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NOTES FROM THE EDITOR

This is the last issue of DIS to come from the University of Oregon. We took over the responsibility of nurturing it from Dr. M. Demerec in 1960 and, during the past eighteen years, have put out twenty issues. During this period of time, quite a number of innovations and, we hope, improvements have been made, but we feel that there is constant opportunity for fresh ideas to be tried out and look forward to the changes that will be suggested under its new management.

Over the years a large number of staff and students have helped in the various aspects of the production of DIS, often donating their time or accepting minimal payment. I would like to express my appreciation to all of them, and particularly to Esther Ellen Novitski, who, for the past ten years, has accepted the responsibility for taking DIS through the various phases of its production, freely volunteering much of her time for this purpose.

Finally, we wish our best to Professor Hedrick who is now assuming the obligations of the editorship, a task being undertaken with full knowledge of the problems inherent in it. Address all future communications to:

Professor Philip Hedrick
Division of Biological Science
University of Kansas
Lawrence, Kansas 66045

E. Novitski

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Colleagues who have submitted stock lists for the last two issues of DIS understand that they were held back in anticipation of the appearance of the first computerized world list. This project proved to be almost too big a job for even a large computer, so it was decided to submit an abbreviated tentative list for this issue while the few remaining "bugs" are worked out. It is hoped that in the near future DIS will distribute a truly comprehensive World Stock List of D. melanogaster chromosomes, mutants and genes. There are no plans at present, however, to so list species stocks or special melanogaster lists, so DIS will carry that information in the usual way.

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A COMPUTERIZED LIST OF MUTANT STOCKS OF DROSOPHILA MELANOGASTER FROM SIX LABORATORIES

List prepared from contributed or published stock lists by

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Department of Biology
University of California, San Diego

Computer program written by C. MAUDLIN with
J. GERSZTYN, D. GREGORY, R. OLDS, AND A. WOOD
and compiled using BDMSALGOL on the BURROUGHS 6700 at the
U.C.S.D. COMPUTER CENTER

Project funded by NSF Grant (GB-41118) to D.L. LINDSLEY

MUTANT STOCKS

More than 4,500 mutant stocks of Drosophila melanogaster maintained in 6 laboratories are included in the following computerized list which has been subdivided into sections for stocks, chromosomes, and genes. Each section has been cross-indexed and arranged in alphanumeric order. Preceding the list are a lab key and a balancer key.

The STOCK LIST section is subdivided in accordance with the component chromosomes of each stock. In stocks homozygous for recessive genes, the recessive genotype is listed once. Stocks segregating for recessives are written as heterozygotes in which the wild-type alleles of the recessives are listed as follows: +1(=wild X); +2(=wild 2); +3(=wild 3); +4(=wild 4). A zero at the beginning of a stock designation means that no free Y's are present. All chromosomes not indicated in a stock designation are assumed to be normal. Each stock entry consists of:

A. The reference number (at far left) for use in cross-indexing from the chromosome list.
B. Symbols for mutant genes and rearrangements. Homologous chromosomes are separated by slash bars and are listed in alphanumeric order. Non-homologous chromosomes are separated by semicolons. Stocks are grouped according to types of chromosomes (i.e., 1,2,3,4, 1-2,1-3,1-4,2-3,2-4,3-4,1-2-3,1-2-4,1-3-4,2-3-4,1-2-3-4) and then listed alphanumerically.
C. Explanatory note enclosed in curly brackets. (Only a few stocks have notes).
D. Two-letter lab symbols (at far right). The lab key identifies each symbol and supplies lab address, telephone number, and date of the most recent list.

The CHROMOSOME LIST section is also subdivided in accordance with the component chromosomes. Each entry includes:

A. The reference number (at far left) for use in cross-indexing from the gene list.
B. The chromosomes, grouped according to chromosome types (1,2,3,4,1-2, etc.), and then listed alphanumerically.
C. Reference numbers (at far right) for use in cross-indexing to the stock list.

The GENE LIST section includes the symbols for mutant genes, rearrangements, balancers, and special Y's, arranged in alphanumeric order. Each entry consists of:

A. The gene symbol (at left).
B. Reference numbers (at right) for use in cross-indexing to the left-hand numbers of the chromosome list.

The genetic constitution of each balancer used in the stock list, chromosome list, and gene list is given in an alphabetized balancer key.

Stock centers under federal support are located at the CT and BG labs. Many of the standard mutant stocks are kept at these labs, and orders should be sent to them whenever possible. In ordering a stock, please give its complete genetic constitution, as indicated by the entry in the stock list. Numbers serve only for cross-indexing within a list and will change with each updating.

LAB KEY

AX=Austin, Texas 78712: The University of Texas at Austin, Department of Zoology, Genetics Foundation. Tel (512) 471-4128. {1977}
BG=Bowling Green, Ohio 43403: Bowling Green State University, Mid-America Drosophila Stock Center, Department of Biology, Genetics Research Group. Tel (419) 372-2631. {1972}
CT=Pasadena, California 91125: California Institute of Technology, Division of Biology. Tel (213) 795-6811. {1977}
DV=Davis, California 95616: University of California, Department of Genetics. Tel (916) 752-6295. {1977}
LJ=La Jolla, California 92093: University of California, San Diego, Department of Biology. Tel (714) 452-3109. {1977}
MS=Madrid 34, Spain: Centro de Biologia Molecular, C.S.I.C., Facultad de Ciencias, C-X, Universitad Autonoma de Madrid. Tel 7 34 01 00. {1977}

KEY TO BALANCER CHROMOSOMES

Basc = In(1)$sc^{S1}L_{sc^{8}R}$ In(1)S, sc^{8} sc^{S1} w^{a} B
Binsc = In(1)$sc^{S1}L_{sc^{8}R}$ In(1)dl-49, sc^{8} sc^{S1} B
Binscy = In(1)$sc^{S1}L_{sc^{8}R}$ In(1)dl-49, y sc^{8} sc^{S1} B
Binsn = In(1)$sc^{S1}L_{sc^{8}R}$ In(1)dl-49, sc^{8} sc^{S1} sn^{X2} B
C(1)M4 = C(1)M4, In(1)w^{m4} In(1)AB, w^{m4} bb -- In(1)FM7a, y^{-} w^{a} v^{Of} bb
C(2)EN = 2R2L•2L2R
C(3)x = In(3L)P In(3R)P {probably not same as Payne inversions}
ClB = In(1)Cl, sc 1(1)C_t^{2} v sl^{1} B
CyO = In(2LR)O, Cy dp^{lvI} pr cn^{2}
CyO^{nA} = In(2LR)O, Cy dp^{lvI} Adh^{nA} pr cn^{2}
CyO^{nB} = In(2LR)O, Cy dp^{lvI} Adh^{nB} pr cn^{2}
FM7Y = y^{+}YSX•YLB^{S}, In(1)FM7a^{L}EN^{R}, y y^{+} B^{S}
FM1 = In(1)sc^{8} In(1)dl-49, y^{31d} sc^{8} w^{a} lz^{s} B
FM3 = In(1)FM3, y^{31d} 1(1)$J1^{FM3}$ sc^{8} dm B 1(1)?
FM4 = In(1)FM4, y^{31d} sc^{8} dm B
FM6 = In(1)FM6, y^{31d} sc^{8} dm B
FM7 = In(1)FM7, y^{31d} sc^{8} w^{a} sn^{X2} v^{Of} g^{4} B
FM7a = In(1)FM7, y^{31d} sc^{8} w^{a} v^{Of} B
FM7b = In(1)FM7, y^{31d} sc^{8} w^{a} lz^{s} B
FMO = FM1 inversions with markers y^{31d} sc^{8} dm cv v m^{2} g^{4} f B
Insc = In(1)$sc^{S1}L_{sc^{8}R}$ In(1)dl-49, sc^{8} sc^{S1}
Inscy = In(1)$sc^{S1}L_{sc^{8}R}$ In(1)dl-49, y sc^{8} sc^{S1}
LVM = In(3L)P In(3R)P, 1(3)LVML 1(3)LVMR
Maxy(a) = In(1)$sc^{S1}L_{c}{}^{8R}$, y sc^{8} sc^{S1} pn w ec rb cm ct^{6} sn^{3} ras^{2} g^{2} f os^{s} os^{o} car 1(1)? {multiply marked X from Maxy stock}
Maxy(b) = In(1)$sc^{S1}L_{sc^{8}R}$, y sc^{8} sc^{S1} pn w ec rb cm ct^{6} sn^{3} ras^{2} v dy g^{2} f os^{s} os^{o} car 1(1)? {multiply marked X from another Maxy stock}
MKRS = Tp(3)MKRS, M(3)S34 kar ry^{2} Sb
MRS = Tp(3)MRS, M(3)S34 ry^{2} Sb
SM1 = In(2LR)SM1, al^{2} Cy cn^{2} sp^{2}
SM2 = T(2;3)SM2, al^{2} Cy lt^{v} cn^{2} sp^{2}
SM5 = In(2LR)SM5, al^{2} Cy lt^{v} cn^{2} sp^{2}
TM1 = In(3LR)TM1, Me ri sbd^{1}
TM2 = In(3LR)Ubx^{130}, Ubx^{130} e^{s}
TM3 = In(3LR)TM3, y^{+} ri p^{p} sep bx^{34e} e
TM6 = In(3LR)TM6, ss^{P88} bx^{34e} Ubx^{P15} e

CORRECTIONS FOR GENE LIST

These corrections are found on the last page of the GENE LIST. They also apply to listings of these genes in the stock list and chromosome list.

Triantaphyllidis, C.D. Aristotelian University of Thessaloniki, Greece.

Esterase and leucine aminopeptidase isozymes of D. auraria.

Starch gel electrophoresis was used to examine the esterase (Est-6 and Est-C locus) and leucine aminopeptidase (Lap-D and Lap-A locus) allozyme patterns in D. auraria stocks. D. melanogaster (Oregon R) and D. simulans stocks were used as reference material. In order to facilitate species comparisons, the allozyme controlled by the most common allele of D. melanogaster was assigned a mobility of 1.00; all other allozymes of the three species used were compared to that standard and named accordingly. Thus, Est-6^S of D. melanogaster is designated here as Est-$6^{1.00}$, Est-C^F of the same species is designated as Est-$C^{1.00}$, while Lap-D^F is designated as Lap-$D^{1.00}$.

1. Esterases: A total of six regions (A to F) of esterase activity were observed in the zymograms from single D. auraria individuals. (In Figure 1 only Est-C, Est-D and Est-A are shown.) These regions correspond to the A to F esterase regions of D. melanogaster (Beckman and Johnson, 1964). D. auraria exhibited five esterase zones in the C and D regions

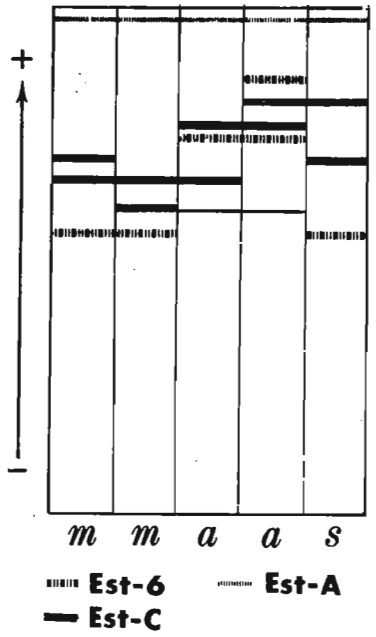


Figure 1. Schematic representation of the relative positions of Est-6, Est-C and Est-A in D. melanogaster (m, genotype $6^{1.00}/6^{1.00}$; $C^{1.00}/C^{1.07}$ and $6^{1.00}/6^{1.00}$; $C^{\cdot 91}/C^{1.00}$), D. auraria (a, genotypes $6^{1.35}/6^{1.35}$; $C^{1.00}/C^{1.18}$ and $6^{1.35}/6^{1.57}$; $C^{1.18}/C^{1.25}$) and D. simulans (s, genotype $6^{1.00}/6^{1.00}$; $C^{1.07}/C^{1.25}$).

(cf. Fig. 1). Of these, two were colored red (D region, as Est-6 of D. melanogaster) and were called Est-6, while the rest were colored blue-black (C region, as Est-C of D. melanogaster) and were called Est-6 (Triantaphyllidis and Christodoulou, 1973). Matings were performed to determine the mode of inheritance of the Est-6 and Est-C variants. The data obtained suggest that the variants are controlled by codominant alleles at two different autosomal loci.

Comparisons of the electrophoretic mobilities of the Est-6 and Est-C zones of D. auraria with those of D. melano-

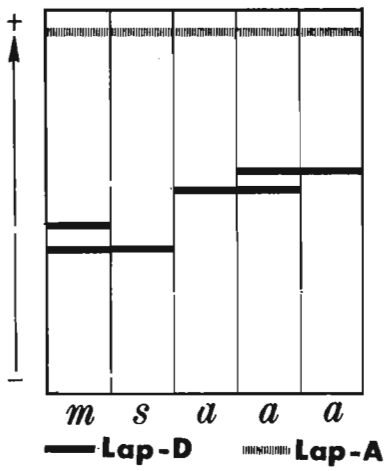


Figure 2. Schematic representation of the relative positions of leucine aminopeptidase in D. melanogaster (m, genotype $D^{\cdot 85}/D^{1.00}$), D. simulans (s, genotype $D^{\cdot 85}/D^{\cdot 85}$) and D. auraria (a, genotypes $D^{1.16}/D^{1.16}$, $D^{1.16}/D^{1.28}$ and $D^{1.28}/D^{1.28}$).

gaster show that the D. auraria zones have relative mobilities 1.35 and 1.57 for Est-6 and 1.00, 1.18 and 1.25 for Est-C. From this and Fig. 1 it becomes evident that there are some common zones among the three species studied.

