and recentrifuged at 27,000 x g for 10 minutes. (5) The supernatants were removed for enzyme assays. All steps in the isolation were carried out at 4°C. The pellets of the centrifugation steps contained almost no GOT activity.

Total GOT activity was determined using the method of Amador and Salvatore (1971) in which (1) 0.1 ml of the enzyme source was incubated for 15 minutes with 0.5 ml of buffered substrate (0.639 g L-aspartate; 0.146 g α -ketoglutaric acid; 0.726 g Tris; 0.05 g EDTA; 0.5 g NaOH in 50 ml of distilled-demineralized water - the pH was adjusted to 7.6 with 2N HCl). Control tubes contained 0.1 ml of distilled-demineralized water in place of the enzyme source. (2) Following incubation with the substrates, 0.5 ml of the dye, Fast Ponceau L (200 mg/50 ml 0.1 M lactic acid, pH 3.0), was added for a period of 8 minutes. (3) The staining reaction was stopped by the addition of five ml of a solution containing nine parts of one percent Tween-80 and one part concentrated HCl. (4) The optical density was determined at 467 nm, and the micromoles of oxalacetate formed determined by comparison to a standard curve. (5) Protein determinations were carried out following the procedure of Lowry, et al. (1951).

In this study, one unit of enzyme activity is equal to the amount of enzyme needed to produce one micromole of oxalacetate per minute at 30°C, and specific activity (S.A.) is defined as the number of enzyme units per milligram of protein.

In order to determine the proportion of the total enzyme activity which is localized to the mitochondrial and soluble fractions, single organisms of the desired developmental age were homogenized in 20 μ l of distilled-demineralized water and this crude extract was inserted into wells of 0.7% Agarose gels for electrophoresis using the EBT buffer system of Ursprung and Leone (1965). Electrophoresis was carried out at a constant 300 volts for one hour. Staining for GOT was performed according to the procedure of DeLorenzo and Ruddle (1970) for a period of 20 minutes. From densitometric recordings of the stained gels, the proportion of the total activity due to soluble and mitochondrial fractions was determined.

The maximum activity in three-day-old adults was achieved at a pH of 8.0, with a linear increase in activity from pH 6.0 to pH 7.8. All subsequent analyses were performed at pH 7.6, and the following specific activities were found at various pupal and adult ages:

Hours in Pupal Development	S.A. \pm S.E.	Adult Age in Days	S.A. \pm S.E.
0	1.63 \pm 0.02	0	0.95 \pm 0.05
12	1.65 \pm 0.04	1	1.38 \pm 0.01
24	0.55 \pm 0.07	2	1.42 \pm 0.03
48	1.08 \pm 0.02	3	1.41 \pm 0.02
72	0.81 \pm 0.01	4	1.23 \pm 0.02

The percentage of total activity ascribable to the mitochondrial and soluble GOT's at selected developmental stages were as follows:

Developmental Stage	% Mitochondrial	S.A. Mitochondrial	% Soluble	S.A. Soluble
12-hour pupae	20.3	0.335	79.7	1.315
72-hour pupae	41.7	0.338	58.3	0.472
2-day-old adult	60.8	0.857	39.2	0.554

As can be seen, it appears that the activity of the mitochondrial enzyme stays relatively constant throughout pupal development with an increase following eclosion. The activity of the soluble form drops during pupal development with a slight increase following eclosion.

References: Amadore and Salvatore 1971, Amer. J. Clin. Path. 55:6; DeLorenzo and Ruddle 1970, Biochem. Genet. 4:259; Lowry, et al. 1951, J. Biol. Chem. 192:265; Ursprung and Leone 1965, J. Exp. Zool. 160:147.

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Kakpakov, V.T. and L.G. Polukarova.
Kurchatov Institute of Atomic Energy,
Moscow, USSR. Effect of Concanavalin
A on established cultures of *Drosophila*
and mosquito diploid embryonic cells.

Becker (1) has found that Con A promotes fusion of *Drosophila* embryonic cells in a culture. To find out the possibilities of fusing cells of various insect species and different sublines of *Drosophila* cells in a culture, the action of Con A on established cultures of diploid embryonic cells of *Drosophila* (2) and mosquito (3)

was studied in the present work. The addition of 50 µg/ml Con A twice suppressed the growth of cells. High concentrations of Con A (100-200 µg/ml) have almost completely inhibited cell division. By cultivation of cells in Con A supplemented medium (100 µg/ml) for eight hours, the number of binucleate cells remained at the control level (2-4%). 24 hours after Con A addition, cell aggregation is observed, and 50-70% of binucleate and 1-3% of trinucleate cells are revealed. The maximum number of binucleate cells (approximately 80%) is detected at Con A concentration of 200 µg/ml. However, these cells treated at a high concentration of Con A are unable to divide further even after Con A has been removed from the medium. After the change to fresh medium, a sharp increase in percentage of tetraploid cells and decrease in percentage of binucleate cells is observed. This testifies to the fact that in binucleate cells formed by Con A treatment synchronous mitoses occur resulting in the formation of tetraploid cells. The decreased number of binucleate cells was accompanied by an increased percentage of tetraploid cells from 4% to 70%. By repeated treatment of Con A, one can obtain up to 20% of actively dividing octoploid cells.

The question was whether binucleate cells were formed by Con A action as a result of cell fusion or by the disturbance of the cell division being followed by polyploidization. To answer this question, two portions of cells of the same subline were mixed, one of them being labelled by ³H-thymidine for 43 hours. In this case 96% of the cells contained labelled nuclei. Then equal numbers of labelled (washed out from thymidine) and non-labelled cells were mixed and treated by Con A, after which the percentage of binucleate cells containing labelled and non-labelled nuclei was calculated. By mixing the cells of one subline, it has been found that 90% of binucleate cells contain either two labelled nuclei or two non-labelled ones and that only 9-10% of binucleate cells contain labelled and non-labelled nuclei both resistant and sensitive to ecdysterone sublines (4). When the cells of two different sublines were jointly cultivated, the number of binucleates containing only one labelled nucleus did not exceed 1% (see Table). While analyzing karyotypes of mixed culture of two cell sublines, hybrid cells containing parent marker chromosomes were not revealed among a thousand analyzed tetraploid metaphases.

Table: The formation of binucleate cells by joint cultivation of *Drosophila* labelled and non-labelled cells of two sublines in medium containing 100 µg/ml Con A (in any variant 100 binucleate cells were analyzed).

Mixed cultures	Types of binucleate cells in per cent		
	both nuclei labelled	both nuclei non-labelled	one nucleus labelled
X* plus X	43	47	10
X* plus Y	47	53	0
Y* plus X	34	65	1
Y* plus Y	53	38	9

Symbols: X-subline Da sensitive to ecdysterone; Y-subline Da T(X;3) resistant to ecdysterone; ³H-thymidine labelled cultures are asterisked.

Thus the problem of the possibility of fusion of *Drosophila* cells of two different sublines with the help of Con A remains to be solved. It was noted that mosquito cells as well as *Drosophila* cells had formed about 40% of binucleate cells under Con A action. However, mosquito cells did not fuse with *Drosophila* cells. The results obtained suggest the idea that when *Drosophila* and mosquito cells are treated with Con A, the formation of binucleate cells (dikaryons) may be induced, not by a fusion of two cells (1), but by a more complicated mechanism of Con A action upon insect cells. The bulk of binucleate cells (80%) may possibly be formed through reversible disturbance of cell division.

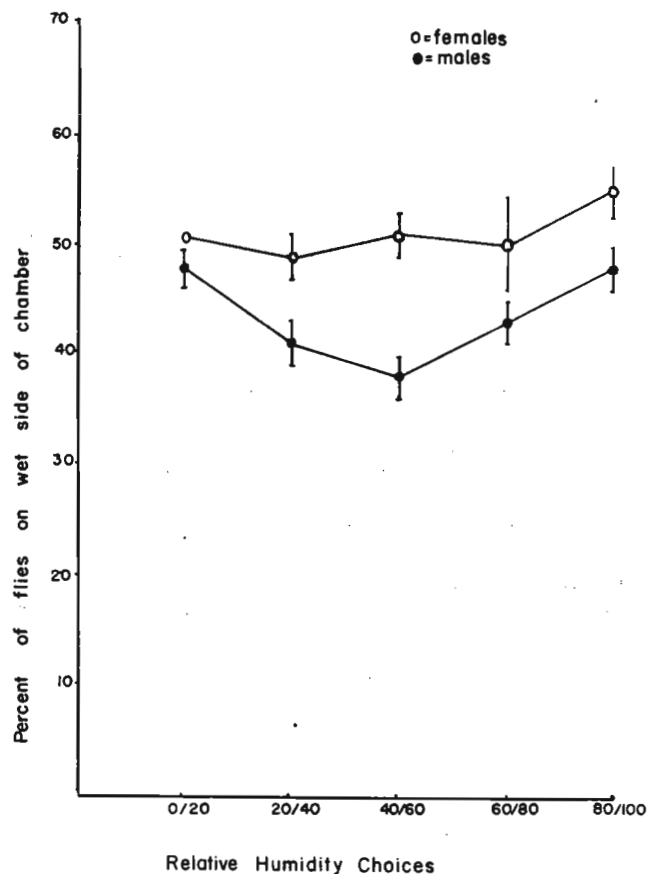
References: 1) Becker, J.L. 1972, C.R. Acad. Sci., Paris 275:2969; 2) Kakpakov, V.T., V.A. Gvozdev, T.P. Platova and L.G. Polukarova 1969, Genetika 5:67-75; 3) Varma, M.G.R. and M. Pudney 1969, J. Med. Entomol. 6:432-439; 4) Gvozdev, V.A., V.T. Kakpakov et al. 1974, Ontogen. 5:33-42.

Vowell, J. and G. Hooper. Marist College, Poughkeepsie, New York. The humidity behavior of a geographic strain of *Drosophila pseudoobscura*.

Reaction of male and female flies to a series of humidity environments was determined for a strain of *Drosophila pseudoobscura* collected at Payson, Arizona, in 1962. The humidity apparatus consisted of an organdy-floored, circular lucite chamber, 6.8 cm in diameter by 3 mm

which was placed on a glass Felsen culture dish containing two humidity-controlling sulfuric acid solutions. Humidity preference for a sample of 20 flies was allowed to develop in the dark for six consecutive 15-minute intervals. At the end of each interval, distribution of flies in the chamber was photographically recorded. The response for a sample was obtained by summing the number of flies on each side of the chamber for the six periods and then calculating the percentage of flies on the side with higher relative humidity. Flies were randomized after each reaction period. Experiments were carried out at 21°C with 4-8-day-old flies from a laboratory culture maintained at 18°C under a 12-12 light-dark cycle.

Humidity choices were 0-20, 20-40, 40-60, 60-80 and 80-100. The number of male and female samples for each choice in humidity ranged from 10 to 15 (except in the instance of 0-20, where there were only two female samples). Mean responses with standard errors were calculated for each of the choices (above exception excluded). Results are shown in Figure 1.



Significant differences in the response of male and female flies were detected for the range of choices surveyed. Males consistently preferred lower relative humidity, while females showed a random response except for the 80-100 choice. Results were unexpected, since we anticipated that both sexes would show a wet response over the range of choices, with the response being most intense in males. Boyer (1953) observed a wet response in 7-10-day-old *D. pseudoobscura* males from

Figure 1. Mean responses with standard errors to five relative humidity choices of samples of male and female flies from a strain of *Drosophila pseudoobscura* collected at Payson, Arizona.

Mather, California, for a similar series of humidity choices, with the exception of the 80-100 choice which was random. A wet response was also observed in females from another Payson strain for the same choices (unpublished observations). This wet response of *D. pseudoobscura* was interpreted by Hooper (1956) and Pittendrigh (1958) as an adaptive form of behavior for flies exploiting arid areas, resulting in a decrease in water-loss through transpiration. We anticipated that males would show a

stronger preference for higher humidities because of a tendency to lose water at a faster rate than females (Hooper 1956) and consequently remain at a distinct disadvantage in maintaining normal water relations. A possible intraveneing factor in our results may relate to the age of our culture which was brought into the lab in the summer of 1962. Subsequent adaptation to laboratory conditions could have resulted in a change of humidity behavior. Further studies with other and new strains are planned.

References: Boyer, D.D. 1953, Ph.D. Thesis, Princeton University; Hooper, G.B. 1956,

Ph.D. Thesis, Princeton University; Pittendrigh, C.S. 1958, Chap. 18 in: Behavior and Evolution, A. Roe, G.G. Simpson, eds. (Yale Univ. Press).

This research was supported, in part, by a Marist College Faculty Research Grant.

Takanashi, E. and O. Kitagawa.
Tokyo Metropolitan University, Japan.
Quantitative analysis of genetic
differentiation among geographical
strains of *D. melanogaster*.

materials used here consist of 5 Japanese and 5 French strains. All flies were maintained at 25°C under uncrowded conditions. From these cultures of each strain, 25 females and 25 males

Metric characters of ten geographical strains of *D. melanogaster* were analyzed to investigate genetic differentiation between Japanese and French strains. It has been shown that Japanese and French strains are accurately distinguishable in metric characters on the basis of discriminating function (Teissier, 1958). The characters measured were femur length, tibia length, wing length, wing width, a/b , b/c , d/c , f/e , $3CBr/b$ (Figure 1), wing

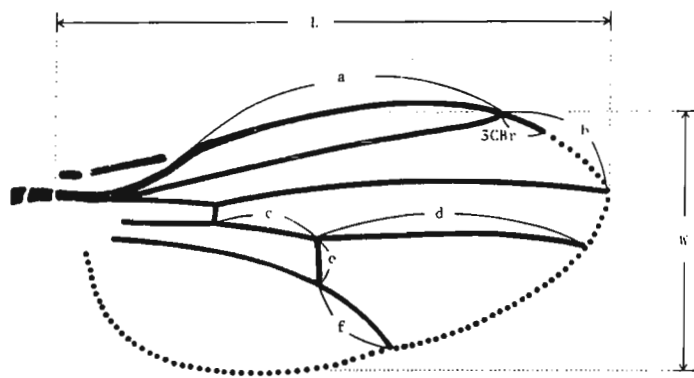
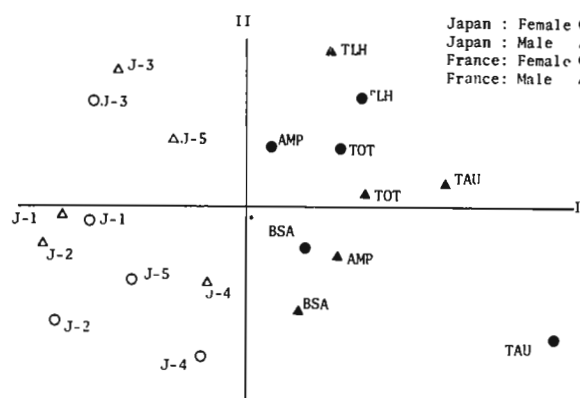


Figure 1.

length/wing width, wing length/femur length, fresh body weight, and bristle number for both sexes, and ovariole number for females. In Japanese strains, the average wing length is shorter, fresh body weight is heavier,

femur length is longer, and ovarioles are fewer. These differences are significant in analysis of variance. Most characters are significantly different between sexes, countries, strains within country, and in their interactions.



Based on the means of ten strains, the principal component analysis was made. The eigenvector for the first component indicates that a strain having larger wings has shorter

Figure 2.

femurs and lighter weight, and a strain having small wings has longer femurs and heavier weight. For the second component, Burla's rule is observed; a/b increases with the decrease of b/c , d/c and f/e , and wing length increases with the increase of a/b and the decreases of the other three indices.

From eigenvectors and means, scores of each strain were calculated for the first and second components. For the first component the difference between the average score of 5 Japanese and 5 French strains is significant at 1% level in females and at 0.1% level in males. For the second component no significant difference is detected. Figure 2 shows the distribution of scores of ten strains, clearly indicating that they are classified into two types, Japanese and French.

McCrady, W.B. University of Texas at Arlington, Texas. Studies of the Dly locus in *D. melanogaster*.

The idea that the Dly locus which determines delayed-recovery from CO₂ in *D. melanogaster* is an integrated form of virus sigma, the causative agent of CO₂-sensitivity, was introduced by McCrady and Sulerud in 1964. Since that time

some work has supported that hypothesis (Fielding 1971; McCrady 1968, 1974, 1975; Sulerud 1968); however, certain experiments which, if successful, would have provided strong evidence for the episomal hypothesis have turned out negatively. This report describes some of these experiments.

1. The Dly locus appears to be non-inducible. C.L. Fielding, working in this laboratory developed a strain of delayed-recovery flies called TDR-BC₃ by back-crossing delayed-recovery males to Oregon (resistant) females for three generations, then selecting heterozygous Dly progeny for mating to restore homozygosity for Dly in the stock. He injected 988 Oregon flies with concentrated extract from this strong delayed-recovery stock. When the injected flies were exposed to CO₂ approximately one month later, only 11.9% (S.E.=3.4) failed to recover within 15 minutes. This, compared to 15.6% \pm 1.1 non-recovery of Oregon (control) flies injected with Oregon extract, led to the conclusion that TDR-BC₃ flies do not contain infectious material. Flies of this stock were subjected to X-irradiation (as larvae or young adults) or EMS treatment (as young adults), were aged 25-30 days and then were used as the source of extract which was injected into Oregon flies. After 25-30 days the injection recipients were exposed to CO₂; the results are shown in Table 1. There were no significant differences in recovery behavior between the control groups and any of the experimental groups. Obviously, no infectious virus was induced.

Table 1. CO₂ tests of Oregon flies injected with various extracts from flies treated with X-rays or EMS

Oregon injected with:	Number Tested	Number non-recovered after -- minutes				% Flies non-recovered after 15 minutes (\pm S.E.)
		15	30	45	60	
Oregon extract after 1500 R	757	128	58	41	21	17.1 \pm 1.7
TDR-BC ₃ extract after 1500 R	791	98	37	21	9	13.2 \pm 2.8
Oregon extract after 3000 R	614	104	68	53	37	15.8 \pm 1.6
TDR-BC ₃ extract after 3000 R	679	127	92	69	47	18.6 \pm 1.3
Oregon extract after 6000 R	432	64	42	26	18	14.1 \pm 2.7
TDR-BC ₃ extract after 6000 R	549	108	82	56	38	19.6 \pm 1.2
Oregon extract after EMS	586	117	73	51	33	21.1 \pm 2.1
TDR-BC ₃ extract after EMS	386	88	52	30	24	18.9 \pm 3.4

2. Gene Dly apparently has not replaced any normal third chromosome material. Delayed-recovery stocks were crossed with the following third chromosome recessive stocks: cp (45.3), as^{hg} (46), in (47), eg (47.3), roe (47.6), p^P (48), ma (49.7), cu (50), kar (51.7), ry (52.0), red (53.6), cv-c (54.1), jvl (56.7), sbd (58.2), ss^a (58.5). In every cross all of the progeny were wild-type. Since Dly maps at 52.7, some of the heterozygous offspring would have expressed the mutant phenotype(s) as a result of pseudodominance had the origination (integration) of Dly involved replacement of a part of the third chromosome.

3. Dly is not a large addition to the *Drosophila* chromosome. V.A. Patwardhan (1975), working in this laboratory, was unable to demonstrate any differences in the Dly region of salivary gland chromosomes of Oregon, delayed-recovery, or Oregon-delayed-recovery hybrids using light microscopy.

These experiments do not necessitate dropping of the episomal hypothesis since explanations for each negative result can be formulated. For example, TDR-BC₃ flies may be inducible but defective (non-infective) viruses may be the only products. The observation that nothing appears to have been replaced by the possible integration of Dly could mean that integration was accomplished by single crossing-over between the *Drosophila* chromosome and a circular viral genome. As each salivary gland chromosome band may include enough DNA for as many as 100 genes, it is not unexpected that light microscope studies would fail to show differences between "integrated" and "non-integrated" chromosomes.

The episome hypothesis is still considered tenable; a model based on a causal relationship between the agents responsible for delayed-recovery and CO₂-sensitivity has been formulated. This model will be the subject of a paper soon to be submitted for publication.

References: Fielding, C.L. 1971, M.S. Thesis, University of Texas at Arlington; McCrady, W.B. 1968, DIS 43:82; McCrady, W.B. 1974, Genetics 77:42-43; McCrady, W.B. 1975, Genetics 80:55-56; McCrady, W.B. and R.L. Sulerud 1964, Genetics 50:509-526; Patwardhan, V.A. 1975, M.S. Thesis, University of Texas at Arlington; Sulerud, R.L. 1968, Ph.D. Thesis, University of Nebraska.

Svobodová, M., J. Poruba and J.K. Benedik.
University J.E. Purkyne, Brno, Czechoslovakia. The frequency of multiple mating in two laboratory strains of *D. melanogaster*.

The possibility of more than one mating in *Drosophila* females was suggested by Fuerst, Pendlebury and Kidwell (1973) and Ehrman (1974) working with natural populations. Two mutant strains of *D. melanogaster*, bw e and vg, were used in our experiments. The number of matings,

in particular females (approximately 200 of each strain in two replications) of the two strains was studied along with the speed and duration of the first copulation.

Three day old virgin females of tested strains were collected and mated separately in single pair culture successively with one male of the same stock for 24 hours. Then the males were discarded and replaced with two males of the Cy/L strain and after another 48 hours the Cy/L males were replaced by three males of the standard strain Oregon-R for 96 hours. The occurrence of progeny of the three possible phenotypes was taken as a proof of multiple mating.

Dependence of the frequency of matings on the speed and duration of the first copulation

Stock	No of females	begining of copulation	duration of copulation	No of matings
vg	58	16	14	1
	14	45	14	2
	69	5	15	1
	34	55	17	1
	35	55	17	3
	37	17	18	1
	23	15	18	2
	46	86	22	1
	15	84	24	2
bw e	60	33	9	3
	14	39	11	2
	30	22	13	2
	28	15	14	3
	49	17	16	2
	51	47	17	1
	35	12	17	2
	38	38	18	3
	17	5	19	2
	57	19	24	2
	4	5	20	2
	21	26	33	2
	2	51 95	5 8	2
	16	24 85	8 15	3
	23	13 39	9 25	3

In 74 percent of females of the strain vg only one mating took place, while in the strain bw e, in only 21 percent. This difference was shown to be significant by means of a two-way analysis of variance for unequal subclass number (Harvey, 1968). The difference between stocks was significant ($F = 57.72$, $P < 0.01$), while the difference between the replications was not significant.

During the first two hours after getting together with the males of the same strain, all females studied were observed for the speed and duration of the first copulation. In the vg strain only about 4.5 percent of females copulated, while in the bw e strain about 14 percent. Three females from this strain (Nos. 2, 16 and 23) copulated even twice during the first two hours (Table).

Conclusions: Multiple mating seems to be a common phenomenon in laboratory strains vg and bw e. The difference between these strains in the number of matings is suggested to be of genetic character, and also the speed of copulation seems to depend on the genotype. It cannot be generally said that the speed or duration of the first copulation affects the number of successive matings even

if in the bw e strain the ready mating was followed by higher number of matings.

References: Ehrman, R.C. 1974, *Experientia* 30:369; Fuerst, P.A., W.W. Pendlebury and J.F. Kidwell 1973, *Evolution* 27:265; Harvey, W.R. 1968, *Agr. Res. Serv., US Dept. Agric.*

Costa, R., G.A. Danieli and E. Morbini.
Università di Padova, Italy. Exceptional
three-band heterozygotes at the Esterase
6 locus in *D. melanogaster*.

Polymorphism at the locus Est 6 has been extensively studied in *Drosophila*; in particular in *D. melanogaster* six different isozymes have been detected and identified (Wright, 1963; Rodinò and Martini, 1971; Rodinò and Danieli, 1972) (Figure 1); these electrophoretic variants are

codominant, and give a characteristic two-band pattern in the heterozygote.

In spite of this definition, an extensive study made on about 4000 wild flies revealed in some individuals the presence of exceptional three-band patterns (Figure 2). These cases account for about 0.5% of the total number of individuals in the sample.

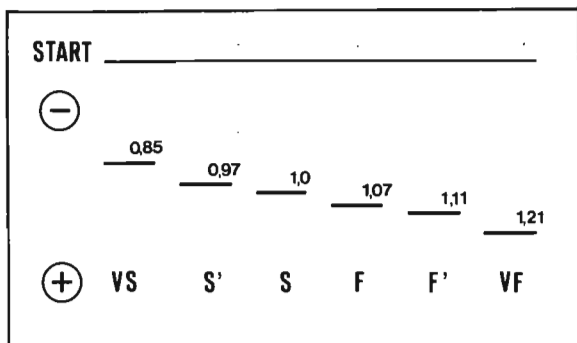


Figure 1. Electrophoretic variants for the locus Est 6 in *D. melanogaster*.

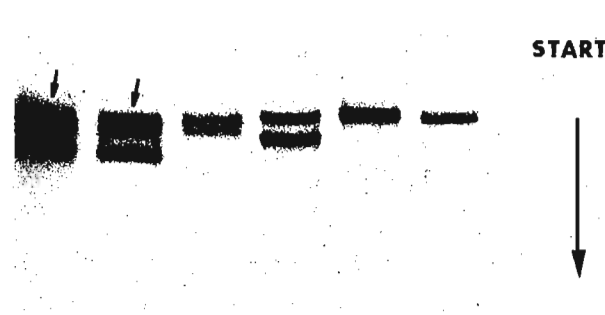


Figure 2. Exceptional three bands homozygous for the locus Est 6.

From a large sample of wild flies, several lines of breeding have been followed up; in two of these we succeeded in isolating exceptional two-band homozygotes. From the progeny of these flies homozygous stocks were obtained and carefully analyzed (Figure 3).

Two-band homozygotes are indistinguishable from a normal two-band heterozygote, but the two bands segregate together as a single trait. In crosses with VF homozygous, three-band heterozygotes are formed. The inbreeding of these heterozygotes gives the expected results of segregation (Figure 4).



Figure 3. *D. melanogaster* two bands homozygous for the locus Est 6.

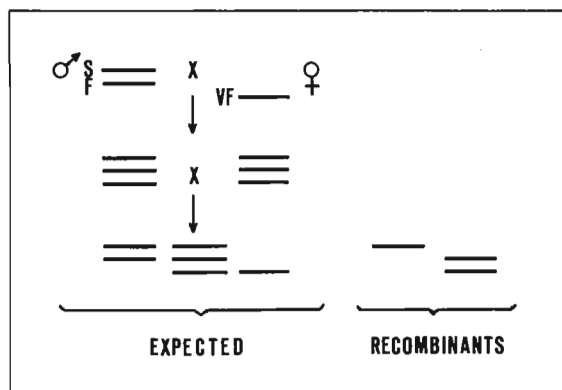


Figure 4. Results of a SF/SF x VF/VF cross.

In a few cases recombinational events were observed: whereas in the P generation S and F were segregating together, after the F_1 inbreeding, in the F_2 , F and VF segregated together, independently of S.

In these exceptional stocks, the F band apparently is inherited linked to the S and, by

recombination, may leave the S and become linked to VF. As far as substrate specificity is concerned, this F band shows an α -specificity in an α - β mixture, when the β -specificity of Est 6 alleles is well known (Johnson et al., 1966). Work is in progress in order to map the position of the cistron which codes for this exceptional variant.

References: Johnson, F.M., C.G. Kanapi, R.H. Richardson, M.R. Wheeler and W.S. Stone 1966, Univ. Texas Publ. 6615:517-532; Rodinò, E. and A. Martini 1971, DIS 46:139; Rodinò, E. and G.A. Danieli 1972, DIS 48:77; Wright, T.R.F. 1963, Genetics 48:787-801.

Hilliker, A.J.*, W.G. Gibson, T.C. Yeomans and D.G. Holm. University of British Columbia, Vancouver, Canada. The localization relative to the centromere of proximal loci in chromosome two.

Coincident with the genetic analysis of deficiencies generated in the proximal heterochromatin of chromosome two (Hilliker and Holm 1975), attempts were made to verify the map assignments of proximal genetic markers relative to the position of the centromere. In previous experiments (Holm et al. 1969) the position of the

centromere in chromosome three, relative to the genetic markers eagle (eg) and Deformed (Dfd), was demonstrated through the construction of C(3L) and C(3R) chromosomes. Similarly, we generated C(2L) and C(2R) chromosomes from *Drosophila* strains heterozygous for dominant or homozygous for recessive markers whose reported map positions were in the proximal region of chromosome two.

If, for example, the expression of the gene's mutant phenotype is associated with the recovery of newly induced compound-left autosomes, the locus is to the left of the centromere. The expression of a mutant phenotype of a gene to the right is recovered with a newly generated C(2R) chromosome. Through this procedure we have confirmed the assignment of light (lt) purple (pr), and Bristle (Bl) to 2L, and the assignment of rolled (rl), straw (stw), apterous (ap), Minute (2) of Schultz-2 (M(2)S2), prickly (pk), tufted (tuf), lightoid (ltd) and cinnamon (cn) to 2R.

However, we were surprised to discover that roughish (rh), the mutant recessive allele of which is expressed by a rough-eye phenotype, which had been tentatively mapped as a marker on 2L (distal to lt and Bl at 2-54.7) was associated with the recovery of newly generated C(2R) autosomes. 355 rh/rh virgin females were treated with 4250 rads of gamma radiation (from a ^{60}Co source), single pair mated in shell vials with C(2L)P, b;C(2R)VHK1, rl cn males, and brooded for 7 days. From this experimental cross, 44 exceptional F_1 progeny were obtained (Table 1). Of these exceptional progeny, 2 were matroclinous; 33, patroclinous; and 9 carried newly induced compound-2 autosomes. The rh phenotype was associated with all 4 newly induced C(2R) chromosomes, but none of the 5 newly generated C(2L) chromosomes, clearly demonstrating that rh is on the right arm of chromosome 2. Moreover, it is evident that rh is distal to ap as it is not uncovered either by Df(2R)M-S10 or by Df(2R)M-S4.

Table 1. Exceptional progeny recovered from irradiated rh/rh virgin females crossed to C(2L)P, b;C(2R)VHK1, rl cn males.

Phenotype	Number recovered	Phenomenon
rh (matroclinous)	2	Nondisjunction
b;rl;cn (patroclinous)	33	Nondisjunction or chromosome loss
cn	5	Newly induced C(2L)
b;rh	4	Newly induced C(2R)

Note that all 5 of the newly induced C(2L) chromosomes gave a rl^+ phenotype in combination with C(2R)VHK1, rl cn. Many of the C(2L) chromosomes generated from a variety of strains in our laboratory have been found to carry rl^+ duplications of 2R. These and other observations have prompted us to conclude that in *Drosophila* females the radiation induced formation and detachment of compound autosomes are translocation events (Hilliker and Holm 1975).

References: Hilliker, A.J. and D.G. Holm 1975, Genetics 81:705-721; Holm, D.G., M. Baldwin, P. Duck and A. Chovnick 1969, DIS 44:112.

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Sen, S. and J.P. Gupta. Banaras Hindu University, Varanasi, India. Some studies on the systematic position of *D. andamanensis* Gupta and Ray-Chaudhuri.

Recent studies on the species comprising the ananassae subgroup of the melanogaster species group have revealed that two species complexes may be recognised within the ananassae subgroup, the ananassae complex and the bipectinata complex. The species of the former complex possess

non-bifid and apically hirsute aedeagus while of the latter complex have bifid, bare and apically hooked aedeagus (Kaneshiro and Wheeler, 1970; Bock, 1971). Furthermore, six species, namely *D. ananassae*, *D. pallidosa*, *D. phaeopleura*, *D. nesoetes*, *D. atripectus* and *D. varians* have been included in the ananassae complex, while *D. bipectinata*, *D. malerkotliana*, *D. parabiptinata* and *D. pseudoananassae* constitute the bipectinata complex.

Gupta and Ray-Chaudhuri (1970) added one more very interesting species, *D. andamanensis* from Andaman Is., India, to this subgroup. Bock and Wheeler (1972), based on purely morphological observations, pointed out that the position of *andamanensis* is less clear, since its non-bifid and apically hirsute aedeagus resembles that of the ananassae complex species, while its small anterior parameres resemble those of the bipectinata complex species, and its sex-comb is closest to bipectinata and parabiptinata.

In the present studies an attempt has been made to get some information from chromosomal homology as well as interspecific hybridization tests so as to establish its identity with either of the two species complexes.

Material and method: Preparations of mitotic and salivary gland chromosomes were made by the conventional squash techniques.

For interspecific hybridization, reciprocal crosses between *andamanensis* and all available species from both the complexes were made through mass matings involving 30 females and 30 males, all aged for four days prior to mating.

Observations: The squash preparations from the neuroblast cells showed 3 pairs of V-shaped and one pair of rod-shaped chromosomes (Figure 1). The salivary gland nuclei showed 7 arms radiating from a common chromocenter.

Altogether eight interspecific crosses were made involving three species from the bipectinata complex and one species from the ananassae complex. None of the crosses could yield hybrids indicating that *andamanensis* is a well established and perfectly isolated species (Table 1).

Discussion: Based on purely morphological observations, Bock and Wheeler (1972), while reviewing all available information on the melanogaster species group, pointed out that the position of *D. andamanensis*,

the latest addition to the ananassae subgroup, is difficult to define clearly, since it partially resembles the species of both complexes of the subgroup.

However, the highly restricted geographic distribution of this species does resemble that of *D. pallidosa*, *D. nesoetes* and *D. phaeopleura*. Each species has so far been recorded only

from one or a few small islands. Moreover, the present few interspecific hybridization tests involving species from both complexes have not provided any clue regarding its genetic affinity with any of these species.

Bock and Wheeler (1972) encountered another very interesting situation with *D. varians* whose anal plate shows some differentiation of the lowermost bristles, characteristic of the species of the *suzukii* subgroup, and on the other hand it does not have single toothed secondary clasper, characteristic of all other members of the anan-

assae subgroup. Nevertheless *variens* has been given a place in the ananassae subgroup just on the basis of chromosomal homology.

The situation with *andamanensis* is quite different. Since it fits well with the ananassae subgroup, the problem arises only at the complex level. If chromosomal homology is taken as a criterion for its placement, then it should be kept in the ananassae complex and closer to *D.*



Figure 1. Mitotic chromosomes of *D. andamanensis*.

Table 1. Details of interspecific crosses

S.No.	Parental Crosses		F ₁ hybrid
	♂	♀	
1.	ananassae	x andamanensis	No
2.	andamanensis	x ananassae	"
3.	bipectinata	x andamanensis	"
4.	andamanensis	x bipectinata	"
5.	parabipectinata	x andamanensis	"
6.	andamanensis	x parabipectinata	"
7.	malerkotliana	x andamanensis	"
8.	andamanensis	x malerkotliana	"

nesoetes (sp. 3 of Kaneshiro and Wheeler, 1970). Both of these species have identical mitotic chromosomes represented by 3 pairs of V-shaped and one pair of rod-shaped chromosomes.

The present authors are still of the opinion that in order to get much further information on this relationship, a very comprehensive cytological and genetical analysis of all the species constituting the ananassae subgroup will have to be done.

References: Bock, I.R. 1971, Texas Univ. Pub. 7103:273-280; Bock, I.R. and M.R. Wheeler 1972, Univ. Texas Pub. 7213:1-102; Gupta, J.P. and S.P. Ray-Chaudhuri 1970, Orient. Insects 4:169-175; Kaneshiro, K. and M.R. Wheeler 1970, DIS 45:143.

Kosuda, K. Josai University, Sakado, Saitama, Japan. Femaleless, a new case of sex ratio anomaly in *Drosophila virilis*.

In order to detect anomalous sex ratio variants, *Drosophila virilis* flies were collected from a natural population in a beer factory in Tokyo, Japan. A number of isofemale lines were established and maintained by full sib pair matings

every generation. Two or four replicate crosses were made so as not to lose these inbred lines. After nine generations of successive pair matings, progeny from one of the replicates of a given line, designated as 65a, were exclusively males (0 ♀♀; 46 ♂♂). Offspring from the remaining replicate (65b), however, normally consisted of both males and females. Accordingly this extraordinary sex ratio anomaly could be retained and the hereditary nature could be examined by crossing males from 65a with virgin females from 65b by single pair matings (Experiment I). The results of these matings are shown in the upper part of the table. In the next generation, males from sex ratio deviants were individually mated with single females recovered from normal cultures in Experiment I (Experiment II). The results of Experiment II are also presented in the table. Cultures which produced entirely males among more than eight

Sex ratio of deviants recovered from Experiments I and II.

	No. of Cultures	Normal	Deviant	Sex Ratio of Deviant (♀:♂)		
Exp. I	11	5	4(2)	0:104*	0:16**	0:11
				0:10	0: 3	0: 3
Exp. II-a	32	11	21	0:232	0:181	0:32
				2:203	1:135	1:96
				2:111	2:177	0:43
				0:103	0:126	0:90
				0:35	0:77	0:67
				0:94	0:30	0:92
				0:59	0:48	0: 9
Exp. II-b	10	4	4(2)	0:166	0:116	0:19
				0:10	0: 6	0: 4

Figures in parentheses represent the number of cultures producing less than 8 progeny which consist entirely of males. Males marked with * and ** were used in Experiments II-a and II-b, respectively.

progenies were tentatively classified as sex ratio deviants. Much smaller numbers of progenies were recovered from sex ratio deviants in comparison to normal cultures. Females from normal cultures in the above matings always produced both males and females when they were mated with males which had no relation to line 65 (male progeny/total = $.476 \pm .006$). And normal sex ratio ($.515 \pm .013$) was obtained when males from deviants were mated with females which were independent of line 65. These experimental results suggest the following unique nature of this sex ratio anomaly which was named femaleless. The anomaly is heritable. The deviation from the expected 1:1 sex ratio is extreme and almost all progeny of deviants are males. All female parents derived from this sex ratio anomaly do not necessarily produce sex ratio deviants, as shown in the table. This anomaly seems not to be pre-zygotic, as with meiotic drive, but to be zygotie, although the exact nature is not known yet.

Siddaveere Gowda, L., M.R. Rajasekarasetty and N.B. Krishnamurthy. University of Mysore, India. Studies on the *Drosophila* fauna of peninsular India.

Drosophilidae is a large family of acalypterate flies of world-wide distribution and it has nearly 1254 named species of *Drosophila* (Wheeler and Hamilton 1972). Of these, only eight species, namely *busckii*, *melanogaster*, *simulans*, *hydei*, *ananassae*, *repleta*, *immigrans* and *fun-*

bris are ubiquitous in almost all parts of the world and have been called "cosmopolitan" species. The remaining species come under either widespread or endemic categories. This can be viewed as an indication of differences in the potentialities of species and/or specialization to exploit the varied ecological conditions.

Some valuable information has been collected with regard to the *Drosophila* fauna of South India (Reddy and Krishnamurthy 1973-74; Siddaveere Gowda and Krishnamurthy 1972; Ranganath and Krishnamurthy 1972) but their investigations are confined to a few areas of Karnataka and Tamilnadu States and hence the major areas of this part of the country still await exploration. In view of this, an attempt has been made by the authors to add to the knowledge on *Drosophila* of peninsular India.

Drosophila flies have been sampled from 14 geographically distant places of peninsular India. They are Poona, Hyderabad, Pileru, Soundathi, Karwar, Madras, Thirupathi, Coorg, Hassan, Mysore, Lalgondana Halli, Nandi Hills, Tellicherry and Coonoor (Figure 1). Flies were trapped in forests, plantations and gardens in the above mentioned areas during the months of July to October by the routine method of fermenting banana bait.

In all, eighteen different species of *Drosophila* were recorded. Of these, nine species belong to the subgenus *Sophophora*, six to the subgenus *Drosophila*, two to the

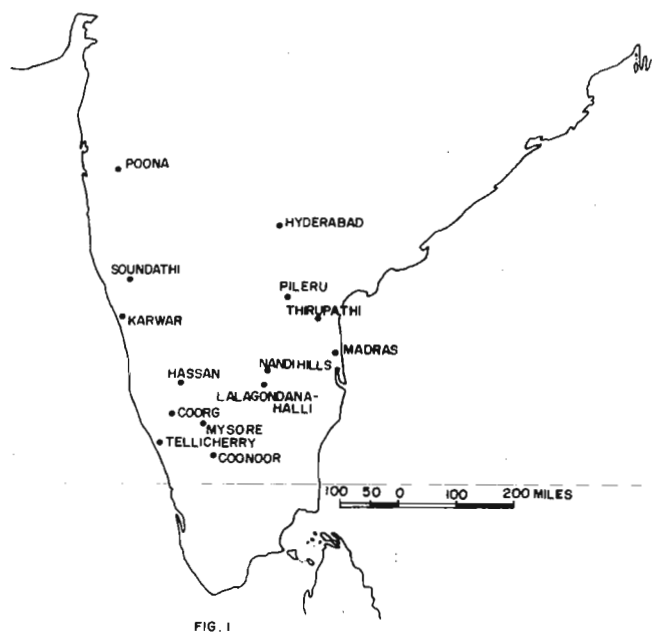


Figure 1. Map of Peninsular India illustrating the localities of collections of *Drosophila* populations.

subgenus *Scaptodrosophila* and one species to the subgenus *Dorsilopha*. The details of the collection record are presented in Table 1. Qualitative and quantitative differentiations in the *Drosophila* fauna in different areas of South India are striking. Mysore population has the highest number of species with eleven while only four species have been obtained from Hyderabad,

Soundathi and Hassan.

D. rajasekari, *malerkotliana* and *D.n. nasuta* can be adjudged as 'versatile' species as they are present in almost all the areas of South India under study and also numerically they dominate the other species. Perusal of the literature reveals that *immigrans* is restricted to higher elevations where cool and humid climate prevails (Reddy and Krishnamurthy 1973-74, Ranganath and Krishnamurthy 1972). But the present report on *immigrans* from Mysore and Hassan points out that this is not a universal phenomenon. The authors feel that larger populations are built up at higher elevations than at lower elevations as evidenced by the present data. Further, another important feature of the present investigation is the first report of *hypocausta* Osten-Sacken, 1882 from India. Characteristic of these flies is the strong sexual dimorphism in body colour, males being much darker than the females.

It is a unique phenomenon that in the present collections, members of the *melanogaster* species group of the subgenus *Sophophora* and the *immigrans* species group of the subgenus *Drosophila* dominate other members in all the areas of Peninsular India. This is in conformity with the earlier observations made by Reddy and Krishnamurthy (1973-74). Further, these findings support the view of Bock and Wheeler (1972) that both these species groups have orig-

inated in South-east Asian area and speciated widely within this region. More extensive explorations of Indian as well as the neighbouring islands' fauna are warranted, which may throw more light on the speciation in these groups.

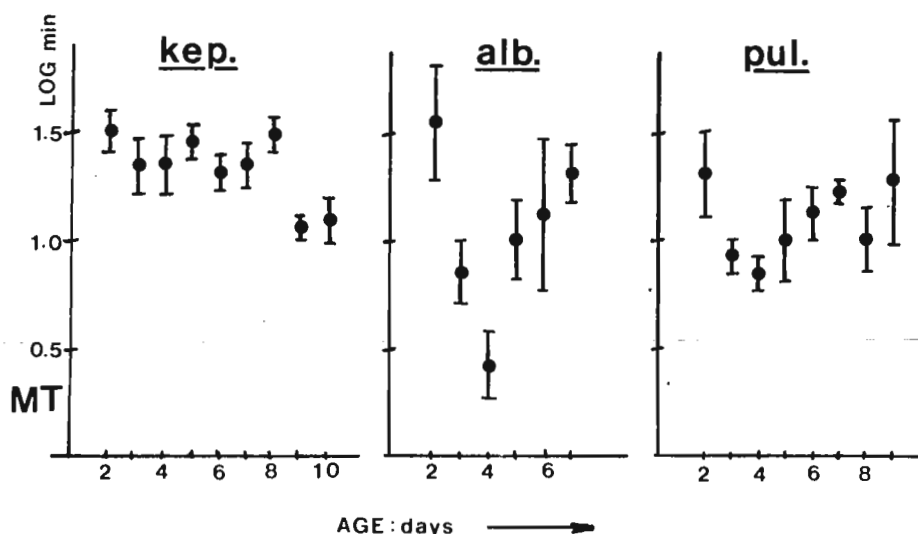
The financial assistance from the Department of Atomic Energy, Bombay, is gratefully acknowledged.

References: Bock, I.R. and M.R. Wheeler 1972, In Studies in Genetics, VII, Univ. Texas. Publ. 7213, pp. 1-102; Ranganath, H.A. and N.B. Krishnamurthy 1972, DIS 48:132-133; Reddy, G.S. and N.B. Krishnamurthy 1973-74, Sci. J. Mys. Univ. XXVI:54-64; Siddaveere Gowda, L. and N.B. Krishnamurthy 1972, DIS 48:38; Wheeler, M.R. and N. Hamilton 1972, In Studies in Genetics VII, Univ. Texas Publ. 7213:257-268.

Wright, R.G. University of Edinburgh, Scotland. Receptivity and mating time in *nasuta* flies.

Single pair matings of virgin flies (aged 1 to 9 days) were timed within 3 hr of dawn (L/D : 12/12) on 4 different days. Probit plots of mating time (excluding latency) were linear suggesting a log-normal distribution so log

values were used in all statistics of this parameter. Bliss, C.I. (Statistics in Biology 1967, 1:162-163) describes the statistical treatment of data such as collected here. Analysis suggests that my 1 hr observation period was sufficient to divide the flies into receptive and non-receptive populations at each age. Most flies were receptive by day 2. Mean log mating time of receptive flies showed no significant heterogeneity in *D. kepulauanana* or *D. pulaua*



Mean (+ - 1 standard error) log mating time with age for *D. kepulauanana*, *D. albomicans*, and *D. pulaua*.

(using single classification analysis of variance). This suggests a "switch on" of full receptivity in these females (cf. Manning, A. 1967, Anim. Behav. 15:239-250). *D. albomicans* was significantly heterogeneous. Student Newman-Keuls test (Sokal and Rohlf, Biometry. Freeman, San Francisco 1967) showed that mating time on day 2 was longer than on days 3, 4, 5 and 6 ($p < 0.05$) suggesting a gradual reduction of the threshold quality and quantity of male courtship required by females for acceptance. Perhaps females who can afford to be "choosy" on day 2 change strategy by day 3 if still uninseminated (an unlikely situation unless density of mates is very low) lowering their threshold in order to maximise their investment: accepting shared capital with an inferior male with the possibility of producing young in an under-exploited environment.

Davis, B.K. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Rejoining Y-autosome translocations to produce single autosomes.

A set of Y-autosome translocations is available from which a series of duplications and deficiencies can be produced which span most of the two major autosomes (Seattle-La Jolla Drosophila Laboratories, 1971; Lindsley et al., 1972). As Lindsley et al. have pointed out, the

duplications and deficiencies would be much more convenient if they were carried by single rather than translocated chromosomes. Here I report the successful rejoining of translocated chromosomes to produce single chromosomes. This technique may also produce useful insertional translocations of bb^+ or male fertility genes in an autosome.

Females, heterozygous for a balancer chromosome and a translocation, were X-irradiated to produce breaks in both Y chromosome portions of the two translocation elements, followed by a rejoining of the two autosomal elements to produce a single autosome. This chromosome should segregate from the Y chromosome fragment at first meiotic anaphase. Since both Y chromosome arms are capped with a dominant marker (y^+ for Y^S and B^S for Y^L), progeny with a rejoined chromosome are phenotypically distinct. They do not carry y^+ , B^S or the balancer chromosome.

This technique was used for two deficiency translocation combinations of the second chromosome. The L62-R93 combination is deficient for 56EF to 57A and R104-H158 is deficient for 58A to 58E. Since all four translocations have breakpoints in Y^S , both deficiency combinations are y^+ and B^S . Virgin C(1)RM, $y/T(Y;2)def/In(2L+2R)Cy$, $Cy\ cn^2$ females from 4 to 48 hrs old were irradiated 5.6 minutes at 535.1 r per minute for a total of 3000 r. The irradiation was kindly done by R.R. Rinehart at San Diego State University. Females were immediately mated to $Y^S X \cdot Y^L$, $In(1)EN$, $y/O; In(2LR)SML$, $al^2\ Cy\ cn^2\ sp^2/Sco$ males. Matings consisted of about 21 females and 30 males in each half pint bottle. The flies were transferred to produce four broods. Broods 1, 2 and 3 were 24 hours each and brood 4 was 96 hours. Adults were then discarded.

Regular progeny are $y\ Cy\ Sco$ females, $B^S\ Cy$ males, and $B^S\ Sco$ males from alternate disjunction; $y\ B^S\ Cy\ Sco$ males from adjacent I disjunction since the terminal duplication is viable for both of these translocations; and $y\ Cy\ Sco$ males from 3:1 segregation. Progeny carrying a rejoined chromosome 2 are either $y\ Cy$ or $y\ Sco$ and either male or female. Progeny carrying the complementary rejoined Y chromosome are $B^S\ Cy\ Sco$ and either male or female. The following table shows the progeny from 645 irradiated females carrying the L62-R93 deficiency translocation.

Progeny from irradiated L62-R93 females				
Brood	Regular	$y\ Cy$ and $y\ Sco$	$B^S\ Cy\ Sco$	Total
1	23	0	0	23
2	145	1	5	151
3	-	0	-	-
4	-	16	-	-

Complete counts were done only for the first two broods. In broods 3 and 4 the progeny were examined for putative cases of rejoining but counts were not done. A total of 17 putative cases were recovered, including 1 $y\ Sco$ male in brood 2. The other cases, all from brood 4, were 10 $y\ Sco$ females, 3 $y\ Sco$ males and 3 $y\ Cy$ females. The $y\ Sco$ females were discarded since the recovered chromosome was not balanced. The 4 $y\ Sco$ males were each mated to $Y^S X \cdot Y^L$, $In(1)EN$, $y/In(1)d1-49$, $y\ Hw\ m^2\ g^4; In(2LR)SML$, $al^2\ Cy\ cn^2\ sp^2/Sco$ females. One male produced no offspring. A second male proved to carry $In(2L+2R)Cy$ without the dominant marker Cy . The unmarked chromosome in the other two males behaved genetically as a single chromosome 2, segregating regularly from either a chromosome 2 balancer or a chromosome carrying Sco and independent of the sex chromosomes. Salivary gland analysis confirmed that the chromosome was single and included part of the Y chromosome at the deficiency region.

The 3 $y\ Cy$ females were each mated to $Y^S X \cdot Y^L$, $In(1)EN$, $y/O; In(2LR)SML$, $al^2\ Cy\ cn^2\ sp^2/Sco$ males. The first produced all $Cy\ Sco$ offspring indicating that Sco was present but had not been expressed. The second still had a translocation which now lacked y^+ and B^S . The third female proved to carry a rejoined chromosome by the same genetic and cytological criteria described above for the males. In summary, three rejoined chromosomes were recovered for this deficiency.

The finding of all but one of the putative rejoined chromosomes in brood 4 was unexpected

since late stages are most sensitive to ionizing radiation (e.g. Koch et al., 1970) and these should occur in the early broods. This is only partly explained by the small number of progeny in the early broods.

Attempts to rejoin a second deficiency translocation (R104-H158) failed. Irradiated females were much less fertile than the L62-R93 females. A second factor explaining the failure might be the amounts of Y chromosome capping the autosomal arm and lying between the Y centromere and the autosomal cap. A translocation in which either is small should be more difficult to rejoin.

References: Lindsley, D.L. et al. 1972, Genetics 71:157-184; Koch, E.A., P.A. Smith and R.C. King 1970, Chromosoma 30:98-108; Seattle-LaJolla Drosophila Laboratories 1971, DIS 47 supplement.

Ives, P.T. and P.A. Simmons. Amherst College, Amherst, Massachusetts. Distribution of lethal loci on the second chromosome of South Amherst D. mel.

Nine inversion-free non-lethal chromosome lines from the South Amherst natural population on 73j31 were used to determine the distribution of newly induced lethal loci along the standard genetic crossover map. Individual males were given a single drink of a .025 M EMS sucrose

solution and the sperm they produced 24 to 48 hours later were used for the extraction of lethals by SM5 or Cy B1 L⁴. The loci of 81 non-allelic lethals were mapped by one or more of three stocks: Sp B1 L², S^x Sp vg^U, and Sp vg^U If, and were tabulated in Left (0.0-49.9 map units), Middle (50.0-69.9), and Right (70.0-108 ±) sections of the chromosome. Distributions of groups of non-allelic lethal loci, extracted from this population in 1971, 1972 and 1973, and of a group induced by EMS in inbred Oregon-R, had been determined similarly earlier by Ives. Following is a summary of all of these distributions:

Distribution of lethal loci along the South Amherst second chromosome.

Sample	No. of Lethals	% Left	% Middle	% Right
1. 1971 June	46	50.0	39.1	10.9
2. Nov.	50	64.0	28.0	8.0
3. 1972 June	40	45.0	30.0	25.0
4. late Oct.	79	44.3	31.7	24.0
5. 1973 June	43	32.6	27.9	39.5
6. late Oct.	73	39.8	30.1	30.1
7. South Amherst EMS	81	42.0	42.0	16.0
8. Oregon-R EMS	80	33.8	31.2	35.0
9. Salivary Chrom. bands		30 ±	40 ±	30 ±

The shift in the distribution of natural population lethal loci in 1971-1973 was, in comparison with the Ore-R EMS data (item 8, done in 1971), thought to be towards a more "normal" distribution. However, in comparison with the 1974 South Amherst EMS data (item 7), this same shift appears to be away from "normal."

The significant difference (at the 2% level) between items 7 and 8 suggests (1) that Oregon-R is not a useful "control" for a study of this kind and (2) that comparisons of the distributions of lethal loci in different stocks or from widely separated natural gene pools cannot be made accurately without some knowledge of the current distribution of mutable loci in their respective chromosomes. In addition other "point" mutagens should be used to achieve a broader spectrum of the distributions of mutable loci.

The Oregon-R proportions in item 8 are close to those of the salivary gland chromosome bands in item 9. The South Amherst distribution in item 7 is sufficiently different from both of those to suggest that natural selection may influence that genetic component of natural gene pools as well as the proportionate distribution one finds in lethals collected some generations after the mutations themselves occurred, items 1-6.

McDonald, J. and F.J. Ayala. University of California, Davis. Substrate specificities of ADH, ODH and ALDOX in *D. melanogaster*: A preliminary survey.

We have made a preliminary survey to determine in *Drosophila melanogaster* the ability of three distinct enzyme systems to catalyze a variety of alcohol substrates. The results are shown in the accompanying table. The methods are as follows:

100 mg of adult (5-10) days post eclosion) *Drosophila melanogaster* are homogenized in 1 ml of deionized H₂O. The homogenate is centrifuged at 5°C and 28,000g for 20 minutes. Starch

Presence (+) or absence (-) of activity for three enzyme systems in *Drosophila* acting on a variety of alcohol substrates.

Substrate	Zone of Activity		
	1 (ADH)	2 (Aldox)	3 (ODH)
allyl alcohol	-	+	-
allyl alcohol + NAD ⁺	+	+	+
amyl alcohol	-	+	-
amyl alcohol + NAD ⁺	+	+	+
n-butyl	-	+	-
n-butyl + NAD ⁺	+	+	+
ethanol	-	+	-
ethanol + NAD ⁺	+	+	-
methyl	-	(+)*	-
methyl + NAD ⁺	(+)*	(+)*	-
octanol	-	+	-
octanol + NAD ⁺	+	+	+
1-pentene-3-ol	-	-	-
1-pentene-3-ol + NAD ⁺	+	-	-
iso-propenol	-	+	-
iso-propenol + NAD ⁺	+	+	-
benzaldehyde	-	+	-
benzaldehyde + NAD ⁺	-	+	-

* Slight activity detectable when allowed to stain overnight.

gel electrophoresis is carried out according to the techniques of Ayala et al. (1) utilizing Sigma starch and the discontinuous buffer system of Poulik (2). Activity is detected by tetrazolium deposition in a medium containing 100 ml Tris-HCl 55.5 mM, pH 8.6, 25 mg nitroblue-tetrazolium (NBT), 1 mg phenazine monosulfate (PMS) and 20 mg NAD⁺ where indicated (see Chart). Under these conditions three separate areas of activity are apparent each of which is under separate genetic control (see Figure).

The area of activity nearest to the origin (Zone 1) is due to alcohol dehydrogenase

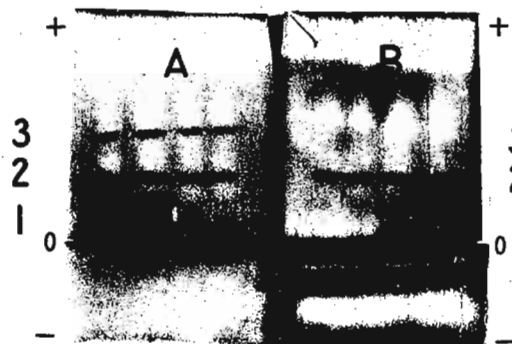


Figure 1. Zymograms displaying the three zones of enzyme activity discussed in the text. Gel A utilized Amyl alcohol as a substrate; Gel B - benzaldehyde. Zone 1 - displays allozymes of alcohol dehydrogenase; Zone 2 - aldehyde oxidase; Zone 3 - octanol dehydrogenase.

(structural locus: II 50.1). A second area of activity (Zone 2) anodal to ADH is due to aldehyde oxidase (structural locus III 56.6). Anodal to Aldox there appears a third area of activity (Zone 3) which is due to octanol dehydrogenase (structural locus III 49.2). Both ADH and ODH are NAD^+ dependent while Aldox has no cofactor requirement. Aldehyde oxidase is known to convert a variety of aldehyde substrates (3,4). Our results indicate that it is also able to utilize a variety of alcohols. This fact may be of importance for the interpretation of adaptive responses of *Drosophila melanogaster* flies to natural or experimental environments containing alcohol.

References: 1) Ayala, F., J. Powell, M. Tracey, C. Mourão and S. Perez-Salas, 1972, *Genetics* 70:113; 2) Poulik, M. 1957, *Nature* 180:1477; 3) Dickinson, W.S. 1970, *Genetics* 66:487; 4) Madhavan, K.M., F. Conscience-Egli, F. Sieber, H. Ursprung 1973, *Jour. Insect Phys.* 19:235.

Timner, K. Freie Universität Berlin, Germany. Influence of narcosis on the progeny of *Drosophila melanogaster*.

In order to determine the influence of ether and CO_2 narcosis usually used for experimental research, the following experiments were performed. Wild type *D. melanogaster* (Berlin wild +K) were used. After eclosion, virgin females and males were separated and kept in culture vials until the age of 3 days. Then they were allowed to copulate in an empty culture bottle. After copulation, females were separated by narcosis; each female was transferred into a new

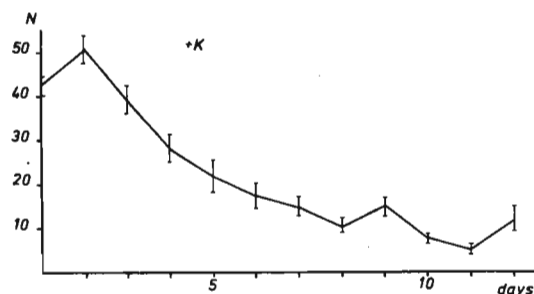


Figure 1. Average number of offspring (N) per +K female ($n = 27$) after a single copulation with +K male. Parents were treated with CO_2 and ether narcosis (experiment 1). Ordinate: mean values and standard errors of the means ($\bar{x} \pm 1m$) of offspring per female. Abscissa: days after copulation.

culture vial each day until the 12th day after copulation. These replacements were carried out without narcosis. In experiment 1, CO_2 was used for harvesting virgin flies and ether narcosis was used for the first transfer of mated females into the culture vials. In experiment 2,

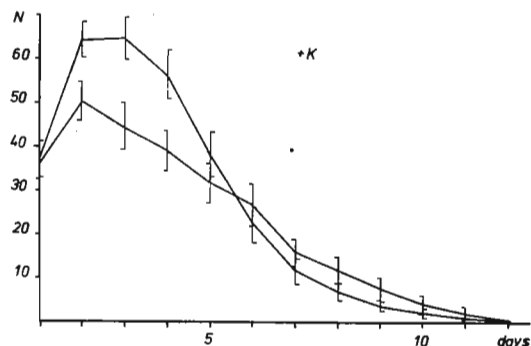


Figure 2. Average number of offspring (N) of CO_2 treated +K females ($n = 47$) and of ether treated +K females ($n = 35$) after a single copulation with +K males which had previously been treated with CO_2 and ether. Ordinate: mean values and standard errors of the means ($\bar{x} \pm 1m$) of offspring per female. Abscissa: days after copulation.

CO_2 was the only narcotic. And in experiment 3 ether was used for anaesthetic.

The results presented here in Figures 1 and 2 indicate that the ether narcosis applied in experiment 3 reduced the average number of offspring per female on the 2nd, 3rd and 4th days after copulation compared to the numbers from CO_2 narcotized females in experiment 2. The same tendency is obvious in the results of experiment 1, where mated females were anaesthetized by ether. This may be due to the ether narcosis.

Basden, E.B. Leyden Park, Bonnyrigg, Midlothian, EH19 2DE, Scotland. On the name *Drosophila disticha*.

In 1921 O. Duda gave the new name *disticha* to a palearctic species of *Scaptomyza* with two rows of acrostichal bristles. He was careful to point out that *Scaptomyza* was only a subgenus of *Drosophila*, and repeats this on p. 196 of

his 1924 paper.

In 1965 D.E. Hardy described the new species, *Drosophila disticha*, from the Hawaiian Islands but as this specific name is preoccupied in *Drosophila*, I am proposing the new name *waddingtoni* for Hardy's species. The name commemorates Professor C.H. Waddington of Edinburgh, who died 26 September 1975, just before his 70th birthday.

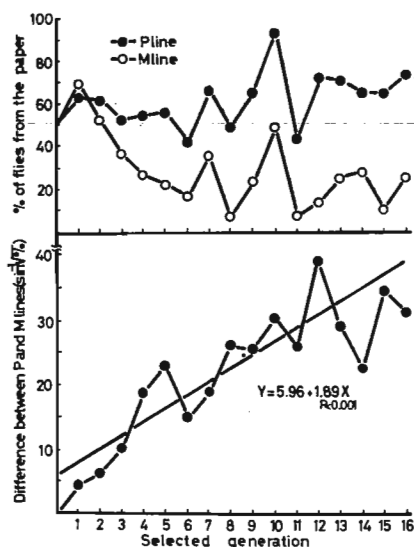
Reference: Duda, O. 1921, *Jahr. Ver. schles. Insekt. Breslau* 13:57-69; _____ 1924, *Archiv Naturgesch.* 90A:172-234; Hardy, D.E. 1965, *Insects of Hawaii Vol. 12 Drosophilidae*: 814 pp.

Takamura, T. and Y. Fuyama. Tokyo Metropolitan University, Japan. Selection for oviposition site preference in *D. melanogaster*.

There are not many reports about the choice of oviposition site of *Drosophila* (Del Solar and Palomino, 1966; Del Solar, 1968; David and Herrewage, 1969, 1970). Here we report another case of oviposition site preference of *D. melanogaster* which seems to be genetically controlled.

When female flies were provided with medium into which a piece of paper (strawboard) was stuck, they laid eggs both on the surface of the medium and on the paper. However, the relative abundance of eggs deposited on these two oviposition sites varied considerably but consistently with strains, which suggests the existence of genetic variation in the behavior of the choice of oviposition site. In order to demonstrate this, a selection experiment was started. F_2 flies of two strains, one of which laid the highest proportion of eggs on the paper, the other the lowest, were used as the base population of the selection. Thirty inseminated females, three days old, were introduced into a vial (3 cm in diameter, 10.5 cm in height) containing killed yeast medium (20% yeast, 0.8% agar) into which a piece of paper (1 mm thick) was stuck vertically. The paper stands so as to provide the same surface area as

the medium surface and is fully wet by the water oozing from the medium. After about two hours' oviposition in the dark at 25°C, the flies were removed and the paper, with the eggs on it, was transferred into another vial containing fresh medium. The flies which emerged from these eggs were used as the parents of the next generation of a line preferring paper to medium, the paper (P) line, while the flies from the eggs deposited on the surface of the medium were the parents of the line preferring medium to paper, the medium (M) line. The selection was started with two replicate lines in each direction, paper and medium, and each line consisted of four replicate vials. At each generation, the number of flies from eggs on both sites were counted separately. The upper half of the figure shows the proportion of the flies from the eggs on the paper to the total number of flies. Each direction, P and M, was represented by the mean value of two replicate lines. From generation to generation the proportion fluctuated considerably but the difference between the paper and the medium lines became larger with subsequent generations as shown in the lower half of the figure. (The differences were calculated after the angular transformation.) The regression coefficient of the difference by generation deviates significantly from 0 ($p < 0.001$). Sensory basis of the site discrimination and



the genetic mechanisms underlying this behavior are now under investigation.

References: Ayala, F.J. and M. Ayala 1969, *DIS* 44:120; David, J. 1970, *Rev. Comp. Anim.* 4:70-72; David, J. and J. van Herrewage 1969, *C.R. Acad. Sci. Paris*, 268D:1778-1780; _____ 1970, *Rev. Comp. Anim.* 4:82-84; Del Solar, E. 1968, *Genetics* 58:275-282; Del Solar, E. and H. Palomino 1966, *Am. Nat.* 100:127-133.

Monclús, M. University of Barcelona, Spain. *Drosophilidae* attracted to light after dusk.

We have been collecting *Drosophilidae* in Peramola (Spain, near the Pyrenées) at 600 m. above sea-level since 1956. The collections were carried on: 1) in xerophytic mediterranean woods with *Pinus* and *Quercus*, 2) near the river

with *Salix*, *Ulmus* and *Populus*, 3) in cultured fields with olive trees, 4) in orchards, and 5) indoors.

The technique of trapping with fermenting banana was used mostly and some samples were also obtained from grasses with a net. Over 5000 individuals distributed in 18 species (17 *Drosophila* and *Parascaptomyza disticha*) were captured.

With these techniques we never trapped *Phortica variegata* and *Gitona distigma* in this locality. However, in summer of 1971 when collecting insects attracted to electrical lights after dusk, some specimens of these genera were found. Since then we have observed regularly samples of insects attracted to the light, and these species have always been present. We infer that these *Drosophilidae* have nocturnal activity and positive phototropism.

Pinsker, W. Institut für Biologie II, Tübingen, Germany. Associative balance in the *Mdh*-locus of *D. subobscura*.

In a laboratory strain of *D. subobscura*, an enzyme polymorphism of *Mdh* could be detected by means of starch gel electrophoresis. There exist two variants of *Mdh* with different electrophoretic mobility, which were called S

and F. The same alleles S and F occur also in natural populations of *D. subobscura* with frequencies of 97% for S and about 1% for F (Saura et al. 1973). In our laboratory strain, however, the F-allele frequency was much higher and remained almost constant at 32% for 11 generations after the transfer of the strain into a population cage (population C). A constant deviation from the expected Hardy-Weinberg distribution was found among adults with a small but significant excess of 1.7% for the heterozygotes. It was therefore concluded that the *Mdh* polymorphism is balanced by heterozygote advantage.

To prove this hypothesis, two different experimental populations were started in two population cages: population A with initial allele frequencies of 90% S and 10% F, and population B with 10% S and 90% F respectively. Population C was used as a control with the genetic composition of the original strain where the allele frequencies were already in a constant equilibrium. Every generation, egg samples were taken from the three populations and the genotypes of 300 flies from each cage were determined electrophoretically with respect to *Mdh*. The observed frequencies of the S-allele are shown in the following table:

	A	B	C
start	0.900	0.100	0.665
1 gen.	0.843	0.363	0.675
2 gen.	0.767	0.402	0.653
3 gen.	0.699	0.392	0.668
4 gen.	0.557	0.565	0.768
9 gen.	0.522	0.580	0.677

It can be seen that the frequencies changed very quickly until the fourth generation in both populations with the artificial allele distribution (population A and B). From the fourth to the ninth generation, however, the frequencies kept constant. In the control population, on the contrary, there was no remarkable change of the allele distribution between any of the generations. From this result it can be concluded that the frequency of the two *Mdh*-alleles is strongly balanced by euheterosis, and that under laboratory conditions a population has its highest

fitness when the frequency proportion between the S and F alleles is about 0.6 to 0.4.

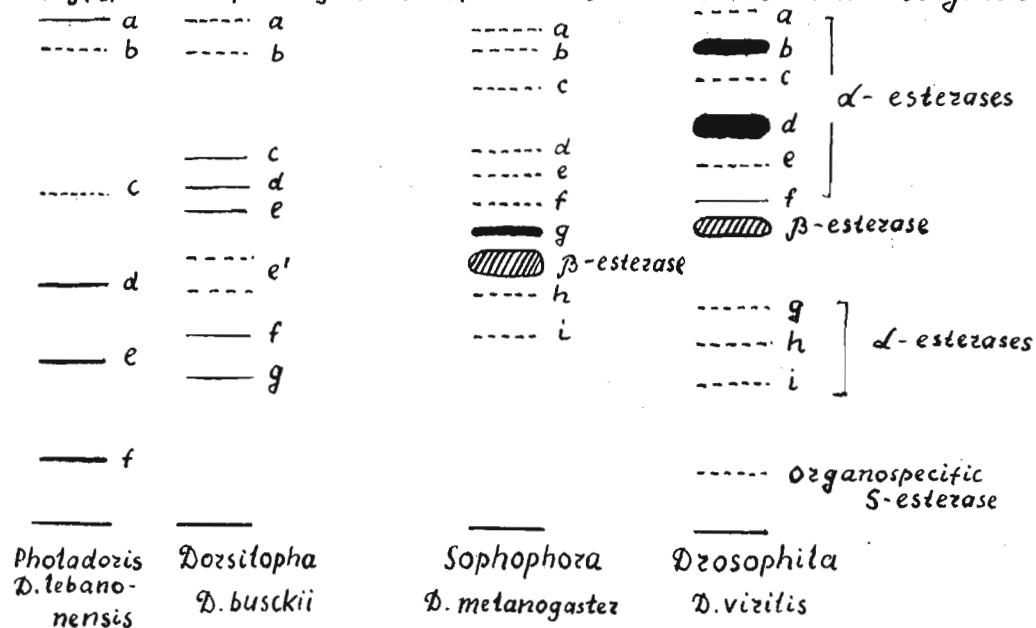
Our next question then was whether the *Mdh* locus itself is responsible for this heterotic effect or is only hitch hiking with other heterotic systems, like inversions. Since *Mdh* has been localized on the U-chromosome by crossings with visible marker strains, we looked for inversions simultaneously with the result that the U-chromosome polymorphism for the two different gene arrangements U_{1+2} and U_{1+2+8} , and the enzyme polymorphism for *Mdh* F and S were found to be connected with each other. The U_{1+2+8} inversion arrangement is always combined with the F-allele without exception, and U_{1+2} with the S-allele. This complete association between the enzyme and the inversion polymorphism seems to be the correct explanation for the heterozygous advantage of the *Mdh* S/F heterozygotes. Hence, the heterotic effect is most probably not caused by the enzyme locus itself, but rather by the coadapted genblocks in the inversions.

Korochkin, L.I., I.G. Kiknadze and A.N. Pahomov, Institute of Cytology and Genetics, Novosibirsk, USSR. Comparative analysis of esterases in genus *Drosophila*.

In different groups of genus *Drosophila* (subgenuses *Pholadoris*, *Dorsilopha*, *Sophophora* - groups *melanogaster* and *obscura*; subgenus *Drosophila* - groups *virilis*, *hydei*, *mulleri*, *quinaria*, *guarani*) the esterases with wide substrate specificity (which have been referred to as carboxyl esterases) and with narrow one (acetylcho-

line esterase - ACHE) were identified. ACHE has three fractions, which are nonvariable, keeping identical electrophoretic activity in all groups investigated. Three types of distribution of ACHE fractions were described (Figure 1): type A - intensive staining of slow and

Fig. 1. Electropherograms of esterases of some of investigated *Drosophilidae*



middle fractions - fast fraction is practically not detected; type B - traces of fast fraction are detected, intensive slow and middle fractions; type C - all three fractions are distinct. The characteristics of studied stocks and species are depicted in Table 1.

Carboxyl esterases are divided into two groups with respect to substrate specificity, α - and β -esterases (depending on which type of a substrate it mainly splits). Both esterase

Table 1. Types of distribution of ACHE fractions of investigated stocks and species.

Species	Larvae		Pupae		Imago	
	type	number of individuals	type	number of individuals	type	number of individuals
<i>D. virilis</i>	A	200	A	200	A	400 ♂ & 400 ♀
<i>D. busckii</i> 1763	A	200	B	200	B	200 ♂ & 200 ♀
<i>D. busckii</i> 2374	B	200	B	200	B	200 ♂ & 200 ♀
<i>D. hydei</i>	-	-	-	-	A	100 ♂ & 100 ♀
<i>D. imeretensis</i> 10F	-	-	-	-	A	200 ♂ & 200 ♀
<i>D. imeretensis</i> 1010a	-	-	-	-	A	200 ♂ & 200 ♀
<i>D. imeretensis</i> 1010b	-	-	-	-	B	200 ♂ & 200 ♀
<i>D. imeretensis</i> 1006	-	-	-	-	C	100 ♂ & 100 ♀
<i>D. lebanonensis</i>	-	-	-	-	A	100 ♂ & 100 ♀
<i>D. funebris</i>	A	200	A	100	B	200 ♂ & 200 ♀
<i>D. texana</i>	A	200	B	200	B	200 ♂ & 200 ♀
<i>D. littoralis</i>	-	-	-	-	B	200 ♂ & 200 ♀

types are characterized by high variability in electrophoretic mobility (Figure 1) and differ significantly between various groups of *Drosophilidae*. β -esterase is not detected in the representatives of subgenera *Pholadoris* and *Dorsilopha*. It was shown immunochemically, that antiserum to β -esterase of *D. virilis* gave cross-reaction with corresponding fraction of esterase in eight studied species of virilis group. This antiserum has no affinity to β -esterase of all other investigated species of *Drosophila*. The cross-reaction between antiserum to esterase-6 of *D. melanogaster* and esterases of other species was not observed.

The evolution of genetic system, regulating the organospecific (bulbus ejaculatorius) S-esterase was investigated. The existence of this regulatory system was demonstrated in virilis-repleta branch of subgenus *Drosophila*. On the basis of our data on the direction of variability of the system, regulating expression of S-esterase, the virilis group can be divided into two branches. One branch includes *D. texana*, *D. littoralis*, *D. novamexicana*, which have rarely or never S-esterase; *D. ezoana*, *D. montana*, *D. imeretensis* with active S-esterase form the second branch.

We are grateful to M. Reveley for supplying us with flies used in this study.

Mohan, J. Haryana Agricultural University, Hissar, India. Differential activity of ribosomal DNA in *Drosophila melanogaster*.

Various tissues of an organism exhibit different rates of rRNA synthesis yet most of them contain the same amount of rDNA (Ritossa et al., 1966; Mohan et al., 1969; Mohan, 1976a). To see whether there is differential utilization of

rRNA genes in *Drosophila* tissues, we have studied rRNA synthesis in two adult and one larval tissue. Table 1 gives the results of in vitro experiments with various tissues of two genotypes after 30 minute pulse with ^3H -uridine. Most of the rRNA synthesized during this period serves as a precursor to 18S and 28S RNA and is known to have a sedimentation coefficient of 38S and a molecular weight of 2.9×10^6 daltons (Greenberg, 1969; Mohan and Ritossa, 1970; Mohan, 1975). Results demonstrate that the rates of rRNA synthesis vary a great deal between

Table 1. In vitro synthesis of ribosomal RNA in various tissues of two genotypes*

Genotype	Percent rDNA	Specific activity (CPM/ μg rRNA) in:		
		Male reproductive organs	Ovaries	Imaginal discs**
bb ⁺ /bb ⁺ ♂	0.352	52 \pm 7	185 \pm 11	410 \pm 48
bb ⁺ /bb ⁺ ♀	0.330			
car bb/Y ^{bb} ♂	0.112	41 \pm 9	107 \pm 14***	165 \pm 24***
car bb/car-bb ♀	0.137			

* Description of genotypes and procedural details have been described elsewhere (Mohan 1975)

** Males and females employed together

*** Significantly different from wild-type at 1.0 percent probability level

various tissues. The differences between various tissues of the same genotype are visible at the rRNA precursor level (Mohan, 1975; 1976b). Differences between wild and bobbed genotypes were significant in ovaries and imaginal discs but not in male reproductive organs. In spite of reduced rRNA synthesis, bb flies maintain a normal amount of 4S RNA synthesis and the same RNA/DNA ratios as phenotypically wild flies. However, 5S RNA synthesis is coordinately reduced (Mohan, 1975).

The specific activity expressed as CPM/ μg of rRNA varies over a magnitude of eight times from nearly 50 to above 400 in in vitro experiments. Obviously, rRNA genes are not being utilized to their potential capacity in each tissue. As the differences in the amount of precursor to rRNA synthesized can be observed not only in flies of varying rDNA content but also in various tissues of the same genotype, it suggests that there is a control at the primary gene product level itself. In addition, there must also exist a post-transcriptional control since total ribosome content in genotypes deficient in rRNA synthesis is comparable to wild-type flies.

References: Greenberg, J.R. 1969, J. Mol. Biol. 46:85; Mohan, J. 1975, Genetics 81: 723; 1976a, DIS (this issue); 1976b (in preparation); Mohan, J. and F. Ritossa 1970, Devel. Biol. 22:495; Ritossa, F.M., K.C. Atwood, D.C. Lindsley and S. Spiegelman 1966, 23: 449.

D'Alessandro, A., F. Ritossa and F. Scalenghe. University of Bari, Italy. Cytological localization of the "ebony" locus in *Drosophila melanogaster*. II.

We report here the new mutants at the ebony locus obtained with a selective scheme different from that previously described (M. Fortebraccio, F. Scalenghe and F. Ritossa: DIS, this issue). Wild type *Drosophila melanogaster* males of the strain Canton-S (aged 3-4 days) are given 3,000

r X-rays and immediately mated to virgin females (aged 2 days) of genotype Tm1, Me cu/ru h th st cu sr e^s ca. The mating is interrupted after 3 hours by discarding the males. The females are allowed to lay eggs and transferred to vials containing fresh food every three days. The F₁ generation is scored for flies exhibiting only the ebony phenotype. Among a progeny of about 50,000 individuals, we have found 7 ebony (5 males and 2 females) which will have the genotype ru h th st cu sr e^s ca/ex.

Single ebony male flies are then crossed to Tm1, Me cu/H Pr females. Sons are selected which show the Moiré phenotype only and crossed inter se.

If the progeny of this cross does not segregate ebony individuals then it constitutes a stable line. If it does the ebony progeny is crossed inter se. The cytology of the ebony mutations obtained has been analyzed (for polytene chromosome band identification reference is made to F. Scalenghe e F. Ritossa: "Controllo dell' attività genica in *Drosophila*. Il puff al locus ebony e la Glutamina Sintetasi I". Atti dell' Accademia Nazionale dei Lincei 1976 in press) with the following results:

In(3R)e^{D12}: this mutant is homozygous viable and appears to be a small inversion on the right arm of chromosome 3. The left breakpoint (with respect to the centromere) of the inversion presumably is near band 92E12-13 (which remains in place). The right breakpoint is in the interband between bands 93D1 and 93D6-7, or in the very left portion of band 93D6-7, since at least the majority of this band remains in place.

In(3R)AFA, e: this mutant is homozygous viable and is an inversion with the left breakpoint in section 86C. The right breakpoint must be in close proximity of the left side of band 93D6-7 or in the interband between bands 93D1 and 93D6-7, as for In(3R)e^{D12}.

T(2;3)e^{D8}: this mutant is homozygous lethal and appears to be a reciprocal translocation involving chromosome arms 2R and 3R. Chromosome arm 3R is broken between bands 93D1 and 93D6-7, while chromosome arm 2R is broken near the centromeric heterochromatin.

Df(3R)e^{D7}: this mutant is homozygous lethal and is a deletion of the region comprised between bands 93D1 and 93E4-5.

Taken together, these data and those previously presented (M. Fortebraccio, F. Scalenghe and F. Ritossa: DIS, this issue) strongly argue in favour of a cytological localization of the ebony locus in the interband between bands 93D1 and 93D6-7, or in the very left portion of band 93D6-7.

Faccio Dolfini, S. University of Milan, Italy. Karyotype evolution in two cell lines of *D. melanogaster* maintained in different culture conditions.