In addition to the above mentioned zones a faint blue-black zone was observed mostly in pupae and sometimes in adults of D. auraria having a relative mobility of 0.91 when compared to Est-$C^{1.00}$ of D. melanogaster.

The Est-6 and Est-C bands of D. auraria exhibit the same activity as Est-6 and Est-C of

D. melanogaster (Beckman and Johnson, 1964a) and D. simulans (Triantaphyllidis, 1973) in all developmental stages studied.

Also, in all three species studied the Est-6 zones have greater activity in the males while the Est-C zones have greater activity in the females.

2. Leucine aminopeptidases: In the zymograms from single pupae of D. auraria, two electrophoretic bands were observed in the Lap-D and one in the Lap-A region (Fig. 2). The relative mobilities of the two Lap-D zones in reference to Lap-D$^{1.00}$ of D. melanogaster (Beckman and Johnson, 1964b) were 1.16 and 1.28; Lap-A exhibits the same mobility in all three species studied. Crosses within and between stocks exhibiting these bands showed that the Lap-D variants are controlled by a pair of codominant autosomal alleles.

Pupal homogenates of D. auraria exhibit strong Lap-A and Lap-D zones. For the remaining developmental stages the situation for Lap-A and Lap-D was similar to that described by Sakai et al. (1969) for D. melanogaster.

References: Beckman, L. and F.M. Johnson 1964a, Hereditas 51:212-220; Beckman, L. and F.M. Johnson 1964b, Hereditas 51:221-230; Sakai, R.K., D.A. Tung and J.G. Scandalios 1969, Mol. Gen. Genetics 105:24-29. Triantaphyllidis, C.D. 1973, J. of Heredity 64:69-72; Triantaphyllidis, C.D. and C. Christodoulou 1973, Bioch. Genetics 8:383-390.

Ivanov, Yu.N., M.D. Golubovsky and I.D. Erokhina. Institute of Cytology and Genetics, Novosibirsk 90, USSR. Maintaining of instability of some singed alleles in D.m. without any selection.

The sharp increase of mutation rate in the sex linked singed gene has been observed in geographically isolated populations since 1973. Many regions from Crimea to the Far East were involved[1,2]. Most of the alleles arisen under laboratory conditions in the Muller-5 test or isolated directly from natural populations had an extraordinary ability to mutate back to normal or pseudonormal alleles. The unstable mutations sn77-27, sn63-15 (from Tashkent 1973), sn84-6, sn26-7 (from Krasnodar 1974), sn49-5 (from Far East 1975) produce unstable phenotypically normal alleles which are capable of producing the unstable sn condition again. The property of instability and its character are allelic specific and persist without any selection for many generations in mass laboratory cultures. The data are given in Table 1. The persistance of the unstable condition of these and other alleles was also found by M.M. Green who is investigating this phenomenon in detail

Table 1. The instability of singed alleles determined immediately after isolation and after one year of maintainance without any selection.

Crosses: 1 ♂ sn x ♀♀ XX/Y

Population and year	Allele	Immediate testing			After about 10 generations		
			reverses*			reverses	
		sn	incomplete	complete	sn	incomplete	complete
Tashkent 1973	77-27	674	0	64	322	0	8**
Krasnodar 1974	84-6	115	0	14	7006	2	6
Canton-S 1974	73-8(M5)	196	3	5	1594	64	11
Krasnodar 1974	50-18	133	0	1	761	15	17
Krasnodar 1974	26-7	+	+	+	5059	11	9
Krasnodar 1974	90-9	103	2	1	5300	9	11
Tashkent 1973	63-15	+	+	+	1817	6	5

*Two types of reversions to normal condition were observed: complete (wild phenotype) and incomplete (slightly mutant); sign "+" indicates the presence of named phenotype when exact count was not made; Canton-S - laboratory cage population; **underlined cases where complete reversions were also unstable.

(personal communication). The hypothesis is suggested that some infectious agent widespread throughout the continent is capable of being inserted as episome in the region of singed locus and leaving it, inducing the unstable condition.

References: [1]Berg, R.L. 1974, DIS 51:100; [2]Ivanov, Yu.N. 1974, DIS 51:74.

Schäfer, U. University of Düsseldorf, Germany. Analysis of two heterosome-autosome translocations in D. hydei.

In D. hydei a reciprocal translocation between the Y chromosome and one of the four telocentric autosomes was induced by X ray treatment (Hess, 1963). The results were T(A;Y^L) which includes the distal part of the long arm of the Y together with the autosomal centromere and surrounding heterochromatin and T(Y^S;A) consisting of the short arm, the centromere, and the proximal part of the long arm of the Y combined with the entire euchromatic part of the autosome. As a result of the particular location of the autosomal breakage point in the centromeric heterochromatin both elements of the translocation can segregate independently without causing morphogenetic defects. Therefore, these two translocation chromosomes could, for instance, be used to build the so-called combination stocks which were important tools in the analysis of the relationship between Y chromosomal lampbrush loops and male fertility (Hess, 1970).

With the aid of a special stock in which all five pairs of autosomes carried recessive genetic markers ("multi^5"), the autosome involved in the translocation could be identified. Females of multi^5 carrying, in addition, an attached-X ($\overline{XX}$) and the translocation chromosome T(Y^S;A) but no free Y chromosome were crossed to males with an X-Y translocation. The T(X;Y) chromosome is complementary to the other translocation element with respect to the Y chromosomal male fertility genes, so that the male progeny receives a full set of the Y chromosomal fertility factors. The F_1 males were then crossed to multi^5 females with either an $\overline{XX}$ or two wild type X chromosomes. Because the T(X;Y) and the T(Y^S;A) segregate almost regularly, the phenotype of the F_2 indicates what autosome is translocated since the T(Y^S;A) carries one of the five markers and crossing over does not occur in D. hydei males. In the cross with the $\overline{XX}$-females all F_2 females and no F_2 males (with rare exceptions only) showed the phenotype vestigial. The other autosomal markers were statistically distributed among the female and male progeny. In the second cross the reversed result was expected. In fact, apart from a few exceptions no F_2 females but all F_2 males carried the mutation vestigial. This clearly demonstrates that chromosome 5 according to the classification of Berendes (1963) or chromosome 3 in Spencer's classification (1949) is involved in this Y-autosome translocation.

When $\overline{XX}$-females carrying the T(Y^S;A) chromosome were X irradiated, X-autosome translocations were comparatively often induced (Hess, 1976). One of these translocations (listed as 703/33) was analysed in a similar manner as described above. $\overline{XX}$-multi^5 females were crossed to 703/33 males and the F_1 males were then crossed to either $\overline{XX}$-multi^5 or X/X-multi^5 females. In both cases the distribution of autosomal markers among the F_2 was identical to the crosses which were made to analyse T(Y^S;A). Furthermore, repeated backcrossing of 703/33 males to $\overline{XX}$-multi^5 females does not result in any male homozygous for the mutation vestigial. This confirms the expectation that the X-autosome translocation induced by detachment of $\overline{XX}$/T(Y^S;3) females is an X-3 translocation.

We became interested in the T(X;3) translocations because it was found that the males in the translocation stocks exhibited a high tendency to accumulate two Y chromosomes. Therefore, we supposed that T(X;3) is correlated with a high percentage of spontaneous nondisjunction (ND) of the sex chromosomes. To test this hypothesis 703/33 males with only one Y chromosome were produced by X ray induced ND. The males were crossed to C(1)Tp(1)/Y females (kindly provided by H. Beck). The compound chromosome of these females consists of twice the euchromatic part of the X chromosomal transposition white-mottled^1. This transposition results in a shift of rDNA into the euchromatic X arm and, therefore, this C(1) chromosome carries ribosomal RNA genes (Beck, 1976). As a consequence, females with C(1)Tp(1) and without a Y chromosome or a fragment thereof are viable in contrast to females with a normal $\overline{XX}$ deficient for the ribosomal cistrons. In the above mentioned cross, 13.9% (142 out of 1020) of the female progeny showed a clear-cut bobbed phenotype. They turned out to be C(1)Tp(1) females without a Y chromosome as a consequence of spontaneous ND in the T(X;3) males. These C(1)Tp(1)/O females were crossed to wild type males and in complete accordance with the expectations all male progeny was X/O.

A similar technique for the recovery of C(1)Tp(1)/O females and their use for the mass production of X/O males was independently developed by Beck (1976). Our method described here takes advantage of the high rate of spontaneous ND in the T(X;3) males which may be due to the absence of ribosomal genes in the T(X;3) chromosome and has been used in our institute for routine production of X/O males of D. hydei with continuously good results.

References: Beck, H. 1976, Genet. Res. 26:313-317; Berendes, H.D. 1963, Chromosoma 14: 195-206; Hess, O. 1965, Chromosoma 16:222-248; ___ 1970, Molec. Gen. Genet. 106:328-346; ___ 1976, in: The Genetics & Biology of Drosophila (Ashburner & Novitski, eds.) Vol. 1c:1343-1363; Spencer, W.P. 1949, in: Genetics, Palaeontology & Evolution (G.L. Jepsen et al. eds.) pp 23-44.

Pasteur, G. Ecole Pratique des Hautes Etudes, Montpellier, France. Affinities of Zaprionus and chromosomal evolution.

Wilson et al. (1969) stated that African species of Zaprionus are "fairly certainly" related to the immigrans group of Drosophila, and included the non-African species into a lineosa subgroup of the D. immigrans group. Such a partition of the genus Zaprionus may seem arbitrary, since reasons for it were not given and the Wilson et al. lineosa subgroup species were said to be "similar to Zaprionus, s.s., in having prominent silvery to chalky-white longitudinal stripes on the mesonotum", indeed the striking character of Zaprionus flies. In fact, the partition does not seem tenable on chromosomal grounds.

The immigrans group mitotic chromosome configuration (Figure 1) is characterized by a NF of 8 and two of the four haploid arms being linked in a rather large metacentric. This implies two independent changes from the five-rods primitive haploid karyotype of Drosophila.

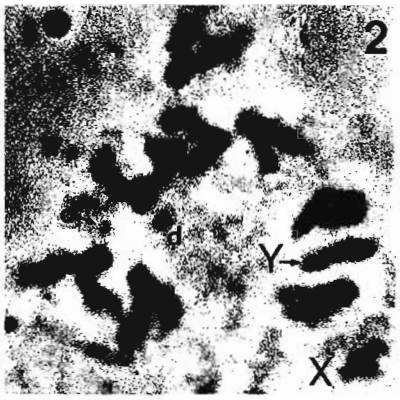


Figure 1. Larval brain mitotic chromosomes in an African species of the Drosophila nasuta complex of the immigrans group.

Figure 2. Larval brain mitotic chromosomes in a widespread African species of Zaprionus, Z. vittiger (for another species see Tsacas et al., 1977).

d. dot chromosome.

One change is the centric fusion that gave rise to the metacentric chromosome. The other, more significant change is the process that led from five to four pairs of arms.

Mitotic chromosome configurations of the following species of African Zaprionus groups have been investigated: from the inermis group, Z. ghesquieri and Z. inermis; from the tuberculatus group, Z. sepsoides and Z. tuberculatus; from the vittiger group, Z. ornatus and Z. vittiger. The same primitive set of ten rods (NF = 10) and two dots has been uniformly observed, with a heteropycnotic Y (Figure 2). Clearly, flies with such karyotypes can hardly be closely related to the immigrans group. Now, Zaprionus multistriatus Sturtevant, of southeast Asia, has the same karyotype; it is even farther off since its Y is not differentiated cytologically (Sciandra et al., 1974). Therefore, ranging multistriatus in the immigrans group of Drosophila is no longer warranted.

Karyotypes of two other members of the Wilson et al. 1969 lineosa subgroup are known - those from Drosophila argentostriata Bock, 1966, and D. silvistriata Bock and Baimai, 1967. Bock (pers. comm.) no longer thinks these species belong to the subgenus Sophophora as he described. Actually they should be called Zaprionus argentostriatus (Bock) and silvistriatus (Bock and Baimai), knowing that the former has just the same chromosome set as Z. multistriatus and the latter only differs from other chromosomally known Zaprionus species by the centric fusion of two shorter acrocentrics. Z. silvistriatus retains the NF of 10, and the loss of one shorter acrocentric is clear between such a karyotype and a karyotype of the immigrans sort. The gap is important, and investigations are prepared to test the working hypothesis that members of the so-called lineosa subgroup, whether once classified as Zaprionus or not, are all Zaprionus species.

References: Bock, I.R. 1966, Univ. Queensland Pap. Zool. 2:269-276; Bock, I.R. & V. Baimai 1967, Univ. Queensland Pap. Zool. 3:17-25; Sciandra, R.J., F. Andel-Hameed & J. Bennett 1974, J. Hered. 64:31-34; Tsacas, L., J. David, R. Allemand, G. Pasteur, M.T. Chassagnard & S. Derridj 1977, Ann. Soc. Entom. France 13:391-415; Wilson, F.D., M.R. Wheeler, M. Harget & M. Kambysellis 1969, Stud. Genet. 5:207-253.

Research supported by RCP 318 of C.N.R.S., Paris.

Golubovsky, M.D. Institute of Cytology and Genetics, Novosibirsk 90, USSR.

Unstable lozenge and "fine bristle" mutations from Far East population.

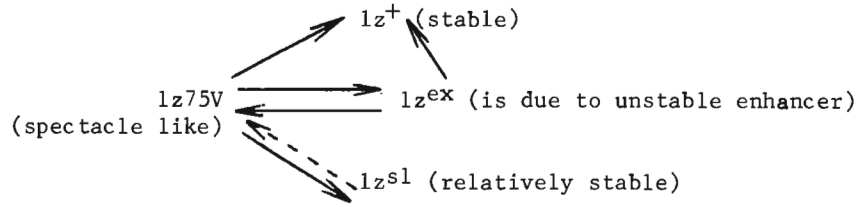
In 1975 we analyzed the phenotype of flies from one Far East population (near Vladivostok). Among 3050 wild males we found a relatively high frequency of males with fine, small or partly reduced bristles (0.65%). Sex linked inheritance was tested by crossing of anomalous males with $\overline{XX}$ females. Most of the males do not transmit the "fine bristle" feature to the F_1 sons. The progeny of some males were partly mutant (so the parents were gonadal mosaics). Only one out of 20 tested anomalous wild males gave all F_1 sons having the phenotype "fine bristle" (or fbr). In the F_2 of mass crossings of F_1 males with ♀♀ $\overline{XX}$, two lozenge males were discovered. The results of further crosses were as follows: $F_2 \rightarrow F_3$: 1♂ lz fbr x ♀♀ $\overline{XX}$ → 53 ♂ lz fbr; 2♂ normal eyes fbr; 1♂ lz with normal bristles. $F_3 \rightarrow F_4$: 1♂ lz (normal bristles) x ♀♀ $\overline{XX}$ → 82 ♂ lz; 4♂ lz^+; 2♂ $lz^{extreme}$. So it appears that both lozenge and fine bristle are unstable. The last sex-linked mutation was named fbr and was precisely located in position 3.3 m.u. The lozenge allele designated as lz75V belongs to spectacle group of alleles of that locus; eye size reduced, oval, no true facets, red brown, creamy in combination with vermilion, claws are vestigial, female sterile. The mode of instability of lz75V allele was tested in individual crosses with $\overline{XX}$ ♀♀ in a series of successive generations. The results are given in Table 1. The original allele may mutate to three conditions: 1) absolutely normal phenotype (females are fertile), 2) lz^{slight} derivative - eyes almost normal in shape and

Table 1. The direction and frequency of mutations of lz75V unstable allele and its derivatives. Crosses with $\overline{XX}$ ♀♀.

Phenotype of males	Number of males	Phenotype of F_1 progenies: lz75V	lz^+	lz^{sl}	lz^{ex}	Total
lz 75V	109 (40)*	4119	121	9	1	4250
	mass crosses	1633	35	0	1	1669
		5752	156	9	2	5919
lz^+(revert.)	68	0	6072	0	0	6072
lz^{sl}	5 (1)	1	0	275	0	276
	mass crosses	0	0	970	0	970
lz^{ex}	mass crosses	**	7	0	251	258

* number of individual crosses in which reverses occurred.
** reversions to lz75V were found in other series of mass crosses.

colour, with rough facets on the rim, claws are yellow and reduced, female sterile, 3) $lz^{extreme}$ - eyes are oval, glossy, pink brown or pale, partly male sterile, viability very poor. This derivative is unstable and mutates to original and absolutely normal phenotype. It appears that lz^{ex} condition is connected with the recessive enhancer of lozenge located in 23.4 m.u., or 4.3 units from the lz locus. The en(lz) is also unstable so we observe a change from lz^{ex} to lz75V. The en(lz) have no special phenotypic expression and when the original lz75V allele mutates, it is possible to see reversions from lz^{ex} to lz^+. The picture of mutational changes is the following:



The original mutant lz75V and its derivatives are absolutely normal in phenotype and female fertile in compounds with lz^{50} allele. The biochemical nature of unstable lz75V is under investigation. It is possible to note here that unstable singed allele sn49V was found in this population simultaneously.

Sasaki, M. and S. Narise. Josai University Sakado, Saitama, Japan. Molecular weight of esterase isozymes of Drosophila species.

The molecular weight of esterase isozymes in eleven species belonging to melanogaster group of Drosophila was determined by means of gel filtration. The laboratory strains of these species were obtained from Dr. T.K. Watanabe of the National Institute of Genetics, Japan. The esterase zymograms on agar gel electrophoresis of fly extracts used in this study showed one major band for either α- or β-esterase in each species, except D. lutescens which possessed three bands for β-esterase locus (Figure 1). These two esterases controlled by α- and β-esterase loci, respectively, were isolated and molecular weights were determined in the following ways: Five grams of adult flies were homogenized in 0.1 M Tris buffer at pH 7.4 and the homogenate was centrifuged at 10,000 rpm and the precipitate was discarded. 40 to 80 per cent saturated amonium sulfate fractions of the supernatant were collected, dissolved in a small amount of 20 mM phosphate buffer pH 7.4 and applied to a Sephadex G-150 column (2.5 x 80 cm or 2.5 x 50 cm) equilibrated with the same buffer. Sephadex G-150 column for the estimation of molecular weight was calibrated with myoblobin (18,000), chymotrypsinogen (25,000), ovalbumin (45,000), BSA (67,000) and BSA dimer (134,000). All procedures were conducted at 4°C. The enzyme activity was assayed by a colorimetric method as previously reported[1], using α- or β-naphthyl acetate as substrate. Elution patterns of the esterases through the gel filtration showed two peaks of activity; one fast migrating was β-esterase, the other slow migrating was α-esterase. Aliquotes of each peak fraction were subjected to agar gel electrophoresis, and the mobility and color of bands on gel were compared with those of the extract of flies of the species. In elution patterns of D. melanogaster and D. simulans, only one peak of activity was found, corresponding to α-esterase of other species and electrophoretic analysis revealed that both α- and β-esterases were included in the peak. This means that in both species, the molecular weights of the two esterases are equal and the same as those of α-esterase of other species. The molecular weights of the two isozymes were estimated from elution volume of enzymes using the calibration curve mentioned before. The results were given in Table 1. As indicated in this table, molecular weights of α-esterase of all species examined seemed to be equal. The same tendency was found in β-esterase except two species. The molecular weight of β-esterase in melanogaster subgroup, as described above, was 51,300 daltons, which corresponded to α-esterase.

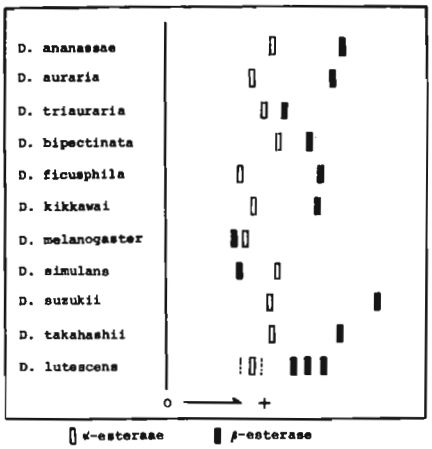


Figure 1. Esterase zymograms of Drosophila species.

Table 1. Molecular weight of esterase isozymes of Drosophila species estimated by gel filtration.

Species	α-esterase	β-esterase
melanogaster group		
ananassae subgroup		
D. ananassae	51,900	111,000
D. auraria	51,000	102,300
D. triauraria	50,200	111,000
D. bipectinata	51,900	110,000
ficusphila subgroup		
D. ficusphila	50,200	111,000
D. kikkawai	51,000	102,300
melanogaster subgroup		
D. melanogaster	51,300	51,300
D. simulans	51,300	51,300
suzukii subgroup		
D. suzukii	52,500	------
takahashii subgroup		
D. takahashii	51,000	102,000
D. lutescens	52,500	104,000
Means	51,300 ± 793	106,200 ± 1,440*

* The mean was obtained from the values of β-esterases except melanogaster subgroup.

Narise previously described the molecular weight of β-esterase of D. pseudoobscura as about 100,000[2], that of D. virilis as about 140,000[1] and that of α-esterase of D. virilis as 75,000[1]. In order to confirm the discrepancy between D. virilis and other species, molecular weights of these two esterases were determined again with the column used in this study. The results have shown that the β-esterase has molecular weight of 102,300 and the α-esterase,

51,000, both being identical to those of melanogaster group and that misestimation of molecular weights of D. virilis esterases was due to use of inadequate proteins as markers for calibration.

References: 1) Narise, S. 1973, Japan. J. Genetics 48:119; 2) Narise, S. and J.L. Hubby 1966, Biochem. Biophys. Acta 112:281.

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Genetic and cytological localization of "aldox".

As part of a larger project we have investigated the localization of aldox, the structural gene for the enzyme aldehydeoxidase (1), both on the meiotic as well as on the cytological map.

Recombinant males were recovered from females heterozygous for cu red sbd sr e/aldox^{n}, a negative allele lacking aldehydeoxidase activity. These recombinants were individually mated to aldox^{n}/aldox^{n} virgin females, and 8 offspring of each cross were tested with the "rapid spot assay" (1) for aldehydeoxidase activity. Based on 64 crossovers between red and sbd, the locus for aldox was found to be just to the left of sbd, at position 57.08-57.4.

To determine the cytological localization of aldox, wildtype males of strain "Sevelen"

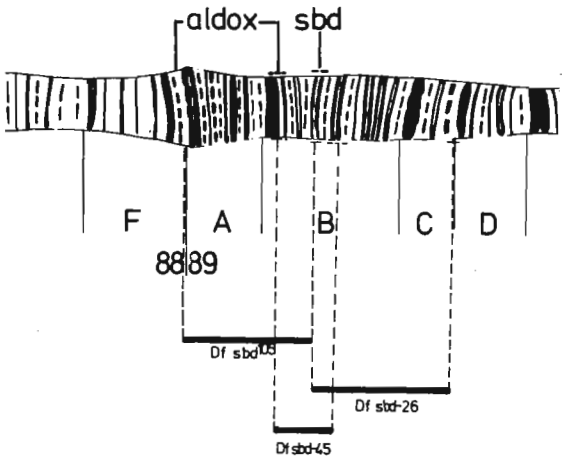

Figure 1. Cytological localization of aldox.

were irradiated with 4000 r (50 kV, 25 mA, 0.3 mm Al, 1000 r/min) and then mated to cu red sbd sr e virgins. Newly induced sbd phenotypes were selected and the treated chromosomes were introduced into balanced stocks (TM2). A cytological analysis was carried out with those sbd mutants that were recessive lethals, assuming that some of them would be deficiencies including or not the locus for aldox. Using three deficiencies (Figure 1), we could assign aldox to a short region ranging from 88F9-89A1 (left end of Df(3R) sbd^{105}) to 89B1-4 (left end of Df(3R)sbd-45). The locus for aldox is deleted in Df(3R)sbd^{105}, but is still present in Df(3R)sbd-45 as well as in Df(3R)sbd-26.

We want to thank Dr. E.B. Lewis for valuable help in identifying the chromosome rearrangements.

Reference: Dickinson, W.J. 1970, Genetics 66:487-496.

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An immunochemical analysis of 1(2)gl in D. melanogaster.

The general pattern of water soluble antigens of homozygotes for locus 1(2)gl in D. melanogaster (an allele causing death in the third instar larvae) was studied to compare it with the pattern of normal heterozygous individuals in the general representations of the third instar larvae by means of two-dimensional immunoelectrophoresis. The antigenic pattern of homozygous larvae contains 39 precipitation peaks, while 46 were found in the immunoelectropherograms of extracts from heterozygous individuals. The differences of qualitative character of some of them were also observed with comparative analysis of two-dimensional immunoelectropherograms. Two homo- and nine heterozygous-specific antigens were detected by analysis of electrophoretic mobilities of the antigens from both patterns. Fourteen antigens identical to the eight from no-allelic forms of enzymes of D. melanogaster (Dickinson and Sullivan, 1975;

Karakin et al., 1975) were identified in the immunoelectropherograms with the help of immunohistochemical reactions.

Only small differences were detected between homo- and heterozygous individuals by comparisons in activity of immunoprecipitate forms of the enzymes: alcohol dehydrogenase, α-glycerophosphate dehydrogenase, two forms of malate dehydrogenases, three forms of phenol oxidases, two forms of leucine aminopeptidases, two (from three) forms of immunochemically non-identical forms of esterases (antigens-enzymes of wild type of D. melanogaster, see Karakin et al., 1975). The disappearance of the fast migrating molecular form of the fast α-naphtylacetate specific (brown) esterase from homozygous larval extracts was detected, and big differences were established in both acid and alkaline phosphatases with respect to activity and quantity of the enzyme proteins. Acid phosphotase of homozygous individuals have, perhaps, an electrophoretic "pupal" high active form of enzyme, which is not active in heterozygous third instar larvae. On the contrary, alkaline phosphatase of heterozygous individuals have a more active form of enzyme and more enzyme protein than do homozygous larvae.

Our data indicate that both acid and alkaline phosphatases, and two homozygous-specific antigens were the biochemical markers of this allele of 1(2)gl of D. melanogaster.

References: Dickinson, W. and D. Sullivan 1975, Gene Enzyme Systems in Drosophila; Karakin, E., L. Korochkin and S. Sviridov 1975, DAN SSSR 222:960 (In Russian).

Karakin, E., V. Kokoza, Leonid Korochkin and S. Sviridov. Institute of Cytology and Genetics, Novosibirsk, USSR. A two-dimensional immunoelectrophoretic study of D. melanogaster development.

It was shown (Karakin et al., 1975) that 93-96 precipitation peaks of water-soluble antigens may be counted in Laurell-immunoelectropherograms of extracts from whole embryos and larvae of D. melanogaster Canton-S (0-120 hrs after oviposition). Roberts (1971) has shown the differences between instar-specific patterns of antigens in this period by means of one-dimensional immunoelectrophoresis.