The *Drosophila* cell lines, regularly maintained in a medium supplemented with 20% fetal bovine serum (Mosna and Dolfini, 1972), multiply also in absence of serum (Mosna, 1972). The analysis of the karyotype situation of two cell lines, GM₂ and GM₃, growing in different cul-

ture conditions, i.e. presence or absence of serum in the medium, suggests some preliminary conclusions:

- 1) A karyotype evolution occurs in both culture conditions.
- 2) A karyotype polymorphism is observed in the presence of serum; in serum-free medium, on the contrary, chromosomal homogeneity is maintained. A relationship probably exists between the karyotypic situation and the presence or absence of the serum in the culture medium. The serum-free medium may represent a more controlled environment where selective forces are stronger.
- 3) The chromosomal rearrangements are mainly translocations and deletions involving heterochromatin. Moreover, in the different karyotypes observed, the euchromatin of the pairs II and III remains quantitatively and topographically constant; any variation of the euchromatin, modifying both gene dosage and gene position, is presumably incompatible with cell life in vitro.

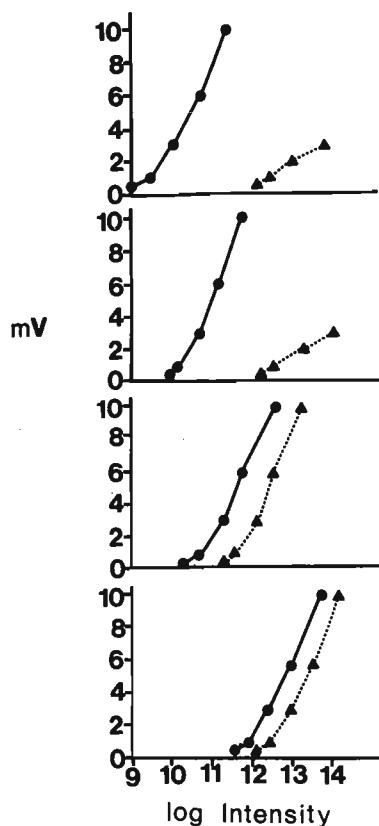
References: Mosna, G. and S. Dolfini 1972, *Chromosoma* (Berl.) 38:1; Mosna, G. 1972, DIS 49:60.

Stark, W.S. The Johns Hopkins University, Baltimore, Maryland. Diet, vitamin A and vision in *Drosophila*.

Vitamin A deprivation dramatically alters light adaptation in the *Drosophila* retina. The R1-6 receptors of normally reared flies (6 of 8 retinula cells per ommatidium with peripheral rhabdomeres) are reversibly inactivated by

bright adaptation with wavelengths below 500 nm; this inactivation is mediated by photo-conversion of the 470 nm absorbing rhodopsin to a stable 570 nm absorbing metarhodopsin during which receptors depolarize fully and are incapable of further transduction (Harris, Stark and Walker 1976; Stark, 1975; Minke, Wu and Pak, 1975). This depolarization lasts after termination of the bright adapting short wavelength stimulus and is reflected in a long-lived electroretinogram (ERG) negative afterpotential. Bright adaptation with wavelengths above 520 nm immediately reactivates R1-6 receptors, reconverts metarhodopsin to rhodopsin and repolarizes the ERG. Vitamin A deprivation blocks the R1-6 inactivation and the associated afterpotential (Stark and Zitzman, 1976); a smaller component of long vs. short wavelength adaptation, reflecting rhodopsin levels remains.

Determining the effects of standard laboratory diet with known diets is therefore important for those doing research in *Drosophila* vision. The synthetic (mostly defined) diet used in these studies was Sang's medium (Doane, 1967) with or without beta-carotene (an effective source of vitamin A for insects) supplemented at very high levels (1.25 mg/ml, or 2.0×10^3 units of vitamin A activity per ml). My standard laboratory diet consists of 8 g agar, 40 g brewer's yeast, 100 g yellow corn meal, 100 ml of syrup (part corn syrup, part molasses), 3.2 g Carolina mold inhibitor, and live yeast (on the surface) in about 1000 ml water. Watt and Merrill's (1963) data suggest that the yellow corn meal (but not white) is the major or only vitamin A source in the standard diet. Quaker enriched degerminated corn meal has 100 units of vitamin A activity per oz yielding a final dietary activity of 0.28 units per ml. Although most *Drosophila* researchers seem to prefer yellow corn meal, an experimental examination of different corn meals in standard diet seemed worthwhile. Thus, the effects of Sang's medium with and without vitamin A and of the standard diet made with yellow and white corn meal on the ERG receptor component sensitivity were examined and compared.



The figure shows intensity-response functions for the white-eyed *Drosophila* retina after 570 nm (long wavelength) bright adaptation (circles, solid lines) and after 470 nm (short wavelength) bright adaptation (triangles, dotted line): 1) top for Sang's with vitamin A; 2) second for standard with yellow corn meal; 3) third for standard with white corn meal; 4) bottom for Sang's without vitamin A; top and bottom redrawn from Stark and Zitzmann (1976). Prior 570 nm adaptation should reactivate R1-6 to near maximal responsivity. Thus, sensitivity (inverse intensity to elicit a given response) is highest in high vitamin A medium, about .75 log units lower in yellow corn meal diet, about .75 log units lower still in white corn meal diet, and about 1 log unit lower in zero vitamin A medium. Bright adaptation with 470 nm inactivates R1-6 in the former two dietary conditions but not in the latter two as shown by the altered intensity-response function shapes in the top two graphs in the figure (triangles, dotted lines, see Stark and

Zitzmann, 1976). Quantitatively, sensitivity on the yellow and white corn meal standard diets is intermediate between high and zero vitamin A medium; qualitatively, bright adaptation is similar in white corn meal diet and zero vitamin A medium.

These results suggest that the vitamin A supplementation in the supplemented medium is above the maximal usable level and that the vitamin A level in standard diet (4 orders of magnitude lower), while low, is sufficient to cause little quantitative sensitivity change and no qualitative adaptation change. However, further diminution of vitamin A levels causes qualitative adaptational changes in visual receptors. Researchers concerned with vision in *Drosophila* should be aware of the importance of diet from a standpoint of control as well as experimental

manipulations.

In tests of the effectiveness of several sources of vitamin A to supplement the larval diet, beta-carotene and vitamin A ester were fully effective, vitamin A palmitate partially effective, and vitamin A acetate, trans-retinol and trans-retinal ineffective. Furthermore, maintaining adults for one week on carrot juice (a rich vitamin A source) completely reversed the effects of larval vitamin A deprivation. This latter finding supercedes a previous report and is the first evidence that effects of vitamin A deprivation on insect vision can be reversed in adult life.

References: Doane, W.W. 1967, in *Methods in Developmental Biology* (ed. W.K. Wessels), New York, Thomas N. Crowell Co.; Harris, W.A., W.S. Stark and J.A. Walker 1976, *J. Physiol.* 256:415-439; Minke, B., C.-F. Wu and W.L. Pak 1975, *J. Comp. Physiol.* 98:345-355; Stark, W.S. 1975, *J. Comp. Physiol.* 96:343-356; Stark, W.S. and W.G. Zitzmann 1976, *J. Comp. Physiol.* 105:15-27; Watt, B.K. and A.L. Merrill 1963, *Composition of Foods, Agricultural Handbook No. 8*, Washington, D.C., U.S.D.A.

Supported by NSF Grant GE-M574-12817. I thank A. Ivanyshyn, W. Zitzmann, G. Pransky, K. Hu, D. Lakin, R. Greenberg and M. Chapin for assistance.

Arthur, C.G. and E.S. Goldstein. Arizona State University, Tempe. Rate of *Drosophila melanogaster* development at 15°C.

A major benefit obtained by the use of D.m. for both genetic and developmental studies is the speed with which this organism completes its embryological stages. The study of very early development in D.m., however, is hampered by

this speed. In order to increase the amount of time available for experimental manipulations, such as incorporation of label, incubation of embryos at 15°C was examined for its effects on

the rate of development in D.m. Figure 1 indicates that the rate of development at 15°C is approximately one-half as rapid as at 25.5°C through gastrulation. Viability at 15°C was comparable to controls (>90%).

Reference: Bownes, M. 1975, *J. Embryol. Exp. Morph.* 33 (3):789-801.

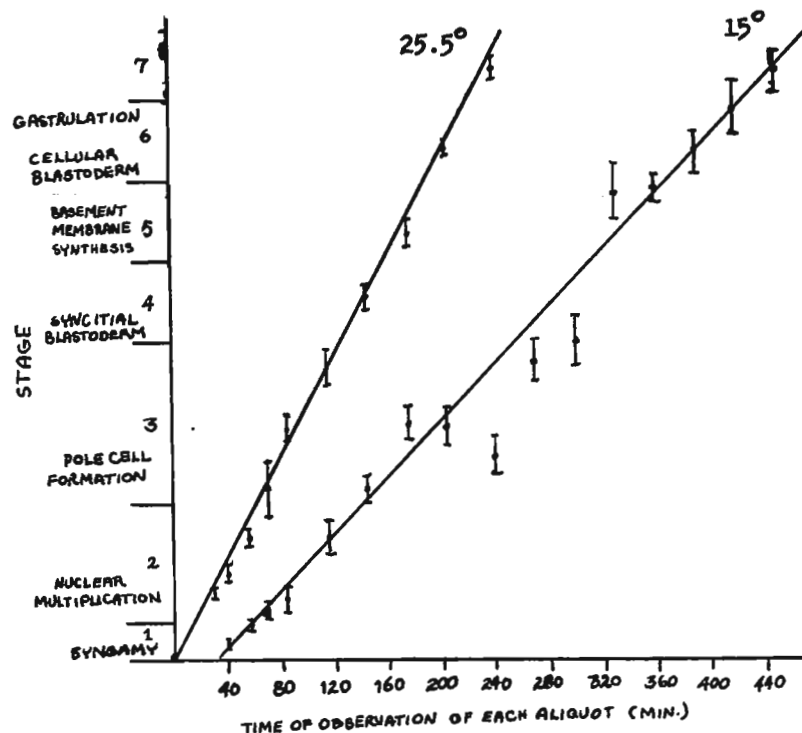


Figure 1. Eggs are collected on agar planchets for 20 min (10 ± 10 min old embryos) and rinsed off the planchets with 15°C water. The eggs are immediately dechorionated (15°C) and maintained in a 15°C incubator. Aliquots are removed at timed intervals and staged, using the descriptive morphology of Bownes (1975). The points represent the mean time of development of the eggs in each aliquot (vertical bars represent \pm standard error of the mean). The same procedure is used for the controls, maintained at room temperature (25.5°C).

Barrett, J.A. University of Cambridge, England. Distinguishing matings by breeding tests.

The determination of the matings which have taken place in population cages or wild populations of *Drosophila* species is often made by progeny testing females of known genotypes.

This method is especially suited to electrophoretic markers. There are, however, certain drawbacks to this method; if two few offspring are tested, the type of mating cannot be determined with any accuracy; if a large number of offspring are tested, to ensure an accurate determination of the type of mating, fewer progenies can be tested. Use of the following tables (Tables I and II) enables the number of offspring per progeny tested to be minimised if the genotype of the female parent is known. The tables also give an indication of the accuracy with which the matings have been determined.

A single locus, two allele system in which the heterozygote is distinguishable is assumed. If the female parent is homozygous, the presence in the progeny of heterozygotes and the female parental genotype is sufficient to differentiate between all possible matings:

		female parent	
		<u>AA</u>	<u>aa</u>
male parent	<u>AA</u>	AA	Aa
	<u>Aa</u>	50% AA, 50% Aa	50% Aa, 50% aa
	<u>aa</u>	Aa	aa

(the body of the table contains the expected offspring from the possible matings between homozygous females and males of any genotype).

Except for matings with heterozygous males, all matings produce progenies of single genotypes. The presence of at least one of the respective homozygotes and the heterozygote in the progeny of homozygous females is sufficient to differentiate the matings. The probability of obtaining at least one of each genotype in a homozygous female x heterozygous male mating is given by:

$$v = 1 - \text{probability of all homozygotes in a sample of } n \\ - \text{probability of all heterozygotes in a sample of } n$$

$$v = 1 - 0.5^n - 0.5^n \quad \text{where } n \text{ is the number of offspring tested.}$$

$$\therefore v = 1 - 2(0.5^n)$$

Table I gives the values of v for different values of n . If eight or more offspring are tested and they are all of the same genotype, the probability that the mating was with a heterozygous male is very small (< 0.01).

If the female is heterozygous, the offspring of the different possible matings are:

		female parent	
		<u>Aa</u>	
male parent	<u>AA</u>	50% AA, 50% Aa	
	<u>Aa</u>	25% AA, 50% Aa, 50% aa	
	<u>aa</u>	50% Aa, 50% aa	

(the body of the table contains the expected frequencies of the progeny genotypes from different matings).

The presence of at least one individual of each genotype or both homozygotes only is sufficient to distinguish heterozygote female x heterozygote male matings from the other two. Hence in a sample of n offspring:

$$v = 1 - \text{probability all AA} - \text{probability all Aa} - \text{probability all aa} \\ - \text{probability of AA, Aa only} - \text{probability of Aa, aa only.}$$

$$\therefore v = 1 + p_{Aa}^n - (p_{AA} + p_{Aa})^n - (p_{Aa} + p_{aa})^n$$

$$\therefore v = 1 + 0.5^n - 2(0.75^n)$$

Table II consists of values of v for different numbers tested. Thus, thirteen individuals must be tested if the different matings are to be distinguished with a probability of error of < 0.05 .

If the genotypes of the females are determined first, the number of offspring required

to differentiate between the matings, with a given degree of accuracy, can be read from the tables.

Table I

<u>n</u>	<u>v</u>
1	0.000
2	0.500
3	0.750
4	0.875
5	0.938
6	0.969
7	0.984
8	0.992
9	0.996
10	0.998
11	0.999

Table II

<u>n</u>	<u>v</u>	<u>n</u>	<u>v</u>
1	0.000	11	0.916
2	0.125	12	0.937
3	0.281	13	0.953
4	0.430	14	0.964
5	0.557	15	0.973
6	0.660	16	0.980
7	0.741	17	0.985
8	0.804	18	0.989
9	0.852	19	0.992
10	0.888	20	0.994

Koenig, J. and J.R. Merriam. University of California, Los Angeles. Autosomal ERG mutants.

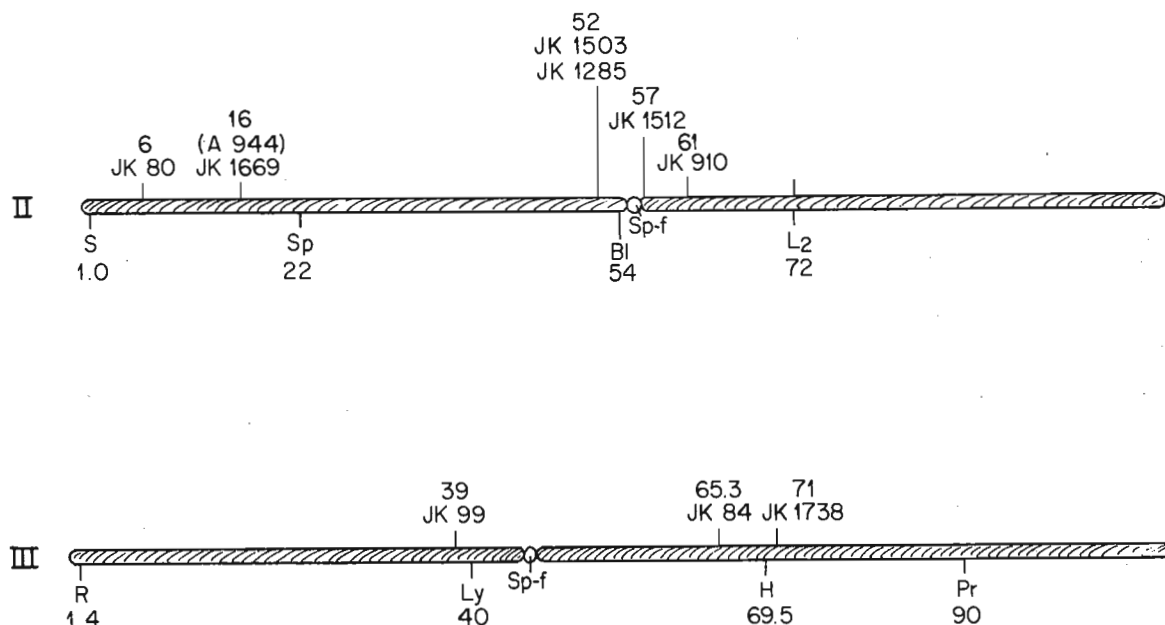
Nine autosomal mutants with visual abnormalities have recently been obtained. To avoid the problem of making homozygous newly induced lethal mutants along with behavioral mutants we used the scheme of "free recombination" sug-

gested by Dr. Dan Lindsley. Canton-S males treated with EMS were mated to females carrying SMI and TM2 and a 2:3 translocation. Single pair matings were made between those F_1 males and females which received both the SMI and TM2 chromosomes. In the F_2 generation those virgin females and males without the balanced chromosomes were selected to yield the next generation. In each line the selected females carry one normal and one mutagenized X, two independently mutagenized second chromosomes and two independently mutagenized third chromosomes. Brothers carry the same mutagenized second and third chromosomes as their sisters.

A behavioral mutant carried heterozygously in the F_2 generation in any line appears homozygously in some fraction of the F_3 generation of that line. We used Benzer's (1967) counter current distribution of the phototactic response to separate putative mutant homozygotes from their normal sibs. Individual males which ran away from light were crossed to females carrying SMI and TM2 and their ERG's examined after they had mated. Of approximately 1000 lines examined in this way nine autosomal mutants and six sex-linked mutants have so far been recovered. Some mutant properties are summarized in the following table.

	Chromosome	ERG		Histology	Optomotor response to wide stripes	Remarks
		"RP"	"LP"			
JK 80	II	+	-	+	-	Pleiotropic uncoordinated
JK 1669	II	+	-	+	+/-	
JK 1503	II	+	-		+/-	
JK 1285	II	+	-		+/-	
JK 1512	II	+	-		+/-	
JK 910	II	+	-		+/-	
JK 99	III	+	-		+/-	
JK 84	III	+/-	-	+/-	-	
JK 1738	III	+/-	-	+	+/-	dominant

Recombination mapping and complementation tests on the nine autosomal mutants indicates that they represent a total of eight loci, five on the second chromosome and three on the third chromosome. Their genetic locations are shown in Figure 1. All the mutants complemented each other except JK 1503 and JK 1285 which both mapped to the same location. (A944, obtained from M. Heisenberg, is an allele of JK 1669.) In all of these mutants the on and off transients of the ERG are either missing or greatly reduced. Two of the mutants with interesting phenotypes have so far been more extensively examined: JK 84, a 3rd chromosomal



recessive mutant, displays an ERG which appears to represent a normal receptor response lacking all presumed laminar components. The amplitude of the response is noticeably reduced, however (5MV). Histology reveals that the rhabdomeres of the 1-6 receptor cells apparently never form, thus isolating the 7-8 system, which appears to remain intact. Behaviorally, JK 84 flies are almost blind, exhibiting little optomotor or phototactic response. The 3rd chromosomal mutant JK 1738 is unusual in that it is dominant. The ERG of homozygous flies lacks the on and off transients and exhibits a slow return to baseline. Flies heterozygous for the mutation possess a more "normal-appearing" ERG response, in that a delayed off response may be elicited in these flies with light adaptation. The time course of the return to baseline also appears more normal under conditions of light adaptation. Light adapted triploid females carrying 1 mutant chromosome and 2 normal chromosomes exhibit a fairly normal ERG, possessing both on and off transients. However, with dark adaptation in those flies the on transient becomes reduced in amplitude, the off transient becomes delayed, and the return to baseline is slowed. Although this last effect of light adaptation on the ERG is not understood, it seems probable that the JK 1738 mutation has some effect on the receptor cell function. The genetic data, i.e., the triploid studies, clearly indicate the mutant effect stems from an altered gene product instead of the absence of a regular gene product.

Sheldon, G. University of South Florida, Tampa, Florida. The effects of butylated hydroxy-toluene and related antioxidants on longevity, development time, and X-ray sensitivity in *D. melanogaster*.

Longevity was observed on diets containing .1% of one of the antioxidants butylated hydroxy-anisole, propylgallate, or butylated hydroxy-toluene. The BHA diet was significantly ($P=.05$) detrimental, while the others had no appreciable effect.

The development time was recorded for larvae raised on diets containing various concentrations of the antioxidants. All were found to have a retarding effect on development and BHA was again found to be most detrimental.

BHT was tested for radio-protection on recently eclosed male flies and was found to be effective at the .05% concentration.

A .1% concentration of BHT, BHA or PG added to the larvae diet did not provide significant radio-protection to the adults. The loss in larval viability at the .1% concentration is almost total for the BHA diet.

Additional information on this work is available as a copy of my master's thesis from the University of South Florida Library, 4202 Fowler Ave., Tampa, Florida.

Krishnamurthy, N.B. and H.A. Ranganath.
University of Mysore, India. Adaptedness
in six species of the immigrans group of
Drosophila.

Dobzhansky (1968) has defined "adaptedness" as
the ability of the carriers of a genotype or a
group of genotypes to survive and reproduce in
a given environment. A possible measure of
adaptedness of a population to its environment
is given by the ability of the population to

transform the available resources into living matter. This provides means for comparing the
overall biological performance of one gene pool with another where both are maintained under
similar environmental conditions. In the present investigations, the productivity and popu-
lation size, as suggested by Ayala (1965) have been used to estimate the adaptedness of six
closely related species of the immigrans group of *Drosophila*.

D. nasuta nasuta (Coorg, Mysore), *sulfurigaster neonasuta* (Coorg, Mysore), *nasuta albom-
icana* (University Texas Stock No. 3045.11), *sulfurigaster bilimbata* (University Texas Stock
No. 3071.6), *pulaua* (University Texas Stock No. 3121.5) and *hypocausta* (Coorg, Mysore) have
been employed to measure the two parameters of the adaptedness. Experimental populations were
maintained at 21°C by adopting the serial transfer technique of Ayala (1965). Each population
was built with 25 pairs of flies. The culture of each species was maintained for 30 weeks.
The mean values for productivity and population size were calculated from 7th week to the 30th
week (24 counts). 4 replicates were set up for each population. Measurements were made on
the adaptedness of six closely related species, maintained under similar conditions in the
laboratory. The mean values for productivity and population size of the six species under
study are given in Table 1. Analysis of variance for the data in Table 1 has revealed homo-
geneity among the replicates of each population and significant heterogeneity between popu-
lations of different species under study. The species which maintain the larger population
size may be said to be performing better from the biological point of view than species having
a smaller population size. The species having larger population size may be said to have a

Table 1. Mean values along with the standard errors of the productivity
and population size in the populations of six species.

Population	Species	Productivity	Population size
5	<i>D.n. nasuta</i>	70.00 ± 12.78	165.87 ± 16.35
6	"	71.20 ± 12.17	177.58 ± 18.93
7	"	67.91 ± 12.92	163.70 ± 21.66
8	"	85.75 ± 15.70	199.37 ± 24.04
	Average	73.71	176.63
13	<i>D.s. neonasuta</i>	52.54 ± 14.04	129.87 ± 16.33
14	"	46.20 ± 9.61	120.25 ± 18.08
15	"	42.00 ± 10.92	107.20 ± 17.11
16	"	40.54 ± 9.77	105.54 ± 14.81
	Average	45.32	116.71
17	<i>D.n. albomicana</i>	36.70 ± 7.85	74.04 ± 8.81
18	"	36.00 ± 7.91	68.79 ± 10.22
19	"	27.12 ± 5.35	56.95 ± 6.73
20	"	34.00 ± 7.76	70.20 ± 9.04
	Average	33.45	67.45
21	<i>D. pulaua</i>	45.25 ± 9.49	74.66 ± 12.13
22	"	34.95 ± 6.91	65.37 ± 8.88
23	"	32.83 ± 7.12	62.58 ± 8.15
24	"	37.79 ± 6.29	68.45 ± 8.43
	Average	35.20	67.76
25	<i>D.s. bilimbata</i>	32.75 ± 8.01	61.66 ± 7.75
26	"	31.66 ± 7.30	60.00 ± 8.32
27	"	36.54 ± 7.45	62.33 ± 8.52
28	"	35.33 ± 8.72	64.37 ± 8.93
	Average	34.07	62.09
29	<i>D. hypocausta</i>	32.71 ± 4.84	64.28 ± 6.06
30	"	56.14 ± 8.87	97.00 ± 12.88
31	"	68.57 ± 9.94	102.28 ± 13.87
32	"	72.57 ± 10.26	111.43 ± 16.27
	Average	57.49	93.74

collective genetic endowment which enable it to exploit the experimental resources. The adaptedness of the experimental populations under study is shown to be highly variable. Differences in productivity or population size between any two populations are in most cases significant. Thus the differential ability of the species to exploit the experimental environment is striking. The statistical comparisons of the parameters in the experimental populations can be summarized as follows: for productivity = *D.n. nasuta* > *D. hypocausta* > *D.s. neonasuta* > *D. pulaua* = *D.n. albomicana* = *D.s. bilimbata*; and for population size - *D.n. nasuta* > *D.s. neonasuta* > *D. hypocausta* > *D. pulana* = *D.n. albomicana* = *D.s. bilimbata*. Essentially the ability of a population to increase in numbers depends upon the birth rate and the survival rate of the animals. There exists a positive correlation between population size and productivity in the experimental populations under study. Those populations which have more productivity have larger population size with the exception of *D. hypocausta*.

Thus, the differences in the genetic potentialities of the six species under study to survive and reproduce in a similar ecological situation is striking and this could reflect different levels of species/genotypic - environmental interactions.

Authors are grateful to Prof. M.R. Rajasekarasetty, University of Mysore, for his constant help and encouragement. Thanks are due to University of Mysore, Mysore and C.S.I.R., New Delhi, for financial assistance.

References: Ayala, F.J. 1965, *Genetics* 51:527-544; Dobzhansky, Th. 1968, *On some Fundamental Concepts of Darwinian Biology*. In *Evolutionary Biology*, Vol. 2. Eds. Th. Dobzhansky, M.K. Hecht and W.C. Steere, pp. 1-34.

Russell, M.A. University of Alberta, Edmonton, Canada. The frequency of cell-autonomous mutants among EMS-induced temperature-sensitive lethals.

By the use of genetically marked somatic mosaics Demerec (1936) found that 10 out of 24 X-ray induced sex-linked lethal mutants were cell lethal. I have isolated a series of 49 EMS-induced sex-linked temperature-sensitive lethals and screened them for cell autonomy of the

lethal effect at two temperatures (29° and 22°), by a method adapted from that of Stern and Tokunaga (1971). Larvae of the genotype *wsn³ lts/y* were treated with 1,500 r of X-radiation at 3 days after oviposition, and then placed either at 22° or 29° to complete development. Marked clones resulting from somatic crossing-over were scored in the median triple row bristles on the anterior margin of the wing. I took as a criterion of cell lethality, a statistically significant deficiency in the frequency of singed clones relative to yellow ones. The results are given in Table 1, where the mutants are divided into three classes defined as follows:

Table 1

Mean frequency of singed clones relative to yellow clones (%)

Class	No. of mutants	at 29°	at 22°
1	20	62	96
2	7	77	78
3	22	92	94

Class 1. A significant deficiency of singed clones at 29°, but not at 22°. Class 2. A significant deficiency of singed clones at both temperatures; Class 3. No reduction in the frequency of singed clones at either temperature. All mutants fall into one of the three classes.

It is clear that the method used for estimating mutant effects

on cell viability will underestimate the true frequency. This is because: (i) A single tissue was used as the test for cell lethality. (ii) The temperature sensitive period of a given mutant may not persist into the late third instar when cells first become homozygous for the mutant in this method. (iii) Mutants which map close to the centromere would sometimes be proximal to the position of a somatic cross-over, and therefore might not lead to a significant reduction in the recovery of singed clones.

The results therefore indicate that out of 49 lethals at least 27 have a detectable effect on cellular viability. This frequency is even higher than that obtained by Demerec (1936), and shows that non-autonomous mutants are a surprisingly infrequent class among EMS induced temperature-sensitive lethals.

References: Demerec, M. 1936, *Proc. Nat. Acad. Sci. Wash.* 22:350-354; Stern, C. and C. Tokunaga 1971, *Proc. Nat. Acad. Sci. Wash.* 68:329-331.

Basden, E.B. Leyden Park, Bonnyrigg, Midlothian EH19 2DE, Scotland. *Drosophila nigricornis* Loew of a New York cellar.

In the 38th Annual Report on (sic) the New York State Museum of Natural History, 1885, Charles H. Peck in his Report of the Botanist (pp. 77-138) describes on p. 96 the micro-fungus, *Appendicularia entomophila* n. gen. et sp. It was found on small flies, *Drosophila nigricornis*,

in the cellar of the Rev. J.L. Zabriskie at Nyack, Rockland County. The fungus was discovered on the flies in March, 1884, it growing on the head, thorax, abdominal rings, costae of the wings but most frequently on the legs. Beautifully mounted specimens on microscope slides were sent by Zabriskie to Peck. Attached to one leg were seven well developed specimens and one or two imperfect ones of the fungus, and this leg is illustrated on plate 3. The affinities of the fungus were not clear.

The name *nigricornis* has never since been used validly in *Drosophila* sens. strict. and is omitted from catalogues. So what is *Drosophila nigricornis*? There is no description of this fly but Peck's drawing of the leg thus gives the name availability. If correctly drawn the leg, if the first leg, cannot be that of *D. melanogaster* or *D. funebris*, common inhabitants of cellars, as the metatarsus of these species is obviously shorter than tarsal joints 2 and 3 together, whereas the drawing shows it to be the same length.

Possibly Zabriskie's slides are still in the New York Museum and an examination of these might solve the identify of the fly. Sturtevant, 1921, North American *Drosophila*, p. 106, claims *D. nigricornis* Loew as only a manuscript name and gives further references, but not the one above. An additional reference is M.C. Cooke, 1892, "Vegetable Wasps and Plant Worms", London, pp. 250, 345, and who calls the fungus *Appendiculina entomophila*, the American Fly Hanger, and who also refers to Saccardo's "Sylloge", 8, no. 3617, of 1889.

Hasty, J.H. Davidson College, Davidson, North Carolina. Apparent stability of Bristle Lobe over Curly with respect to cross-over type progeny.

In an attempt to illustrate crossing-over in *Drosophila melanogaster*, BIL/Cy virgin females were mated with wild type males, but significantly fewer recombinant type progeny than expected were scored. Curly, Bristle and Lobe are located on the second chromosome at loci

6.1, 54.8, and 72.0, respectively. Were these mutations simply dominant alleles, with no additional factors acting, rather frequent crossing-over would be expected. In fact, Curly is associated with a large inversion which inhibits survival of most gametes which would exhibit cross-overs between Curly and Bristle (those occurring in what I called region 1). One would still expect 15-20% of the progeny to show evidence of crossing over between Bristle and Lobe (in region 2). The experimental data are as follows:

	No cross-over		Cross-over region 2		Cross-over region 1		Double cross-over		Totals
	BIL	Cy	Bl	CyL	+	CyBIL	CyBl	L	
Brood 1	254	284	0	0	0	0	0	0	538
Brood 2	282	288	3	2	1	3	0	0	579
Totals	536	572	3	2	1	3	0	0	1117

These data show very little evidence of crossing-over in either region. This indicates either an actual genotypic stability (recombinant type progeny appear in the offspring only infrequently), or an apparent stability (recombinant offspring appear in the expected ratio, but due to phenotypic phenomena are not recognized as recombinants).

An actual stability could be accounted for by either or both of two factors. First, the large inversion may make synapsis difficult, thus greatly reducing crossing-over. Second, it is stated in Lindsley and Grell that the presence of Bristle may cause a reduction in crossing over. On the other hand, the case for apparent stability is supported by the fact that the phenotype of heterozygous Lobe (L/+) overlaps wild type (+/+), which could cause CyL and L to be scored as Cy and +, respectively. More work is needed to determine which factor or factors are acting here, and if the observed stability is actual or apparent.

Reference: Lindsley, D.L. and E.H. Grell 1968, Genetic Variations of *D. melanogaster*.

Vaidya, V.G. and N.N. Godbole, University of Poona, India*. A new species of genus *Stegana* (Drosophilidae) from India: *Stegana* (*Steganina*) *subexcavata* sp. nov.

Genus *Stegana* has not yet been reported from the Indian subcontinent. A new species of this genus is described here.

DESCRIPTION OF MALE IMAGO: General features and head: Body black, about 2.6 mm in length. Head dorsally flat. Eyes dark reddish brown. Antenna dark brown, third segment black and pubescent. Arista with about nine branches above and four below including the terminal fork. Basal 1/3 of arista yellow. Palpus yellowish, pubescent and with few prominent setae. Second oral about 1/2 the first. Front shining black, periorbits yellowish brown. Carina narrow, black below, yellow above. Cheek white, about 3/5 greatest diameter of eye. Second orbital about 1/3 the first and about 1/5 the third.

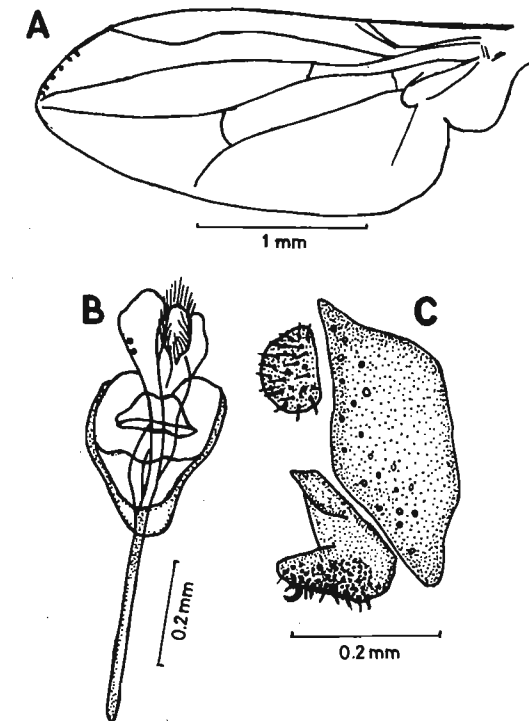


Figure: *Stegana subexcavata* sp. nov. A. Wing; B. Phallic organs; C.Periphallallic organs

Post-verticals small. Occiput black.

Thorax: Scutum brown with a broad dark median longitudinal band throughout its length and narrower longitudinal stripes on its lateral sides. Scutellum dark brown. Thoracic pleura yellowish with a black broad longitudinal band. Humerals 2, lower longer. Acrostichal hairs in 8 rows. Dorsocentrals in 2 pairs. Cross distance between dorsocentrals about four times the length distance. Prescutellars well developed. Anterior scutellars divergent. Posterior scutellars crossing each other. Sterno-index about 1.0.

Legs: White, black at knee joints. Preapicals on all three tibiae and apicals on second. Forefemur with a row of three especially long bristles. Middle and hind tarsae with two rows of minute cuneiform bristles enclosing two rows

of stout black bristles.

Wings: Wings held obliquely over the abdomen and slanting sideways. Wings grey, anterior half darker. Veins R 4+5 and M apically strongly convergent. Third costal section with about 8-10 thornlike warts. Costal index about 2.2. 4th vein index about 1.9. 4C-index about 1.1. 5X-index about 1.7. C1 bristles 2, equal. C3 bristles on about basal 4/5. Halteres pale yellow.

Abdomen: Shining black.

Periphallallic organs: Genital arch dark brown, broad below. Anterior margin convex. Heel narrow, blunt, lower than toe. Genital arch with about 21 bristles including 6 on posterior margin and 4 on undermargin. Clasper dark brown, roughly triangular. Lower half of clasper with many small conical warts and with about 25 bristles. A strongly developed black tooth on the ventral border. Anal plate oval, separate from genital arch and with about 23 long bristles.

Phallic organs: Phallic organs brown. Aedeagus with many slender processes apically. Anterior paramere nearly as long as aedeagus and with 2 sensillae. Apodeme of aedeagus darker, slender, rodlike. Ventral fragma longer than broad, rounded proximally. Novasternum with a pair of submedian processes and articulated with ventral fragma. PI about 1.

DESCRIPTION OF FEMALE IMAGO: Similar to male. Egg guide: Lobes dark brown, entirely fused to each other. Each lobe with several setae, 2 of which are especially long.

Internal characters: Spermatheca dark brown, pearshaped, apically pointed. Ventral receptacle with about 120 coils.

MATERIAL: Holotype: Male: Poona (India); July 1972 (Vaidya). Deposited with Depart-

ment of Zoology, University of Poona, Poona - 411 007, India.

Paratypes: 2 males, 1 female collected together with holotype. 1 male deposited with Professor Dr. Toyochi Okada, Department of Biology, Tokyo Metropolitan University, Tokyo, Japan.

HABITAT: The flies were collected on decomposing leaves in vegetable garden by sweeping with net. They appear only in wet season. It was not possible to rear them in the laboratory.

RELATIONSHIP: This species resembles closely *Stegana excavata* Okada from Japan. However, it differs from the latter in having distal black band of face narrower, apex of palpal dark and no distinct lateral setae on aedeagus.

ACKNOWLEDGEMENT: The authors are grateful to Professor Dr. Toyochi Okada of the Tokyo Metropolitan University for checking the description and for confirming the identification of the species.

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Kan, P. Pui-Hung*. University of Nebraska, Lincoln. Winglessness and increased inter-pulse-intervals in male courtship sounds of several *Drosophila* species.

Although sounds produced by courting males accompany extension and vibration of the wings, similar sounds are also detected from males that have had their wings largely removed (Waldron, 1964, for *D. persimilis*; Miller, Goldstein, and Patty, 1975, for *D. athabasca*). A preliminary

effort has been made to determine any differences in sound quality that winglessness might cause, employing two pairs of sibling species, *D. melanogaster* and *simulans* and *D. pseudoobscura* and *persimilis*, and one other, *D. athabasca* (data provided by Drs. R.B. Goldstein and D.D. Miller). The procedure of sound amplification, recording, and analysis was like that of Patty, Goldstein and Miller (1973), including the preparation of sonograms, from which numerical data were obtained. Flies were made wingless by teasing off the wings of one day old males with dissecting needles. In *D. melanogaster* the mutant vestigial was also used. Observations and recordings, at $25 \pm 0.5^\circ\text{C}$, were done after two days for *D. melanogaster* and *simulans*, after one week for the three *D. obscura* group species. The sounds of wingless males did not seem qualitatively different from those of normal males of their species. However, sonograms revealed that inter-pulse-intervals were always greater for wingless than for normal males. Although only a few clear observations were made of each kind and the numbers of intervals to measure were small, the consistency of results suggests an involvement of a common mechanism. The table gives ranges and means of inter-pulse-intervals (i.p.i.) in milli-seconds, n being the number of intervals measured in each case:

<i>D. melanogaster</i> (Oregon R)	
normal wings ($n = 13$):	28.86-44.80 ($\bar{x} = 37.04 \pm 4.67$)
wings removed ($n = 5$):	75.95-100.25 ($\bar{x} = 86.28 \pm 9.79$)
vestigial ($n = 5$):	47.85-68.35 ($\bar{x} = 59.39 \pm 8.62$)
<i>D. simulans</i> (Eastabuchie, Mississippi)	
normal wings ($n = 21$):	35.7-45.57 ($\bar{x} = 47.55 \pm 10.38$)
wings removed ($n = 11$):	34.94-72.15 ($\bar{x} = 55.10 \pm 9.88$)
<i>D. pseudoobscura</i> (Pinon Flats, California; Standard)	
normal wings ($n = 6$):	91.14-127.59 ($\bar{x} = 111.77 \pm 15.15$)
wings removed ($n = 6$):	142.78-220.25 ($\bar{x} = 183.04 \pm 33.18$)
<i>D. persimilis</i> (Mather, California)	
normal wings ($n = 5$):	39.49-45.57 ($\bar{x} = 42.68 \pm 2.18$)
wings removed ($n = 5$):	56.96-78.23 ($\bar{x} = 67.52 \pm 7.79$)
<i>D. athabasca</i> (Skokie, Illinois)	
normal wings ($n = 11$):	15.95-31.89 ($\bar{x} = 21.68 \pm 4.99$)
wings removed ($n = 5$):	51.65-121.52 ($\bar{x} = 74.74 \pm 27.62$)

References: Patty, Goldstein and Miller 1973, DIS 50:67; Waldron 1964, Sci. 144:191-193; Miller, Goldstein and Patty 1975, Evol. 29:531-544.

* Presently at University of Texas Medical Branch, Galveston; further information can be obtained from Dr. D.D. Miller, School of Life Sciences, University of Nebraska, Lincoln, Neb. 68508.

Aaron, C.S. Louisiana State University, Baton Rouge, Louisiana. Homosexual behavior induced in *Drosophila* by sodium tungstate.

Homosexual behavior in insects has been observed before (Chapman, R.F. in "The Insects", Cha. XVI English Universities Press, London, 1974). We also have observed a peculiar effect of sodium tungstate that leads to a very rapid onset of marked and widespread homosexual behavior in

vials of treated virgin male flies. This abnormal behavior begins within 20 minutes after the males are placed on the solution and persists for some time with repeated aggressive advances by virtually every male upon any other male nearby.

The conditions under which it was first noted were as follows: Males of the type described previously for treatment (Lee, et al., Mutation Res. 16:195-201, 1972) were generated without females. Only a slight amount of homosexual tendency is noted in control samples of these males. The males were partitioned 50 per vial and fed on glass fiber filter (Aaron, et al., DIS, this issue) impregnated with 1% sucrose solution containing $10^{-4}M$ sodium tungstate ($10^{-3}M$ is also effective).

The time of onset of the condition is variable but usually appears within 20 minutes. The approach of one male to another leads to the usual dancing behavior and in numerous instances males were observed to mount the back of other males in pseudo-copulation. The abdomen was distended and curled under the lower male just as though copulation were possible. Randomly selected control males sham-treated have not been observed by us to exhibit pseudo-copulation.

We have only tentative explanations for this effect. The apparent loss of sexual orientation is probably neural in origin. The usual tactile and sensory responses from the approached animal are apparently unnecessary for continued advances or attempted copulation. The effect is not limited to males although the effect in the females is less marked.

Acknowledgement: Support for this study was provided by USPHS Grant ES00320-09 and ERDA Grant AT-(40-1)-3728.

Hatsumi, M. and O. Kitagawa. Tokyo Metropolitan University, Japan. The crossability between *D. melanogaster* and *D. simulans*.

The sexual isolation between *D. melanogaster* and *D. simulans* has been investigated as to some effective factors. In this report the crossability of ten strains of *D. melanogaster* (eight wild type strains: four Japanese, one Taiwan, Egypt, Yugoslavia and France; two mutant

stocks, bw and e) with one strain of *D. simulans* (Ogasawara, Japan) was examined. A single 0-day-old virgin *D. melanogaster* female was placed with two 5-day-old *D. simulans* males for five days at 25°C under the constant light condition (Cross I). Reciprocal crosses (Cross II) were carried out in the same manner. A hundred and sixty crosses were made in each strain. The results are shown in Table 1. For the comparison of the crossability of each strain, χ^2 test and Fisher's exact probability test were applied for Cross I and II, respectively. In Cross I, 15 out of 36 combinations were significantly different at the 0.1% level, 7 at 1%, and

Table 1. The number of fertile crosses.

Cross	Strains of <i>D. melanogaster</i>										Total
	Katsunuma Japan	Matsuyama Japan	Ogasawara Japan	Iriomote Japan	Taiwan	Egypt	Yugo-slavia	France	brown	ebony	
I	50	94	63	63	64	37	75	62	39	25	572/1600 (35.75%)
II	3	7	5	4	3	1	9	6	5	4	47/1600 (2.94%)

one at 5%. The other 13 combinations were not significantly different. On the other hand, in Cross II, only one combination was significant at the 1% level and the other three at the 5% level. From these results it seems to be safe to conclude that the crossability of different geographic strains of *D. melanogaster* with Ogasawara *D. simulans* were controlled by some genetic factors, most probably by the polygenic systems.

Van Valen, L. University of Chicago, Illinois. The nature of positional information in development.

Wolpert's theory of positional information by the local interpretation of spatial coordinates is well known. In 1970 (Dev. Biol. 23:456-477) I independently proposed another theory and gave some evidence against a mechanism equivalent to

Wolpert's. The evidence is that, in complex serially homologous structures, morphological gradients of different components are often directly visible but the gradients cannot always be equated even topologically. Therefore different components of the same structures are probably determined in part separately, rather than by response to a single positional signal. My evidence was from mammals but I predicted that the mechanism would hold for organisms generally. (It reduces to Wolpert's when all observable gradients are equivalent topologically.) Goethe had a theory for plants that could be interpreted rather similarly, although the similarity is not evident in the caricature of his theory that is usually given. Recently Sonneborn (1975, *Année Biol.* (4)14:565-584) has found evidence against Wolpert's mechanism in ciliates, but mine is compatible with his results.

In *Drosophila*, determination of the polyclones that give rise to compartments needs no more than Wolpert's mechanism in the blastoderm or an alternative on the same level of simplicity. However, there are definite although small correlations between the intensities of morphological gradients on different parts of a fly's body (Van Valen 1962, *Evolution* 16:125-142). The gradients I looked at come from different polyclones, as they occur on head, thorax and abdomen. The relationship is independent of body size. Therefore there is some sort of developmental integration among distant compartments, even though such integration is lacking for their degree of resistance to developmental noise, and is even lacking between the same gradients on opposite sides of the body. This should hardly be surprising, but because of the independence between sides it does suggest that a branching-tree model of progressive determination represents only a subprogram, although perhaps the major one.

I thank W.K. Baker for discussion.

Milkman, R. University of Iowa, Iowa City. Temperature shock selection at three allozyme loci.

Using an approach similar to that of Johnson and Powell (PNAS 71:1783), we treated adult *D. melanogaster* at 40°C for 10 minutes. As the flies regained their feet over the next few minutes, they were sequestered until they

numbered about half the sample. The rest were classified as "twitching" or "dead". Data in these two latter categories are pooled. All flies (cf. Johnson and Powell) were electrophoresed on cellulose acetate, using the Adamkewicz applicator (DIS 45:192). The flies were the progeny of individuals taken from a 5-year-old 25th cage, long at linkage equilibrium for the loci studied. The flies' ages were controlled at 0-1, 1-2, 3-5, or 6-8 days. The age differences appear unimportant. The results, given in Table 1, indicate two things: 1. At two loci there is an overall deficiency of heterozygotes. At the malate dehydrogenase locus,

Table 1

Locus	"Living" Flies					Twitching and Dead Flies				
	F	H	S	p*	het**	F	H	S	p*	het**
α -Gpdh	179	460	320	0.426	-10	181	489	273	0.451	+22
Mdh	96	253	313	0.336	-42	79	259	315	0.319	-25
Adh	19	171	769	0.109	-15	20	156	767	0.104	-20

* frequency of the fast allele

** excess (+) or deficiency (-) of heterozygotes over Hardy-Weinberg expectation.

$\chi^2 = 13.85$, $p < 0.0005$; at the alcohol dehydrogenase locus, $\chi^2 = 4.18$, $p < 0.05$. Such deficiencies have never been observed in the cage populations but have been seen before in progeny. 2. At the alcohol dehydrogenase locus, no selective effects are evident, in contrast to Johnson and Powell's observations on some samples from natural populations and in agreement with others. One of several possible conclusions is that enzyme mobility classes frequently represent more than one allele in substantial frequency, and that these frequencies differ among populations. Supported by NSF (DEB-76-01903).

Evgen'ev, M.B. Institute of Developmental Biology, Academy of Sciences, Moscow, USSR. New method of cytological localization of genes in "virilis" group of *Drosophila*.

In spite of the fact that rather recently new ways of cytological localization of repeated genes have been developed (Jones and Robertson, 1970; Prensky et al., 1973) precise cytological location of different non-repeated structural genes is usually still performed by means of

deficiency mapping. Everyone who has ever tried to localize genes to individual bands in *Drosophila* knows how difficult this procedure is and how much time it needs. Having this consideration in mind, we worked out a combined genetic and cytological approach to determine precise position of genes in the "virilis" group of *Drosophila*.

This approach may be of interest since *D. virilis* and other species belonging to the group are now widely used in genetical and biochemical studies.

In a previous report (Evgen'ev, 1971) we showed that when crossing *D. virilis* by *D. texana* the fifth chromosome of F_1 hybrids exhibited poor conjugation in salivary glands in spite of quite similar band patterns in the chromosomes of parental species. Moreover, a small section of *D. texana* 5th chromosome transferred by back-crosses into the genome of *D. virilis* in heterozygous condition, exhibited quite similar modes of conjugation in the F_1 hybrid between the species and in the *D. virilis* genome. We conclude that frequency of conjugation is an autonomous character and solely depends on the intrinsic properties of chromosome loci. Based on this assumption it is possible to study crossing-over in the FB_1 generation ($\varnothing F_1$ (*virilis* x *texana*) x σ^7 *D. virilis*) cytologically. The place of crossing-over occurring in the distal end of the fifth chromosome of F_1 hybrids was easily observed by the change in the typical polytene chromosome pairing scheme when studying salivary gland chromosomes in FB_1 (Figure 1, 2).

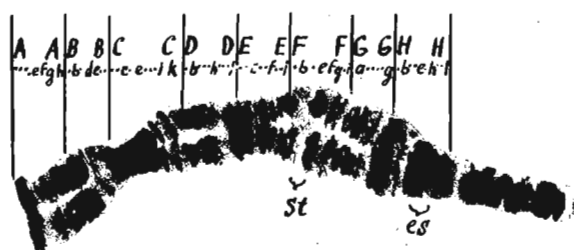


Figure 1. The typical picture of fifth chromosome conjugation in *D. virilis* x *D. texana* F_1 hybrids. (The position of the gene markers is indicated.) The fifth chromosome subdivided into sections (Hsu, 1952).



Figure 2. The cytologically observed crossover in FB_1 resulted from exchange in C_{ab} region. (The arrow indicates the place of exchange.)

Since we have two recessive genes scarlet (*st*) and eosinoid (*es*) eye colours in the distal region of the fifth chromosome, we can obtain the following two types of crossovers resulting from exchange in F_1 hybrid female:

$$\frac{st +}{st es} \quad \text{and} \quad \frac{+ es}{st es}$$

These phenotypically observed crossovers were crossed to *D. virilis*, and polytene chromosomes of the progeny from the cross (FB_2) were analysed (Figure 3a, b). A broad scale investigation showed that in the progeny of + *es*/*st es* crossovers, cytologically observed places of crossing-over were never located more proximally than the H_{e-h} locus. We use cytological map after Hsu (1952). Thus we concluded that the *es* gene is located in the region mentioned. A study of progeny of the reciprocal class of cross-overs (*st* +/*st es*) enables us to conclude that cytologically observed places of crossing-over were never located more distally than the F_{e-f} locus, i.e. *st* gene is localized in the F_{e-f} region. These mapping data coincide with induced aberration analysis. In our case the whole procedure took only about two months. However, it is necessary to emphasize that general utility of the above method is restricted

to cases in which genes are localized in the regions with poor conjugation of polytene chromosomes in interspecies hybrids but not within heterozygous species-specific inversions.



Figures 3a,b. The cytologically seen results of exchange observed in the progeny of + es/st es crossovers (FB_2). Both crossovers (a,b) resulted from a double crossing-over. Arrows indicate places of exchange.

References: Evgen'ev, M.B. 1971, T.A.G. 41:249-254; Fujii, S. 1942, Cytologia 12:435-459; Hsu, T.G. 1952, Univ. Texas Pub. 5204:35-72; Jones, K.W., F.W. Roberts 1970, Chromosoma 31:331-345; Prenskey, W., D.M. Steffensen, W.L. Hughes 1973, Proc. Nat. Acad. Sci. USA 70:1860-1864.

Ménsua, J.L. and M. Pérez. Autonomous University of Barcelona, Spain. Hybridization in nature of *D. melanogaster* and *D. simulans*.

In order to estimate the percentage of hybridization between *D. melanogaster* and *D. simulans* in nature, two collections were made in two different localities (different as regards the abundance of *Drosophila*), obtaining 810 and 35 females, respectively. The females collected

were immediately separated from the males, and then distributed in individual vials in the laboratory. The results were as follows:

1st Locality: Of 810 females collected, 784 yielded normal progenies (529 melanogaster and 255 simulans), 3 yielded only females and 1 yielded 25 females and 2 males.

The females of the three unisexual progenies were individually tested with males of the two species, and all were sterile. The dissection of these females gave no ovarioles at all. Presumably they were the result of an interspecific cross. The females of the latter progeny were also individually tested as before, and were 9 females with normal melanogaster progenies and 16 females that did not yield progenies. As before, these females did not have ovarioles. They were probably the result of a cross between a melanogaster female with males of the two species.

The 22 females that had not yielded progenies were crossed with melanogaster and simulans males and yielded 7 melanogaster progenies and 15 simulans progenies. The ratio of females not fertilized in nature was 1.29% for melanogaster and 5.55% for simulans.

2nd Locality: Of 36 females collected, 35 yielded normal melanogaster progenies and 1 yielded only females. These females were tested as before. The ratio of hybridization (the two samples pooled) is 6.1×10^{-3} between melanogaster females and simulans males. Crosses between simulans females and melanogaster males were not found. The percentage of that cross in the laboratory is lower (melanogaster females x simulans males = 81.94 ± 2.55 , simulans females x melanogaster males = $11.39 \pm 2.06^*$). It seems that the size of the two populations does not have an important influence on the rate of hybridization. We are taking monthly samples to verify these previous results.

*Unpublished data.

Milkman, R. and R. Zeitler. University of Iowa, Iowa City. Recombination frequencies in cage populations.

phosphate dehydrogenase, malate dehydrogenase, and alcohol dehydrogenase. From 20 to 138 progeny were genotyped from each of the 22 females that proved to be triple heterozygotes. The results (Table 1) indicate recombination frequencies of 14.8 and 6.9, where about 23.4

Culture vials were removed from two D.m. 25° cages of 4 years' standing. Virgins were collected and mated to males homozygous for the fast alleles (or for the slow alleles) at each of these second chromosome loci: α -glycerol phosphate dehydrogenase, malate dehydrogenase, and alcohol dehydrogenase. From 20 to 138 progeny were genotyped from each of the 22 females that proved to be triple heterozygotes. The results (Table 1) indicate recombination frequencies of 14.8 and 6.9, where about 23.4 and 8.9, respectively, were anticipated from Grell's localizations. There were 4 double crossovers (interference = 0.73) and so the number of doubles within either region is negligible. A simple Chi-square test ($df = 4$) indicates no differences between the cages ($0.25 < P < 0.50$). There is no evidence of heterogeneity among the progenies.

Table 1

Sample	N	R ₁	R ₂
Cage 1	653	99 (0.152)	53 (0.081)
Cage 2	844	122 (0.145)	50 (0.059)
Total	1497	221 (0.148)	103 (0.069)

Linkage disequilibrium values in laboratory populations are often compared to expected values, in attempts to discern selection. It is unsafe to use standard map distances in these calculations, even when generation times are known. For example, after 20 generations without selection, beginning with a trihybrid cross, the observed distances predict linkage disequilibrium (D) values of 0.054 and 0.121 for regions 1 and 2, respectively, while the distances of 23.4 and 8.9 map units predict 0.021 and 0.101. Moreover, the map distances used may be decisive in determining the apparent significance of the deviation from equilibrium. Similarly the half times predicted from the observed recombination frequencies are 9.0 and 19.7 generations, as opposed to 5.6 and 15.2.

Grell has mapped the loci at 2-17.8, 2-41.2, and 2-50.1, respectively but points out (personal communication) that these positions were established with reference to nearby points on the standard map, which appears to overstate distances, especially on 2L.

Supported by NIH Grant GM-18967.

Marcos, R. Autonomous University of Barcelona, Spain. Selection for inter-ocular bristles.

The response of this character to selection is being studied in order to discover and to place the genotype which governs it. The character is located in the area limited by the three ocelli. Their function is not known, but it is

possible that it is to keep the ocelli clean. The first work with these bristles (Wolsky, 1958, and Ménsua, 1966) show that this character has a mild response to selection.

We are working at 19°C in two populations (AR and BR) of the same origin. Each population was divided into five lines (two high, two low and one control). The response we have found is fairly strong:

Origin			19th Generation		
	males	females		males	females
AR	6.72 ± 0.02	7.02 ± 0.02	Low	0.60 ± 0.10	0.30 ± 0.10
			Control	7.06 ± 0.12	7.46 ± 0.10
			High	16.84 ± 0.38	16.89 ± 0.33
BR	6.90 ± 0.02	7.20 ± 0.02	Low	0.48 ± 0.10	0.48 ± 0.09
			Control	6.77 ± 0.10	6.78 ± 0.10
			High	16.02 ± 0.25	16.22 ± 0.23

In one high line (AR population), the mutant hairy appeared at the 6th generation and disappeared at the 11th generation. A mutant with a polychaetoid-like phenotype appeared in two high lines (BR population) and actually is in the population. The selection for this character is in progress.

Hegde, S.N. and N.B. Krishnamurthy.
University of Mysore, India. Mating
behaviour in the members of the
bipectinata complex of *Drosophila*.

Pattern of mating behaviour is one of the parameters which is a very useful tool in tracing evolutionary relationship of closely related species of animals. As a part of a long range project on certain members of the melanogaster species group, preliminary studies have been

made to analyse mating behaviour in members of the bipectinata complex. The stocks used are *D. bipectinata*, *malerkotliana*, *parabipectinata* and *pseudoananassae*. The observation of the courtship pattern was made directly and also with the help of a hand lens. A minimum of 10 pairs were observed for each stock.

In *bipectinata* the male initiates the courtship slowly by tapping the abdomen of the female or sometimes other parts by his front tarsi. In this process, he vibrates the wings, occasionally scissoring or by waving action. The nonreceptive female moves rapidly away from the male while the receptive female spreads her wings to accommodate the male, which immediately copulates with the female. Copulation lasts for ten to fifteen minutes, when the male rests his front legs on the sides of the abdomen of the female. If the copulation is incomplete, the male becomes more active and repeats the courtship activities. Sometimes, it displays circling movement around the female to copulate again. If the copulation is completed, the female kicks the male with her last pair of legs simultaneously fluttering her wings.

The pattern of mating behaviour in *D. malerkotliana*, *parabipectinata* and *pseudoananassae* is similar to the pattern described above for *bipectinata*. However, certain minor differences are noted. For instance, *malerkotliana* and *parabipectinata* exhibit circling movement even at their first courtship, while in *bipectinata* circling movement is exhibited only when copulation is incomplete. In *pseudoananassae* the males display semicircular movements around the female. *D. malerkotliana* licks the head of the female if she is non-receptive. Such a behaviour is not manifested by others. In general the behavioural pattern showing maximum similarities between the various species of the bipectinata complex suggests close relationship of these species.

The authors are grateful to Prof. M.R. Rajasekarasetty for his help and encouragement. This work is financially supported by U.G.C. Research Grants.

Mather, W.B. and M. Clyde. University of
Queensland, Brisbane, Australia. Inver-
sions in *D. kohkoa* from the Philippines.

During 1974 ten isolines of the relatively rare
nasuta complex species *D. kohkoa* were estab-
lished and examined for inversions in January
and July from Hidden Valley Springs (H.V.S.),
Luzon and in July from the central Philippines

island of Cebu (see table). Inversions B,D,E,H,I,P and Y have previously been recorded (Mather and Thongmeearkom 1972, DIS 48:40, and Mather, Thongmeearkom, Clyde and Lambert, DIS 51:86). J_3 , S_3 , T_3 and U_3 are new inversions (see figure). They are all proximal to the chromocentre on chromosome III. J_3 and T_3 are simple, whilst S_3 and U_3 are complex.

Inversion	H.V.S.		Cebu
	Jan. 1974 (1 isoline)	July 1974 (6 isolines)	July 1974 (3 isolines)
B		X	X
D		X	
E		X	X
H			X
I	X	X	X
P	X	X	X
Y		X	
J_3			X
S_3	X		
T_3		X	
U_3		X	

The material was collected and the isolines established by W.B.M. The chromosomes were prepared, analysed and photographed by M.C. (See figure on facing page.)

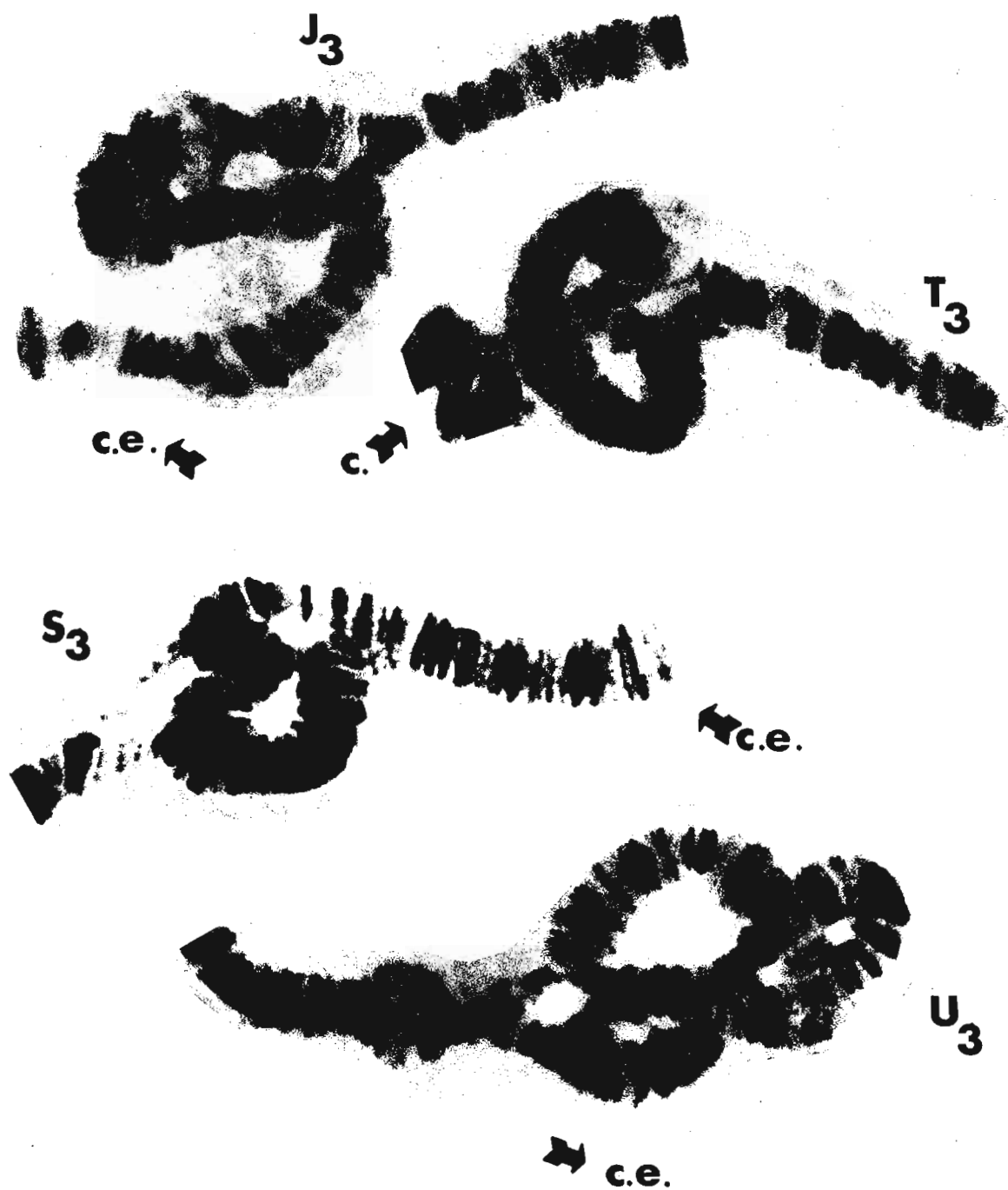


Figure of W.B. Mather and M. Clyde (see facing page).
 c. chromocentre c.e. chromocentre end

Aaron, C.S. and W.R. Lee. Louisiana State University, Baton Rouge, Louisiana. Rejection of ethyl methanesulfonate feeding solution by *D. melanogaster* adult males.

The principal method of treatment of adult *Drosophila* used by many workers is the feeding method of Lewis and Bacher (DIS, 43:193, 1968). We have recently made a quantitative examination of this procedure and found that serious errors in interpretation of EMS concentrations vs. genetic response can occur if consumption is

not monitored.

Our procedure for monitoring consumption is as follows: 50 ♂♂ (3-5 days old) are placed into each of several feeding vials containing various concentrations of feeding solution impregnated in tissue paper. The males are allowed to remain on the solutions for various times up to 24 hours (see Figures 1 and 2). The feeding solutions consist of 1% (1g/100ml) ^{14}C -sucrose (uniform radionuclide concentration within experiment) containing .025M, .0025M, and 0.0M ethyl methanesulfonate (EMS). Differences in counts per minute (CPM)/5 ♂♂ between experiments (Figures 1 and 2) can be attributed to differences in initial specific activity of the ^{14}C -sucrose solutions (136 $\mu\text{C}/\text{mM}$ for experiment 1, Figure 1 and 34 $\mu\text{C}/\text{mM}$ for experiment 2, Figure 2). The flies are sacrificed after the times shown in the figures. The males are anesthetized, transferred to sealable ampoules and flushed with dry nitrogen. The ampoule is plunged into liquid nitrogen and then quickly flame sealed while the flies are still frozen. The flies are stored at -20°C until prepared for liquid scintillation counting. We have found in separate experiments (Aaron, et al., DIS, this issue) that glass fiber filter does not result in differences in consumption and is superior to tissue paper as a support for the treatment solutions because 1) the flies are not trapped in folds as with tissue, 2) the glass filter can be acid cleaned, and 3) the glass does not react with most organic chemicals.

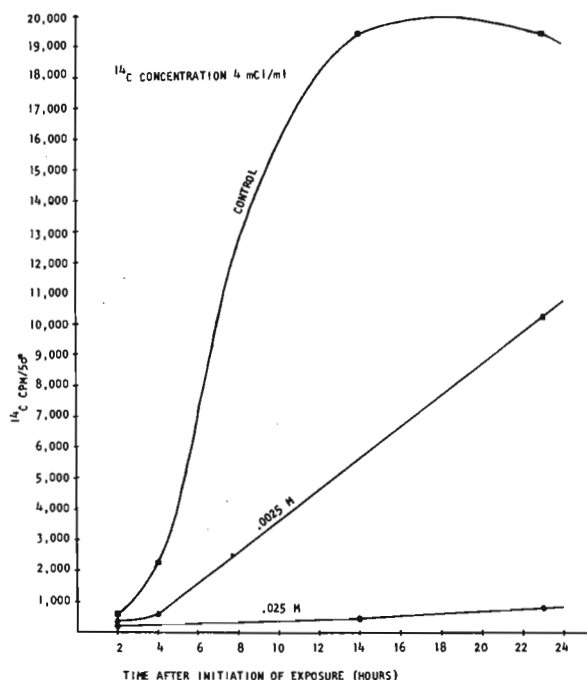


Figure 1. Effect of EMS Concentration of Feeding Behavior, Experiment #1.

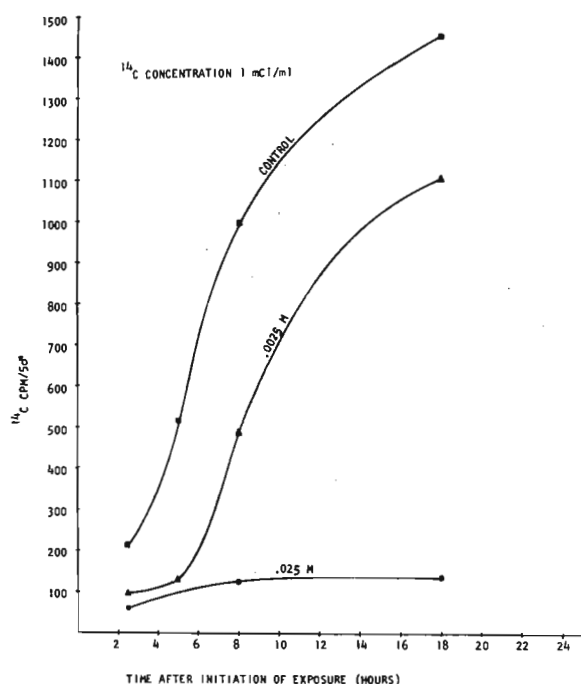


Figure 2. Effect of EMS Concentration of Feeding Behavior, Experiment #2.

Groups of 5 flies are selected at random from the frozen samples identified as to exposure conditions and washed to remove external radionuclide contamination. The washing procedure consists of swirling each group in H_2O , then in 0.1N HCl solution, and then absolute ethyl alcohol and finally with ethyl ether. Flies are briefly air dried and prepared for scintillation counting by a modification of the procedure of Mahin and Lofberg (1966). Each

group of washed flies is placed in a scintillation vial and 100 μ l of 70% perchloric acid and 100 μ l of 30% hydrogen peroxide are added to each vial. The vial is capped tightly and heated to 70° - 80°C and maintained at that temperature until the carcass is completely digested. The solution should be colorless. The contents of the vial is frozen on dry ice (Sega, Gary, Ph.D. dissertation) before opening so as to minimize loss of volatile radioactive breakdown products. Scintillation fluor (Insta-gel, Packard) is added, the cap is replaced and the mixture allowed to warm to room temperature. The vial is shaken well and placed in the scintillation counter for counting.

The results of two experiments are shown in Figures 1 and 2. The consumption behavior of the flies is markedly affected by the concentration of EMS. The apparent saturation kinetics of the accumulation of 14 C in the 24 hours of feeding can be understood if one considers the metabolic rate of 14 C-sucrose in the fly and the hydrolysis of this mutagen. The sucrose is metabolized into 14 CO₂ and eliminated by the normal mechanisms of the fly and does not accumulate indefinitely. The plateaus in these curves therefore reflect the equilibrium between the rate of elimination by metabolism of sucrose. EMS has a half life of just over 48 hours in aqueous solution at 25°C (Osterman-Golkar, et al., 1970). For example, flies placed on an initial concentration 0.025M are still being exposed to approximately 70 percent of that concentration 24 hours later. The delay in 14 C uptake in these figures at 2 and 4 hour feeding periods may be due to a delay in onset of feeding.

It is significant that the normally employed treatment concentrations are inhibitory with respect to feeding behavior. As measured by the 14 C counts, the amount imbibed per fly in 18 hours (Figure 1) and 23 hours (Figure 2) at 0.025M concentration is 1/10 the amount imbibed at 0.0025M concentration. Those employing the feeding procedures for administering mutagens should evaluate the consumption pattern over the concentration range of interest if meaningful exposure vs. response correlations are desired.

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Acknowledgement: Support for this study was provided by USPHS Grant ES00320-09 and ERDA Grant AT-(40-1)-3728.

Dudai, Y. California Institute of Technology, Pasadena, California. Molecular states of acetylcholinesterase from *Drosophila melanogaster*.

The cholinergic system, of which acetylcholinesterase (AChE) is an essential component, is believed to play a major role in the central nervous system of *Drosophila* (Pitman, 1971; Dewhurst et al., 1972). The enzyme is membrane bound in various vertebrate tissues and can be

solubilized in several molecular forms, which can be interconverted (Silman and Dudai, 1975). Studies were made in order to reveal molecular properties of AChE in *Drosophila*. All studies were carried out with C-S flies unless otherwise indicated.

It was found that most of the AChE in the homogenates of *Drosophila* is associated with particulate fractions when homogenization is performed in the absence of detergent, both in low and in high ionic strength (Table 1). The particulate-bound enzyme sediments on iso-

Table 1. Subcellular distribution of *Drosophila* AChE.

Fraction	Homogenization medium			
	Buffer + 0.5% Triton		Buffer	
	AChE activity μ m/min/ml	AChE activity %	AChE activity μ m/min/ml	AChE activity %
Total homogenate	0.178 \pm 0.025	100	0.150 \pm 0.013	84
1,500 g supernatant	0.180 \pm 0.029	101	0.105 \pm 0.015	59
100,000 g supernatant	0.149 \pm 0.022	84	0.045 \pm 0.005	25

Groups of 25 flies were frozen in liquid N₂ and immediately homogenized in 1 ml of 0.02 M tris-Cl, pH 7.4, in the presence or in the absence of 0.5% Triton X-100. The activity of the Triton homogenate was taken as 100%.

density centrifugation to the interfaces between 0.32 and 0.8 M sucrose, and between 0.8 and 1.0 M sucrose. These fractions contain both membrane vesicles and membrane sheets, as revealed by electron microscopy. The enzyme which remains in the supernatant of 100,000 g in the non-detergent homogenates has a sedimentation coefficient of $\sim 7S$ (Figure 1).

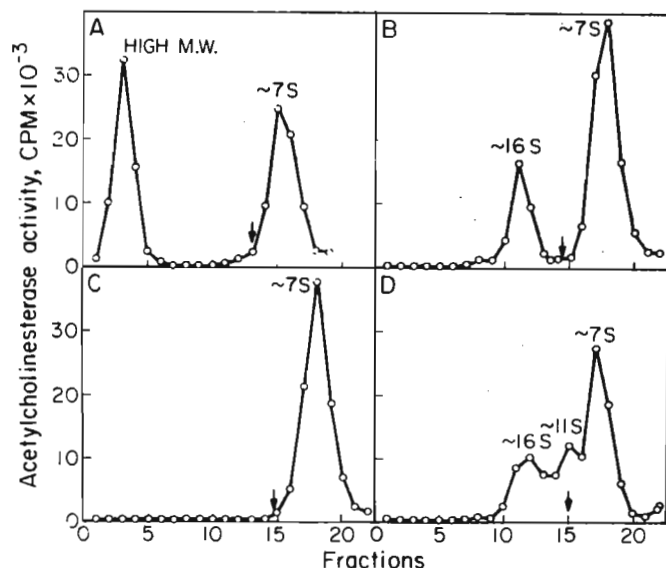


Figure 1. Sedimentation profile of *Drosophila* AChE on a 5-20% sucrose gradient, with a cushion of 60% sucrose at the bottom. The gradients were performed in 0.02 M Na-phosphate, pH 7.4 with 0.2% Triton (B-D) or in the absence of Triton (A).

A) Homogenization performed in the absence of Triton.

B) Homogenization performed in the presence of 0.5% Triton.

C) Recentrifugation of the 7S component isolated from (B) and dialysed against the buffer overnight at 4°C.

D) Recentrifugation of the 16S component isolated from (B) and dialysed as above. The arrows indicate the position of a catalase (11.4S) marker. The bottom of the gradient is on the left-hand side.

All the apparent activity in the homogenate can be solubilized by the addition of Triton X-100 (Table 1), as a mixture of $\sim 7S$ and $\sim 16S$ forms. The 7S form can be readily isolated from the gradient and further purified, whereas the 16S form is readily degraded during isolation into the 7S form, either directly or via an 11S form (Figure 1). The latter conversion occurs without a significant change in the overall activity of the preparation. The 11S component can be derived only from the 16S form and was not generated from samples of isolated 7S form. The 16S and the 11S forms were also not observed when the homogenate had been treated with trypsin (2% w/w tissue protein for 6 hr at 22°C) prior to application to the gradient.

On chromatography on Bio-Gel P-300 or Sepharose 6B molecular sieves, in the presence of Triton X-100, the 7S form displayed a Stokes radius similar to that of globular 11S eel AChE (MW = 300,000). This indicates that the *Drosophila* enzyme is not a typical globular protein. Thus taken together, the above observations indicate that *Drosophila* AChE can exist in several molecular forms, which can be interconverted, and at least part of which are asymmetrical, as in the case with vertebrate AChE (Silman and Dudai, 1975).

The molecular forms of the enzyme were also found to be present in *Df(3R)126d/+* flies, i.e., flies heterozygous for a deficiency covering the putative AChE structural gene locus (Kankel and Hall, in preparation). This observation is consistent with the assumption that the forms contain common polypeptides. The above flies were found to display a specific activity of 0.032 ± 0.007 μ moles acetylcholine split per min per mg protein, vs. a specific activity of 0.069 ± 0.012 in wild-type flies. The behavior of the AChE deficient flies was found to be normal, as judged by tests for phototaxis, geotaxis, flight, shock-sensitivity (Benzer, 1973), and learning (Quinn et al., 1974). Such flies were also found to have the same sensitivity toward the AChE inhibitor neostigmine (fed in sucrose solutions) as wild-type flies. Thus not all the AChE activity revealed in the fly homogenate appears crucial for normal function.

I thank Dr. S. Benzer for the hospitality of his laboratory. This work was supported by an EMBO long-term fellowship, and by NSF grant No. BMS 73-01972-A01 to Dr. S. Benzer.

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Gvozdev, V.A., T.I. Gerasimova,
Yu.M. Kovalev and E.V. Ananiev. Kurchatov
Institute of Atomic Energy, Moscow, USSR.
Cytogenetic structure of the 2D3-2F5 region
of the X-chromosome of *D. melanogaster*.

Some data concerning the fine genetic structure
of the 2D3-2F5 region of the X-chromosome were
reported by us earlier (1,2). We have saturated
this region with lethals of the total number of
101, a bulk of which were induced by ethyl meth-
anesulfonate. In several cases the mutants were
induced by nitrosomethylurea (NMU) and Co^{60}

irradiation (see Table). Selection of the lethals was performed by the use of the $\text{Df}(1)\text{Pg}d\text{-kz}$
with the 2D3-2F5 region deleted. The deficiency uncovers no more than 12 bands. The minimum
number of the deleted bands can be no more than eight: 2D5-6, 2E1-3, 2F1-3. Approximately
1% of the tested X-chromosomes carried a lethal in the 2D3-2F5 region. The mutation frequen-
cies per band or DNA content in this region and the whole X-chromosome were approximately
equal. Complementation analysis revealed no more than 12 functional units (9 units are shown
in Table) including the non-essential pn locus where no lethals were obtained. Three EMS-
induced lethals, 27, 72 and 90 (not included in Table) were found to be complementary to each

Table. Location and complementation map of lethals in
the 2D3-2F5 region of the X-chromosome.

Bands	2D3-6			2E1-3	2F1-5				
	< 0.05 >				< 0.031 >				
						< 0.01 >		< 0.006 >	
1	2	3	4	5	6	7	8	9	
Pgd	wap	pn	kz		< 0.026 >				
13	3*		4	69*	16	2	14	1*	
35	25		5	70*	18	8	15	46	
39	29		17	81	22	30	19	54	
45	33		21	107+	24	51	20	62	
50	37		32		28	59	23	75	
71	38		34		57	66	26	89	
93	40		36		58	76	31	98	
94	44		41		68	83	42		
100	46		47		78	85	43		
109	49		55		82	91	53		
111+	52		61		96	92	60		
	56		63		99	106+	84		
	73		65		102		103		
	74		67		108		105		
	79		77						
	86		80						
	87		88						
	95		97						
	110+		C115						
			RA34						
					----- Df(1)TEM-304 -----				
					----- Df(1)TEM-1 -----				
					----- Df(1)TEM-501 -----				
					----- Df(1)64c18 -----				

Note: lethals 1, 2, 58 and 109 were obtained by treatment of the wild type D32
($\text{Pg}d^A$) stock; lethals 77, 78, 84-89, 100 and 103 were originated from $\text{y Pg}d^{Bw}$,
the rest from the $\text{br Pg}d^{Apn}$ stock; asterisk and cross-marked lethals were induced
by NMU and by Co^{60} irradiation respectively; each solid line shows the segment
which is deleted in the chromosome indicated; lethals C115 and RA34 were obtained
from Dr. G. Lefevre.

other and to the lethals taken as representatives of the other complementation groups. The obtained results however may reflect the phenomenon of interallelic complementation. At any rate a satisfactory correspondence was demonstrated between the number of bands and complementation groups of lethal mutations. The order of complementation of the genetic map has been established by means of deletion mapping and recombination analysis. Deficiencies Df(1)TEM-1, Df(1)TEM-304, Df(1)TEM-501 were kindly supplied by Dr. J. Lim and Df(1)64c18 by Dr. G. Lefevre. The leftmost position of the region studied is occupied by the Pgd and wap loci. Because of altered wing phenotype in semilethals the vital locus to the right of Pgd was named wap (wings apart). Recombinational analysis shows that the wap locus is situated to the right of Pgd and separated from it by 0.05 map units. The 190, mentioned above, was also mapped in the 2D3-6 region. At least 6 complementation groups are apparent in the 2E1-2F5 region which includes 7-10 bands. The kz locus was localized earlier in the same region. Lethals were obtained in the kz locus. It was demonstrated that the lethals 47, 55, 63, 77 from the group N4 expressed kz phenotype in compound with kz. Two lethals, C115 and RA34 which appear to be allelic with kz (personal communication from Dr. G. Lefevre) were non-complementary to the 147. Deletion mapping localizes the 172 in the immediate vicinity of the kz locus and the 127 near the N5 locus. The precise order of these lethals on the genetic map has not been determined. Recombinational analysis shows that the locus N9 (11 and 175) is separated from the locus N8 (115 and 184) by 0.006 map units and is located to the right of the locus N8. The rest of the loci were located to the left of the N8 locus. Cytological analysis shows that the 2E1.2 doublet is preserved in the Df(1)TEM-501 which is complementary with the lethals from the loci N4. Therefore the kz locus may be attributed to the 2E1.2 doublet.

The relative DNA content along the 1A1-3C8 region of the X-chromosome was determined by densitometry of Feulgen-stained chromosomes (3). The 2EF and 3A3-3B4 regions contained 7.3% and 8.4% of DNA of the 1A1-3C8 region respectively. The amount of crossing over in the 2EF region (no more than 0.05 map units) calculated per DNA content was shown to be approximately ten times decreased as compared to the 3A3-3B4 region studied by Judd et al. (4). The average frequency of crossing over between the adjacent loci (complementation groups) in the 2EF region (0.01%) was decreased by 6-8 times when compared to the 3A3-3B4 region (4) and an average amount of crossing over (0.06-0.08%) that can be attributed to an "average" band in the X-chromosome (5).

References: 1) Gvozdev, V.A. et al. 1973, DIS 50:93; 2) Gvozdev, V.A., S.A. Gostimsky et al. 1975, Genetika (Russ.) 11:73; 3) Ananiev, E.V. and V.E. Barsky 1975, Mol. Biol. (Russ.) 9:752; 4) Judd, B.H. 1972, Genetics 71:139; 5) Lefevre, G. 1971, Genetics 67:497.

Harris, W. California Institute of Technology, Pasadena, California.
The tip of 3R onto the tip of X.

The following scheme was undertaken in order to translocate the Acph-1⁺ gene (normally located between ca and bv on 3R) to the tip of the X-chromosome. Such a chromosome makes it possible to generate X-linked behavioral mutant

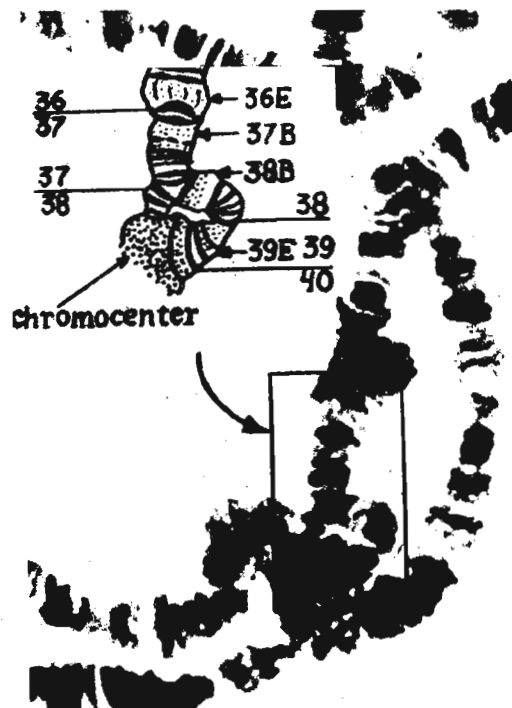
mosaics (using pal, mit, or somatic crossing-over) in which the internal tissues, especially the nervous system, can be scored by histochemical techniques.

FR1 y⁺, y cv v f (a normal sequence X with the basal heterochromatin deleted and Y material translocated to the tip), and T(Y;A)H163 (breakpoint 98B) were used to construct a FR1 y⁺, y cv/FR1 y⁺.y cv/o; ca bv/T(Y;A)H163 stock. Approximately 3000 such virgin females were X-rayed at 3000r and mated to y;ca bv males for 4 days. Only 1 of approximately 15,000 progeny was a y cv ca⁺ bv⁺ phenotype male. This male must have lost the y⁺ from the T(Y;A)H163 Y^S y⁺ 98B-3R tip yet it must have inherited the 98B-3R tip itself which contains ca⁺ bv⁺ (and in between Acph-1⁺). It must also have lost y⁺ from the tip of Fr-1 y⁺, y cv. The most likely explanation for this exceptional male is a translocation of the 98B-3R tip from the T(y;A) to the tip of X. Examination of the salivary chromosomes and genetic mapping of the ca⁺ bv⁺ markers showed this explanation to be correct.

Oocytes rather than sperm were X-rayed so that the meiotic process in the egg would sort out the desired translocation from the unwanted y⁺ containing Y fragment. Thus we now have an entire normal sequence X-chromosome, with the Acph-1⁺ gene translocated to the distal tip.

Thanks go to B. Butler, J. Hall, D. Lindsley, G. Lefevre and E. Lewis, who helped with the work, and to the Gordon Ross Medical Foundation and NSF Grant GB 27228 (to S. Benzer) which supported it.

Khesin, R.B., B.A. Leibovitch and V.N. Bashkirov. I.V. Kurchatov Institute of Atomic Energy, Moscow D-182, USSR. More precise cytogenetic localization of the gene pr.



Lindsley et al. (1) obtained a set of translocations between Y chromosome and autosomes by combinations with which one could form deficiencies and duplications for most of the autosomal regions. Using these translocations, we have investigated the effect of the deficiency of histone structural genes on the X chromosome template activity and on the V-type position effect (2,3). It is known from in situ hybridization of sea urchin histone mRNA with *Drosophila* polytene chromosomes, that histone genes were localized in the region 39DEF (4,5). The obtained deficiencies were checked cytologically and genetically by the expression of pr mutations (2-54,5) in heterozygotes pr/Df. The gene pr was localized earlier only approximately in the region 36B-40 (6). Deficiencies 38B-40, 38B-39C, 38C-39C were obtained by crossing the stocks of Lindsley et al. (1) $\phi P57 \times \delta B190$, $\phi P57 \times \delta L138$, $\phi B110 \times \delta L138$ respectively. Cytological analysis confirmed the existence of deficiencies in these regions (see Figure 1 - deficiency 38B-40). Mutation pr was expressed in heterozygotes only against the deficiencies 38B-40 and 38B-39C but not 38C-39C. It leads to a conclusion that the gene pr localizes in the region 38B-39C.

References: (1) Lindsley, D.L. et al. 1972, *Genetics* 71:157; *DIS Suppl.* 47 (1972); (2) Khesin, R.B. 1975, *Genetika* (USSR), 11(8):154; (3) Khesin, R.B. and B.A. Leibovitch 1976, *Molec. Biol. (USSR)* 10:1; (4) Pardue, M.L. et al. 1972, *J. Cell Biol.* 55:199a; (5) Pardue, M.L. 1975, *Genetics* 79:Suppl. 159; (6) Lindsley, D.L. and E.H. Grell 1968, *Genetic Var. in Dros.*

Mengual, V. Autonomous University of Barcelona, Spain. High frequency of eye colour mutants in a natural population of *D. melanogaster*.

A sample of *D. melanogaster* was captured in April 1974. The place was a farm room near Figueras (Gerona). The room had many wine and vinegar barrels. 20 females of *D.m.* were collected for the purpose of searching for eye colour mutants. The F_2 of eleven pairs from the

F_1 generation of each wild female was analyzed.

Ten eye colour mutants were detected in eight different females. Two of them turned out to be heterozygotic in two different mutations. One of these mutants could not be selected because of the sterility of the individuals. They showed a Plum-like colour as well as changes in the shape of the wings and in the colour of the body.

One of the nine remaining mutants was located in the second chromosome, and turned out to be a new allele of the cinnabar gene (cn, 2-57,5). Another of the mutants, not located as yet, was of a reddish-brown colour, darker than the wild type, different from the sepia colour, and with pigmented ocelli.

The seven remaining ones were found in the third chromosome. Phenotypically, four of the seven mutants showed the red eyes more brilliant than the wild type, having colourless ocelli. Two of them were alleles.

Another one of these seven mutants appeared to be a sepia colour, although it turned out not to be an allele of se (3-26,0), and had pigmented ocelli. The two remaining ones, very much alike, had eyes of a more uniform and pinkish shade than the wild type, although becoming darker with age. One of these had pigmented ocelli, while the other did not.

With respect to eye colour, we can see a great genetic variability in the sample (30% single heterozygotic and 10% double heterozygotic females). This variability can be related to the special habitat where the capture was made. The study of these mutants is in progress.

Russell, J.S., B.L. Ward and W.B. Heed.
University of Arizona, Tucson, Arizona,
and Wayne State University, Detroit,
Michigan. Sperm storage and hybridiza-
tion in *D. nanoptera* and related species.

Ward and Heed (1970) attempted hybridization of *D. nanoptera* with related species, *D. pachea*, *D. acanthoptera*, and the undescribed species W. All attempts failed because of sexual isolation.

At a later date Herman Spieth observed that the mating behavior of *nanoptera* and species W were quite similar and that *nanoptera* males were very aggressive. He suggested a second attempt at hybridization with these two species. This is of interest because *nanoptera* females normally store sperm in the ventral receptacle and rarely in a spermatheca (4 out of 70 dissections) while species W females always store sperm in the spermathecae but not the ventral receptacle (15 dissections). These observations suggest the possibility of an isolating mechanism. The species are homosequential and sympatric in southern Mexico.

A total of 64 "pair matings" and 20 mass matings were made with species W females x *nanoptera* males. The parents of the mass matings averaged 50 females and 75 males each. In the reciprocal cross a total of 20 "pair matings" and 16 mass matings were made. The parents of the mass matings averaged 30 females and 50 males each. Different combinations of young and old individuals were attempted. In the majority of "pair matings" an extra female was added which belonged to the same species as the male in hope of stimulating the male sufficiently to outcross. All tests were run from 10 to 14 days before the females were examined for sperm. 100 females in the mass and pair matings combined in each cross were dissected and no sperm was present either in the ventral receptacle or spermathecae of either species. Many eggs but no larvae were present in any of the mass matings.

Virgin *pachea* females were added to 4 of the above mass matings having species W males and 3 having *nanoptera* males. *D. pachea* males were added to 4 of the above mass matings having species W females and 1 having *nanoptera* females. Dissections were made 6-10 days later. Dead sperm of species W was found in both spermathecae of *pachea* in 14 out of 21 dissections. Dead sperm of *nanoptera* was found in *pachea* spermathecae in 3 out of 15 dissections. No sperm was present in species W females x *pachea* males in 24 dissections.

These data show that *pachea* females are not sexually isolated from the other two species probably because *pachea* is geographically isolated in the Sonoran Desert of northwestern Mexico. The fact that the sperm entered the spermathecae and not the ventral receptacle in the crosses with *nanoptera* males is interesting even though the lumen of *pachea* ventral receptacle is .05mm width compared to .1mm in *nanoptera*. This would suggest a mechanical problem except that the lumen of species W is the same as *nanoptera* and it is also not used for sperm storage.

Reference: Ward, B.L. and W.B. Heed 1970, *J. Hered.* 61:248-258.

Dow, M.A. University of Edinburgh,
Scotland. Density and the mating
success of yellow mutant males.

The mating success of yellow mutant males with wild-type females is low, but is increased substantially by an increase in the number of males per female (Dow, M.A. 1975, *Nature* 255:172). I have examined whether this increase is due to

an increase in the density of flies.

The procedure followed is that in Dow (loc. cit.). Density was varied by altering the amount of food placed in the mating vials and by altering the number of pairs of flies placed in the vials. As no differences were found between these two methods, the combined results are reported here.

Density (flies/cc)	0.25	0.30	0.49	0.74	0.99	1.23	1.33
Sample size	249	74	68	75	100	75	50
Mating success (%)	0.8	1.35	7.35	5.33	4.00	12.0	4.00

As shown in the table, mating success does increase with increasing density. However, a 1:3 sex-ratio (density = 0.49) increases mating success to 21.3% (Dow, M.A., loc. cit.) and only 7.4% can be attributed to a density effect. Therefore, density cannot entirely explain the mating facilitation that occurs at a 3 male:1 female sex-ratio with yellow mutant males and wild-type females.

Hegde, S.N. and N.B. Krishnamurthy.

University of Mysore, India. Studies on the isozyme variability in twelve members of melanogaster species group of *Drosophila*.

Alkaline phosphatase (APH), acid phosphatase (ACPH) and esterases (EST) have been analysed in twelve species of melanogaster species group of *Drosophila*. Of these twelve, six species - *D. malerkotliana*, *bipectinata*, *parabipectinata*, *pseudoananassae*, *ananassae* and *nasoetes* - belong

to *ananassae* subgroup; three - namely, *mysorensis*, *jambulina* and *anomelani* - belong to *montium* subgroup; one - *eugracilis* - to *eugracilis* subgroup; one - *rajasekari* - to *suzuki* subgroup and one species - *melanogaster* - to *melanogaster* subgroup.

A comparative study of the zymograms of these species shows that alkaline phosphatase (APH) exhibits three zones of activity, A, B and C. Zones A and B have two variant phenotypes each, while zone C has four. Among the species under study, *malerkotliana*, *ananassae* and *rajasekari* show highest polymorphism at zone C, whereas all other species except *bipectinata* are less polymorphic to all zones. *D. bipectinata* is monomorphic in zone B represented by a single band while A and C are silent zones.

For acid phosphatase (ACPH) four zones of activity have been recognised, zone A and B have two variant phenotypes, zone C has four and zone D has three. Among the twelve species analysed, *malerkotliana* and *ananassae* are highly polymorphic at zone C and D, while *bipectinata* is monomorphic with one band in zone B. All three species of *montium* subgroup have only A and B zones of activity and they show greater resemblance in their zymogram patterns than any other species.

Esterases (EST) show six zones of activity, A, B, C, D, E and F. In all species studied the zones A and B are monomorphic with one band each. Zone C has three variants, zone D has two and zone E and F, each has three. The members of the *bipectinata* complex show no activity in the F zone, while in *mysorensis* and *jambulina*, both E and F zones are silent. But other species show activity in all the zones.

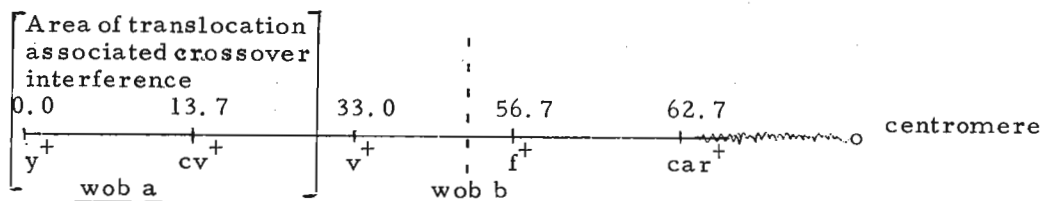
The present data reveals that the members of the same subgroup show similarity in their zymogram patterns. Thus among six species of the *ananassae* subgroup, four species, namely, *malerkotliana*, *bipectinata*, *parabipectinata* and *pseudoananassae*, are more closely related than *ananassae* and *nasoetes*. The three members of *montium* subgroup show close similarity in the zymogram pattern particularly for ACPH. The other three species do not fit in with any of these groups in their phenotypes. The evolutionary implications are being studied.

The authors are grateful to Prof. M.R. Rajasekarasetty for his help and encouragement. This work is financially supported by U.G.C. Research Grants.

Schmidt-Nielsen, B. and L.M. Hall.
Massachusetts Institute of Technology,
Cambridge, Mass., USA. An abnormal
walking mutant associated with a
translocation.

Adult wobbly (wob) flies cannot coordinate the proper sequence of leg movements for normal walking and frequently legs on one side of their bodies become entangled (Grigliatti, et al., 1973). The mutation was originally isolated in a translocation bearing stock and we wanted to determine if it could be separated from the

associated translocation. Based on the results of our experiments, we developed the model presented below for the wobbly chromosome that suggests the wobbly phenotype is due to two loci. One locus maps as a point mutation between *v* and *f* and the second is inseparable from the translocation.



In order to test this model and to quantitate the differences in locomotor ability among the various progeny classes, we tested the progeny from the mapping cross

$y\ cv\ v\ f\ car/T(X \rightarrow 2R \rightarrow 3R \rightarrow X)wob\ \text{♀♀} \times y\ cv\ v\ f\ car/Y\ \text{♂♂}$

in a countercurrent distribution device (Benzer, 1967). All progeny from the mapping cross were aged 12-48 hours after eclosion and then run towards light and against gravity in a six place countercurrent device. A twenty second run was allowed in each tube and at the end of the run, the phenotypes of the flies in each tube were scored. The mean number of times flies of a given phenotype ran toward the light was calculated by assigning a value of from 0 to 5 depending on where flies were at the end of the run. Thus, a value of five was assigned to flies in the last tube indicating they had run five out of five times. To eliminate differences in running ability due to the mapping markers, females (which are all heterozygous for wobbly) were used as internal controls and the final score was calculated as the difference between the male and female scores for a given phenotype. The results are summarized in the table:

Phenotype	Average position in countercurrent device (number of flies scored in parentheses)		Net score $\delta - \text{♀}$	Observed recombination frequency (expected)
	δ	♀		
y cv v f car	4.37 (284)	3.92 (303)	+0.45	
+ + + + +	0.55 (359)	4.47 (346)	-3.92	
+ + v f car	2.29 (51)	4.29 (59)	-2.00	8.5
y cv + + +	2.05 (37)	4.26 (38)	-2.21	(19.3)
+ + + f car	1.73 (119)	4.47 (141)	-2.74	23.4
y cv v + +	2.52 (124)	4.13 (148)	-1.61	(23.7)
+ + + + car	0.60 (30)	4.76 (41)	-4.16	6.2
y cv v f +	4.15 (34)	4.14 (36)	+0.01	(6.0)
Totals	2.22 (1038)	4.26 (1112)	-2.04	

There was no crossing over between y and cv, and crossing over was reduced between cv and v. Males which would not be expected to carry either of the proposed wobbly loci (i.e., y cv v f car and y cv v f +) ran somewhat better than the corresponding females. Males which carry both wobbly loci ran very poorly as reflected by the scores of -3.92 for the + + + + flies and -4.16 for the + + + + car flies. Flies which were presumably either wob a alone (+ + v f car) or wob b alone (y cv + + +) had intermediate locomotor ability. When all of these flies were observed directly, we could distinguish three phenotypic classes of abnormal locomotor ability. The full wobbly phenotype was characterized by uncoordinated walking in which the legs frequently crossed. The wobbly a phenotype exhibited only slightly uncoordinated walking. The wobbly b phenotype did not show lack of coordination, but the flies were weak, walked slowly, and climbed poorly.

The wobbly a phenotype was not separated from the region in which the translocation caused crossover interference, but the wobbly b phenotype seemed to map between v and f. The running scores of the y cv v + + and + + + f car males should depend on the relative proportions of wob b and wob b⁺ in each. Since the ratio of the two genotypes depends on the genetic location of wob b, a calculation can be made to determine its map position. The y cv v + + males with an average score of -1.61 should be a mixture of y cv v wob b + + with a score of -2.21 (like y cv + + +) and y cv v wob b⁺ + + with a score of +0.01 (like y cv v f +). This gives a proportion of y cv v wob b + + flies of $(+0.01 - 1.61) / (+0.01 - 2.21) = 0.73$. That is, wob b is more closely linked to f than v. The analogous calculation can be made for the + + + f car class of flies. The proportion of wob b⁺ $[(-4.16 - (-2.74)) / (-4.16 - (-2.00))] = 0.66$ again suggests that wob b is more closely linked to f than v. These two values are independent and in excellent agreement. On the basis of this model, the wob b locus is 0.7 of the distance between v and f or in the vicinity of 50 on the standard map. This procedure is of general use in mapping locomotor mutants whose phenotype might otherwise be difficult to score.

References: Benzer, S. 1967, Proc. Nat. Acad. Sci. U.S. 58:1112; Grigliatti, T.A., L. Hall, R. Rosenbluth and D.T. Suzuki 1973, Molec. Gen. Genet. 120:107.

This work was supported by NIH grant GM21106 to L.M.H.

Kimura, M.T. and E. Momma. Hokkaido University, Sapporo, Japan. Drosophilid species breeding on decayed leaves.

Drosophilid species which utilize decayed leaves as their breeding sites were examined on herbaceous plants in a field in Hokkaido. The substrate suspected to contain larvae were taken into the laboratory. The culture of the larvae

in decayed leaves was carried out by placing substrate in a milk bottle (180ml) with tissue paper at the bottom.

Decayed leaves of eleven plant species were observed as their breeding sites. Three species of *Scaptomyza* and nine species of *Drosophila* were reared from the decayed leaves as shown in the table. They are commonly found by sweeping on undergrowth plants in forests in Hokkaido. Of the 12 species, *D. auraria*, *D. nigromaculata*, *D. brachynephros* and *D. testacea* are collected best by traps using fermenting fruits.

Numbers of drosophilid flies reared from decayed leaves.

Plant species	A	B	C	D	E	F	G	H	I	J	K	Total
<i>Scaptomyza pallida</i>	44	17	-	70	45	82	328	15	-	-	-	601
<i>S. consimilis</i>	-	-	-	1	-	-	6	2	-	-	-	9
<i>S. okadai</i>	-	-	-	1	2	1	1	-	-	-	-	5
<i>Drosophila</i> (<i>Lordiphosa</i>)												
<i>collinella</i>	2	-	7	-	-	3	8	11	-	15	1	47
<i>D. (Sophophora) mommai</i>	-	-	3	-	-	-	1	-	-	-	-	4
<i>D. (S.) nipponica</i>	-	-	-	8	34	28	10	11	-	-	2	93
<i>D. (S.) magnipectinata</i>	-	-	-	1	-	1	11	6	6	41	-	66
<i>D. (S.) auraria</i>	-	-	-	-	-	-	34	-	-	-	-	34
<i>D. (Drosophila)</i>												
<i>nigromaculata</i>	24	-	-	60	5	93	111	29	1	-	1	324
<i>D. (D.) brachynephros</i>	-	-	-	-	-	5	-	-	-	-	-	5
<i>D. (D.) testacea</i>	1	-	-	8	-	12	32	20	-	-	-	73
<i>D. (D.) tenuicauda</i>	-	-	17	-	-	-	12	-	4	42	-	75

A - *Petasites japonicus*; B - *Trifolium repens*; C - *Cryptotaenia japonica*; D - *Heracleum dulce*; E - *Osmorhiza aristata*; F - *Anthriscus sylvestris*; G - *Anemone flaccida*; H - *Lilium cordatum glehni*; I - *Smilacina japonica*; J - *Trillium* sp.; K - *Maianthemum dilatatum*.

Nissani, M. University of Wisconsin, Madison. Some observations on *D. melanogaster* gynandromorphs.

Gynandromorphs of the constitution $y\ sn^3\ v\ un^4/In(1)w^{VC};bw/bw$ were obtained and the intensities of pigmentation of their ocelli were recorded. Unexpectedly, it was noted that in all gynandromorphs all 3 ocelli had approximately the same

intensity of pigmentation. This effect was most striking in all 22 bilateral head mosaics which had one $bw;v^+$ eye and one colorless eye since all these 22 gynandromorphs had 3 colorless ocelli. It was concluded therefore that when kynurenine is not produced in the fat body or the Malpighian tubes (Beadle, 1937), wild type ocelli lack pigmentation. The same effect was also observed in 4 bw^+ gynandromorphs and hence it is not due to the presence of the bw mutation. In order to rule out the possibility that all 3 ocelli originate from the same site on the blastoderm, 10 bilateral head mosaics of the constitution $y\ sn\ oc/In(1)w^{VC}$ and 7 bilateral head mosaics of the constitution $y\ w/In(1)w^{VC}$ were examined. As expected, in all 17 cases one lateral ocellus showed the mutant characteristic (white or ocelliless), and the other lateral ocellus was wild type. This fact also indicates that the expression of oc is autonomous.

In a separate experiment, gynandromorphs of various genetic constitutions were tested for sexual attractiveness by placing them in a mating chamber with 2 wild type males. Two gynandromorphs with female external genitalia and which exhibited normal male sexual behavior (courtship of females and violent wing flicking when courted) copulated after a minimal courtship period of 45 minutes. This fact lends indirect support to the suggestion that forced matings occur in *Drosophila* (Dow and von Schilcher, 1975).

References: Beadle, G.W. 1937, *Genetics* 22:587; Dow, M.A. and von Schilcher, F. 1975, *DIS* 51:71.

Trippa, G., G.A. Danieli*, R. Costa* and R. Scozzari. Università di Roma and *Università di Padova, Italia. A new allele at the Pgm locus in *D. melanogaster*.

In the course of a study carried out to detect possible linkage disequilibrium at the Pgm and Est⁶ loci in natural populations of *Drosophila melanogaster*, a new Pgm allele, Pgm^G, has been found with a frequency of 0.0004 (Figure 1 and G. Trippa et al., New Mutants, this issue).

From this mutant pure stock has been obtained since all males collected in the wild were crossed with homozygous females before being analysed by electrophoresis. In addition to the two common alleles, four new Pgm variant alleles were reported in 1972 by Trippa et al. (DIS 49:35). At

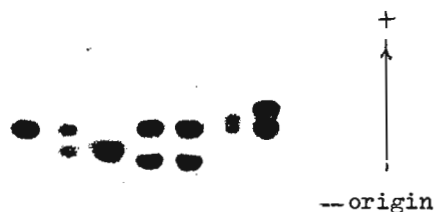


Figure 1. The PGM pattern of heterozygote for the new Pgm^G variant allele and the Pgm^A common allele in comparison with the most significant ones.

A AB B AE AE AG AC

present seven allelic variants of the Pgm locus are known. In view of possible identification of new electrophoretic mutants, we propose a different classification of the Pgm alleles, based on the relative electrophoretic mobility of the allozymes. The mobility of the most common allele is 1.00 (see below).

Known alleles at Pgm locus

Pgm ^{0.55}	: phosphoglucomutase ^{0.55}	(formerly Pgm ^E)
Pgm ^{0.70}	: phosphoglucomutase ^{0.70}	(" Pgm ^B)
Pgm ^{0.85}	: phosphoglucomutase ^{0.85}	(" Pgm ^F)
Pgm ^{1.00}	: phosphoglucomutase ^{1.00}	(" Pgm ^A)
Pgm ^{1.10}	: phosphoglucomutase ^{1.10}	(" Pgm ^G)
Pgm ^{1.20}	: phosphoglucomutase ^{1.20}	(" Pgm ^C)
Pgm ^{1.50}	: phosphoglucomutase ^{1.50}	(" Pgm ^D)

Miller, D.D. University of Nebraska, Lincoln. New Inversions in the X- and C-chromosomes of *D. athabasca*.

Nine inversions, listed below, have been found in *D. athabasca* since the report of Miller and Voelker, 1972 (Journ. Hered. 63:3-10), so the total number known to cause variation in this species is now 82. One inversion, X-short IV,

was found in a strain of "western-northern" *athabasca*; all others were in strains of the "eastern A" semispecies. The following kindly provided strains in which these inversions were found: Dr. George Carmody, Miss Cynthia Hodges, Dr. John Jaenicke, Dr. Max Levitan, Dr. Rollin Richmond, Dr. Eliot Spiess and Dr. Robert Voelker.

X-long. MXIV: 1-3'11-13'6-10'15 14'4 4'16--- (superimposed on MI-MII sequence, included in MI but overlapping MII; Orland, Maine). MXV: 1-3'11-15'10-4'16--- (superimposed on MI-MII, included in MI, independent of MII; Rogers Mt., Virginia).

X-short. IV: 1-5 6d'9d 8 7 6p'9p 10--- (based on Sequence I, included in II, proximal break close to that of II; Orland, Maine). MVII: 1 2d'25 24p'18-24d'17-12'6-11'5-2p'26 (superimposed on MI-MII, included in MI but independent of MII; Dedham and Old Town, Maine; Netcong, New Jersey). MVIII: 1 2d'25 24p'10 11'5-2p'26d'23p 24d'9-6'19-12'20-23d'26p (superimposed on MIII-MIV sequence, overlapping MIII but independent of MIV; Skokie, Illinois). MIX: 1 2d'25-23p'26d'2p-5'11-7'20-12'6'21-23d'26p (superimposed on MIII sequence, but not MIII-MIV, included in MIII but including MIV; Acadia National Park, Maine; Gatineau Park, Quebec). MX: 1 2d'19p 20-25'19d 18-12'6'11'5'2p'26 (superimposed on MI-MII, included in MI, independent of MII, overlapping new inversion MVII; Dedham, Maine).

C-chromosome. MXXXI: 1-3'8 7'14'5 4'9-13'6'15--- (superimposed on MII sequence, included in MII, overlapping MV; Orland, Maine). MXXXII: 1-5'14 13p'30d'25-27'29'24--- (superimposed on MXX sequence, included in MXX, overlapping MXXII; Rogers Mt., Virginia).

Trippa, G., A. Loverre and A. Micheli.
Istituto di Genetica, Città Universitaria,
Rome, Italy. Genetic localization of a
recessive spontaneous mutation *fs(2)TLM*
affecting female fertility in *Drosophila*
melanogaster.

During the analysis of a sample of second chromo-
somes, extracted from a natural population of
Drosophila melanogaster from Ranna (Sicily,
Italy), a new SD factor, named *SD^{Ra}* (= *SD^{Ranna}*),
associated with the complete sterility of *SD^{Ra}/*
SD^{Ra} females, was found. These homozygous
females are externally normal; however they do
not lay eggs.

In order to ascertain whether the *SD^{Ra}* gene itself, or one or more genes, were involved
in female sterility, we undertook the genetic localization of the gene(s) on the second chromo-

Table 1. Testing the fertility of *F₁* *SD^{Ra}/*
different crossover females obtained by the
cross *Sp Bl L Pin/SD^{Ra}* ♀♀ with *SD^{Ra}/SM5* ♂♂.

Type of Crossover	Fertile	Sterile
Sp	-	4
Bl L Pin	14	-
Sp Bl	-	21
L Pin	21	1*
Sp Bl L	28	31
Pin	35 + 37**	41 + 31**
Sp L Pin	4	-
Bl	-	12
Sp Bl Pin	1	3
L	5 + 16**	1 + 17**
Bl L	4	7

* A possible double crossover in the L-Pin region.

** Data obtained by a successive count of the same
cross; the individuals were classified only for
the Lobe or Pin phenotype.

sosome. Virgin females of *Sp Bl L Pin/*
SM5 constitution were crossed with *SD^{Ra}/*
SM5 males. Heterozygous *F₁* *Sp Bl L Pin/*
SD^{Ra} females were backcrossed with *SD^{Ra}/*
SM5 males. Females heterozygous for
one crossover and the *SD^{Ra}* chromosome
have been analysed for the presence of
dominant markers and classified for the
presence of the sterility factor(s) on
the basis of the fertility. The data
shown in Table 1 indicate the presence
of a gene, named *fs(2)TLM* (New Mutants,
this issue) responsible for the female
sterility between the dominant markers
L (2:72.0) and Pin (2:107.3). A more
precise localization of the gene is pos-
sible by using the data of crossovers in
that region. The percentages of recom-
bination between Lobe and Pin give the
distance of the gene equivalent to 17.7
(129:257 = x:35.3) map units from Lobe
and to 17.6 (128:257 = X:35.3) map units
from Pin. Therefore the locus of *fs(2)*
TLM gene is at 89.7 ± 1 on the genetic
map of the second chromosome.

Dow, M.A. University of Edinburgh,
Scotland. Mating interference in
wild-type *D. melanogaster*.

I have examined the mating speed and duration
of copulation of a recently collected wild-type
strain of *D. melanogaster* at two sex-ratios, 1:1
and 1:3 (female:male) to determine whether
mating interference occurs.

Virgin females were aged singly and males were aged singly or in groups of three in vials
(20 x 76 mm) with 5 ml of propionic acid medium for 3 days. Males were introduced into the
female's vials without anaesthetization and observed for 40 min. Three replicates were run

Source of Variation	df	MS	F
Between Sex-Ratios ¹	1	71.875	4.93
	1	0.016	0.07
Among Replications,	4	14.594	1.19
Within Sex-Ratios	4	0.224	1.98
Within Replications	216	12.314	
	264	0.115	

¹upper row - duration of copulation
lower row - mating speed

(N = 45 for mating speed, N = 37 for duration of
copulation). Mating speed was normalized by
logarithmic transformation and both measures
were analyzed by nested analysis of variance.

The F-ratios shown in the table indicate
that sex-ratio had no effect on either measure.
For the 1:1 ratio, the mean mating speed was
 1.05 ± 0.03 log min, the mean duration of copu-
lation was 18.7 ± 0.3 min and 16 females did
not mate. For the 1:3 ratio, the mean mating
speed was 1.04 ± 0.03 log min, the mean duration
of copulation was 19.9 ± 0.3 min and 15 females
did not mate.

Thompson, J.N., Jr. University of Oklahoma, Norman. Region-specific interactions in venation mutants.

Many of the mutants affecting wing venation in *Drosophila* interact with each other, and these interactions have been studied by various workers (for example, House, V.L., 1953 Genet. 38:199-215, 1953 Genet. 38:309-327, 1954 J.

Exp. Zool. 127:53-74). I would like to report an interesting interaction between veinlet and two other venation mutants. These interactions are unusual in that the mutants interact superadditively upon some parts of the phenotype, but additively upon others. In veinlet all longitudinal veins are shortened, while in radius incompletus only the L2 vein is affected, and in cubitus interruptus the L4 and sometimes the L5 are shortened. In the double mutant *ve ri*, however, the L2 vein is almost completely eliminated, although the proximal region of the L2 is always present in the single mutants, even in lines selected for very extreme expression (Thompson, 1974 Hered. 33:373-387). The L3, L4, and L5 veins are apparently not



Wing vein double mutants in *D. melanogaster*. Right, *ve ri*; left, *ve;ci*.

affected at all by the second mutant. In the double mutant *ve;ci* the L3, L4, and L5 veins are almost completely erased, even though the L3 is long in veinlet and not affected by *ci*, and the L4 and L5 veins are usually expressed in the proximal part of the wings of the single mutants, even in highly selected lines. The L2 vein in the *ve;ci* double mutant, however, is not affected by the interaction. For this vein the two mutant phenotypes are additive. Although these interactions are quite striking, it is unfortunately not clear what they can tell us at the moment about the developmental effects of these mutants.

I am very grateful to Mr. Brian Curtis for the photography.

Dow, M.A. University of Edinburgh, Scotland. Artificial selection for yellow mating success.

As yellow male *D. melanogaster* have a lower than normal mating success with wild-type females (Dow, M.A. 1976, Behav. Biol. 16:233-239), artificial selection for increased success has been attempted. The first generation matings were

between inbred yellow stock males and inbred wild-type stock females. Yellow males from the inbred stock were again used in the second generation. For all later generations the selection lines were closed, and all matings were between heterozygous wild-type females and yellow males. Two mating regimes were used; single pair matings (2 replicates) and 4 male:1 female matings (3 replicates). Virgin flies were placed in shell vials with 10 cc of standard propionic acid medium and left together for 9 days. Mating success was scored by the presence of progeny.

By generation 12, the mating success of the 1:1 lines had risen from 1% to 52% and that of the 4:1 lines had risen from 22% to 81%. Control matings (1:1) were never more than 2% successful. The mean difference in mating success over the 12 generations of selection between the two different sex-ratio regimes was $24.3 \pm 2.8\%$. As this is the same as the difference found in the unselected stocks (22%) it would appear that selection at the 4:1 sex-ratio has been equivalent to single pair selection plus an additive effect, due to the extra males, of 24%.

Band, H.T. Michigan State University, East Lansing. Genetic drift, natural selection or both?

Charles Darwin (1872) argued that natural selection is important in the maintenance of adaptation and suggested that constitutional changes might be associated with geographical differences over the range of a species. How-

ever, Darwin (ibid.) also argued that variation occurs by chance and that some polymorphisms could be neutral.

The discovery of numerous biochemical polymorphisms in natural populations has precipitated anew the controversy over drift versus selection in the maintenance of genetic variability. Using heterozygote analysis on data collected from many sources, Yamazaki and Maruyama (1974) concluded that enzyme polymorphisms are neutral but blood group polymorphisms are not.

However, Band (1975) noted that in two species of *Drosophila*, *D. melanogaster* and *D. robusta*, heterozygosity among glucose-metabolizing isozymes tends to increase as one moves northward. No such relationship between heterozygosity and latitude was evident among non-glucose metabolizing isozymes. Glucose-metabolizing isozymes (also called group I) are associated with the burning of sugars, with energy production; non-glucose-metabolizing isozymes (group II) are involved in a variety of other biochemical pathways. Table 1 presents the data on average heterozygosity among group I and group II isozymes and their ratio in relation to the latitude of the populations surveyed for each of the two *Drosophila* species. Table 2 shows the regression coefficients for each species and isozyme group and the combined species' results.

Table 1. Average heterozygosity among group I and group II isozymes and their ratio according to locality in two species of *Drosophila*.

Locality	Latitude*	Average heterozygosity		Ratio I/II
		I	II	
<i>Drosophila melanogaster</i> **				
Amherst, Mass.	42.23	0.14	0.18	0.78
Raleigh, N.C.	35.47	0.08	0.20	0.40
Katsunuma, Japan	35.39	0.09	0.16	0.56
Brownsville, Texas	25.54	0.04	0.24	0.17
<i>Drosophila robusta</i> ***				
Chadron, Nebr.	42.29	0.05	0.21	0.41
Williamstown, Mass.	42.42	0.06	0.15	0.40
Englewood, N.J.	40.53	0.05	0.14	0.36
Lincoln, Nebr.	40.49	0.05	0.13	0.26
St. Louis, Mo.	38.37	0.03	0.15	0.20
Raleigh, N.C.	35.46	0.02	0.19	0.10
Myrtle Beach, S.C.	33.42	0.03	0.16	0.19
Asotr, Fla.	29.09	0.01	0.19	0.15

*Latitudes for localities in which *D. melanogaster* has been sampled are taken from the Rand-McNally Atlas (1969). Latitudes for localities in which *D. robusta* has been sampled are given by Prakash (1973).

**Sources of the isozyme data: Band (1975) - Amherst, Mass.; Kojima et al. (1970) - Raleigh, N.C. and Katsunuma, Japan; Gillespie and Langley (1974) - Brownsville, Texas.

***Isozyme survey data for *D. robusta* are from Prakash (1973).

Clearly, the relationship between latitude and heterozygosity among group I isozymes is significant in each species, that between latitude and heterozygosity in group II isozymes is not. This confirms our previous observation (Band, 1975). It also indicates that natural selection may be more important in the maintenance of some biochemical polymorphisms than in others, as Darwin (1972) himself suggested concerning the occurrence of polymorphisms in populations.

Whether or not our results are general for all widely distributed species, for poikilothermic organisms only or merely for these two species of *Drosophila* is not known at present. Gillespie and Langley (1974) have commented on the disproportionate occurrence of group II

isozymes in polymorphism surveys and have argued that it may be grossly misleading to talk about "total genomic heterozygosity" from such survey data.

Table 2. Regression coefficients and their 95% confidence intervals.

Species and isozyme group	$b_i \pm t_{.05, d.f., b_i}$
<i>Drosophila melanogaster</i>	
group I isozymes	0.0058 \pm 0.0040*
group II isozymes	-0.0039 \pm 0.0096
ratio I/II	0.0359 \pm 0.0103*
<i>Drosophila robusta</i>	
group I isozymes	0.0034 \pm 0.0014*
group II isozymes	-0.0018 \pm 0.0057
ratio I/II	0.0203 \pm 0.0143*
Combined species' results via an analysis of variance	
group I isozymes	0.0045 \pm 0.0023*
ratio I/II	0.0277 \pm 0.0035*

* indicates that the regression coefficient is significantly different from zero.

Our current study would confirm their argument. Our results also suggest that data may be inherently biased in favor of "neutrality" if (1) biochemical surveys are largely made on populations collected from warmer latitudes or (2) if mostly group II isozymes are surveyed in population studies done along a north-to-south gradient.

Despite the expense involved in assay work, clearly more attention should be paid to the glucose-metabolizing isozymes. Work on these may be of greater importance to crop scientists or agricultural geneticists faced with the prospect of retailoring important commercial crops to meet the demands of a coming colder and/or more variable climate, for which evidence has been accumulating (Bryson, 1974; NCAR Quarterly, 1974).

References: Band, H.T. 1975, *Genetics* 80:761-771; Bryson, R. 1974, *Science* 184:753-760; Darwin, C. 1872, *Origin of Species*, 6th ed. (paperback, New Amer. Lib. 1958); Gillespie, J.H. and G.H. Langley 1974, *Genetics* 76:837-848; Kojima, K-I., J. Gillespie and Y.M. Tobari 1973, *Genetics* 75:627-637; Rand McNally, 1969, *The International Atlas*, Rand McNally & Co., Chicago; Yamazaki, T. and T. Maruyama 1974, *Science* 183:1091-1092.

Cafagna, C. and F. Ritossa. University of Bari, Italy. Construction of particular compound X chromosomes.

It is rather difficult to obtain, by genetic methods, a female free progeny of phenotypically bobbed males. One such method has employed temperature-sensitive mutations coupled to exposure to non-permissive temperatures (1). The method we devised is that of crossing C(1)RM, 1(1)/1(1)⁺ bb.Y^L females to Xbb/Ybb⁺ males. The lethal mutation we have used is 1(1)Jl, but any other may be used to condition the particular developmental time when female death is desired. If 1(1)Jl is used, 1(1)Jl⁺.Y^L fragments are available (or easily constructed) which carry bb loci of any desired redundancy (bb, bb^{ds}, bb^l). To construct the compound X we used an X.Y^S, bb chromosome which revealed two bb loci, one on the X and one on the Y^S arm, which are eucentric with respect to the centromere (2). Recombination at the rDNA level between the two loci, leads to formation of X.X (RM) and Y^S.Y^S chromosomes. To increase the frequency of such recombinants, we exploited the observation that recombination at the rDNA level is strongly increased during rDNA magnification (3). 1(1)Jl y v bb.bb Y^S/y⁺ sc⁸ bb^l.Y^L males, in which rDNA magnification occurs, were constructed and crossed to C(1)RM, y²su(wa)w^a/y⁺sc⁸bb⁺.Y^L females. 3 vermilion females, derived from independent events, were selected among 10,783 females. One was a nondisjunctional product; two were the C(1)RM, 1(1)Jl y v bb type.

References: (1) Williamson, J.H. et al. 1975, *MGG* 139:33; (2) Ritossa, F. et al. 1975, *Atti Ass. Gen. Ital.* 20:154; (3) Ritossa, F. 1973, *PNAS* 70:1950.

Chadov, B.F. Research Institute of Medical Radiology, Academy of Medical Sciences of USSR, Obninsk. A non-homologous pairing of chromosomes and spontaneous formation of translocations in *Drosophila melanogaster*.

One can speculate that non-homologous pairing of chromosomes is accompanied by non-homologous exchanges. Such exchanges between two chromosomes can be detected by the appearance of half-translocations.

In our experiments XXY and XX females $sc^8.Y/y/y;C(2L)RM,b\ pr;C(2R)RM,cn$ and $y/y;C(2L)RM,b\ pr;C(2R)RM,cn$ containing compounds 2L

and 2R, were mated to $b\ j\ pr\ cn/T(Y;2)C$ males, bearing translocation $T(Y;2)C$ with the break-point near the centromere of chromosome 2. It was supposed that in XXY females non-homologous pairing of the Y and $C(2L)$ had taken place resulting in a half-translocation $Y\cdot 2L$, bearing the left arm of the chromosome 2 and some material of the Y-chromosome. Males $b\ j\ pr\ cn/T(Y;2)C$ produce four types of gametes, two of them are aneuploid and complementary to oocytes containing half-translocations with a break-point near the centromere of chromosome 2. Unfortunately, we could not detect half-translocations with other break-points of the compound $C(2L)RM,b\ pr$ or half-translocations arising as a result of interchanges between the $C(2R)RM,cn$ and the Y-chromosome. We would not expect production of individuals with $Y\cdot 2L$ from females without a Y-chromosome.

The progeny of XXY females consisted of 500 individuals, and that of XX females of 326 individuals. They were mostly of phenotypes $b\ pr\ cn$ and $b^+pr^+cn^+$. The genetic analysis of these flies showed that they are produced as a result of two processes, the spontaneous transformation of compounds into structurally normal autosomes 2 and the formation of diploid oocytes. In addition to these progeny, XXY females have produced 42 individuals, $b\ pr$ males and cn females. As the analysis proved, these individuals originated from oocytes containing half-translocations $Y\cdot 2L$. In 15 cases the half-translocations contained a long arm of the Y-chromosome with marker y^+ and in 22, short arm of this same chromosome. In all cases the left arm of the autosome 2 with genes b and pr was included. It should be noted that XXY females also produced one $b\ pr$ female. She had the half-translocation containing 2L, $b\ pr$ and the left arm of the X-chromosome with y . XX females produce no half-translocations. The individuals with $Y\cdot 2L$ half-translocation arise in clusters.

It may be concluded from our data that in mitosis in *D. melanogaster* non-homologous pairing of chromosomes takes place, sometimes accompanied by interchange. These spontaneous interchanges can be detected in flies with genotypes allowing such non-homologous pairing. These data suggest that the interchanges resulting in oocytes with translocations produce individuals both with complete translocations and with half-translocations. The former are the result of non-disjunction and escape detection, the latter result from disjunction of the two parts of the translocation in meiosis and are detected by our method.

Harshman, L.G. University of California, Riverside. The *Drosophila* attracting flowers of *Calycanthus occidentalis*.

Calycanthus occidentalis, spice bush, is a streamside shrub found in California's North Coast Ranges and Sierra Nevada. Wild *Calycanthus occidentalis* blooms from May to September and produces an abundance of solitary, reddish-

brown flowers having an odor that may be described as wine-like or fruity. In May, 1975, a number of flies were observed hovering about the open flowers of *Calycanthus occidentalis* specimens in the Botanic Garden at U.C. Riverside. The flies were numerous on the flowers in the early evenings and present, but less abundant, in the mornings. Collections revealed the fly fauna of spice bush to be largely (approx. 90%) composed of a variety of *Drosophila* species. *D. immigrans*, *busckii*, *pseudoobscura*, *melanogaster/simulans*, and species of the *repleta* group were found on the flowers. Up to twenty *Drosophila* could be observed on the imbricated petals of a single flower, sometimes in mating pairs.

Calycanthus occidentalis shares a common range with the much studied *D. pseudoobscura* whose natural ecology is still something of a puzzle. *Calycanthus fertilis* and *Calycanthus floridus* are two North American relatives of *Calycanthus occidentalis*, which have aromatic flowers, that may also attract *D.*

Vacek, D.C., W.T. Starmer and W.B. Heed.
University of Arizona, Tucson. The Y-2 strain of yeast at the Texas stock center is *Candida krusei*.

Wagner (1944) isolated 8 types of yeast from *Opuntia* fruits but did not identify them. One of these strains (Univ. of Texas strain Y-2) has been routinely sprayed on the surface of *Drosophila* media for the proliferation of many strains at the University of Texas (Austin)

stock center (Wheeler, 1967). We have identified the Y-2 strain as *Candida krusei* (Cast.) Berhout. The strain was identified by the procedure described by Van der Walt (1970). Assimilation tests of 28 different carbon compounds were conducted by inoculating onto plates with yeast nitrogen base agar, containing .5% of a carbon source. Tests for growth on vitamin free agar, nitrate and elevated temperatures were conducted in addition to tests for the fermentation of glucose, pseudomycelium formation and spore formation. The strain agrees with the standard description of Van Uden and Buckley (1970) except for the assimilation of citric acid which is positive for the Y-2 strain. *C. krusei* is considered the imperfect form of *Pichia kudriavzevii* Boidin, Pignal et Besson.

Candida krusei has been isolated frequently from the crops of species of *Drosophila* in temperate and tropical regions. Four out of seventeen strains of yeasts isolated by Hedrick and Burke (1950) from the fecal pellets of Hawaiian *Drosophila* (*D. crucigera* and *D. pilimana*) were *C. krusei*. Shehata, Mrak and Phaff (1955) recovered *C. krusei* (5 out of 118 isolates) from the crops of *Obscura* group *Drosophila* in southern and central California. Studies in the Yosemite region of California (Phaff et al., 1956) revealed *C. krusei* was also present in the crops of *D. pseudoobscura*, *D. persimilis*, *D. azteca*, *D. pinicola* and *D. occidentalis* (10 out of 242 isolates). Forty isolates (out of 394) of *C. krusei* were taken from crops of *D. paulistorum*, *D. willistoni*, *D. capricorni*, *D. griseolineata*, *D. guaramunu*, *D. polymorpha*, *D. atrata*, and members of the tripunctata species group, in the São Paulo region of Brazil (da Cunha, Shehata and de Oliveira, 1957).

The natural habitats of *C. krusei* and related species in the genera *Candida* and *Pichia* are mainly spoiling fruits (Nakase, 1971).

References: da Cunha, A.B., A.M. El Tabey Shehata and W. de Oliveira 1957, *Ecol.* 38(1): 98-106; Hedrick, L.R. and G.C. Burke 1950, *Jour. Bact.* 59:481-484; Nakase, T. 1971, *J. Gen. Appl. Microbiol.* 17:383-398; Phaff, H.J., M.W. Miller, J.A. Recca, J. Shifrine and E.M. Mrak 1956, *Ecol.* 37:533-538; Shehata, A.M. El-Tabey, E.M. Mrak and H.J. Phaff 1955, *Mycol.* 47:799-811; Van der Walt, J.P. 1970, In: J. Lodder (ed), *The Yeasts - A Taxonomic Study*, North-Holland Publ. Co., Amsterdam, 891-1087; Van Uden, N. and H. Buckley 1970, In: J. Lodder (ed) *The Yeasts - A Taxonomic Study*, North-Holland Publ. Co., Amsterdam 891-1087; Wagner, R.P. 1944, Univ. of Texas Pub. No. 4445:104-128; Wheeler, M.R. 1967, In: U.F.A.W. Handbook: The care and management of laboratory animals, 3rd ed. E. & S. Livingstone, Ltd., Edinburgh 906-924.

Petri, W.H., A.R. Wyman and S. Henikoff.
Harvard University, Cambridge. Synthesis of "heat shock" mRNA by *Drosophila* melanogaster follicle cells under standard organ culture conditions.

As a part of our studies on eggshell development in mass dissected *Drosophila* follicles we have examined the patterns of RNA synthesis in this tissue in culture. We find by in situ hybridization of the follicle cell RNA to salivary gland chromosomes that the follicles incorporate ³H-uridine into RNA which hybridizes primarily

at the major "heat shock" loci; i.e., 87A, 87C and 93D. This result indicates that the tissue was in a "heat shock" condition even though temperatures never exceeded 22° and the tissue was shaken for aeration during incubation. The tissue was chilled in a *Drosophila* ringers solution (Ephrussi & Beadle, *Amer. Nat.* 70:218-225, 1936) to approximately 4°C during the one and a half hour mass dissection procedure (Petri, Wyman & Kafatos, *Develop. Biol.* 49:185-199, 1976) and perhaps "shocked" either during the procedure or upon warming up to 22°C for incubation. This is currently under study. However, our purpose here is to caution others that the organ isolation and culture conditions used by many may result in evoking a "heat shock" response in the tissue of interest and possibly causing abnormal patterns of RNA and protein synthesis.

Ranganath, H.A. and N.B. Krishnamurthy.
University of Mysore, India. Chromosomal
polymorphism in *Drosophila nasuta*.
VI. Non-random association of inversions.

which one is in the X chromosome, ten in the second chromosome and twenty-two in the third chromosome. The present note incorporates the findings on the non-random association of two independent inversions 3A and 3C in the various natural populations of *D.n. nasuta*. Populations of *D. nasuta nasuta* have been sampled from nine geographically distant places in South India - viz., Poona, Hyderabad, Karwar, Soundathi, Hassan, Coorg, Mysore, Coonoor and Tellicherry, at different altitudes of Biligirirangana Hills (820 m, 1040 m and 1300 m) and Sampaje Ghats (150 m, 800 m and 1100 m) and of different months of the year - November 1971 to October 1972. Chromosomal constitution of these populations have been analysed by adapting the egg sample technique of Strickberger and Wills (1966).

Of all the combinations of heterozygous inversions, 3A and 3C combination is more frequent than others. The frequencies of these two inversions occurring independently and in combination in all the analysed populations are set forth in Table 1. In almost all of the

Table 1. Non-random association of 3A and 3C heterozygous inversions in the natural populations of *D.n. nasuta*.

	Total number	Inversions		
	larvae scored	A	C	AC
I. <u>Geographical Populations</u>				
Poona	71	-	-	4
Hyderabad	90	-	-	-
Soundathi	106	-	3	7
Karwar	163	-	-	42
Hassan	104	-	-	26
Coorg	85	1	3	15
Mysore	167	3	4	64
Coonoor	59	-	-	10
Tellicherry	130	-	-	34
II. <u>Populations of different altitudes</u>				
<u>Biligirirangana Hills:</u>				
820 mts.	106	1	1	7
1040 mts.	138	-	2	18
1300 mts.	95	1	6	11
<u>Sampaje Chats:</u>				
500 mts.	68	-	2	8
800 mts.	65	-	2	21
1100 mts.	89	-	-	34
III. <u>Populations of different months</u>				
November	19	1	4	5
December	45	3	5	-
January	50	-	2	-
June	61	3	6	-
July	111	6	9	6
August	338	2	9	36
September	122	2	2	34
October	74	-	1	14

Exuberant chromosomal polymorphism due to inversions as well as their ecological and adaptive significance in the natural populations of *D. n. nasuta* have been well documented (Ranganath and Krishnamurthy 1973, 1974, 1975). *D. nasuta* has in all th ty-three inversions, of

populations, the double heterozygotes of these inversions exceed the frequency of single heterokaryotypes of either 3A or 3C irrespective of their geographical altitudinal and monthly variations.

Each chromosomal arrangement possesses groups of genes organized by selective forces to produce an adaptive phenotype either in itself or in combination with other gene arrangements of the same chromosome (Spiess, 1957). Further, the selective favouring of particular linkages (intrachromosomal) and independently assorting compounds (interchromosomal) have been well documented (cf. Spiess, 1962). In *D. nasuta nasuta* under study, the two independent inversions 3A and 3C coexist most of the time. The prevalence of this combination suggests some evolutionary advantage. The selective advantage conferred upon by the compounds of linked inversions have also been recorded by Levitan (1954) in *D. robusta*, by Brncic (1961) in *D. pavani* and Nirmala and Krishnamurthy (1972) in *D. sulfurigaster neonasuta* (earlier called *D. neonasuta*). Thus, the occurrence of non-random association of 3A and 3C inversions in the natural populations of *D. nasuta nasuta* suggests the existence of an adaptive interaction between these gene orders.

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Am. Nat. 88:419-423; Nirmala, S.S. and N.B. Krishnamurthy 1972, DIS 48:34; Ranganath, H.A. and N.B. Krishnamurthy 1973, DIS 50:106; _____ 1974, Sci. J. Mys. Univ. XXVI:65-69; _____ 1975 J. Hered. 66:89-96; Spiess, E.B. 1957, Evol. 11:84-93; _____ 1962, Intro. in Papers of Animal and Population Genetics, Ed. E.B. Spiess (Methuen and Co., London) pp. xi-IXXI.

Maddern, R.H. University of Leiden, The Netherlands. Distal X linked lethals.

exposing two lethal loci. $l(1)^d$ (a lethal commonly present in stocks of dor^1) and $l(1)2804$ (of spontaneous origin) were both exposed by $l(1)403$ but complement each other.

The doubly marked Y-chromosome $y^{31d} Y^{BS}$ was found to be lethal in combination with $l(1)403$ and $l(1)^d$ but viable with $l(1)2804$ and $l(1)J1$. If the order of these loci is tentatively taken as $l(1)^d l(1)2804 l(1)J1 y^+$ then this Y-chromosome is deficient for the most distal but not for the other two lethal loci. The $y^+ Y^{BS}$ chromosome covers all of the distal X linked lethals.

Cytologically the tip of the $l(1)2804$ chromosome is abnormal, the bands 1A5-6 to the telomere frequently being unpaired. By comparison with the X-chromosome of Oregon-K, the telomeric band is thinner and one band (presumably 1A4) is missing. The frequent failure to pair may however indicate a more complex rearrangement than the interstitial deletion of a single band.

Reference: Maddern, R.H. 1972, DIS 49:48.

Lambert, D.M.* University of Queensland, Brisbane, Australia. D. kohkoa and D. albomicans from the Philippines and Western Malaysia.

In the case of D. kohkoa a total of eleven heterozygous inversions were detected. Of the seven inversions found in the Kota Tinggi cultures, inversions Y_2 , Z_2 and G_3 have appeared in previous publications (Mather, Thongmeearkom, Clyde and Lambert 1975, DIS 51:86 and Thongmeearkom 1975, DIS 52:117).

Inversion	Type	Position	Locality
Y_2	Sim	IIL D	K.T.
Z_2	Sim	III C	K.T.
G_3	Sim	III P	K.T.
M_3	Com	IIL C	K.T.
Q_3	Sim	III C	K.T.
K_3	Sim	III P	K.T.
Z_4	Com	III P	K.T.
F	Com	III P	C.
A	Sim	III P	C.
L	Sim	III P	C.
J_3	Sim	III P	C.
E	Sim	IIL C	Pen.
C	Sim	III D	Pen.
E_3	Com	III C	Pen.
I_3	Sim	III C	Pen.
P_3	Com	III C	Pen.
L_3	Sim	III C	Pen.

Sim = simple, Com = complex, D = distal
C = central, P = proximal to centromere
KT = Kota Tinggi, C = Cebu; Pen = Penang

It was previously reported that $l(1)J1$ and $l(1)403$ represented two separate lethal loci located distal to y^+ on the X-chromosome. It now appears that $l(1)403$ is itself a deficiency

exposed by $l(1)403$ but complement each other. In January 1973, two iso-female lines of D. kohkoa from Kota Tinggi, Malaysia and one iso-female line of D. albomicans from Penang, Malaysia were established. In August 1973 another two iso-female lines of D. kohkoa from Cebu, Philippines were also established.

The remaining four inversions, M_3 , Q_3 , K_3 and Z_4 have not previously been detected (see figure). Four heterozygous inversions were detected in the iso-female lines collected from Cebu; three of these, F, A and L, have appeared previously (Mather and Thongmeearkom 1972, DIS 48:40). The remaining inversion, J_3 , is new (see figure).

Six heterozygous inversions were detected in the one iso-female line of D. albomicans collected. Three of these inversions, E, C and E_3 , have previously appeared (Mather and Thongmeearkom 1972, DIS 48:40 and Thongmeearkom 1975, DIS 52:117). The new inversions I_3 , P_3 and L_3 are presented (figure).

It should be noted that the highly polymorphic populations of D. kohkoa from both the Philippines and Western Malaysia have completely different inversion compositions. Inversions Y_2 , Z_2 and E, C recorded here from populations of D. kohkoa and D. albomicans, respectively, have previously been recorded from populations of D.s. albostrigata (Mather, Thongmeearkom, Clyde and Lambert 1975, DIS 51:86).

Details of inversions are given in the Table. The material was collected and iso-female lines established by Dr. W.B. Mather, Genetics Laboratory, University of Queensland. (See Figure 1 on facing page.)

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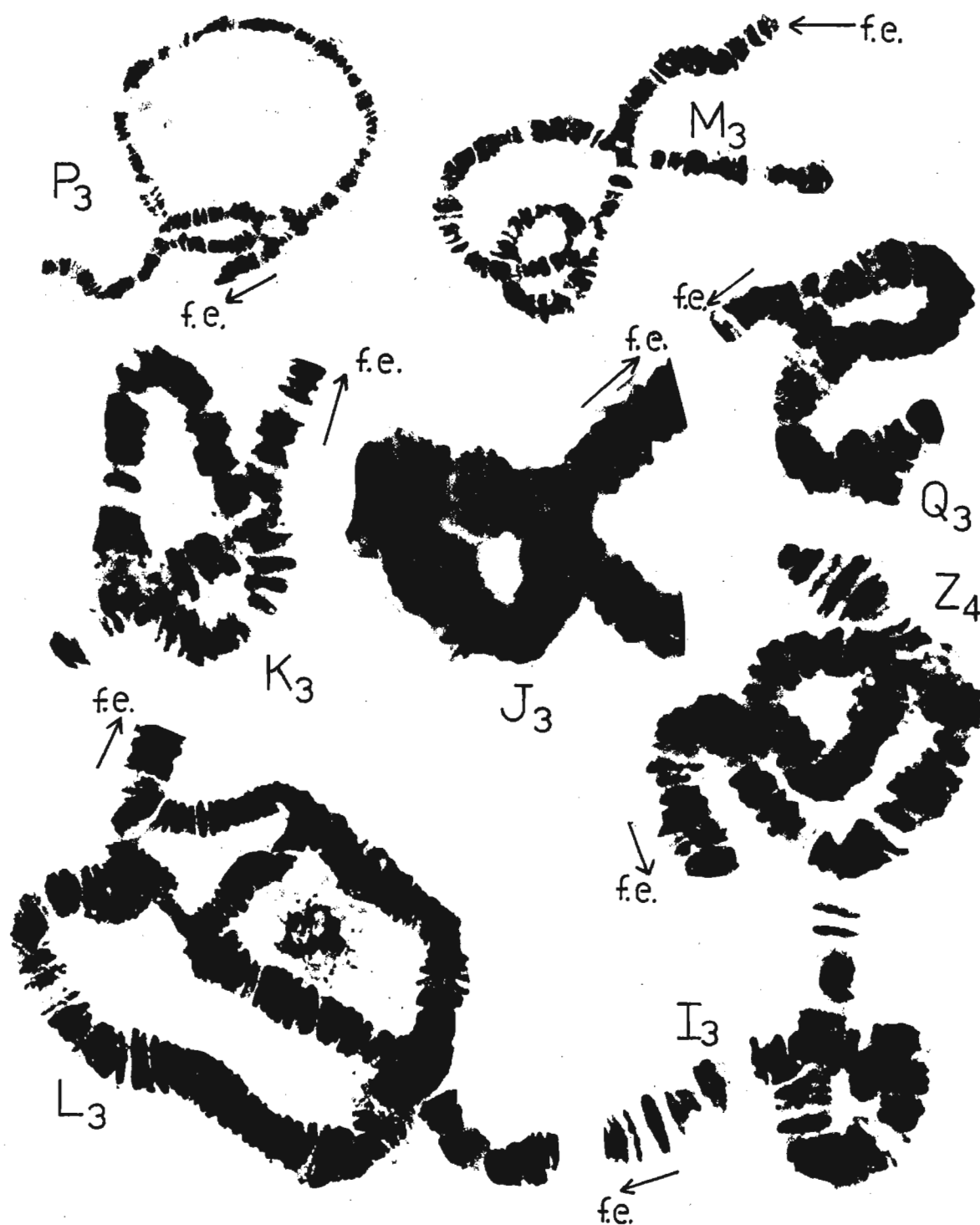


Figure 1. Inversions found in iso-female lines of *D. kohkoa* and *D. albomicans*.
(Lambert, D.M., *D. kohkoa* and *D. albomicans* from the Philippines and Western Malaysia.)

Gibson, P.A.K. and W.W.M. Steiner.
University of Illinois at Urbana, Illinois.
A note on enzyme variation found in
Drosophila americana americana.

Five lines of *Drosophila americana americana* collected in July, 1975, near Des Moines, Iowa, were analyzed using starch gel electrophoresis. Details on the systems and techniques are described by Steiner and Johnson (1973). The superscripts at the Malate dehydrogenase (Mdh)

and Hexokinase (Hk) loci represent different banding regions with increasing numerical values indicating increasingly anodal migrating zones. This is similar to the designations of banding regions in *D. melanogaster* (Madhavan, Fox and Ursprung, 1972). In no case did the fast forms at Mdh¹ and Mdh² segregate together. There appears to be no correlation in the variability between the banding regions at the Hk locus.

Locus	Number of alleles	Number of organisms analyzed	Number of lines sampled
α -Esterase (α -Est)	3	51	5
β -Esterase (β -Est)	2	51	5
Leucine aminopeptidase (Lap)	3	32	4
Alcohol dehydrogenase (Adh)	2	32	4
α -glycerophosphate dehydrogenase (α -Gpdh)	2	32	4
Isocitrate dehydrogenase (Idh)	2	32	4
Malic enzyme (Me)	1	32	4
Malate dehydrogenase ¹ (Mdh ¹)	2	32	4
Malate dehydrogenase ² (mdh ²)	2	32	4
Glutamate oxaloacetate transaminase (Got)	2	32	4
Hexokinase ¹ (Hk ¹)	2	32	4
Hexokinase ² (Hk ²)	3	14	4
Hexokinase ³ (Hk ³)	3	18	4
Adenylate kinase (Adk)	1	24	5
Phosphoglucomutase (Pgm)	3	28	5
Aldehyde oxidase (Aldox)	2	16	4
Glyceraldehyde-3-phosphate dehydrogenase (G-3-pdh)	2	15	4
Xanthine dehydrogenase (Xdh)	2	16	3
Octanol dehydrogenase (Odh)	1	16	4
Alcohol dehydrogenase (Adh)	2	28	4

Research supported by a grant from the Research Board of the University of Illinois and a University Biomedical Sciences Support Grant to W.W.M. Steiner.

References: Madhavan, K., D.J. Fox and H. Ursprung 1972, J. Insect Physiol. 18:1523-1530; Steiner, W.W.M. and W.E. Johnson 1973, U.S. I.B.P./IRP Tech. Rep. 30:1-21.

Ramón, M. Autonomous University of Barcelona, Spain. Study of the matching of the third chromosome in the hybrids of *D. melanogaster* and *D. simulans*.

A study of the matching of the two left arms of the third chromosome of the hybrids has been made. It has allowed us to record a degree of variability greater than that admitted until now (Horton, 1939; Ashburner, 1968) in the third chromosomes of these species.

The most prominent points are as follows: 1. The presence of extra bands which mark a difference between the two species, due to deficiencies or duplications of one band. Those found in *D. simulans*, which do not show *D. melanogaster*, are: 61A1 (it was also found by Ashburner), 74A or B1 and with a frequency less than 100% in 61E1.2 and 62E. Those found in *D. melanogaster* are: 61C2 or C3, 62C or D1 and 68B.

2. Two small inversions have been detected. The first in region 68E and the other in region 75 which ranges from A3 to B1.

We find that as the frequency with which a duplication may appear can vary from 20% to 100%, we are led to suppose that an intraspecific polymorphism due to deficiencies or duplications exists in each of the species under examination. It is possible that some of this variability is due to technical deficiencies.

Kaneshiro, K.Y., A.T. Ohta and H.T. Spieth*
University of Hawaii, Honolulu. Mushrooms
as bait for *Drosophila*.

The endemic Hawaiian *Drosophila* species respond poorly to collecting techniques which utilize fermenting bananas and/or other fruits, wine, or Lakovaraara's (DIS 44:123) fermenting malted barley. Members of the picture-winged species

group and a few other species are attracted in modest numbers to some fermenting fruits, but not to barley bait. Most species of most species groups simply ignore all such baits. One of us (HTS), after studying the Western American fungivorous species *Drosophila flavopinicola* and *D. pinicola*, suggested the use of rotting commercial mushrooms, *Agaricus campestris*, as bait. Field tests were quite successful and the following procedure is now routinely employed:

Fresh mushrooms are moistened with water, placed in a tightly closed container (we use plastic bags) and then "ripened" in a relatively warm site (27° - 30°C). After 3 - 5 days the mushrooms turn dark brown, exude a moderate amount of dark liquid and give off a pungent odor. Such "ripened" bait is suitable for field use.

Hawaiian drosophilids are behaviorally unique in that a resting or feeding individual when disturbed does not fly upward or laterally to escape but rather dives sharply and swiftly downward. It is non-productive therefore, to place bait in the bottom of a can or similar container. When a net is swept over the top of such a container, or if the container is sharply disturbed, any Hawaiian drosophilid that has been attracted to the bait typically becomes enmeshed in the bait substances on the bottom of the container.

For the Hawaiian flies we use rectangular (3" x 7" x 1/8"), smooth surfaced sheets of cellulose sponge (DuPont Sponge Cloth). Red or salmon colored sheets are the most effective, yellow the least effective. In the field, these sheets are thoroughly moistened with the mushroom liquid or by rubbing a rotting mushroom over the sponge surface. The individual sheets are then thumbtacked onto branches or trunks of trees, shrubs, and tree ferns. We also smear banana bait (Gerber's Baby Banana Food that has been inoculated with yeast 12 - 24 hours earlier) on tree branches and trunks near the sponge baits. The combined odors appear to have a synergistic effect on the drosophilids.

The most productive sites are those where (1) the air movement is gentle, (2) there are leaves in close proximity above, below, and lateral to the bait, and (3) the collector can approach the bait sponge without casting a shadow on it. A few species prefer the placement of the bait close to the ground but most come readily to baits located at about eye level. The baits should be visited every 30 minutes to one hour, and the flies collected by using a glass tube aspirator. The collector's movements in approaching the bait and in using the aspirator must be deliberate and smooth. Any jerkiness or exhalation through the aspirator is disastrous.

Hawaiian drosophilids will readily visit the baits during light to moderate rainfall. It is therefore helpful to carry 2 - 3 aspirators and also a supply of pipe cleaners for drying and cleaning the interior of the aspirator tubes. The sponges can be re-used repeatedly by washing with a detergent and then sequentially rinsing first in water, then in 25% alcohol and finally again in water.

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Dow, M.A. University of Edinburgh,
Scotland. Activity of yellow females.

Bastock (1956 *Evol.* 10:421-439) has suggested that the yellow gene causes female *D. melanogaster* to be less active than wild-type when courted. Since yellow males court more

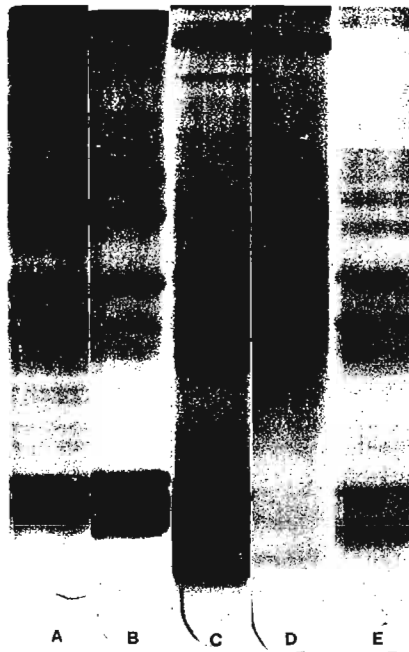
normally when the female is stationary, this may be the cause for their mating success with yellow females. This has been tested with females from a strain that has been segregating for the yellow allele for 41 generations.

Three to five day old virgin females were timed as they traversed a distance of 10 cm in a glass tube (diameter 2 cm). Females that moved in both directions were discarded.

The results show that yellow females (mean = 57.5 sec., N = 11) are significantly less active (sign test, $P < 0.05$) than wild-type females (mean = 24.3 sec., N = 11). This supports Bastock's suggestion that yellow females are less active than wild-type and localises the behavioral difference to the yellow gene itself, as the females used in this experiment can be expected to differ at the yellow locus alone.

Andrews, P.W. and D.B. Roberts. University of Oxford, England. A comparison of *Drosophila* chromatin from different sources.

embryos was 10%, 2nd instar larvae 35% and 3rd instar larvae 15%. Non-histone proteins were prepared from chromatin by acid extraction of histones followed by digestion of DNA with



DNase. The non-histone proteins were analysed by SDS gel electrophoresis with 10% polyacrylamide gels (Figure 1).

The gel patterns showed more than 30 bands with molecular weights ranging from 20,000 - 100,000 daltons. Different preparations from the same source gave reproducible patterns. A comparison of the patterns from different sources showed a similar general pattern but superimposed on this were a few striking differences. 2nd and 3rd in-

Figure 1. A Gm3 cells; B embryos; C 2nd instar larvae; D 3rd instar larvae; E fat body.

star larvae showed an intense complex of bands of high molecular weight not found in embryos or cultured cells (M). Cultured cells showed a light molecular weight band (N) about 25,000 daltons not found in other material. Cultured cells were generally similar to embryos from which the cells were derived (note especially O, P and Q). It is worth noting that a similar comparison of nuclear sap proteins showed much greater differences between the different source materials.

The different chromatin preparations were also analysed immunologically³. With one exception they were only distinguished by the different relative concentrations of the antigens. 2nd instar larval chromatin however did lack an antigen found in all other preparations.

The general similarity of the chromosomal proteins from the different developmental stages and cell types is in agreement with other results for *Drosophila*^{4,5} and for other organisms^{6,7}, although a few unambiguous differences were observed.

References: 1) Mosna, J. and S. Dolfini 1972, *Chromosoma* 38:1-9; 2) Andrews, P.W. and D.B. Roberts 1974, *Nucleic Acids Res.* 1:979-997; 3) Roberts, D.B. and P.W. Andrews 1975, *Nucleic Acids Res.* 2:1291-1303; 4) Elgin, S.C.R. and L.E. Hood 1973, *Biochem.* 12:4984-4991; 5) Helmsing, P. and O. van Eupen 1973, *Biochem. Biophys. Acta* 308:154-160; 6) Seale, R.L. and A.I. Aronson 1973, *J. Mol. Biol.* 75:633-645; 7) MacGillivray, A.J. and D. Rickwood 1974, *Europ. J. Biochem.* 41:181-190.

Rendel, J.M. C.S.I.R.O., Sydney, Australia. Cytological location of stripe and an allele for extra bristles.

A section of chromosome III in *D. melanogaster* between 89C and 90E is haploid in some flies when T(3:Y)B116 90E males are crossed with T(3:Y)L142 89C females, (Lindsley, Sandler et al. 1972, *Genetics* 71:157-184). This region

contains Ubx and Ix. I have used this region against a third chromosome marked with the recessive gene stripe (sr) which contains a recessive gene or region linked to stripe that increases bristle number in wild type flies. The number of scutellars may be as high as seven or eight instead of four, and there are extra dorsocentrals. The flies, heterozygous for the deficiency between 89C and 90E and this chromosome III, were stripe and had extra scutellar and dorsocentral bristles. Hence these two genes lie in the region between 90E and 89C. In addition all flies heterozygous for the deficiency had their wings rotated round the long axis with the costal margins up. Since no such gene existed in the sr chromosome III, this is a dominant effect and may have some connection with Rf of Waddington.

Lemke, D.E. and J. Tonzetich. Bucknell University, Lewisburg, Pennsylvania. Observations of inversions in polytene chromosomes using the alkali-urea technique.

slide, and covered with several drops of a 1:1 mixture of 1N sodium hydroxide and a saturated aqueous solution of urea. After fifteen minutes

this solution is carefully drawn off with absorbent paper and replaced by several drops of aceto-orcein. The glands are allowed to stain for an additional fifteen minutes, after which the aceto-orcein is removed and a drop of 50% acetic acid placed over them. A cover glass is then applied, the glands squashed gently, and the entire preparation made semi-permanent by sealing the edges of the cover glass.

Chromosomal aberrations become particularly apparent in such alkali-urea treated preparations, as can be seen from the accompanying photomicrographs. Figure 1, taken through Nomarski optics, clearly shows the core fibers of the chromosomes as being distinct from the uncoiled chromomeres radiating out to every side. Two large inversion loops are also apparent in this spread. Much detail has been obscured, however, owing to the long exposure times and consequent unavoidable vibration necessitated by the Nomarski optics; the actual preparations are much sharper when viewed directly. Figure 2, taken through standard light optics, reveals the double stranded nature of the central core and clearly demonstrates the structure of the inversion loop in a structural heterozygote. Utilizing alkali-urea treatment, the exact configuration of the chromosome strands in more complex aberrations can be easily worked out, and since the chromosomes seem to uncoil completely during alkali-urea treatment, the method is especially useful for visualizing the very small inversions which may often be confused with areas of twisting and coiling in ordinary chromosome spreads.

References: Kodani, M. 1941, J. Hered. 32:146-156.

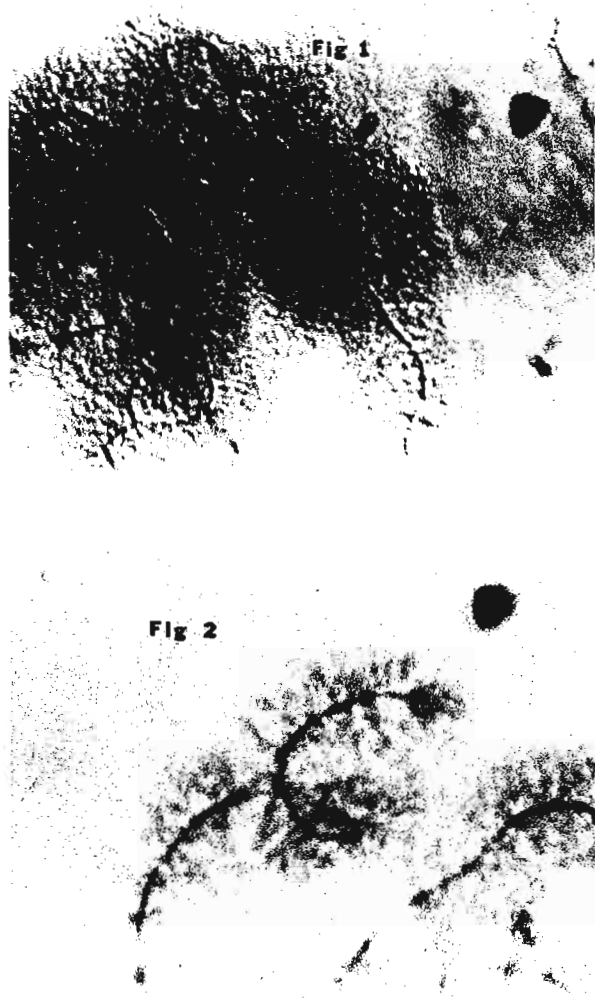
Panopoulou-Diamantopoulou, E. Agricultural College of Athens, Greece. A gene for the dot chromosome of *D. subobscura*.

chrom.), pp pl (E chrom.), ch cu (O chrom.), abb (U chrom.). It was found unlinked with these and also not sex linked. Thus it seems that shv is located in the dot chromosome.

The existence of a nearly similar mutant, the ci (cubitus interruptus) in *D. melanogaster* located in the fourth chromosome, the dot, is a good indication that shv is also located in the dot.

The mutant short vein, shv (fourth longitudinal vein not reaching wing margin) was unplaced according to Burla's report (DIS 43:76).

Crosses were made for its chromosomal localization using the mutants ma int (J



Choi, Y. Yonsei University, Seoul, Korea.
Inversions in a Korean natural population
of *Drosophila melanogaster*.

Samplings from wild populations were made on a
day in the beginning of July and August in 1975, at
a local population at Chungju, about 70 km south
of Seoul. Wild caught males were crossed with

Oregon-R-S standard virgin females. Each hybrid larvae from this cross was examined.
According to the standard chromosome maps of Bridges (1935), thirteen different inversions
were found in this study. The seven inversions were identified as cosmopolitan types:
In(2L)t, In(2R)NS, In(3L)P, In(3L)M, In(3R)C, In(3R)MO, In(3R)P. In(2L)Ka was a new
single paracentric inversion, with the breakage points at 22D and 24A. In(2L)Kb was a new
small paracentric inversion, with one breakage point at 32B and the other one at 34D.
In(2R)Kc was a new single paracentric inversion between 55E and 57D. In(2R)Kd was a new
small paracentric inversion between 57C and 58F. Additionally, two other inversions were of
interest, since these two types, namely In(2R)L and In(3R)J, were probably identical to those
which had been found only once in Katsunuma (1964) and Kofu (1963) natural populations of
Japan by T. Watanabe. In(2R)L was a single paracentric inversion between 43D and 49F.
In(3R)J was a single paracentric inversion between 96E and 98F. The number of flies tested
and the frequencies of the various gene arrangements are given in Table 1. 154 wild males in

Table 1. The relative frequencies of chromosomal inversion occurrences in
D. melanogaster collected from a Korean natural population at Chungju

Inversion	July N=154*	August N=150	Total N=304	%	Homogeneity test
In(2L)t	13	14	27	8.9	P > 0.7
In(2L)Ka	1	0	1	0.3	
In(2L)Kb	0	2	2	0.7	
In(2R)NS	26	31	57	18.8	P > 0.7
In(2R)L	1	0	1	0.3	
In(2R)Kc	1	0	1	0.3	
In(2R)Kd	0	2	2	0.7	
In(3L)P	4	6	10	3.3	P > 0.7
In(3L)M	8	7	15	4.9	
In(3R)C	4	5	9	3.0	P > 0.9
In(3R)P	15	16	31	10.2	
In(3R)MO	7	8	15	4.9	
In(3R)J	5	6	11	3.6	

* N indicates the number of flies tested.

in June and 150 in August were tested. Homogeneity tests (χ^2) between these two samples show
no significant differences. So these two data were pooled again in the next column.

Pyle, D.W. North Carolina State
University, Raleigh, North Carolina.
The longevity of divergent geotactic
maze strains of *Drosophila melanogaster*
in a dry environment.