In this investigation the dynamics of the formation and qualitative characteristics of the antigenic patterns were studied by means of Laurell-immunoelectrophoresis at definite points of D. melanogaster development: 1) egg laying (0 hrs); 2) hatching (20 hrs); 3) 1st larval moulting (72 hrs); 4) spiracle eversion (120 hrs after oviposition). The comparative analysis of antigenic patterns demonstrated maximum translational activity in the 2nd instar larvae (see Table).

hours of development	total antigen number
0	31
20	42
48	43
72	58
120	48

The decrease in the number of antigens at the end of the third instar larvae is associated with increased electrophoretic heterogeneity of a number of antigen fractions which is tentatively explained by the appearance of salivary gland secretion in the material studied, which unspecifically inhibits the formation of precipitation peaks (Perkowska, 1963).

Three classes of antigens with different characteristics at the period of development studied were established by the systematization of our data: 1) antigens were found at all stages of development, perhaps, species-specific antigens of mammals (Vyazov, 1962); 2) antigens were found at definite stages of development, and were also found at all of the following stages of development, perhaps, hetero-organic and/or organ-specific antigens; 3) antigens were found only at definite stages of development, stage-specific antigens.

A new type of antigens was found in our experiments. They are the antigens which demonstrate immunochemically identical, but electrophoretically different, forms of the same antigens at different stages of development. These antigens, from their characteristics of stage-specific differences, may be included in any of three classes mentioned above.

The differences of detected antigens during ontogenesis possibly reflect the differences of degree of their synthetic activity in different tissues and organs of developing D. melanogaster. From this point of view, two phases of the ontogenesis are critical: The first is the embryonic period, and the second one is the second instar larvae.

References: Karakin, E, L. Korochkin and S. Sviridov 1975, DAN SSSR 222:960 (in Russian); Perkowska, E. 1963, Exptl. Cell Res. 32:259; Roberts, D. 1971, Nature 233:394; Vyazov, O.E. 1962, Immunology of Embryogenesis (in Russian).

Lossinsky, A.S. and H.M. Lefever. University of Maryland School of Medicine, Baltimore, Maryland, and Emporia State University, Emporia, Kansas. Ultrastructural banding observations in region 1A-10F of the salivary gland X-chromosome of Drosophila melanogaster.

Band counting and map preparations of the salivary gland chromosomes of Drosophila melanogaster have undergone considerable criticism in recent years attributable either to artifacts created by acetic acid fixation (Sorsa and Sorsa, 1967a; Sorsa, 1969), by squashing techniques (Sorsa and Sorsa, 1968) or perhaps both. The reliability of such map preparations becomes understandably questionable and presents the investigator with a dilemma. Ultrastructural maps of the polytenes based on serial section information of immersed fixed salivary glands is impractical and appears virtually impossible (Skaer and Whytock, 1976). In spite of the disadvantages of the squash technique, however, it is the easiest and most practical method available for ultrastructural banding studies of the polytenes.

The purpose of this report is to present band counts obtained from transmission electron micrographs of acetic acid fixed, squashed salivary gland X-chromosomes of Drosophila melanogaster. Regions presented are: a). region 1A-4E for the purpose of comparison to the cytological maps of Bridges (1938) and Berendes (1970) using acetic acid fixative employed by Bridges (1968) compared to aldehydes used by Berendes (1970). The need to identify acetic acid fixed bands in region 1A-4E at the ultrastructural level has been presented recently (Skaer and Whytock, 1976), although the earlier report by Berendes (1968) indicated no observable differences comparing acetic acid and aldehyde fixatives of squashed salivary gland X-chromosomes, and; b). region 4F-10F, a previously unidentified region of the X-chromosome.

Chromosome preparation for electron microscopy: Salivary glands of late third instar larvae of both male and female D. melanogaster (Oregon-R, wild type stock) were excised following a previously described method (Demerec and Kaufmann, 1967). Each salivary gland was then cleaned of fatty material, bisected and placed on a siliconized (Silicad^{R}) glass slide in a modified acetic acid fixative for 2-5 minutes (Ellison, 1971). The fixative consisted of 20.0 ml H_2O, 11.5 ml lactic acid and 8.5 ml glacial acetic acid, i.e., 21.3% acetic acid. After fixation, a glass coverslip was placed over the tissue and squashed by direct thumb pressure.

Squashed X-chromosomes were then identified under the phase microscope and their location marked. Randomly selected, well puffed X-chromosomes were then sketched to facilitate later band locations in electron micrographs of similar chromosomes. The squash preparations were frozen in liquid N_2, coverslips were then removed and the preparations were dehydrated through an ethanol-acetone series. Staining was accomplished with uranyl acetate saturated in acetone.

The squash preparations were flat embedded in Araldite using Beem cylinder preparations following modifications of a number of previously described techniques (Sparivoli, Gay and Kaufmann, 1965; Sorsa and Sorsa, 1967b; Berendes, 1968; Ellison, 1970).

After polymerization of the block assemblies for 48 hours at 59°C, blocks were removed from the glass slides on a hot plate and the desired chromosomes were relocated under the phase microscope. Blocks were then trimmed, sketched and sectioned at 800-1500 Å thickness in interference color (Peachy, 1958). Both serial and randomly selected sections were collected on a variety of collodian coated, carbon stabilized grids. Photomicrographs were made using either the Hitachi HS-8 or RCA EMU 3G transmission electron microscopes at 50 kV.

Representative electron micrographs from which band counts were obtained in region 1A-10F are shown in Figures 1-10 and were labelled according to the cytological maps of Bridges (1938) and Berendes (1970). Bank observations were based on mid-region sections of 3-10 different chromosomes investigated in region 1A-10F. Chromosome tips were observed frequently (6-10 different chromosomes in regions 1A-4E) compared to areas approaching 10F (3 different chromosomes). This was attributed to a high degree of chromosome coiling and overlap in the later areas of the chromosome. Table I is a comparison of our electron microscopic observations to those of the ultrastructural map of Berendes (1970) and to the bands of the revised, light microscopic map of Bridges (1938) in region 1A-4E. Table II completes the region investigated, i.e., from region 4F-10F. Table III is a tabulation of Tables I and II compared to the bands of Bridges (1938) and shows percent calculations.

The calculations shown in Table III, suggest the following: a) Electron microscopy indicates that the band counts of the salivary gland X-chromosome of D. melanogaster appear to remain essentially unchanged using either acetic acid or aldehyde fixatives. This is based on the similarity of band reduction observed in region 1A-4E comparing the present report (68%) to the ultrastructural map by Berendes (1970) (67%), both of which were compared to the

revised map of Bridges (1938); b) It appears conceivable that the data observed in region 4F-10F (41%) and 1A-10F (50%) compared to the map of Bridges (1938), and perhaps strengthened by autoradiographic data (Kerkis, et al., 1975), are reliable; c) Finally, these results appear to support the current concept of an overall band number reduction employing electron optics compared to the revised map of Bridges (1938) and cast further doubt on the one band-one gene hypothesis of Muller and Prokofyeva (1935).

Table I. Comparison of transmission electron microscopic observations from Berendes (1970) and from the present report to the bands of the revised light microscopic, cytological map of Bridges (1938) in region 1A-4E. *Band number count of a single observation.

	Bridges (1938)			Electron microscopic observations of Berendes (1970)				Present report		
Chromosome region	Single bands	Double bands	Total bands	Single bands	Double bands	Granular areas	Total bands	Granular and single bands as one	Double bands	Total bands
1 A	6	1	8	5	-	1	6	6	-	6
B	6	4	14	6	(1)	1	8/9	4	1	6
C	1	2	5	2	-	-	2	2	-	2
D	2	1	4	-	1	1	3	3	-	3
E	1	2	5	-	1	1	3	1	1	3
F	2	1	4	3	-	1	4	4	-	4
2 A	-	2	4	4	-	-	4	1	1	3
B	6	6	18	14	-	1	15	15*	-	15
C	4	3	10	2	-	1	3	3	-	3
D	2	2	6	2	-	1	2	4	-	4
E	1	1	3	2	1	-	2	3	-	3
F	6	-	6	4	-	-	4	3	-	3
3 A	8	1	10	8	-	1	9	5	2	9
B	2	1	4	4	-	1	5	5	-	5
C	4	4	12	5	-	1	6	6	-	6
D	4	1	6	2	-	1	2	3	2	7
E	2	3	8	4	-	1	5	5	-	5
F	3	3	9	6	-	-	6	6	-	6
4 A	2	2	6	4	-	-	4	2	1	4
B	2	2	6	3	-	-	3	2	1	4
C	-	8	16	8	1	1	11	6	1	8
D	3	2	7	6	-	-	6	4	1	6
E	1	1	3	1	-	1	2	1	1	3
Totals	68	53	174	94	4	14	116	94	12	118

(Note continues through page 131)

Table II. Comparison of transmission electron microscopic observations to the bands of the revised light microscopic, cytological map of Bridges (1938) in region 4F-10F.

	Bridges (1938)			Present report		
Chromosome region	Single bands	Double bands	Total bands	Granular and single bands as one	Double bands	Total bands
4 F	-	7	14	6	-	6
5 A	6	4	14	3	2	7
B	2	4	10	3	-	3
C	4	3	10	2	1	4
D	-	4	8	2	1	4
E	2	3	8	4	-	4
F	2	2	6	2	-	2
6 A	-	2	4	1	1	3
B	-	2	4	2	-	2
C	7	3	13	5	-	5
D	2	3	8	3	-	3
E	2	2	6	3	-	3
F	1	5	11	3	-	3
7 A	2	3	8	1	1	3
B	6	1	8	1	1	3
C	3	3	9	2	-	2
D	8	7	22	4	-	4
E	5	3	11	5	-	5
F	4	3	10	5	-	5
8 A	1	2	5	2	1	4
B	4	2	8	4	1	6
C	7	5	17	9	-	9
D	4	4	12	3	-	3
E	2	5	12	4	-	4
F	2	4	10	3	-	3
9 A	5	-	5	3	1	5
B	1	7	15	3	-	3
C	-	3	6	3	-	3
D	2	1	6	3	-	3
E	2	4	10	3	-	3
F	5	4	13	1	-	1
10A	1	5	11	4	1	6
B	5	6	17	5	1	7
C	-	5	10	9	-	9
D	6	1	8	5	-	5
E	2	2	6	3	-	3
F	5	3	11	3	-	3
Totals	110	127	364	127	12	151

Table III. Tabulation from Tables I and II showing percentage calculations. **These figures present doublets included in the calculation as observed by transmission electron microscopy.

Investigator	Region investigated	Total bands	Percent of Bridges (1938)
Bridges (1938) Light microscopy	1A-4E	174	100
Berendes (1970) Electron microscopy	1A-4E	116	67
Present report	1A-4E	118**	68
Bridges (1938) Light microscopy	4F-10F	364	100
Present report	4F-10F	151**	41
Bridges (1938) Light microscopy	1A-10F	538	100
Present report	1A-10F	269**	50

References: Berendes, H.D. 1968, DIS 43:115; _____ 1970, Chromosoma 29:118; Bridges, C. B. 1938, J. Hered. 29:11; Demerec, M. and B.P. Kaufmann 1967 Dros. Guide Wash., D.C.:1; Ellison, J.R. 1970, DIS 45:117; _____ 1971, Pers. Comm.; Muller, H.J. and A.A. Prokofyeva 1935, Proc. Nat. Acad. Sci. 21: 16; Peachy, L. 1958, J. Biophys. Biochem. Cytol. 4:237; Sorsa, M. 1969, Ann. Acad. Sci. Fenn. Ser. A IV Bio. 151:1; Sorsa, M. and V. Sorsa 1967a, Chromosoma 22:32; _____ 1967b J. Ult. Res. 23:302; _____ 1968, Ann. Acad. Sci. Fenn. Ser. A. IV Bio. 127:1; Sparivoli, C.P., H. Gay and B.P. Kaufmann 1965, Stain Tech. 40(2):83; Skaer, R.J. and S. Whytock 1976 J. Cell Sci. 20:221; Kerkis, A.Yu., I.F. Zhimulev and E.S. Belyaeva, 1975, Tsitologiia 17(11):1330.

I would like to thank Dr. H. Michael Lefever, my research advisor at Emporia Kansas State College for his guidance throughout this study. The use of equipment at the University of Maryland, School of Medicine, Department of Pathology is gratefully acknowledged. The work was done in partial fulfillment for the masters of science degree at EKSC, Emporia, Kansas.

Figure 1. X-chromosome regions 1A-2A; X12,000.

Figure 2. X-chromosome regions 2A-3A. Single arrow represents a thin singlet, double arrow a granular puff; X12,000.

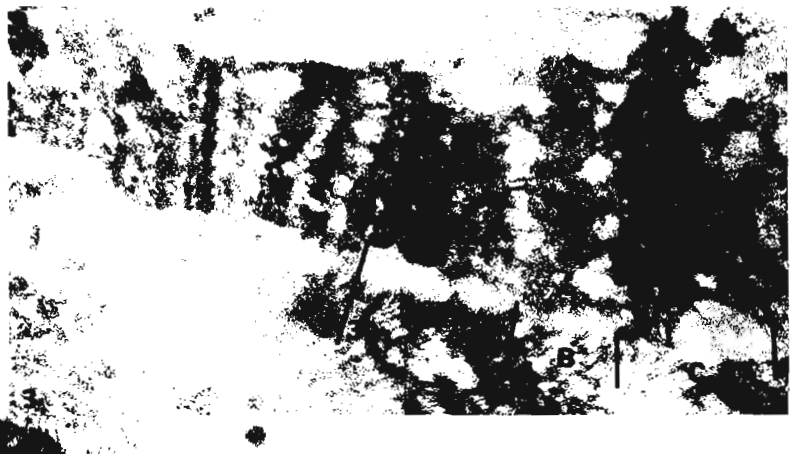

Figure 3. X-chromosome regions 3A-3C; X12,000.

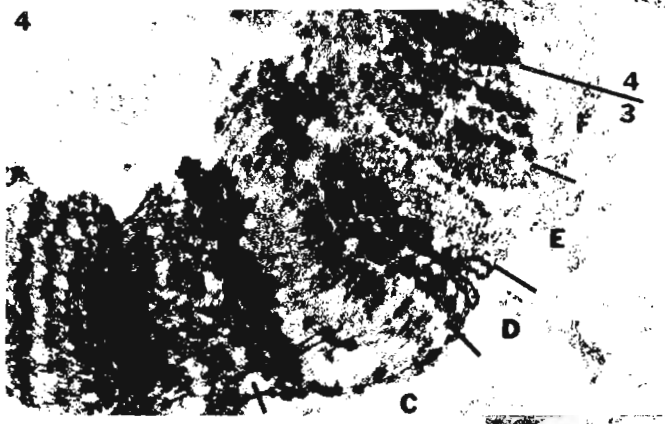

Figure 4. X-chromosome regions 3C-3F; X12,000.

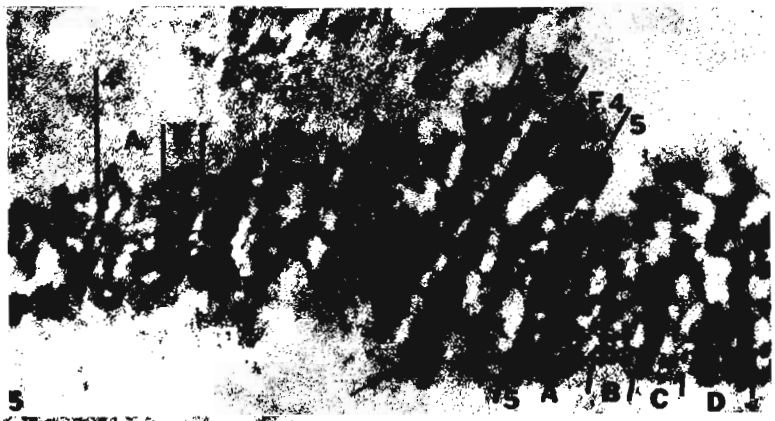

Figure 5. X-chromosome regions 4A-5D. Arrow represents a thick singlet; X12,000.

Figure 6. X-chromosome regions 5D-6F. Arrow represents a doublet; X12,000.

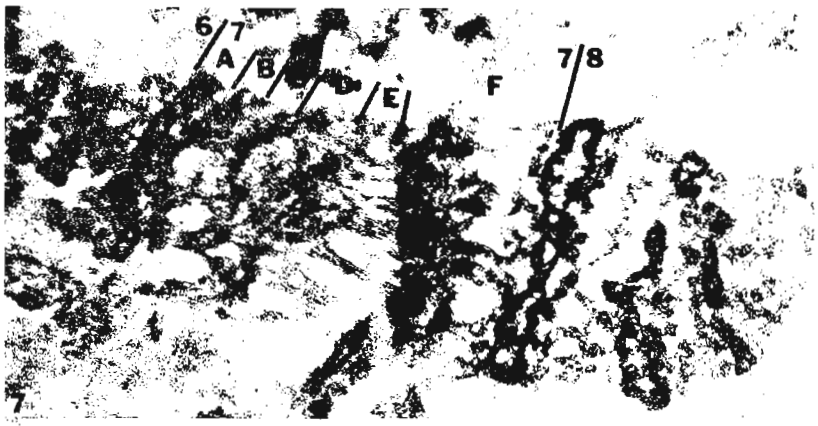

Figure 7. X-chromosome regions 7A-7F; X12,000.

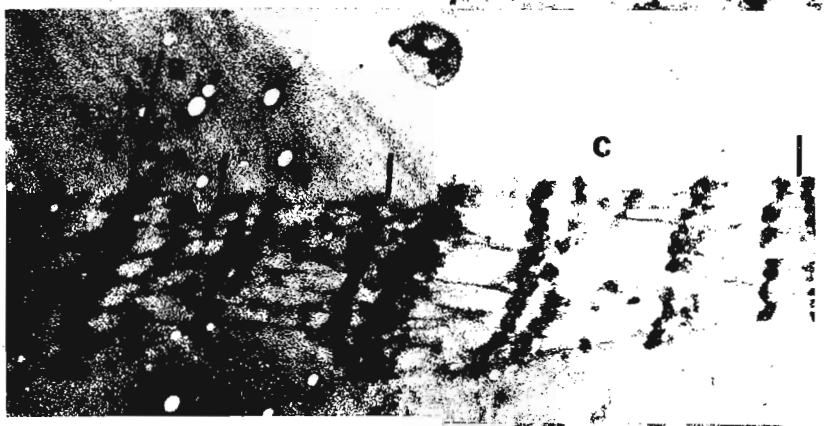

Figure 8. X-chromosome regions 8A-8C; X10,000.

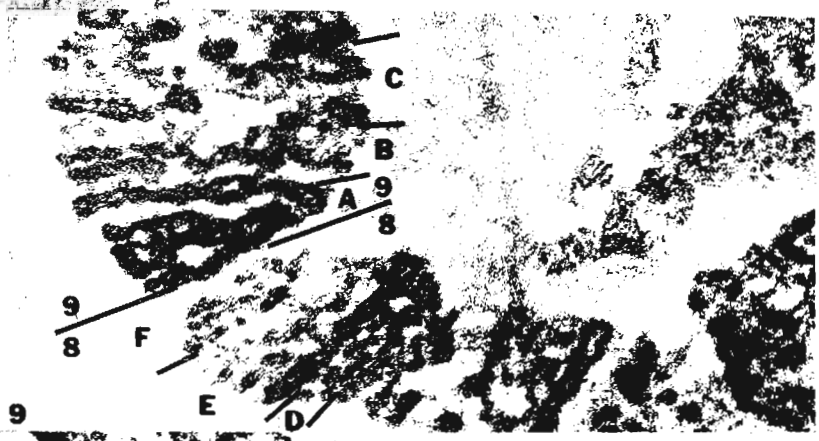

Figure 9. X-chromosome regions 8D-9C; X12,000.

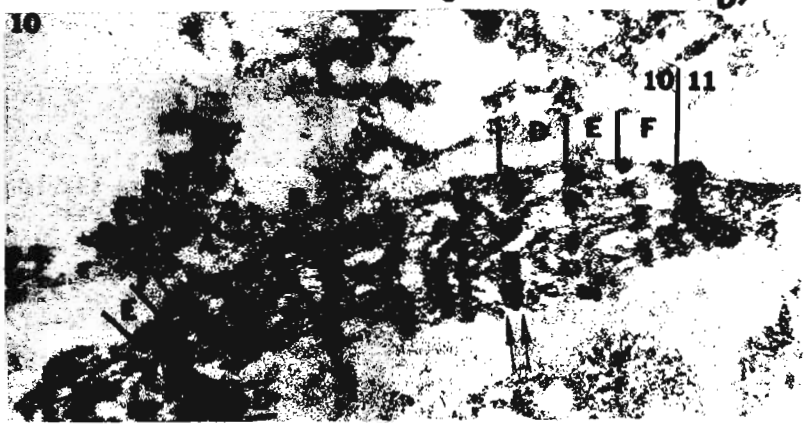

Figure 10. X-chromosome regions 9D-10F. Single arrow is an example of a double band with an apparent artifact. Double arrow represents a band composed of a series of granules. Such areas were counted as single bands; X10,000.

von Wyl, E. and P.S. Chen. University of Zurich, Switzerland. Efficient methods for isolation of X-linked male sterile mutations in Drosophila melanogaster.

We were interested in mutations affecting the accessory gland (paragonial) proteins to clarify the function of these proteins. We speculated that some of these mutations might belong to the group of X-linked male sterile mutations which interfere with sperm transfer, and from which the very low frequency of 3/192 (among the X-linked male sterile mutations) has been reported (Denell, 1973). An economic screening system should facilitate their isolation.

Our system is shown in Figure 1. Muller's standard procedure for isolation of X-linked recessive lethals and sterile factors (Muller, 1928; Spencer and Stern, 1948) was modified so that it offers in addition the following two advantages: (1) No virgin females

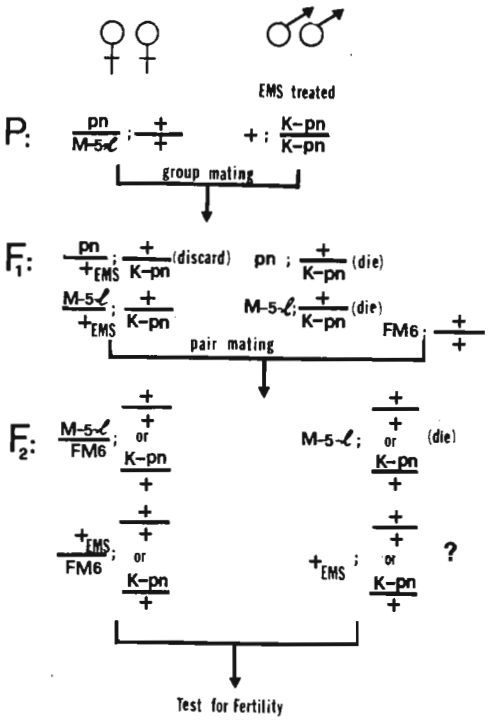

Figure 1. Mating scheme employed for isolation of X-linked male sterile mutations. In case a mutation was found, $^{+}$EMS/FM6 females were crossed to FM6 males for maintaining the mutant stock.

have to be collected in the F_1 and (2) the fertility test can be carried out very easily. For the production of virgin females in the F_1 we utilized the Killer-prune system (Sturtevant, 1955, 1956). The fertility test was simplified by introducing a lethal into the Muller-5 balancer. This M-5-1 chromosome is principally a ClB chromosome (Muller, 1928) however it offers a slight advantage in that fewer double crossing overs occur due to a larger extension of the inverted region (Spencer and Stern, 1948). The fertility test can be accomplished by simply transferring all male producing F_2 cultures into fresh vials and watching for growing larvae. FM6, used for maintaining male sterile stocks, provides a fully balanced system (FM6/FM6 females are sterile due to dm; therefore separation of female genotypes becomes unnecessary). It is also a somewhat better crossing over suppressor than Muller-5 (Grell and Lewis, 1956; Lindsley and Grell, 1968).

Ethyl methane sulfonate (EMS) was used as mutagenic

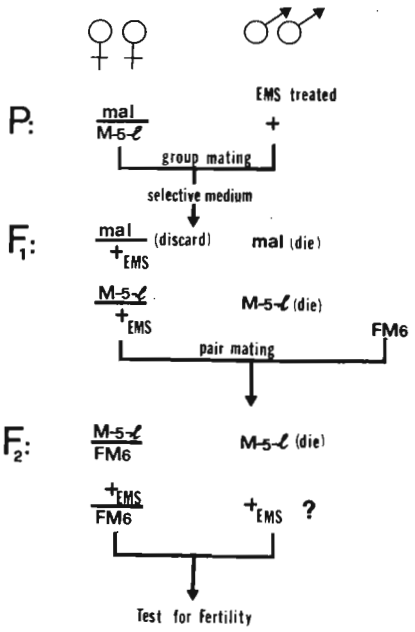

Figure 2. Hypothetical mating scheme for isolation of X-linked male sterile mutations utilizing the maroonlike system.

agent (Lewis and Bacher, 1968). Flies were fed with 10 ml of a 0.5% (v/v) EMS solution in 1% sucrose. The treatment resulted in lethal factor frequencies of between 28% and 37%. Among 9527 X chromosomes tested, 171 male-sterile mutations (1.8%) were found. With regard to mutations affecting the paragonial proteins, we were fortunate to find just one mutation. In flies bearing such mutation, a main protein band (SDS polyacrylamide gel; von Wyl, 1976) is reduced to a very low level.

Alternative systems, not realized by us, are proposed

in Figures 2 and 3. In the mating scheme presented in Figure 2, K-pn and pn genes are substituted by maroonlike (mal). Virgin females can be produced by adjusting the purine concentration in the medium to a level not tolerated by mal flies but tolerated by + flies (Glassman, 1965; Finnerty et al., 1970 a,b). Another possibility is the utilization of temperature-sensitive lethals (Suzuki et al., 1967). Among EMS treated X chromosomes bearing a single lethal, at least 10.7% can be expected to carry a heat-sensitive lethal and 1.5-3% a cold-sensitive lethal (Suzuki et al., 1967; Mayoh and Suzuki, 1973). Once realized, there is a slight advantage to this system, since separation of females in the F_1 fall away.

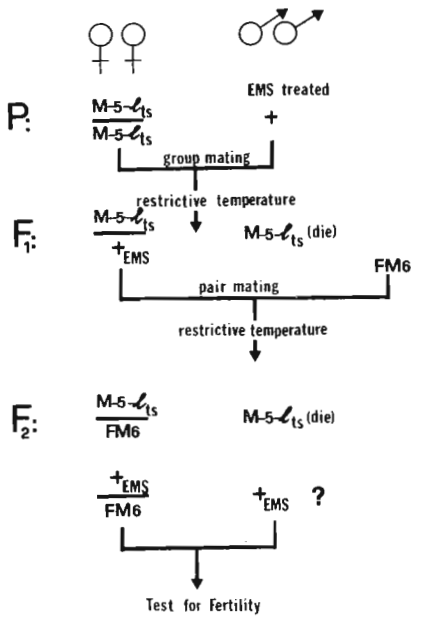

Figure 3. Hypothetical mating scheme for isolation of X-linked male sterile mutations utilizing Muller-5 balancers carrying temperature sensitive lethals.

Acknowledgement: We wish to thank Cathrine Labhart for critical reading of the manuscript and helpful discussion.

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Golubovsky, M.D. and L.A. Kulakov. Institute of Cytology and Genetics, Novosibirsk, USSR. Regulator gene in Drosophila?