Murphey and Hall (1969) investigated correlated
responses in males of *Drosophila melanogaster*
selected for negative geotactic maze behavior.
They determined that these geonegative males
were better able to survive in a dry environment
than males from an unrelated control strain.

Moreover geonegative males were found to be less
active than the control males. They concluded that selection for negative geotactic maze be-
havior may also entail selection for "maze hardness", that is, an increased ability to with-
stand desiccation and starvation. A lower level of locomotor activity, resulting from nega-
tive geotactic selection, was hypothesized to facilitate survival in a dry environment because
it was shown that increased activity in a dry container results in increased mortality.

If selection for geotactic maze behavior includes selection for "maze hardness", other divergent strains of *D. melanogaster* should also show increased survivorship. In this study I have repeated the desiccation-survival experiment similar to Murphey and Hall (1969) to determine if males and females from both positive and negative geotactic maze strains unrelated to the geonegative strain used previously also showed increased survivorship compared to the unselected control strain from which they were derived.

The strains of *D. melanogaster* used in this study have been selected for 50 generations on 15-unit classification mazes (Hirsch, 1959). In these mazes geotactic scores may vary from 1 to 16. A score of 1 represents the most negative geotactic response possible, while the maximum positive response results in a score of 16. The strains used here had mean geotactic scores of 1.80 for the geonegative strain, 8.17 for the control strain and 14.92 for the geopositive strain. The geonegative males used by Murphey and Hall (1969) were taken from the University of Illinois geotactic strains supplied by Professor Hirsch.

The two selected strains and the control strain were tested here for their ability to survive in a dry environment. The flies were collected from population bottles 24 hours before the desiccation-starvation experiment, sexes separated and placed in 6 half pint milk bottles with the standard molasses-cornmeal-agar medium. The flies were approximately 3 days old when tested. Two hundred flies of each sex from each of the 3 strains were used; 8 flies were aspirated into each of 25 dry glass, 8 dram shell vials for each of the 6 groups. The vials were fitted with cotton plugs. After a 24 hour desiccation-starvation period the number of living flies were counted. This test was conducted at room temperature (24°C) at 10% relative humidity.

The results presented in Table 1 show the proportion of flies surviving after 24 hours in the dry container. The number of surviving males from both geopositive and geonegative strains are not significantly different, the

same holds true for the selected females. The control strain males however are significantly better able to survive in the dry container as compared to the selected strain males. Control strain females likewise showed a significantly higher proportion survivorship than females from the selected strains. These results were analyzed by using a Clopper and Pearson chart (found in Steel and Torrie, 1960) giving the 99% confidence limits for proportions. Females from each of the 3 strains show a higher proportion survivorship than the males from their strain. It is likely that the larger size of the female is responsible for a more favorable surface area to volume ratio facilitating increased longevity in a dry environment. Superior female resistance to desiccation has been found in other studies of *D. melanogaster* (see Parsons, 1973, for references).

The method of determining the effects of desiccation on longevity in this study is comparable to that used by Murphey and Hall (1969). Survivorship of the control males is similar in both studies. The proportion of control males surviving in this study is 0.590 while the proportion of control males surviving in the previous experiment was 0.567.

The results presented here do not correspond with the findings of Murphey and Hall (1969). "Maze hardness" was not observed in males or females from either the positive or negative geotactic maze selected strains. In fact a decrease in longevity in a dry environment appears in males and females from both selected strains. This decrease in longevity is not unexpected following artificial selection for many generations.

"Maze hardness" or the ability to survive in a dry environment does not seem to be an indispensable correlate of selection for divergent geotactic behavior. The increased longevity observed by Murphey and Hall (1969) can best be explained as a genetic correlate due to pleiotropy, founder effect or linkage. In polygenically controlled traits such as geotactic maze behavior in *D. melanogaster* (Erlenmeyer-Kimling et al., 1962) it is not unexpected that different sets of polygenes would be selected by chance in different selection experiments, leading to observed differences in correlated responses.

References: Erlenmeyer-Kimling, L., J. Hirsch and J.M. Weiss 1962, *J. Comp. Physiol. Psychol.* 55:722-731; Hirsch, J. 1959, *J. Comp. Physiol. Psychol.* 52:304-308; Murphey, R.M. and C.H. Hall 1969, *Anim. Behav.* 17:181-185; Parsons, P.A. 1973, *Behavioral and Ecological Genetics: A Study in Drosophila* (Clarendon Press, Oxford); Steel, R.G.D. and J.H. Torrie 1960, *Principles and Procedures of Statistics* (McGraw-Hill, New York).

Table 1. Proportion of flies from the geopositive, geonegative and control strains surviving a 24 hour desiccation-starvation period (n=200).

Strain	Males	Females
Geopositive	0.260	0.635
Control	0.590	0.835
Geonegative	0.300	0.595

Roberts, D.B. and P.W. Andrews. University of Oxford, England. Immunofluorescence analysis of salivary gland chromosomes.

ized as having antibodies against single or double stranded DNA. The chromosome preparations were washed with saline and incubated with

Salivary gland chromosomes, prepared by fixing and squashing glands in 20% acetic acid, were air dried and incubated with serum from patients suffering from systemic lupus erythematosus (SLE). These sera had previously been characterized as having antibodies against single or double stranded DNA. The chromosome preparations were washed with saline and incubated with fluorescent sheep anti-human immunoglobulin G. The preparations were again washed with saline and mounted in Farrant's medium. They were examined with a Leitz dialux microscope using the U.V. lamp with a BG 12 filter and a K530 barrier filter.

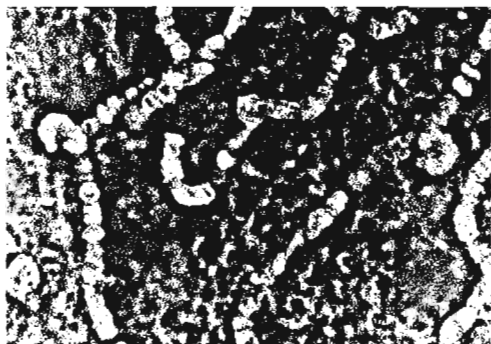


Figure 1a. Phase contrast photograph of polytene chromosome preparation after treatment with SLE serum against double stranded DNA.

than the interbands. The SLE serum against single stranded DNA gave a weak stippled fluorescence on polytene chromosomes. The stippling always occurred but the pattern with reference to the banding pattern varied from experiment to experiment. We conclude that the stippling does reveal regions of single stranded DNA but

The SLE serum, characterised as having antibodies against double stranded DNA, associated with the polytene chromosomes (Figure 1). The fluorescence reflects the concentration of DNA along the chromosome, the bands fluorescing more than the interbands. The SLE serum against single stranded DNA gave a weak stippled fluorescence on polytene chromosomes. The stippling always occurred but the pattern with reference to the banding pattern varied from experiment to experiment. We conclude that the stippling does reveal regions of single stranded DNA but that these are an artefact of preparation rather than having biological significance.

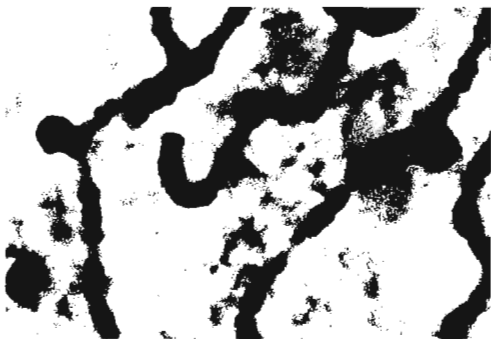


Figure 1b. Same as Figure 1, showing the fluorescence. Preparations incubated with normal human serum showed no fluorescence. The fluorescent antiserum was first incubated with pig liver powder to reduce non-specific association with the preparation.

vation was not reproducible. It seems likely that the techniques used to prepare the chromosomes remove or denature the associated proteins and that occasionally, due to vagaries of technique, some proteins remain.

A similar experiment was carried out with antisera prepared against *Drosophila* chromatin and various chromatin fractions. Occasionally fluorescent chromosomes were observed but this observation was not reproducible. It seems likely that the techniques used to prepare the chromosomes remove or denature the associated proteins and that occasionally, due to vagaries of technique, some proteins remain.

Sewell, D.F. The University, Hull, England. Effect of temperature variation on locomotor activity in *D. melanogaster*: A comparison of behavioural measures.

amplifier (Kaplan and Trout, 1969), and activity measured by a time sampling technique (Hay, 1972). Although a number of methods have been adopted, few of these have been compared. Recently, Angus (1974) compared the open-field and time-sampling methods, and concluded that the two measures reflected a similar, and overlapping, array of components of locomotor

In investigations into locomotor activity in *Drosophila*, a variety of measurement methods have been used. These include open-field methods (Connolly, 1966); maze activity (Ewing, 1963); locomotion measured by the number of buzzing events recorded using a microphone and

activity. However, behavioural phenotypes are not just influenced by genotypic variation, as in Angus' study, but are also influenced by environmental variation. In the investigation reported here, locomotor activity measured by the open-field and time sampling methods was examined under conditions of temperature variation.

The flies used came from a laboratory inbred stock of *D. melanogaster*, reared under standard laboratory conditions on an oatmeal-agar-molasses medium, on a light-dark cycle of 14 hours light and 10 hours darkness, at a rearing temperature of $25 \pm 1^\circ\text{C}$. Three experimental temperatures were used, these being 12°C , 17°C and 25°C . The nature of the data provided by the activity measures was:

Open-field: number of 1 cm squares entered in a square perspex arena measuring 10 cm x 10 cm in a 1 minute test period. The data obtained showed a positive correlation between the means and variances, and a logarithmic transformation (base 10) was applied to render the data suitable for parametric analysis.

Time-sampling: this method involved observing individual flies in glass tubes measuring 10 cm x 3.5 mm, and classifying each individual as active or inactive. Over a 1 minute test period, 5 observations were made on each individual, and thus the activity score generated could range from 0 out of 5, to 5 out of 5. Using this method few individuals had scores of zero, and so each individual's activity score could be expressed as a proportion. To render the data suitable for parametric analysis, the data were transformed to an angular scale.

Table 1 gives the means and variance expressed in the transformed scales for locomotor activity at each temperature when measured by the two methods used.

Table 1. Means and variances of the locomotor activity scores obtained using the open-field and time-sampling methods of measurement.

Temperature $^\circ\text{C}$		Open-Field (log transform)		Time-Sampling (angular transform, expressed in radians)	
		Male	Female	Male	Female
	N	20	20	50	50
12	Mean	1.2123	1.0752	1.8397	1.7109
	Variance	0.1210	0.1398	0.3041	0.2964
17	Mean	1.4615	1.3014	1.8349	1.7374
	Variance	0.0941	0.0651	0.3263	0.2764
25	Mean	1.5683	1.4541	1.8891	1.5544
	Variance	0.0601	0.1064	0.4217	0.3790

Analysis of the data by means of 2-way ANOVA designs revealed that when measured by the open-field method, locomotor activity increased with temperature increase ($F = 14.2070$, $df = 2,114$, p less than 0.01), with there also being an overall sex difference ($F = 5.7720$, $df = 1,114$, p less than 0.05). On the time sampling method there was no consistent temperature effect ($F = 0.3562$, $df = 2,294$), but there was a significant sex effect ($F = 7.8584$, $df = 1,294$, p less than 0.01). On neither measure was there evidence of a significant interaction factor.

These data suggest that under the conditions of environmental variation used, the two methods are not similarly sensitive, and do not necessarily reflect similar components of activity. Both measures appear to be sensitive to genotypic differences as reflected by the presence of a sex difference on both measures. However, the open-field measure appears to be the more sensitive to environmental variation. This implies that in investigations examining locomotor activity in *Drosophila* it is important to specify the measuring device used, as these data indicate that the conclusions drawn may vary according to the nature of the measurement. The data also throw doubt on whether these two methods do reflect similar and overlapping components of activity, and raise the question as to what is the nature of locomotor activity in *Drosophila*.

References: Angus, J. 1974, *Animal Behaviour* 22:890-898; Connolly, K. 1966, *Animal Behaviour* 14:444-449; Ewing, A. 1963, *Animal Behaviour* 11:369-378; Hay, D.A. 1972, *Hered.* 28:311-336; Kaplan, W.D. and W.E. Trout 1969, *Genetics* 61:399-409.

Costa, R., G.A. Danieli and E. Rodino'.
Universita di Padova, Italy. A new allele
at the ODH locus in *Drosophila melanogaster*.

Polymorphism at the ODH locus has been studied
by several authors in many species of *Drosophila*.
Some species, like *D. albirostris* show a
multibanded ODH zymogram which is interpreted
as indicative of a high degree of polymorphism

(Ogonji, 1971).

In *D. melanogaster* the ODH pattern is usually given by a single cathodical band, referred to as F (Fast). Less frequently, individuals show a less cathodical band, named S (Slow). Heterozygous S/F show a characteristic three-band pattern. According to Courtright et al., (1966), these three ODH forms represent the three possible dimers formed by interaction of two subunits, each controlled by its ODH allele.

Possible polymorphism at the ODH locus in *D. melanogaster* has been investigated in our laboratory by sampling wild populations of a vineyard near Padua (Italy) in the fall of 1974.

Electrophoresis of crude extracts was performed on acrylamide gel vertical slabs according to Wright (1963) and Ayala (1972).

In acrylamide gel, the most common form migrates slowly toward the anode; on the contrary, the so-called S form is more anodical.

In addition to the phenotypes described above, an alternative, more cathodical form was detected as heterozygous.

In order to avoid possible misunderstanding, we refer to the "Fast" migrating form in starch gel as M (most common form), to the "Slow" form as L (less common), and to the new form as R (rare).

The situation is summarized in Figure 1. The results of the sampling are given in Table

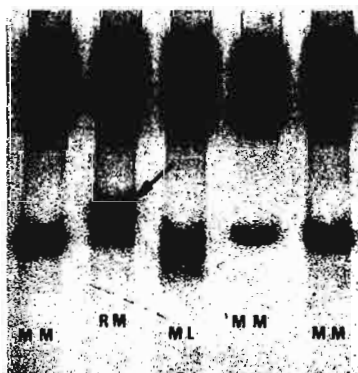


Figure 1. ODH patterns showing the presence of a slower form (R).

ODH PHENOTYPES		GENE FREQUENCIES	
RR	-	ODHR	1,62
MM	903	ODHM	97,47
LL	-		
ML	17		
RL	-	ODHL	0,89
RM	31		

Table 1. Results of the sampling for ODH locus variants.

1. Further investigations on a larger sample and on samples from different places should help to find out the real extent of ODH locus polymorphism in *D. melanogaster*.

References: Ogonji, G.O. 1971, J. Exp. Zool. 178:513-522; Courtright, J.B., R.B. Imberski, H. Ursprung 1966, Genetics 54:1251-1260; Wright, T.R.F. 1963, Genetics 48:787-801; Ayala, F.J., J.R. Powell 1972, Biochem. Genet. 70:331-345.

Atkinson, W. University of Leeds, England. The effect of aging on the attractiveness of bananas to domestic species of *Drosophila*.

During August 1973 flies were trapped around the buildings of a wholesale fruit market near Leeds using bananas of different ages as bait. pH was used as a measure of the physiological age of the banana.

Seven species of *Drosophila* were captured in the following frequencies: *D. melanogaster* 441; *D. simulans* 28; *D. immigrans* 49; *D. funebris* 38; *D. hydei* 66; *D. busckii* 9 and *D. subobscura* 90. Figure 1 shows the mean pH of bananas at each age together with the mean numbers of *D. melanogaster* per trap for each age and

Table 1 shows the mean age and pH of banana on which each species was captured. The number of flies captured rose with age of banana, reached a peak at an age which depended on the species, then fell.

Table 1

Species	Mean age of banana \pm 2SE (in weeks)	Mean pH \pm 2SE
<i>D. melanogaster</i>	2.38 ± 0.13	4.03 ± 0.05
<i>D. immigrans</i>	2.43 ± 0.44	4.17 ± 0.19
<i>D. simulans</i>	2.96 ± 0.62	4.01 ± 0.25
<i>D. subobscura</i>	3.02 ± 0.29	4.06 ± 0.08
<i>D. busckii</i>	3.11 ± 0.91	4.31 ± 0.44
<i>D. hydei</i>	3.15 ± 0.44	4.51 ± 0.22
<i>D. funebris</i>	3.18 ± 0.43	4.16 ± 0.19

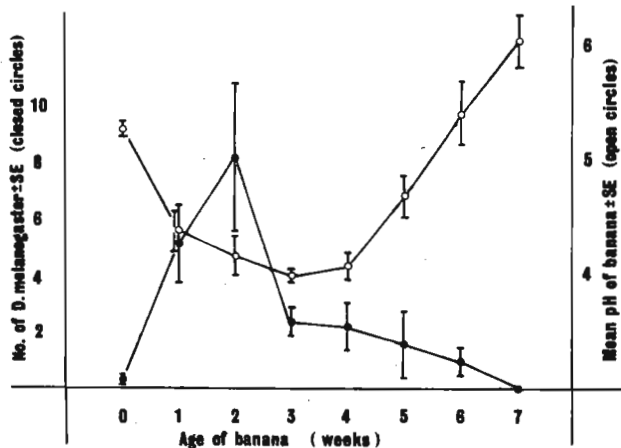


Figure 1

A comparison among the means using the SNK test is shown below. Species with means not significantly different from each other have been underlined.

Rank mean age						
1	2	3	4	5	6	7
<u>melanogaster</u>	<u>immigrans</u>	<u>simulans</u>	<u>subobscura</u>	<u>busckii</u>	<u>hydei</u>	<u>funebris</u>
Rank pH						
1	2	3	4	5	6	7
<u>simulans</u>	<u>melanogaster</u>	<u>subobscura</u>	<u>funebris</u>	<u>immigrans</u>	<u>busckii</u>	<u>hydei</u>

The species can broadly be separated into two groups with respect to age of banana visited, those that are attracted most before three weeks (*D. melanogaster*, *D. immigrans* and *D. simulans*) and those that are attracted after three weeks. Three weeks is an important age biochemically in the decomposition of banana as its pH then is at a minimum. The *Drosophila* species are readily separable on the basis of mean pH of banana visited and knowing which species are attracted before the minimum pH is reached and which are attracted after a chronological sequence of *Drosophila* species visiting bananas can be suggested:

immigrans - *melanogaster* - *simulans* - *subobscura* - *funebris* - *busckii* - *hydei*

The fall in pH of bananas may well be associated with the formation of ethanol and its conversion by bacteria to volatile acids, the subsequent rise in pH being due to some unknown process, possibly the evaporation of acids or the deamination of proteins.

D. melanogaster, *D. simulans* and *D. immigrans* are usually described as being bred from fermenting fruits and *D. hydei*, *D. funebris* and *D. busckii* from decaying plant matter. Though in this experiment the use of bananas as feeding or breeding sites was not distinguished, the association with fermentation or decay appears to be confirmed.

Tsuchiyama, S. and B. Sakaguchi. Kyushu University, Fukuoka, Japan. Relationship between the killing action of the Sex-Ratio spirochete and the rRNA gene deficient mutant of *D. melanogaster*.

Sex-Ratio (SR) spirochete (identified with spiroplasma at present) selectively kill male zygotes, but not females, in *Drosophila*. Attached-XY females, intersexes of 3N autosome and 2N X chromosome and phenotypic males of attached-XY, *tra/tra* are not killed by the SR spirochetes (Sakaguchi and Poulson, 1963).

In order to get further information on the relationship between the gene constitution of the X chromosome and killing action of SR spirochetes, a deficient strain of rRNA gene was used. Males and females of the genotype are both present in the stock designated *In(1)sc^{4L}sc^{8R}, y sc^{4L}sc^{8R} cv v B/C(1)DX, y f/BS^y*, obtained from the collection at Oak Ridge National Laboratory.

To test the effects of SR spirochetes on the progenies from the strain of deficient rRNA genes, hemolymph of the SR strain of *D. nebulosa* was introduced into five young adult *C(1)DX/Y* females and mated with *In(1)sc^{4L}sc^{8R}/Y* males. Counts of eggs and adults from the injected females were made for the every-three-days broods. As a control experiment, hemolymph of the normal strain of *D. nebulosa* was introduced into five young adult *C(1)DX/Y* females. Results of these experiments are shown in Table 1.

Table 1. Hatchabilities and sex-ratio of progenies from *C(1)DX, y f/BS^y* injected with hemolymph of the SR strain of *D. nebulosa* and mated with *In(1)sc^{4L}sc^{8R}, y sc^{4L}sc^{8R} cv v B*. (First generation from injected females.)

Brood (days)		1-3	4-6	7-9	10-12	13-15	16-18	19-20
Injected	No. of eggs	271	637	1485	1659	1815	1863	360
	No. of ♀ or ♂ (Expected)	136	319	743	830	908	932	180
	No. of ♀ or ♂ (Observed)	45:20	108:31	245:30	245:18	231:0	156:0	21:0
	Hatchability of ♀ and ♂ (%)	33:15	34:10	33:4	30:2	25:0	17:0	12:0
Control	No. of eggs	300	1088	1626	1777	1952	726	200
	No. of ♀ or ♂ (Expected)	150	544	813	899	976	381	100
	No. of ♀ or ♂ (Observed)	41:26	126:63	205:58	205:52	110:30	56:7	15:2
	Hatchability of ♀ and ♂ (%)	27:17	23:12	25:7	23:6	11:3	15:2	15:2

Hatchabilities were calculated from the expected number of females or males derived from the number of eggs and the observed number of adults. Rates of the hatchabilities of female progenies from the female injected with the SR hemolymph were slightly higher than that of the control throughout every brood. On the other hand, rate of the hatchabilities of male progenies from the female injected with the SR hemolymph were somewhat lower than that of the control. Complete disappearance of males from the 13th day brood in the progenies of the female injected with SR hemolymph was observed.

Rates of the hatchabilities of male and female progenies from both the control and the injected series in this experiment were very low compared with about 80% of the normal strain of *D. melanogaster* in any case. In particular, hatchabilities of males were distinctly poor in both the experimental and the control groups. It can be said from the results that the deficiency of rRNA genes of X chromosomes remarkably affect hatchability or viability. For this reason, the effect of the SR agent on the deficient strain of rRNA genes was hidden by the striking effect of the deficiency of rRNA genes on hatchability or viability.

Reference: Sakaguchi, B. and D.F. Poulson 1963, *Genetics* 48:841-861.

Bates, D., L. Ehrman and I. Perelle. State University of New York, Purchase. Intersemispecific hybridity and egg hatchability in *D. paulistorum*.

Hybrid male sterility in *D. paulistorum* appears to be determined by nonagreement of the semi-species of the mother of the female and that of the donor of the sperm, i.e., an incompatibility of the male genotype with mycoplasma-like organisms (MLO) unique to each semispecies and con-

tained in their cytoplasm. Additionally, abnormal pole cell development, often leading to embryo disintegration has been found in hybrids again in the presence of MLO (Daniels, S. and

L. Ehrman 1974). To determine if those factors responsible for hybrid male sterility resemble those occurring in aberrant embryo development differences in egg hatchability were investigated by recording the proportions of eggs hatching into first instar larvae in pure strains and in intersemispecific F_1 and BC_1 hybrids of Uaica, Brazil, Andean semispecies (U) with Mesitas, Colombia, Andean semispecies (M) and New Llanos, Colombia, close to Interior semispecies (L) with Georgetown, Guyana, Orinocan semispecies (O). For classification details concerning L and O see Dobzhansky, Pavlovsky and Powell 1976. Hatchability scores were subjected to an analysis of variance to determine which factors or interaction of factors were significant. Methods: Virgin females (1-5 days of age) and males were placed together for four days. One hundred gravid females (immobilized with CO_2) were then placed in 1/2 pint culture bottles containing a spoon filled with blue food and left undisturbed 3-6 hours whereupon 200 eggs for pure strains and 100 eggs for all other crosses were gently removed from the medium and placed in tissue culture dishes, twenty per dish, on a thin layer of blue medium. Forty eight to sixty hours later all larvae were scored by microscopic inspection of slides with thin spreads of well dampened medium. The increased moisture facilitated scoring by stimulating larval movement and final flooding of tissue culture dish "picked up" those remaining by causing floating and/or swimming.

Means and standard deviations of larvae per tissue culture dish considering all possible F_1 and BC_1 crosses of U with M and L with O.

U with M	Mean	S.D.	L with O	Mean	S.D.
$(U_{\text{♀}} \times U_{\text{♂}})_{\text{♀}} \times U_{\text{♂}}$	13.00	2.79	$(L_{\text{♀}} \times L_{\text{♂}})_{\text{♀}} \times L_{\text{♂}}$	2.60	1.35
$(U_{\text{♀}} \times U_{\text{♂}})_{\text{♀}} \times M_{\text{♂}}$	9.60	3.30	$(L_{\text{♀}} \times L_{\text{♂}})_{\text{♀}} \times O_{\text{♂}}$	6.60	2.30
$(U_{\text{♀}} \times M_{\text{♂}})_{\text{♀}} \times U_{\text{♂}}$	0.80	0.83	$(L_{\text{♀}} \times O_{\text{♂}})_{\text{♀}} \times L_{\text{♂}}$	4.00	1.58
$(U_{\text{♀}} \times M_{\text{♂}})_{\text{♀}} \times M_{\text{♂}}$	10.00	0.71	$(O_{\text{♀}} \times L_{\text{♂}})_{\text{♀}} \times L_{\text{♂}}$	4.60	1.34
$(M_{\text{♀}} \times U_{\text{♂}})_{\text{♀}} \times U_{\text{♂}}$	7.40	3.65	$(L_{\text{♀}} \times O_{\text{♂}})_{\text{♀}} \times O_{\text{♂}}$	1.40	1.52
$(M_{\text{♀}} \times U_{\text{♂}})_{\text{♀}} \times M_{\text{♂}}$	3.60	1.82	$(O_{\text{♀}} \times L_{\text{♂}})_{\text{♀}} \times O_{\text{♂}}$	0.60	0.55
$(M_{\text{♀}} \times M_{\text{♂}})_{\text{♀}} \times U_{\text{♂}}$	8.40	2.88	$(O_{\text{♀}} \times O_{\text{♂}})_{\text{♀}} \times L_{\text{♂}}$	4.40	1.52
$(M_{\text{♀}} \times M_{\text{♂}})_{\text{♀}} \times M_{\text{♂}}$	14.80	2.29	$(O_{\text{♀}} \times O_{\text{♂}})_{\text{♀}} \times O_{\text{♂}}$	8.30	2.36

Little ethological isolation has been found between L and O and they are thought to be closely related (Dobzhansky and Pavlovsky 1974; Dobzhansky, Pavlovsky and Powell 1976). The sperm storage organs of 50 of the U females were dissected in physiological saline and only 2 were found uninseminated by M males; 3 out of 50 $F_1(M_{\text{♀}} \times U_{\text{♂}})_{\text{♀}}$ dissected were found uninseminated by U males. From this we assume little ethological isolation between U and M.

Results of analysis of variance applied to hatchability scores.

Factors	U with M				L with O			
	D.F.	S.S.	M.S.	F.	D.F.	S.S.	M.S.	F.
Mother of female (A)	1	0.08	0.08	0.07	1	1.36	1.36	2.62
Father of female (B)	1	0.02	0.02	0.02	1	1.71	1.71	3.29
A x B	1	72.00	72.00	64.67**	1	15.96	15.96	30.76*
Donor of sperm (C)	1	8.82	8.82	7.92*	1	0.21	0.21	0.41
A x C	1	1.28	1.28	1.14	1	0.28	0.28	0.54
B x C	1	64.98	64.98	58.36**	1	0.21	0.21	0.41
A x B x C	1	0.72	0.72	0.64	1	26.28	26.28	50.66**
Error	42	46.76	11.13		42	21.79	51.88	

* $p < 0.01$

** $p < 0.0001$

No consistent single factor was found significant. Various interactions of factors were significant with A x B consistent between series. There appears to be no concurrence with those conditions known to determine hybrid male sterility. Thus the genetic architecture of egg hatchability in this superspecies is unlike that of its hybrid male sterility and seems to differ among these evolving semispecies.

References: Daniels, S. and L. Ehrman 1974, J. Inv. Path. 24:14-19; Dobzhansky, Th. and O. Pavlovsky 1974, Evol. 29:242-248; Dobzhansky, Th., O. Pavlovsky and J. Powell 1976, Evol. (in press).

Köhler, W. Institut für Genetik der Freien Universität Berlin, Germany. Selection for positive and negative phototactic behavior in presence of a multiply marked X-chromosome in D.m.

the males carried either a wild-type (+K) or a marked (106) X-chromosome (1:1). The phototactic behavior of about 500 males and virgin females was tested separately in Hirsch-Hadler mazes (Hadler, 64). The 30 most photopositive or most photonegative males and females were used as the parents of the next generation. A random sample of 30 pairs out of the unselected hybrid strain constituted the control line. To each strain there existed a parallel line in a second maze. The mazes were constructed according to the design of Hadler (64) and each maze consists of 13 consecutive Y-units. The light intensity varied from 900 - 1100 Lux, the temperature from 24 - 27°C both measured on the upper surface of the maze.

The selection responses of the different lines are shown in Figure 1 (uppermost). The control line is omitted as there was only a slight difference from the population selected for positive phototaxis (POS). It is obvious that the hybrid population and its foundation populations were extremely photopositive and therefore the selection on positive phototaxis did not succeed and the difference from the control line was not significant. The response in the opposite direction (NEG) is unequivocal and nearly linear. There were no different results in the parallel series.

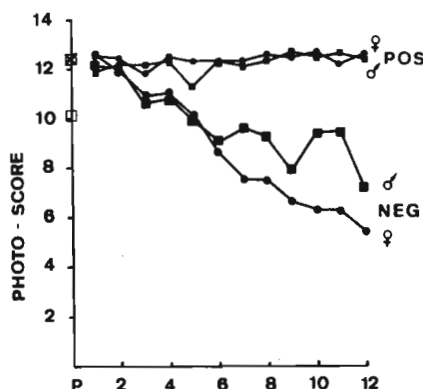
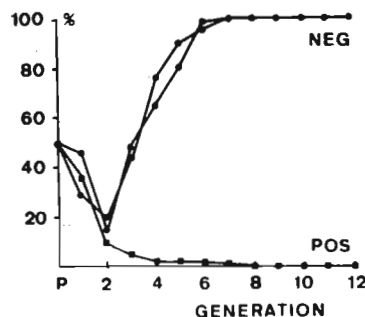


Figure 1. The response of the hybrid strain over 12 generations of selection for positive (POS) and negative (NEG) phototactic behavior (uppermost) and the corresponding frequencies of the v, g - linkage group (bottom). Open squares indicate the mid-parent values of +K (⊗) and 106 (□).



In addition to the test on phototactic behavior we recorded the markers of each male in each generation and line. Figure 1 (bottom) demonstrates the outcome of the relative frequencies of the linkage group v, g in the different selected lines and their repetitions. After 6 generations of selection, the linkage group was lost in the photopositive strains (POS) while in the photonegative strains (NEG), after an analogous decline in the first two generations, v and g were fixed in the 6th and 7th generation, respectively, of selection.

In a second experiment the 22nd generation of the negative line NEG of maze 2 homozygous for v and g was crossed with a strain of wild-type eye colour selected 38 generations for negative phototaxis in a Hirsch-Hadler maze by Pulvermacher (NEPU). Starting with the F₂, these hybrid flies were selected for negative phototactic behavior and the frequencies of v and g were recorded. In the F₁ we observed a strong influence of the X-chromosome on the phototactic behavior of the males and a decrease in negative phototaxis below the mid-parent values in females of both crosses (Table 1). While the unselected control (CON) varied about these scores, the selection in the negative direction (SEL) was successful (Figure 2, uppermost). Figure 2 (bottom) shows the outcome of the relative frequencies of the v, g - linkage group during 14 generations. In the line SEL selected for negative phototaxis v and g were fixed and in the unselected control (CON) they were lost.

The results may be summarized as follows: In our Hirsch-Hadler mazes wild-type D.m. proved to be extremely photopositive. Therefore only directional selection for negative phototactic behavior was successful. All selected hybrid populations indicated the character-

istic of an interaction between phototaxis and the v, g - linkage group. Besides a possible hitch-hiking effect (Maynard Smith and Haigh, 74) of favourable genes close to v and g, this

Table 1. Photoscores of the negative phototactic strains NEG and NEPU and their reciprocal hybridizations.

strain	♀♀ (maze 2)		♂♂ (maze 1)	
	\bar{x}	SD	\bar{x}	SD
NEG	4.13	4.10	4.86	4.46
NEPU	7.93	4.57	7.02	4.30
NEG (♀) × NEPU (♂) ^a	8.84	3.57	4.60	4.28
NEPU (♀) × NEG (♂) ^a	9.36	3.48	7.91	4.29

^a pooled data out of two repetitions

may be an effect of the eye pigmentation (Hengstenberg und Götz, 67; Wehner et al., 69) or a hint at a possible biochemical component of phototactic behavior (Pak et al., 69). The advantage of this linkage group for negative phototaxis was strong enough to compensate for the antagonistic influence of recombination and a disadvantage of the linkage group because of the presence of inferior fitness alleles. The decrease in the frequencies of v and g may be a hint to this disadvantage. It was absent in the selected hybrid population of the two negative phototactic strains (Figure 2, bottom). The results of the

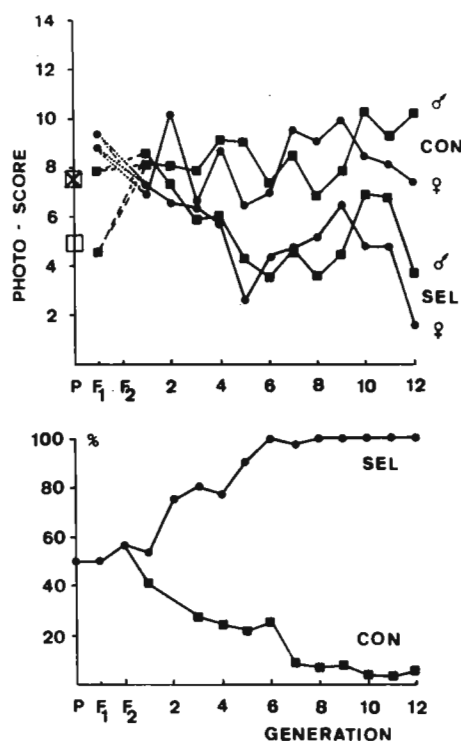


Figure 2. The response of the hybrid strain NEG/NEPU over 12 generations of selection for negative (SEL) phototactic behavior and its unselected (CON) control (uppermost). The second figure (bottom) shows the corresponding frequencies of the v, g - linkage group. Open squares indicate the mid-parent values of NEG (□) and NEPU (⊠).

selection of these hybrids indicate that different factors for negative phototactic behavior were selected in both preceding experiments. Furthermore, the reciprocal hybridization and the selection experiments revealed the importance of the X-chromosome especially in connection with the linkage group v, g (Table 1).

References: Hadler, N. 1964, DIS 39:131-133; Hengstenberg, R. und K.G. Götz 1967, Kybernetik 3:276-

285; Maynard Smith, J. and J. Haigh 1974, Genet. Res. 23:23-35; Wehner, R., G. Gartenmann and Th. Jungi 1969, J. Insect Physiol. 15:815-823.

Mark, H.F.L.*, Brown University, Providence, Rhode Island. Nonrandom disjunction in no exchange tetrads?

Novitski (1951) postulated that given a pair of heteromorphic homologues and exchange creating asymmetric dyads, nonrandom genetic recovery of the reciprocal crossover products will result in the *Drosophila* female. The biological basis for

this nonrandom recovery is hypothesized to be due to the preferential inclusion of the shorter elements of the asymmetric dyads into the two outer meiotic products, one of which invariably becomes the egg. Since all dyads from no exchange tetrads (NET) are symmetric, no nonrandom

genetic recovery will be predicted. Subsequent work (for example, Weltman, 1954; Zimmering, 1955a, 1955b; Novitski, 1967; Mark, 1974) in the female has confirmed the original observations of Novitski (1951), mainly that nonrandom recovery of the shorter elements is observed where asymmetric dyads are created, and that no nonrandomness has been reported from situations where only NET's are produced.

Zimmering and Bendbow (1973) reported the first instance of nonrandom genetic recovery due to production of asymmetric dyads in the male. The above authors considered the experimental data to be entirely consistent with the hypothesis of NRD (Novitski, 1951). Additional support for the hypothesis of NRD in the male based on the data of Mark (1974) will be discussed elsewhere. The present note concerns itself with the clarification of an ambiguous point raised by the data of Zimmering and Bendbow (1973) where a statistically significant difference in the reciprocal noncrossovers was found. The question arises whether there is nonrandomness in NET's as well as in ET's, although the degree and direction of nonrandomness may be different in the two kinds of tetrads.

To test such a possibility, $Px^{-2}/V4$ Dr^{Mio} males were crossed to $V4/Cy$ females. The use of dominant markers in the male enables the two crucial modes of disjunction (alternate versus adjacent I) to be distinguished. The use of the Cy inversion in the female serves as a crossover suppressor, ensuring that all products are NCO's..

Figure 1 gives the pairing configuration of the male and the female parents. Table 1 gives the consequences of different kinds of disjunction in the male and the female parents. It can be seen from Table 1 that only six out of the possible sixteen products contain bal-

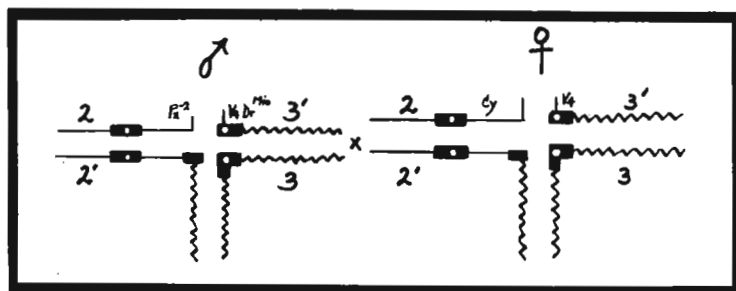


Figure 1. Pairing configuration in the male and female parents.

anced amount of chromosomal material. And since $V4$ Dr^{Mio} $V4$ flies are almost always lethal due to homozygosity for $V4$, only five fully viable classes are available for comparison.

Under the hypothesis of no non-randomness in NET's in the male as well as in the female, the following results would be predicted: $f(Px^{-2}Cy) = f(Px^{-2}V4) = f(V4 \text{ } Dr^{Mio}Cy)$; and $f(V4 \text{ } Cy) = f(Px^{-2}V4 \text{ } Dr^{Mio})$, given that viability differences caused by markers are taken into account. f is the frequency of recovery of flies of various phenotypes. Under the alternate hypothesis, however, the following would be predicted: $f(Px^{-2}Cy) \neq f(Px^{-2}V4) \neq f(V4 \text{ } Dr^{Mio}Cy)$; and $f(V4 \text{ } Cy) \neq f(Px^{-2}V4 \text{ } Dr^{Mio})$, given that viability differences are taken into account.

The results of the above experimental cross are given in column 2 of Table 2. From inspection, it can be seen that the first three classes are roughly equal, as are the fourth and fifth. The close agreement between the numbers in the first three classes and the next to last two are further supported by the data on viability effects of mutant markers presented in Table 3.

From Table 3, it can be concluded that the viability of $V4$ is roughly equal to that of Cy which is in turn roughly equal to that of Px^{-2} . Furthermore, the viability of $V4 \text{ } Dr^{Mio}$ is approximately .8750 that of $TM6$. If one can assume that the viabilities of $V4$, Cy and Px^{-2} are approximately equal to that of wild type, and that the viability of wild type is equal to or slightly higher than that of $TM6$, then the viability of $V4 \text{ } Dr^{Mio}$ is approximately .8750 that of any of the other genotypes mentioned above. Therefore, from Table 3, it can be concluded that Dr^{Mio} does depress viability somewhat and that the slight depression in the numbers of $Cy \text{ } V4 \text{ } Dr^{Mio}$ and $Px^{-2} \text{ } V4 \text{ } Dr^{Mio}$ flies is probably due to the presence of Dr^{Mio} . When this is corrected, $f(Cy \text{ } V4 \text{ } Dr^{Mio})$ and $f(Px^{-2} \text{ } V4 \text{ } Dr^{Mio})$ become 3348.6 and 832, respectively. The results of Chi-square tests of the null hypothesis of no difference in the frequency of the various classes after viability corrections are given in the last column of Table 2. The

inference from the above is that the prediction of the hypothesis of Novitski (1951) on NRD is again supported: NRD occurs only as a consequence of exchange and creation of asymmetric dyads. The possibility that there is NRD in NET's in the male and in the female but that the nonrandomness is in different directions in the two sexes and therefore cancelling each other out exactly is extremely improbable, although this possibility cannot be ruled out experimentally in the present setup.

Table 1. Consequences of different kinds of disjunction in the male and the female.

Male		Female			
		Alternate & Adj. II disjunctions		Adjacent I disjunction	
		(2+3)or(2+2'): Cy	(2'+3')or(3'+3): V4	(2+3'): CyV4	(2'+3): +
Alternate and	(2+3)or(2+2'): Px-2	Px-2Cy	Px-2V4	Px-2V4Cy (lethal)	Px-2 (lethal)
Adjacent II disjunctions	(2'+3')or(3'+3): V4DrMio	V4DrCy	V4DrV4 (rarely survive)	V4DrCyV4 (lethal)	V4Dr (lethal)
Adjacent I disjunction	(2+3'): Px-2V4DrMio	Px-2V4DrCy (lethal)	Px-2V4DrV4 (lethal)	Px-2V4DrV4Cy (lethal)	PxV4Dr
	(2'+3): +	Cy (lethal)	V4 (lethal)	V4Cy	+ (lethal)

Table 2. Results and analyses of experimental cross described in Figure 1.

Phenotypes	No. F ₁ observed	No. F ₁ after viability correction	Expected No. F ₁	Chi-square tests*
PxCy	3231	3231	3263.2	Chi-square = 3.14;
PxV4	3210	3210	3263.2	d.f. = 2; p is
CyV4DrMio	2930	3348.6	3263.2	between .10 & .25
PxV4DrMio	728	832	849	Chi-square = 0.68;
CyVr	866	866	849	d.f. = 1; p is
V4DrMioV4	42	-	-	between .25 & .50

* H₀: numbers of F₁'s are equal for all classes.

Table 3. Data on viability effects of mutant markers used.

Parental crosses	No. F ₁ produced	Relative viabilities (W's)
V4/Cy ♀♀ x +/+ ♂♂	f(V4) = 2775 f(Cy) = 2791	W(V4) : W(Cy) ≈ 1:1
Px-2/Cy cn ² L ⁴ sp ² ♀♀ x V4DrMio/TM6 ♂♂	f(Cy) = 1142 f(Px-2) = 1149	W(Cy) : W(Px-2) ≈ 1:1
Px-2/Cy cn ² L ⁴ sp ² ♀♀ x V4DrMio/TM6 ♂♂	f(V4DrMio) = 1379 f(TM6) = 1576	W(V4DrMio) : W(TM6) ≈ .8750:1

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References: Mark, H.F.L. 1974, Ph.D. thesis, Brown University, Providence, R.I.;
Novitski, E. 1951, Genetics 36:267-280; Novitski, E. 1967, Ann. Rev. Genet. 1:71-86; Weltman, A.S. 1954, DIS 28:166; Zimmering, S. 1955a, Genetics 40:809-825; Zimmering, S. 1955b, DIS 29:174; Zimmering, S. and E. Bendbow 1973, Genetics 73:631-638.

Davis, B.K. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Cytological mapping of the meiotic mutant, mei-S332.

Lindsley et al. (1972, see also Seattle-La Jolla Drosophila Laboratories, 1971) have described the use of a series of Y-autosome translocations for producing autosomal duplications and deficiencies. Here I describe the use of these

translocations to test the effects of dosage of the meiotic mutant, mei-S332 and to map it cytologically. The recessive mutant has been shown to cause precocious centromere division which results in frequent second divisional nondisjunction for all chromosomes in both sexes (Davis, 1971). It maps genetically at position 95 on the right arm of chromosome 2.

By crossing appropriate translocations, six deficiencies were generated which collectively span the region between 54F and 58E. Each was then made heterozygous with mei-S332. Four of the six were tested in males which were YSM·Y^L, In(1)EN, y/T(Y;2)def/mei-S332;spa^{Pol}/+ where T(Y;2)def refers to the deficiency combination. Males were singly mated to either C(1)RM, y v bb/B^SYy⁺ females or C(1)RM, y pn v/Y;C(4)RM, ci ey^R/O females. The only deficiency which produced a significant number of exceptional progeny was the combination of R104 and H158 which is deficient for 58A to 58E. Among 408 total progeny from crosses to C(1)RM, y v bb/B^SYy⁺ females were 42 y⁺ v⁺ B^S females resulting from diplo-X sperm and 7 triploid females. In addition, there were 98 y v females which could have resulted either from 3:1 segregation of the translocation or from second divisional nondisjunction of YSM·Y^L. Almost all probably resulted from the latter since control crosses in which the deficiency was heterozygous with mei-S332⁺ produced only 1 y v female among 234 progeny. Control crosses produced no triploid or y⁺ v⁺ B^S females.

Five of the six deficiencies were tested in females which were y/YSM·Y^L, In(1)EN, y; T(Y;2)def/mei-S332;spa^{Pol}/+. They were mated singly to YSM·Y^L, In(1)EN, v f B/O;C(4)RM, ci ey^R/O males. Again the only deficiency which produced frequent exceptional progeny was the R104-H158 combination. Among 59 progeny, there were 5 v f B males from nullo-X eggs, 6 y females from diplo-X eggs, 3 spa^{Pol} flies from diplo-4 eggs and 18 ci ey^R flies from nullo-4 eggs.

Measurement of the rates of nondisjunction is confounded by the various segregation patterns of the translocations. This can be avoided by rejoining the two elements of the translocation to produce a single chromosome. This technique is described in a second report in this issue.

To confirm the results of the deficiency tests, 4 stocks were constructed which carried terminal duplications for 2R varying from 58E to the tip to as long as 56C to the tip. These duplications are the elements from the reciprocal translocations marked by either y⁺ or B^S and with an incomplete Y chromosome capped by the autosomal tip. Males which were y/y⁺Y/Tdp; mei-S332/mei-S332;spa^{Pol}/spa^{Pol}, where Tdp represents the terminal duplication, were crossed to C(1)RM, y pn v/Y;C(4)RM, ci ey^R/O females. The terminal duplication from the translocation H158 (58E to the tip) does not include the mei-S332 locus since the phenotype was mutant. Among 99 progeny were 2 y females from diplo-X sperm, 9 spa^{Pol} progeny from diplo-4 sperm, 14 ci ey^R progeny from nullo-4 sperm, and 1 intersex.

The next longest duplication was 57E to the tip (A120). Males which were homozygous for mei-S332 and carried the duplication produced no exceptional progeny. Thus, the duplication must have contained mei-S332⁺. The other two stocks with yet longer duplications produced the same results.

It is concluded that: 1) the mei-S332 locus lies between 58A and 58E, 2) a deficiency for the mei-S332 locus is mutant, and 3) one wild type allele with two mutant alleles has wild type phenotype.

References: Davis, B.K. 1971, Molec. Gen. Genetics 113:251-272; Lindsley, D.L. et al. 1972, Genetics 71:157-184; Seattle-La Jolla Drosophila Laboratories 1971, DIS 47 supplement.

Michutta, A., C. Pulvermacher and J. Krause
Institut für Genetik der Freien Universität
Berlin, Germany. Mating behavior in different strains of *Drosophila melanogaster*.

The strains used in the experiment were: +K (Berlin wild), f (forked), y (yellow), y w (yellow, white), w (white). In order to get the same autosomal background in the strains, there was used a balancer system (inversions and dominant markers with recessive lethal effect) to

substitute the autosomes for those of a wild population, captured during 1973 in Berlin. The

elements of mating behavior were investigated, i.e. o (orientation), v (vibration), l (licking), ac (attempted copulation) and cc (completed copulation) (Bastock and Manning 1955).

After eclosion females and males were separated and stored in culture vials for three days. For observation of mating behavior, one male and one female of the same strain were introduced without anesthetization into a glass cylinder of 2.5 cm diameter and 0.4 cm height.

The activities of each couple were observed under a binocular for 250 seconds, using light intensities of 800-1000 lux. Every 5 seconds the courtship actions were recorded. The usual observation period was between 6 and 10 p.m. The laboratory temperature was about $25 \pm 1^{\circ}\text{C}$.

The differences between the 5 strains can be found in the number of observations showing no mating behavior at all and in the number of couples which copulated within 6.5 minutes. Couples showing less than 3 courtship actions during the time of 250 seconds were not taken into consideration.

From 87 observations of +K flies, only 7 couples showed no mating behavior at all; this number is much higher in flies of phenotype y w and w (Table 1). The same tendency can be seen in the frequency of completed copulations.

Table 1. The numbers and proportions (%) of mating behavior elements for each strain

Phenotype	Number of observations	Precopulatory mating behavior occurrences (o+v+l+ac)	Copulation	Possible mating behavior	Observed mating behavior (o+v+l+ac)	Observed mating activity
+K	87	80 (92%)	40 (50%)	2926	3166	108.2%
f	88	68 (77.3%)	32 (36.4%)	2632	2390	90.8%
y	89	72 (80.9%)	11 (12.4%)	3317	2616	78.9%
w	67	35 (52.2%)	3 (4.5%)	1673	468	28.0%
y w	62	31 (50.0%)	3 (4.8%)	1497	416	27.8%

In mating activity (all mating actions taken collectively) there are clear differences among the strains. "Possible mating behavior" describes the number of actions, which are possible during the observation time of 250 seconds (50 actions), not considering that several elements of mating can be found at the same time. Comparing the number of actions which are possible to those which actually occurred, the "mating activity" can be defined (Table 1). Values from more than 100% can be explained in supposing the occurrence of several mating actions at the same time. Mating activity is high in flies of the phenotype +K, low in y w.

Separating the observed mating actions into the components o, v, l, ac, there can be found distinct differences among the strains (Table 2).

Table 2. The numbers and proportions (%) of particular mating behavior elements for each strain.

- 1) Proportions of mating behavior elements of observed mating activity.
- 2) Relative proportions of mating behavior elements within the strains.

Phenotype	o		v		l		ac	
	1	2	1	2	1	2	1	2
+K	76.5	70.7	26.3	24.3	3.7	3.4	1.8	1.6
f	64.0	70.5	22.3	24.6	3.5	3.8	1.0	1.1
y	56.8	72.0	18.8	23.8	3.0	3.8	0.3	0.3
w	20.0	71.4	6.6	23.7	1.1	3.8	0.3	1.1
y w	23.0	82.7	3.8	13.7	0.9	3.4	0.1	0.2

Analysing the mating behavior elements within the strains and comparing these data with one another, differences are not seen except for y w, where o and v show differences. This leads to the following conclusions: a) there are distinct differences among the strains regarding the frequency of time spent in particular mating activity elements; b) if mating behavior occurs, the proportions among the mating behavior elements are nearly the same in all five strains.

Reference: Bastock, M. and A. Manning 1955, Behaviour 8:85-111.

Fortebraccio, M., F. Scalenghe and F. Ritossa. University of Bari, Italy. Cytological localization of the "ebony" locus in *Drosophila melanogaster*. I.

The "ebony" mutant of *Drosophila melanogaster* is reported to map at position 70.7 on chromosome 3 and to be cytologically localized between bands 93B7 and 93F9.

We have recently obtained a series of new mutants at the ebony locus by X-ray mutagenesis.

Males Sb H/In(3R)C, cd (aged 3-4 days) are irradiated with a dose of 3,000-4,000 r and immediately mated to virgin females (aged 3-4 days) homozygous for the markers e^4 wo ro. The mating is interrupted after 4-5 hours by discarding the males. Females are then allowed to lay eggs and transferred to vials containing fresh food every five days.

The progeny of such a cross is scored for individuals exhibiting the "ebony" phenotype. These can be of two types: type 1, e^4 wo ro/Sb H e^x and type 2, e^4 wo ro/In(3R)C, cd e^x which are phenotypically distinguishable.

Once produced, the new mutant is stabilized as follows: single ebony flies of type 1 are crossed with individuals of genotype In(3R)C, cd/ e^4 wo ro. Progeny of genotype In(3R)C, cd/Sb H e^x are selected and crossed inter se. Due to the extremely low viability of flies homozygous for our In(3R)C, cd chromosome, they constitute a stable line.

Single ebony flies of type 2 are crossed with flies of genotype In(3R)C, cd/Sb H e^4 wo ro. Progeny of genotype In(3R)C, cd e^x /Sb H e^4 wo ro are selected and crossed inter se. They constitute a stable line.

Using this selective scheme, among a progeny of about 25,000 flies we have recovered a total of 7 ebony flies (3 of type 1 and 4 of type 2). Two of these mutations have successfully been lost. The cytological analysis (owing to the non-perfect correspondence between Bridges' map and our photographic map of the region, reference is made here for band identification to: F. Scalenghe e F. Ritossa "Controllo dell' attività genica in *Drosophila*. Il puff al locus ebony e la Glutamina Sintetasi 1". Atti dell' Accademia Nazionale dei Lincei, 1976 in press) on polytene chromosomes of the remaining five has given the following results:

Df(3R) e^{F1} : this mutant, which is carried on an In(3R)C, cd chromosome, appears to be a deletion of the region comprised between bands 93B6-7 and 93E1-2.

Df(3R) e^{F2} : this mutant, carried on an In(3R)C, cd chromosome, is a deletion of the region comprised between bands 93B1-2 and 93E1-2.

Df(3R) e^{F3} : this mutant is carried on a Sb H chromosome and is a deletion of the region comprised between bands 93B6-7 and 93E4-5.

Df(3R) e^{F4} : this mutant is carried on an In(3R)C, cd chromosome and is a deletion of the region comprised between bands 93C1-2 and 93F1-2.

e^{F6} : this mutant is carried on a Sb H chromosome and does not appear to be associated with any cytologically detectable chromosomal rearrangement.

Based on these data the gross cytological localization of the ebony locus can be restricted to the interval between bands 93C1-2 and 93E1-2. In an accompanying note we present further results which allow a more definite localization of this locus.

Waddle, F.R. and I.I. Oster. Fayetteville State University, Fayetteville, North Carolina and Bowling Green State University, Bowling Green, Ohio. Third chromosomal suppressors of Segregation-distorter.

At least 9, probably 10 of 62 tested stocks of the Mid-America Stock Center carry third chromosomes that partially suppress the effect of Segregation-distorter. The SD-bearing chromosome used has a high average K ($\approx .98$) when heterozygous in the absence of suppressors but lacks St(SD). The average K values of SD/+;Su/+

males for the 10 lines ranged from .55 to .90. It is not known whether the differences in average K values among lines reflect real genetic differences or simply variability in test conditions. The lines that carry suppressors (tentatively and collectively symbolized as Su(SD)3) are: b248 sd;(se), h2 aa h, h72 h, h82 in, h125 Mio (= Dr^{Mio}), h155 rsd, h195 sr, h221 su-ve ru ve bv and j421 Y^S.X InEn y.Y^L;bw;e;ci ey^R (this line also carries the new mutant e^4). The line that probably carries Su(SD)3 is b191 m.

Douglas, L., A. Geurts v. Kessel,
R. Douglas and A. Sinnege. Katholieke
Universiteit, Nijmegen, The Netherlands.
Effect of outcrossing on phenotypes of
spa^{pol}/spa^{pol} (= pol/pol) in males and
females of *D. melanogaster*.

In attempting to determine whether heterochro-
matin suppresses development of black pigment in
eyes of pol/pol as Morgan (1947) reported for
spa/spa, we seem to have accidentally discovered
a new effect: a surprisingly large number of
the pol/pol offspring from backcrossed pol/pol⁺
F₁'s had black pigment spots in the eyes (not
greyish-black as in the parental pol/pol used

to make the F₁). That is, the fraction of pol/pol with observable dark pigment spots is much
higher than in the parent pol/pol stock (Table 1) regardless of: (1) whether the pol/+ F₁ was
a male or female, (2) whether male, female or pooled offspring are used as a criterion for
measuring the fraction, or (3) which of the four pol⁺/pol⁺ stocks were used for obtaining the
F₁'s. Since the crosses used (Table 1) do not conspicuously change the amount of heterochro-
matin to levels above or below that in the pol/pol stock, we conclude that other factors are
responsible for the increased fraction of flies with black spots.

Table 1. pol/pol⁺ x pol/pol: Counts of pol/pol⁺-, pol/pol without- and pol/pol
with black pigment spots (= +, 0 and 1 respectively). pol is written
for spa^{pol} and all other abbreviations are standard.

pol/+ parent		offspring					
		♂♂			♀♀		
		+	0	1	+	0	1
sc, cv, v, f	♂	229	33	173	245	7	200
sc, cv, v, f/X ⁺	♀	175	52	103	201	19	127
f	♂	42	6	12	37	6	33
f/X ⁺	♀	48	18	28	46	18	40
se/+	♂	58	25	24	44	56	23
se/+	♀	88	37	58	99	17	67
wild t.	♂	67	59	11	56	26	16
wild t.	♀	79	12	48	69	14	55
Totals		786	242	457	797	163	561
Total pol/+	♂	396	123	220	382	95	272
Total pol/+	♀	390	119	237	415	68	289

pol/pol x pol/pol: 16 ♀♀ with black (= 1) in 1852 total

A further test suggests that pol/+ ♀♀ F₁'s produce roughly 65-75% black offspring in all
four kinds of backcrosses (homogeneity χ^2_3 on black-not black counts \approx 7.2 in male offspring
and 8.7 in females), whereas the % blacks from pol/+ ♂♂ is impressively heterogeneous over the
same four kinds (χ^2_3 corresponding to the above are 114.7 and 173.9, respectively). Also, as
suggested by spa/spa results (Morgan 1947), blackening seems to be more readily induced in
pol/pol ♀♀ than in ♂♂ (the standard $2 \times 2 \chi^2_1 \approx 25.7$).

Reference: Morgan, L.V. 1947, Genetics 32:200-219.

Jeffery, D.E. Brigham Young University,
Provo, Utah. Further attempt to produce
position effect by rearrangement of the
h allele.

Position effects are usually expressed as an
apparent deactivation of a dominant gene. In
such cases, when the rearranged dominant is
placed in heterozygous combination with a reces-
sive allele, the recessive phenotype is permitted
to be expressed in at least some of the relevant

cells. While the mechanisms by which position effects occur are still not understood (see
Spofford 1976 for review), the general concept of inactivation seems to have been sufficiently
pervasive that very few attempts have been made to obtain the opposite situation: the as-
sumption of 'dominance' by a normally recessive allele when its position in the genome is
altered.

Nevertheless, a few such cases are known in *D. melanogaster*. Glass (1933) reported certain rearrangements of *bw* which expressed the *bw* phenotype, variegated, when in heterozygous combination with *bw*⁺, the usually dominant allele. Similarly, Stern and Kodani (1955) found specific rearrangements of *ci* which expressed a variegated *ci* phenotype when combined with *ci*⁺. In the course of studies of position effects of the *h* locus (Genetics, in press), an attempt was made to produce rearrangements of *h* which would express position effect phenotypes when combined with *h*⁺. Homozygous *se h* males were X-rayed with 5000-7000 r acute radiation, at rates of approximately 105 r/minute. These males were mated immediately to wild-type tested virgins. The resulting F₁, all with non-sepia eyes, were scored for the presence of such hairs as would indicate a position effect of *h*. Careful screening was accomplished on the scutellum, mesopleurae, and wings of 47,694 such F₁, with negative results; all appeared wild-type. From a similar attempt, Stern (1944) reported comparable results from a screening of 17,922 progeny. Thus, a total of 65,616 offspring from the above cross have been screened with no positive results. It would appear that, unlike *ci* and *bw*, it is extremely difficult or impossible to produce by rearrangement a situation wherein the recessive mutant *h* allele assumes 'dominance' over the wild-type.

References: Glass, H.B. 1933, J. Genet. 28:69-112; Spofford, J.B. 1976, In: The Genetics of *Drosophila*, vol. 1, M. Ashburner and E. Novitski, eds., in press; Stern, C. 1944 DIS 18:56; Stern, C. and M. Kodani 1955, Genetics 40:343-373.

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Dow, M.A. University of Edinburgh, Scotland. Light regime and temperature effects on mating success.

The mating success of male yellow *D. melanogaster* with wild-type females has been shown to be reduced (Bastock, M., 1956 *Evol.* 10:421-439). Barker (1962 *Gen.* 47:623-640) has studied the effects of age, sex ratio, density and

mating period on the success of yellow males. As temperature and lighting regime have strong influences on metabolism, their effects on the mating success of yellow males were examined in order to determine if an altered general metabolism might be a cause of their behavioral deficiencies.

An inbred wild-type and yellow strain of *D. melanogaster* were used in these experiments. In each test sixty virgin three day old females and males were placed in a 200 ml. bottle containing 40 ml. of propionic acid medium. After 5 days the flies were etherised, the males discarded and the females placed singly in vials to determine whether they had been inseminated. The conditions used are set out in the table:

Light Regime (12 hr:12 hr)		LL	LL	LL	LD
Temperature (°C)		15	20	25	25
Male	Female				
wild	wild	47%	88%	77%	84%
yellow	yellow	90%	88%	66%	100%
yellow	wild	0%	0%	1.7%	3.3%
yellow	wild	0%	0%	1.7%	3.6%
yellow	wild	0%	0%	1.7%	3.4%

The only significant effect was due to the decreased mating success of wild-type at 15°C versus wild-type at 20°C ($P < 0.005$) and versus yellow at 15°C ($P < 0.005$), for which no explanation can be offered. There also appears to be a general trend for increased mating success under alternating 12 hour light:dark conditions (5 of 5 comparisons). As Hardeland (1972 *Anim. Behav.* 20:170-174) has shown that *D. melanogaster* has the peak of its courting rhythm in the dark, this may indicate that a high concentration of courtship activity is beneficial for mating success and that courting pairs stimulate each other. However, this point is equivocal, as others (Averhoff, W.W. and Richardson, R.H., *Behav. Gen.* 1974 4:207-225) have found the peak to be in the light.

One can conclude that temperature and light regime do not have major effects on the mating success of yellow males with wild-type females.

Sreerama Reddy, G. and N.B. Krishnamurthy.
University of Mysore, India. Distribution
of different species of *Drosophila* in
Jogimatti Hills, Chitradurga District,
Karnataka, India.

Evaluation of the collection records from India
indicates that the genus *Drosophila* is widely
distributed in this subcontinent. In spite of
the growing interest in the field observation
of *Drosophila* during recent years, many parts
of the country still remain to be explored as
to the occurrence and distribution of *Drosophila*

fauna. Therefore, a survey of Jogimatti Hills was undertaken. It is located 7 miles south of
Chitradurga Town. The peak of the hill is 3803 feet high, characterised by thick and bushy
vegetation. The average rainfall during the year ranges from 650 mm to 750 mm. A maximum
temperature of 36°C to 38°C has been recorded during summer months. The maximum percentage of
humidity varies from 80 to 85 during monsoon months of June through October.

Two collection trips were made in the months of October and November 1974, soon after the
monsoon period. Each time, four spots were chosen from the base of the hill to the top at
different elevations. The collections were made by usual banana trap method. A total of 1363
flies were collected comprising 10 species. Most of the species collected belong to two sub-
genera, viz., *Sophophora* and *Drosophila* and two species, one of which belongs to *Scaptodro-*
sophila and the other to subgenus *Dorsilopha*. Among the species trapped, *D. rajasekari*, *mal-*
erkotliana, *jambulina*, *mysorensis*, *nasuta nasuta* and *brindavani* are abundant. These and other
species collected are listed in Table 1. The most interesting feature of the collection is

Table 1. Distribution of different species of *Drosophila* in
Jogimatti Hills (Chitradurga), Karnataka, India

Altitude:	Thimmannanayakana tank area 1850 ft	Himoth- kethara 2400 ft	4th mile stone to Jogimatti Hills 3000 ft	Jogimatti hill top 3600 ft	Total
Subgenus: <i>Sophophora</i>					
<i>melanogaster</i> species group					
<i>D. rajasekari</i>	4	8	110	185	307
<i>D. malerkotliana</i>	25	42	84	101	252
<i>D. jambulina</i>	27	35	85	130	277
<i>D. bipectinata</i>	-	2	10	8	20
<i>D. parabipectinata</i>	-	-	4	-	4
<i>D. takahashii</i>	-	-	10	-	10
<i>D. mysorensis</i>	-	19	45	61	125
Subgenus: <i>Drosophila</i>					
<i>immigrans</i> species group					
<i>D.n. nasuta</i>	-	9	15	62	86
<i>D. brindavani</i>	3	20	60	86	169
<i>D. repleta</i>	54	-	-	-	54
Subgenus: <i>Scaptodrosophila</i>					
<i>D. krishnamurthyi</i>	-	-	-	15	15
Subgenus: <i>Dorsilopha</i>					
<i>D. busckii</i>	-	4	33	7	44
Total:	113	139	456	655	1363

the gradual ascendancy in the number of individuals of almost all the species from a lower
elevation characterised by sparse vegetation with dry and hot climate to a higher elevation
with thick vegetation and cooler climate. This is also evidenced by the fact that nearly 4/5
of the total flies lured come from the two highest elevations indicating the favourable con-
ditions for colonization. The taxonomic composition of the collection data also reveals a
total dominance in the number of individuals belonging to two species groups - *melanogaster*
species group of the sub-genus *Sophophora* and the *immigrans* species group of the sub-genus
Drosophila.

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Alahiotis, S., A. Zacharopoulou and M. Pelecanos. University of Patras, Greece. The effect of two ecological factors upon the inversion frequencies in *D. melanogaster* cage populations.

The problem of the maintenance of the genetic polymorphisms in Mendelian populations has been approached from two opposite and controversial points of view. Thus, two different theories have been developed, the theory of the balance school on the one hand (Ayala, Powell and Dobzhansky 1971, and others), and on the other, the

neutral theory (Kimura and Ohta 1971, and others).

A tool for approaching the previously mentioned problem is the study of chromosomal polymorphisms. Changes in the gene arrangement frequencies have been correlated with the influence of environmental factors and are usually considered to be an extremely rapid process of adaptation whether seasonal, altitudinal, or geographical variations are concerned; a given arrangement is considered to be better adapted to a given environmental condition (for a review see P.A. Parsons 1973). In contrast with the above mentioned findings, Krimbas (1967) has not been able to show any important effect of dryness and temperature on the gene arrangement frequencies of *Drosophila subobscura*. Moreover, Dobzhansky (1963) has come to the conclusion that none of the environmental changes in *Drosophila pseudoobscura* and *Drosophila persimilis* are clearly correlated with genetic changes; the latter has developed (Dobzhansky and Pavlovsky 1967) the hypothesis according to which the process of speciation may be initiated by the establishment of new symbiotic relationships between *Drosophila* and a virus or other microorganisms.

In view of the previously mentioned situation, and realizing that environmental factors can not be really under control in nature, we have thought it of interest to test for the possible selectional effect of two ecological factors using cage populations. Thus, we have chosen "food medium" and "humidity" as the variable ecological factors, and we have tried to study the reaction, if any, of the same gene pool using as markers of the possible selectional effect, the inversion frequencies in *Drosophila melanogaster*.

Three cage populations produced from six hundred common parents (300 virgin females and 300 males) were tested. The previously mentioned 600 parents originated from 100 isofemale lines, derived from recently collected (summer 1973) individuals on the island of Cephalonia (one of the Ionian Islands). Six (3 virgin females and 3 males) 4-6 day old individuals were taken from each of the isofemale lines and thus, the 300 virgin females and the 300 males gathered were allowed to mate randomly in the cage. Consequently, we may regard the derived populations as possessing practically the same gene pool.

Cage population 1A was put under a temperature of $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, 43% (mean) relative humidity (R.H.) and in a cornmeal-sugar-agar food medium. Cage population 1D was treated under the same as previously mentioned food medium and temperature conditions but in 90% R.H., while population 1B was put in $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ 43% (mean) R.H. and in a dead-yeast-food medium. More details of the origin and maintenance of these populations are provided by Alahiotis (1975).

Twelve generations after the origin of the cage populations, males from each population were individually mated with virgin Oregon-K (Or-K) females homozygous for the standard gene arrangement. Break points of the inversions were identified on the basis of the salivary gland chromosome maps provided by Lindsley and Grell (1967).

Our results are gathered in two tables. Table 1 shows the inversions found as well as their respective frequencies in both artificial and natural populations. Among the inversions found In(2R)46A-56A and In(3R)91D-95A are reported for the first time in *Drosophila melanogaster*. The changes found in the inversion frequencies of the artificial populations are really dramatic. Impressive differences were found in the frequencies between the populations 1A versus 1B and 1D versus 1B, while no such differences were detected between the population 1D versus 1A and 1B versus the natural population of Cephalonia, with the exception of the case of the In(2R)52A-56F (see Table 1); however, this discrepancy may very well be attributed to a sampling error due to the small number of the individuals examined (N=32).

In order to detect any significant differences in the gene arrangement frequencies, the variation of the genic proportions among the populations was subjected to a contingency chi-square analysis, according to Workman and Niswander (1970).

The results of the analysis are given in Table 2. No differences were found between the populations 1A/1D while in most cases, the chi-square values obtained from the comparison of 1A versus 1B and 1B versus 1D were significant at the .01 and .001 levels. Populations 1A and 1D were obviously influenced by the restrictive environment of the "poor food medium", while the 1B from the optimum "rich food medium". Hence, it appears that the factor "food medium"

plays an important role whilst "humidity" does not seem to influence the inversion frequencies. We should point out here that from further data (not published yet) linkage disequilibrium was revealed between the allozymes of alcohol dehydrogenase (Adh) and the polymorphic inversions In(2L)22D-34A and In(2R)52A-56F in the same populations. Furthermore, corresponding changes in the allozyme frequencies of alcohol dehydrogenase (Adh) and α -Glycerophosphate dehydrogenase (α -Gpdh) were detected. On the other hand, the study of the prementioned allozyme frequencies using two cage populations in order to control the effect of each factor (Alahiotis 1975) revealed that the observed dramatic changes are under influence of the environmental factor "food medium"; consequently, the role of the genetic drift in this case should be considered as very much limited.

Table 1. Frequencies of inversions in the natural population of Cephalonia as well as in the 1A, 1B, 1D cage populations.

Gene arrangement	Nat. pop. of Cephalonia (2N=64)	Population 1A (2N=122)	Population 1B (2N=180)	Population 1DD (2N=118)
Second chromosome				
2L (Standard)	0.9531	0.4672	0.9389	0.5593
In(2L)22D-34A	0.0469	0.5328	0.0611	0.4407
2R (Standard)	0.9219	0.9262	0.9889	0.9153
In(2R)52A-56F	0.9781	0.0656	0.0111	0.0847
In(2R)46A-56A	-	0.0082	-	-
Third chromosome				
3L (Standard)	1.0000	0.9180	0.9333	0.9476
In(3L)63A-72F	-	0.0738	0.0611	0.0254
In(3L)67A-72C	-	0.0082	0.0056	-
3R (Standard)	0.9531	0.8443	0.9389	0.8475
In(3R)92D-100F	0.0313	0.1393	0.0500	0.0847
In(3R)89D-96A	0.0156	0.0164	0.0111	0.0593
In(3R)91D-95A	-	-	-	0.0085

Table 2. Chi-square values resulting from contingency χ^2 analysis of inversion frequencies among the populations 1A versus 1B, 1B versus 1D and 1A versus 1D.

Gene arrangement	Populations 1A-1B	Populations 1B-1D	Populations 1A-1D
Second chromosome			
2L (Standard)	85.9116 ^c	61.6047 ^c	2.0366
In(2L)22D-34A	85.9114 ^c	61.6004 ^c	2.0366
2R (Standard)	8.1462 ^b	9.9997 ^b	0.0984
In(2R)52A-56F	6.7453 ^b	9.9962 ^b	0.3155
Third chromosome			
3L (Standard)	0.2529	2.5442	3.7506
In(3L)63A-72F	0.1896	1.6600	2.9580
In(3L)63A-72C	0.0744	-	-
3R (Standard)	7.2752 ^b	6.7807 ^b	0.0049
In(3R)92D-100F	7.3713 ^b	1.4381	1.7916
In(3R)89D-95A	0.1563	5.6564 ^a	3.0591

a=.01 < P < .05

b=.001 < P < .01

c=P < .001

The Hardy-Weinberg control was carried out according to (Johnson et al. 1969) method. No significant deviation from panmixia was detected either towards the direction favouring the heterozygotes or the homozygotes in both artificial and natural populations.

Our findings do not permit definitive conclusions; however, they provide enough evidence to allow us the following assumptions. 1. The observed dramatic changes of the gene arrangement frequencies as well as those of the allozymes in the 1A and 1D cage populations in combination with the existence of linkage disequilibrium (between the allozyme of Adh and the

second chromosome polymorphic In(2L)22D-34A and In(2R)52A-56F on the one hand, and between the polymorphic inversions of the II and III chromosomes on the other), can more easily be attributed to the selectional effect of the "food medium" than to random genetic drift. In favour of this view is the fact that many other research workers have reported the importance of food media and particularly that of yeast considering the latter as a controller ecological factor in *Drosophila* (Dobzhansky and Cuhna 1955, Ayala 1967).

2. The environmental factor "humidity" does not seem to have any effect on the differentiation of the gene arrangement frequencies, at least for the time of the 12 generations tested.

3. The changes of the gene arrangement frequencies detected in the populations 1A, 1D may very possibly be the reflection of a process through which the selection acts upon co-adapted large blocks of genes. A general explanation of the observed changes may be based on the two types of selection K and r proposed by McArthur and Wilson (1967), where K stands for the carrying capacity of the environment and r for the innate capacity of increase. When the density of population is low r selection occurs, as in newly founded populations periodically reduced in numbers by seasonal climatic changes. As the population density increases, K selection occurs. Each genotype in a population is subjected to either r or K selection or to a combination of both, depending on the population size. While a population is growing both types of selection are in operation (King and Anderson 1971).

In the case of our experiments we had under control two parameters, food medium and humidity, of the environment of the populations 1A, 1B, and 1D. Thus, the 1B population was under the effect of "rich food medium" and its environment was considered as optimum, while the populations 1A and 1D were under the effect of "poor food medium" and consequently, their environment was considered as very restrictive. The ecological factor "high humidity" seems to decrease slightly, the restrictiveness of the environment in the population 1D without affecting significantly the gene arrangement frequencies. Therefore it appears that the r selection acts on the 1B cage population, while the K type occurs in the 1A and 1D populations. We think that the action of these two types of selection is a process of coadaptation of large blocks of genes, where possibly certain chromosomal structure, i.e. the In(2L)22D-34A, are advantageous in the particular environment with a high density of population. On the contrary, other chromosomal structures, e.g. St(2L) may be favoured in an environment with low density of population. Birch (1955) reported that in uncrowded populations of *Drosophila pseudoobscura* the inversion Chiracahua was favoured over the standard; however, when larvae only, or larvae and adults were crowded at a high density, the standard arrangement of the genes was favoured over Chiracahua. On the basis of the previously mentioned rational, the natural population density of *Cephalonia* should be low; hence, there is good evidence that the same gene acts differently under different environmental conditions.

Finally, it should be pointed out that the observed changes in the gene arrangement frequencies of the second chromosome and especially those of In(2L)22D-34A after 12 only generations of selectional stress (that is before the population reached its equilibrium), are proportionally higher than those of the third chromosome. This observation raises a question: Could it be that the second chromosome and particularly the In(2L)22D-34A play an important role in the process of coadaptation in *Drosophila melanogaster*? Further investigations would probably provide an answer to this problem.

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Parsons, P.A., I.R. Bock, D.A. Hay and J. Grossfield. James Cook University, Queensland, Australia and City University of New York. Endemic *Drosophila* species in Victoria, Australia.

In spite of the comparatively large size of Australia, about equal to that of the continental United States, few Australian species had been reported until a survey of museum specimens (Bock, 1976), which contained 81 species including 40 that are new.

Detailed collecting work in Victoria began during the winter of 1974 by Grossfield and Parsons (1975) in isolated fern gully habitats. They reported 33 *D. inornata* Malloch and 16 flies of two other species to be described in

Bock (1976). The habitats are sheltered locations, generally isolated mountain gullies, and given the Mediterranean climate of Victoria, represent "insular patches of vegetation".



Figure 1. Map of Victoria indicating localities in Table 1.

During the summer months, November 1974 - January 1975, numerous collections were made in the insular patches of vegetation at localities indicated in Figure 1 - the distance between localities 1 and 10 being about 500 miles. Within collection sites, the distribution of flies depended upon temperature/humidity relationships (Parsons, 1976). As shown in Table 1, the most common species is *D. inornata*

Malloch, but 11 additional species were recorded, of which only 3 have been previously described. Including *D. inornata*, all but one of the 12 Victorian species belong to subgenus

Table 1. Number of *D. inornata* and other *Drosophila* collected in various Victorian sites from November 1974 - January 1975.

Locality	<i>D. inornata</i>		Other <i>Drosophila</i>		No. of Species
	♀♀	♂♂	♀♀	♂♂	
1. Otway Ranges	137	120		1	1
2. Lerderberg Gorge	17	22			
3. Mt. Macedon	23	21			
4. Dividing Range	47	50	1	3	2
5. South Gippsland	25	58	16	22	1
6. Wilson's Promontory	46	22	21	13	5
7. Den of Nargun	3	2			
8. Alfred National Park	37	23	1	3	2
9. Mallacoota Inlet region	21	15	8	4	8
10. Howe Ranges	22	28			

Scaptodrosophila - a subgenus which on present evidence is uncommon elsewhere in the world (Spieth and Heed, 1972).

References: Bock, I.R. 1976, Aust. J. Zool. (in press); Grossfield, J. and P.A. Parsons 1975, Proc. Roy. Soc. Vict. 87:235; Parsons, P.A. 1975, DIS 52; Spieth, H.T. and W.B. Heed 1972, Ann. Rev. Ecol. Syst. 3:269.

Kakpakov, V.T., L.G. Polukarova and E.M. Cherdanzeva. Institute of Atomic Energy, Kurtschatova Street, Moscow, USSR. Some new embryonic cell lines in *Drosophila melanogaster*.

To date, there exist about 20 embryonic cell lines from wild-type strains of *Drosophila melanogaster* (Hink, 1975). In this paper we describe the isolation and characterization of new embryonic cell lines which were obtained from *Drosophila melanogaster* vg. II - 67. In order to isolate new cell lines, embryos of

vestigial flies were collected over a period of 1 to 18 hours. The embryos were then washed carefully with water, sterilized with 70% ethanol for 3 minutes and with 96% ethanol for 12 minutes. The sterilized material was then homogenized (Gvosdev and Kakpakov, 1968). 2×10^6 cells/ml were suspended in medium S-47 (Shields and Sang, 1970), containing 15% foetal calf serum and 2% larval *Drosophila* extract, and kept at 28°C in small air-tight boxes of 0.4 content. The medium was renewed every 7 - 8 days. Seven cell lines were totally isolated and designated as 75 e 7 vg 1 - 75 e 7 vg 7. These seven lines can be distinguished in terms of their morphology, growth behaviour and karyotypes (see Table). Line 75 e 7 vg 1, 2 and 5 are mainly formed by a monolayer of rounded cells. Line 75 e 7 vg 3 consists of a mixture of spindle-like and round cells which, when grown for 2 - 3 days, give rise to aggregates capable of growing in suspensions. Line 75 e 7 vg 4 and 6 consist of a mixture of epithelial, fibroblastic, and round cells. Line 75 e 7 vg 7 consists mainly of fibroblastic cells which form solid colonies with a monolayer of round cells in their center. After 10 - 20 passages, the cells of line 75 e 7 vg 4 were capable of growing in a medium which did not contain any longer larval *Drosophila* extracts.

The growth of all lines can be suppressed by adding 0.1 µg/ml ecdysone. Cells of all lines can be re-suspended in a 1:3 to 1:10 ratio 7 days after onset, and can be kept in liquid nitrogen at -196°C.

Karyotypes of new cell lines of *Drosophila melanogaster*

Line	Runs	Number of metaphases (in %)						
		XX	XY	XO	4XX	4XY	4XO	Others
75 e 7 vg1	10	8	24	60	-	-	8	-
75 e 7 vg2	10	-	-	92	-	-	8	-
75 e 7 vg3	15	-	42	15	-	3	-	40
75 e 7 vg4	13	12	84	-	-	4	-	-
75 e 7 vg5	9	100	-	-	-	-	-	-
75 e 7 vg6	12	15	22	45	10	8	-	-
75 e 7 vg7	9	-	100	-	-	-	-	-

All lines were found to be of the diploid type. The pure lines 75 e 7 vg 2 (XO), 75 vg 5 (XX) and 75 e 7 vg 7 (XY) were obtained from a mixture of cells without special cloning.

References: Gvosdev, V.A. and W.T. Kakpakov 1968, *Genetika* 2:129; Hink, W.F. 1975, The Second Compilation of Invertebrata Cell Lines and Culture Media (Ohio); Shields, G. and J.H. Sang 1970, *J. Embryol. Exp. Morph.* 23:53.

Maddern, R.H. University of Adelaide, South Australia*. Recombination within the suppressor of sable in *D. melanogaster*.

Four mutant suppressor alleles were crossed in all possible heterozygous combinations and the progeny screened for male *su(s)*⁺ recombinants. A lethal selector system was employed to eliminate approximately 99.9% of the non-recombinant

male progeny, while all females were eliminated by using the daughterless mutant. The distal lethal marker was either lethal(1)J1 or lethal(1)403* while lethal(1)160* or deep orange lethal(*dor*¹) was used as the proximal lethal marker. All stocks were homozygous for *v*¹ so that the suppressor phenotype could be ascertained, and the visible markers yellow (*y* or *y*²), scute (*sc*) and prune (*pn*) were present as indicated in Table 1. Further details of the mutants used can be found in Lindsley and Grell, 1968. Of the suppressor alleles used the weak acting *su(s)*⁶⁸¹ and strong acting *su(s)*^{51c} were of spontaneous origin while the two strong suppressors, *su(s)*^{68h} and *su(s)*^{69f}, were recovered after treatment with ethyl-methane sulphonate and X-rays respectively.

Twenty pairs of parents were used per culture without removal or brooding. In 4% of the cultures the selector system was altered to allow half of the male progeny to live, and this, together with counts of *dor*¹ carrying larvae where appropriate, was used to estimate the number of gametes screened. The system was such that the double mutant suppressor recombinant could not be recognized and progeny arising from a single gene conversion would die.

Table 1. Recombination between *su(s)* alleles.

Cross	Chromosome A			Chromosome B			su(s) ⁺			Estimated progeny size
	genotype			genotype			recombinants			
	l ₁	su(s)	pn	y, sc	su(s)	l ₂	sc (or y, y ²)	y ⁺	sc ⁺	
1	1(1)J1	68h	pn	y ²	51c	dor ¹	0	0	1.8 x 10 ⁵	
2	1(1)403	51c	pn ⁺	sc	68h	1(1)160	0	1	6.6 x 10 ⁵	
3	1(1)J1	68h	pn	y	68i	dor ¹	0	2	8.6 x 10 ⁵	
4	1(1)403	68i	pn	sc	68h	1(1)160	2	3	16.8 x 10 ⁵	
5	1(1)403	51c	pn ⁺	y ²	68i	dor ¹	0	0	3.5 x 10 ⁵	
6	1(1)403	68i	pn	y ²	51c	dor ¹	0	1	5.7 x 10 ⁵	
7	1(1)J1	69f	pn	sc	68h	1(1)160	0	0	0.6 x 10 ⁵	
8	1(1)J1	68h	pn	y ²	69f	dor ¹	0	0	0.14 x 10 ⁵	
9	1(1)J1	69f	pn	y ²	51c	dor ¹	0	1	5.9 x 10 ⁵	
10	1(1)403	51c	pn ⁺	y ²	69f	dor ¹	0	0	6.4 x 10 ⁵	
11	1(1)J1	69f	pn	y	68i	dor ¹	0	1	2.6 x 10 ⁵	
12	1(1)403	68i	pn	y ²	69f	dor ¹	0	50*	2.4 x 10 ⁵	

* These 50 arose from one culture.

The results of the twelve crosses are listed across Table 1, and the twelve types of heterozygous females carried both of the X-chromosomes listed in columns A and B. The *su(s)*⁺ progeny can be divided into two classes depending on the visible markers carried.

One class, in column four showed the distal visible marker (*sc*) and on progeny testing were found to carry the proximal marker *pn* (progeny testing was necessitated because the *pn* phenotype in the males was masked by other eye colour markers). These flies can be accounted for by a single exchange between the *su(s)* alleles. Both of these recombinants from cross 4 were tested against *vk* and *s* and for tryptophan pyrrolase levels in the *v*¹ strain. All the results were negative indicating no difference from *su(s)*⁺.

The second class of *su(s)*⁺ progeny, in column five did not express the distal visible marker and all failed to reproduce and so could not be tested for *pn*. Of these ten presumptive recombinants nine arose as independent events and the tenth as a cluster of fifty, which under the conditions in the crosses is as many progeny as a female produces, and so probably arose during an early mitosis to give gonad tissue which was *su(s)*⁺ in genotype. A number of these sterile males, and the females with which they had been allowed to mate were dissected but no motile sperm could be found. No satisfactory explanation of the origin of these sterile *su(s)*⁺ flies is offered. Sterility was not common in the eighty recombinants between the flanking markers which were not recombinant for the *su(s)* gene. The spontaneous occurrence of back mutation or of a gene which suppresses the action of *su(s)* is unlikely because it would have to occur simultaneously with an exchange between flanking lethals. Such mutations were not observed during stock keeping. Simultaneous gene conversion and the failure of a flanking lethal to act (lethal escapes) will explain the occurrence of *su(s)*⁺ progeny but their frequency is much higher than the product of the frequencies of the separate events.

It is concluded that a low frequency of classical recombination ($1.1 \times 10^{-4}\%$) was demonstrated between only two alleles, *su(s)*^{68h}, a strong suppressor, and *su(s)*⁶⁸ⁱ, a weak suppressor. The data suggest that recombination frequencies at *su(s)* are comparatively low and similar in magnitude to recombination frequencies reported at maroon-like⁽¹⁾. The low frequency of recombination also makes it unlikely that *su(s)* is redundant, that is present as a number of repeated copies.

* These were non-autonomous lethals kindly made available by Professor E. Novitski.

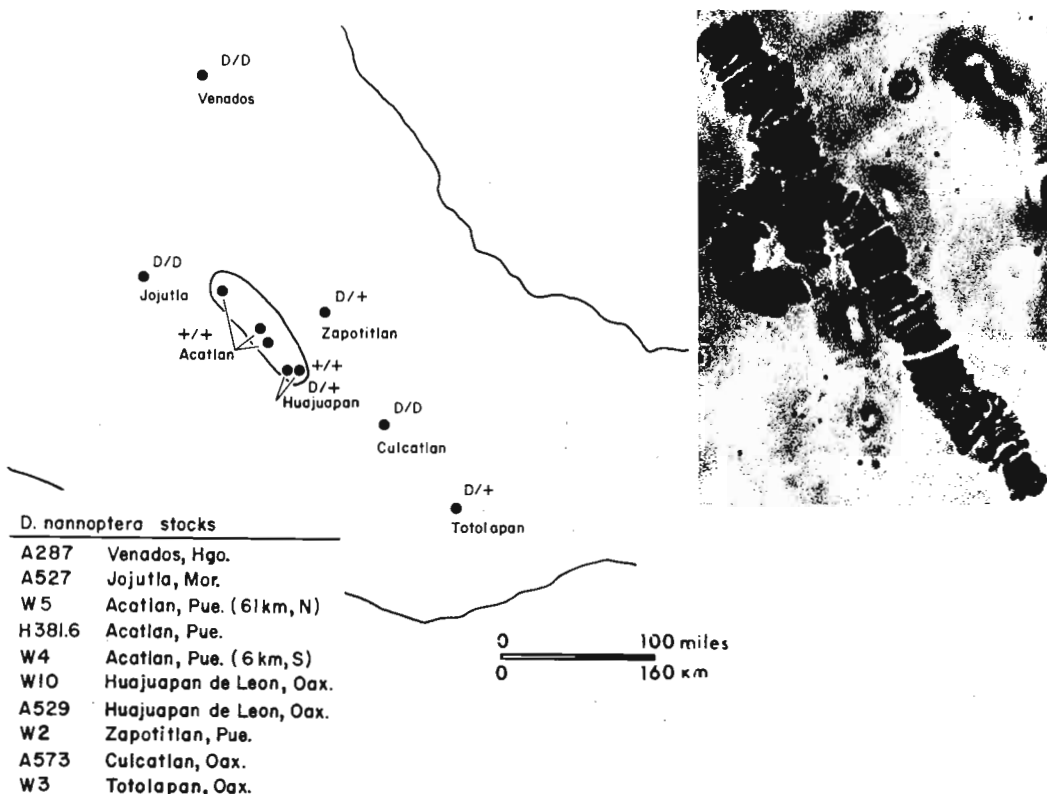
** Present address: Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, The Netherlands.

Reference: (1) Finnerty, V.G., P. Duck and A. Chovnick 1970, Proc. Nat. Acad. Sci. U.S. 65:939-946.

Russell, J.S., B.L. Ward and W.B. Heed.
University of Arizona, Tucson, Arizona,
and Wayne State University, Detroit,
Michigan. Inversion polymorphism in
D. nanoptera.

Ward and Heed (1970) reported a single heterozygous sub-terminal inversion in chromosome 7 of *D. nanoptera* in 2 out of 3 cultures examined. The species is otherwise homozygous. Since then 7 more cultures have been collected and examined. The collecting localities from which each culture was derived are shown in the map along with

the cytological condition of each culture and a photomicrograph of the inversion heterozygote. We wish to point out that the ancestral sequence (+/+) exists in the homozygous condition in 4 out of 5 cultures north and south of Acatlan, Puebla, and that the derived condition (D/D) exists in the homozygous condition in 3 out of 5 cultures surrounding this area. The southern part of the area is part of the famous Tehuacan Desert that contains a variety of arborescent cacti in several of which *D. nanoptera* is known to breed. The pattern of small



inversion number with fixed differences within short distances has also been found in *D. pachea*, a close relative of *D. nanoptera*, and it may be a general characteristic of desert adapted *Drosophila* (Ward, et al. 1974). We thank R.L. Mangan, R. Richardson and M. Wasserman for recent collections.

References: Ward, B.L. and W.B. Heed 1970, *J. Hered.* 61:248-258; Ward, B.L., W.T. Starmer, J.S. Russell and W.B. Heed 1974, *Evol.* 28:565-575.

Kaplan, W.D. City of Hope Medical Center, Duarte, California. The choice of markers for mosaic studies of behavioral phenotypes.

Mosaics of behavioral mutants are studied in order to identify the developmental focus for mutant behavior. We originally used *y w* and *f* as markers in order to identify the behavioral focus of the abnormal jump response of the

mutant, *Hyperkinetic*¹. Males carrying the X chromosome *y w Hk*¹ *f* were crossed to females

heterozygous for the ring X chromosome, In(1)w^{vc}. Gynandromorphs produced by the loss of the ring X early in embryology were mosaic for female tissue heterozygous for y w Hk¹ f and male tissue, hemizygous for y w Hk¹ f.

Hyperkinetic flies jump and frequently fall over when a hand or a mechanically driven vane moves above a vial containing them. When scored individually, the mutant flies show an average response of about 42 out of 50. Wild-type Canton-S flies exhibit a response close to zero (Kaplan and Trout, 1969).

In a study of mosaics it was found that there must be mutant tissue in the head for the response to be elicited and that half a male head was a sufficient condition. Although one would expect some cases of male tissue in mosaics which did not respond to movement because of a discordance between the cuticle of the head and the underlying nervous tissue, there was a rather high frequency of mosaics with whole or half male heads which did not respond (32%). Upon testing patroclinus y w Hk¹ f males from the crosses producing mosaics it was found that 21% of these flies did not respond and scores of the positive responders were generally lower than males that were Hk¹ only. This was true also of y w Hk¹ f males taken from stock. In addition, only rarely did an Hk¹ male fail to respond in the many experiments that we have completed over many years. Since it was known that neither y nor w nor y w have an inhibiting effect upon the jump response of Hk¹ (Williamson et al., 1974) we tested to see whether sn³, because it is a bristle mutant, would lower the response. However, 100% of y w sn³ Hk¹ patroclinus and stock males respond to movement (50 males of each tested), and 21 of 22 mosaics with male tissue in the head have responded positively.

It, therefore, appears that forked, for reasons yet unknown, has an inhibitory effect upon a behavioral mutant phenotype.

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Barker, J.S.F. and I.R. Bock. University of Sydney and James Cook University of North Queensland, Australia. *Drosophila quadrilineata* de Meijere in Sulawesi, Indonesia.

The recorded distribution of *D. quadrilineata* is Java, Philippines, Marianas Is., Admiralty Is., and Solomon Is. (Wheeler and Takada, 1964) and ? new Guinea (? = *D. tetrachaeta* Angus) (Angus, 1964, 1967). Bohart and Gressitt (1951) found this species to be "one of the most abundant

drosophilids on the island" (of Guam), and noted that adults were common on all sorts of decaying fruits. Adults were reared from breadfruit, bananas, mangoes, and papayas. There seems to be no other information on the natural ecology of the species.

During May 1975, a number of stands of cactus species in 10 localities in South Sulawesi were investigated for presence of soft rots, potential breeding and feeding sites for *Drosophila*, and for any life stages of *Drosophila* species, particularly *D. buzzatii* (Barker and Mulley, 1976) and *D. aldrichi* (Mulley and Barker, 1976). Four species of cactus were seen, but only one (*Opuntia eliator*) was common. All species appear to have no natural enemies (other than man), and *O. eliator* is often used as a hedgerow. Small numbers of decaying cladodes were found in each locality, but these were generally inhabited by various species of ants, and evidence of *Drosophila* was observed at only one locality, in the village of Amparita in Kabupatén (district) Sidrap.

Large numbers of *D. quadrilineata* were observed on two decaying cladodes. One had about 12-13 sq. cm of fresh, moist decay, and some 200 adult flies were feeding closely packed on this area. Some courtship and mating activity occurred around the periphery of the decay during some 10 minutes' observation. When an attempt then was made to collect by aspiration, the flies rapidly dispersed and collection was difficult. Some 50 were collected in about one hour. The rotting sections were dissected, but no immature stages of *Drosophila* were detected. Either this species does not use the cactus niche as a breeding site, or the rotting cladodes observed had only just become suitable for oviposition.

(Work supported by University of Sydney Overseas Research Travel Grant.)

References: Angus, D.S. 1964, Qld. Univ. Papers, Dept. Zool. 2(8):155-159; Angus, D.S. 1967, Qld. Univ. Papers, Dept. Zool. 3(3):31-42; Barker, J.S.F. and J.C. Mulley 1976, Evolution (in press); Bohart, G.E. and J.L. Gressitt 1951, B.P. Bishop Museum, Honolulu, Hawaii, Bull. 204, 152 pp; Mulley, J.C. and J.S.F. Barker 1976, DIS 52:151; Wheeler, M.R. and H. Takada 1964, B.P. Bishop Museum, Honolulu, Hawaii: Insects of Micronesia 14(6):163-242.

Wright, C.P. Western Carolina University, Cullowhee, North Carolina. Development of Glyful-1, 1(1)EN10a, a lethal mutant of *Drosophila melanogaster*.

Glyful-1, 1(1)EN10a, is a sex-linked, lethal mutant of *Drosophila melanogaster* which was X-ray induced by Novitski (1963). Death in this mutant occurs in the pupal stage. Both weight and oxygen consumption measurements were made on individual male larvae and pupae from the

first-instar larval stage until the time that oxygen uptake ceased. Oxygen consumption measurements were made with small respirometers in a 25°C water bath. A 20% NaOH solution was used to remove CO₂ from the respirometers, which caused movement of the NaOH drop. By measuring the volume of the droplet displacement, the oxygen consumption of a larva or pupa was determined. Control larvae and pupae were y, w, spl, sn males from the stock of Novitski (1963) in which the lethal mutant was induced. Experimental data were tested statistically against control data by use of the Mann-Whitney U nonparametric test (Tate and Clelland, 1957).

In 1(1)EN10a larvae both weights and rates of oxygen consumption in cu. mm./larva/hour (Table 1) were generally less than in controls (Table 2). When calculated per unit dry weight, rates of oxygen consumption in 1(1)EN10a larvae (Table 1) were not significantly different from those in control larvae (Table 2). Control larvae formed puparia at about 110-116 hours after oviposition. Puparium formation in 1(1)EN10a occurred at about 123-202 hours after oviposition. About 97% of 1(1)EN10a larvae formed puparia. Because puparium formation occurred later than in controls, measurements for 1(1)EN10a larvae were made until 120 hours after oviposition. This lengthened larval stage probably accounts for the fact that 1(1)EN10a larvae (Table 1) were actually larger than control larvae (Table 2) just prior to the time of puparium formation. Gross examination revealed no visible morphological abnormalities in 1(1)EN10a larvae.

Table 1. Average weights and rates of oxygen consumption for 1(1)EN10a larvae.

Age in hr. after oviposition	Fresh weight/ larva in mg.		Dry weight/ larva in mg.		O ₂ consumption in cu. mm./ larva/hr.		O ₂ consumption in cu. mm./hr. dry wt./mg.	
	n	M. ± S.E.	n	M. ± S.E.	n	M. ± S.E.	n	M. ± S.E.
30 hr.		Did not weigh	4	0.0037 ± 0.0002	8	0.135 ± 0.011	8	36.588 ± 2.774
48 hr.		Did not weigh	6	0.017 ± 0.001	8	0.374 ± 0.016	8	21.993 ± 0.962
72 hr.	8	0.19* ± 0.02	8	0.04** ± 0.01	8	0.829** ± 0.060	8	20.195 ± 0.648
96 hr.	8	1.03** ± 0.05	8	0.20** ± 0.01	8	3.650** ± 0.248	8	18.135 ± 0.728
120 hr.	7	1.74 ± 0.10	7	0.46 ± 0.03	7	4.328 ± 0.223	7	9.721 ± 0.932

* Significant at .05 level

** Significant at .01 level

Table 2. Average weights and rates of oxygen consumption for y, w, spl, sn control larvae.

Age in hr. after oviposition	Fresh weight/ larva in mg.		Dry weight/ larva in mg.		O ₂ consumption in cu. mm./ larva/hr.		O ₂ consumption in cu. mm./mg. dry wt./hr.	
	n	M. ± S.E.	n	M. ± S.E.	n	M. ± S.E.	n	M. ± S.E.
30 hr.		Did not weigh	4	0.0039 ± 0.0001	8	0.137 ± 0.004	8	35.192 ± 1.021
48 hr.		Did not weigh	4	0.018 ± 0.001	10	0.390 ± 0.015	10	21.689 ± 0.851
72 hr.	10	0.29 ± 0.02	10	0.07 ± 0.01	10	1.456 ± 0.059	10	19.698 ± 0.236
96 hr.	9	1.36 ± 0.05	9	0.31 ± 0.01	9	5.108 ± 0.282	9	16.382 ± 0.441

Measurements for 1(1)EN10a pupae (Table 3) are given until 200 hours after puparium formation. This is done because 1(1)EN10a pupae failed to develop to the adult stage, but lived as incompletely developed pupae until about 200 hours after puparium formation. Measurements for control pupae (Table 4) stop at 80 hours after puparium formation because soon after this age they emerged as adults. Throughout the time during which normal pupal development should

occur, fresh weights of 1(1)EN10a pupae (Table 3) were about the same as in controls (Table 4) and dry weights of 1(1)EN10a pupae (Table 3) were somewhat greater than in controls (Table 4). Fresh weight of 1(1)EN10a pupae (Table 3) was fairly constant until 80 hours, after which it began to decrease, until at 200 hours the pupae were almost completely desiccated. Dry weight in 1(1)EN10a was fairly constant throughout the pupal stage (Table 3). Rates of oxygen consumption in 1(1)EN10a pupae (Table 3) were less than in controls (Table 4). Oxygen uptake in 1(1)EN10a pupae was at a fairly high level at 3 hours, but decreased to quite a low level at 20 hours after puparium formation. After this point the rate of oxygen uptake decreased until at 200 hours no oxygen uptake could be detected in 1(1)EN10a pupae.

Table 3. Average weights and rates of oxygen consumption for 1(1)EN10a pupae.

Age in hr. after puparium formation	Fresh weight/ pupa in mg.		Dry weight/ pupa in mg.		O ₂ consumption in cu. mm./ pupa/hr.		O ₂ consumption in cu. mm./mg. dry wt./hr.	
	n	M. ± S.E.	n	M. ± S.E.	n	M. ± S.E.	n	M. ± S.E.
3 hr.	8	1.37 ± 0.07	8	0.39 ± 0.03	8	2.547 ± 0.455	8	6.553** ± 0.898
20 hr.	8	1.20 ± 0.04	8	0.37 ± 0.03	8	0.321** ± 0.035	8	0.898** ± 0.112
40 hr.	8	1.20 ± 0.06	8	0.41 ± 0.02	8	0.371** ± 0.023	8	0.936** ± 0.079
60 hr.	8	1.18 ± 0.06	8	0.39 ± 0.02	8	0.336** ± 0.044	8	0.773** ± 0.147
80 hr.	10	1.18 ± 0.04	10	0.46* ± 0.03	10	0.238** ± 0.033	10	0.525** ± 0.081
104 hr.	10	1.02 ± 0.07	10	0.43 ± 0.02	10	0.217 ± 0.042	10	0.509 ± 0.106
128 hr.	8	0.91 ± 0.11	8	0.41 ± 0.02	8	0.357 ± 0.126	8	0.844 ± 0.293
152 hr.	8	0.84 ± 0.07	8	0.43 ± 0.02	8	0.281 ± 0.091	8	0.669 ± 0.230
176 hr.	4	0.58 ± 0.09	4	0.39 ± 0.03	4	0.114 ± 0.096	4	0.343 ± 0.292
200 hr.	4	0.44 ± 0.02	4	0.39 ± 0.03	4	0.000 ± 0.000	4	0.000 ± 0.000

* Significant at .05 level

** Significant at .01 level

Table 4. Average weights and rates of oxygen consumption for y, w, spl, sn control pupae.

Age in hr. after puparium formation	Fresh weight/ pupa in mg.		Dry weight/ pupa in mg.		O ₂ consumption in cu. mm./ pupa/hr.		O ₂ consumption in cu. mm./mg. dry wt./hr.	
	n	M. ± S.E.	n	M. ± S.E.	n	M. ± S.E.	n	M. ± S.E.
3 hr.	10	1.38 ± 0.02	10	0.38 ± 0.01	10	3.092 ± 0.076	10	8.244 ± 0.187
10 hr.	10	1.19 ± 0.03	10	0.36 ± 0.01	10	2.213 ± 0.066	10	6.261 ± 0.211
20 hr.	10	1.16 ± 0.02	10	0.34 ± 0.01	10	1.114 ± 0.037	10	3.340 ± 0.162
40 hr.	10	1.17 ± 0.04	10	0.35 ± 0.01	10	0.842 ± 0.083	10	2.433 ± 0.097
60 hr.	10	1.16 ± 0.02	10	0.35 ± 0.01	10	1.266 ± 0.049	10	3.620 ± 0.098
80 hr.	10	1.14 ± 0.02	10	0.34 ± 0.02	10	1.864 ± 0.070	10	5.542 ± 0.194

Gross examination revealed some morphological development in the 1(1)EN10a pupae. At 80 hours after puparium formation most 1(1)EN10a pupae showed some development, varying from presence of the head, legs, and wings in some individuals to only the head in others. Some of the 1(1)EN10a pupae had dark spots at the anterior region. Some 1(1)EN10a pupae showed no development whatsoever. At 176 and 200 hours all 1(1)EN10a pupae looked completely desiccated, this being indicated by shrivelling of the organisms. None of the 1(1)EN10a individuals ever emerged as adults. By 200 hours after puparium formation all the 1(1)EN10a pupae had apparently died.

References: Novitski, E. 1963, DIS 37:51-53; Tate, M.W. and R.C. Clelland 1957, Non-parametric and Shortcut Statistics in the Social, Biological, and Medical Sciences (Interstate, Danville, Illinois).

Rajasekarasetty, M.R., S.R. Ramesh and N.B. Krishnamurthy. University of Mysore, India. A comparative study on the giant chromosomes in a few members of *Drosophila nasuta* subgroup.

Interspecific hybrids provide a means by which information is obtained concerning the extent to which inversions have been involved in the past with regard to evolutionary divergence within a species group. The interspecific hybrids also provide material for comparison with intraspecific polymorphism and information con-

cerning the phylogenetic longevity of various chromosomal rearrangements. Studies of this nature have been made on a variety of species groups within the genus *Drosophila* (cf. Bock, 1971). The present report deals with the fixed inversion differences encountered in the hybrids of a few members of the *nasuta* subgroup, namely, *Drosophila nasuta nasuta*, *D.n. albomincana*, *D.n. kepulauana*, *D. kohkoa*, *D. sulfurigaster sulfurigaster*, *D.s. neonasuta*, *D.s. albos-trigata* and *D.s. bilimbata*. Crosses were made between these members. The homologies of the chromosomes were analysed in the salivary gland chromosomes of the hybrid larvae wherever the species were compatible, while the larval salivary gland chromosomes of the parents were compared if the cross was unsuccessful. Hybrid progenies of *D.n. nasuta* x *D.n. kepulauana* revealed the presence of fixed inversions, namely, Beta R¹ and Gamma¹, while all the hybrid larvae of *D.s. neonasuta* x *D.s. sulfurigaster* and *D.s. neonasuta* x *D.s. bilimbata* carried the heterozygous inversion Beta L¹. The cross between *D.n. nasuta* and *D.s. neonasuta* is incompatible and hence comparison of salivary gland chromosomes of the larvae of these two parental species was made. This revealed that the X and the second chromosomes are homo-sequential while the third chromosome differs by two inversions, one at the base and the other in the middle part. The basal one is called Gamma² and the middle one is called Gamma³.

Studies on the chromosomal polymorphism of *D.n. nasuta* (Nirmala and Krishnamurthy, 1974; Ranganath and Krishnamurthy, 1975) and of *D.s. neonasuta* (Nirmala and Krishnamurthy, 1972) have revealed the presence of 44 and 3 inversions in these two species, respectively. Of the five fixed inversions herein reported, two namely Beta L¹ and Gamma³ are identical with the inversions 2LB and 3C reported earlier in natural populations of *Drosophila nasuta* by Nirmala and Krishnamurthy (1974) and Ranganath and Krishnamurthy (1975). The other three inversions Beta R¹, Gamma¹ and Gamma² are not identical with any of the earlier reported inversions.

The authors are thankful to the Department of Atomic Energy, Government of India, for the financial support.

References: Bock, I.R. 1971, *Chromosoma* 34:206-229; Nirmala, S.S. and N.B. Krishnamurthy 1972, *DIS* 48:34-35; Nirmala, S.S. and N.B. Krishnamurthy 1974, *The Egyptian J. Genet. and Cytol.* 3:211-228; Ranganath, H.A. and N.B. Krishnamurthy 1975, *The J. Hered.* 66:90-96.

Dow, M.A. University of Edinburgh, Scotland. Multiple male courtship in *D. melanogaster*.

The mating success of yellow mutant males with wild-type females is increased from 1% to 21% by altering the sex-ratio from 1:1 to 1:3 (female:male) (Dow, M.A. 1975, *Nature* 255:172).

I have examined whether this increase is due to

the occurrence of multiple male simultaneous courtships of the female.

Virgin wild-type females and yellow males were placed in vials (20 x 76 mm) containing 5 ml of propionic acid medium, at various sex-ratios, without etherization and observed for 5 min periods. As there was no decrease in the mean percentage of time spent courting after 7 hr, only the data from the first 5 min period is reported here.

Percent Female Courted by:

Sex-Ratio	1 male	2 males	3 males	Total	N
1:1	65.2	-	-	65.2	12
1:2	48.6	1.9	-	50.5	7
1:3	43.7	5.4	0.2	49.3	7
1:4	52.8	12.1	0.9	65.8	7

As shown in the table, increasing the number of males per female did not result in an increase in the total percentage of time that the female was courted. However, simultaneous courtships by 2 or more males did increase with increasing sex-ratio. A Spearman rank correlation between the amount of simultaneous courtship and mating success (data from Dow, loc. cit.) is

significant ($r_s = 1.0$, $P < 0.05$). Thus, the mating facilitation that occurs at a 1:3 sex-ratio with yellow males and wild-type females can be attributed, at least partially, to the effects of simultaneous courtships by 2 or more males.

Thongmeearkom, P.† University of Queensland, Brisbane, Australia. The nasuta complex in Western Malaysia.

In addition to *D. sulfurigaster albostrigata* from Western Malaysia collected in January 1973 (Mather, Thongmeearkom, Clyde and Lambert, 1975), three other species of the nasuta complex namely *D. albomicans*, *D. kepulauan* and *D.*

kohkoa were also detected. However, only one isoline from each species was established. Details of inversions possessed by each species are shown (see table and figure).

It should be noted that inversions C,E,Y₂ and Z₂ have also appeared in *D.s. albostrigata*.

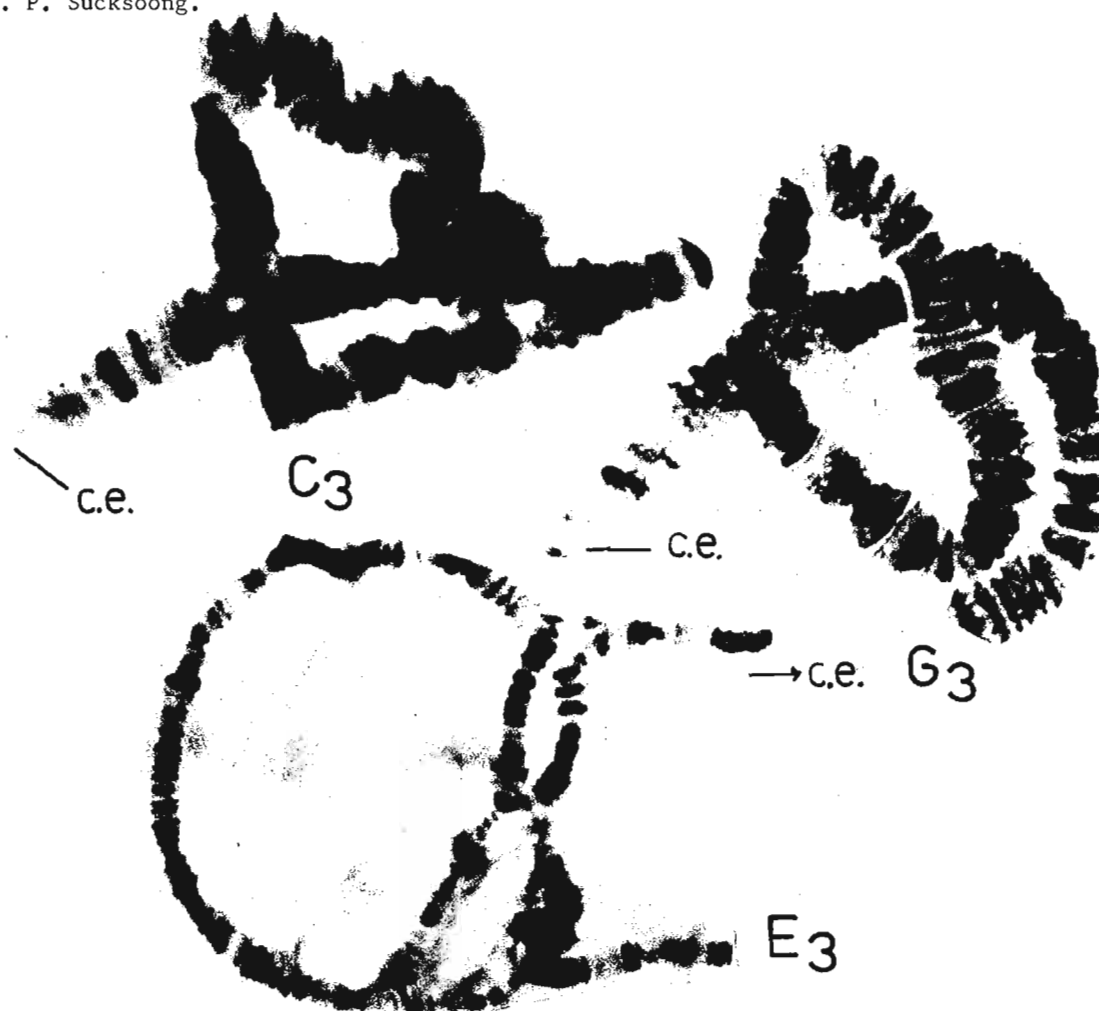
Species	Locality	Inversion	Type	Position	
<i>D. albomicans</i>	K.L.	C*	Sim	III D	Note: Sim = simple Com = complex D = distal C = central P = proximal to centromere K.L. = Kuala Lumpur K.T. = Kota Tinggi c.e. = centromere end
		E ₃	Com	III C	
<i>D. kepulauan</i>	K.L.	E*	Sim	IIL C	
<i>D. kohkoa</i>	K.T.	K*	Sim	III D	
		Y ₂ **	Sim	IIL C	
		Z ₂ **	Sim	III C	
		C ₃	Com	III P	
		G ₃	Sim	III P	

* See Mather & Thongmeearkom 1972, DIS 48:40

** See Mather, Thongmeearkom, Clyde & Lambert 1975, DIS 51:86

The material was collected and the iso-lines established in the genetics laboratory by Dr. W.B. Mather.

† Mrs. P. Sucksoong.



ANNOUNCEMENTS II

The Genetics of Behavior, by Lee Ehrman, State University of New York, and Peter A. Parsons, La Trobe University, was published by Sinauer Associates, Sunderland, Massachusetts, in 1976. It applies principles of genetics to behavioral traits in *Drosophila*, rodents and Man.

Handbook of Genetics, Volume III, edited by Robert King, was published by Plenum Press in November, 1975. It is titled "Invertebrates of Genetic Interest," with chapters 17 through 21 dealing with the genus *Drosophila* and chapters 22 through 32 with *D. melanogaster* specifically.

Roy, S. and S.C. Lakhotia.* Gujarat University, Ahmedabad, India. Photomap of salivary gland chromosomes of *Drosophila kikkawai*.

A map of salivary gland polytene chromosomes of *Drosophila kikkawai* has not been made so far. We are studying replication patterns in these chromosomes, and as such a map would be of great importance. We are presenting here a photomap of salivary gland polytene chromosomes of *D.*

kikkawai. Conventional aceto-orcein-aceto-carmin squash preparations from late third instar larvae were used and the photomicrographs were taken under oil immersion phase contrast optics.

The salivary gland chromosome complement of *D. kikkawai* reveals five long arms which are recognized on the basis of identification made by Lakhotia and Mukherjee (1972). They represent the X-chromosome (one arm), and the left and right arms of the second and third chromosomes, respectively. The fourth chromosome in polytene nuclei is very small and U-shaped, with both ends merging into the chromocentre.

The photomap of the salivary gland chromosomes (Figure 1) is divided into 102 sections on the basis of certain major landmarks. Each of the longer chromosome arms is divided into 20 sections and the fourth into two. Each section is again divided into a number of divisions (maximum 7, A - G) with each division consisting of several bands. The numbering of the sections is continuous, starting from the tip of the left arm and ending at the tip of the right arm in case of the metacentric autosomes; on the X-chromosome arm, the numbering is from tip to chromocentric end. The fourth chromosome being U-shaped, does not show any proximal or distal end; thus the two sections on this are arbitrarily numbered. (See Figure facing page.)

The X-chromosome is the shortest arm of the complement with sections 1 - 20. The tip is narrow and ends bluntly. It is easily identified by a prominent puff in the region 2AB. It can be further distinguished by three markedly thick bands at 18DE, by two thick bands at 19E. There is a prominent interband region at 6A and some prominent puffs at 5C, 6E, 7C. The junction of X-euchromatin with the chromocentre heterochromatin is usually stretched or broke during squashing.

2L is longer than the X-chromosome with sections 21 - 40. It has a thick band at its rounded tip. The other diagnostic features are the presence of prominent puffs (in late third instar) in divisions 23D, 26BC, 28C, 34C and a hat-shaped region at 25A-C.

2R has nearly the same length as the 2L and has sections 41 - 60. The tip is rounded, followed by three prominent bands. It is also marked by the presence of easily identifiable bands at 60D, 59A, 58C, 56A, 52C, 41ABCD. The section 42D is a weak point and the chromosome is often broken or bent at this point during squashing.

3L is the second longest arm with sections 61 - 80. It has a fan-shaped tip followed by a prominent band at 61A. The notable features are the two dark bands at 61E and 62A. The enlarged and darkly stained region at 63C is very notably present in late third instar as well as prepupal stages and forms an important landmark in the identification of this arm.

3R is the longest arm of the chromosome complement. It is easily recognized by the presence of remarkable puffs in the section 90. Other characteristic features are the puffs at 92D, 89C and the bands at 100F, 99A, 91ACDE, 88A, 85AB. The tip is blunt, followed by a prominent band and a series of thin bands.

The fourth chromosome is quite large in mitotic nuclei, but only a small region has a euchromatic banded appearance in polytene nuclei. This forms a U-shaped structure close to the chromocentre. It is divided into two sections (101 and 102). The sections are recognized by a prominent interband at 101B and two thick bands at 102E.

Reference: Lakhotia, S.C. and A.S. Mukherjee 1972, Proc. Zool. Soc. Cal. 25:1-9.

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PHOTOMAP OF SALIVARY GLAND CHROMOSOMES OF *Drosophila kikkawai*.

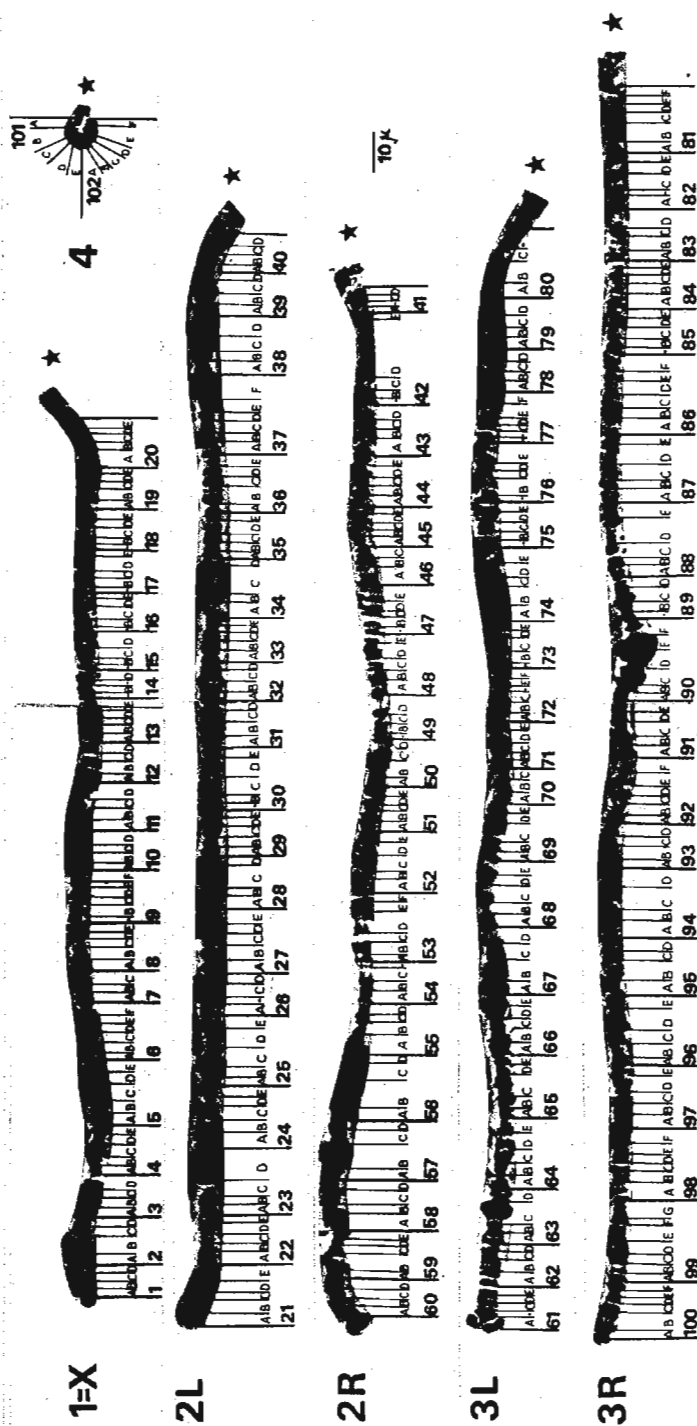


Figure 1. Photomap of salivary gland chromosomes of *D. kikkawai*, after Roy and Lakhotia.

Pfriem, P. Institut für Biologie II, Tübingen, Germany. The effect of radiation on linkage equilibrium in experimental populations of *D. melanogaster*.

Males from a *dp b* strain of *D. melanogaster*, homozygous for a second chromosome containing the visible markers *dp* and *b* were exposed to 9000r X-radiation and mated to wild type females, also homozygous for the second chromosome. Heterozygous *dp b/+ + F₁* males and

females were used to start two population cages (numbers 1 and 2). Two other population cages (3 and 4) were started in the same way except that the *dp b* males had not been irradiated. The second chromosomes of the populations 1 to 4 were originally derived from a single *dp b* and a single *+ +* chromosome and are consequently composed of only two different second chromosomes. They may be hence designated as dichrosomal populations. Another set of two populations (5 and 6) were started with *dp b/+ +* flies having their *dp b* and *+ +* chromosomes respectively from many different unirradiated sources and may hence be designated as polychrosomal populations. Egg samples were taken from the population cages at a time corresponding approximately to generations 3, 7 and 11 respectively. From each sample about 300 males were individually back-crossed to females of the *L Cy-Pm* strain which contains *dp* and *b* in the *Pm* chromosome. From these crossings the genotype of the males could be determined.

With respect to the single loci *dp* and *b* an equilibrium seems to be approached for *b* and *+b* at a frequency of $q = 0.3$ and $p = 0.7$ in the dichrosomal populations, whereas the *dp* allele seems to be inferior to *+dp* in all populations. The main purpose of the experiment, however, was to investigate the combination of the two alleles of the loci by linkage. The populations were started in complete linkage disequilibrium with the *cis* types *dp b* and *+ +* only. The *trans* types *dp +* and *+ b* are produced by crossing over every generation (the actual crossover value *c* is 0.26 in our case in the females and has to be divided by two because of the lack of exchange in males) and the frequencies of *dp b*, *+ +*, *dp +* and *+ b* chromosomes are expected to eventually reach a linkage equilibrium which corresponds to theoretical expectations. Deviations from the equilibrium can be numerically expressed by the *D'*value (Lewontin, 1964). These *D'*values of the various populations are shown in the table together with the theoretically expected *D'*values corresponding to $c = 0.13$ and no selection. It is obvious

<u>Generation</u>	<u>Dichrosomal Populations</u>				<u>Polychrosomal Populations</u>		<u>D' value</u>
	<u>irradiated</u>		<u>non-irradiated</u>		<u>non-irradiated (Sperlich, unpub.)</u>		<u>Expected</u>
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
3	.664	.583	.736	.753	.669	.579	.560
7	.319	.322	.744	.422	.412	.417	.320
11	.296		.488	.470	.445	.500	.183

that irradiation of the original chromosomes has an effect on the combination of the alleles of the two loci. Whether this deviation between the irradiated and non-irradiated populations is due to independent selection on the single loci *dp* and *b*, or to a mutational disturbance of interacting genes can not be clearly derived from our present data. Further studies with other visible and enzyme markers, however, may help to get an answer to this very important question.

The work has been supported by the grant Sp 146/1 from Deutsche Forschungsgemeinschaft.

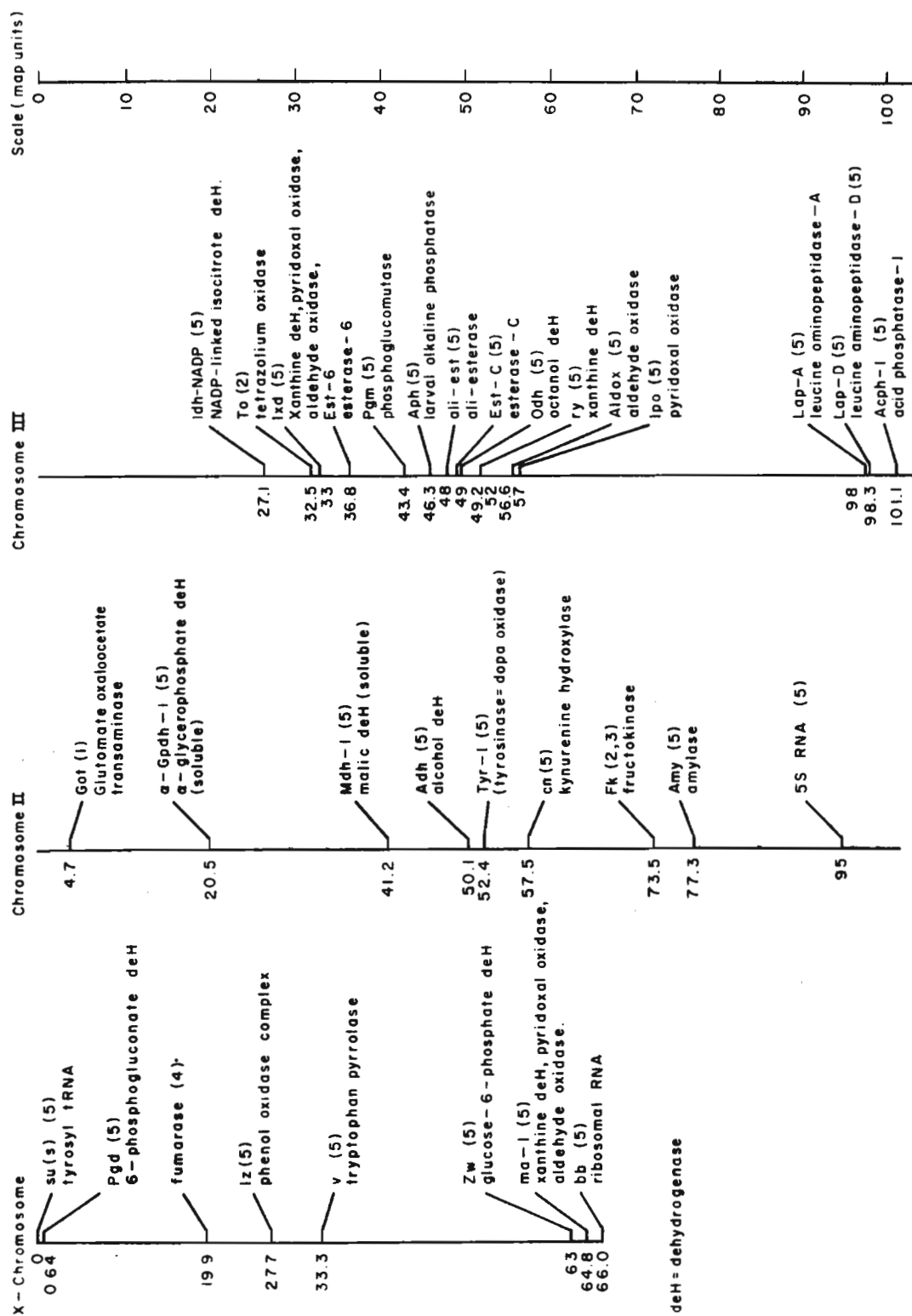
Cavener, D.R. Brown University, Providence, Rhode Island. An enzyme and general protein genetic map of *Drosophila melanogaster*.

O'Brien and MacIntyre (1971) presented a genetic map of those loci which effect or control the expression of enzymatic and general proteins of *Drosophila melanogaster*. Since that time, four additional loci have been mapped (fumarase, glutamate oxaloacetate transaminase, fructo-

kinase and tetrazolium oxidase). This note presents an updated map of enzyme loci. —→

The original sources are cited for only those loci mapped since 1971. The convention of listing a reference number adjacent to the locus designation has been followed.

Supported in part of NSF Grant DEB76-01987 to M.T. Clegg.



Rajasekarasetty, M.R. and S.R. Ramesh.
University of Mysore, India. On the
occurrence of new inverted gene
arrangement in *Drosophila nasuta nasuta*.

The third chromosome is the longest in the karyotype and harbours a majority of known inversions. The authors in the course of their investigations on the inversion polymorphism in natural populations of Tirupati Hills (India) found an inverted gene order in heterozygous condition in the third chromosome. A close scrutiny of its break points revealed that this is a new gene arrangement of overlapping type (Figure 1) thus far not reported. The two inversions of this

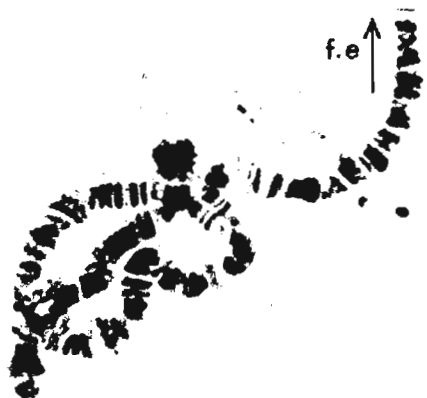


Figure 1. Overlapping inversions 3Y + 3Z.

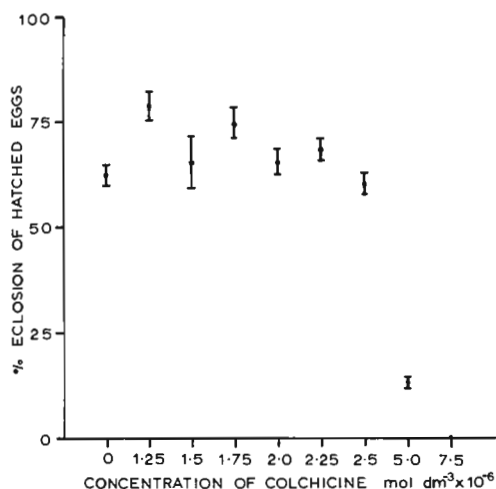
overlapping complex are named as 3Y and 3Z. The break points of these are localized by the authors on the standard photomap of *D.n. nasuta* made by Ranganath and Krishnamurthy (1973-74). The break points for 3Y are 84-119/136-119/136-137 and for 3Z are 84-112/127-112/127-137.

The authors are thankful to the Department of Atomic Energy, Government of India, for the financial assistance.

References: Nirmala, S.S. and N.B. Krishnamurthy 1974, *The Egyptian J. Genet. and Cytol.* 3:211-228; Ranganath, H.A. and N.B. Krishnamurthy 1973-74, *J. Mys. Univ.* 26:65-69; Ranganath, H.A. and N.B. Krishnamurthy 1975, *J. Hered.* 66:90-96.

Hannah, S.M. University of York, England.
Effect of colchicine on lethality and
sterility.

previously autoclaved yeast-agar-sucrose vial food (Carpenter, 1950) in increasing concentrations. Each vial contained 6 mls of medium and was inoculated with 40 eggs collected over



The suitability of the antimicrotubular drug colchicine as an agent for the selection of mutants affecting microtubules in *D. melanogaster* has been investigated by colchicine feeding experiments. Colchicine was added to concentrations of colchicine below $5 \text{ mol dm}^{-3} \times 10^{-6}$ do not appear to impair normal development, while toxic levels cause larval death within 24 hours from the time of egg hatch. Sublethal concentrations do not slow down the development rate or have any effect on egg hatching.

However, the adults which eclose from larvae grown on sub-lethal concentrations of colchicine are invariably sterile and do not become fertile even after transfer to standard vial food. Male adults reared on standard vial food become irreversibly sterile after 12 hours feeding on food containing as little as $1 \times 10^{-8} \text{M}$ colchicine. This sterility effect means that a simple one-step selection for colchicine resistant mutants is not feasible.

Reference: Carpenter, J.M. 1950, *DIS* 24:96-97.

Thongmeearkom, P., M. Clyde and W.B. Mather.
University of Queensland, Brisbane, Australia. Key to members of the *Drosophila* nasuta subgroup.

As part of the continuing research project "Evolution in the Genus *Drosophila*" some hundreds of isolines of the nasuta subgroup have been established from various regions of Southeast Asia (Philippines, Taiwan, Malaysia and Thailand).

Since 1971, by banana baiting methods six of the so far known nine species of the nasuta subgroup have been detected. Of these *D. sulfurigaster albostrigata*, *D. kohkoa* and *D. albomicans* are prevalent in at least some localities but *D. nasuta*, *D. kepulauana* and *D. pulaua* are rare in all localities. Since females of these species are similar externally, species differentiation is therefore based on male characters. The external traits used are the extent of white markings on the dorsal surface of the head and the presence or absence of a lateral longitudinal dark stripe on the thorax. When external morphological differences are not available as in the extreme sibling species, *D. nasuta* and *D. kepulauana*, the banding patterns of their salivary gland chromosomes are employed. The following key is based on the above characters:

1. Males with white orbits. 2
- Males with white frons 3
2. White orbits distinct (Fig 1) *D.s.albostrigata*
- White orbits indistinct *D.pulaua*
3. Males with white on frons in form of cross (Fig 2) 4
- Males with full white frons (Fig 3) 5
4. Males with lateral longitudinal dark stripe on thorax (Fig 4) *D.albomicans*
- Males with no such stripe on thorax *D.kohkoa*
5. Centromere end of the third salivary gland chromosome type A (Fig 5a) *D.nasuta*
- Centromere end of the third salivary gland chromosome type B (Fig 5b) *D.kepulauana*

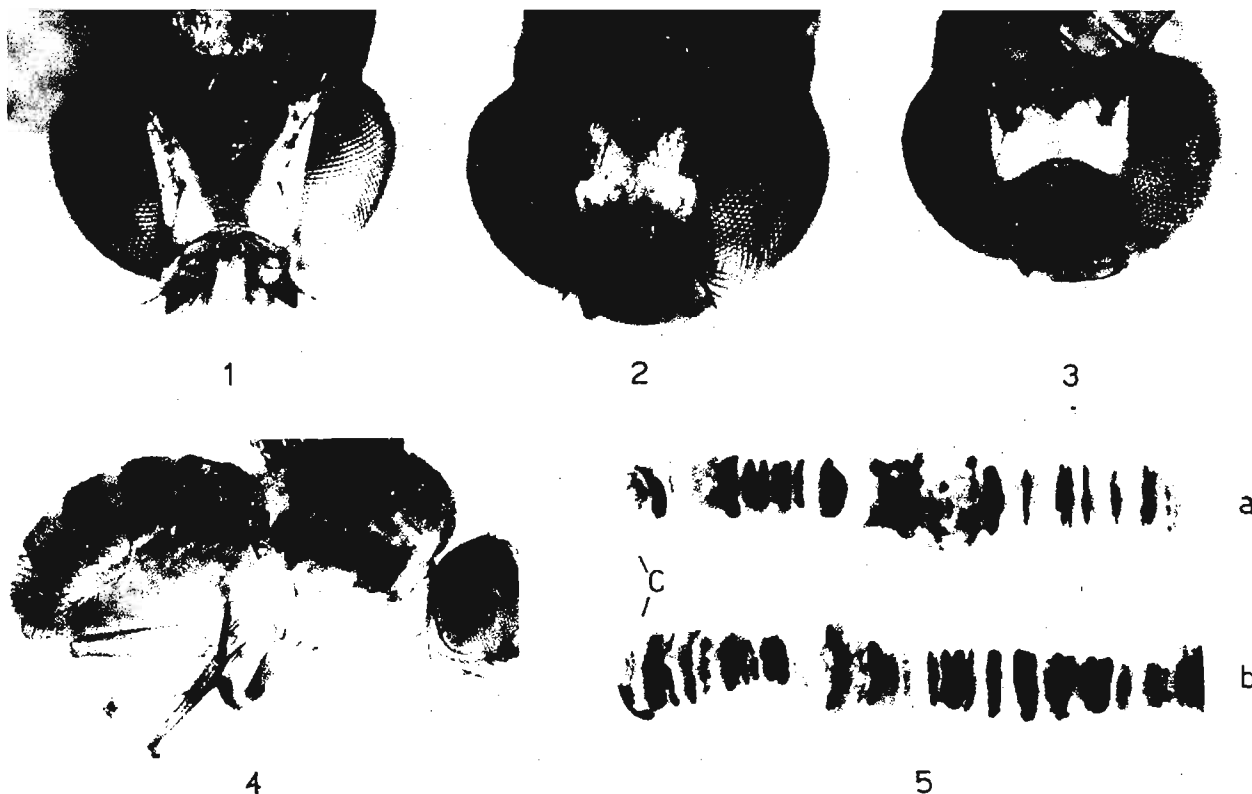


Figure 1) *D. sulfurigaster albostrigata* male showing white orbits; 2) *D. kohkoa* male showing white on frons in form of cross; 3) *D. kepulauana* male showing full white frons; 4) *D. albomicans* male showing the lateral longitudinal dark stripe on thorax; 5) Third chromosome centromere end of (a) *D. nasuta*, (b) *D. kepulauana*, C - centromere end.

Jeffery, D.E. Brigham Young University, Provo, Utah. Developmental times of selected species of Hawaiian picture-winged *Drosophila*.

The existing literature contains very little specific information regarding the developmental biology of the Hawaiian *Drosophila*. In a recent study aimed at interspecific comparisons of puffing patterns of salivary gland chromosomes, it became evident that the flies exhibit an extra-

ordinary variability in developmental timing. In one species, for example, eggs laid all within a four-day span produced larvae some of which began to pupate on the 26th day after initial egg laying; pupation continued to occur in the culture in significant numbers for over two additional weeks, then occurred in diminishing numbers until at least the 80th day, at which time active larvae were still observed in the culture. It is thus evident that eggs laid the same day may vary by as much as 50 days in the time required to reach maturation for pupation. It was noticed, however, that a significant number of the late-maturing larvae died without pupating. Experiments were not conducted to resolve whether this was due to specific lethal genotypes acting in the pre-pupatory stages, or to degeneration of the quality of the medium.

It is a somewhat standard rule of thumb among workers of the Hawaiian *Drosophila* Project that the flies (picture-wings specifically) require roughly one month from oviposition to pupation, three to four more weeks to reach eclosion, and approximately another month to reach sexual maturity, thus producing a generation time of approximately three months. In the interests of the above-mentioned study, it was desirable to produce better synchronization of development. To date, no really satisfactory method has been found.

Females prove to be rather unpredictable in their egg-laying habits, often requiring several days of familiarization with a specific culture vial before beginning ovipositing. The same females, in another culture vial, may begin oviposition within a day or two. In many cases no precise pattern relating to age, condition of culture medium, etc., has been definable.

Though the flies can remain healthy and productive for two to three months or even longer, it is necessary to transfer them to new culture vials every seven days. Larvae produced in the vials are given periodic supplemental food; after they reach maturity they begin to migrate out of the culture medium in search of an appropriate sand or soil mixture in which to pupate. On finding suitable medium, they will pupate usually within a few hours. The migration behavior thus provides a readily-identifiable landmark of larval maturity.

The above characteristics introduce a certain amount of imprecision into the developmental times given below; the first two columns thus list numbers of days after the parents were first introduced into the culture vials, and indicate periods possibly one or more days longer than the actual time measured from oviposition.

<u>Drosophila species</u>	<u>Time in Days</u>		
	<u>Vial entry to migration</u>	<u>Vial entry to eclosion</u>	<u>Eclosion to fertile mating</u>
grimshawi subgroup			
balioptera	28	50	26
crucigera	25	47	27
formella	--	58	22
grimshawi	28	52	16
gymnobasis	30	52	16
hawaiiensis	33	59	16
silvarentis	28	51	20
planitibia subgroup			
differens	24	54	30
hemipeza	24	45	33
heteroneura	27	52	30
plantibia	26	52	28
silvestris	24	50	18

The above times are the minima recorded to date; average times are several days or a week longer. Currently on-going work by other investigators indicates that under special conditions the time between eclosion and breeding may be reduced still further, for at least some of the above species.

(Research supported by NSF grants BMS 74-20117 to D.E.J. and GB 27586 to H.L. Carson.)

Rajasekarasetty, M.R., S.R. Ramesh and N.B. Krishnamurthy. University of Mysore, India. Preliminary report on the isozymes of acid phosphatase and alkaline phosphatase in a few members of *Drosophila nasuta* subgroup.

Acid and alkaline phosphatases in a few members of the *nasuta* subgroup of immigrans species group have been analysed by polyacrylamide gel electrophoretic technique of Davis (1964). Staining procedure employed is after Ayala (1972) with slight modification involving fast blue RR salt as the dye coupler instead of fast blue BB salt.

About one-day-old male flies of *Drosophila nasuta nasuta*, *D.n. albomicana*, *D.n. kepulauana*, *D. kohkoa*, *D. sulfurigaster neonasuta* and *D.s. sulfurigaster* are used for the above investigation. The zymograms of each of the above enzymes exhibit three zones of activity named A, B and C from the anodal end. Further in both APH and ACPH enzyme systems, the zone A is represented by a single broad band, which is invariably present in all the members studied, while B and C zones exhibit a number of variant phenotypes.

Considering the zymograms of ACPH, zone C has three variant phenotypes and zone B has two. All the variant types together are not present in any single individual of any species. In *D.n. nasuta*, the zone B and C are polymorphic. In *D.n. albomicana* and *D. kohkoa* zone C is found in 10% of the individuals. In *D.n. albomicana* zone B exhibits least variability. In *D.n. kepulauana*, both B and C zones are less polymorphic. In both *D.s. neonasuta* and *D.s. sulfurigaster* the zone B is monomorphic.

A study of the zymograms for APH activity in these members has revealed that zone B has four variant phenotypes and zone C has five. All of them are not found together in any single individual. *D.n. nasuta*, *D.n. albomicana* and *D. kohkoa* are polymorphic for both zones B and C, while *D.n. kepulauana* is least polymorphic for these zones of activity. Null activity is observed in the zone C of *D.s. neonasuta* and *D.s. sulfurigaster* while the zone B is less variable in *D.s. neonasuta* than in *D.s. sulfurigaster*. The allelomorphous implications with regard to the different variants are being analysed.

The authors are thankful to the Department of Atomic Energy, Government of India, for the financial assistance.

References: Ayala, F.J., J.R. Powell, M.L. Tracey, C.A. Murao and S. Perez-salas 1972, *Genetics* 70:113-139; Davis, B.J. 1964, *Ann. N.Y. Acad. Sci.*:121.

Grossfield, J. City College of New York, New York City. Sexual isolation between *D. auraria* and *D. quadraria*.

The *auraria* complex of species in the *melanogaster* group shows a high degree of behavioral isolation. Two of the species can mate in darkness while a third species cannot. On morphological grounds a fourth species was added

to this group (Bock and Wheeler, UTP 1972). Tests of this new species with the most widespread member of the complex, which can mate in darkness (Table 1) shows that *D. quadraria*

Table 1. Degree of insemination of *D. auraria* (A) and *D. quadraria* (Q) under conditions of constant light (LL) and constant darkness (DD). Both light conditions tested for 2h and 24h exposure periods. N = number of females dissected; % = percentage inseminated. All tests performed in vials of 10 females and 5 males.

		2h				24h			
		LL		DD		LD		DD	
♀	♂	N	%	N	%	N	%	N	%
A	A	120	84.2	50	8.0	100	98.0	50	68.0
Q	Q	100	85.0	70	0.0	100	84.0	80	0.0
A	Q	120	0.0	60	0.0	100	0.0	50	0.0
Q	A	100	81.0	60	0.0	100	68.0	50	26.0

does not mate in darkness. Unidirectional isolation is present but these two species show a higher degree of isolation than that reported for any other pairwise combination of species in this complex (Kurokawa, Jap. J. Genet. 35:161).

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Iwamoto, R.N. Washington State University, Pullman. Chromosome substitutions and ovariole numbers.

A chromosomal interchange technique (Moree 1971) was used to substitute second and third chromosome sets of three different strains of *D. melanogaster*. Females of the six resulting interchange lines in addition to the three

original strains used in their synthesis were examined for ovariole numbers. The direction and magnitude of interchromosomal interactions and individual chromosomal contributions could then be evaluated by analyzing changes in relation to chromosomal constitution.

The three original strains differ in geographical origin and length of time maintained in captivity. The histories of Canton-S (S;S) and Kalahari (K;K) are well documented. Orchards (O;O) is a relatively new strain collected in the vicinity of Lewiston-Orchards, Idaho in 1974.

Females were collected as virgins from 60 ml culture bottles containing 20 ml of medium and no more than 40 larvae. They were dissected approximately four days after emergence. Because records of individual ovarioles undergoing active vitellogenesis were not kept, the ovariole counts represent total numbers.

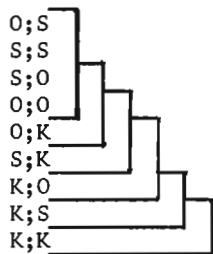
Means, coefficients of variation, and confidence intervals for individual strain means are presented in the accompanying table. Significant differences were based on non-overlap

Table. Ovariole numbers of original and interchange lines

Strain	n ^a	\bar{X}	\pm	$(t.01\bar{S}\bar{X})$	CV
O;O	35	41.20	\pm	2.82	0.15
K;K	51	29.35	\pm	1.97	0.18
S;S	24	43.58	\pm	2.26	0.09
O;K	34	39.06	\pm	1.73	0.09
K;O	43	35.16	\pm	2.54	0.18
O;S	36	45.06	\pm	2.44	0.12
S;O	34	42.47	\pm	1.79	0.09
K;S	40	33.00	\pm	2.22	0.16
S;K	35	38.06	\pm	2.00	0.11

^a = number of females dissected.

Comparisons - [within brackets, no significant differences]



fewer ovarioles relative to Canton-S and Orchards and more ovarioles relative to Kalahari. All other interchange lines either demonstrated increases in ovariole numbers or statistically insignificant changes. Lines involving Orchards chromosomes and those involving Canton-S chromosomes were not significantly different (\bar{X} = 40.59 and 40.43, respectively). Kalahari lines on the other hand averaged six ovarioles less per female.

These results indicate that significant interchromosomal interactions were generally present although the extent and direction of such interaction effects were difficult to predict. Canton-S, primarily because of its longer laboratory history and theoretically reduced fitness, was expected to possess fewer ovarioles. That Canton-S females had among the highest ovariole numbers recorded and also that coefficients of variation for all strains were consistently high appear to be further evidence that numbers of ovarioles may not be directly related to reproductive fitness.

In experiments with isogenic lines, Seiger (1966) reported that the replacement of one chromosome of a basic strain generally resulted in body weight changes in the negative direction, perhaps the result of alterations of co-adapted gene complexes. Ovariole numbers in this experiment were not similarly affected. Perhaps this may be reflective of overall lower selection pressures and consequently, reduced coupling of the particular gene complexes involved in the manifestation of the character.

References: Moree, R. 1971, DIS 47:82; Seiger, M.B. 1966, Genetics 53:237-248.

between confidence intervals. For the original strains, the number of ovarioles in Kalahari females was significantly lower than that of both Orchards and Canton-S between which no significant differences were detected. The grand mean of the three original strains was virtually equivalent to that of the six interchange lines (38.04 vs 38.80).

When contributions of second and third chromosome sets were determined relative to the combination of chromosomes in the original strains, the following results were noted: Interaction effects from second chromosome substitutions were more pronounced regardless of whether the resultant effects were positive or negative. Interchange lines with either Kalahari second or third chromosome sets possessed

Kalisch, W.-E. and K. Hägele. Ruhr-Universität Bochum, Germany. Determination of ^3H -thymidine background labeling over polytene chromosomes in *Drosophila melanogaster*.

Autoradiographic analysis of replication behavior in ^3H -thymidine labeled polytene chromosomes presumes low background labeling. For the analysis of labeling frequencies a 'tight' cluster of four, or rather three silver grains is deemed as the minimum of significant labeling in 'low' background autoradiographs

(Howard and Plaut 1968; Arcos-Terán 1972). For the analysis of labeling intensities, however, this demarcation between background and labeling is insufficient, as has been shown in Kalisch and Hägele 1974.

In each of 10 ^3H -thymidine labeled autoradiographs with relatively high background we have analysed five unlabeled nuclei. According to Hägele and Kalisch 1974, unlabeled nuclei are characterized by no silver grains over prominent puffs 2B, 68C, 74EF, 75B and the chromocenter. Autoradiographs are made from 3rd instar wild type (Berlin) *D. mel.* female larvae (10 min. of incubation at $17 \pm 2^\circ\text{C}$; $20 \mu\text{Ci/ml}$; spec. act. 15.1 Ci/m mol ; Feulgen-staining; Kodak stripping film AR10; 20 days of exposure at 4°C ; further methods used in Kalisch and Hägele 1973; Hägele and Kalisch 1974). Polytene nuclei were slightly spread so the bulk of chromosomes filled a square of $2500 \mu\text{m}$ (magnification: $100 \times 2 \times 12.5$). In Table 1 (A) - values show arithmetic means and standard deviations of background silver grains over the chromosomes within the five squares of each autoradiograph; (B) - values are arithmetic means and standard deviations of corresponding chromosomeless areas immediately next to each of the nuclei. Differences in background labeling between chromosomeless areas and polytene chromosomes $[(B-A) \times 100/B]$ are represented by (C). On the average, background labeling is 32.4 percent lower over the polytene chromosomes.

Table 1. Intensities of background labeling (standard deviations in brackets).

Slide No.	1	2	3	4	5	6	7	8	9	10	M
A	31.8 (7.6)	44.2 (13.8)	54.0 (8.4)	50.6 (9.0)	33.4 (9.8)	30.8 (8.1)	18.6 (2.0)	65.2 (20.0)	61.6 (6.8)	64.0 (16.5)	45.4 (15.8)
B	61.6 (10.9)	62.4 (7.8)	75.6 (18.1)	68.4 (5.7)	44.0 (4.1)	50.2 (6.3)	31.6 (7.9)	94.2 (19.0)	84.8 (4.6)	99.0 (8.0)	67.2 (20.7)
C	48.4	29.2	28.6	26.0	24.1	28.6	41.1	23.1	27.3	35.3	32.4

The arithmetic mean of background silver grains over squares with unlabeled chromosomes [M of the (A) values in Table 1] can serve, along with its standard deviation (S), as background determination [i.e., background: $M(50) = 45.4$; $S = 15.8$].

Distribution of background silver grains over unlabeled chromosomes was analysed (by the same method used for Table 1) over ten different autoradiographs (Table 2), which were suitable for labeling intensity analysis because of their low background [background: $M(50) = 24.3$; $S = 11.8$]. The grid consisted of $100 \times 25 \mu\text{m}^2$ single squares ($25 \mu\text{m}^2$ covers an average spot-labeling section over polytene chromosomes). Even for the range of 0-5 silver grains per single square, data in Table 2 do not fit the Poisson-distribution [$\chi^2(5) = 98.4$; $p < 10^{-10}$] because 'tight' clusters of background silver grains are to be found.

Table 2. Distribution of background silver grains over unlabeled chromosomes.

	Squares with 0 1 2 3 4 5 6 8 12 20 silver grains										
Expected (Poisson-distribution)	3952.6	930.5	109.5	8.6	0.5	0.0					
Observed	3998	862	108	22	4	1	2	1*	1*	1*	
Percent	79.96	17.24	2.16	0.44	0.08	0.02	0.04		0.06		

* Linear and starlike arrangements of silver grains

References: Arcos-Terán, L. 1972, *Chromosoma* (Berl.) 37:233; Hägele, K. and W.-E. Kalisch 1974, *Chromosoma* (Berl.) 47:403; Howard, E.F. and W. Plaut 1968, *J. of Cell Biol.* 39:415; Kalisch, W.-E. and K. Hägele 1973, *Chromosome* (Berl.) 44:265; Kalisch, W.-E. and K. Hägele 1974, *Nature* 249:862.

Gateff, E., D.M. Golubovsky and K.S. Sokolova*. Biologisches Institut I, Albert-Ludwigs-Universität, Freiburg, i. Br., W. Germany and *Institute for Cytology and Genetics, Novosibirsk 90, U.S.S.R. Lethal phase, morphology and developmental capacities of the presumptive adult optic centers in the larval brain and the imaginal discs of fifteen 1(2)gl alleles and a net 1(2)gl deficiency.

More than 50 independent 1(2)gl have been found since the discovery of the first 1(2)gl allele by Bridges (Lindsley and Grell, 1968; Gateff and Schneiderman, 1969a; Golubovsky and Sokolova, 1973 and unpublished data). In two of these alleles, namely the 1(2)gl and 1(2)gl⁴ alleles, Gateff and Schneiderman (1969b, 1974) demonstrated neoplastic imaginal discs and a malignant neuroblastoma in the presumptive adult optic centers of the larval brain (Table 1). The affected organs showed drastic morphological and histological aberrations and the

abnormal growths exhibited all characteristics of truly benign and malignant neoplasms (Gateff and Schneiderman, 1969b, 1974).

The present study compares the lethal phase, the morphology and the developmental capacities of thirteen additional 1(2)gl alleles and of a net 1(2)gl deficiency (Table 1). All 1(2)gl alleles are spontaneous, independent mutations, and were collected in isolated populations throughout the Soviet Union by Golubovsky and Sokolova (1973, and unpublished results). The designations of the different 1(2)gl alleles are preliminary. The net 1(2)gl deficiency was x-ray induced by Golubovsky. The methods and criteria used to identify and characterize neoplastic benign and malignant growth are described elsewhere (Gateff and Schneiderman, 1969b, 1974).

Table 1 summarizes the results of this study. It shows that the lethal phase of the different alleles ranges from predominantly larval to predominantly pupal. It shows further considerable variations in size and morphology of the brain and the imaginal discs. The size and the morphology of these organs extend from normal over very enlarged to rudimentary small. The table demonstrates finally, that the capacity for neoplastic growth of the affected tissues relates closely to the extent of the morphological changes. Excepting the 1(2)gl 558 allele, in which the brain and the imaginal discs are morphologically and developmentally normal, the brains and imaginal discs of all remaining 1(2)gl alleles vary in size and morphology. It was generally observed that the more enlarged and deformed an organ, the more severe was the neoplastic growth in situ as well as in culture in vivo in the adult abdomen. For instance, brain pieces from 1(2)gl alleles with extremely large brains, such as 1(2)gl, 1(2)gl⁴, 1(2)gl 150, 1(2)gl 110 or Df(2)net 1gl, when implanted into the abdomens of female flies showed very fast growth and killed the host in one week. 1(2)gl alleles like 1(2)gl 138, 1(2)gl M119 or 1(2)gl 334, whose brains did not exceed significantly the size of a wild-type brain, grew slower and needed longer to kill the adult host. In respect to the morphology and the neoplastic qualities of the imaginal discs, three classes of 1(2)gl alleles could be distinguished: 1. 1(2)gl alleles whose imaginal discs represent lethal, benign and transplantable neoplasms, 2. 1(2)gl alleles which possess rudimentary small imaginal discs and 3. one 1(2)gl allele with normal imaginal discs.

The present work and genetic complementation studies (Golubovsky and Sokolova, unpublished results) indicate strongly the complex structure of the 1(2)gl locus.

References: Gateff, E. and H.A. Schneiderman 1969a, *DIS* 44:46-47; Gateff, E. and H.A. Schneiderman 1969b, *Nat. Cancer Inst. Monogr.* 31:365-397; Gateff, E. and H.A. Schneiderman 1974, *Wilhelm Roux' Arch. Entwicklungsmech. Organismen* 176:23-65; Golubovsky, M.D. and K.B. Sokolova 1973, *DIS* 50:124.

Work supported by Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 46.

l(2)gl allele	Lethal phase, %		Size and morphology of:		Capacity for neoplastic growth in culture in vivo of:	
	Larvae	Pupae	Brain in situ	Imaginal discs in situ	Adult optic neuroblasts	Imaginal discs
558	97.2	2.8	Normal	Normal	-	-
1(2)gl	98.0	2.0	Very enlarged deformed	Very enlarged clumped	+++	+++
1(2)gl ⁴	89.1	10.9	Very enlarged deformed	Very enlarged clumped	+++	+++
D 150	97.3	2.7	Very enlarged deformed	Very enlarged clumped	+++	+++
138	98.0	2.0	Small deformed	Small clumped	+	+
M 119	85.0	15.0	Small deformed	Small clumped	+	+
705	89.7	10.3	Slightly enlarged deformed	Only wing disc present slightly enlarged	++	++
271	81.4	18.6	Small deformed	Small clumped	+	+
275	79.1	20.9	Small deformed	Slightly enlarged clumped	+	++
309	54.0	46.0	Slightly enlarged deformed	Slightly enlarged clumped	++	++
314	57.3	42.7	Slightly enlarged deformed	Rudimentary small	++	-
110	53.6	46.4	Very enlarged deformed	Very enlarged clumped	+++	+++
334	18.4	81.6	Small deformed	Small clumped	+	+
353	19.5	80.5	Small deformed	Rudimentary small	+	-
351	17.5	82.5	Slightly enlarged deformed	Slightly enlarged clumped	++	++
Df net 1(2)gl	-	-	Very enlarged deformed	Very enlarged clumped	+++	+++

Table 1. Lethal phase, morphology and capacity for neoplastic growth in culture in vivo of the neuroblasts in the presumptive adult optic centers in the larval brain and the imaginal discs of fifteen l(2)gl alleles and a net l(2)gl deficiency. Key: capacity for neoplastic growth in culture in vivo: + = present, slow growth; ++ = enhanced growth; +++ = very fast growth; - = no growth.

Boehm, K.D. and F. DeMarinis. Cleveland State University, Cleveland, Ohio. Uptake of acetamide-1-C¹⁴ by the 70-hour stage imaginal eye discs of the Bar series.

storing a genetically Bar to a phenotypically normal or wild-type eye. Furthermore it was shown that in effect this compound had three distinctive critical periods in the development of the larval eye. The authors gave a new interpretation of the regulatory mechanism of Bar. The so-called B-substance which had been envisioned by the early workers of Bar took on the new interpretation as a repressor substance; the early interpretation of Bar dosage took on the new interpretation as repeated operons. The action of glutaramide and amides in general, were given the role of inducers or depressors. The model presented at that time (DeMarinis and Sheibley 1965) was based on the general model for the prokaryotes (Jacob and Monod 1961).

Collecting significant data to prove or disprove this hypothesis has been most difficult. The empeding factor has been in collecting enough of the same tissue in which Bar has its prime effect, that is, the imaginal eye discs.

In this experiment we have collected enough imaginal eye discs to give a significant brief report at this time.

The present studies were done to determine the relationship between Bar dosage and the quantity of uptake of radioactive acetamide and radioactive uracil by the imaginal eye discs at the most sensitive of the larval stages (70-hour). Radioactive tracers in the form of acetamide-1-C¹⁴ and uracil-2-C¹⁴ were fed to reverted Bar (+), Bar (B) and double Bar (BB)

larvae during the sensitive period for a period of one hour. The larvae were then immediately frozen. Later, samples of imaginal eye discs and equivalent samples of larval cutical and fat bodies were removed and scintillation counts were made in males and females. Average counts per minute (CPM) were made on some 2000 eye discs for each sample. Males treated with acetamide-1-C¹⁴ wild (+) = 112

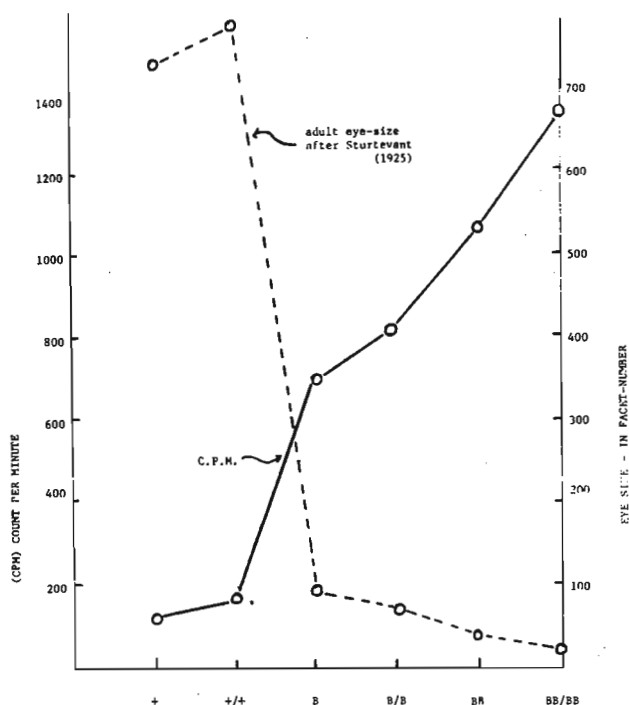


Figure 1. Relationship between uptake of radioactive acetamide-1-C¹⁴ by 70-hour larval imaginal eye discs and Bar dosage. This is contrasted with eye-size and Bar dosage of Sturtevant (1925).

± 3.3; Bar (B) = 715 ± 16.6, double Bar (BB) = 1089 ± 6.6; females, wild (+/+) = 153 ± 3.8 Bar B/B = 814 ± 7.2, double Bar BB/BB = 1302 ± 6.2.