In one mating of irradiated wild male with females of marker stock SM1/net al ex ds S, the F_1 "non Cy" female appeared with closed crossveins and wide wings. This female (no. 81) was gonadal mosaic on net mutation, so two sublines were established:

$\frac{81A}{\text{net al ex ds S}}$ "ds" phenotype; $\frac{81B}{\text{net al ex ds S}}$ "ds net" phenotype

The homozygotes 81/81 were received. They had very poor viability: 81A/81A - 1.5% and 81B/81B - only 0.25%. But the phenotype of these weakly viable homozygotes was absolutely unexpected: 81A/81A - strong expression of "al ex ds" mutations; 81B/81B - strong expression of "net al ex ds" mutations.

So the question arises as to why heterozygotes 81/al ex ds have no "al ex" phenotype on the background of these recessive markers. 81A/+ and 81A/1(2)gl flies were absolutely normal. But flies 81A/Df(2)al of Lewis were poorly viable (0.5%) and have once more extreme expression of genes "al ex ds" (the chromosome with Df(2)al of Lewis is deficient for genes al, ex, ds). We compared the length of aristae and number of side branches on it in +/net al ex ds and 81A/net al ex ds flies and found weak but significant reduction of these structures in the second genotype. The main conclusions about the nature of the "81" mutation are the following:

1) 81A influences the expression of three closely linked genes in the region about 0.3 m.u., but 81A is not a deficiency on these genes because homozygotes 81/81 are viable and because of the absence of any cytological abnormalities as investigated by L.S. Korochkina.

2) We suggest that three closely linked genes al ex ds are functionally connected in some manner and there exists a special locus which can regulate the expression of the named genes.

3) If we name this locus by the letter "R", then 81 mutation will correspond to R^- condition and the genotype 81A/al ex ds would be + + + (R^-)/al ex ds (R^+) and 81A/81A would be + + + (R^-)/+ + + (R^-). It can be seen that in homozygous condition the action of all three genes is almost blocked. In heterozygotes R^+ is partially dominant in relation to R^-, so weak effect of al and ds is observed. The attempt to localize this locus was made and preliminary results show that it is located left of S gene. In another series of X-ray experiments two chromosomes were obtained, no. 12 and no. 14, which had the same feature as 81A, but 14 had a rearrangement in 21D region where the suggested R locus may be located.

Bogdanova, E.S. Kurchatov Institute of Atomic Energy, Moscow, USSR. Differences of H1 histones from a fruitfly Drosophila melanogaster and a mosquito Aedes aegypti.

H1 histones of the two studied representatives of Diptera: Drosophila melanogaster and Ceratitis capitata are known to differ markedly from mammalian H1 histones in the amino acid composition. Molecular weight of Drosophila H1 histone was determined and found to be 5 - 10% higher than the mol. wt. of mammalian H1. It has been suggested that an unusual structure of Dipteran H1 histone is a prerequisite for the polytenization of chromosomes (Cohen & Gotchel, 1971; Oliver & Chalkley, 1972; Aflageme et al., 1974; Franco et al., 1974). In order to test this hypothesis, we examined histones in another representative of Diptera, a mosquite Aedes aegypti.

We compared electrophoretic mobilities of histones isolated from cell cultures of Drosophila melanogaster (2n) and of Aedes aegypti (4n) (Polukarova et al., 1975) using polyacrylamide gel electrophoresis in the presence of 1.25 M, 2.5 M, 4.0 M, 6.25 M and 8 M urea according to Panyim & Chalkley (Panyim & Chalkley, 1969). Great similarity of H3, H2A, H2B and H4 histones of both insect species was found. H1 histone of Aedes, however, under all conditions of electrophoresis used migrated markedly faster than Drosophila H1 but differed only slightly from mouse and hamster H1 (histones were isolated from cultured mammalian cells) (Figure 1).

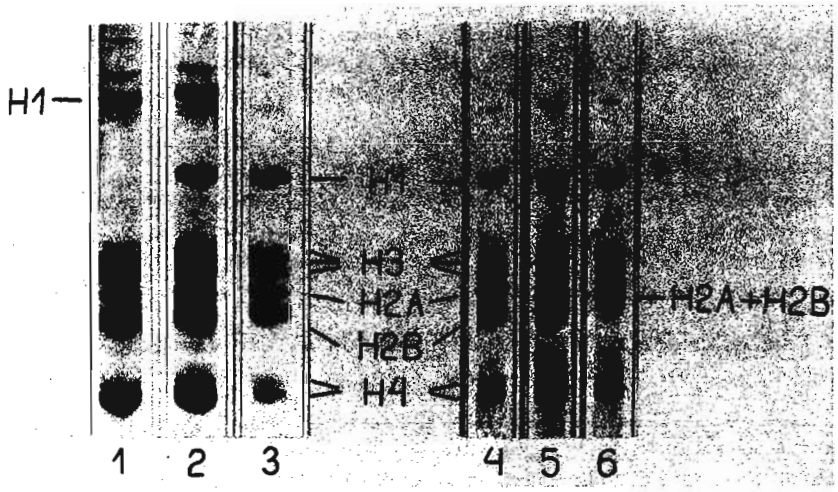

Figure 1. Electrophoretic mobility of histones from Drosophila, Aedes and mouse at 8.0 M urea. Electrophoresis was conducted in 15 cm long gels for 9 hours at ambient temperature and 200 v.
1 - Drosophila histones
2 - mixture of Drosophila and Aedes histones
3 & 4 - Aedes histones
5 - mixture of Aedes and mouse histones
6 - mouse histones

As can be judged from the electrophoretic mobility in the presence of sodium dodecyl sulphate (Panyim & Chalkley, 1971) the mol. wt. of Aedes H1 is by 6% smaller than that of Drosophila H1, but is equal to the mol. wt. of the main component of mouse H1 (Figure 2).

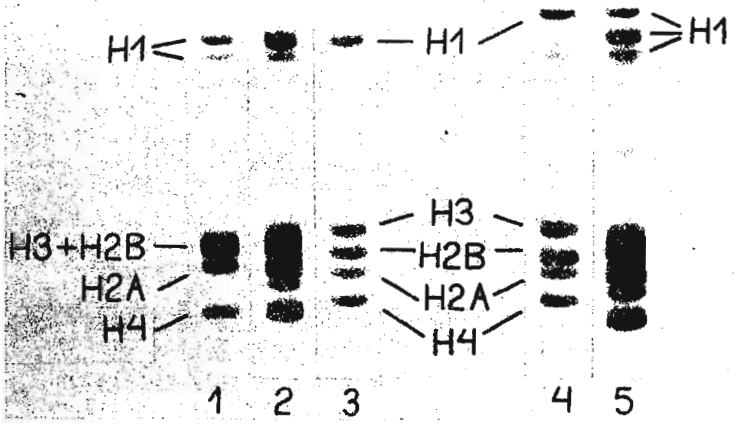

Figure 2. Electrophoretic mobility of Drosophila, Aedes and mouse histones in the presence of sodium dodecyl sulphate at pH 10. Electrophoresis was conducted in 10 cm long gels for 12 hours at 160 - 180 v.
1 - mouse histones
2 - mixture of mouse and Aedes histones
3 - Aedes histones
4 - Drosophila histones
5 - mixture of histones from Drosophila, Aedes and mouse.

Electrophoresis of Drosophila histones in 25 cm long gels in 6.25 M urea reveals heterogeneity of the H1 histone. This heterogeneity is due to the phosphorylation of a part of H1 molecules since it disappears after the treatment of H1 preparations by the alkaline phosphatase. Phosphorylated components were not found in the H1 of Aedes (Figure 3).

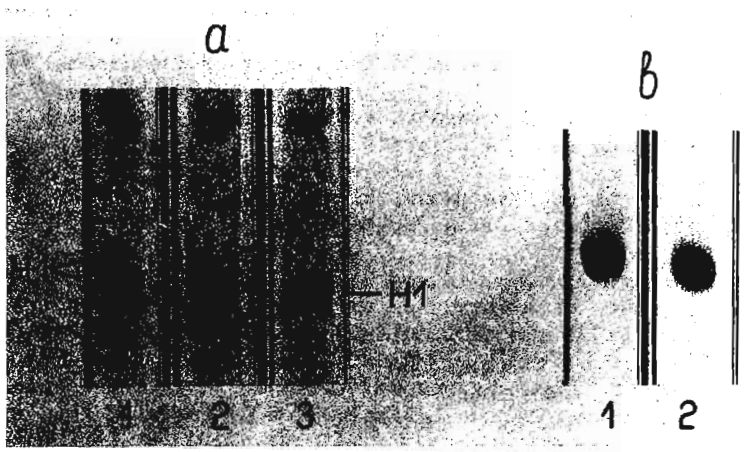

Figure 3. Effect of alkaline phosphatase treatment on the mobility of histones H1 from Drosophila and Aedes. (a) Drosophila H1 histone. 14 μg of Drosophila total histone were incubated without the enzyme (1,2) and with 0.7 μg of alkaline phosphatase (3). Electrophoresis was performed in 25 cm long gels in 6.25 M urea for 90 hours at 4° and 200 v. (b) Aedes H1 histone. 12 μg of the total Aedes histone were incubated without the enzyme (1) and with 0.6 μg of alkaline phosphatase (2). Electrophoresis conditions as in (a) but for 72 hours.

Thus two representatives of Diptera, Aedes and Drosophila possessing polytene chromosomes at the larval stage of development have H1 histones with markedly different primary structures. This fact demonstrates that the polytenization of chromosomes may occur in species with markedly different H1 histones.

I want to express my deep gratitude to Prof. R.B. Khesin for many helpful discussions and to L.S. Kukharkina and L.G. Polukarova for their help with tissue cultures and histone isolation.

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Note added in proof: Recently Dr. O. Chertov determined amino acid composition of my preparations of H1 histones. Drosophila and Aedes H1 histones contain in moles/100 moles: lysine - 25.0 and 28.0, aspartic acid - 5.1 and 3.4, threonine - 7.4 and 4.6, serine - 11.0 and 4.3, proline 6.0 and 9.0, glycine - 5.5 and 7.4, alanine - 19.0 and 27.2, valine - 5.3 and 2.8, respectively.

Frutos, R. de and A. Aguilar *, University of Valencia and *University of Barcelona, Spain. The lack of association between the distinct interchromosomal arrangements of Drosophila subobscura.

A series of χ^2 tests have been carried out in order to prove the possible association between the arrangements of the different chromosomes (designated by A, the sex chromosome, J, U, E and O) that make up the polymorphic system of Drosophila subobscura. The tests were applied to the data which was obtained from the chromosomic analysis carried out on eight experimental populations of the above species which were kept in population cages. The cages were started with four Spanish natural populations from which two replicas for every initial population were established. The chromosomal frequencies were obtained from the original population and afterwards from the samples taken from the cages during a period of one year (replicas G) up to four years (replicas P). The method followed to obtain the chromosomal frequencies of each one of the samples was the following: The males from the population cages were mated with virgin females from the K99 strain, which is homozygous for the standard order in all the chromosomes. From the progeny only one larva was analysed. While the chromosomes A and J turned out to have a clearly erratic behaviour, the chromosomes U, E and O developed in a similar way in all of the populations, showing a rise in only one arrangement, U_{1+2+8}, E_{st} and O_{st} respectively, with the consequent decrease of the rest of the arrangements that make up the polymorphic system of each one of the mentioned chromosomes. Only in two G replicas the arrangement U_{1+2+8} has the tendency to diminish (Frutos, in press).

The number of distinct chromosomal arrangements in the initial populations is 26, practically the same in all of them, of which 3 correspond to A, 2 to J, 5 to U, 6 to E, and 10 to O. This supposes that the possible number of chromosomal sets (n) is about 1800, if only the individuals XX are considered and about 600 if only the XY are considered, not taking into account the chromosome A (X), making a total of 2400 possible combinations of 5 and 4 arrangements respectively, which is a superior amount compared to the number of chromosomal sets analysed in each sample.

Therefore: 1) The arrangements that make up the polymorphic system of each one of the chromosomes were grouped together in the following way: A_{st} versus $A_{o.a.}$, U_{1+2+8} versus $U_{o.a.}$, E_{st} versus $E_{o.a.}$, and O_{st} versus $O_{o.a.}$ (o.a. means "all other arrangements"). The chromosome J only presents two arrangements in the analysed populations, J_{st} and J_1. The denomination $A_{o.a.}$ is practically integrated by the inversion A_2. The denominations $U_{o.a.}$, $E_{o.a.}$ and $O_{o.a.}$ respectively, include the arrangements U_1, U_{st}, U_{1+2}, U_{1+2+6}; E_{1+2}, E_{1+2+9}, $E_{1+2+9+12}$, $E_{1+2+9+4}$ and E_8; O_{3+4}, O_{3+4+7}, O_{3+4+8}, O_{3+4+2}, O_{3+4+1}, O_{3+4+22}, O_{3+4+17}, O_{3+4+16} and O_7. The arrangements U_{1+2+8}, E_{st} and O_{st} have been chosen as regards to all the other ones for their special behaviour in the cages, as we are particularly interested in proving whether or not there exists any association between them. 2) Contingency tables of the frequencies of the different combinations between the five chromosomes taken by two's, have been obtained with the help of a computer (Frek programme, in the Laboratory of Calculus of the University of Barcelona, Spain).

In general the results obtained show that there is a lack of association between the different analysed interchromosomal combinations. The adjoined table only points out those chromosomal combinations which give a significantly higher frequency than expected in relation to each one of the samples and populations. The blank columns mean that all the interchromosomal combinations corresponding to a sample or a group of given data seem to happen at random. It can be seen that the chromosomal combinations that appear to be quite associated change from one sample to another and even change in the same population. The chromosomes that tend to be associated with more frequency are the A and J, which are the same ones that showed an erratic behaviour in the populations. The given information clearly indicates the lack of association between the arrangements U_{1+2+8}, E_{st} and O_{st}. There was a higher frequency than expected in only one sample of one population, the combination O_{st}-U_{1+2+8} (sample 3^a of population G1). It is curious to underline that it is precisely in the populations G that the arrangement U_{1+2+8} tends to diminish. That is that the evolution of the chromosomes U, E and O, in the analysed populations, doesn't seem to have been affected by a tendency to association between the three arrangements stated. (See table next page.)

Reference: Frutos, R., Genetica (in press).

The interchromosomal combinations that present a higher frequency than expected.

Populations	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
P1	n = 147 E_{st}-$U_{o.a.}$ $P < 0.05$	n = 91 J_1-E_{st} $P < 0.05$	n = 53 J_1-U_{1+2+8} $P < 0.001$	n = 59 A_2-U_{1+2+8} $P < 0.05$	n = 95 -	n = 66 J_{st}-E_{st} $P < 0.05$	n = 39 -	n = 95 -	n = 98 -	n = 92 -
P2	n = 133 -	n = 106 -	n = 64 -	n = 99 -	n = 97 A_{st}-J_{st} $P < 0.05$	n = 91 -	n = 75 $A_{o.a.}$-U_{1+2+8} $P < 0.01$ $E_{o.a.}$-U_{1+2+8} $P < 0.05$			
T1	n = 111 A_{st}-$U_{o.a.}$ $P < 0.05$ E_{st}-$U_{o.a.}$ $P < 0.05$	n = 109 -	n = 64 -	n = 98 -	n = 99 $A_{o.a.}$-U_{1+2+8} $P < 0.01$	n = 98 -	n = 88 -	n = 110 A_{st}-$U_{o.a.}$ $P < 0.01$ E_{st}-$U_{o.a.}$ $P < 0.05$		
T2	n = 94 -	n = 61 -	n = 73 O_{st}-$U_{o.a.}$ $P < 0.05$	n = 78 -	n = 109 -	n = 97 -	n = 67 -			
H1	n = 146 -	n = 63 -	n = 95 -	n = 105 -	n = 71 A_{st}-O_{st} $P < 0.05$	n = 100 J_{st}-U_{1+2+8} $P < 0.01$				
H2	n = 90 E_{st}-$U_{o.a.}$ $P < 0.05$	n = 90 -	n = 105 J_1-U_{1+2+8} $P < 0.05$	n = 90 E_{st}-$A_{o.a.}$ $P < 0.01$	n = 106 -					
G1	n = 78 -	n = 84 -	n = 105 A_{st}-J_{st} $P < 0.05$ $A_{o.a.}$-U_{1+2+8} $P < 0.05$	n = 56 O_{st}-U_{1+2+8} $P < 0.05$	n = 89 -					
G2	n = 59 J_1-O_{st} $P < 0.05$	n = 59 -	n = 90 A_{st}-E_{st} $P < 0.05$ O_{st}-U_{1+2+8} $P < 0.05$							

Craddock, E.M. and W.E. Johnson. New York University, New York and Western Michigan University, Kalamazoo. Multiple insemination in natural populations of Drosophila silvestris.

Females of the Hawaiian picture-winged fly D. silvestris have been found to show some propensity for multiple mating in nature. This phenomenon has previously been recorded in wild populations of D. melanogaster (Milkman and Zeitler, 1974) and D. pseudoobscura (Anderson, 1974), as well as in laboratory cultures of these and one other Drosophila species (D. paulistorum; Richmond and Ehrman, 1974). The evidence in D. silvestris is based on progeny testing of females collected from nine populations on the island of Hawaii. The markers used were the four linked paracentric inversions of chromosome IV ($4k^2$, 4t, $4l^2$ and $4m^2$): several enzyme loci were also used in some instances for confirmation.

Amongst 245 larval families scored, 10 showed segregations for the fourth chromosome inversions which did not agree with expectations derived from the assumption of insemination by a single male, i.e. the progeny included more than four karyotypes. In a few cases, the additional unexpected progeny classes could have resulted from recombination. Since it has been found that crossing over between the fourth chromosome inversions in D. silvestris is extremely rare (Craddock and Johnson, 1976), it seems difficult to accept recombination as the cause of the observed abnormal segregations, particularly in view of the recombination frequencies required (up to 29% of gametes from certain females). Moreover, the fact that some segregations cannot be accounted for at all by recombination between inversions allows us to reject this as a general explanation. Further support for the occurrence of multiple insemination is provided by electrophoretic data on allozyme segregations in these and other families of larval progeny reared from field-collected females of D. silvestris. The finding of more than four alleles segregating at a single locus when the female parent was heterozygous, or more than three segregating alleles when the female was known to be homozygous at that locus provided conclusive evidence of insemination by more than one male. The frequency of electrophoretic segregations reflecting multiple paternity was low compared with that of chromosomal segregations, undoubtedly because very few of the loci analysed in D. silvestris are polymorphic for more than three alleles in a single population (Craddock and Johnson, 1977).

The actual frequency of multiple insemination in natural populations of D. silvestris probably exceeds the estimate of 4% of females detected via the inversion markers. The observed cases were from only three of the populations (Kilauea Forest, Kipuka 9 and Kipuka 14), all of which are polymorphic for all four of the chromosome IV inversions. Other populations polymorphic for only two inversions (most often $4k^2$ and 4t) contain fewer different chromosome IV karyotypes, and the probability of detection would thus be much lower, since females inseminated by two or more males of the same karyotype cannot be recognized. In addition, where only 10 (or fewer) larvae were scored, the chances of recovering sperm from two male parents are small, particularly if the sperm were present in disproportionate amounts. Despite these factors, it would appear that multiple insemination is a limited phenomenon in D. silvestris, by contrast with the situation in D. pseudoobscura (Anderson, 1974), or in D. melanogaster where the frequency in one population has been estimated at 0.47 (Milkman and Zeitler, 1974). An equivalent frequency could pertain in D. silvestris, but be obscured by a different pattern of sperm utilization. This is considered unlikely since eggs oviposited in a one-week period were found to have been fertilized by gametes from two or more males. Stored sperm originating from different males had been used concurrently rather than sequentially.

It is interesting to note that D. silvestris females can store viable sperm for extended periods. Field-collected individuals continued to produce progeny even after 6 weeks isolation in the laboratory without males. The closely related and homosequential species D. heteroneura and D. planitibia were found to store viable sperm for at least 8 and 10 weeks respectively. These are minimum estimates, since it is unknown how long before capture these females had been inseminated, and secondly, females were electrophoresed shortly after successful progeny production rather than being maintained until exhaustion of their sperm reserves. Extended sperm storage may not be an unusual phenomenon in female Drosophila. Kambysellis and Heed (1974) reported that D. macroptera females contained viable sperm throughout their winter diapause of several months, even though these females remained reproductively immature with respect to their ovarian development. The large picture-winged Hawaiian Drosophila have an adult longevity of many months, maintaining active oviposition throughout this period without any reproductive diapause. Thus it is not surprising that females occasionally mate more than once.

Work done at the University of Hawaii and supported by NSF grants GB-29288 and GB-27586 to Professors D.E. Hardy and H.L. Carson.

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Korochkina, L.S. and L.I. Korochkin.
Institute of Cytology and Genetics, Novosibirsk, USSR. Some characteristics of endocrine system in mutants 1(2)gl.

It is known that the moulting process and metamorphosis are chiefly controlled by two hormones, the juvenile hormone (JH) produced by corpora allata and ecdysone secreted by cells of the prothoracic gland. There were many attempts made to elucidate the role of hormones in growth and metamorphosis. We tried to clear up the nature of hormonal changes in homozygotes for 2 alleles of the 1(2)gl gene, out of which one causes death of homozygous individuals at the larval stage and the other at the prepupal stage. The prothoracic gland was found to be underdeveloped in homozygotes for both alleles (34 and 334). By contrast nuclei and cells of corpora allata of non-pupating larvae are twice as large as the corresponding cells of pupating larvae. As a result of these changes in pupating larvae, the nuclei of prothoracic cells are 12-14 times larger than cell nuclei of corpora allata; in pupae the figures are lower - about 4-5.

However the critical hormonal situation is dependent upon the relation of the activity of the endocrine system and the corresponding esterase system splitting preferentially the juvenile hormone (Whitmore et al. 1974). The analysis of the esterase pattern with the help of starch gel electrophoresis shows that a full set of fast esterases is not expressed in non-pupating homozygotes 1(2)gl/1(2)gl (stock 34). These esterases are obtained 2-5 hours before pupation in normal larvae. This set of fast esterases is formed in pupating homozygotes of stock 334 too. The increase of the activity of these esterase isozymes can be seen on 7-8th days after egg laying (Figure 1).

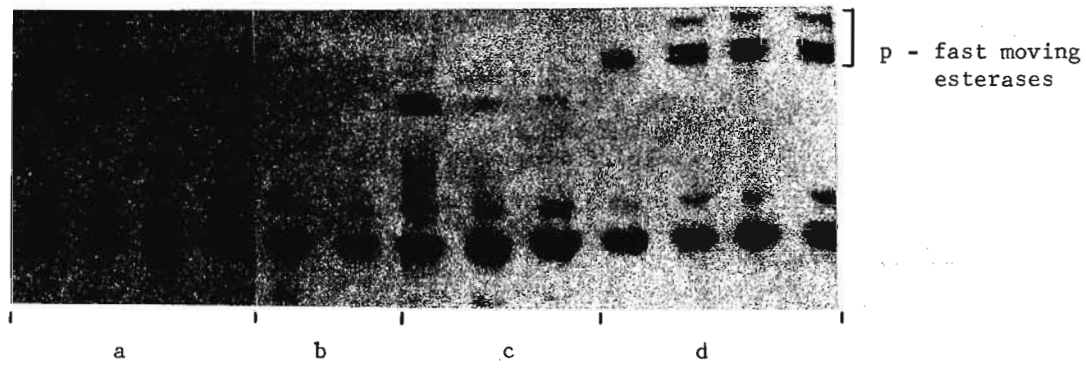


Figure 1. Electropherograms of esterases in different stocks of D. melanogaster: a - stock 334, 8 days; b - stock 334, 7 days; c - stock 34, 8 days; d - Oregon stock, white prepupa.

We suppose that unpupating homozygotes are characterized by the high level of juvenile hormone due to the higher function of corpora allata and slow degradation of enzyme (because fast moving JH esterases are absent).

References: Whitmore, D., E. Whitmore and I. Gilbert 1972, Proc. Nat. Acad. Sci. USA 69: 1592.

Shorrocks, B. and P. Charlesworth,
University of Leeds, England. The larval niche of the fungal breeding species - D. phalerata, D. cameraria, D. subobscura, D. confusa and D. transversa.

During 1974 and 1975 collections of fungi were made from 6 woodlands in the vicinity of Leeds, England. Individual fruiting bodies were identified, given an approximate age and measured. Each one was placed in a separate jar containing a layer of damp sawdust and placed in an outdoor insectory. Emerging flies were removed each day and identified.

In 1974 a total of 712 specimens comprising 83 species of fungi were collected. Only 37 species produced Drosophila and only 5 species (Phallus impudicus, Polyporus squamosus, Lactarius quietus, Aminata rubescens and Paxillus involutus) produced 80% of the 6,842 Drospohila reared. In 1975, although more fungal specimens were collected (727), these comprised only 39 species. Only 11 species produced any Drosophila and Phallus impudicus produced 1,195 (79%) of the 1,506 Drosophila reared.

These fungal species are therefore quite selective in their choice of breeding site, a feature even more clearly seen when individuals of different species are compared (Table). Intuitively one feels that breeding sites are not a limiting factor since many unoccupied sites are collected, even of species widely utilised. For Phallus impudicus there is also no correlation between the size of the individual fungal bodies and the numbers of emerging adults.

Table. % emergence for four common Drosophila species.

D. phalerata	63.38%	Phallus impudicus
	10.45%	Polyporus squamosus
	4.34%	Melanoleuca melaleuca
D. cameraria	24.48%	Lactarius quietus
	11.67%	Phallus impudicus
	11.00%	Amanita rubescens
	6.74%	Paxillus involutus
	5.29%	Boletus erythropus
	4.25%	Russula ochroleuca
D. confusa	90.08%	Polyporus squamosus
D. subobscura	82.22%	Phallus impudicus

Preliminary analysis of the emergence data from Phallus impudicus alone shows interesting differences between the two years. For 1974 the total number of Phallus collected was 63, producing the species listed below:

	number of Phallus	%
no Drosophila	4	6
D. phalerata	55	87
D. cameraria	15	24
D. confusa	12	19
D. subobscura	18	29
D. transversa	1	2
	63	

The observed frequencies of all combinations of these species compared to the expected frequencies on the basis of simple compound probabilities are not significant: $\chi^2_{16} = 4.61$.

	number of Phallus	%
no Drosophila	60	45
D. phalerata	60	45
D. subobscura	24	18
D. cameraria	20	15
D. transversa	2	1
	133	

However, for 1975 we have a very different picture. In this case a comparison of expected and observed frequencies yields a significant result, $\chi^2_7 = 19.62$, with an excess of observed over expected of the no Drosophila class and the 3 species class, suggesting aggregation of species.

Further analysis is in progress on the relationship between Drosophila species and other Diptera emerging from the fungi collected, and the relationships between age and size and numbers emerging. In the case of Phallus impudicus more detailed information is available on microdistribution within woodlands and these are also in process of being analysed.

Diamantopoulou, E. Agricultural College of Athens, Greece. Sterility genes in natural population of D. subobscura.