Figure 1 shows a plotting of these data. It compares increasing dosage of Bar with the quantity of acetamide uptake by cells of the imaginal eye discs at 70-hour larval stage. It also shows a comparison of gene dosage and

adult eye size in terms of facet-number (Sturtevant 1925). It is clear from these results that there is a direct relationship between Bar dosage and the uptake of radioactive acetamide while an inverse relationship exists between Bar dosage and adult eye size. These data lend more support to the original hypothesis (DeMarinis and Sheibley 1965) which predicts that Bar and double Bar produce more repressor and in turn would show a higher degree of uptake of the acetamide inducer than the wild genotype.

The cyclic amide, uracil-2-C¹⁴, also shows similar results as acetamide-1-C¹⁴. Larval cuticle from each of the above group of larvae never reach a value higher than 59 ± 3.0 CPM. The fat bodies give average count 72 ± 3.1 CPM.

References: DeMarinis, F. and F. Sheibley 1965, Proc. Symp. Mutational Proc., 303, Academic Press, Prague; Jacob, F. and J. Monod 1961, J. Mol. Biol. 3:318; Sturtevant, A. 1925, Genetics 10:117.

Sampsel, B.M. and R.D. Milkman.
University of Iowa, Iowa City. Alcohol dehydrogenase thermostability variants: genetic localization and occurrence in a natural population of *D. melanogaster*.

Recently it has been shown that heat treatments can be used to distinguish among allozymes with the same electrophoretic mobility (1, 2, 3). Examination of *D. melanogaster* laboratory strains has revealed that both the fast and slow mobility classes of Adh allozymes are heterogeneous and that 2 slow forms and 2 fast forms

can be separated on the basis of their inactivation by heat treatments (4, 5).

We have surveyed a natural population and have found all four thermostability variants. Wild males captured in Cedar Rapids, Iowa, were crossed to a balanced lab stock, and strains homozygous for wild second chromosomes were obtained.

Using the Adamkewicz applicator (DIS 45:192), 4 flies from each strain were homogenized individually. The crude extract was applied to cellulose acetate strips and electrophoresed for 45 min at 210 V. in a deluxe Gelman chamber. Control strips were stained immediately after electrophoresis, while strips to be treated were placed in a Saran envelope and immersed in a water bath.

176 strains were tested in this fashion, with the results shown in Table 1.

Table 1

Number of Strains	% of Total	Adh Electrophoretic Mobility	Adh Thermostability
78	44.3	Fast	Moderate (Inactivated by 20 sec at 43°C)
3	1.7	Fast	Resistant (Unaffected by 20 sec at 43°C)
94	53.4	Slow	Moderate (Unaffected by 15 sec at 40°C)
1	0.6	Slow	Sensitive (Inactivated by 15 sec at 40°C)

Mapping experiments were performed to determine whether the variant isozymes were produced by different alleles at the *Adh* locus.

Flies with the fast resistant form of Adh were crossed to a *n b cn bw* lab stock which is homozygous for the "moderate" fast form. F₁ flies were backcrossed to *n b cn bw*. Progeny showing both parental phenotypes, as well as recombinants between *b* and *cn* were tested. All 102 parental types gave the expected results in terms of mobility and thermostability. The behavior of the 104 tested recombinants located the difference in heat resistance of the 2 fasts at 50.75 on the second chromosome. This strongly suggests that the altered resistance is due to an allelic difference at the *Adh* locus (II-50.1).

The slow sensitive form was mapped in the same manner and placed at 49.9 on the basis of 176 offspring recombinant for the same flanking markers, *b* and *cn*. In another experiment, females heterozygous for the "slow, sensitive" and "fast, resistant" forms of Adh were crossed to males homozygous for the "moderate fast" form. 406 progeny were tested - of which, 212 were homozygous fast with respect to mobility and resistant to a treatment of 20 seconds at 43°C. 194 offspring were mobility heterozygotes and in every case, the slow allozyme was inactivated by a heat treatment of 15 seconds at 40°C.

The fact that no recombination was observed between the properties of mobility and thermostability suggests that they are controlled by closely linked genes, if not - as seems more likely - by the same gene. There are, therefore, a minimum of 4 alleles at the *Adh* locus in the population samples, apparently.

Supported in part by NSF Grant No. DEB-76-01903. B.M.S. is a trainee on NIH Training Grant No. T32-GM07091.

References: (1) Wright, T.R.F. and R.J. MacIntyre 1965, J. of the Elisha Mitchell Sci. Soc. 81:17-19; (2) Bernstein, S.C., H.L. Throckmorton and J.L. Hubby 1973, Proc. Nat. Acad. Sci. U.S. 70:3928-3931; (3) Singh, R.S., J.L. Hubby and L.H. Throckmorton 1975, Genetics 80: 637-650; (4) Thörig, G.E.W., A.A. Schoone and W. Scharloo 1975, Biochem. Genetics 13:721-731; (5) Milkman, R.D. 1976, Biochem. Genetics 14:383-387.

Mohan, J. Haryana Agricultural University, Hissar, India. Multiplicity of ribosomal RNA genes in *Drosophila melanogaster* during growth and development.

It has been seen that in *Drosophila* all the DNA sequences do not replicate uniformly (Gall et al., 1971). In polytene chromosomes of salivary glands, rDNA is underreplicated (Hennig and Meer 1970; Spear and Gall, 1973). They (Spear and Gall, 1973; Spear 1974) have suggested that (i)

rDNA level in salivary glands is independent of the genotype and (ii) large nuclear DNA content may be associated with underreplication of rRNA genes. We have studied rDNA levels in various tissues of several genotypes and our observations do not fully support their suggestions. Results are summarized in Table 1. It is clear that except for underreplication of ribosomal genes in larvae and salivary glands, no other tissue examined displayed any change in rDNA redundancy. Decrease in rDNA content observed in third-instar larvae might be partly accounted for by underreplication of rDNA in salivary glands. Other tissues analysed, i.e. imaginal discs, male genitalia, carcass and pupae showed no change in rDNA content in comparison to adult level (Mohan, 1976).

Table 1. Percentage of ribosomal DNA in various tissues of *D. melanogaster**

Genotype	Adult	Larva	Salivary glands	Ovaries
bb ⁺ /bb ⁺ ♀ bb ⁺ /bb ⁺ ♂	0.342 ± 0.011	0.282 ± 0.008**	0.073 ± 0.006***	0.317 ± 0.012
bb ⁺ y ² eq/bb ⁺ y ² eq ♀ bb ⁺ y ² eq/bb ⁺ ♂	0.526 ± 0.011	0.436 ± 0.006***	0.095 ± 0.005***	0.541 ± 0.017
car bb/car bb ♀ car bb/ybb- ♂	0.124 ± 0.006	0.108 ± 0.003	0.046 ± 0.005***	0.153 ± 0.015

* Description of genotypes and procedural details have been already described (Mohan, 1975; Mohan and Ritossa, 1970).

** Significantly different from the adult at 1.0% probability level.

*** Significantly different from the adult at 0.1% probability level.

The first suggestion of Spear and Gall was based on the observation that rDNA level in two wild stocks was equal in salivary glands though it differed in adults. Since the level of percent hybridization is very low in this tissue, it is not very easy to verify their statement. Still, the rDNA level in the bobbed genotype is significantly reduced in comparison to one wild genotype (bb⁺ y² eq/bb⁺ y² eq ♀ and bb⁺ y² eq/bb⁺ ♂) but in no other case the differences appeared to be significant. Therefore, more data should be obtained to ascertain whether rDNA content in polytenic salivary glands is really independent of the genotype. The second suggestion of Spear and Gall that large nuclear DNA content may be associated with underreplication of rRNA genes is not compatible with normal amounts of rDNA in polyploid/polytenic ovaries of various genotypes. Underreplication of rDNA has been observed in polytenic suspensor cells (Lima-de-Faria et al. 1975). On the contrary, White (1974) has obtained evidence in favour of rDNA amplification in polyploid tapetal cells of onion and lily. So it appears that polyploidy or polyteny and consequently large nuclear DNA content need not necessarily be correlated with underreplication of rRNA genes. Sirlin (1972) has argued that in nurse cells of *Drosophila*, the nucleolar masses originate from NO (nucleolar organizer) rDNA that has undergone amplification. He has suggested that this exceptional somatic counterpart of germinal amplification would be due to the fact that the nurse cells supply almost all rRNA to the oocyte. Our results show no marked increase in rDNA content in ovaries over the level of adults in various genotypes. Since most of the DNA present in ovaries is contributed by nurse cells, it is likely that nurse cells do not show amplification of rDNA to any marked degree. These results are in agreement with the interpretation (Pardue et al., 1970; Wimber and Steffensen, 1974) that the nucleolar masses (micronucleoli) represent ramifications of NO rDNA.

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Biology of RNA (Academic Press, N.Y., London); Spear, B.B. 1974, *Chromosoma* 48:159; Spear, B.B., J.G. Gall 1973, *Proc. Nat. Acad. Sci.* 70:1359; White, R.L. 1974, Quoted in Wimber and Steffensen; Wimber, D.E. and D.M. Steffensen 1974, *Ann. Rev. Genet.* 7:205.

Charlesworth, B., D. Charlesworth and M. Loukas. University of Sussex, Brighton England. Linkage disequilibrium studies on *D. subobscura* populations.

O and J chromosomes were extracted from wild males by crosses to recessive marker stocks. Each chromosome was characterised with respect to its allelic content at a number of electrophoretically detected loci and also with respect to gene arrangements. The following O chromo-

some loci were studied: Est 5, ODH, AO, ME, XDH and LAP. The J loci were Est 3, Est 7, APH and PGM (see Zouros et al. 1974 for chromosomal locations of these loci, apart from LAP, PGM and ME, which are discussed below).

The first population studied was captured at a woodland site near the University of Sussex in May 1975. 152 O chromosomes and 141 J chromosomes were analysed. The following O gene arrangements were detected: ST (42%), 3 + 4 (36%), 3 + 4 + 8 (18%), 3 + 4 + 16 (3%) and 3 + 4 + 12 (1%). None of the O genes showed significant associations with these inversions, except for LAP, where a highly significant linkage disequilibrium was found ($\chi^2_4 = 30.7$). The association is such that O_{ST} contains a higher proportion of LAP allele 1.11 than the gene arrangements involving O_{3+4} , which are predominantly 1.00. This association is very similar to that reported by Zouros et al. (1974) for Greek populations and Prevosti (unpublished) for Spanish populations. Our linkage data indicates that LAP is located either inside or very close to the breakpoints of O_{3+4} : out of 503 backcross progeny of O_{3+4}/O_{ST} females heterozygous for LAP, no recombinants were detected. This corrects an earlier report of appreciable crossing over between LAP and O_{3+4} (Zouros et al. 1974). The linkage disequilibrium may therefore be due to a hitch-hiking effect (Maynard Smith and Haigh 1974), due to the spread of the O_{3+4} gene arrangement in association with the LAP allele contained in the gamete in which the chromosomal rearrangement producing O_{3+4} originally occurred. No significant associations between O chromosome loci were detected; the total χ^2 for pairwise disequilibrium is 56.5 for 54 d.f. This is in contrast to the data of Zouros and Krimbas (1973), who found strong linkage disequilibrium between AO and XDH in two Greek populations. With respect to the J chromosome, two gene arrangements were detected: ST (30%) and 1 (70%). No pairwise disequilibria between electrophoretic loci, or between electrophoretic loci and inversions, were detected. The J map has been extended by locating PGM with respect to ma and int (with which it shows free recombination), and with respect to APH, with which it gave 37% recombinants out of 142 backcross individuals. PGM is therefore 46.1 map units to the right of APH, using Haldane's correction for double crossovers.

A second population was collected in September 1975 at a site in suburban North London, and 124 O chromosomes were extracted. These were scored for gene arrangement and for all the above loci except ODH. 2 of the chromosomes carried the mutant cherry (ch). The spectrum of gene arrangements was similar to that of the Sussex population: ST (38%), 3 + 4 (46%), 3 + 4 + 8 (12%), 3 + 4 + 16 (2%) and 3 + 4 + 12 (2%). The frequencies do not differ significantly from the Sussex ones. A highly significant association was detected between LAP and gene arrangements, of the same type as in the first population. The only other significant pairwise association was between XDH and ME: $\chi^2_3 = 11.0$. There was no evidence for this association in the Sussex population, which is the only other one for which data are available at present. The chromosomal location of ME has been investigated further by five-point mapping using cu, AO, ME, XDH, ch. We have confirmed that ME lies between AO and XDH, as stated by Zouros et al. (1974), but find that it is located close to XDH (4.3% recombination with 170 backcross individuals).

References: Maynard Smith, J. and J. Haigh 1974, *Genet. Res.* 23:23-35; Zouros, E. and C.B. Krimbas 1973, *Genetics* 73:659-674; Zouros, E., C.B. Krimbas, S. Tsakas and M. Loukas 1974, *Genetics* 78:1223-1244.

This research was supported by a grant from the Science Research Council.

Mertens, M. Zoologisches Institut der Universität, München, W. Germany. Larval and imaginal defects after pricking of blastoderm stages: Experiments complementary to morphogenetic fate maps of *Drosophila*.

The spatial distribution of presumptive adult disc cells in the blastoderm stage of *Drosophila* has been mapped by means of analysis of gynandromorphs (Garcia-Bellido and Merrian, 1969; Ripoll, 1972; Hotta and Benzer, 1972; Janning, 1974a, 1974b). A study was set up to verify whether these fate maps of adult structures correspond to the actual distribution of the prospective

cell groups in the egg. For that reason egg material was removed from blastoderm stages at specific sites by pricking the eggs with a glass needle. Defects in larvae and adults which were found as a result of pricking were examined according to their number, kind and distribution.

The eggs had been pricked in various planes of the long axis of the egg (0.5, 0.66, 0.8, 0.9 and P, 0.0 being equivalent to the anterior pole and 1.0 = P being equivalent to the posterior pole of the egg) as well as on various egg sides (dorsal, lateral, ventral). Table 1 gives the total data as compared to the controls. The following results have been found: Survival rates of all eggs pricked and defect rates of larvae and adults were dependent on the sites of pricking; survival rates showed a lower value after pricking in middle and posterior egg regions (0.5, P), whereas they were higher if the eggs had been pricked in the regions in between (0.66, 0.8, 0.9). Defect rates were generally of higher value after pricking of the egg plane 0.66; a conspicuous increase in the defect rate was found in adults stemming from eggs that had been pricked at the lateral sides of egg plane 0.66.

Table 1. Development of eggs pricked at blastoderm stage.

	total no. of eggs	no development	aberrant development	1. larvae hatched	1. larvae defective	2. larvae	3. larvae	pupae	adults	adults defective
experiment	997	74	105	818	187	629	576	547	528	143
	100%	7.4%	10.6%	82.0%	18.8%	63.1%	57.8%	54.9%	53%	14.3%
control	435	20	21	394	19	373	356	340	336	10
	100%	4.6%	4.8%	90.6%	4.4%	85.7%	81.8%	78.2%	77.2%	2.3%

The number of surviving larvae and adults as well as the number of defects found in larvae and adults was found to be dependent on the amount of the egg material lost. The treated eggs had been divided into three classes according to loss of egg material; class I comprised all eggs with a loss of less than 0.1% of the egg volume, class II was made up of eggs that showed a loss of 0.1% - 1% of the egg volume and class III included all eggs with a loss of egg material that was higher than 1% of the egg volume. The highest ratio of defect rate to survival rate for adults was found in class II (loss of egg material between 0.1% and 1% of the egg volume) with 22% of the pricked eggs showing defects as adults. With a loss of egg material greater than 1% of the egg volume (class III) the rate of survival decreased, with a loss of egg material smaller than 0.1% of the egg volume (class I) the defect rate decreased markedly.

Defective embryos showed a developmental arrest in different stages, largely dependent on the egg plane in which they had been pricked. Embryos pricked in the middle regions of the egg were arrested earlier in development (predominantly through organogenesis) (0.5, 0.66), while most embryos pricked in posterior egg regions (0.8, 0.9, P) showed an arrest only in the hatching phase.

Defects were generally correlated to the sites of pricking, defects in embryos and larvae not as closely so as defects in adults. Reasons for a less close correlation of the former may be first, that it is more difficult to examine larval and embryonal defects properly and, second, defects in embryos and larvae usually cover a larger area and range than they do in adults.

Table 2 shows kinds and distribution of adult defects as they were found after pricking

of the various blastoderm sites. The defects corresponded well to the sites of pricking. Pricking in the middle of the egg (0.5) resulted in defects of thorax structures such as legs, wings and halteres and in defects of sternites and tergites of the first abdominal segments. Pricking in the egg plane 0.66 led to defects ranging predominantly from the third to the sixth abdominal segments. After pricking in the egg plane 0.8, abnormalities in the last abdominal segments as well as in inner and outer genitalia were detected. A similar picture was found in the 0.9 series where, in addition, irregular Malpighian tubules could be seen. Pricking at the posterior pole resulted mainly in defects of the hindgut, the Malpighian tubules and the genitalia. Exceptional animals not fitting into these correlations may have arisen either by chance (defects were also found among control animals) or possibly by an accidental injury during handling. Abnormalities in the head structures of adults could not be detected at all.

Table 2. Adult defects found after pricking the listed regions.

No. of Flies	L	L	L	W	H	T	T	S	T	S	T	S	T	S	T	S	T	S	P	B	HG	M	iG	oG
	1	2	3			1	2	2	3	3	4	4	5	5	6	6	7	7						
0.5																								
vent.	4	1	-	1	-	-	3	2	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
dor.	9	-	1	1	1	2	3	6	1	5	1	-	-	-	-	-	-	-	-	-	-	-	-	-
lat.	10	1	2	7	5	4	-	1	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
0.66																								
vent.	10	-	-	-	1	1	-	-	-	1	3	1	4	-	4	-	1	-	-	-	-	-	-	-
dor.	16	-	-	-	-	-	-	1	-	3	-	10	2	8	3	8	1	1	1	-	-	-	-	-
lat.	38	-	-	1	-	-	-	-	-	3	2	7	15	21	30	11	9	-	-	-	-	-	-	-
0.8																								
vent.	10	-	-	-	-	-	-	-	-	-	1	1	-	2	1	1	2	-	-	-	-	-	2	3
dor.	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
lat.	8	-	-	-	-	-	-	-	-	-	1	-	1	-	2	-	3	4	-	-	-	-	1	4
0.9																								
vent.	4	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	1	1
dor.	4	-	1	1	1	1	-	-	-	1	-	2	-	1	-	1	-	-	1	1	-	-	1	-
lat.	11	-	-	-	-	-	-	-	-	-	1	1	1	1	-	-	-	-	-	-	-	8	3	-
P																								
post.																								
Pole	17	-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	12	2	5	1
total	143	2	4	12	9	8	7	10	5	15	8	23	23	34	41	23	12	6	5	1	1	12	10	14
contr.	10	-	-	-	1	-	-	-	3	-	6	2	-	1	1	1	-	-	-	-	-	-	-	-

L1 = 1st leg, L2 = 2nd leg, L3 = 3rd leg, W = wing, H = haltere, T1 = 1st tergite, T2 = 2nd tergite, S2 = 2nd sternite, T3 = 3rd tergite, S3 = 3rd sternite, etc., P = supra-anal plate, B = sub-anal plate, HG = hindgut, M = Malpighian tubules, iG = inner genitalia, oG = outer genitalia.

On the basis of these data, conclusions were drawn about the presumptive sites of adult disc cells in the blastoderm stage of *Drosophila*. Allowance was made for the fact that a given site of the egg could not always be hit at the very spot due to technical inaccuracy in defining the identical site of pricking for each egg. The positions of presumptive adult disc cells in the blastoderm, as they could be derived here from kind and distribution of adult defects, were largely in agreement with the fate map of adult structures drawn by Janning (1975) on analysis of internal and external structures of gynandromorphs.

References: Garcia-Bellido, A. and J.R. Merriam 1969, J. Exp. Zool. 170:61-75; Hotta, Y. and S. Benzer 1972, Nature 240:527-535; Janning, W. 1974a, W-Roux Arch. 174:313-332; Janning, W. 1974b, W-Roux Arch. 174:349-359; Janning, W. 1975, Habilitationsschrift, University of Münster; Ripoll, P. 1972, W-Roux Arch. 169:200-215.

Wright, R.G. University of Edinburgh, Scotland. Diurnal variation in frequency of courtship behavior in nasuta group flies.

D. melanogaster is believed to exhibit peaks in courting activity in the morning and evening. Other species have different activity patterns (for review see Spieth, H.T. 1974, Ann. Rev. Ent. 19:385-406). I investigated the pattern in nasuta group flies.

24 hr prior to observation, 5 pairs of virgin flies were introduced to 90 x 15 mm plastic petri dishes seeded with standard medium and ventilated to disperse condensation. For 30 hr the number of courting interactions occurring in a 2 min observation period was recorded at

30 min intervals. During the light part of the 12/12 L/D cycle the flies were observed by dim incandescent light (these species were found to be sensitive to high light levels) and in the dark period by red light. The experiment was replicated twice for *D. albomicans* and three times for *D. kepulauana* and *D. pulaua*, always at 25°C. The figure shows the unweighted mean value of the frequency of courting behaviors for all replicates (expressed as a percentage of the total activity in

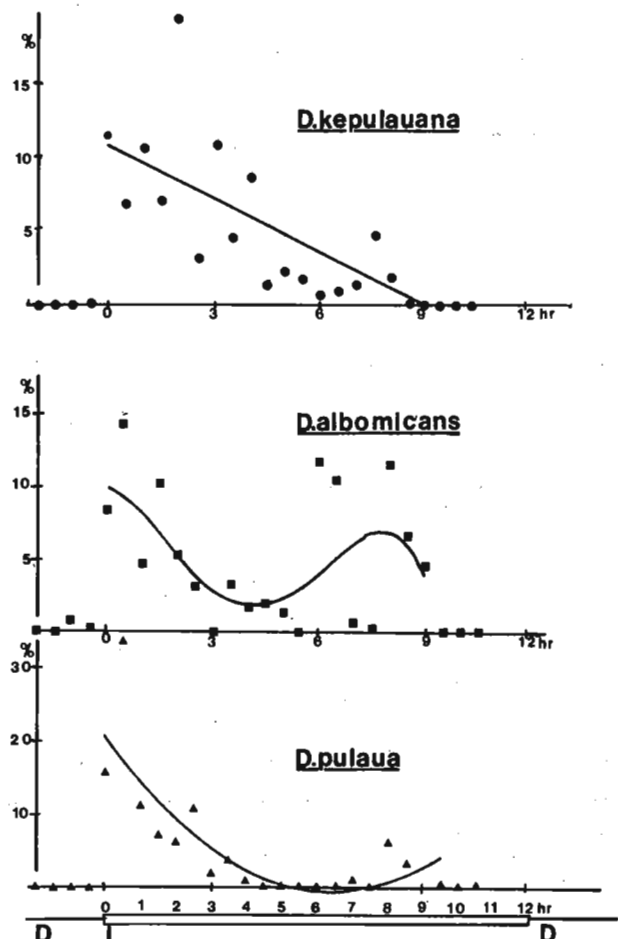


Figure: Unweighted mean percentage of 24 hr courting activity seen at the corresponding observation period.

	Significance of regression	Variance explained
<i>D. kepulauana</i>	$p = 0.0013$	46.46%
<i>D. albomicans</i>	$p = 0.117$	31.68%
<i>D. pulaua</i>	$p = 0.00009$	66.52%

24 hr). This activity was entirely restricted to the L period except in *D. albomicans* where < 1% occurred in the D period, so values between dawn and the last observed event were fitted to a least squares polynomial regression (using the multiple regression technique). These regression lines are plotted with the figure. All 3 species show a clear peak in courting activity immediately after light-on, presumably this is timed to coincide with the feeding peaks when environmental parameters are least demanding and the density of conspecific mates is enhanced around food.

Single courting males were observed to attract

others, often resulting in several males courting one female. The additional stimulation of females must be considerable but little is known of this situation as single pair courtship observations are the norm.

The light dependence of the courting of these flies (also noted by Spieth, H.T. 1969, Univer. of Texas Publ. 6918:255-270) implies the importance of visual components of courtship; the striking displays of these species and the iridescent frons (particularly in *D. albomicans* and *D. kepulauana*) corroborate this view. Light dependence is not, however, obligate: all species were 100% fertile within 9 days when kept on a 0/24 L/D cycle (10 pairs per vial, fertility scored on a vial basis).

Bélo, M. and A.J. Gallo. Fac. de Med. Vet. e Agro. de Jaboticabal and Fac. de Fil. Ciên. e Letras de S.J. do Rio Preto. Domestic *Drosophila* species. I. Flies collected in Olímpia, SP, Brazil.

Data were obtained through collections carried out in Olímpia, São Paulo State, Brazil, using naturally yeasted banana baits, the collecting period covered from May 24th, 1970 to May 23rd 1971. The time interval between successive collecting days was 13 days. In each day collections were carried out at 9, 10, 11 o'clock

in the morning and 2, 4, 6 o'clock in the afternoon.

Table 1 shows the mean number of flies per species captured monthly, total number of captured individuals (55,121 flies), total number and the percentages of each species captured compared to the total of flies collected and the total number compared to the percentages of individuals captured monthly. The numbers in parentheses represent the number of collecting days per month. We name "repleta" group those specimens that may belong to three different unidentified species, the "willistoni" group contains specimens that possibly belong to *D. willistoni*, *D. tropicalis* and *D. paulistorum* (cf. Spassky et al., 1971).

Table 1. Numerical data of *Drosophila* collected in Olímpia, SP, Brazil.

Species	Jan. (3)	Feb. (2)	Mar. (2)	Apr. (2)	May (3)	June (2)		
simulans	110.0	525.5	407.5	329.5	278.3	126.0		
latifasciaeformis	2379.3	862.5	2800.0	397.5	122.7	63.5		
ananassae	426.7	225.5	518.0	594.0	544.3	563.0		
kikkawai	190.0	23.5	50.0	30.0	136.7	162.0		
willistoni group	102.3	214.0	281.0	66.5	12.3	7.0		
repleta group	44.7	27.5	48.5	4.5	10.7	16.0		
polymorpha	33.3	65.5	87.5	24.5	16.7	16.0		
nebulosa	101.3	55.5	111.0	26.5	15.0	11.5		
cardinoides	37.7	7.5	72.5	10.0	43.0			
sturtevantii	73.0	41.5	146.0	9.0	5.0	15.5		
other species	0.7	2.5	7.0	1.0	4.0	2.0		
Total	13470	4102	9058	2986	3566	1965		
%	24.4	7.5	16.4	5.4	6.5	3.5		

continued below:

	Jul. (2)	Aug. (3)	Sept. (2)	Oct. (2)	Nov. (2)	Dec. (2)	No. of flies	%
	896.5	677.0	1385.5	642.5	1058.5	439.5	17791	32.3
	37.0	41.0	64.5	118.5	76.5	260.0	16989	30.8
	224.0	97.7	116.0	145.0	271.5	223.0	8966	16.2
	337.0	258.7	242.0	148.0	48.0	56.0	3949	7.2
	7.5	17.7	14.5	51.0	57.5	23.0	1841	3.3
	37.0	49.3	94.0	178.0	85.5	9.0	1314	2.4
Table 1 continued:	49.5	28.0	49.0	106.0	108.0	17.0	1276	2.3
	3.5	5.3	15.0	61.0	51.5	37.0	1110	2.0
	41.0	44.0	56.5	39.5	11.0	6.0	862	1.6
	1.5	2.0	4.5	2.5	1.5	14.0	712	1.3
	18.0	8.0	27.5	66.0	9.0	3.5	311	0.6
	3305	3686	4134	3116	3557	2176	55121	
	6.0	6.7	7.5	5.7	6.5	3.9		100.0

The frequencies of *D. simulans* and *D. melanogaster* were recorded separately (the Gallo method, 1973, was used to identify the females) during seven months; as *D. melanogaster* frequencies compared to *D. simulans* were always very low, we decided to treat these two sibling species collectively as *D. simulans*. Figure 1 shows *D. melanogaster* frequencies.

According to the dynamics of their populations (Bélo, Gallo & Mourão, not published) and the species frequency during the year, the flies were classified in three groups: Group I - species with occurrence in every month of the year and a dominance period in relation to all other species and a final frequency more than 10% (*D. simulans*, *D. latifasciaeformis* and *D. ananassae*); Group II - species with final frequency between 1 and 10% and occurrence in the

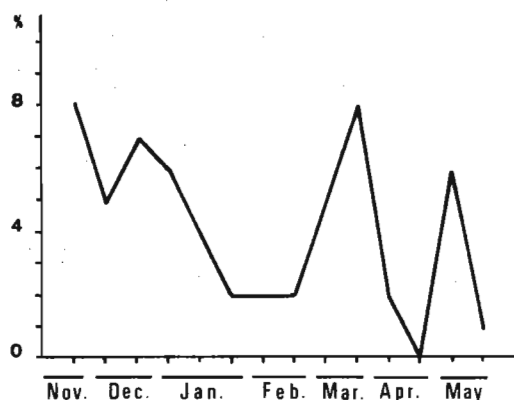


FIG. 1. *D. melanogaster* frequencies compared to *D. simulans*

Mourão, unpublished; Gallo, A.J. 1973, *Ciênc. e Cultura* 25:341-345; Spassky, B., R.C. Richmond, S. Perez-Salas, O. Pavlovsky, C.A. Mourão, A.S. Hunter, H. Hoenigsberg, Th. Dobzhansky & F.J. Ayala 1971, *Evolution* 25:129-143.

Kenney, J.A. and A.S. Hunter. University of the Pacific, Stockton, California. Effect of elevated temperature on salivary chromosomes of *D. virilis*.

Temperature shock has been used on *D. virilis* in an attempt to induce abnormal puffing as well as to check for effects on structural developmental changes in the larval or adult organs. Larvae were cultured at 15°C in order to have larger chromosomes for easier study. Third instar larvae were selected on the basis of the number of teeth in the mandibular hooks. The salivary glands were removed in isotonic Ringer solution and the fat cleaned away. Then fifteen glands were placed in a shallow glass with 25 ml of Ringer solution as used by Ephrussi and Beadle (7.5 g NaCl, 0.35 g KCl, 0.21 g CaCl₂ per liter of tap water, pH 6.4-6.8). The dishes were covered and placed at 37°C for 45 minutes. After this time the glands were removed, stained with aceto-orcein and squashed in a temporary mount. The slides were studied under phase contrast and photographs were taken for permanent data and easier study.

In order to test for possible developmental effects of the temperature shock treatment, third instar larvae were placed in conditions identical to those of the *in vitro* studies. After the treatment with 37°C they were put into *Drosophila* media in 150 ml plastic vials. The vials were kept at 25°C with 12 hours light and dark cycles.

Our results show an abnormal puffing on the #1 arm between B/C and D-h-D lines (identified according to Patterson and Stone). The puffs were quite obvious and seen in every cell on numerous slides. Additional puffs are still under study. Based on Ritossa's report (1962) that *in vitro* and *in vivo* puffing patterns are identical, we checked for other effects in temperature-shocked larvae. They all survived the treatment; the development of the treated larvae appeared to be equivalent to that of the controls and all flies hatched. The test flies appeared to be completely normal. No external structural differences nor any behavioral differences were observed.

It is hypothesized that the abnormal puffing induced by temperature shock has no drastic effect on development of the adult flies. This may be related to the fact that *D. virilis* is a hardy, cosmopolitan species. Further experimentation is in progress on the effect of temperature shock on larval survival when treated in earlier instars.

References: Ellgaard, E.G. 1972, *Chromosoma* 37:417-422; Ephrussi, B. and G.W. Beadle 1936, *Amer. Nat.* 70:218; Leenders, H.J. 1972, *Jour. Cell Biol.* 55: #2, part 1; Ritossa, F. 1962, *Experientia* 18:571-573.

majority of the months (*D. kikkawai*, "willistoni" and "repleta" groups, *D. polymorpha*, *D. cardinoides* and *D. sturtevantii*); Group III - species with or without occurrence during each month, their final frequencies not reaching 1% with respect to each other. In Table 1, these species are grouped under the name of "other species"; they are *D. guaramunu* (207 flies), *D. mediotriata* (41), *D. pallidipennis* (39), *D. mirassolensis* (7), *D. crocina* (5), *D. immigrans* (4), *D. busckii* (3), *D. mediopunctata* (2) and three flies likely to belong to another unidentified species. Thus about 24 species were captured during the experimental period.

More details will be published elsewhere in a series of works about the collecting area, environmental conditions, and fly behavior during the year or during the collecting hours.

This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil).

References: Bélo, M., A.J. Gallo & C.A.

Abnormal puff induction by temperature shock has been reported for *D. melanogaster* (Ellgaard, 1972) and *D. busckii* (Ritossa, 1962). A possible physiological mechanism for the inductions of puffs by temperature shock is discussed by Leenders (1972) in his report on *D. hydei*.

Klapholz, S. and R.J. MacIntyre. Cornell University, Ithaca, New York. Studies on the suppression of putative nonsense mutants at the Acid phosphatase-1 locus with suppressor of Hairy-wing-2 in *D. melanogaster*.

The recessive allele specific, locus non-specific suppressor of Hairy-wing-2 (3-54.8) has been considered by many to be a good candidate for a nonsense suppressor (Lee, 1970 and 1973; Hartman and Roth, 1973). $su(Hw)^2$ was examined for its ability to suppress three putative nonsense mutations at the Acid phosphatase-1 locus (3-101.1). The EMS induced, null

activity mutants ($Acph-1^n$; $n = 8, 13, 15$) chosen for this study are non-leaky, CRM (cross reacting material) minus and exhibit no intragenic complementation (Bell, et al., 1972).

Crosses were made to construct stocks with both $su(Hw)^2$ and an $Acph-1$ null on the third chromosome, balanced over the inversion T_M1, Me. $cm\ ct^6; su(Hw)^2\ bx\ bxd\ Acph-1^n/TM1, Me$ stocks were then used to generate $cm\ ct^6; su(Hw)^2\ bx\ bxd\ Acph-1^n$ homozygotes. These flies are female sterile and have altered thoracic segments due to the bithorax mutation. Both bxd and ct^6 are suppressed by $su(Hw)^2$, which provides a check for the presence of the suppressor in these homozygotes.

Three methods were used to study suppression at the gene product level - a spot test for enzymatic activity, an acrylamide gel based assay, and an immunological assay for CRM.

The spot test, described by Bell, et al. (1972), was used as a preliminary qualitative assay for acid phosphatase activity. Single $su(Hw)^2\ Acph-1^n$ and $su^+(Hw)^2\ Acph-1^n$ homozygotes were compared. No enzyme activity was observed in either group. The spot test detects only down to about 10% of wild type acid phosphatase activity.

For a more sensitive assay, concentrated crude extracts were prepared from homozygous $su(Hw)^2\ Acph-1^n$ and $su^+(Hw)^2\ Acph-1^n$ flies. They were electrophoresed in 5% acrylamide gels, along with a diluted (1:20) wild-type ($Acph-1^A/Acph-1^B$) control extract which has the dimeric enzymes AA, AB and BB. The gels were overstained (72 hours at 30°C) to allow even the slightest amount of acid phosphatase activity to be detected. (See Bell, et al., 1973 for details of the procedure.) No bands were observed in the acid phosphatase-1 region of the gel for either $su^+(Hw)^2\ Acph-1^n$ or $su(Hw)^2\ Acph-1^n$ genotypes.

The last study employed the CRM test described by Bell and MacIntyre (1973). This technique would enable one to detect suppression in the absence of an enzymatically active protein. The $su(Hw)^2\ Acph-1^n$ and $su^+(Hw)^2\ Acph-1^n$ homozygotes were compared with controls generating 0%, 10% and 100% CRM levels, in 5% acrylamide gels. The gels were subsequently stained for acid phosphatase activity of the enzyme from *D. simulans* added to the second incubation (antigen excess), and the amount of this enzyme present in the gel was quantified by densitometry. The experimental extracts were not significantly different

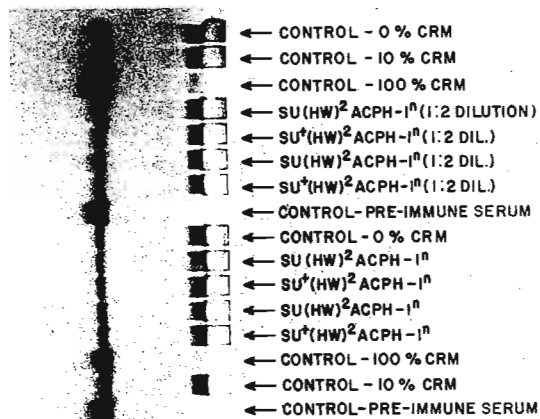


Figure 1. Acrylamide gel showing the results of a typical CRM test.

* Uncomplexed *D. simulans* acid phosphatase whose activity is measured by a densitometer.

** Soluble, enzymatically active enzyme-antibody complexes.

from the 0% control, and thus no suppression was evident (see Figure 1). This assay will reliably detect CRM levels down to 5%.

None of the three putative nonsense alleles $Acph-1^{n8}$, $Acph-1^{n13}$ or $Acph-1^{n15}$ is suppressed. Therefore, this study presents no support for the contention that $su(Hw)^2$ is a nonsense suppressor. However, many more null mutants at various loci need to be examined before a stronger conclusion can be drawn.

References: Bell, J.B. and R.J. MacIntyre 1973, *Biochem. Genetics* 10:39; Bell, J.B., R.J. MacIntyre and A.P. Olivieri 1972, *Biochem. Genetics* 6:205; Hartman, P.E. and J.R. Roth 1973, *Advances in Genetics* (Academic Press, N.Y.) 17:1; Lee, G.L.G. 1970, *Australian Jour. Bio. Sci.* 23:645; Lee, G.L.G. 1973, *Australian Jour. Bio. Sci.* 26:189.

Costa, D., F. Ritossa and F. Scalenghe.
University of Bari, Italy. Production
of deletions of the puff-forming regions
87A and 87B in *Drosophila melanogaster*.

It has not been possible to produce deletions
of the puff-forming regions 87A and 87B in
Drosophila melanogaster using curled (50.0) as
selected marker, owing to the presence of a
Minute locus in the interval between curled and
the region of interest (see Lindsley, Sandler

et al., Genetics 71:157, 1972).

We have been successful instead by using the mutant karmoisin. This mutant has been mapped at 51.7 on the third chromosome of *Drosophila melanogaster* and Lefevre (DIS 46:40, 1971) presents evidence for its cytological localization between bands 87C2-3 and 87D1-2. Males homozygous for the In(3R)AFA, e chromosome (see A.D'Alessandro, F. Ritossa and F. Scalenghe, DIS this issue) are aged 3-4 days and then irradiated with 3,000-4,000 r X-rays. They are immediately mated with virgin females (aged 3-4 days) homozygous for the markers cu and kar. The mating is interrupted after 3-4 hours by discarding the males. The females are allowed to lay eggs and the progeny scored for individuals exhibiting the kar phenotype. In this way we have recovered 4 kar flies among a progeny of about 20,000 individuals. The new mutations have all been balanced against a cu kar Sb chromosome by proper crossing.

None of the stocks so established has segregated ebony flies, indicating that a lethal mutation had been produced on the In(3R)AFA, e chromosome.

The cytological analysis (for the banding pattern of the region reference is made here to F. Scalenghe and F. Ritossa: "Controllo dell' attività genica in *Drosophila*. Il puff al locus ebony e la Glutamina Sintetasi 1". Atti dell' Accademia Nazionale dei Lincei, 1976 in press) has given the following results:

Df(3R)kar^{D1} and Df(3R)kar^{D2}: these mutants are deletions which, besides involving the kar locus, involve the puff-forming region 87B but not the puff-forming region 87A. They show a very similar cytology, both lacking regions 87B and 87C. The breakpoints are presumably in sections 87A1-2;87B1-2 and 87C2-3;87D1-2.

Df(3R)kar^{D3}: this mutant is a deletion which involves both the puff-forming regions 87A and 87B. The chromosome appears to lack regions 86F-87A-87B-87C-87D. The breakpoints are provisionally placed in sections 86E1-2;86F1-2 and 87D1-2;87E1-2.

Df(3R)kar^{D4}: this mutant is a deletion lacking sections 87C and 87D but not the puff-forming regions 87A and 87B. The breakpoints are presumed to be in sections 87B11;87C2-3 and 87E1-2;87F1-2.

Timmer, K. Freie Universität Berlin, Germany. Methods for the estimation of age dependent drosopeterin concentration in eyes of wild type and vermilion mutants of *Drosophila melanogaster*.

Material: Wild type (Plus) and vermilion mutants (v) of *D. melanogaster* were raised under constant conditions on a standard medium in 250 ml culture bottles at 25°C. Newly emerged flies were collected, using a mild ether narcosis, from the culture bottles every 15 minutes, if these flies were to be tested within the fol-

lowing 24 hours. If not, the flies were harvested every 1 - 2 hours after eclosion. The collected flies were kept in small culture vials containing the standard medium until the test.

Method I: Flies were etherized until death, after which males and females were separated and decapitated. The extraction of the drosopeterins was carried out with 30% ethanol, acidi-

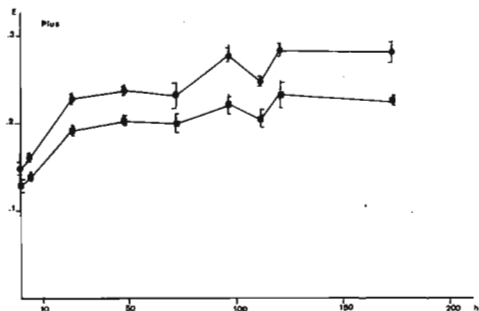


Figure 1. Developmental profiles of drosopeterin formation in Plus females (○—○) and Plus males (●—●) estimated by method I. Ordinate: mean values and standard errors of the means ($\bar{x} \pm 2m$) of the extinctions at 485 nm of 2.5 heads per ml. Abscissa: age of flies in hours after eclosion.

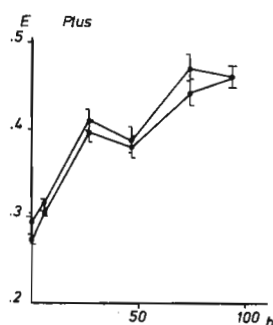


Figure 2. Developmental profiles of drosopterin formation in Plus females (●—●) and Plus males (■—■) estimated by method II. Ordinate: mean values and standard errors of the means ($\bar{x} \pm 2m$) of the extinctions at 485 nm of 10 heads per ml. Abscissa: age of flies in hours after eclosion.

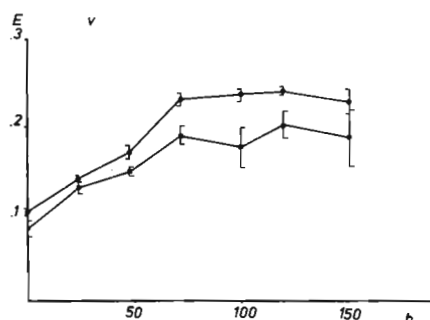


Figure 3. Developmental profiles of drosopterin formation in v females (●—●) and v males (■—■) estimated by method I. Ordinate: mean values and standard errors of the means ($\bar{x} \pm 2m$) of the extinctions at 485 nm of 2.5 heads per ml. Abscissa: age of flies in hours after eclosion.

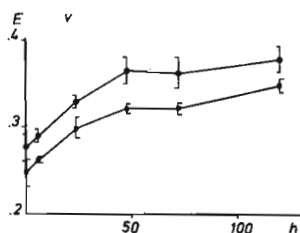


Figure 4. Developmental profiles of drosopterin formation in v females (●—●) and v males (■—■) estimated by method II. Ordinate: mean values and standard errors of the means ($\bar{x} \pm 2m$) of the extinctions at 485 nm of 10 heads per ml. Abscissa: age of flies in hours after eclosion.

fied with concentrated HCl to pH 2. This solvent was designated as AEA by Ephrussi and Herold (1944). Five heads were homogenized in 2 ml AEA with 3 strokes of a homogenizer (method of Potter and Elvehjem), using a teflon pestle. This homogenate was spun at 2000 rpm for 15 minutes with a labcentrifuge. The supernatant, having a drosopterin concentration equivalent to 2.5 heads/ml, was decanted into glass cuvettes. The drosopterin content was estimated as the light extinction at 485 nm using a Zeiss PMQ II spectrophotometre.

Method II: Flies were killed by ether narcosis. Twenty-five males or females were put into a 5 ml glass vial and 2.5 ml AEA added. The vials were sealed with a polyethylene cap and kept in dark at room temperature for 4 weeks. After that time the extract, with a concentration equivalent to 10 heads per ml, was decanted into glass cuvettes and the extinction of the drosopterins was determined photometrically at 485 nm.

The results presented in Figures 1 to 4 indicate that the samples obtained by method I exhibit more drosopterins than those of method II. This must be due to the reduced time of extraction, which precludes the destruction of drosopterins in the extraction solvent. Comparing the developmental profiles resulting from method II, there is no difference between the drosopterin formation in eyes of wild type (Plus) and vermilion (v) flies. This is in agreement with the observations of Ephrussi and Herold (1944) and Nolte (1952). But there is a difference between strains Plus and v, when the drosopterin concentration was determined by method I. Here v females exhibit 13%, and v males 15% less drosopterins than Plus females and Plus males respectively.

References: Ephrussi, B. and J.L. Herold 1944, Genetics 29:148; Nolte, D.J. 1952, J. Genet. 51:130.

Benner, D.B. East Tennessee State University, Johnson City, Tennessee. The unusual segregation properties of a chromosome fragment.

This report deals with a y^+ marked chromosome fragment which segregated regularly from an attached X-Y chromosome, but had no other Y chromosome properties. The fragment was derived from the Dp(1:4)193, $y^+ ac^+ ci^+ ey^+ spa^{pol}$ (Williamson, Parker and Manchester 1970, Mut.

Res. 9:299-306) by irradiating C(1)RM, $y v bb/Y/y^+ \cdot spa^{pol}/ci ey^R$ females (Parker, personal communication). The resulting fragment did not cover the fourth chromosome mutants spa^{pol} or $ci ey^R$ suggesting the loss of all fourth chromosome markers. Tests with the fertility mutant stocks of Brosseau were completely negative, and the fragment would not rescue C(1)DX, $y f$ females who lack the nucleolus organizer. These observations suggest that little if any Y chromosome material was present, or if present it was silent.

Despite the lack of Y chromosome genetic activity the fragment showed regular segregation from the attached X-Y chromosome. In the cross of XY/0; y^+ fragment males to C(1)RM, $y v bb/Y$ females the fragment segregated with a frequency of 0.998 (Table 1a). Segregation from the compound-X chromosome in females was less frequent (0.795, Table 1b), but it was still not random.

Table 1. Segregation of a y^+ fragment in males and females with and without a free Y chromosome.

Cross	Freq. y^+ seg.
a. XY/ y^+ frag. ♀♀ x C(1)RM, $y v bb/Y$ ♂♂ <u>females:</u> $y^+ - 3,096$; $y - 9$ <u>males:</u> $y^+ - 6$; $y - 3,252$	0.998
b. C(1)RM/ y^+ frag. ♀♀ x X, $y pn/Y$ ♂♂ <u>females:</u> $y^+ - 37$; $y - 162$ <u>males:</u> $y^+ - 159$; $y - 46$	0.795
c. X, $y v/BSY/y^+$ frag. ♀♀ x C(1)RM, $y v bb/Y$ ♂♂ <u>females:</u> $y^+ B^S - 477$; $y^+ - 664$; $y B^S - 440$; $y - 3$ <u>males:</u> $y^+ B^S - 0$; $y^+ - 545$; $y B^S - 623$; $y - 652$	0.708
d. C(1)RM, $y v bb/BSY/y^+$ frag. ♀♀ x X, $y pn/Y$ ♂♂ <u>females:</u> $y^+ B^S - 0$; $y^+ - 118$; $y B^S - 2$; $y - 72$ <u>males:</u> $y^+ B^S - 55$; $y^+ - 3$; $y B^S - 114$; $y - 0$	0.362
e. X, $y v/X, y v/y^+$ frag. ♀♀ x In(1)49, BMI/Y ♂♂ <u>Total females:</u> 111 <u>No. exceptions:</u> 0	
f. X, $y v/In(1)49, B^{MI}/y^+$ frag. ♀♀ x X, $y B/Y$ ♂♂ <u>Total females:</u> 387 <u>No. exceptions:</u> 0	

In males with a free BSY chromosome present the y^+ fragment segregated from the X chromosome with a frequency of 0.708 (Table 1c). The BSY segregated from the X chromosome with an observed frequency of 0.619. The analysis is clouded by the fact that B^S was recovered with a frequency of only 0.451 instead of the expected 0.50, and there was a significant excess of male progeny recovered, due primarily to the decrease in the presence of B^S females. The y^+ fragment appeared to segregate from the X and not as an independent element because within the male progeny there was a deficiency of y^+ progeny and an excess of y progeny.

When a free Y chromosome was present in females the fragment preferentially segregated with the compound-X chromosome. It segregated from the compound chromosome with a frequency of only 0.362 while the BSY chromosome segregated with a frequency of 0.988 (Table 1d). In this case the y^+ fragment behaved as the most extreme $y^+ \cdot spa^{pol}$ fourth chromosome, including Dp(1:4)193, reported by Williamson, et al. (1970) where segregation from the compound-X chromosome showed a highly significant deviation on the high side without a free-Y chromosome and

a highly significant deviation on the low side with a free-Y chromosome present.

The behaviour of the fragment in the female is within the interpretation of Williamson, et al. that the y^+ fragment might be the smallest element in a distributive pool leading to its being regularly directed to the same pole as the largest element - the compound-X chromosome. In males, however, the evidence suggests at least random segregation of the three elements. The segregation frequencies in the male show preferential segregation of y^+ from the X chromosome, but the reduced number of B^S progeny may account for the increase over the random expectation.

The influence of this y^+ fragment on the disjunction of free X chromosomes was also measured. Females of the X, $y\ v/X, y\ v/y^+$ fragment and X, $y\ v/In(1)49, B^M/y^+$ fragment genotypes were tested, and in neither case did the fragment interfere with normal X chromosome disjunction (Table 1e and f). The same genotypes with the B^S chromosome present gave nondisjunction values approaching those reported from other studies.

This y^+ fragment was lost before a cytological examination was completed, so its exact structure was not determined. The presence of y^+ suggests that that portion of the original duplication was retained along with the centromere. The fourth chromosome markers had been lost and possibly replaced by a portion of the Y chromosome. The portion of the Y chromosome involved could have been from a region distal to the fertility factors, or it might have included some fertility factors which were inactive in the rearrangement. The segregational properties of the fragment in the female suggest the behaviour of a small element in a distributive pool, but its more regular disjunction from the X chromosome suggests that some factor other than size, possibly X chromosome homology, was involved in the segregational event.

Hall, J.C. Brandeis University, Waltham, Massachusetts. Recombination influenced by two alleles of the meiotic mutant $c(3)G$.

Mutants at the $c(3)G$ locus nearly eliminate meiotic crossing over in *D. melanogaster* females and cause up to 40% nondisjunction. The effects of $c(3)G$ mutants on segregation have been more amenable to analysis than the effects on recombination since recombinants from $c(3)G$ are so rare. However, several components of crossing over influenced by the mutations have been established, in rather unorthodox circumstances.

$c(3)G/+$ females actually have increased levels of crossing over (e.g. Hinton, C.W. 1966 Genetics 53:157). This has been confirmed for the original allele of $c(3)G$, in that $c(3)G^{17}/+$ females yield a total X chromosome map expanded ca. 11%, and a map for the right arm of chromosome 2 expanded ca. 24% (Table 1). The newer allele of $c(3)G$ has less of a stimulatory effect on crossing over when heterozygous: $c(3)G^{68}/+$ females yield the same X chromosome map length as the control, and only about an 8% increase in 2nd chromosome crossing over. There appears to be no simple relation between the severity of segregational disruption and the heterozygous effect on recombination, because homozygous $c(3)G^{68}$ has higher nondisjunction frequencies for all chromosomes (Hall, J.C. 1972, Genetics 71:367).

Table 1. Recombination in heterozygous $c(3)G$ females. Crossing over was measured in X chromosome regions $pn-v$ (1) and $v-y^+$ (2), and in 2nd chromosome regions $b-cn$ (1), $cn-c$ (2), and $c-bw$ (3).

3rd chromosome constitution	Map units, Region:			Total progeny	Exchange rank			
	1	2	3		E_0	E_1	E_2	E_3
$+/+, X$	32.9	32.7	-	6813	.025	.639	.336	-
$+/+, 2nd$	4.9	16.7	25.3	19680	.183	.698	.116	.003
$c(3)G^{17}/+, X$	37.2	35.2	-	7033	.006	.538	.456	-
$c(3)G^{17}/+, 2nd$	7.2	20.0	31.0	17968	.092	.673	.217	.019
$c(3)G^{68}/+, X$	32.6	32.9	-	6277	.045	.599	.356	-
$c(3)G^{68}/+, 2nd$	4.9	17.0	28.7	18628	.147	.701	.142	.010

The possibility that $c(3)G/+$ females have slightly increased frequencies of non-exchange tetrads - that is, simply a much less severe decrease in exchange than homozygous $c(3)G$ - was examined. This could lead to a non-random loss of noncrossover chromosomes through non-

homologous segregation among, for example, non-exchange X and 2nd chromosome, which would yield aneuploid, lethal zygotes (there is much nonhomologous segregation in $c(3)G$ homozygotes, Hall, 1972). Thus, there would be an apparent increase in crossing over. However, tetrad analysis (Table 1) revealed that there is an absolute increase in exchange in $c(3)G/+$ females, in that the proportion of multiple exchange tetrads is markedly increased (especially for the 2nd chromosome data from $c(3)G^{17}/+$ females). Moreover, no X-2nd chromosome non-homologous segregation was revealed in crosses of $c(3)G/+$ females to attached-2nd chromosome bearing males: 6 progeny were recovered from 537 $c(3)G^{17}/+$ females; none of the progeny came from ova in which the 2 X's had segregated from the 2 2nd chromosomes; 7 progeny were recovered from 513 $c(3)G^{68}/+$ females, again, none of which were X-2nd chromosome nonhomologous segregants. In the control, 25 progeny were recovered from 1036 females, and there were 2 cases of simultaneous X-2nd chromosome nondisjunction, one of which was a diplo-X; nullo-2 egg, and the other a nullo-X; diplo-2 egg.

The possibility was examined that homozygous $c(3)G$ leads to a substantial amount of sister chromatid crossing over - by removing the usual inhibition of such exchange. This might allow a replicated chromosome to have a necessary condition for its meiotic behavior to be satisfied, so that homologs would not have to pair and undergo regular, non-sister chromatid exchange. Inversion heterozygosity for a given chromosome pair increases nondisjunction for other chromosome pairs in homozygous $c(3)G$ (Hall, 1972). This could be due to the interchromosomal effect of inversion heterozygosity, which increases crossing over on the other, non-inverted chromosomes. So in $c(3)G$ females carrying inversions, there would be even more sister-chromatid crossing over, and thus more mis-pairing and nondisjunction of homologs. This possibility cannot be dismissed out of hand, because homozygous $c(3)G$ increases loss of ring-X chromosomes (especially compound rings - Sandler, L. 1965, Nat. Cancer Inst. Monogr. 18:243), which could be due to sister chromatid exchange and resultant dicentric rings. But the hypothesis fails in a test of $R(1)2/X$; $c(3)G^{17}/c(3)G^{17}$ females. The proportion of ring-X eggs among all X-regular eggs (corrected for the fact that double crossover rings are not recoverable from homozygous $c(3)G$) is .45 from the control. This value is actually higher from $c(3)G$, i.e. .47 (Table 2). Heterozygosity for $In(2LR)Sml$ leads to 27% more X nondisjunction in $R9(1)2/X$; $c(3)G^{17}/c(3)G^{17}$ females, and does increase ring loss slightly - $R(1)2$ ova: total X regular ova = .44 (Table 2). Apparently, this is not enough of a loss increase to account for the nondisjunctional increase.

Table 2. Results of crossing $R(1)2$, cv v f/y females to y B/Y males.

2nd & 3rd chromosome constitution of females	Progeny types (nco = noncrossover; dco = double crossover)								Total
	R/X	X/X	matroc. female	patroc. male	nco R male	nco C male	dco R male	dco X male	
+/+ ; +/+	3250	4302	2	350	2960	3852	134	420	15270
+/+ ; $c(3)G^{17}/c(3)G^{17}$	2528	2827	586	956	2093	2802	0	0	11792
$Sml/+$; $c(3)G^{17}/c(3)G^{17}$	973	1250	324	548	800	1193	0	0	5088

Sister chromatid exchange influenced by $c(3)G$ was examined from a different point of view. B^+ crossover products can be recovered from B/B ; $c(3)G^+$ females, and a fraction of these are apparently due to unequal sister chromatid crossing over (e.g. Peterson, H.M., and J.R. Laughnan, 1963, P.N.A.S. 50:126). Should such B^+ chromosomes be recovered from B/B ; $c(3)G$ females, they might be due only to sister chromatid crossing over, since non-sister chromatid crossing over is almost non-existent. A preliminary test of this notion resulted in 2 B^+ chromosomes recovered among 4581 X's from females homozygous for B and $c(3)G^{17}$; the corresponding test with respect to $c(3)G^{68}$ yielded 2 B^+ in 8759 X's. 8 of 33320 X's were B^+ in the control (from females homozygous for $c(3)G^+$), yet these females have about 600-fold more crossing over than do $c(3)G$ females (Hall, 1972). It could not be determined if the B^+ chromosome from B/B $c(3)G$ were not, in fact, due to non-sister chromatid crossing over, since there were no flanking markers. However, a subsequent experiment resulted in 2/2832 B^+ chromosomes from females homozygous for $c(3)G^{17}$ and heterozygous for B and a deletion of this locus; the corresponding results with respect to $c(3)G^{68}$ were 3/8361; but in the control there were no B^+ chromosomes in 11399 X's. Thus - whereas B^+ chromosomes from $c(3)G$, B homozygotes or hemizygotes are rare - they may indicate that sister chromatid crossing over in $c(3)G$ meioses is frequent. Only the unequal crossovers of this type, within the confines of the B duplication, would yield these products.

Alahiotis, S. University of Patras, Greece. α -Glycerophosphate dehydrogenase and the locomotor activity in *Drosophila melanogaster*.

The production of energy in insect muscles during flight and other activities (Zebe and McShan 1967; Sacktor and Dick 1962) is one of the functions of the α -Glycerophosphate cycle in *D. melanogaster*. Thus, flies genetically deficient in α -Glycerophosphate dehydrogenase

(α -Gpdh) lose the ability to sustain flight, an observation consistent with the enzyme's function in energy production (O'Brien and MacIntyre 1972). In addition, many biochemical differences between the α -Gpdh allozymes have been detected (Miller et al. 1975).

In view of the above mentioned data, an attempt was made to assess whether the level of locomotor activity of *D. melanogaster* is related to the genotypes of the α -Gpdh locus. Such relationship may reflect some different selectional alloenzymic behaviour with respect to this trait. The behavioural trait locomotor activity has often been considered as a mechanism for adaptation to needs. Connolly (1966a,b) also succeeded in selecting active and inactive strains of *D. melanogaster* as well as revealing the effect of food deprivation on the rhythm of locomotor activity. Hence, we have thought it of interest to investigate the possible selectional effect of the ecological factors "food medium" and "humidity" upon the level of the locomotor activity of *melanogaster* cage populations maintained by random mating under competitive conditions. Thus, three populations kept in 41 x 41 x 16 cm cages as well as some inbred lines for the α -Gpdh locus genotypes (Fast and Slow) were tested. These cage populations, possessing practically the same original gene pool were kept under the selectional effect of the previously mentioned ecological factors "food medium" (poor and rich) and "humidity" (43% and 90%); (for details see Alahiotis 1975). All of these populations and inbred lines were on a light/dark cycle of 12 hr. In order to determine the locomotor activity Connolly's method (1966a) was followed. Each fly (17 generations after the origin of the cage population) was examined for its locomotor activity and enzyme genotype; the latter was detected by means of starch gel electrophoresis (Alahiotis 1975). The observed locomotor activity (Table 1) is generally high, in comparison with that which was found by Connolly

Table 1. Distribution of the locomotor activity per genotype of α -Gpdh in *D. melanogaster*.

Populations	Genotypes	Mean	S.D.	Number of individuals examined
1A	FF	67.50	19.33	80
	FS	61.22	14.21	21
1B	FF	65.32	11.24	49
	FS	63.37	18.16	60
	SS	67	10.05	14
1D	FF	60.4	15.08	55
	FS	60.53	11.71	25
Or-K	FF	72.56	14.28	62
Inbred lines	α -Gpdh ^{SS}	67.29	13.55	40
	α -Gpdh ^{FF}	61.97	10.85	44

(1966a). The highest value was obtained by the Oregon-K stocks which were maintained in the laboratory for an extremely high number of generations. These high values are perhaps due to the action of directional selection for a high level of activity (Angus 1974). Comparison (with t criterion) between the mean of the level of locomotor activity per genotype of the α -Gpdh did not reveal any differences. There were also no differences between the mean locomotor activity among cage populations. It seems that it is difficult to show differences, if any, in the

locomotor activity of *melanogaster* which is related to the genotype of the α -Gpdh. This may be due to the fact that the level of locomotor activity could be affected by many loci. There were many enzymes known to be active in energy metabolism (Gillespie and Kojima 1968). However, in progress experimentation with the behaviour trait "flying ability", more suitable for this type of study, may reveal such a relationship. Finally, the two ecological parameters examined did not seem to have any effect upon the level of the locomotor activity of *D. melanogaster* for at least 17 generations.

Acknowledgments: I am grateful to Prof. M. Pelecanos for providing the laboratory facilities as well as for corrections of the manuscript.

References: Alahiotis, S. 1975, DIS 51:63, 88; Angus 1974, Behav. Gen. 4(4):317-329; Connolly 1966a, Anim. Behav. 14:444-449; 1966b, Nature 209:224; Gillespie and Kojima 1968, Proc. Nat. Acad. Sci. 61:582; Miller et al. 1975, Biochem. Gen. 13:175-188; O'Brien and MacIntyre 1972, Genetics 71:127-138; Sacktor and Dick 1962, J. Biol. Chem. 237:3259-3263; Zebe and McShan 1957, J. Gen. Physiol. 40(5):799-790.

Szabo, P. and V. Donofrio. University of Illinois, Urbana. Cytological RNA:DNA hybridization to ovarian nurse cells of *Drosophila*.

to assay the amount of a specific DNA sequence. These cells undergo a series of duplications of the DNA content by endomitosis as described by King (1970).

In an attempt to determine whether cytological RNA:DNA hybridization yields quantitative data which accurately reflects DNA content, ^{125}I labelled 5S RNA from *D. melanogaster* was hybridized to ovarian squashes. The nurse cells of *Drosophila* ovaries provide an excellent system

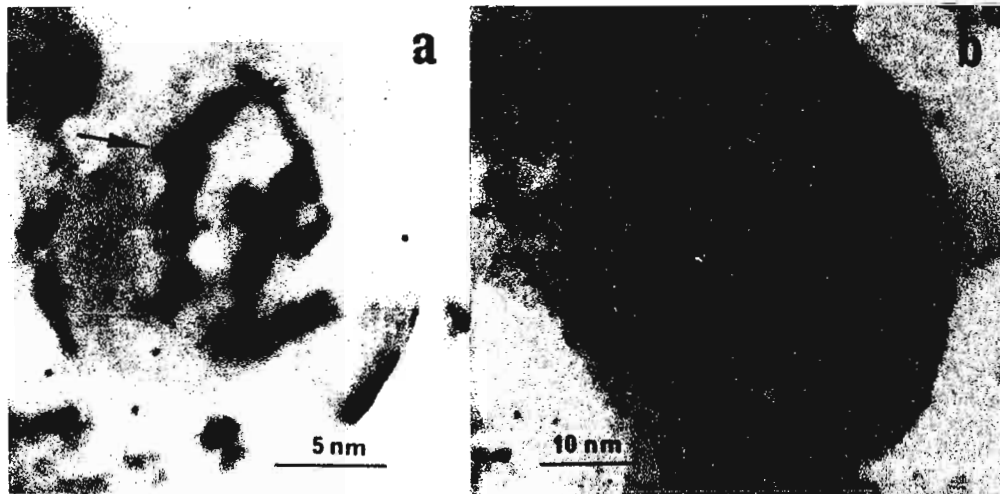


Figure 1(a). Stage 3 nurse cell - the arrow marks the position of the 5S RNA gene site in a region which appears puffed. (b). Stage 6 or 7 nurse cell - grains are diffusely distributed because the chromatin strands have partially separated. The areas marked by the dotted line enclose the bulk of the 5S genes. The number of grains over the nucleus has increased by a factor of about 8, in accordance with the increase in DNA content.

The ovarian squashes were prepared and hybridized with ^{125}I /5S RNA (sp. act. 1.5×10^8 dpm/ μg) using the procedure of Wimber et al. (1974). The slides were stained with Giemsa and scored after development of the autoradiographs.

Polytene nuclei from stage 3 nurse cells can be identified easily, so they served as a standard. Nurse cells at this stage have a DNA content of $16 \times n$ (Figure 1a). Cells from later stages were also scored which had higher strand numbers (Figure 1b). These cells could only be distinguished by size, with the later stages having progressively larger nuclei.

Figure 2 shows the grain distribution over stage 3 nuclei; the mean number of grains is about 3. Based on this mean number of grains, the expected positions of the means for higher ploidy levels are marked by the arrows (stage 4 cells - $32 \times n$ were not scored in this experiment). The

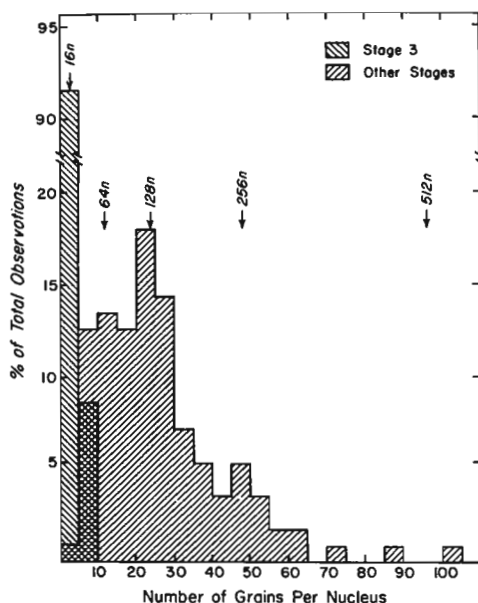


Figure 2. Histogram showing the distribution of grains over nurse cell nuclei. The arrows mark the observed position of the mean main number of stage 3, which corresponds to $16 \times n$ nuclei and the expected positions of the means for stages 5-8 using stage 3 as a standard.

observed trimodal distribution has individual means corresponding to ploidy levels from 64 to 256n as expected for stages 5 to 8. The number of grains increased as a function of cell size. Very few of the larger nuclei were scored because many were broken or overlapped, prohibiting accurate scoring.

These data confirm that in situ hybridization can measure DNA content accurately. The progressive doubling of DNA is verified by the in situ method. Conversely, the numerical distribution of silver grains is proportional to ploidy levels, so cytological hybridization can be used for quantitative DNA studies. There is no evidence for the amplification of 5S DNA. Since nurse cells are known to produce ribosomes in large quantities, it is comforting to see that stage 3 nuclei are puffed at the 5S gene sites (Figure 1a).

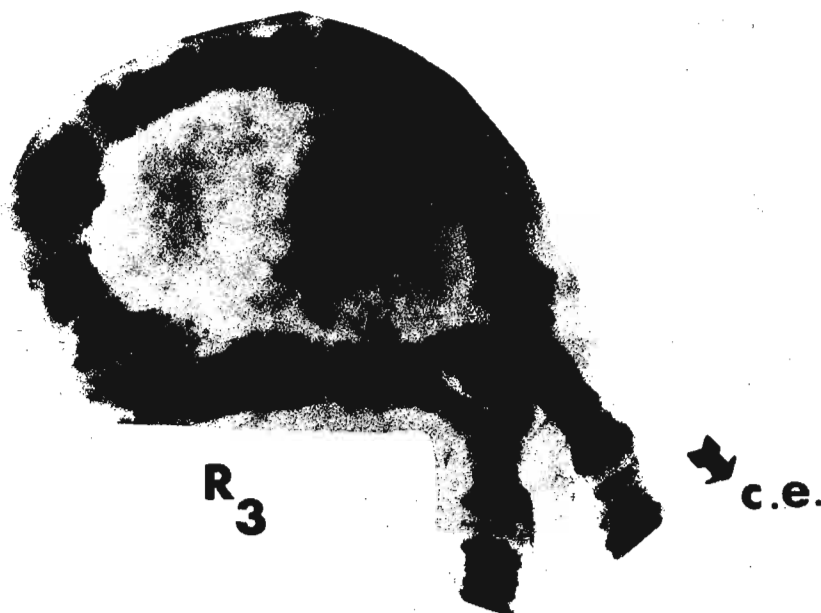
References: King, R.C. 1970, *Ovarian Development in Drosophila melanogaster*, Academic Press, New York; Wimber, D.E., P. Duffey and D.M. Steffensen 1974, *Chromosoma*, 47:353-359.

This work was partially supported by HEW grant GM 211-23-01.

Mather, W.B. and M. Clyde. University of Queensland, Brisbane, Australia. Inversions in *D. sulfurigaster albostrigata* from the Philippines.

During 1974 one hundred and fifty eight isolines of the species *D. sulfurigaster albostrigata* were established and examined for inversions in January and July from Hidden Valley Springs (H.V.S.), Luzon and in July from the central Philippines Island of Cebu (see Table). Inver-

sions C, E, G and W_2 have previously been recorded (Mather and Thongmeearkom 1972 DIS 48:40 and Mather, Thongmeearkom, Clyde and Lambert 1974 DIS 51:86). R_3 is a new simple inversion in the central region of chromosome III (see Figure).



c.e. chromocentre end

Inversions	% Het. Freq.			
	Jan. 1974	July 1974		
	H.V.S.% (79 isolines)	Cebu % (42 isolines)	H.V.S.% (37 isolines)	
C	51	76	43	
E	84	76	86	
G	8	26	5	
W_2	0	2	3	
R_3	1	0	0	

The material was collected and the isolines established by W.B.M. The chromosomes were prepared, analysed and photographed by M.C.

Halfer, C. University of Milan, Italy.
Cell fusion induced by Lysolecithin (LL)
in *Drosophila melanogaster*.

Since inactivated Sendai virus is inefficient in producing cell fusion in *Drosophila melanogaster* (G. Echallier, 1971), an attempt was made using a chemical fusion inducer, the lysolecithin, already successfully employed in mam-

malian cells (Howell and Lucy, 1969; Lucy, 1970; Poole, Howell and Lucy, 1970; Croce, Sawicki, Kritchevsky and Koprowsky, 1971). The present communication constitutes a preliminary report of this investigation carried out on two different established cell lines cultured in vitro, 1B5 and GM1, with the aim of determining the optimal concentration of lysolecithin for inducing cell fusion. The 1B5 line is characterized by a female karyotype and a variable percentage of tetra- and polyploid cells; the GM1, on the contrary, is fundamentally diploid and characterized by a male karyotype. The first results obtained demonstrate that the two lines considered differ in their sensitivity to the cytotoxic activity of lysolecithin: GM1 cells are more sensitive and less efficient in the fusion (the concentration of LL used is 100 - 150 $\mu\text{g}/\text{cc}$ for 3 - 5 min) than 1B5 cells (exposed to LL at a concentration of 200 $\mu\text{g}/\text{cc}$ for 30 - 40 min). Concentrations of LL greater than 150 $\mu\text{g}/\text{cc}$ and 200 $\mu\text{g}/\text{cc}$ respectively cause a drastic membrane damage with the lysis of all the treated cells. To avoid this different sensitivity effect the first fusion experiments were performed only within the cell line. The production of homokaryons, which generally contain 2, rarely 3 or 4 nuclei (Figure 1), was demonstrated by autoradiographic analysis. A cell population

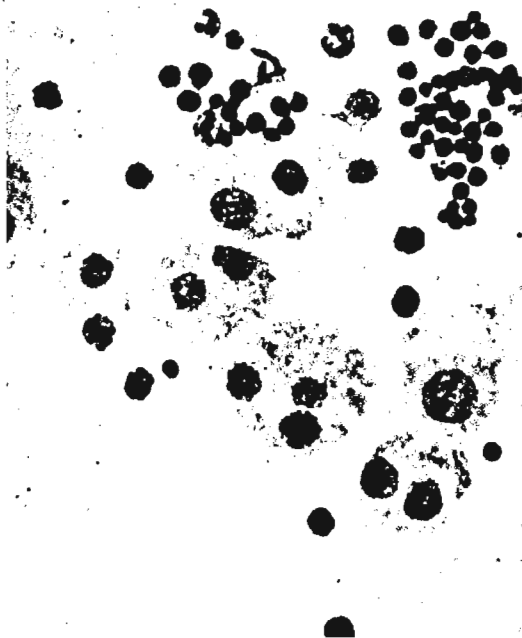


Figure 1: Multinucleated cells and lysed cells of 1B5 line following incubation with lysolecithin for 40 min at 200 $\mu\text{g}/\text{cc}$.

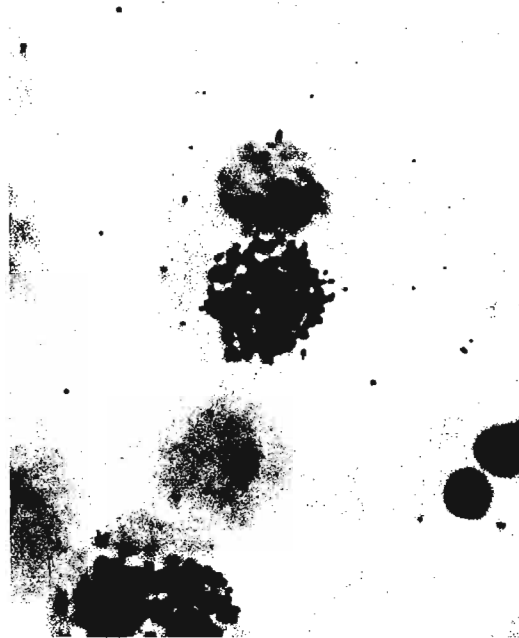


Figure 2: Autoradiograph of homokaryon of GM1 line where one nucleus is $^3\text{HTDR}$ labelled and one is unlabelled.

composed of non labelled and H^3 tritiated thymidine labelled cells (exposed for 16 or 24 hrs) in roughly equal proportions, was treated with LL, and immediately the slides were made following the air-drying technique (Figure 2). These preliminary findings confirm the results obtained with LL in mammalian cells, even if nothing is known at the moment about the possibility of obtaining heterokaryons and cell hybrids between different cell lines in *D. mel.*

References: Croce, C.M., W. Sawicki, D. Kritchevsky and H. Koprowski 1971, *Exp. Cell Res.* 67:427-435; Echallier, G. 1971, *Arthropod Cell Cultures and Their Application to the Study of Viruses*, Springer Verlag (Berlin-Heidelberg-New York) 220-227; Howell, J.I. and J.A. Lucy 1969, *F.E.B.S. letters* 4:147-150; Lucy, J.A. 1970, *Nature* 227:815-817; Poole, A.R., J.I. Howell and J.A. Lucy 1970, *Nature* 227:810-813.

Pulvermacher, C. and K. Timmer. Freie Universität Berlin, Germany. Influence of double matings on the offspring of *Drosophila melanogaster*.

bottles with one genotype of males. After copulation, each female was transferred into a culture vial and one male of a different genotype was added for the next 24 hours. This second male was removed and the females were transferred into a new vial. The vials were replaced every 24 hours until the 12th day after the first copulation. Carbon dioxide narcosis was used for harvesting virgin females and for separating mated females from males. The following mating scheme was used:

Experiment	Genotype females	Genotype males	
		first mating	second mating
Ia	+K	+K	f
Ib	+K	f	+K
IIa	f	f	+K
IIf	f	+K	f

The results are presented in Figures 1 and 2. In experiment Ia and Ib only the total number of offspring can be estimated, because the phenotypes of the daughter cannot be differentiated. It is obvious that double matings in the altered sequence of males had no influence on the total number of offspring. The maximum of offspring per female was produced during the 2nd and

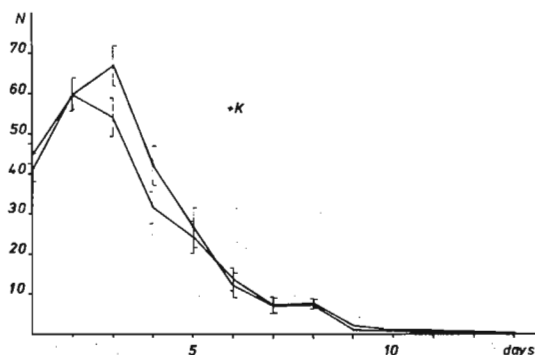


Figure 1. Average number of offspring (N) per +K-female in experiment Ia (—, n = 59) and in experiment Ib (---, n = 45). Ordinate: mean values and standard errors of the means ($\bar{x} \pm 1m$) of offspring per +K-female. Abscissa: days after copulation.

3rd day after copulation.

Comparing the number of offspring produced by double matings to that from single matings (+K σ x +K σ) no enhancement can be noticed after double matings (Timmer, 1976). This corresponds to the results of Lefevre and Jonsson (1962).

As in Figure 1, Figure 2 demonstrates also that double mating of f-females in altered sequence of males did not influence the total number of offspring. The daily rate of offspring does not exhibit a clear cut maximum as compared to +K-females. In contrast to the experiments Ia and Ib, it is possible in the experiments IIa and IIf to distinguish the daughters' phenotypes and to estimate the success of the succeeding males. Furthermore the number of

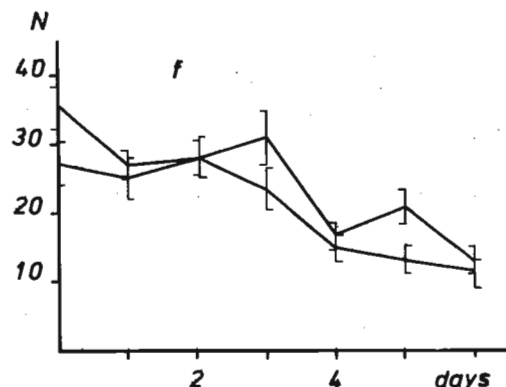


Figure 2. Average number of offspring (N) per f-female in experiment IIa (—, n = 39) and in experiment IIf (---, n = 48). Ordinate: mean values and standard errors of the means ($\bar{x} \pm 1m$) of offspring per f-female. Abscissa: days after copulation.

double matings can be estimated. In experiment IIa, 36 single and 20 double matings were observed. In

experiment IIb, 38 single and 25 double matings were noticed. The frequencies of +-daughters and f-daughters of double matings are summarized in Table 1. The ratio of +-daughters and f-daughters seemed to depend on the sequence of their fathers. In experiment IIa the second

Table 1. Number of different daughters of double mated f-females in experiment IIa (n of P-females = 20) and in experiment IIb (n of P-females = 25)

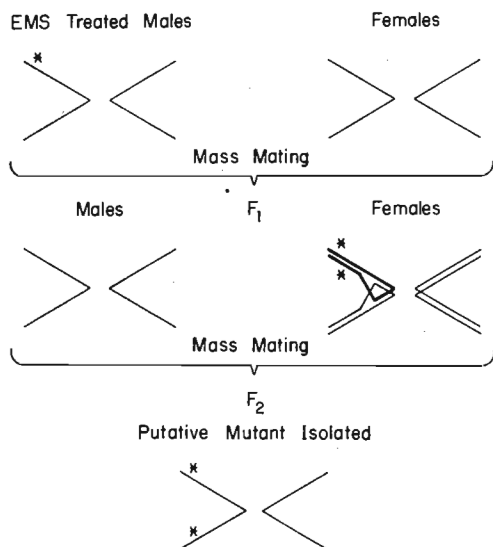
Experiment	Phenotype	Days after copulation									
		1	2	3	4	5	6	7	8	9	10
IIa	+	66	143	194	213	90	84	45	20	15	9
IIa	f	215	88	85	85	48	57	36	18	11	13
IIb	+	219	199	163	103	36	31	22	0	0	0
IIb	f	43	158	177	127	134	100	74	3	0	0

male (+K) seemed to produce more daughters than the f-male which mated first. In experiment IIb, where f-males were the second mating males, this result was not observed. This may be due to a dominance of wildtype males, as Lefevre and Jonsson (1962) mentioned. The experiments IIa and IIb indicate that the offspring phenotype is determined by the first mating male on the first day after copulation. From the second or the third day on, the number of daughters per day of the second male exceeds those of the first male. Analyzing the total number of daughters gained by double matings and by single matings, no difference in the number is found.

References: Lefevre, G. and U.B. Jonsson 1962, Genetics 47:1719; Timmer, K. 1977, DIS 52.

Williamson, R. City of Hope National Medical Center, Duarte, California. A convenient method for generating visible fertile autosomal recessive mutants.

COMPOUND AUTOSOME SCREENING METHOD



A single crossover between nonsister chromatids of a compound reversed metacentric chromosome renders the genetic material distal to the crossover homozygous in the zygote. By this mechanism, mutations induced by the treatment of males with EMS, may be rendered homozygous in F₁ females. Homozygotes may then be recovered from the F₂. A small test of this method has been conducted.

The stocks were obtained from the Pasadena collection, DIS 47:17. Flies were reared at approximately 22°C on the standard Cal Tech medium.

C(2L),+; C(2R),+ flies were examined to confirm their normal phenotype. Males were fed 0.025 M EMS for 12 hrs (Lewis and Bacher, DIS 43:193) and were then placed with virgins in two half-pint bottles (30 pairs/bottle). After 6 days the parents were discarded. Over a four day period with large scale adult emergence, the F₁ were shaken into fresh bottles. The F₁ flies were left to brood for 2 to 5 days before being discarded.

Putative mutants were collected from the F₂. Inheritance of phenotypes was demonstrated by crosses to C(2L),+; C(2R),+. Linkage was determined by crosses to C(2L) j⁶³; C(2R), px and C(2L), dp; C(2R), px.

Examination of the 1702 F₂ yielded the true-breeding mutants shown in the table.

The total fecundity of the F₁ females was not

Number of mutants found	Phenotype	Chromosome
2	Legs shake under ether anesthesia.	X (Dominant)
1	Similar to jaunty, but transverse creases in the wings are more prominent.	2R
1	Wings lifted slightly, but curve downward like an umbrella. Inner margins of wings are shrivelled.	2R
1	Flightless. Wings often maintained in raised or lowered position, becoming fixed with age.	2L

exploited and only a few obvious phenotypes were looked for, thus the potential productivity of this screen is probably greater than this test indicates.

Supported by NIH, NS-0814-08, to William D. Kaplan.

Mulley, J.C. and J.S.F. Barker. University of Sydney, Australia. The occurrence and distribution of *Drosophila aldrichi* in Australia.

D. aldrichi (Patterson and Crow, 1940) previously has been recorded only in the southern United States and Mexico. During our continuing studies of genic variation in *D. buzzatii* in Australia (Barker and Mulley, 1976), a number of populations of *D. aldrichi* have been

located. Both species are members of the mulleri subgroup and both breed in rot pockets in a number of species of the genus *Opuntia*.

All species of *Opuntia* in Australia were introduced. As some of them became major pests of agricultural and grazing land, and are still declared noxious weeds, their past and

present distribution is well recorded (Mann, 1970). Therefore, assuming complete specificity of *D. buzzatii* and *D. aldrichi* to the *Opuntia* niche, their possible geographical distribution in Australia is readily defined. Figure 1 shows the distribution of the main *Opuntia* infestations at the beginning of the biological control

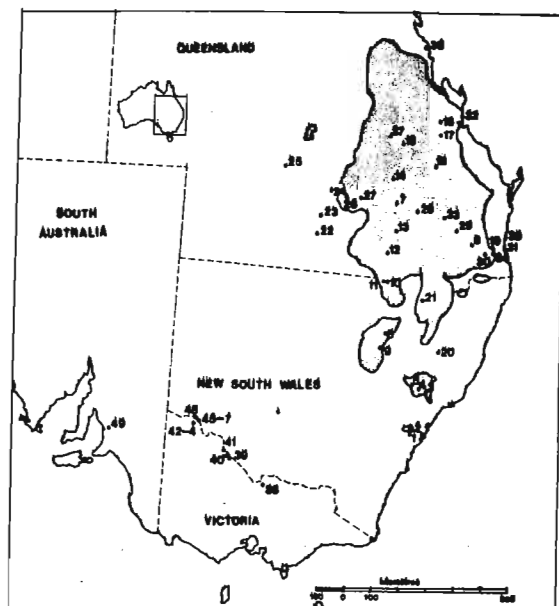


Figure 1. Distribution of the main *Opuntia* infestations in 1920 (shaded areas), and the localities from which *D. buzzatii* populations were sampled.

program in 1920. Following the success of *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) as a biological control agent, *Opuntia* now exists within the areas of major infestation, and elsewhere in eastern Australia, as isolated patches ranging in size from less than one hectare to a few hundred hectares.

D. buzzatii is suspected to exist throughout the entire *Opuntia* distribution in Australia, and the populations that have been sampled are shown in Figure 1. Table 1 gives, for all collections that included *D. aldrichi*, the numbers of each species collected and the percentage *D. aldrichi*. The distribution of *D. aldrichi* is entirely within the *D. buzzatii* distribution, and apart from the one population found in New South Wales, is concentrated in the northern part. In those localities where both

species exist, adults of both species have been collected from the same rot, both in the natural populations, and as emergences from rotting cladodes brought back to the laboratory.

For both species, occurrence of *Opuntia* is the primary factor determining their distribution and abundance, but for *D. aldrichi* secondary factors, presumably climatological, add additional constraints. Just what factors determine the ecological boundary of *D. aldrichi* is unknown. The single population (locality 5) in the Hunter Valley of N.S.W. is separated by some 900 km from the main distribution area. However, the species has not been found in nearby *Opuntia* stands (17 km and 40 km distant) where extensive collecting has been done.

Therefore in *D. buzzatii*, although the distributional margin is vast, little or no ecological margins may exist. In contrast, *D. aldrichi* populations inhabiting the marginal ecological boundary may be subject to strong selection, so that this species may present an excellent opportunity for studies of genotype-environment associations and of selection in natural populations.

In addition, the two species can be compared for levels of variation, genetic divergence between populations and temporal variation within populations for polymorphic loci. Locality 5 presents an excellent opportunity for the study of selection following perturbation of gene frequencies, since preliminary electrophoretic analyses have detected a number of low frequency alleles in populations in the northern part of the species distribution, but these are absent from locality 5.

Finally, since *D. aldrichi* usually coexists with *D. buzzatii* under natural conditions, an ideal system of defined populations is available for the study of ecological displacement

Table 1. Numbers of each species collected and percentage of *D. aldrichi*

Locality	Collection Date	Numbers collected		Per cent aldrichi
		aldrichi	buzzatii	
5	28.2.74 - 9.10.75	Monthly collections 3-2083 9-1254		2.73 - 79.22
7	18. 8.73	1	234	0.43
	26.11.73	2	131	1.50
	17. 8.74	1	127	0.78
13	17. 8.73	1	169	0.59
14	18. 8.73	4	27	12.90
15	19. 8.73	4	204	1.92
	18. 8.74	4	50	7.41
16	19. 8.73	36	21	63.16
	22. 8.74	4	10	28.57
17	20. 8.73	28	31	47.46
18	20. 8.73	100	21	82.64
	23. 8.74	57	95	37.50
23	24.11.73	2	15	11.76
24	24.11.73	1	31	3.13
26	26.11.73	4	83	4.60
28	26.11.73	2	125	1.57
32	22. 8.74	5	1	83.33
34	24. 8.74	1	151	0.66
37	3.11.74	3	3	50.00

and interspecific competition in the natural environment.

(Work supported by Australian Research Grants Committee.)

References: Barker, J.S.F. and J.C. Mulley, 1976, Evolution (in press); Mann, J. 1970, Cacti naturalised in Australia and their control, S.G. Reid, Government Printer, Brisbane; Patterson, J.T. and J.F. Crow 1940, Univ. Texas Publ. 4032:251-256.

Lin, F.-J.¹, H.-C. Tseng² and W. Chiang¹.

1. Academia Sinica, Nankang, Taipei, Taiwan. 2. University of Nebraska, Lincoln. Chromosomal polymorphisms in *Drosophila albomicans*.

Heterozygous chromosomal inversions from 113 stocks of *Drosophila albomicans* iso-lines from various localities in Taiwan have been analysed. From the number of inversions we found that *D. albomicans* in Taiwan can be divided into 3 main population centers, one located in the northern, one in the southern and one in the eastern part

of Taiwan. 91 heterozygous inversions were detected (see Table). Of them two common inversions, In(1)A₁A₄ and In(2L)B₁D₅ were found from every locality. In(2R)A₁A₅, In(2R)A₁C₁, In(2L)A₂B₄, In(2L)B₁C₄, In(3)A₁B₁, In(3)A₁B₄ and In(3)M₅O₄ were found only in the population centers but did not occur in the population peripherals. 45 out of the 91 inversions were

Heterozygous chromosomal inversions of *D. albomicans* from Taiwan.

In(1)		In(2R)		In(2L)		In(3)	
A ₁ A ₄ *	A ₄ D ₁	A ₁ A ₂	B ₁ C ₁	A ₂ B ₄ **	B ₃ C ₅	A ₁ A ₄	F ₅ G ₃
A ₁ B ₂	A ₅ B ₅	A ₁ A ₅ **	B ₃ C ₁	A ₂ C ₂	B ₄ D ₂	A ₁ B ₁ **	F ₅ J ₄
A ₁ B ₃	A ₅ C ₁	A ₁ B ₂	B ₅ D ₁	A ₂ D ₁	B ₅ D ₄	A ₁ B ₄ **	H ₃ J ₁
A ₁ B ₅	A ₅ C ₃	A ₁ C ₁ **	C ₁ D ₄	A ₄ B ₂	C ₁ C ₃	A ₁ C ₃	H ₃ J ₃
A ₁ C ₁	B ₄ C ₃	A ₂ B ₂	C ₁ F ₃	B ₁ C ₄ **	C ₁ C ₅	B ₁ D ₄	J ₂ J ₄
A ₁ C ₃	C ₁ E ₃	A ₂ B ₅	C ₃ D ₄	B ₁ D ₅ *	C ₂ D ₄	B ₄ C ₃	K ₁ L ₂
A ₁ C ₄	C ₃ D ₃	A ₂ C ₂	D ₁ F ₄	B ₂ C ₂	C ₅ D ₂	B ₄ D ₄	K ₅ N ₄
A ₂ B ₁	D ₃ E ₁	A ₂ F ₂	D ₃ E ₅	B ₃ B ₅	D ₁ E ₁	C ₃ D ₁	K ₅ P ₄
A ₃ B ₅	E ₃ E ₅	A ₃ B ₁	D ₄ E ₁	B ₃ C ₁	D ₃ D ₅	D ₁ G ₃	L ₂ M ₃
A ₄ A ₄	F ₂ F ₅	A ₃ C ₁	D ₅ F ₂			D ₁ H ₃	M ₅ N ₄
		A ₅ B ₅	E ₂ F ₃			D ₁ M ₅	M ₅ O ₄ **
						D ₃ F ₁	O ₄ P ₅
						D ₄ G ₂	P ₁ Q ₅
						D ₄ J ₄	P ₄ Q ₄
						D ₅ E ₅	Q ₁ Q ₅
						F ₃ J ₄	
20 inversions		22 inversions		18 inversions		31 inversions	

* common inversion

** only occur in population center

found only from a single locality and the frequencies are very low which may serve as specialized adaptabilities to the micro-environmental condition. From this analysis we could conclude that the species distributional pattern of a population can not only refer to the geographical two-dimensions but also to the altitude dimension. For example, flies from Chi-tou (1,200 to 1,500 m elevation) where there is a geographical center in Taiwan, but from the chromosomal inversions detected show that the Chi-tou's population have only one inversion on the X chromosome (total of 20 inversions found in Taiwan), one on 2R (total of 22 inversions), two on 2L (total of 18 inversions) and none on 3 (total of 31 inversions). Although we have not analysed that the changes of the inversion number and frequency with the time, but it has been proved (Dobzhansky et al., 1966, on *D. pseudoobscura*). So here we may say that species distributional pattern should be referred to four dimensions which are longitude, latitude, altitude and time. The correspondent inversions between *D. albomicans* and other species of *nasuta* sub-group of flies please refer to Wilson et al. (1969) and Mather and Thongmeearkom (1973). For the standard salivary chromosome see Lin et al. (1974).

References: Dobzhansky, Th., W.W. Anderson and O. Pavlovsky 1966, *Evol.* 20:418; Lin, F.J., H.C. Tseng and T.C. Wang 1974, *DIS* 51:42; Mather, W.B. and P. Thongmeearkom 1973, *DIS* 50:60; Wilson, F.D., M.R. Wheeler, M. Harget and M. Kambysellis 1969, *Univ. Tex. Publ.* 6918: 207.

Thongmeearkom, P. University of Queensland, Brisbane, Australia. Chromosome map of *D. sulfurigaster albostrigata*.

A photographic chromosome map of *Drosophila sulfurigaster albostrigata* is here presented so that inversions previously detected in natural populations (Mather and Thongmeearkom 1972 and Mather et al. 1974) can have their break points

assigned.

Inversion	Locality	Chromosome	Breakpoints	Inversion Photograph Reference
G	C., L.	I(X)	14.3 - 18.1	1
A ₃	Pen., K.L., K.T.	I(X)	10.3 - 21.0	2
E	C., L., Pen., K.L., K.T.	IIL	6.6 - 21.1	1
Y ₂	K.L., K.T.	IIL	9.4 - 15.8	2
D ₃	K.L., K.T.	IIR	8.5 - 16.0	2
C	C., L., Pen., K.L., K.T.	III	6.0 - 10.0	1
Y ¹	K.T.	III	24.8 - 31.3	Fig. 2
Z ₂	L., K.T.	III	18.5 - 22.5	2
W ₂	L., Pen., K.L., K.T.	III	32.7 - 36.4	2
X ₂	L., Pen., K.L., K.T.	III	W ₂ + F ₃	2
F ₃	K.L.	III	32.7 - 36.6	2
R ₃	L.	IIL	12.3 - 22.4	3

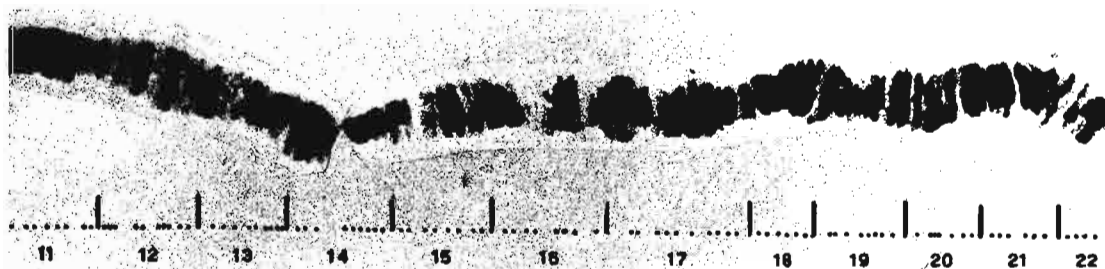
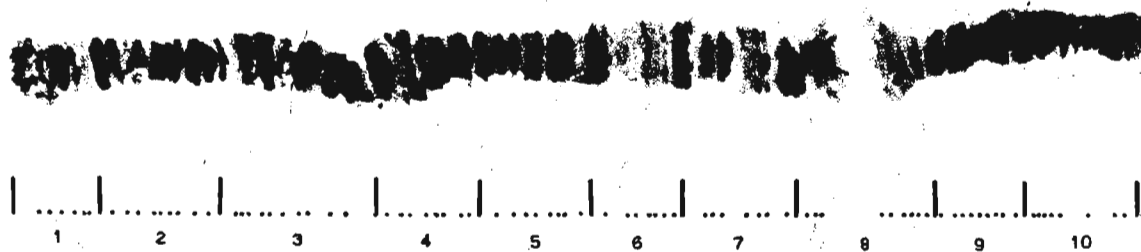
Note: C. - Cebu; L. - Luzon; Pen. - Penang; K.L. - Kuala Lumpur; K.T. - Kota Tinggi.
1. Mather, W.B. and P. Thongmeearkom 1972, DIS 48:40; 2. Mather, W.B., P. Thongmeearkom, M. Clyde and D. Lambert 1974, DIS 51:86; 3. Mather, W.B. and M. Clyde 1977, DIS 52:

Figure 1. Giant chromosome photographic map of *Drosophila sulfurigaster albostrigata*. (On following five pages)

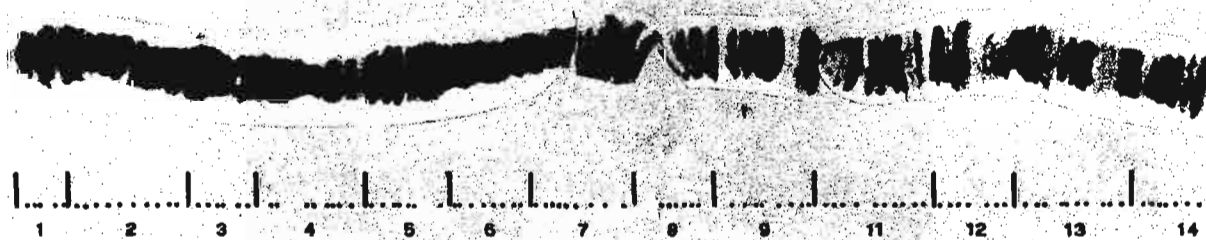


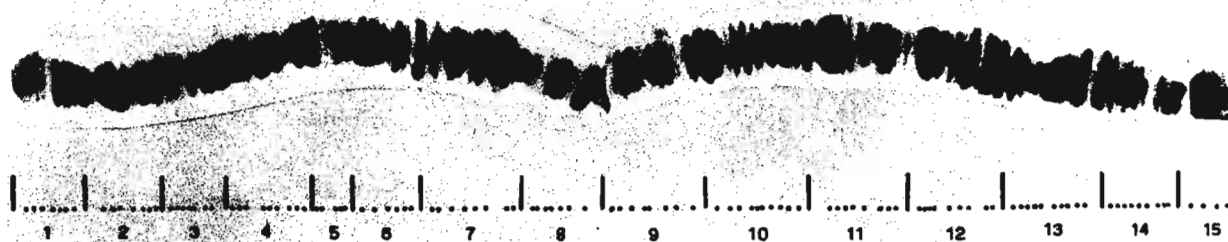
Figure 2. Inversion Y¹, f.e. - free end.

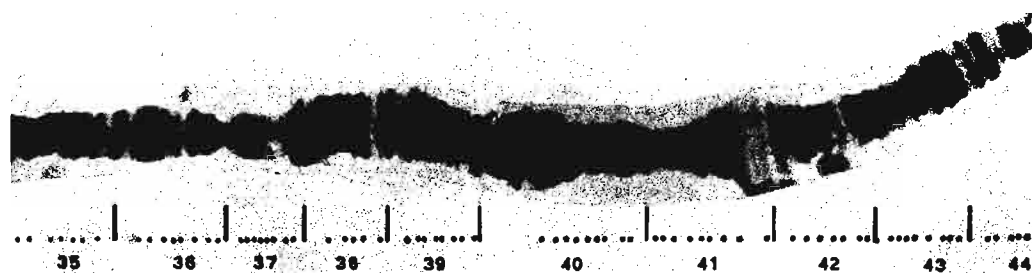
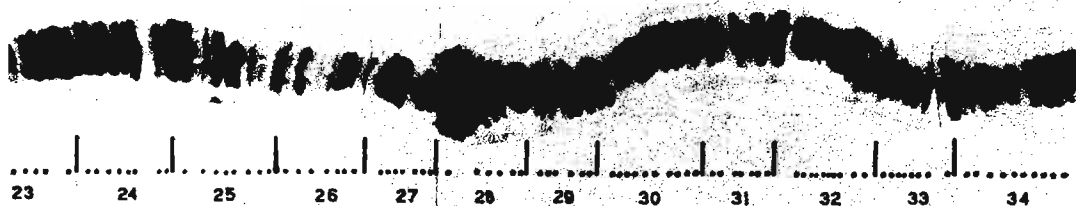
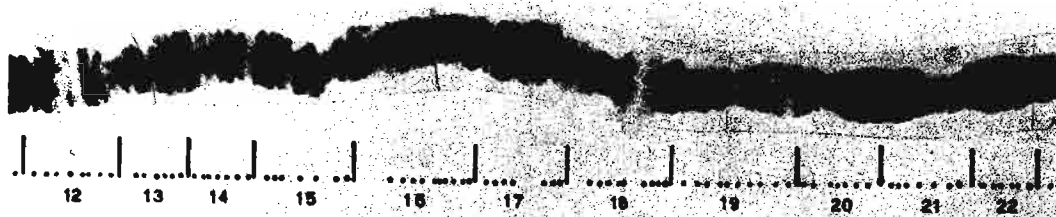
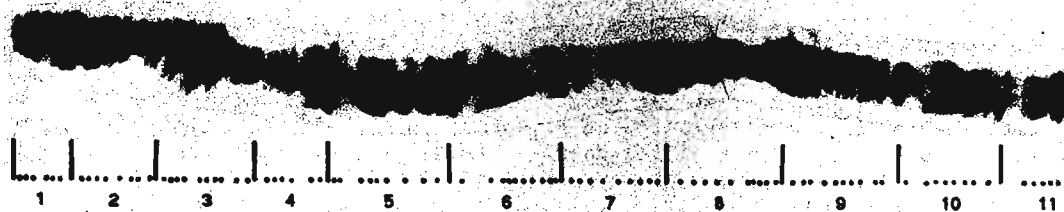
Chromosome I



Chromosome II L



Chromosome IIR**Chromosome IV**

Chromosome III

Peñafiel, T. and J.L. Ménsua. Autonomous University of Barcelona, Spain. Selection by asymmetry in the dorsocentral bristles of *D. melanogaster*.

From a strain of thirteen wild females, three lines of selection have been made in duplicate. The criteria of selection were: a) Directional asymmetry in that two lines of flies having extra dorsocentral bristles only on the left side (1-L, 2-L), and two lines of flies having

extra bristles only on the right side (1-R, 2-R) were selected. b) Fluctuating asymmetry in that two lines of flies with a maximum difference of bristles between the two sides were selected (1-D, 2-D). A control line was also drawn in duplicate (1-C, 2-C).

The results obtained are illustrated in Figures 1 and 2. In the lines selected by directional asymmetry, there is a slight response to selection. The heritability obtained is only significant in line 2-R ($h^2 = 0.063 \pm 0.005^*$).

In the lines selected by fluctuating asymmetry, the response was greater and clearer. The heritability obtained was as follows: $h^2 = 0.063 \pm 0.032^*$ (1-D) and $h^2 = 0.061 \pm 0.024^*$ (2-D).

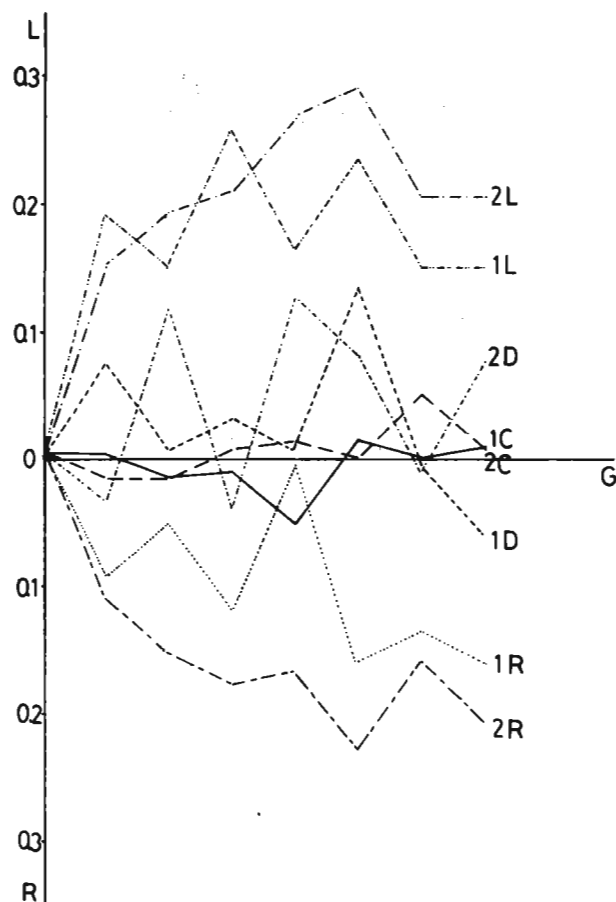


Figure 1. Means by directional asymmetry for all the lines.

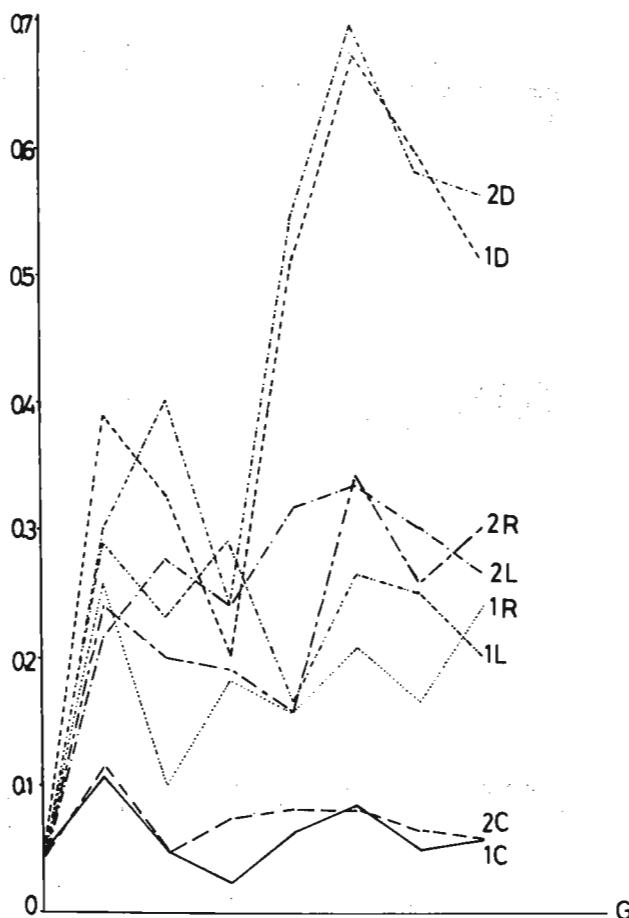


Figure 2. Means by fluctuating asymmetry for all the lines.

The values of the regression between generations and increase in directional and fluctuating asymmetry in all the lines are shown:

<u>Lines</u>	<u>Directional Asymmetry</u>	<u>Fluctuating Asymmetry</u>
1-R	→ 0.017 ± 0.007~	0.014 ± 0.010
2-R	→ 0.022 ± 0.006***	0.027 ± 0.010*
1-L	0.011 ± 0.011	0.009 ± 0.013
2-L	0.024 ± 0.010~	0.026 ± 0.010*
1-D	-0.005 ± 0.009	0.066 ± 0.021*
2-D	0.008 ± 0.010	0.073 ± 0.019***
1-C	0.0009 ± 0.003	-0.0007 ± 0.004
2-C	0.005 ± 0.002	-0.0004 ± 0.003

~ 0.10 > P > 0.05

* 0.05 > P > 0.02

*** 0.01 > P > 0.001

It will be noted that regarding directional asymmetry, three of the lines selected for this type of asymmetry are significant as neither lines D nor C. For fluctuating asymmetry all the D lines are significant, as are in two cases the lines selected by directional asymmetry.

After eight generations of selection the following crosses were made between lines:

1-R x 2-R	0.170 ± 0.065	} \bar{x} directional asymmetry
1-L x 1-R	0.005 ± 0.065	
2-L x 2-R	0.000 ± 0.075	
1-D x 2-D	0.530 ± 0.065	} \bar{x} fluctuating asymmetry
2-D x 1-D	0.525 ± 0.065	

These results seem to indicate that directional asymmetry obtained would be of the additive type. The results of the crosses between the D lines indicate that fluctuating asymmetry is being maintained, as in the crosses between R lines.

These results corroborate the data obtained by Beardmore (1963), selecting sternopleural bristles for directional asymmetry.

MATERIAL REQUESTED OR AVAILABLE

Gummed labels: Many manufacturers have discontinued the production of small gummed labels for labeling vials and bottles, according to E.B. Lewis, California Institute of Technology. Dennison Eastman Corp., 3451 Collins Ave., Richmond, Calif. 94804 will make them in any size to special order. Their label with L7 gum (3/4 x 1-1/2 inch) has an excellent writing surface, adheres tightly to glass, and readily comes off during washing.

Frances Clayton, Department of Zoology, University of Arkansas, Fayetteville, Arkansas 72701, would appreciate receiving reprints of papers which include descriptions of metaphases of *Drosophilidae*.

Ch. Malogolowkin-Cohen, University of Haifa, Israel, would appreciate receiving mutations and strains of *Drosophila subobscura* from natural populations.

Van der Meer, J.M. Catholic University, Toernooiveld, Nijmegen, The Netherlands. Optical clean and permanent whole mount preparation for phase-contrast microscopy of cuticular structures of insect larvae.

To study cuticular details of the segment pattern of *Drosophila* (and other insects) larvae with phase-contrast optics, the object should be as transparent as possible in order to gain optimal visualization of details differing only a few thousands of wave-length in optical thickness. Two methods of fixation and clearing are

recommended both of which completely remove the internal larval organs which would normally cause very inconvenient optical disturbances. The first method includes pricking the larva with sharp tungsten needles in an embryo dish containing glycerol (sp. grav. 1.23). The larvae are then transferred to a small tube containing acetic acid : glycerol = 4:1 and the internal organs are dissolved overnight at 60°C. The tubes should be closed tightly to prevent evaporation of acetic acid which would result in incomplete solubilization. The larvae should not stick to the glass wall to prevent them from being burned. Next the larvae should be mounted under a small coverslip in Hoyer's mixture and incubated overnight at 45°C to complete solubilization. The more resistance the internal organs show to solubilization, the longer they should be incubated at 45°C (up to one week). It is also possible to shorten this period by incubation overnight at 60°C, but this results in contraction and sometimes breakage of the coverslip. The medium may be somewhat protruding from under the coverslip, because shrinkage of the larval tissue occurs during incubation. A well-known disadvantage of aqueous mounting media is their hygroscopic character. To prevent eventual deterioration of the preparations by water uptake, the coverslip should be ringed twice by a water insoluble protectant such as Euparal or a commercially available metal paint. Properly mounted and ringed slides will last indefinitely. However, if necessary, the object is easily retrieved after wetting the coverslip with water. The object can then be remounted. Hoyer's modification of Berlese's¹ mixture should be prepared at room temperature as follows²: a) add and mix first: 30g gum arabic (clear crystals) with 50 ml distilled water and stir (magnetic stirrer) overnight until completely dissolved; b) add successively small amounts of 200 g (total) chloralhydrate anhydrous (C₂H₃Cl₃O₂ M.W. 165.4); c) after complete dissolution of the chloralhydrate add: 20 g glycerol, and d) finally clean the mixture thoroughly by filtering through glass wool or cheese-cloth to remove small particles of wood or other impurities of the gum arabic, which would disturb microscopic examination. Gum arabic powder should not be used instead of crystals, because it is very difficult to dissolve and this would result in a medium containing many undissolved gum arabic particles. The whole aforementioned process should be carried out as anhydrous as possible, preferably in a moisture-free room. In any case the erlenmeyer containing the mixture should be kept closed during solubilization of the gum arabic and filtering. When dealing with larvae containing excessive amounts of food or fat droplets, possible remnants of this material after the acetic acid/glycerol step should be removed from the larva by gently pressing the coverslip. The aforementioned procedure has been tested on first instar larvae of *Drosophila melanogaster*, *Callosobruchus maculatus*, *Bruchidium obtectus* and *Leptinotarsa decemlineata* (Coleoptera) and produced excellent results. Neither breakage nor excessive softening of the cuticula did occur. In the second method the acetic acid/glycerol step is replaced by treatment with Nesbitt's fluid. This has an extremely powerful clearing effect and is therefore only recommended for larvae which cannot otherwise be sufficiently cleared or for larvae which have been stored in alcohol for a long time. It has the disadvantage of weakening and eventually digesting chitinous structures too and it should therefore not be used for larvae with a young, thin cuticula or when the relevant cuticular markers normally show little phase contrast. Complete digestion of relevant cuticular details can be prevented by controlling duration and temperature of treatment. For example, each larval instar of *Drosophila* will require a different combination of temperature and duration of treatment to give optimum phase contrast of cuticular markers. The first larval instar e.g. requires a treatment of about one hour at room temperature. Clearing with Nesbitt's fluid is followed by the usual embedding in Hoyer's mixture as described previously. Nesbitt's fluid is prepared as follows³: dissolve 40 g chloralhydrate in 25 ml distilled water and finally add 2.5 ml concentrated hydrochloric acid.

References: 1. Berlese, A. (1882-1903), *Acari, Myriopoda, et corpiones hucusque in Italia reperta*. Fasc. 1-101, Padua; 2. Baker, E.W. and G.W.W. Wharton 1964, *An Introduction to Acarology*, 4th ed., New York; 3. Krantz, G.W. 1971, *A Manual of Acarology*, 3rd ed., Corvallis.

Shields, G. and J.H. Sang. University of Sussex, Brighton, England. Improved medium for culture of *Drosophila* embryonic cells.

We have made further changes in the medium we use for culture of embryonic cells (Shields and Sang, 1970; Shields, Dübendorfer and Sang, 1975), which lead to better development of the cultures. Our latest medium, designated M3, is shown in Table 1. The changes are: a slight

further increase in KHCO_3 , replacement of KCl and NaCl by the glutamate salts, and inclusions of oxaloacetic acid and the organic buffer BIS-TRIS. Antibiotics can also be included if required (Penicillin G, sodium salt, at 3 mg, and Streptomycin sulphate at 10 mg).

Table 1. M3 medium. (Amounts in mg/100 mls).

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	440	Threonine	50	Leucine	40
$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$	150	Serine	35	Tyrosine	25
K.glutamate. H_2O	788	Asparagine	30	Phenylalanine	25
Na.glutamate	653	Glutamine	60	β -Alanine	25
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	88	Proline	40	Histidine	55
(KHCO_3)	50)	Glycine	50	Tryptophan	10
Glucose	1000	α -Alanine	150	Arginine	50
Oxaloacetic acid	25	Valine	40	Lysine.HCl	85
BIS-TRIS	105	Methionine	25	Cysteine.HCl	20
TC Yeastolate (Difco)	100	Iso-Leucine	25	Choline Cl	5
Aspartic acid	30				

Dissolve in about 95 mls doubly-distilled H_2O , adjust pH to 6.6 with 1% NaOH, and then add the KHCO_3 to bring to the final value of 6.8. Make up volume to 100 mls and sterilize by millipore filtration. Add foetal bovine serum to 10%.

This medium gives little improvement to the early differentiation of nerve, muscle, fat-body, chitin-secreting, and macrophage-like cells that occurs in the cultures, but promotes more regular and rapid maturation of the fat-body cells to their final fat-filled form, better growth (enlargement) of the chitin-secreting cells, and an earlier and better sustained multiplication of the macrophage-like cells. Ultimate formation of chitin by the chitin-secreting cells and transformation of the macrophage-like cells to a more advanced larger form also occur more regularly, and so does emergence of several unidentified multiplicative cell types. Appearance of imaginal disc cell vesicles occurs less readily than with the previous medium, and it is believed that this tissue usually grows as condensed lumps of cells in the new situation. This suggests that vesicle formation may be a response to poorer conditions.

A warning should be given about the serum used in the medium. As we stated previously, non-inactivated serum gives the best results for embryonic cultures, and medium should be held for a week in the refrigerator (5°C) after addition of the serum to allow the latter to temper down from an often harmful initial level of lytic activity. Different batches of serum differ widely in quality, and some remain permanently bad for tissue culture purposes. With others, the time of holding to produce the optimal condition may need to be varied somewhat. David Cross, working in our laboratory with single embryo cultures, has recently found that medium with serum may decline again in quality after reaching its optimum. With a particular batch of serum, the optimum condition was reached after about 5 days storage, and a decline was noticeable from about the 10th day. Ideally, therefore, it may be best to use medium only between about the 1st and 2nd week after addition of the serum.

Heat-inactivation of the serum (56°C for 30 min) gives a stable product, but results in a slight loss in quality, affecting particularly the early appearance of the cultures. Use of heat-inactivated serum rather than non-inactivated is necessary in certain situations as, for example, where the medium is used to maintain cell lines; since non-inactivated retains sufficient lytic activity even after prolonged holding of the medium to cause substantial cell disruption.

This work was supported by a grant from the Science Research Council.

References: Shields, G. and J.H. Sang 1970, *J. Embryol. exp. Morph.* 23:53-69; Shields, G., A. Dübendorfer and J.H. Sang 1975, *J. Embryol. exp. Morph.* 33:159-175.

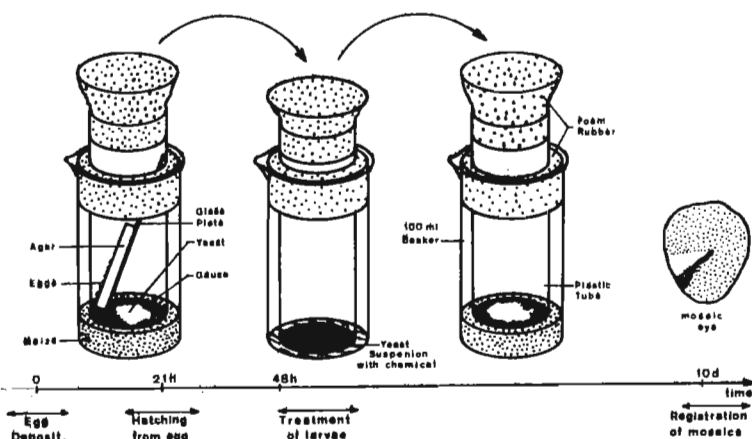
Mollet, P. and W. Weilenmann, Swiss Federal Institute of Technology, Zürich, and Swiss Federal Research Station, Wädenswil, Switzerland. A method for controlled access of *Drosophila* larvae to potential mutagens.

This plastic tube stands in a 100 ml beaker and is held in place by a crepe rubber ring which also prevents contamination by other flies.

The procedure is the following: Eggs are collected as described by Büchi and Bürki (1975). A glass plate supporting an agar block with 75 eggs

For our studies on chemical induction of somatic recombination and mutation (Mollet and Weilenmann, 1975), a special technique for feeding larvae was developed. The set-up used is depicted in Figure 1. It consists of a plastic tube (\varnothing 30 mm, length 90 mm) covered at the upper end and with a crepe rubber stopper and at the lower end with gauze fixed with glue.

SHORT TIME FEEDING OF DROSOPHILA LARVAE WITH POTENTIAL MUTAGENS



of yeast suspension is added. The flies surviving are registered. If larvae with appropriate eye colour markers have been treated with a mutagen, mosaic spots arise in the eyes of the flies developing from these larvae (Figure 1). During the time from incubation of the eggs until hatching of the adults the tubes are kept at 25°C and 80% relative humidity except for the time of exposure to the chemical to be tested. This is done in a hood at room temperature and 60% relative humidity.

In order to examine the adequacy of this method we compared the mortality of individuals kept without any chemical treatment in the manner described (A) to the mortality of individuals raised according to a standard protocol (B, Würzler et al., 1968). We also examined whether individuals from different kinds of crosses ($- w^{sn^+}; se h \times w^{co} sn^2/Y; se h - w^{co} sn^2; se h \times w^{sn^+}/Y; se h -$ Berlin wild stock inter se $-$) show different responses to the two techniques used (Table 1). In addition the influence of the length of egg collection has been investigated (Table 1). Apart from differences in results due to the types of crosses or to the variation of the period of egg collection, it is obvious that with both techniques the mortality is approximately the same. In general, eggs collected within 4 hours are less viable than those collected within 17 hours. Also it can be seen that the vitality of the offspring from the three crosses is different. Whereas Berlin wild individuals show the lowest mortality, the progeny from $w^{co} \varnothing \times w^{co} \sigma$ and especially that from $w^{co} \varnothing \times w^{sn^+} \sigma$ are less viable. Comparing the experiments 1 and 2, it can be recognized that the mentioned tendency becomes more pronounced if egg collection lasts 4 hours only. For the progeny of all crosses it was also noted that the embryos which did not survive, predominantly died at an early stage of development (before blastoderm formation). The mortality of larvae from $w^{co} sn^2; se h \varnothing$ raised with the new technique was higher than expected when compared to all the other data on mortality of larvae (Table 1). The importance of this fact is illustrated by the data from repeated experiments with the new technique, presented in Table 2. It can be recognized that larvae from $w^{co} sn^2; se h \varnothing$ are in general less viable than those from $w^{sn^+}; se h \varnothing$. Nevertheless, the unexpected high mortality of larvae from $w^{co} sn^2; se h \varnothing$ in experiment 2 seems to be rather

Table 1. Mortality of *Drosophila* raised under different conditions.

Cross	Technique	Eggs tested	Embryonic death %	Postembryonic death %	Preimaginal death %
EXPERIMENT 1 Egg collection: 17 hours					
Berlin wild	A	525	3.2	3.4	6.5
	B	625	2.2	5.2	7.4
w sn ⁺ ;se h x w ^{co} sn ² /Y;se h	A	600	6.0	5.7	11.3
	B	575	7.7	7.6	14.6
w ^{co} sn ² ;se h x w sn ⁺ /Y;se h	A	600	26.3	7.7	32.0
	B	601	23.6	6.8	28.8
EXPERIMENT 2 Egg collection: 4 hours					
Berlin wild	A	524	11.5	9.9	20.7
	B	748	9.9	9.8	18.7
w sn ⁺ ;se h x w ^{co} sn ² /Y;se h	A	598	16.7	8.4	23.8
	B	625	19.7	10.0	27.7
w ^{co} sn ² ;se h x w sn ⁺ /Y;se h	A	600	32.7	33.8	55.7
	B	596	34.4	7.7	39.4

Technique A: The individuals were kept as described in this paper. The treatment with the aqueous yeast suspension for two hours was done 44 ± 2 (resp. ± 8.5) hours after egg deposition.

Technique B: The individuals were kept according to a standard protocol (Würgler et al., 1968) on corn meal medium with live yeast.

Table 2. Mortality of *Drosophila* larvae in repeated experiments.

Cross	w sn ⁺ ;se h x w ^{co} sn ² /Y;se h			w ^{co} sn ² ;se h x w sn ⁺ /Y;se h		
Solvent	Exp	Tested	% dead	Exp	Tested	% dead
water	2	498	8.4	2	402	33.8
	3	140	11.4	5	188	26.1
	6	185	9.2	6	161	11.8
1% DMSO	4	237	16.0	4	243	19.8
	7	185	7.0	7	136	20.6
5% acetic acid	4	181	18.8	4	238	17.2
	6	184	12.0	6	167	10.2
	7	171	5.2	7	165	15.2
	8	178	10.7	8	133	22.6

Eggs were collected within 4 hours and raised as described for the new technique. 44 ± 2 hours after egg deposition, the larvae were fed for 2 hours with a yeast suspension containing one of the different solvents.

exceptional since all the other experiments exhibited lower mortality rates.

From the data presented we conclude the new technique is an effective method for a controlled access of *Drosophila* larvae to potential mutagens. If precise estimates of toxicity (mortality of the animals tested) are not needed, e.g. in rapid screening, the eggs can be collected directly into the tubes. If equal numbers of flies are allowed to lay eggs in each tube, rough toxicity estimates are still possible, since each tube will contain approximately the same number of eggs and thus the number of offspring represents a measure of toxicity.

The method described meets the following conditions: 1) the flies hatching are not

externally contaminated by the mutagen; 2) the toxicity of the chemical used can be estimated apart from its mutagenicity; 3) the point of time at which chemical treatment is to begin and the length of time it is to continue can be fixed very exactly.

References: Mollet, P. and W. Weilenmann 1975, 5th ann. meeting of the EEMS, Mutation Res., in press; Büchi, R. and K. Bürki 1975, Archiv für Genetik 48:59-67; Würzler, F., U. Petermann and H. Ulrich 1968, Experientia 24:1293.

Acknowledgements: We are grateful to Bea and R. Cotton-Menzl and Dr. D. Turner for fruitful discussions. The work was supported by grant Nr. 3.7040.72 from the Swiss National Foundation for Scientific Research.

Harshman, L.G. University of California, Riverside. A technique for the preparation of *Drosophila* salivary gland chromosomes.

D. salivary gland chromosomes are usually prepared by squashing the glands between coverslip and slide with an intense straight downward force on the coverslip. The following is a method that relies instead on a light circular motion of the coverslip above the salivary

glands to spread the chromosome arms.

A microscope slide should be wiped clean with silicon paper; we use Scientific Products' lens paper P1057. A lubricant is needed to suspend the coverslip above the slide; hand lotion, vaseline, or especially the sebaceous gland oils from the outside corner of the nose have been used successfully in our laboratory. A ring of the lubricant approximately 1.0 cm wide (across the inside diameter) should be drawn with a forefinger on the slide. The amount of lubricant to be used may take a little experimentation; we find what might be described as a substantial film on the slide to be sufficient.

Enough stain to fill the ring is added. We use a Mainx III lact-aceto-orcein stain, made by adding 2 g. orcein to 100 ml. of 60% glacial acetic acid, refluxing the mixture for two hours, and finally adding 100 ml. of 85% lactic acid. The salivary glands are dissected from a mature third instar larva in "*Drosophila* Ringer's" (7.5 g. NaCl, 0.35 g. KCl, and 0.21 g. CaCl₂ in a liter of distilled water)¹. The glands are cleared of fat and transferred quickly to the stain. A Syracuse watchglass (USBPI watch glass, 27 mm x 8 mm) is inverted over the preparation, which is allowed to stain for 6-9 minutes. Less than 6 minutes may be required for the glands from older, imminently pupal *D.* A coverslip (22 mm sq., No. 1 thickness) is then dropped over the stained glands, and the slide placed on the stage of a bottom-lit dissecting microscope. The remainder of the work is done at a magnification of 7x.

The coverslip should be relatively free-floating on the stain and lubricant. A dissecting needle is now used to gently tap and press the coverslip immediately above the salivary glands, working from the center of the glands outward. This has the effect of separating the cells of the glands. Some of the cells will appear to be relatively well separated from the main body of the gland, which spreads out and appears as a clear, flat zone in the stain. The dissecting needle is now used to gently rotate the free-floating coverslip in small circles above the slide. This rotary motion is repeated in three to five well dispersed locations above the glands, again working from the inside of the glands toward the periphery. An ever-increasing, downward pressure on the coverslip is exerted with each successive rotation, until the coverslip sticks to the slide and the needle tends to slide across the surface of the coverslip instead of rotating it. At this point, the arms should be well spread; however, this should be verified under a compound microscope to see if further rotation of the coverslip above the chromosomes is required. The stain may be cleared from the preparation by inverting the slide on absorbent paper and pressing gently down.

Using this method, our preparations yield a high percentage of good-quality, readable chromosomes from each salivary gland. The arms are extended along one plane exposing the linear order of the bands, and the conformation of inversions can be observed with a minimum of interference from the arms folding back on themselves. The procedure requires little expertise and can be quickly taught. Our work was with *Drosophila* of the *obscura* group, and the technique works much better on their substantial chromosomes than it does on those of *D. melanogaster*. This method was developed coordinately with Dr. Betty C. Moore, with help from Dr. K.W. Cooper.

Reference: ¹Ephrussi, B. and G.W. Beadle 1936, Am. Nat. 70:222.

Wetzel, L.T. and J.F. Barnett. Wyeth Laboratories Inc., Paoli, Pennsylvania. A method for administering test compounds to adult *Drosophila*.

A common method for administering test compounds to adult *Drosophila* is to allow the flies to feed on tissue paper or filter paper saturated with a known volume of a mixture of glucose, water and the compound. Although the test compound is present in a measured quantity, the

amount ingested cannot be determined by weighing because the flies defecate or lay eggs on the paper and water evaporates from it.

To overcome these problems, it is recommended that the compound be fed to the flies in a sucrose-agar medium. This vehicle is prepared with 5.0 gms of sucrose and 2.5 gms of bacteriological agar dissolved in 100 ml of boiling distilled water. An aliquot of a test compound that is heat stable may be mixed immediately with a volume of the vehicle in a conical tube, or the vehicle can be cooled first in water bath to 50°C (the temperature at which the solution begins to gel). The test compound is then added to the conical tube, thoroughly mixed with the stock solution and the bottom of the tube is placed in an ice bath for rapid cooling. Compounds that are heat labile below 50°C cannot be tested with this system.

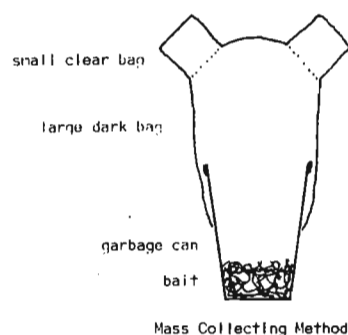
The advantages of this procedure are 1) the amount of compound ingested by a group of flies can be accurately determined by allowing the flies to consume the entire known amount of gel-compound placed in the tube, 2) an accurate determination of the LD₅₀ is made possible by using a series of tubes each with a different drug aliquot and 3) the gel-compound mixture appears to keep the particles of a suspension evenly dispersed indefinitely. Further, in studies requiring the counting of eggs the addition of a dark dye to the agar-gel provides a contrasting background that facilitates the counting.

Bryant, S. and J.S. Jones. University of California, Riverside. *Drosophila* collection methods.

We have found that the common methods of collecting *Drosophila* are neither sufficient nor efficient for collecting large numbers of flies or for collecting all flies coming to a certain trap. We have developed methods that meet

these objectives.

To collect large numbers of flies, we bait a 20-30 gallon plastic garbage can with 6" of rotten bananas and set it out as a trap at the collecting site. The depth of the garbage can appears to provide a trap that is a Markovian sink: the flies that come to the trap do not



leave and large numbers are accumulated. To collect the flies, a large dark-colored plastic garbage can liner is modified as follows: the bottom corners of the liner are cut off and one-gallon clear plastic bags are taped in place to cover the holes. The liner is then flipped over the trap. One person holds the liner upright (with the clear bags at the top) while another holds the edge of the liner against the rim of the trap and beats the trap to agitate the flies. The flies fly up to the clear bags at the top of the inverted liner. The clear bags are then shaken so that the flies fall into the corners of the clear bags. The liner is removed from the trap and the two clear bags are untaped from the liner, tied off and taken to be sorted. New clear bags are taped into place on the liner for another collection. The flies in the two clear bags are then gassed with CO₂ before transfer to the etherizer. This procedure helps equalize the sensitivity to ether of the different

species caught; otherwise, by the time flies of the species least sensitive to ether are quiet, flies of the species most sensitive are dead. Gassing with CO₂ makes all species very sensitive to ether. We have collected over 4000 flies at one time from a single trap in an area where flies were very abundant.

To collect every fly from a standard size trap (coffee can, etc.), we flip a plastic bag of appropriate size (one gallon size for a 3# coffee can trap) over the trap, hold the bag tight against the rim of the trap, and knock the trap until we see that all the flies are in the plastic bag. The bag is shaken so that the flies fall into one corner of the bag, the corner is tied off, and the bag is removed. To sort the flies, we use the same CO₂ and ether procedure as described above.

Aaron, C.S., H.E. Nardin and W.R. Lee.
Louisiana State University, Baton Rouge.
Glass filter supports for treatment of
adult *D. melanogaster* with chemical
mutagens.

filter support for tissue paper. A very important advantage of the modified procedure is the reduction in loss of flies due to physical entrapment as often happens with tissue paper substrate. An additional advantage is the use of a chemically inert support for reactive test substances.

Glass filter paper (Gelman Type A, sheets) is cut into a rectangle (55 mm x 75 mm) and rolled into a cylinder which will just fit into a standard shell vial (Kimble, 25 x 95 mm, 8 dram). (Note: It is possible by careful measurement to completely surround the sides of the vial with glass filter and a disc cut with an appropriate size cork borer can be used to cover the bottom. When properly rolled and uniformly moistened the glass filter adheres to the glass due to effects of capillarity and surface tension. This may be useful when the animals exhibit a marked rejection behavior pattern.) This size of glass filter will support 1 ml of aqueous mutagen solution and the surface will remain moist for a 24 hour feeding period. In comparative tests of feeding behavior using ^{14}C sucrose feeding solutions as described elsewhere (Aaron, Lee and Nardin, EMS effect on feeding behavior, DIS, this volume) no statistically significant difference in consumption was observed between this glass filter method and tissue paper method of exposure.

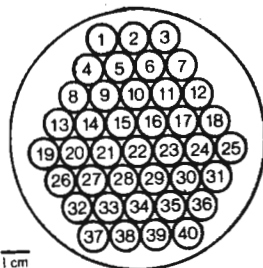
Acknowledgement: Support for this study was provided by USPHS Grant ES00320-09 and ERDA Grant AT-(40-1)-3728.

Nüsslein-Volhard, C. Biozentrum der
Universität, Basel, Switzerland. A
rapid method for screening eggs from
single *Drosophila* females.

In developing methods of molecular dosimetry for environmental mutagens we have examined several methods of treatment (vacuum injection, adult feeding, intracuticular injection). This note will describe briefly a modification of the usual (Lewis and Bacher, DIS 43:193) adult feeding procedure in which we substitute a glass

A method has been developed for rapid examination of eggs laid by large numbers of individual *Drosophila* females, which avoids both dechorionating and mounting of the eggs. Individual flies are put into small test tubes arranged in blocks of 40 tubes. The block is placed on a

transparent agar medium for egg collection. The flies are easily transferred to new plates permitting "replica plating." The chorion is rendered transparent with halofluorocarbon oil, thus allowing inspection of the developing embryos in place, while still permitting normal development.



The fly block is constructed by gluing together small plastic test tubes (1 x 7 cm). The pattern given in the figure fits to standard petri dishes (Ø 9 cm). The bottom of each tube is punctured several times with a hot needle for aeration. The block is covered with a plastic lid with matching holes which can be stoppered individually. Flies are pipetted into the tubes. After filling the block, the flies are shaken down, the lid removed, and a petri dish filled with medium pressed gently onto the block. For a transparent medium we use: 54 g agar in 1.5 l water, autoclaved for 20 min, added to a prewarmed mixture of 0.5 l apple juice, 25 g sucrose and 4 g nipagin. Plates are poured immediately. The medium is usually supplemented with small drops of a fresh yeast suspension distributed in the pattern of the block. A fly lays about 40 eggs/day depending

on genotype and conditions. If the flies are fed well and the medium is changed daily, they can be kept in the tubes for up to four weeks. For inspection of unhatched embryos a drop of Voltalef 3 S oil (Plastimer, 98 bd. Victor Hugo, Clichy, France) is placed onto the food, and the embryos are scored for abnormalities with transmitted light. As the oil does not interfere with normal development, embryos can be observed in successive developmental stages. Egg counts, however, should be done before the oil is added (best on a dark surface), since empty egg cases are barely visible after addition of the oil.

The method proves to be very useful for the genetic analysis of maternal-effect mutants of *Drosophila melanogaster*.

This work was supported by an EMBO longterm fellowship.

Dempsey, B.T. Brock University, St. Catharines, Ontario, Canada. *Drosophila* dumper for removing flies from culture bottles with 'soupy' medium.

When working with *Drosophila* bottles the media frequently becomes 'soupy' (especially at temperatures above 25°C) resulting in a loss of adherency to the bottom of the bottle. On inverting the bottle it is often difficult to shake flies out without the medium plopping

down and killing flies and burying pupae and eggs.

The *Drosophila* dumper is designed to prevent media from falling down while flies are being transferred or anesthetized. The dumper (Figure 1) is simply constructed from a plastic funnel, tubing, a small plastic dish and cotton. Drill a hole through the funnel and feed the tubing through the hole. Attach the plastic dish to end of tubing and stuff dish with

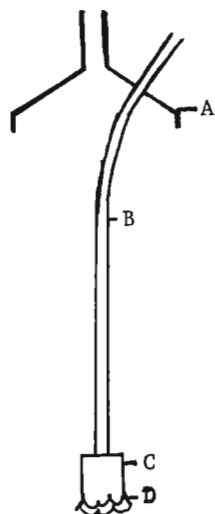


Figure 1. *Drosophila* Dumper. A - funnel; B - plastic tubing (1/4" d. for sufficient rigidity); C - inverted plastic dish; D - cotton.

cotton. When constructing the dumper it is important that the tubing fits the hole in the funnel tightly but can be pushed back and forth through the hole. The tube should be long enough to reach the bottom of the culture bottle.

To use the dumper, place the funnel over the mouth of the bottle and push the tubing down until the cotton rests on the media, all the while holding your thumb over the hole in the funnel. Invert the bottle and vigorously shake the flies into the container.

The food is supported by the device and flies aren't lost by its falling. Shaking can be vigorous enough to remove all flies without the mess of falling media.

Luning, K.G. University of Stockholm, Stockholm, Sweden. Yeast-agar medium.

Cuts in funds forced us 5 years ago to reconsider the use of cornmeal-agar medium as this medium could not be successfully stored. As I had previously used agar cubes and tissue paper soaked

in yeast suspension (Spencer, 1943) I knew the good effects of yeast but also that after 8 to 12 days there was a terrible smell from decaying yeast. By enveloping the yeast cells in agar we got only the positive effects and no smell. We have now 5 years experience of a yeast-agar medium which can be stored in vials, flasks or bottles in a refrigerator at least 10 days. Before use, we adapt it to room temperature for just one hour.

10 liters of medium is prepared in the following way:

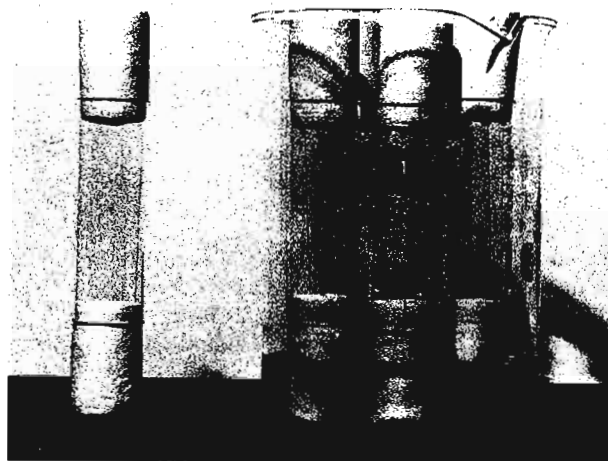
1. 6 liters water
110 g agar
200 g (10% Methylparaoxibenzoas in 70% ethanol)
Boil and stir 20 minutes.
Add 1/2 liter syrup. Boil.
2. 250 g cornstarch is dissolved in 1 liter water and added to the agar solution while stirring.
Boil 3-4 minutes.
3. While the agar is heated, suspend 350 g yeast in 3 liters water (20°C) in a 14 liter pan.
4. Pour the boiling agar solution into the yeast suspension and stir. (This gives max temp. for yeast cells = 70°C).
5. Distribute into vials, bottles or flasks.
6. When cold, down to near room temp., plug and put them in refrigerator.

Before use, we add a piece of tissue paper, which first is used to dry the upper parts of the vials or bottles, and then pressed into the medium. 5 days after the start of egg laying, we add to the flasks and bottles another piece of tissue paper pressed into the medium opposite to the first paper.

For some experiments we prefer a looser medium. Then we add to the bottles or flasks dry coarse oatmeal before pouring the medium on top.

Traut, H. Universität Münster, Germany.
Method of feeding chemical mutagens to
adult Drosophila.

in a glass beaker (size: e.g. 1000 ml). The anaesthetized flies are put into vials (e.g. 20 flies per vial) from which the bottom was removed, or into glass tubes of adequate size.



In order to feed chemical mutagens to *D. melanogaster* we use the following system (Fig. 1) which, however, does not claim originality in all its features. The solution containing the chemical to be tested plus 5% glucose is filled

These vials (tubes) are closed at both ends by cellulose plugs as they are used in bacteriology. Several vials are placed in the glass beaker containing the solution after the flies have awakened from etherization. After a short while the lower plug will be thoroughly moist because it gets soaked with the mutagen-glucose solution. If the upper end of the vials were sealed hermetically instead of being closed by a cellulose plug, undesirable moisture would accumulate in the vials. Also, the transfer of the flies from the vials to the etherizer is facilitated when the vial can be opened at its upper end. The flies can be kept in the vials for several days.

This "maintenance-free" system for feeding mutagens to *Drosophila* is so simple that it has been recommended for school purposes (1). It has also been used to demonstrate that the feeding of a certain drug to females of *D. melanogaster* at extremely low concentrations

produces as many aneuploid offspring as does the X-irradiation with doses ranging from some hundred to some thousand R.

References: (1) Traut, H. 1976, Nat. Math. Unterricht 29:424-430; (2) Traut, H. and U. Sommer 1976, Münchener Med. Wochenschr. 118:1113-1116.

Annest, J.L. University of Hawaii,
 Honolulu. Easily constructed and
 inexpensive population cages.

It seems dubious that a *Drosophila* geneticist would feel the need to go to a "Tupperware" party. But after running population studies in twenty-five discrete- and continuous-generation population cages, I am convinced that Tupperware

(Tupperware Home Parties, Orlando, Florida 32802) is ideal.

Continuous generation cages were constructed from the "Fresh-N-Fancy" cake server (dimensions, 35 cm (l.) x 24.5 cm (w.) x 7.5 cm (ht.)) which has a firm plastic top cover with hard plastic base-plate. Seventeen holes (37 mm in diameter) were cut in each cage with a Circle Cutter (Sears, Roebuck & Co., Chicago, Illinois 60607); that is, fifteen holes in the base-plate to accommodate food vials and one hole at each end of the top cover for access inside the cage. One of these two holes was plugged with a disposable tissue-culture dish cover (35 mm in diam.) to act as a window to view the flies. Large vials (35 mm (diam.) x 100 mm (l.)) were held in the base-plate by the largest-sized diSPo plugs (Scientific Products, 1972 order T1387) which were bored out with a 2 cm cork borer. The major advantages of using the Fresh-N-Fancy are (1) the seal between the top cover and the base-plate is air tight; (2) it is light and comes with a handle which extends securely from one end to the other for carrying purposes; (3) it is easy to clean with soap and warm water.

Discrete generation cages were constructed from the "Bread Server" (dimensions, 25.5 cm (l.) x 13 cm (w.) x 13 cm (ht.)) which has a firm plastic top cover and base-plate. Five holes (37 mm in diam.) were cut in the base-plate for food vials and one hole was made in each end of the top cover for cage access. For these cages medium-sized food vials (28 mm (diam.) x 93 mm (l.)) were used. The seal between the top cover and base-plate was reinforced with strapping tape. The Bread Server is compact and easy to handle.

Johannisson, R. University of Düsseldorf, Germany. A method for making permanent slides of squashed spermatocytes in *Drosophila*.

A simple and fast technique for the preparation of such delicate structures as the Y chromosome lampbrush loops in primary spermatocytes of *D. hydei* is described. The method also allows staining procedures or autoradiographs. Equally good results are obtained with other tissues.

The procedure is as follows:

1. Dissect testes out of larvae, pupae or adults in *Drosophila* Ringer, pH= 7.2, as usual. Larval or pupal testes are prepared as whole organs; from adult testes only the tips, including the meiotic stages, are used.
2. Microscope slides are prepared by dispensing a drop of 0.1% poly-L-lysine over the entire surface. After drying in air, tips of testes are transferred by fine forceps and entire larval or pupal testes are transferred with the aid of a thin pipette onto the slide, on which a drop of Ringer's solution has been placed.
3. Place a little round coverslip (diam. 12 mm or smaller) gently over the drop.
4. Remove surplus Ringer's solution with a sheet of filter paper slightly pressed over the slide. During this procedure the testes rupture and the cells flow out. Strong pressure should be avoided in order to prevent physical damage to the nuclei.
5. Check with microscope to determine whether the spreading appears to be satisfactory. Virtually no cytoplasm should cover the spermatocyte nuclei.
6. Carefully surround the coverslip with some very small drops of fixative (1% glutaraldehyde in 0.05 M sodium cacodylate, pH= 7.2). As soon as the coverslip is lifted, wash it away from the squash preparation by adding more fixative, and remove it gently with forceps. Fix for 30 min.
7. Dip the microscope slide for 1 minute or longer in buffer (0.05 M sodium cacodylate, pH= 7.2).
8. a) Cover the tissue with 2% osmium tetroxide (buffer see above) for 30 minutes. Wash the slide for 5 minutes in buffer solution.
Preparations can be used for autoradiographs. Place stripping film Kodak AR 10 over the squashed and fixed tissue. After air drying, the prepareate may be exposed for weeks without tissue damage.
- or b) Stain for 3 to 5 minutes with a mixture of 0.01% azure B and 0.01% methylene blue (in 0.01% sodium tetraborate) in the proportion 1:1. This step is followed by a short rinsing in buffer.
9. After dehydration in 30% and 70% ethanol for some minutes, and in absolute ethanol for 15 minutes, mount the preparation in euparal (From Chroma-Gesellschaft) or some other adequate medium.
10. For optimal contrast in the phase contrast microscope, firmly attach the coverslip to the slide by placing the entire preparation under a weight for one day.

Miller, D.P.¹ and T.M. Wolf², Washburn University, Topeka, Kansas. (¹Chemistry Dept.; ²Biology Dept.) *Drosophila* head isolation in very large numbers.

While examining a biochemical problem in *Drosophila melanogaster*, we found it necessary to isolate the head from the body. Requiring fresh heads in excess of 100,000, this became an insurmountable task when the heads were decapitated by hand. As a result we have developed a simple,

fast method for decapitating very large numbers of fruit flies and subsequent separation of the heads from the body parts.

The flies are etherized in the usual manner and then placed in an Erlenmeyer flask with an equal volume of glass beads. The flask is then put inside a dry box (dew point—100° C) and liquid nitrogen is added to the flask, slowly and with mild swirling at first, until the flies and beads are covered. The flask is then swirled vigorously for 30 seconds and then the contents are dumped into a set of sieves. The sieves are shaken briefly and allowed to warm up inside the dry box. Subsequently the beads are collected from the top screen while the bodies and heads are separated in lower screens.

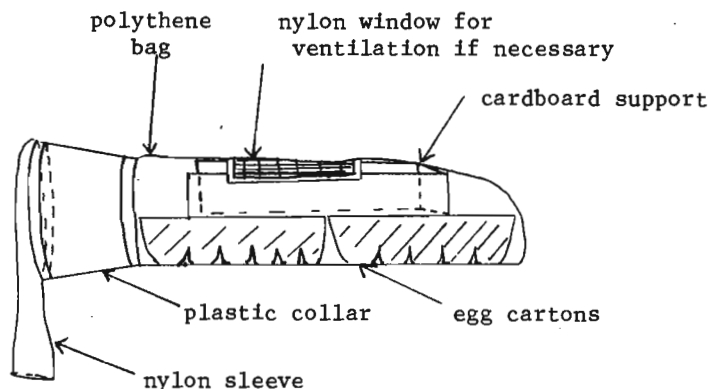
The result is that decapitation is well over 90%. The collected heads have a minor amount of debris consisting of wings and bristle parts. This debris is easily removed with a puff of air or small brush to give pure heads. For all practical purposes, the heads remain in perfect condition using this technique.

Grabowska, F., J.A. Durgo and E.P.M. Candido. University of British Columbia, Vancouver, Canada. Large scale culture of *Drosophila*.

The system consists of a laying cage where adult *Drosophila* lay eggs on trays of food and a series of hatching cages where the eggs develop into adult flies.

Laying Cage

The laying cage has a sturdy frame with a wooden base and wire supports as it must survive food trays being moved in and out daily. The frame is placed in a large, clear polythene bag (30" x 38") which has its opening taped around



a collar cut from a plastic bucket to which a nylon sleeve is attached. About 4 L of cornmeal medium in poured into the dimpled sections of 10 egg cartons, sprinkled liberally with yeast pellets when cool and the cartons placed in the laying cage. To obtain a synchronous yield many flies are needed for the parent generation, at least 20 g are used. On the first laying, 2 days are needed for a good egg lay. If parent flies are added every few days, it is possible to obtain a sufficient egg lay in 24 hours. The same laying cage should be used continuously for up to 4 weeks and then dismantled and cleaned.

Hatching Cage

The hatching cage consists of a polythene bag and a plastic collar with a sleeve, but, instead of a frame, a cardboard support is used which can be inserted or removed through the sleeve. When sufficient egg lay is observed, the egg cartons are removed from the laying cage to the hatching cage.

Collection

When the collar is tapped, the adult flies tend to swarm together at the base of the collar or on the base of the bag and may be sucked up by a vacuum hose leading to a vacuum trap. To remove the flies from the vacuum trap, they are gassed with CO₂, then quickly transferred to a suitable container and frozen. Often, a large number of flies still remain in the bag, and may be collected by removing the egg cartons and cardboard support through the collar, fastening the sleeve and shaking the flies into one corner. The corner and contents may then be tied, cut off, and frozen directly. Yields per hatching cage are typically 25-50 g per generation.

This method enables the daily production of 100 g or more of flies with very little cleaning involved as the egg cartons, cardboard support and polythene bag are disposed of after one use.

Leigh, B. University of Leiden, The Netherlands. 'A useful golfball for fly writing.

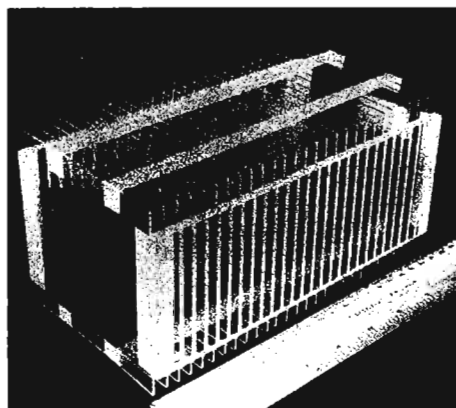
α 2 3 4 5 6 7 8 9 = å ø
 β γ % ? μ ϑ δ () / Å Ø
 q w e r t y u i o p <
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 a s d f g h j k l - æ
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 z x c v b n m , . +
 Z X C V B N M ' : *

The IBM "golfball" typewriters are among the most popular of the new generation of electric machines. They are admirably suited for typing abstracts and manuscripts which are going to be reproduced by photo-techniques. One drawback has been the difficulty of finding a ball with the symbols most often used by biologists. Recently we contacted IBM in Amsterdam and were able to obtain a ball (No. 2428617 Courier 10) with the following letters and symbols:

Hill, R.J. and P. Davis. CSIRO, Sydney, Australia. The bulk transfer of *Drosophila* on a pupation matrix: an aid to the culture of large *Drosophila* populations.

During the course of biochemical studies on embryos of *Drosophila melanogaster* it was necessary to collect eggs from population cages containing some 40,000 adults. Stocking these cages from half-pint bottle cultures is a tedious process. For this reason we raise the animals for a cage in a large plastic box containing agar medium and yeast. They are then transferred to the population cage as pupae by means of a "pupation matrix." This device consists essentially of thin plastic sheets held in a slotted frame and arranged to provide a surface area sufficiently large to render the free surface of the walls of the food container effectively insignificant. The details are as follows.

2.5 g of overnight-collected *Drosophila* eggs are suspended in 10 ml of water and spread on the surface of a 1 inch thick layer of food medium (1500 ml water, 250 g molasses, 2.5 g methyl p-hydroxybenzoate, 18.3 g agar; formula derived from information kindly provided by L.H. Cohen) in a plastic box (11" x 11" x 5 1/2"). After allowing excess water to evaporate, twenty small dabs of yeast paste are applied to the surface. A tightly fitting lid with a 5" x 5" ventilation hole closed by fine nylon mesh is placed on the box which is allowed to stand in a 25°C *Drosophila* room running at 65% humidity. After 24 hours, strips of yeast paste are applied over the food with an icing bag so as to cover approximately two-thirds of the surface. After another 24 hours, 100 gm of compressed yeast is crumbled and applied evenly to the surface. Two pupation matrices are then placed, side by side, on top of the yeast and pressed slightly into it to ensure uniform contact. The boxes are sealed and allowed to stand a further 4-5 days.



A pupation matrix is shown in the figure. Twenty-six plastic sheets (4" x 4" x 1/16") are held 1/4" apart in a slotted perspex frame. The arrangement is stabilised by strips of masking tape and is readily disassembled for cleaning in warm detergent solution. We have utilized sheets of perspex and of polystyrene; both surfaces are effectively of the same efficiency in collecting pupae.

When pupation is complete (ca. day 7 after egg collection) the matrices are removed and transferred to a population cage. Two days later food is provided in the form of yeast paste strips on agar egg-collection plates. These are replaced after a further two days and daily thereafter. Twelve days after the eggs are collected the matrices may be removed from the cage through an air draft produced by a fan directed at the sleeve. Effectively all of the flies remain in the cage.

This procedure has now been in routine use in our laboratory for two years. The procedure has also proven useful for the large scale release of *Drosophila* into wild populations (Dr. I. Franklin, personal communication).

Mittler, S. Northern Illinois University, DeKalb, Illinois. Feeding water insoluble compounds that are alcohol soluble to adult flies.

In a recent set of experiments, young adult males were fed various organic compounds in a screening test for loss of chromosomes and non-disjunction program. The method used was a modification of Lewis and Bacher (DIS 43:193, 1968) in which the dissolved compound was distributed

in aqueous form on to a thin sheet of crumpled "kleenex"-like type paper. To prevent flies from being trapped in drops of fluid that accumulated at the bottom of the bottle, another tissue paper was folded into a pad to fill the bottom. Several of the substances tested were insoluble in water; these were dissolved in 95% ethyl alcohol and diluted to 10% and fed to the adult flies. *Drosophila melanogaster* adult males can survive (probably very happily) for two days on 10% ethyl alcohol, however three days can be lethal! There were no genetic effects from feeding males for 24 hours on 10% ethyl alcohol.

Stanfield, S.W. and D.R. Helinski. University of California, San Diego. An improved method for collecting highly synchronous *D. melanogaster* eggs.

a standard cornmeal-agar-molasses medium sprinkled with live dry yeast. These flies are kept for 2 days on a 12 hr light/dark cycle at 26°C. On the second day, about 3 hrs before the onset of the dark cycle, the males are introduced to the females without the use of anesthesia and the flies are left undisturbed for 2 hr 15 min while mating takes place. The flies are then shaken into community laying cages (24 bottles of flies per cage) and the cages immediately placed over squares of dark green blotting paper soaked in commercial white vinegar diluted 1 to 10 with deionized water. The cage and blotting paper, in turn, are placed over 2 day old yeasted, fermented medium covered with Kimwipes.

The community laying cage is constructed from a hollow, clear plastic cylinder 4½" high by 5" wide (I.D.). The bottom of the cylinder is covered with a single layer of nylon net to which is glued a layer of clear plastic rings 5 mm high with 7/8" inner diameter. The rings serve to maintain an even distribution of flies across the bottom of the cage. The flies are introduced into the cage through a hole in the side. Once in the cage, the hole is sealed with a cork and the flies shaken to the bottom where they are trapped in the rings by a large plunger. The plunger is constructed from an inverted round plastic food container. A snug fit is insured by a narrow band of foam rubber glued around the edge of its lid. The plunger is raised and lowered by means of a knob attached to the top.

The flies lay their eggs on the blotting paper which is removed and replaced by fresh vinegar soaked paper every 20 min. The peak rate of egg laying occurs about 20 min after the onset of darkness and usually lasts up to about 1 hr, after which it decreases noticeably. During the peak rate, each female lays an average of 1 egg every 10 min. To test eggs collected by this method for

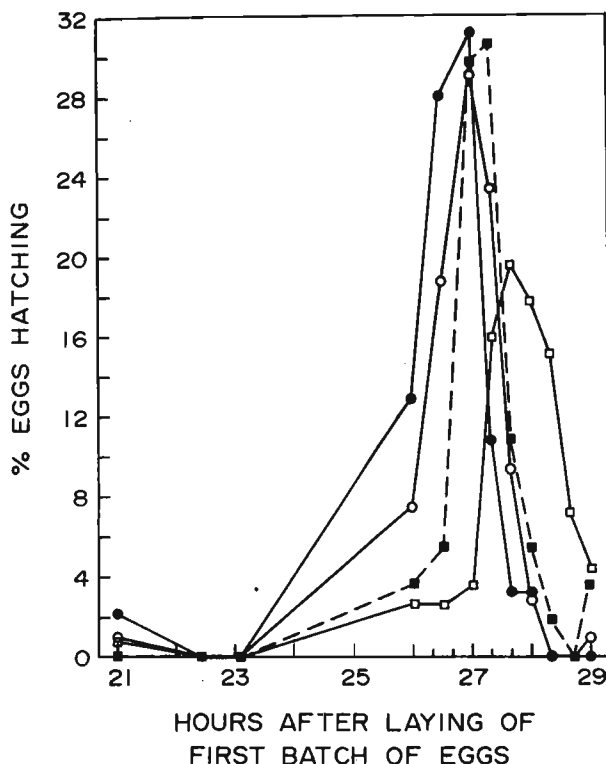


Figure 1. Demonstration of synchrony of *D. melanogaster* eggs collected at successive 20 min intervals. (●—●) 93 eggs collected in the first 20 min laying period; (○—○) 107 eggs collected in the second 20 min laying period; (■—■) 111 eggs collected in the third 20 min laying period; (□—□) 113 eggs collected in the fourth 20 min laying period.

synchrony, several batches of eggs collected at successive 20 min intervals were stored at 26°C, 100% relative humidity, and examined at intervals for hatching. Figure 1 shows the results of this experiment. The peaks of hatching for the 4 batches of eggs were separated by approximately 20 min intervals, with the order of hatching corresponding to the order of laying. The peak of hatching

occurred about 26 hr 50 min after laying, with about 97% of the viable eggs hatching within a 3 hr period. This compares favorably with the 94% hatching over a 3 hr period found by Edney (1969) for eggs known to be collected no later than 30 min after fertilization. When the eggs were examined after 48 hrs, between 90 and 94% of each batch of eggs had hatched, with an average hatching for all 4 groups of 92%. The unhatched eggs were examined under a microscope after crushing between a slide and cover slip. Twenty-eight of the 29 eggs examined

had reached cellular stages. Only one was precellular, indicating that greater than 99% of all eggs collected had been fertilized. The data confirmed that eggs collected by the method described were highly synchronous within 20 min and maintained their synchrony until hatching.

References: Edney, E.B. 1969, *Physiol. Zool.* 42:257; Hildreth, P.E. and C. Brunt 1962, *DIS* 36:128.

Fuyama, Y. Tokyo Metropolitan University, Japan. Triethylamine: an anesthetic for *Drosophila* with prolonged effect.

Ethyl ether and carbon dioxide are most commonly used for anesthetizing *Drosophila*. Anesthesia produced by these chemicals, however, rarely lasts longer than 10 minutes; an introductory student often feels it too short and even

skilled drosophilists do so when they treat heavily marked flies or complicated morphology. Recently it has been reported that the anesthesia produced by Methofane lasts as long as one hour (Binnard, 1974). I report another potential anesthetic with prolonged effect for *Drosophila*.

In the course of searching for chemicals attractive to *Drosophila*, triethylamine was found to be a potent narcotic agent to them. Although triethylamine is a very common reagent, its narcotic effect does not seem to have been noticed. Surprisingly, the narcotism by triethylamine usually continues longer than 30 minutes and even frequently exceeds 1 hour. It was effective for more than 10 species so far tested, so perhaps for all the species of genus *Drosophila*.

For use with a conventional *Drosophila* anesthetizer, a drop (less than 0.1 ml) of triethylamine is enough for a single dose, and since less volatile (bp ca. 90°C) than ether, retains its potency for 1 hour or more; therefore the cost is negligible. The time necessary to immobilize flies is slightly longer than that by ether, but shaking out the flies as quickly as possible is recommended to avoid over-anesthetization. Mortality and fecundity of recovered flies seem to be much the same as with etherization, as tested so far with *D. melanogaster*. Since triethylamine is to a certain extent miscible with water, the solution properly diluted to reduce effects may be used for delicate species. The attitude of flies during anesthesia is fairly good except for slight curling of abdomen and therefore it may be convenient for photographing and class room demonstration.

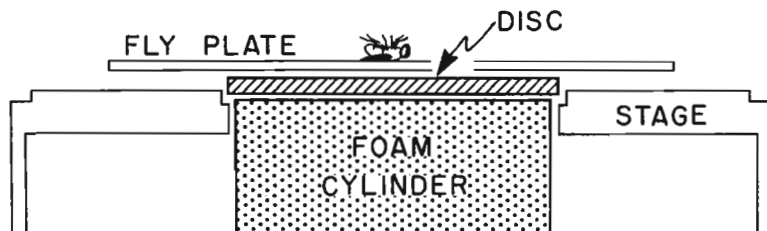
For ordinary use, triethylamine seems to have little deleterious effect for men other than unpleasant ammoniacal odor, though any other possible hazards to *Drosophila* as well as men have to be further assessed. Any information concerning this would be acknowledged.

Reference: Binnard, R. 1974, *DIS* 51:60.

Trout, W.E., III and R. Williamson. City of Hope Medical Center, Duarte, California. A fine-focusing adapter for dissecting microscopes.

While examining flies such as mosaics at higher powers, when the depth of field is small, it is cumbersome and tedious to continually adjust the focusing knob. Therefore for each dissecting microscope we cut out a cylinder of very soft foam rubber about 7 cm in diameter and 3 cm

high, depending on the type of microscope stage, to insert between the removable stage disc and the desk top. The height should be sufficient to lift the disc about 2 mm yet have it still held in position by its socket in the stage. We handle



Cross-section of the fine-focusing adapter as used on a Wild microscope stage.

our flies on a rectangular formica plate, placed on top of the disc in the usual way. The plate can be pushed down to focus on any part of the fly, and can be moved in the horizontal plane, with one hand.

This work was made possible in part by support from the Donald D. Gustafson Research Fund.

Aaron, C.S., W.R. Lee, P.M. Seamster and F. Janca. Louisiana State University, Baton Rouge. A non-aqueous gas exposure technique for microliter quantities of chemical mutagens.

ties of the mutagen is advantageous with specifically radiolabeled mutagens because of the expense of these compounds.

Vacuum injection of flies was previously described using aqueous solutions (Sega and Lee, DIS 45:179) but for reactive mutagens an aqueous solution may be undesirable. Furthermore, the maximum exposure concentration of a compound in the aqueous based vacuum injection procedure is limited to the compound's solubility in water times the maximum concentration of water that can be achieved in the treatment flask (limited by the dewpoint). It is more desirable to use true vapor of the mutagen if it is sufficiently volatile; therefore, we have developed a non-aqueous method of vacuum injection using glass fiber filter discs impregnated with the mutagen. For instance, when using ethyl methanesulfonate (EMS), we dissolve the mutagen in ethyl ether, place the filter disc in the ether at the bottom of a shell vial and carefully evaporate the ether in a stream of dry nitrogen thereby coating the glass filter with mutagen. We then place this filter in the vacuum release line of a special treatment apparatus (described here), thus entraining the mutagen in the air which fills the apparatus. (NOTE: Due to the extreme toxicity of ether to flies, particularly under vacuum injection conditions, it is imperative that no ether remain on the filter.)

The apparatus which we have developed is diagrammed in Figure 1. The equipment used consists of a treatment vessel, high vacuum seal valves, a sintered glass funnel, and O-ring seal joints as well as necessary traps and connections to vacuum pump, air line, etc. The

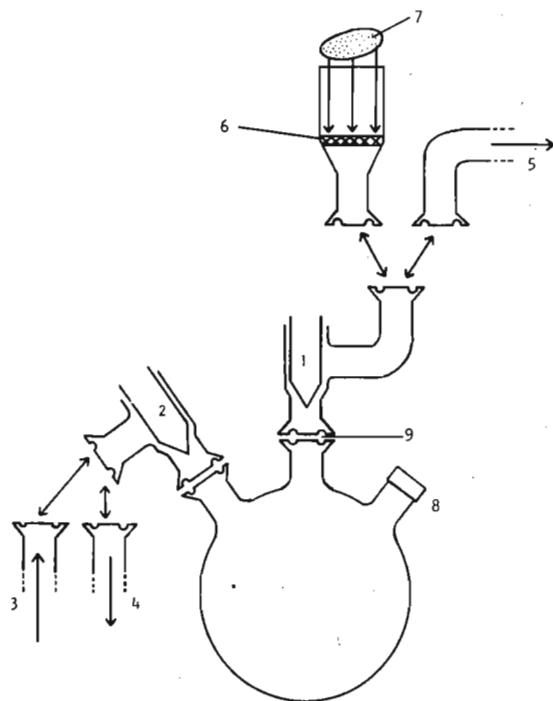


Figure 1. Vacuum injection apparatus. 1) Valve #1; 2) Valve #2; 3) Air or N₂ inlet; 4) Vacuum line connection; 5) Air or N₂ outlet (to appropriate traps); 6) Sintered glass support funnel; 7) Glass fiber filter disc, impregnated with mutagen; 8) Septum for obtaining gas samples for gas chromatographic analysis. All joints (e.g., 9) are "O" ring real; dimensions are selected to present minimum area of contact between vapor and potentially absorbent materials. Either 3 or 4 may be connected to 2, and likewise either 5 or 6 may be connected to 1. Operation is described in the text.

treatment vessel is a modified 250 ml, 3 necked round bottomed flask. One neck was removed and replaced with a septum holder for collecting samples for gas chromatography. The other 2 necks have O-ring seal joints. The high vacuum seal valves (Kontes glass K-826515-0008 Kel-f

seals) are fitted with O-ring seal joints (Kontes K-671750-0007). The sintered glass funnel (Pyrex No. 416320, 15 mm Medium grain) is fitted with an O-ring seal joint.

At the start of an experiment the flies are anesthetized with N₂ and shaken into the treatment vessel. The mutagen is impregnated into a small round piece (the standard size 14 corkborer is a satisfactory diameter) of glass fiber filter (Gelman Type A, # 61701) and placed on the sintered glass funnel which is mounted on the port of the #1 valve. Then, with #1 valve closed, a vacuum is pulled on the vessel (~10 mm Hg) using Valve #2. Valve #2 is closed and by opening Valve #1 the vacuum is released through the funnel, thus evaporating

and entraining the chemical in the air stream. Valve #1 is again closed and the exposure is continued for a period of time (2-8 hrs). At the end of the treatment time, compressed air is admitted through Valve #2, out Valve #1 and subsequently through traps to flush out the system and destroy any remaining mutagen. Nitrogen flow in the same path (into Valve #2, through the flask, out Valve #1) is used to anesthetize the flies for removal from the vessel.

In view of the extremely hazardous nature of radiolabeled mutagens, great care must be taken to ensure the safety of laboratory personnel. The entire apparatus previously described should be contained within a glove box or fume hood. Alkaline mercaptoacetate solution (Lewis and Bacher, DIS 43:193) is used as a chemical trap for EMS in our laboratory. In addition, molecular sieve and cold traps are necessary to avoid radiochemical contamination of other equipment such as the vacuum pump.

The method described here has been used to induce a 6% sex-linked recessive lethal frequency by treating 50 ♂ with 2.0 mg of EMS for 8 hours. In a separate experiment 0.2 mg of radiolabeled EMS was administered to 1,000 males with significant alkylation of DNA observed (Janca, F.C., W.R. Lee and C.S. Aaron, unpublished).

Due to the variability in impregnation of the filter disc with the mutagen, reproducibility of this method of administering mutagens is not sufficient for constructing dosage response curves; however, this is the most economical method we have for treating a large number of males with small quantities of radiolabeled mutagens.

Acknowledgement: Support for this study was provided by USPHS Grant ES00320-09 and ERDA Grant AT-(40-1)-3728.

Colgan, P. and R.F. Rockwell.* Queen's University, Kingston, Ontario, Canada. A Monte Carlo technique for the distribution of the ratio of estimates of two independent added variance components.

Population geneticists often wish to compare the relative sizes of the added component of variance of two populations (cf. Rockwell et al., 1975). However, although estimates (see Sokal and Rohlf, 1969, Chap. 10) and confidence intervals (see Boardman 1974) of these variances can be calculated, the distribution of the ratio of

these estimates is not known, and cannot be assumed, for instance, to be that of an F random variable with appropriate degrees of freedom under the null hypothesis of equal variances. A Fortran computer program was therefore prepared to examine the distribution of this ratio, for specified values of the added and error variances and number of treatments (lines within a population) and replicates, by running a stated number (between 1000 and 10,000) of Monte Carlo trials. In each trial, observations for the two samples were generated via a normal random variable function, the two analyses of variance were carried out, the two added components of variance estimated, and the ratio was formed. The program enables a statement about the probability of the ratio exceeding a stated value under the specified conditions. 1000 trials were found to be sufficient for good definition of the distribution, while 10,000 trials were run if the observed value of the ratio was close to the critical value given the α -level in use.

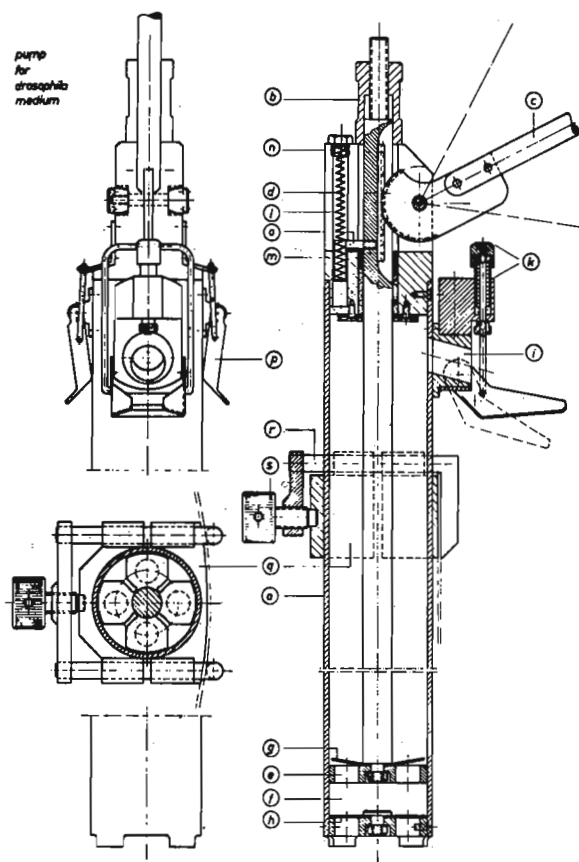
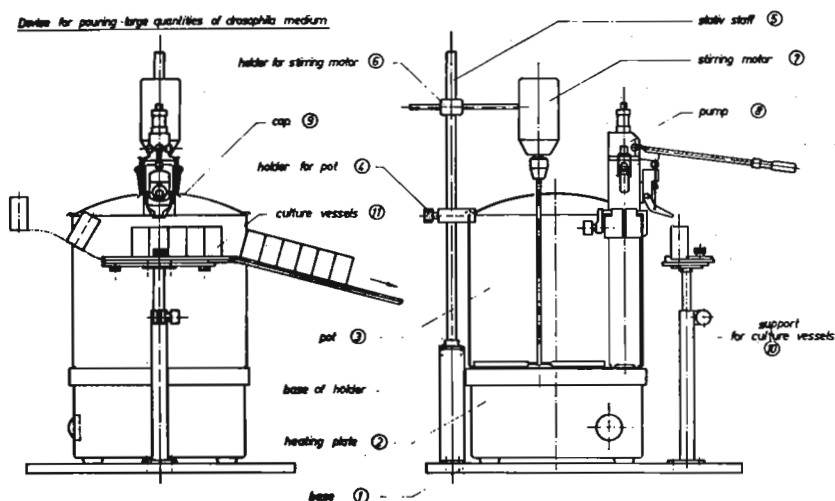
Examples of the application of this system can be found in Rockwell et al. (1975). Examination of the interactions of the various input parameters and of the practical limitations of the model is in progress and will be reported elsewhere. Copies of the program are available from the authors.

References: Boardman, T.J. 1974, *Biometrics* 30:251-262; Rockwell, R.F. et al. 1975, *Behavior Genetics* 5:189-202; Sokal, R.R. and F.J. Rohlf 1969, *Biometry*, Freeman, San Francisco.

*Present address: Department of Biology, City College of the City University of New York, N.Y. 10031.

Schmider, K.H. and E. Gateff. Technischer Bereich der Biologischen Institute II, III and Biologisches Institut I (Zoologie) der Universität Freiburg, Germany. Device for filling large numbers of culture bottles with *Drosophila* medium.

Figure I represents a general view of the device and the various elements of which it consists. These are: 1. a wooden base, 2. a 4000 kW heating plate, 3. a stainless steel pot holding 40 litres of medium, 4. a holder for the pot, 5. a main holder, 6. a holder for the stirring



pump to the side wall of the pot. The left hand delivers the empty culture bottles to the stage (Figure I,10), the right hand operates the handle of the pump (Figure I,8). The pouring device can be fully automated by the installment of a motor operating the handle (c) and a conveyor belt for the continuous delivery of the culture vials. For cleaning purposes the pump can be dismantled easily.

motor, 7. a stirring motor with stirrer, 8. a pump, 9. a lid for the pot, 10. a stage for the culture bottles, 11. culture bottles, 12. base of holder and 13. a sliding plane.

The central element of the device is the pump (Figure I,8). After the medium has been cooked, the pump is placed into the medium and is secured with the screw (Figure II,s) to the side of the pot (3). All parts of the pump are made of stainless steel. The desired amount of medium to be delivered can be

adjusted gradually with the screw (b). It ranges from 10 ml - 100 ml. The handle (c) moves, with the help of the cog wheel (d), the piston (e). During the down movement of the piston (e) (upward movement of the handle (c)) medium enters from the pressure chamber (f) through the teflon valve (g) into the pump's main chamber. At the same time the valves (h) are closed. While the piston (e) moves upwards (downwards movement of the handle (c)), the valves (g) close. Simultaneously the valves (h) open. This allows new medium to enter the pressure chamber (f). The medium rises in the main chamber of the pump until it reaches the runoff spout (i). The down movement of the handle (c) is restricted by the stop (k) which can be adjusted to various positions with the help of the screw (b). The handle (c) applies pressure to the stop (k) causing a system of wheels and springs to lower the spout for pouring. During the replacement of a filled vial with an empty one, the spout moves upward by this preventing excessive dripping of medium. The spring (l) located in the drilling (n) and the case (m) is connected by the peg (o) with the piston rod. This results in the automatic filling of the main chamber with medium. The upper portion of the pump is secured to the cylinder (a) with a clamp (p).

The entire pump in turn is secured to the pot (Figure I,3) by the clamp (Figure II,g,r,s). Rotation of the bolt (s) causes simultaneously the tightening (1) of the two cylindrical halves (q) of the pump and (2) of the entire

Ginevan, M.E.* University of Kansas, Lawrence. A gas exposure system for *Drosophila* and other small insects.

in the case of work on CO₂ and anoxia, primarily designed for dealing with pure gases rather than mixtures (for a review see: Parsons, 1973). In the course of some investigations concerning the genetics of sulfur dioxide tolerance in *Drosophila melanogaster*, I have developed an exposure system which I feel offers some unique advantages in studies involving short term (several minutes to several hours) exposures to controlled gas mixtures.

The exposure chamber is illustrated in Figure 1. It consists of a 350 ml Buchener funnel with a coarse fritted glass disc, two removable nichrome wire mesh shelves, and a 90 mm glass petrie dish cover. The other components of the system are exposure cages made of 20 mm lengths of 20 mm diameter glass tubing with dacron mesh tops and bottoms (Figure 1), a Neptune Dynapump (model 4K), a Dwyer flow meter adjustable from 500 to 2500 ml/min, a humidification chamber consisting of a 250 ml Erlenmeyer flask containing 150 ml of distilled water, a Sage syringe pump (model 341), a 50 cc B-D Multifit syringe with a No. 600 x 3/8 hypodermic needle, and a mixing chamber consisting of a 25 ml gas impinger tube filled with glass boiling beads. The operation of the system is shown schematically in Figure 2).

This system utilizes components which are readily available from scientific supply houses and which require a minimum of modification. The only two pieces which must be built are the exposure chamber and cages (Figure 1).

The chamber requires three steps in its construction. The neck of the funnel is narrowed and bent to accept a gas delivery tube, supports for the shelves are provided by gluing three pieces of glass per shelf to the inside wall of the chamber in a triangular arrangement (I used a silicone rubber adhesive), and the wire shelves are cut out with tinsnips. The petrie dish cover which serves as a lid for the chamber requires no modification since it fits loosely enough to allow unimpeded gas flow.

As for the exposure cages, the glass tubing is first cut into pieces of the desired length. A dacron mesh bottom is then glued on one end of each length of tube, using silicone rubber adhesive. Tops consist of 2.5 cm squares of dacron mesh held on with loops of coat thread. The loops can be tied in advance, using a length of glass tube as a template, and pushed on to hold the mesh square (Figure 1). The exposure chamber will accommodate up to 18 of these cages.

Assembly and operation of the system is straight forward. The components are connected as shown in Figure 2. For sulfur dioxide or other corrosive gases use of teflon tubing is advisable, but for other applications tygon or a similar vinyl tubing should be adequate. Gas concentration is controlled by varying the relative flow rates of air and the experimental gas. For the present study, flow rates of 1400 ml/min air and .7 ml/min SO₂ are used. This results in an SO₂ concentration of about 500 ppm.

The constancy of the air flow rate provided by the pump/flowmeter combination (at 1400 ml/min observable fluctuation was less than 25 ml/min) coupled with the extreme precision of the syringe pump (rated reproducibility $\pm 1\%$ of the flow rate set) insures repeatable gas mixtures. Further, since the syringe pump is variable from .33 ml/hr to 13 ml/min with a 50 cc syringe, a wide range of concentrations are available.

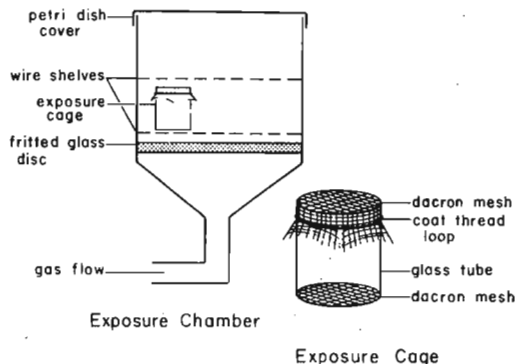


Figure 1. The exposure chamber and cage.

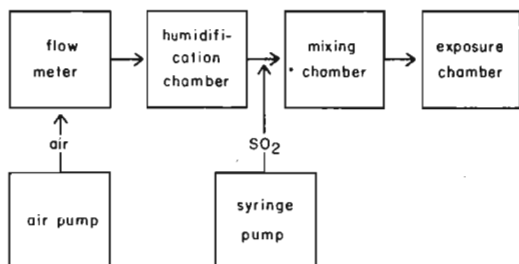


Figure 2. A schematic representation of the exposure system.

Uniformity of exposures is further enhanced by the one pass, high flow rate design of the system, and by the fritted glass bottom of the exposure chamber, which provides an extremely evenly dispersed gas flow. Taken together, these features serve to minimize variations due to area effects within the chamber or absorption of the experimental gas by the materials of the system.

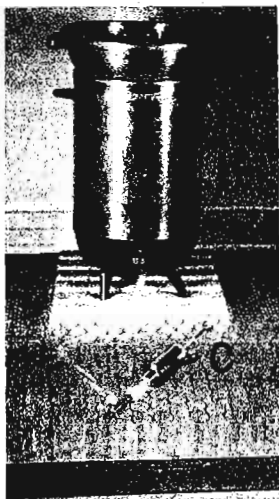
It is my hope that other workers will find this apparatus useful in a variety of studies involving insect responses to gas mixtures. I would appreciate questions or comments from any interested parties.

This work was made possible by a University of Kansas Biomedical Sciences Support Grant #4178-5706-0 awarded to P.W. Hedrick and The Herbert B. Hungerford Memorial Fellowship and a University of Kansas Summer Honors Fellowship awarded to the author. I would like to express my appreciation to B.G. Barr and P.W. Hedrick for the facilities utilized in this study.

References: Gerdes, R.A. 1971, *Atmospheric Environ.* 5:117-122; Mohamed, A.H. 1971, *Second Internat. Clean Air Cong. Proc.* 158-161 (Academic Press, N.Y.); Parsons, P.A. 1973, *Ann. Rev. of Gen.* 3:239-265.

* Department of Entomology, Division of Biological Sciences, University of Kansas.

Smith, P.D. and S.D. Campbell. Emory University, Atlanta, Georgia. An economical and efficient method for preparing culture vials.



During the course of experiments requiring large numbers of culture vials, we have developed a method for the dispensation of medium which is both efficient enough to support the vial requirements of large laboratories but economical enough to be justified for use in any laboratory situation. Our set up depicted in Figure 1 includes (A) a kitchen colander used to strain prepared medium into (B) a 30-cup electric coffee percolater attached to (C), a 5 ml Cornwall pipetter. This method has several advantages: (1) it is easy and economical to assemble, requiring about \$20 and 15-20 minutes; (2) it provides culture vials filled to a uniform volume, adjustable to any number of syringe settings; and, (3) it is rapid, allowing the filling of approximately one vial per second. It is advisable to pre-heat the percolater by "perking" water before use. This can be done conveniently while the medium is being prepared.

Merriam, J., R. Jones, H. Lee and L. Singer. University of California, Los Angeles, California. New plugs from old (a simple cure for mites).

A common problem in *Drosophila* labs is the choice of material to plug bottles. Foam plugs are best in many ways. They do have disadvantages, however. They are expensive and they tend to shrink upon autoclaving. Normally they have to be discarded after

several cycles of use.

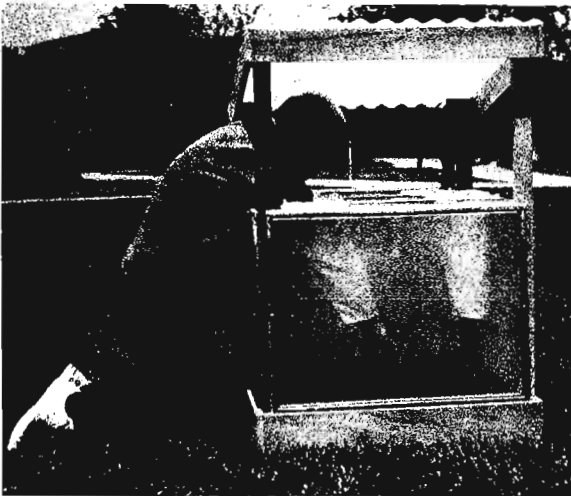
We discovered that treating the plugs with a plasticizing agent puffs them back up to their full size and more. The agent is benzyl benzoate in 95% ethanol. The amount of puffing depends on the concentration of benzyl benzoate. We use 20%; we note that at 30% the plugs become larger than their original size. Our routine is to wash and rinse used plugs in a clothes washer after autoclaving, dry them in air, soak them in benzyl benzoate, squeeze out the mixture, dry again and reuse. Some residue remains on the plugs to make them mite proof; some people find the residue objectionable. Yet our lab lies within a "mite belt," we no longer use caffeine in the medium, and we have not seen a mite for over

2 years. Both puffing and the residue are necessary signs to follow to make sure the treatment is working.

After treatment plugs are softer and hold in the bottle much better than formerly. Softer plugs might wear out sooner but there is no question that the economics of the plugs, at least, favors their treatment and longer use.

Bryant, S., T. Prout and R.W. Gill.
University of California, Riverside.
An outdoor population cage.

to be as large as possible, with the restriction that one must be able to reach every point in the hutch without having to climb inside. More detailed information is available, but the



For the past three years we have been successfully using a large outdoor population cage ("hutch") to conduct studies on the ecology and population genetics of *Drosophila* in a nearly natural environment. The hutches were designed the general design is as follows: a redwood frame consisting of a six-inch high baseboard 31 x 31 inches square, four corner uprights 27 inches high, and a top was built. Panels of aluminum channel and plastic screen were fastened to each of the four faces. The top was made as follows: nine holes were cut into the top in a 3 x 3 pattern. At each corner hole a cloth sleeve was attached. The middle hole on each side was covered with a screen panel, and a plexiglass viewplate was fixed over the center hole. A roof was added.

In use, the hutch is buried in the ground to a depth of five inches; dirt is put in through the top to equalize ground levels inside and out. Rotten fruit (or other medium) and flies are introduced through the sleeves which are then tied off. The medium is changed and the population sampled through the sleeves. We have successfully maintained populations of *D. simulans*, *D. im-*

migrans, *D. buskii*, *D. hydei* and *D. pseudoobscura* outdoors in Southern California with this method. We can now conduct experiments inside and outside the hutches in a field environment and compare the results.

Kochan, J. University of Toronto,
Ontario, Canada. A simple method for
making imprints of fruit fly eyes.

able, relatively non-toxic materials. After determining that wax impressions were difficult to make, the wax being either too brittle or too flexible, I turned to using plastic cement. The simple procedure, which follows, produces satisfactory imprints with minimum practice.

A drop of 70% ethyl alcohol is placed on an etherized fly. After this evaporates, a drop of LePage's plastic cement is placed on the eye. After overnight drying, a razor blade is used to pry the plastic off the eye. The plastic imprint is then mounted on a slide and flattened with a coverslip. Glycerol was used as a mounting fluid.

It was found that more than one imprint could be made from the same eye. This technique can be used on other sites of the integument as well. It may also be possible to reduce the drying time by using a slide warmer or a hair dryer.

The simple, inexpensive method described may be particularly useful in classroom exercises.

There are many situations in which it is necessary to count eye facets, and after it is desirable to make imprints of the eye for future scoring. I searched for an imprinting method which would be simple and require easily avail-

Toda, M.J. Hokkaido University, Sapporo, Japan. Two new "retainer" bait traps.

Two new bait traps which can retain trapped flies for several days were invented. The designs are shown in Figs. 1 and 2. Type I consists of two parts, trap-can and retainer.

Flies in trap-can go to retainer by positive phototaxis and drop into a bottle containing fixative. On collecting the trap is tapped repeatedly to drop flies which are still remaining in trap-can and retainer into the bottle. The bottle is screwed out and removed easily from retainer. Type II is made with cylindrical dry-milk can (13 cm in diameter, 20 cm in depth). The bottom is cut off with a can opener and a funnel painted black is attached there. Flies are retained in a vinyl sack attached to the opposite side.

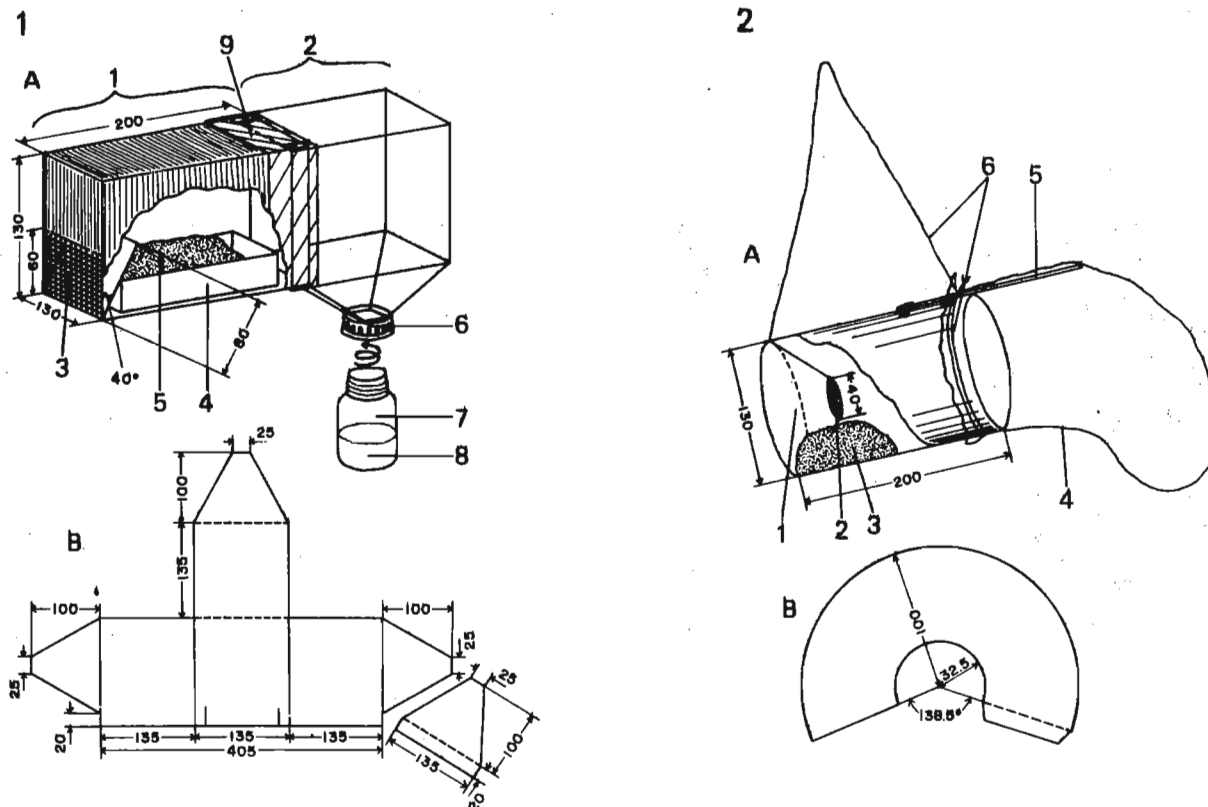


Figure 1.A: "Retainer" Trap Type I. 1) Trap-can. 2) Retainer made with a transparent vinyl chloride plate (0.5 or 1.0 mm thick). 3) Entrance covered with wire net (5 mm meshes) to inhibit the entrance of larger insects, e.g., moths, beetles, etc. 4) Bait container box. 5) Bait. 6) Cap of a polyethylene bottle holed 25 mm square and attached to retainer. 7) Polyethylene bottle (100 cc in capacity). 8) Fixative, e.g., 70% alcohol or Kahle's solution, etc. 9) Broad adhesive paper tape to attach retainer to trap-can. B: Horizontal development of retainer with measurements (in mm). Figure 2.A: "Retainer" Trap Type II. 1) Funnel made with a vinyl chloride plate. 2) Entrance. 3) Bait. 4) Vinyl sack. 5) Stick. 6) String. B: Horizontal development of a funnel with measurements.

The utility of these new traps is obvious on particular occasions, for instance, when the collectors can not frequently visit traps, e.g., at forest canopy. Furthermore, by using Type I, I obtained good results in the study of continuous seasonal population fluctuation, in which collections were continued throughout seasons and the flies retained in traps were removed every seven days (Toda, unpubl.). As to Type II, it is better to remove flies retained within two or three days because the bodies of flies having attached and died on the inner surface of a vinyl sack often begin to decay if they are left for more than four days.

Wright, R.G. University of Edinburgh, Scotland. Light source and driver for vision studies.

rectangular light pulses with rapid rise and fall times (200 nsec) when driven by a pulse generator and require small driving currents (typically 20-100 mA) and produce a negligible stimulus-artifact. LEDs are narrow band sources (circa 20 nm) and are available for a few cents from many manufacturers with λ peak from 560 nm to far infra-red. The most efficient devices I have so far encountered are Hewlett-Packard HI-OP LEDs: red (635 nm), yellow (583 nm) and green (565 nm) (device numbers: 5082 4658, 5082 4558 and 5082 4958). All these have a luminous intensity >16 mcd at 20 mA (manufacturers data sheets). I have found that they may

Light emitting diodes (LEDs) have proved superior to conventional light sources in determining critical flicker fusion frequencies in white-eyed *D. melanogaster* (Cosens and Wright, 1975, J. Insect Physiol. 22:1111-1120). They produce

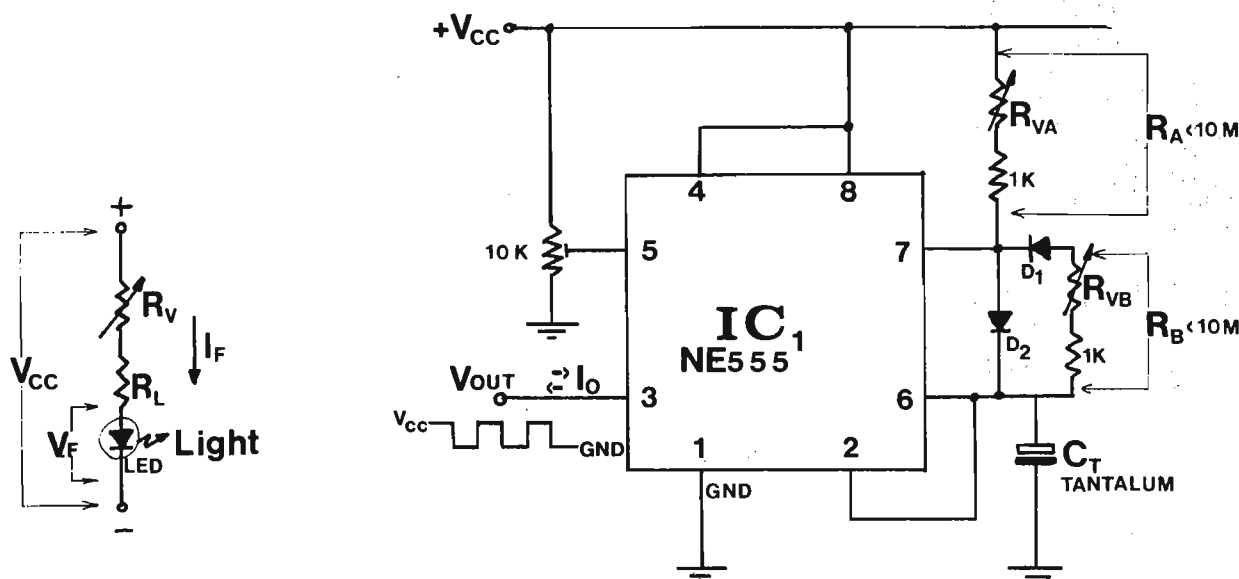


Figure 1. Control of LED light output

Figure 2. Cheap pulse generator/LED driver

be modulated over the range 10^{-2} to 10^3 mW/m² incident at the preparation via a tapered quartz rod (driving current: 50 uA to 75 mA). These diodes are functional as a standard monochromatic test light with readily variable pulse duration (usec to hr). I found log Q (quantal flux) is proportional to log I_F (diode forward current). Figure 1 shows the arrangement used to control I_F . R_L limits current to the desired I_{max}

$$R_L = (V_{CC} - V_F) / I_{max}$$

V_F is the diode forward voltage drop (manufacturer specified: typically 1.9 V at 20 mA) and,

$$I_F = (V_{CC} - V_F) / (R_V + R_L)$$

Figure 2 shows the circuit of a reliable pulse generator (cost \$2) with independently variable mark and space (ratio 1 to 1000 is possible) which is used to drive the light unit. V_{out} switches between approximately V_{CC} (+8 to +15 V) and GND. 100 mA sink or source is available to drive the light unit direct. The 10 K preset trims the timing periods and may be used to match capacitors if these are switched. Diodes D_1 and D_2 are small rectifier type such as 1N914.

$$\text{Mark } (V_{out} = V_{CC}), \text{ time} = 0.8 R_A C_T$$

$$\text{Space } (V_{out} = \text{GND}), \text{ time} = 0.8 R_B C_T$$

$$(10^{-3} \text{ uF} < C_T < 10^2 \text{ uF})$$

Lower, W.R., V.K. Drobney and P.S. Rose.
University of Missouri, Columbia. Design
of an improved multipurpose *Drosophila*
trap.

open bait collecting technique requires the collector to be present during the period of the day when *Drosophila* are active, severely limiting the number of sites one could collect in a single day. A true trap was needed which would attract and retain *Drosophila* until they could

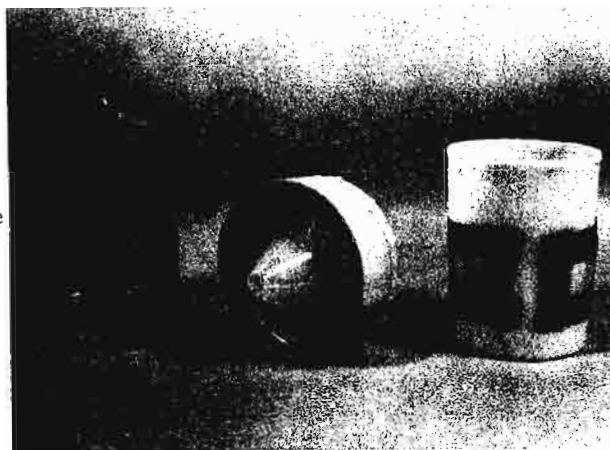


We have been involved with a project in which one person must collect large numbers of *Drosophila* within a period of several days, at numerous sites distributed over a distance of 50 km. Our goals could not be accomplished using the traditional trapping techniques. The trap consists of two chambers, connected by a funnel, inverted over the lower one and extending into the upper. This allows the phototropic flies to enter and be retained in the upper chamber. The lower chamber, containing the bait, is opaque and has coarse screened entry ports. The upper, transparent chamber has two or three air ports covered with fine mesh stainless steel screen. A small hole, for etherizing, is drilled in the top and plugged with a rubber or cork stopper. The trap is suspended from a tree branch by a straightened wire coat hanger hung through an eye bolt in the top of the trap. A thin plastic picnic plate, slipped down over the hanger, serves as a sun and rain shade and allows the trap to be easily seen.

While most trapping techniques are designed to serve a specific purpose, this trap can accomplish a variety of goals. Large numbers of flies, of many species, can be collected in a short time from many sites over a wide geographical area. In two seven-month collecting seasons (April-October) in the southeast Missouri Ozarks, single traps consistently yielded 25 to 200 individuals of 9 to

15 different *Drosophila* species. Once the traps are set, they can be sampled daily or at other intervals, for a week or more, depending on the condition of the bait. Over most of the collecting season approximately three cm of banana bait in the lower chamber lasted five to seven days before drying out or becoming contaminated with pupae. When traps were rebaited, the old bait was completely removed from the chamber and carried from the site in plastic bags. From our experience we agree with Dobzhansky (DIS 6:28-29, 1936) that fermenting banana is the best all purpose bait. We found the bait (mashed banana, peel and brewers' yeast) to be most effective when it was allowed to ferment two to three days before use. In a variety of diverse habitats the traps gave the best results when hung at least one meter apart in bushy understory and 1/3 to 1 1/3 meters above the ground or leaf litter. The time of day the traps were sampled and weather conditions, other than extremes such as rain or high wind, did not appear to affect the species or number of flies collected.

Collecting flies from a trap took only a few minutes. The trap was removed from the hanger, held with hands covering the entry ports, and the lower chamber tapped a number of times to stimulate the flies still on the bait to enter the upper chamber. A wash (squeeze) bottle etherizer was used to etherize flies in the upper chamber. The nozzle of the wash bottle is inserted into the hole in the top after removing the rubber stopper and the ether fumes pumped into the chamber. Air ports may be blocked with masking tape to speed the



etherizing but this is not essential. Anesthetized flies can then be aspirated from the stoppered hole, using flexible rubber tubing, into a five dram plastic vial. Flies up to 1 1/2 cm deep will remain alive for several hours (longer if kept cool in a small ice chest) in the plastic vials, and for several days in a refrigerator.

With these trapping and collecting methods large numbers of live flies can be collected throughout the day and then worked up at the leisure of the collector either in the laboratory or in the field.

MATERIALS

The total cost of materials for a single trap is currently estimated to be \$3.00 at 1977 prices.

Upper chamber: 32 oz. transparent clear plastic canister L23, from Bee Plastic-Consumer Products Division, Amoco Chemicals Corp., Waltham, Massachusetts 02154.

Lower chamber: 30 oz., #112 opaque freezette jars, from Republic Molding Corp., Chicago, Illinois 60648.

Funnels: Polyethylene, #F7450-2 from Scientific Products.

Wire screen: #40 mesh, 010 gauge stainless steel from Flynn & Enslow Inc., 1530 17th St., San Francisco, California 94107.

Plastic screen: Drosophila culture netting, #67-4068 from Carolina Biological Supply Co.

Solid rubber stoppers: Size #51040-1 from Scientific Products.

1/4" eye hooks.

Plastic electricians' tape or masking tape.

EQUIPMENT NEEDED FOR CONSTRUCTION

3/8" drill.

1 1/8" high speed hole saw with a 1/4" shank Mandrel; or adjustable dial saw, 1 to 2 1/2"; or adjustable hole cutter 1 to 1 1/2" or 5/8 to 1 1/2".

1/8" drill bit.

Stanley circle cutter, #040418, 1 1/8" to 4 7/8" or #419, 1 5/8" to 8".

Wire cutters.

Drill press if available—drilling most holes is most easily done with a drill press.

Soldering iron, 3/8" tip, 75 watt and/or pencil type, 42 watt.

Specially made concave and circular soldering tip: This can be easily made by a scientific instrument shop to fit the contour of the plastic container.

Thermogrip electric glue gun and glue.

TRAP CONSTRUCTION

Upper chamber: Use a 1 1/8" high speed hole saw to drill two or three air ports in the sides of a clear Bee Plastic canister. The ports are covered with small pieces of wire screen fused to the plastic with a soldering iron. We found that screen cut in an octagonal shape was easiest to fuse. If a large number of traps are to be constructed, the time required to fuse the screen air ports can be greatly reduced by using the specially made concave circular soldering tip. A 5/8" adjustable hole cutter is used to drill a hole for etherizing in the top of the canister. This hole is plugged with a solid #1 rubber stopper. Use a 1/8" drill bit to drill a small hole in the middle of the top of the canister and screw in the eye hook.

Lower chamber: Using a 1 1/8" hole saw, drill three holes around the sides of an opaque freezette jar for entry ports. To discourage entry by larger insects, small squares of unstretched Drosophila culture netting are taped over the outside of the ports with either plastic electricians' tape or masking tape. Tape is used because it is difficult to solder a firm seal without melting through the plastic of the lower chamber. This is not so for the upper chamber. Both types of tape and the netting held up well under field conditions, but if necessary could easily be replaced.

Lid: The fusion of the lids and funnels requires the most time and care. Using a circle cutter, cut a hole in the opaque lid of the freezette jar (lower chamber) approximately 82 mm in diameter, so that only slightly more than the rim of the lid remains. With a sharp knife remove the stem of the funnel, taking care to leave a smooth edge. A rat tail file or a sharp knife may be used to smooth the surface or ream out the edge. The smooth edge discourages the flies from congregating around the lip of the funnel and facilitates their entrance into the upper chamber. Invert the funnel and carefully fuse it to the top of the freezette lid with a soldering iron. Care must be taken to melt the plastics enough to form a strong bond. In the top of the plastic canister lid (upper chamber) use the circle cutter to cut a hole about 78 mm in diameter. The canister lid is then placed, upside down, over the funnel and firmly fused by melting the outer edge of the canister lid to the outer edge of the

freezette lid with a soldering iron. A strong bond is essential here so that the upper and lower chambers can be put on and taken off easily and frequently in the field without the lids coming apart. Once the funnel and lids are fused together, a glue gun is used to run a small bead of glue around the base of the funnel, filling any gaps between the funnel and the inside bottom of the canister lid. The upper and lower chambers are screwed onto their respective lids and the trap is ready for use.

Note: Noxious fumes are produced when the plastics are melted. Fusing the parts of the trap together should be done under a hood or preferably out of doors.

EQUIPMENT NEEDED FOR FIELD COLLECTING

Straightened wire coat hangers to hang the traps.

Thin plastic plates, slit to fit over the hangers.

Etherizer: Polyethylene wash bottle, Scientific Products, #B7894-250 or #B7894-500.

Clear plastic vials: One inch diameter, five dram, from Owens Illinois Co., Toledo, Ohio.

Aspirator: Insert short pieces of glass tubing through the holes of a two-hole #5 rubber stopper, Scientific Products, #R5150-5. Cover the end of one piece of glass tubing with gauze. Attach to the glass tubing desired lengths of flexible rubber tubing with short pieces of glass tubing for a mouth piece and aspirator tip. The stopper fits into five dram plastic vials and can be easily changed from one to another while collecting.

PERSONAL AND LABORATORY NEWS

S.C. Lakotia has moved to the Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380 009, India (from University of Burdwan, West Bengal).

William Gelbart has moved to Department of Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138 as an assistant professor (from University of Connecticut, Storrs).

Chana Malogolowkin-Cohen, formerly of the Department of Zoology, University of Brazil, has joined the University of Haifa, Israel, where she has been appointed Professor of Genetics at the Department of Biology. She is studying behavior and population genetics of *D. subobscura*.

D. melanogaster stocks are no longer maintained in the Department of Genetics, School of Biology, University of Leicester, England, according to Dr. R. Semeonoff.

Marengo, N.P. C.W. Post College of Long Island University, Greenvale, New York.
A laboratory simulation of natural selection in *Drosophila*.

In an undergraduate course in genetics, a laboratory exercise was devised to illustrate how, in a single generation of *D. melanogaster*, a significant shift in phenotype proportions in a population could be achieved by introducing a selective environmental hazard. This would be

a structure or condition in the environment limiting the viability and reproductive capacity of one of the phenotypes and therefore favoring the viability and reproductive capacity of the other.

The success of the experiment was of such a significant degree, that its communication to the educational and scientific communities appears warranted.

Large numbers of wild type (+) and vestigial (vg) flies were cultured separately in conventional milk bottle cultures of tomato-paste agar (1). After emerging and aging for several days they were issued to students. Some students received five pairs of each phenotype and some received ten. Both

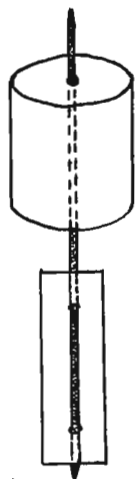


Figure 1. Sketch of the selective hazard consisting of a piece of flypaper attached by a swabstick inserted through bottle plug.

population samples yielded similar results.

The students worked in twos, with one responsible for the "control" culture, and the other the "experimental".

The cultures were identical except that the experimental culture had a 20 mm x 60 mm piece of flypaper (Dars bug-all flycatcher, Dars-Met-All Industries, Inc., Long Island City, New York 11101) pierced twice by a swabstick and held suspended over the food mass by having the top of the swabstick pushed through the foam plug of the bottle (Figure 1).

Each class met twice per week and the experiment was completed in three weeks. The first period was taken up with issuing five or ten pairs of each phenotype to each student. These were issued etherized and were placed in small plugged test tubes until active, at which time they were transferred to the breeding bottles. One period of the second week was used to check the fertility of the cultures and remove the surviving parental phenotypes originally introduced.

At room temperature, the cultures were ready for counting two weeks after the start. Two counts were made of each culture, and each student's totals were entered on a master tally-sheet. The figures were then consolidated and compared. A total of thirty student-cultures provided the

data. The consolidated results are shown in the table.

The striking difference in the proportion of phenotypes in the control and experimental cultures indicates that the introduction of a selective hazard can in one generation change significantly the genetic constitution of a population.

Reference: Lewis, M.T. 1942, Science 96 (No. 2490):282.

Control		Experimental	
+	vg	+	vg
1561	1361	702	2038

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Fleuriet, A. Clermont-Ferrand, France
Fontdevila, A. Santiago, Spain
Forbes, C. Moscow, Idaho
Forrest, H.S. Austin, Texas
Fountatou-Vergini, J. Athens, Greece
Fourche, J. Lyon, France
Fowler, G. Ashland, Oregon
Fox, A.S. Madison, Wisconsin
Fox, D.J. Knoxville, Tennessee
Fox, D.P. Aberdeen, Great Britain
Frankel, A.W.K. Iowa City, Iowa
Frankham, R. Sydney, Australia
Franklin, I.R. Sydney, Australia
French, W.L. Baton Rouge, Louisiana
Frias, D. Santiago, Chili
Friedman, L.D. St. Louis, Missouri
Friedman, T.B. Rochester, Michigan
Fritz-Niggli, H. Zürich, Switzerland
Frutos, R. de Bellaterra, Spain
Frydenberg, O. Aarhus, Denmark
Fuchs, M.S. Notre Dame, Indiana
Fujii, H.M. Fukuoka, Japan
Fukatami, A. Sakado-Machi, Japan
Fukunaga, A. Osaka, Japan
Fullilove, S.L. Austin, Texas
Fuscaldo, K.E. Philadelphia, Pennsylvania
Futch, D.C. San Diego, California
Fuyama, Y. Setagaya-ku, Japan
Gabay, S.J. Urbana, Illinois
Gale, J.S. Birmingham, Great Britain
Gall, J.G. New Haven, Connecticut
Garcia, F.A. Bogota, Colombia
Garcia, M. Bellaterra, Spain
García, R. Barcelona, Spain
Garcia-Bellido, A. Madrid, Spain
Garcia-Pravia, F. Santiago, Spain
Gardner, E.J. Logan, Utah
Garen, A. New Haven, Connecticut
Gartner, L.P. Baltimore, Maryland
Gärtner, S. Basel, Switzerland
Gateff, E. Freiburg, Germany
Gatti, M. Rome, Italy
Geer, B.W. Galesburg, Illinois
Gehring, W. Basel, Switzerland
Gelbart, W. Storrs, Connecticut
Gerresheim, F. München, Germany
Gersh, E.S. Philadelphia, Pennsylvania
Gethmann, R.C. Catonsville, Maryland
Ghysen, A. Pasadena, California
Gibson, P.K. Urbana, Illinois
Gill, K.S. Ludhiana, India
Gill, R.W. Riverside, California
Girard, P. Paris, France
Glätzer, K.H. Düsseldorf, Germany
Gloor, H. Geneva, Switzerland
Godbole, N.N. Poona, India
Godoy, R. Santiago, Chili
Goldstein, E. Tempe, Arizona
Gonzales, F.H. Lima, Peru
Gonzalez, F.W. Upton, New York
Gottlieb, F.J. Pittsburgh, Pennsylvania
Götz, K.G. Tübingen, Germany
Gould, A.B. Newark, Delaware
Goux, J.M. Paris, France
Grabicki, E. New Haven, Connecticut
Grace, D. Eugene, Oregon
Graf, U. Zürich, Switzerland
Granobles, L.A. Bogota, Colombia
Grant, B.S. Williamsburg, Virginia
Green, M.M. Davis, California
Grell, E.H. Oak Ridge, Tennessee
Grell, R.F. Oak Ridge, Tennessee
Grossfield, J. New York, New York
Grossman, A. Jerusalem, Israel
Grossman, A. Iowa City, Iowa
Grünberg, H. London, Great Britain
Guest, W.C. Fayetteville, Arkansas
Guevara, P.M. Lima, Peru
Gupta, J.P. Varanasi, India
Guzmán, J.M.S. Mexico City, Mexico
Hackman, W. Helsinki Finland
Haendle, J. München, Germany
Hägele, K. Bochum, Germany
Hagguist, K. Umeå, Sweden
Hale, G. New York, New York
Halfer, C. Milan, Italy
Halfer Mosna, G. Milan, Italy
Hall, J.C. Waltham, Massachusetts
Hall, L. Cambridge, Massachusetts
Hama, H. Chiba, Japan
Hames, B.D. Colchester, Great Britain
Hammerschmidt, H. München, Germany
Hampel, H. Vienna, Austria
Hanks, G.D. Gary, Indiana
Hannah, S.M. York, Great Britain
Hannah-Alava, A. Turku, Finland
Hara, K. Fukuoka, Japan
Hardy, D.E. Honolulu, Hawaii
Hardy, R. Los Angeles, California
Harrison, B.J. Norwich, Great Britain
Hartl, D.L. West Lafayette, Indiana
Hartmann-Goldstein, I.J. Sheffield, Great Britain
Hauschteck-Jungen, E. Zürich, Switzerland
Hay, D.A. Bundoor, Australia
Haynie, J.L. Seattle, Washington
Hedrick, P.W. Lawrence, Kansas
Heisenberg, M. Würzburg, Germany
Hengstenberg, R. Tübingen, Germany
Hennig, W. Tübingen, Germany

- Henon, B.K. Duarte, California
Herforth, R.S. Minneapolis, Minnesota
Herskowitz, I.H. New York, New York
Hess, O. Düsseldorf, Germany
Hexter, W.M. Amherst, Massachusetts
Hickey, D. Cambridge, Massachusetts
Hihara, F. Matsuyama, Japan
Hildreth, P.E. Charlotte, North Carolina
Hill, R. Sydney, Australia
Hilliker, A. Storrs, Connecticut
Hillman, R. Philadelphia, Pennsylvania
Hinton, C.W. Wooster, Ohio
Hiraizumi, Y. Austin, Texas
Hiroyoshi, T. Osaka, Japan
Hochman, B. Knoxville, Tennessee
Hodgetts, R.B. Edmonton, Canada
Hoekstra, R.F. Haren, Netherlands
Hoenigsberg, H.F. Bogota, Columbia
Hollingsworth, M.J. London, Great Britain
Hollis, R.J. Williamsburg, Virginia
Holm, D. Vancouver, Canada
Holmgren, P. Umeå, Sweden
Homyk, T. Vancouver, Canada
Honda, Y. Nagasaki, Japan
Hooper, G.B. Poughkeepsie, New York
Hoorn, A.J.W. Utrecht, Netherlands
Hotta, Y. Tokyo, Japan
Hubby, J.L. Chicago, Illinois
Hubert, U. Marburg, Germany
Hunt, D.M. London, Great Britain
Huth, A.C. Tübingen, Germany
Ikeda, H. Matsuyama, Japan
Ikeda, K. Duarte, California
Imberski, R.B. College Park, Maryland
Inagaki, E. Hiroshima, Japan
Inocencio, B. Purchase, New York
Ish-Horowicz, D. Basel, Switzerland
Iturra, P. Santiago, Chili
Ives, P.T. Amherst, Massachusetts
Iwamoto, R.N. Pullman, Washington
Jacob, G. Tübingen, Germany
Jacob, R. Tübingen, Germany
Jacobs, M.E. Goshen, Indiana
Jacobson, A.G. Austin, Texas
Jain, H.K. New Delhi, India
James, F.W. Sydney, Australia
Janning, W. Münster, Germany
Jarry, B. Marseille, France
Jeffery, D.E. Provo, Utah
Jha, A.P. Bhagalpur, India
Jinks, J.L. Birmingham, Great Britain
Johannisson, R. Düsseldorf, Germany
Johnsen, R.C. Garden City, New York
Johnson, K. London, Great Britain
Johnson W.W. Albuquerque, New Mexico
Johnston, J.S. Austin, Texas
Jones, G.H. Birmingham, Great Britain
Jong, G. Utrecht, Netherlands
Jordan, B. Marseille, France
Juan, E. Barcelona, Spain
Judd, B.H. Austin, Texas
Jungen, H. Zürich, Switzerland
Kaji, S. Kobe, Japan
Kalicki, H. Garden City, New York
Kalisch, W-E. Bochum, Germany
Kambysellis, M.P. New York, New York
Kaneko, A. Setagaya-ku, Japan
Kaneshiro, K.Y. Honolulu, Hawaii
Kang, E. Seoul, Korea
Kang, Y.S. Seoul, Korea
Kang Song, S.J. Seoul, Korea
Kankel, D. New Haven, Connecticut
Kaplan, M.L. New York, New York
Kaplan, W.D. Duarte, California
Karlik, A. Vienna, Austria
Kastritsis, C.D. Thessaloniki, Greece
Katoh, S. Sakado-Machi, Japan
Kaufman, T. Bloomington, Indiana
Kaufmann, B.N. Philadelphia, Pennsylvania
Kawanishi, M. Misima, Japan
Kearsey, M.J. Birmingham, Great Britain
Keller, E.C., Jr. Morgantown, West Virginia
Kercher, M.D. Lexington, Kentucky
Kertesz, J. Dayton, Ohio
Khishin, A.F. Assuit, Egypt
Kidwell, J. Providence, Rhode Island
Kidwell, M. Providence, Rhode Island
Kiefer, B.I. Middletown, Connecticut
Kiger, J.A. Davis, California
Kijken, F.R. Utrecht, Netherlands
Kim, K.W. Kwangju, Korea
Kimura, M. Misima, Japan
Kimura, M.T. Sapporo, Japan
King, R.C. Evanston, Illinois
Kirshbaum, W.F. Buenos Aires, Argentina
Kitagawa, O. Setagaya-ku, Japan
Klug, W.S. Crawfordsville, Indiana
Kobayashi, Y. Hiroshima, Japan
Kobel, H.R. Geneva, Switzerland
Koch, P. Marburg, Germany
Koelling, C. Duarte, California
Koenig, J.E. Duarte, California
Köhler, W. Berlin, Germany
Koo, D. Vancouver, Canada
Korge, G. München, Germany
Kortselius, M.J.H. Leiden, Netherlands
Kosuda, K. Sakado-Machi, Japan
Kouskounelou, E. Thessaloniki, Greece
Krakauer, E. Bogota, Columbia
Kramers, P.G.N. Leiden, Netherlands
Krause, E. South Orange, New Jersey
Kreber, R.A. Madison, Wisconsin
Kress, H. München, Germany
Kridler, H. Storrs, Connecticut
Krimbas, C. Athens, Greece
Krishnamurthy, N.B. Mysore, India
Krivshenko, J.D. Rochester, New York
Kroeger, H. Saarbrücken, Germany
Kroman, R.A. Long Beach, California
Krunić, M. Belgrade, Yugoslavia
Kubli, E. Zürich, Switzerland
Kunz, W. Düsseldorf, Germany

Kuo, G. New Haven, Connecticut
 Kuroda, Y. Misima, Japan
 Kwan, S.M. Seoul, Korea
 Lachaise, D. Gif-sur-Yvette, France
 Laird, C. Seattle, Washington
 Lakhota, S.C. Ahmedabad, India
 Lakovaara, S. Oulu, Finland
 Lamb, M.J. London, Great Britain
 Lambert, D.M. Johannesburg, South Africa
 Lambertsson, A. Umeå, Sweden
 Lande, R. Cambridge, Massachusetts
 Langley, C.H., Research Triangle Park, N.C.
 Lankinen, P. Oulu, Finland
 LaPushin, R. Houston, Texas
 Laughnan, J.R. Urbana, Illinois
 Lautenburger, J. Chapel Hill, North Carolina
 Lawrence, M.J. Birmingham, Great Britain
 Lechien, J. Namur, Belgium
 Lechner, J.F. Philadelphia, Pennsylvania
 Lee, C.C. Seoul, Korea
 Lee, C.S. Austin, Texas
 Lee, T.J. Seoul, Korea
 Lee, W.H. Misima, Japan
 Lee, W.R. Baton Rouge, Louisiana
 Lefevre, G., Jr. Northridge, California
 Legay, J.M. Lyon, France
 Leibenguth, F. Saarbrücken, Germany
 Leigh, B. Leiden, Netherlands
 Leister, F. Baltimore, Maryland
 Lemeunier, F. Gif-sur-Yvette, France
 Leonard, J.E. Purchase, New York
 Leoncini, O. Tübingen, Germany
 Levitan, M. New York, New York
 Lewis, E.B. Pasadena, California
 Lewontin, D. Cambridge, Massachusetts
 L'Hélias, C. Gif-sur-Yvette, France
 L'Heritier, Ph. Clermont-Ferrand, France
 Libion-Mannaert, M. Namur, Belgium
 Lindsley, D.L. LaJolla, California
 Link, B. Tübingen, Germany
 Lints, C. Louvain-La-Nueve, Belgium
 Lints, F. Louvain-La-Nueve, Belgium
 Littlewood, T. Cambridge, Great Britain
 LoCascio, N. Buffalo, New York
 Lokki, J. Helsinki, Finland
 Lokki, M. Helsinki, Finland
 Lommerse, R. Leiden, Netherlands
 Louis, M. Gif-sur-Yvette, France
 Loukas, M. Athens, Greece
 Loverre, A. Rome, Italy
 Lowy, P.H. Pasadena, California
 Lucchesi, J.C. Chapel Hill, North Carolina
 Luce, W.M. Urbana, Illinois
 Lüers, H. Berlin, Germany
 Lumme, J. Oulu, Finland
 Lüning, K.G. Stockholm, Sweden
 Lyttle, T. Seattle, Washington
 MacIntyre, R. Ithaca, New York
 Madoi, C. Milan, Italy
 Magalhães, L.E. São Paulo, Brazil
 Magma, F. Naples, Italy
 Maher, E.P. Aberdeen, Great Britain
 Mainx, F. Vienna, Austria
 Majumdar, S.K. Easton, Pennsylvania
 Malogowkin-Cohen, Ch. Haifa, Israel
 Manna, G.K. Kalyani, India
 Manosalva, B.J. Lima, Peru
 Mansfield, L. Madison, Wisconsin
 Marcos, R. Bellaterra, Spain
 Marien, D. New York, New York
 Marinković, D. Belgrade, Yugoslavia
 Maroni, G.P. Chapel Hill, North Carolina
 Marsh, J.L. Charlottesville, Virginia
 Marsh, L. Basel, Switzerland
 Martinez, I.N. Lima, Peru
 Marques, E.J. Mato Grosso, Brazil
 Marques, E.K. Pôrto Alegre, Brazil
 Martin, A.O. Cleveland, Ohio
 Martinez, M.N. Pôrto Alegre, Brazil
 Martinez, R.M. Hamden, Connecticut
 Maruyama, T. Misima, Japan
 Massie, H.R. Utica, New York
 Mather, K.C.B.E. Birmingham, Great Britain
 Mather, W.B. Brisbane, Australia
 Matsubara, K. Sakai, Japan
 Matsuda, T. Asamizodai, Japan
 Mayfield, J.E. Pittsburgh, Pennsylvania
 Mazar Barnett, B. Buenos Aires, Argentine
 McCarron, M. Storrs, Connecticut
 McCarthy, P.C. New Wilmington, Pennsylvania
 McCormack, M.K. New Brunswick, New Jersey
 McCrady, E. Greensboro, North Carolina
 McCrady, W.B. Arlington, Texas
 McDonald, J. Bundoora, Australia
 McKenzie, J.A. Bundoora, Australia
 McMurtrey, M. Houston, Texas
 Méndez, J. Santiago, Spain
 Mengual, V. Bellaterra, Spain
 Mensua, J.L. Bellaterra, Spain
 Mercader, J. Mexico City, Mexico
 Merrell, D.J. Minneapolis, Minnesota
 Merriam, J. Los Angeles, California
 Merritt, R. Rochester, New York
 Mertens-Huber, M. München, Germany
 Meyer, G.F. Tübingen, Germany
 Meyer, H.U. Madison, Wisconsin
 Michalopoulou, E. Patras, Greece
 Micheli, A. Rome, Italy
 Michinomae, M. Kobe, Japan
 Miglani, G.S. Ludhiana, India
 Mikasa, K. Sakado-Machi, Japan
 Miklos, G.L.G. Canberra, Australia
 Milkman, R.D. Iowa City, Iowa
 Miller, D.D. Lincoln, Nebraska
 Miller, O.L. Jr. Charlottesville, Virginia
 Milner, M.J. Seattle, Washington
 Minamouri, S. Hiroshima, Japan
 Minato, K. Misima, Japan
 Mindek, G. Zürich, Switzerland
 Mitchell, C. Duarte, California
 Mitchell, H.K. Pasadena, California
 Mittler, S. DeKalb, Illinois

Mitra Nivedita, Calcutta, India
Mizobuchi, K. Chiba, Japan
Mohler, J.D. Iowa City, Iowa
Moisand, R. Buffalo, New York
Momma, E. Sapporo, Japan
Monclús, M. Barcelona, Spain
Monks, G. München, Germany
Montelente, G. Rome, Italy
Montell, I. Umeå, Sweden
Montijn, C. Utrecht, Netherlands
Morea, M. Bari, Italy
Moree, R. Pullman, Washington
Mori, S. Nagasaki, Japan
Moriwaki, D. Setagaya-ku, Japan
Mortensen, M. Copenhagen, Denmark
Moskewski, T. Notre Dame, Indiana
Mossige, J. Oslo, Norway
Mourad, A.O. Alexandria, Egypt
Muckenthaler, F.A. Bridgewater, Massachusetts
Mukherjee, A.S. Calcutta, India
Mulley, J.C. Sydney, Australia
Muñoz, E.R. Buenos Aires, Argentina
Muona, O. Helsinki, Finland
Murakami, A. Misima, Japan
Murata, M. Chiba, Japan
Murnik, M.R. Macomb, Illinois
Myszewski, M.E. Des Moines, Iowa
Nakai, S. Chiba, Japan
Nakai, S. Osaka, Japan
Nakashima-Tanaka, E. Sakai, Japan
Namkoong, Y. Seoul, Korea
Narise, S. Sakado-Machi, Japan
Narise, T. Sakado-Machi, Japan
Nash, D. Edmonton, Canada
Nash, W.G. Bethesda, Maryland
Navarro, J. Santiago, Chili
Nawa, S. Misima, Japan
Neeley, J.C. Portland, Oregon
Nicolosi, R.J. Utica, New York
Nigon, V. Lyon, France
Nilkan, C. Bangkok, Thailand
Nirmala Sajjan, S. Mysore, India
Nissani, M. Madison, Wisconsin
Nix, C.E. Oak Ridge, Tennessee
Nöthel, H. Berlin, Germany
Nöthiger, R. Zürich, Switzerland
Nouaud, D. Paris, France
Novitski, E. Eugene, Oregon
Nuez, F. Valencia, Spain
Nüsslein, Ch. Basel, Switzerland
Nygren, J. Umeå, Sweden
O'Brien, S.J. Bethesda, Maryland
O'Donnell, J. Storrs, Connecticut
Oelshlegel, F.J. Ann Arbor, Michigan
Ofstedal, P. Oslo, Norway
Ogaki, M. Sakai, Japan
Ogita, Z. Osaka, Japan
Ohba, S. Setagaya-ku, Japan
Oikarinen, A. Oulu, Finland
Oishi, K. Kobe, Japan
Oksala, T.A. Turku, Finland
Okubo, S. Osaka, Japan
Olivieri, G. Rome, Italy
Olvera, O.M.S. Mexico City, Mexico
Ondrej, M. Prague, Czechoslovakia
Ono, J.K. Duarte, California
Oram, G. Birmingham, Great Britain
Ortiz, E. Madrid, Spain
Osborn, R. Berkeley, California
Oshima, C. Misima, Japan
Oster, I.I. Bowling Green, Ohio
Oster, P. Bowling Green, Ohio
Ostrowski, R.S. Charlotte, North Carolina
Ouweneel, W.J. Leiden, Netherlands
Pagés, M. Madrid, Spain
Pak, W.L. West Lafayette, Indiana
Palabost, L. Paris, France
Parádi, E. Budapest, Hungary
Parisi, G. Naples, Italy
Park, M.S. Kwangju, Korea
Parker, D.R. Riverside, California
Parsons, P.A. Bundoor, Australia
Paterson, H.E. Nedlands, Australia
Pavan, C. São Paulo, Brazil
Paxman, G.J. Lancaster, Great Britain
Paz, C. Buenos Aires, Argentina
Peacock, W.J. Canberra, Australia
Peers, E. New York, New York
Pelecanos, M. Patras, Greece
Peñefiel, T. Bellaterra, Spain
Pereira, M.A.Q.R. São Paulo, Brazil
Pereira, S.M.F. Mato Grosso, Brazil
Perez, M. Bellaterra, Spain
Periquet, G. Paris, France
Persson, K. Umeå, Sweden
Petersen, N. Duarte, California
Petit, C. Paris, France
Petitpierre, E. Barcelona, Spain
Petri, W. Cambridge, Massachusetts
Petrovich, S.B. Catonsville, Maryland
Pfriem, P. Tübingen, Germany
Phillips, J. Austin, Texas
Picard, G. Clermont-Ferrand, France
Pierce, D. Chapel Hill, North Carolina
Pilaes, G.L. Lima, Peru
Pinsker, W. Tübingen, Germany
Pipkin, S.B. Washington DC
Pleihn, Chr. Bochum, Germany
Plus, N. St. Christol-les-Ales, France
Pohjola, L. Oulu, Finland
Poodry, C.A. Santa Cruz, California
Portin, P. Turku, Finland
Postlethwait, J.H. Eugene, Oregon
Potter, J.H. College Park, Maryland
Poulin, M. Tübingen, Germany
Poulson, D.F. New Haven, Connecticut
Powell, J.R. New Haven, Connecticut
Prakash, S. Rochester, New York
Prevosti, A. Barcelona, Spain
Privitera, E. Milan, Italy
Prout, T. Davis, California
Pruzan, A. Purchase, New York

- Pulvermacher, Ch. Berlin, Germany
 Puro, J. Turku, Finland
 Pyle, D. Raleigh, North Carolina
 Quinn, W.G. Princeton, New Jersey
 Rae, P.M.M. New Haven, Connecticut
 Rahman, S.M.Z. Bhagalpur, India
 Rai, K.S. Notre Dame, Indiana
 Rai-Chauduri, S.P. Calcutta, India
 Rajasekarasetty, M.R. Mysore, India
 Ramel, C. Stockholm, Sweden
 Ramon, M. Bellaterra, Spain
 Ranganath, H.A. Mysore, India
 Ransom, R. Brighton, Great Britain
 Rapport, E. Burnaby, Canada
 Rasmuson, A. Umea, Sweden
 Rasmuson, B. Umea, Sweden
 Rasmuson, M. Umea, Sweden
 Ratnayake, W.E. Gangodawila, Sri Lanka
 Ratty, F.J. San Diego, California
 Ray, C. Atlanta, Georgia
 Reddi, O.S. Hyderabad, India
 Relton, J. Sheffield, Great Britain
 Remondini, D.J. Houghton, Michigan
 Rendel, J.M. Sydney, Australia
 Reno, D. Galesburg, Illinois
 Ribó, G. Barcelona, Spain
 Richards, G.P. Cambridge, Great Britain
 Richardson, R.H. Austin, Texas
 Richmond, R.C. Raleigh, North Carolina
 Rick, J.T. Sheffield, Great Britain
 Rico, M. Valencia, Spain
 Rinehart, R.R. San Diego, California
 Ripoll, P. La Jolla, California
 Ritossa, F. Bari, Italy
 Rivera, M.L. Barcelona, Spain
 Rizki, R.M. Ann Arbor, Michigan
 Rizki, T.M. Ann Arbor, Michigan
 Robbins, L. East Lansing, Michigan
 Roberts, D.B. Oxford, Great Britain
 Roberts, P.A. Corvallis, Oregon
 Roberts, S. Colchester, Great Britain
 Robertson, A. Edinburgh, Great Britain
 Robertson, F.W. Aberdeen, Great Britain
 Rockwell, R.F. New York, New York
 Rodinó, E. Padova, Italy
 Roehrdanz, R. Chapel Hill, North Carolina
 Rokop, S. La Jolla, California
 Rose, R.W. Glenside, Pennsylvania
 Rosenfeld, A. Seattle, Washington
 Rosset, R. Marseille, France
 Roy, S. Ahmedabad, India
 Rubenstein, E. New York, New York
 Ruderer-Doschek, E. Vienna, Austria
 Rudkin, G.T. Philadelphia, Pennsylvania
 Rungger, E. Geneva, Switzerland
 Russell, M.A. Edmonton, Canada
 Rutherford, P. Aberdeen, Great Britain
 Sakaguchi, B. Fukuoka, Japan
 Sakoyama, Y. Osaka, Japan
 Salai, L. Albany, New York
 Salceda, S., V.M. Chapingo, Mexico
 Sander, K. Freiburg, Germany
 Sanders, T.G. Princeton, New Jersey
 Sandler, L. Seattle, Washington
 Sang, J.H. Brighton, Great Britain
 Sanjeeva, R. Hyderabad, India
 Sankaranarayanan, K. Leiden, Netherlands
 Sarker, D.N. Varanasi, India
 Saura, A. Helsinki, Finland
 Saura, M. Helsinki, Finland
 Savontaus, M-L. Turku, Finland
 Sayers, E.R. Tuscaloosa, Alabama
 Scalenghe, F. Bari, Italy
 Schabtach, E. Eugene, Oregon
 Schäfer, U. Düsseldorf, Germany
 Schaffer, H.E. Raleigh, North Carolina
 Schalet, A. Leiden, Netherlands
 Scharloo, W. Utrecht, Netherlands
 Schedl, P. Basel, Switzerland
 Scheid, W. Münster, Germany
 v. Schilcher, F. München, Germany
 Schmid, D. Tübingen, Germany
 Schmolesky, G. Madison, Wisconsin
 Schneider, I. Washington, DC
 Schouten, S.C.M. Utrecht, Netherlands
 Schubiger, G. Seattle, Washington
 Schweizer, P. Zürich, Switzerland
 Schwinck, I. Storrs, Connecticut
 Schwochau, M. Düsseldorf, Germany
 Scowcroft, W.R. Canberra, Australia
 Scozzari, R. Rome, Italy
 Sederoff, R.R. Eugene, Oregon
 Seecof, R. Duarte, California
 Seiger, M.B. Dayton, Ohio
 Semeonoff, R. Leicester, Great Britain
 Sene, F.M. São Paulo, Brazil
 Sernau, R. Madison, Wisconsin
 Serra, L. Barcelona, Spain
 Shafer, S.J. Oakdale, New York
 Sharma, G.P. Chandigarh, India
 Sharma, R.P. New Delhi, India
 Shear, C. Atlanta, Georgia
 Shearn, A. Baltimore, Maryland
 Sheldon, B.L. Sydney, Australia
 Shellenbarger, D. Vancouver, Canada
 Shen, M.W. Austin, Texas
 Shiomi, T. Nagasaki, Japan
 Shorrocks, B. Leeds, Great Britain
 Sick, K. Copenhagen, Denmark
 Siddaveere Gowda, L. Mysore, India
 Sidhu, N.S. Isatnagar, India
 Sillans, D. Lyon, France
 Simmons, J.R. Logan, Utah
 Singh, A. Chandigarh, India
 Smith, B.R. Aberdeen, Great Britain
 Smith, D.A. Birmingham, Great Britain
 Smith, P.D. Atlanta, Georgia
 Sobels, F.H. Leiden, Netherlands
 Södergren, A. Umeå, Sweden
 Sofer, W. Baltimore, Maryland
 Sokoloff, A. San Bernardino, California
 Soliman, H. Louvain-La-Nueve, Belgium

Soll, D.G. New Haven, Connecticut
 Somero, M. LaJolla, California
 Sonnenblick, B.P. Newark, New Jersey
 Sorsa, M. Helsinki, Finland
 Sorsa, V. Helsinki, Finland
 Sourdis, J. Athens, Greece
 Sparrow, J.C. York, Great Britain
 Speers, L. Ottawa, Canada
 Sperlich, D. Tübingen, Germany
 Spiess, E.B. Chicago, Illinois
 Späth, H.T. Davis, California
 Spofford, J.B. Chicago, Illinois
 Sprechman, L. Austin, Texas
 Springer, R. Vienna, Austria
 Steerama Reddy, G. Mysore, India
 Ståhl, G. Stockholm, Sweden
 Stark, W.S. Baltimore, Maryland
 Steffensen, D.M. Urbana, Illinois
 Stein, H. Tübingen, Germany
 Steiner, W.W.M. Urbana, Illinois
 Stern, C. Berkeley, California
 Stewart, B. Los Angeles, California
 Stewart, D. Waltham, Massachusetts
 Strickberger, M.W. St. Louis, Missouri
 Strömman, P. Copenhagen, Denmark
 Suchopova, N. Brno, Czechoslovakia
 Sulerud, R.L. Minneapolis, Minnesota
 Sullivan, D.T. Syracuse, New York
 Suomalainen, E. Helsinki, Finland
 Suzuki, D. Vancouver, Canada
 Svahlin, H. Umeå, Sweden
 Svensson, H. Umeå, Sweden
 Takaya, H. Kobe, Japan
 Takikawa, S. Asamizodai, Japan
 Tallentire, A.C. Kampala, Uganda
 Tantawy, A.O. Alexandria, Egypt
 Tartof, K.D. Philadelphia, Pennsylvania
 Temin, R. Madison, Wisconsin
 Tener, G. Vancouver, Canada
 Teramoto, L.T. Honolulu, Hawaii
 Thalmann, G.J. Oakdale, New York
 The, D. Sydney, Australia
 Thirtle, B. Ann Arbor, Michigan
 Thoday, J.M. Cambridge, Great Britain
 Thompson, C. Johnstown, Pennsylvania
 Thompson, J.N. Norman, Oklahoma
 Thompson, S.R. Ithaca, New York
 Thomson, J.A. Canberra, Australia
 Thongmeearkom, P. Brisbane, Australia
 Thörig, G.E.W. Utrecht, Netherlands
 Throckmorton, L.H. Chicago, Illinois
 Tigerstedt, P. Helsinki, Finland
 Tiivola, A. Helsinki, Finland
 Tobari, I. Chiba, Japan
 Tobari, Y.N. Setagaya-ku, Japan
 Tobler, H. Fribourg, Switzerland
 Toda, M.J. Sapporo, Japan
 Tokunaga, C. Berkeley, California
 Tokuyasu, K. LaJolla, California
 Tonomura, Y. Setagaya-ku, Japan
 Tonzetich, J. Lewisburg, Pennsylvania
 Torres, M.E. Bogota, Columbia
 Torroja, E. Madrid, Spain
 Tung, P.S.-C. University Park, Pennsylvania
 Tracey, M.L. St. Catherine's, Canada
 Traut, H. Münster, Germany
 Triantaphyllidis, C. D. Thessaloniki, Greece
 Trippa, G. Rome, Italy
 Trout, L. San Diego, California
 Trout, W.E. III Duarte, California
 Tsacas, L. Gif-sur-Yvette, France
 Tsakas, S. Athens, Greece
 Tsuno, K. Sakado-Machi, Japan
 Tsusue, M. Asamizodai, Japan
 Tuinstra, E.J. Utrecht, Netherlands
 Twardzik, D.R. Bethesda, Maryland
 Ulrich, H. Zürich, Switzerland
 Urbischek, E.F. Swansea, Great Britain
 Ushioda, Y. Kobe, Japan
 Vaidya, V.G. Poona, India
 Valadé, E. Santiago, Spain
 Valencia, R. Madison, Wisconsin
 Valentin, J. Stockholm, Sweden
 Van Delden, W. Haren, Netherlands
 Van den Haute, J. Namur, Belgium
 Van der Meulen, G. Haren, Netherlands
 Van der Wielen, A.W. Leiden, Netherlands
 Van Herrewege, J. Villeurbanne, France
 Van Valen, L. Chicago, Illinois
 Varga, J. Budapest, Hungary
 Vepsäläinen, K. Helsinki, Finland
 Verburgt, F.G. Leiden, Netherlands
 Viinikka, Y. Turku, Finland
 Vilageliu, L. Barcelona, Spain
 Voelker, R.A. Research Triangle Park, North Carolina
 Vogel, E. Leiden, Netherlands
 Vreezen, W.J. Leiden, Netherlands
 Vyse, E.R. Bozeman, Montana
 Wada, R. Osaka, Japan
 Waddle, F.R. Fayetteville, North Carolina
 Wakahama, K.-I. Matsue, Japan
 Walker, V. McC. Davis, California
 Wallace, B. Ithaca, New York
 Wallace, D. Cambridge, Massachusetts
 Wallace, H. Birmingham, Great Britain
 Ward, C.L. Durham, North Carolina
 Ward, R.D. Swansea, Great Britain
 Wargent, J. Sheffield, Great Britain
 Warren, H. Pittsburgh, Pennsylvania
 Wasserman, M. New York, New York
 Watanabe, T.K. Misima, Japan
 Watson, W.A.F. Aberdeen, Great Britain
 Weber, M. Berlin, Germany
 Wehner, R. Zürich, Switzerland
 Weide, R. Tempe, Arizona
 Weideli, H. Basel, Switzerland
 Welshons, J. Ames, Iowa
 Welshons, W.J. Ames, Iowa
 Wensink, P. Waltham, Massachusetts
 Westerberg, B.M. Umeå, Sweden
 Westerman, M. Bundoorra, Australia
 Wheeler, L. Austin, Texas
 White, B.N. Kingston, Canada
 White, K. New Haven, Connecticut

Whittinghill, M. Chapel Hill, North Carolina	Yakoumi, G. Athens, Greece
Whittle, J.R.S. Brighton, Great Britain	Yamada, M.A. Misima, Japan
Whitty, R.W. Canberra, Australia	Yamazaki, H.I. Setagaya-ku, Japan
Wildeboer-du Pui, M.L.L. Haren, Netherlands	Yamazaki, T. Misima, Japan
Wilkerson, R.D. Oak Ridge, Tennessee	Yanders, A.F. Columbia, Missouri
Williamson, D.L. Stony Brook, New York	Yannopoulos, G. Patras, Greece
Williamson, J.H. Calgary, Canada	Yasuda, N. Chiba, Japan
Williamson, R.L. Duarte, California	Yeh, F. Halifax, Canada
Wimber, D. Eugene, Oregon	Yokokawa, C. Sagamihara, Japan
Wing-Cordeiro, H. Pôrto Alegre, Brazil	Yoon, J.S. Austin, Texas
Wong, P.T.-C. Duarte, California	Yoon, S.B. Madison, Wisconsin
Wood, D.D. Rochester, Michigan	Yoshikawa, I. Nagasaki, Japan
Woodruff, R.C. Austin, Texas	Yost, H.T. Amherst, Massachusetts
Wright, C.P. Cullowhee, North Carolina	Younis, S.A. Assuit, Egypt
Wright, E.Y. Charlottesville, Virginia	Zacharopoulou, A. Patras, Greece
Wright, T.R.F. Charlottesville, Virginia	Zárate, E. Santiago, Chili
Wui, I.S. Kwangju, Korea	Zimm, G. LaJolla, California
Würgler, F.E. Zürich, Switzerland	Zimmering, S. Providence, Rhode Island
Wyman, A. Cambridge, Massachusetts	Zouros, E. Halifax, Canada
Wyman, R.J. New Haven, Connecticut	Zubeldia, A. Valencia, Spain
	Züst, B. Fribourg, Switzerland