One hundred fifty five quasinormal O chromosomes from a natural population of D. subobscura in Greece were examined for sterility genes. A balanced strain for the O chromosome, the Vachcu/Ba was used in order to produce isogenic homozygotes for that chromosome. Fourteen chromosomes were found to cause sterility either in homozygous females or homozygous males and only one was found to make sterile both sexes in homozygotes. So the frequency of chromosomes bearing sterility genes for male and female was .039 ±.015 and .065 ± .019 respectively.

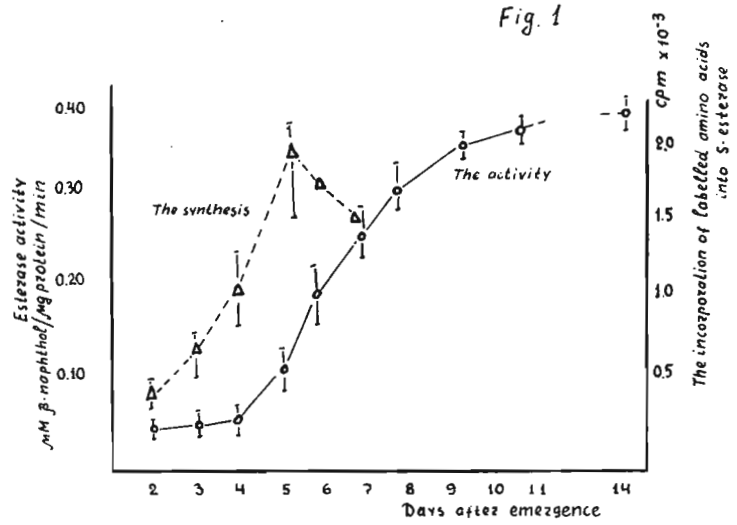
A chromosome also was found that in homozygous state produces male and sometimes female flies fertile when crossed with flies of the oppositite sex of other strains but infertile when the cross was performed within this strain.

This situation is under investigation.

Korochkin, L., B. Kuzin and N. Matveeva. Institute of Cytology and Genetics, Novosibirsk, USSR. The synthesis of organospecific S-esterase in ejaculatory bulb of Drosophila imeretensis.

It is known (Korochkin et al., 1976) that the organospecific S-esterase is synthesized in cells of the ejaculatory bulb in Drosophila of the virilis group but not absorbed from haemolymph. In this investigation the coincidence of the curves of a change of the activity and the synthesis of organospecific S-esterase in the ejaculatory bulb of D. imeretensis was shown. The mixture of ^{3}H-labeled amino acids ("Amersham", England, 1 mCi/ml) was injected into the abdomens of males of D. imeretensis at different times after emergence. 100 nl was injected in each case. 20-40 ejaculatory bulbs were used for each analysis. S-esterase was extracted by polyacrylamide gel electrophoresis. The activity of S-esterase was evaluated according to Van Asparen (1962). The synthesis of an enzyme and the increase of the enzymatic activity are especially expressed on the 4-5th days after emergence (Figure 1). The decrease of the incorporation of the labeled precursors into S-esterase to the moment of the maximal enzymatic activity can be seen.

Fig. 1



Reference: Korochkin, L., E. Belayeva, N. Matveeva, B. Kuzin and O. Serov 1976, Biochem. Genetics 14:161; Van Asparen, K. 1962, J. Insect Physiol. 8:401.

Golubovsky, M.D. and E.B. Kozlovskaya. Institute of Cytology and Genetics, Novosibirsk, USSR. Increase of mutation repertoire of singed locus in D. melanogaster as a result of mutation outburst.

In 1973 there was an outburst of the rate of mutation of singed locus in different natural populations of D.m.[1,2]. Singed alleles isolated from nature and in the Muller-5 test had different phenotypes and were unstable for the most part. In our laboratory of population genetics there is a collection of about 40 singed alleles collected by Dr. Raisa Berg, Yu.N. Ivanov and the first author of this report. Earlier, in 1960, Bender[3] established four phenotypic classes of all known alleles. But the real phenotypic repertoire of sn locus as revealed by the outburst of mutability is much larger. We listed phenotypic diversity of all sn alleles in 6 main classes according to character of bristle abnormalities. Each class can be divided into two subclasses on the basis of the shape and arrangement of hairs on the thorax. For instance:

class I-1: twisted, gnarled, diminished bristles, curved hairs.
class III-2: hooked at ends and shortened bristles, normal hairs.

In Table 1 are given data listing 27 different sn alleles. In front of the specific mutant number there is a letter which indicates the population from which the specific mutant was isolated (see table legend). About half of the mutants of I-1 class cause female sterility in homozygotes. Other mutations are fertile in females. It seems that phenotypic classes

Table 1. Phenotypic classification of singed alleles extracted from natural populations, from Muller-5 test and from some unstable mutants.

Class	Shape and size of bristles	Hairs on thorax	
		1 curved	2 normal or slightly waved
I	extreme mutant: twisted, gnarled, diminished	T77-27, K44-12, K79-22, 73-8(M5), V49-5, V2-2, M7, M8, M9-152, K84-6, 29-11(M5)	M7-55
II	twisted, gnarled, shortened, some bristles hooked at ends	K42-5	D11, K50-18
III	hooked at ends and diminished	G4, K26-7	K79-15, K33-13, K90-9, U9
IV	hooked at ends, partly waved, slightly shortened	-	T63-15, T49-15, K88-9, K42, M7-27
V	waved, normal length	-	K84-6a, K33-13a, T63-15a
VI	normal in males but waved in compounds with Df(sn)	-	some derivatives from unstable T63-15 allele

Underlined alleles of I-1 class are female sterile; M5 in parenthesis indicates that mutation occurred in Muller-5 line; The first letter in front of index of the alleles indicates population: D - Dilizhan (Armenia), G - Gantiady (Georgia), K - Krasnodar (Caucasus), M - Magarach (Crimea), U - Uman (Ukraine), V - Vladivostok (Far East), T - Tashkent (Middle Asia).

IV-1, V-1, VI-1 do not exist because only extreme or strong mutant "bristle phenotype" is connected with abnormal hairs. The different female sterile sn mutations do not complement. In compounds, alleles with more normal phenotype were as a rule dominant. In heterozygotes with Df(sn) some phenotypically normal derivatives have slightly mutant phenotypes.

References: [1]Berg, R.L. 1974, DIS 51:100; [2]Ivanov, Yu.N. 1974, DIS 51:71; [3]Bender, H. A. 1960, Genetics 45:860.

Gvozdev, V.A., T.I. Gerasimova, J.M. Rosovsky, G.L. Kogan and O.Yu. Braslavskaya. Kurchatov Institute of Atomic Energy, Moscow, USSR. Lethal mutations in the Pgd locus and their suppression in Drosophila melanogaster.

The lethal and semilethal mutations affecting the Pgd locus located in the 2D3-4 region of the X-chromosome were described earlier (1,2). Ten mutations were induced by ethyl methane sulfonate (EMS) and a single ($\underline{1}111$) by Co^{60}-irradiation. The Pgd^A and Pgd^B alleles determine the electrophoretically fast and slow forms of 6-phosphogluconate dehydrogenase (6PGD) respectively. Mutations resulted in elimination of the slow and hybrid isozymes of 6PGD in extracts of the $\underline{1}/Pgd^A$ females when the Pgd^B-marked X-chromosomes in males were treated by mutagen; the absence of the fast and hybrid isozymes in the $\underline{1}/Pgd^B$ females was detected if the lethal had been induced in the Pgd^A-marked X-chromosome. The Pgd^- mutations were induced by EMS for a frequency of 2×10^{-4}. The activity of 6PGD was reduced by 30-80% in hemizygous males carrying semilethals and by 40-50% in the $Pgd^-/+$ females as compared to normal ones (Table 1), i.e., in

Table 1. Mutations in the Pgd locus.

Lethals	X-chromosome markers	6PGD activity* in Hemizygous flies	6PGD activity* in $Pgd^-/+$ females
$\underline{1}13$, semilethal female fertility decreased	br Pgd^A pn	40-70	
$\underline{1}50$, semilethal male fertility decreased	br Pgd^A pn	25-50	
$\underline{1}109$, semilethal in females, $\underline{1}109$/Df, lethal in males	Pgd^A	17-20	
$\underline{1}35$, lethal	br Pgd^A pn		57
$\underline{1}39$, lethal	br Pgd^A pn		49
$\underline{1}45$, lethal	br Pgd^A pn		not tested
$\underline{1}71$, lethal	br Pgd^A pn		63
$\underline{1}93$, lethal	y ac sc Pgd^B w		50
$\underline{1}94$, lethal	y ac sc Pgd^B w		58
$\underline{1}100$, lethal	y ac sc Pgd^B w		48-64
$\underline{1}111$, lethal	br Pgd^A pn		not tested

*activity is expressed as percentage of that in normal males or females.

the latter case a gene dosage effect was revealed. The bulk of mutations resulted in lethal effects. In order to check the possibility of interallelic complementation, the following crosses were performed:

♀ $\underline{1}_1$/FM4 x ♂ $\underline{1}_2/w^+$ Y. The number of the $\underline{1}_1/\underline{1}_1$ or $\underline{1}_2/\underline{1}_2$ females eclosed in the offspring of the control crosses (♀ $\underline{1}_1$/FM4 x ♂ $\underline{1}_1/w^+$ Y and ♀ $\underline{1}_2$/FM4 x ♂ $\underline{1}_2/w^+$ Y) did not exceed 0.1-5.0% of the number of the $\underline{1}$/FM4 sisters. These escapers carrying lethals in a homozygous state expressed often abnormal wing phenotype and were characterised by sterility and delayed development. Viability of the $\underline{1}_1/\underline{1}_2$ individuals was essentially the same as was detected for the $\underline{1}_1/\underline{1}_1$ escapers. No restoration of 6PGD activity, i.e., no interallelic complementation was shown in the $\underline{1}_1/\underline{1}_2$ females.

In order to obtain revertants of the Pgd^- lethal mutation the $\underline{1}(1)71$ pn Zw^B/w^+ Y males were treated by EMS and crossed with C(1)RM, y w f females. The Zw^A and Zw^B alleles code for the fast and slow forms of glucose-6-phosphate dehydrogenase (G6PD) (3). Three revertants (viable fertile males with pn phenotype) were obtained for a frequency of 2×10^{-5}. No activity of 6PGD was detected in the extracts of revertants. It was shown that the activity of G6PD coded for by the Zw locus was drastically decreased in all revertants, i.e., the Zw^- mutations suppressed the lethal effects of the Pgd^- mutations. These Zw^- mutations were denoted as the su_1 Pgd, su_2 Pgd and su_5 Pgd.

Activity of G6PD was compared in the $\underline{1}(1)71$ pn Zw^B/w^+ Y and the $\underline{1}(1)71$ pn su Pgd males. The data shown in Table 2 demonstrate that the suppressor mutations caused approximately 30-60 fold and 2-7 fold decrease of the affinity of G6PD for substrate and NADP respectively. Polyacrylamide gel electrophoresis revealed in extracts of all suppressors the isozymes of

intermediate electrophoretic mobility as compared to the A and B isozymes. Thus all the suppressor mutations altered the electrophoretic mobility of G6PD, i.e., affect the structural gene of the Zw locus. Explanation of the biochemical mechanism of suppression requires particular consideration of the relationship of the pentose phosphate cycle and glycolysis.

Table 2. The K_m values for glucose-6-phosphate and NADP in extracts of mutant and normal flies.

Flies	K_m for G-6-P	K_m for NADP
Wild type, Zw^B	9.0×10^{-5}M	7.4×10^{-5}M
Zw^- mutants:		
su_1 Pgd	2.6×10^{-3}M	14.9×10^{-5}M
su_2 Pgd	3.6×10^{-3}M	23.7×10^{-5}M
su_5 Pgd	5.9×10^{-3}M	33.0×10^{-5}M

The obtained recessive suppressors were transposed by crossing over from the l(1)71 pn X-chromosome to the X-chromosomes carrying the other 10 Pgd^- mutations. It was shown their allele non-specific effects: all the Pgd^- su Pgd(Zw^-) males and the corresponding homozygous females were viable and fertile. Assays of the activity of G6PD in stocks carrying suppressors and semilethals were in a good accord with the data shown in Table 1. No, or only traces of, 6PGD activity was detected in extracts of flies carrying the lethals NN35, 39, 45, 71, 93, 94, 100, 111. The presence of enzymatically inactive 6PGD molecules (cross-reacting material) in these flies was checked by antiserum against the 2000-fold purified enzyme. A complement fixation reaction showed that antiserum titrated specifically the molecules of 6PGD since the quantity of antigen was dependent on the Pgd^+ gene dosage. In extracts prepared from flies carrying the wild type Pgd^A or Pgd^B alleles or the 13, 45, 50 and 109 mutations no differences were detected by the Ouchterlony immunodiffusion tests. The 6PGD-like material in extracts of flies carrying the 39, 71, 93, 94, 100 and 111 lethals showed complete immunological identity but the properties of the cross-reacting material in these flies were shown not to be identical to the wild type antigen. The quantity of this cross-reacting material was also dependent on the dose of the Pgd^+ gene, i.e., two fold decrease of antigen quantity was revealed in the Df(1)Pgd^-/Pgd^- su Pgd females as compared to the homozygous Pgd^- su Pgd/Pgd^- ones. It was suggested that the 39, 71, 93, 94, 100 and 111 mutations resulted in formation of drastically altered polypeptides or their fragments. The results suggest that all the studied Pgd^- mutations affect the structural gene coded for 6PGD, because only the alterations of polypeptide structure were observed as a result of the Pgd^- mutations. These observations were taken as evidence of a monocistronic nature of the Pgd locus, because a substantial part of mutations in a polycistronic structure (for example, polar mutations) would be led to elimination or sharp decrease in the quantity of 6PGD.

Table 3. Activity of 6PGD and CRM material in the Pgd^- su Pgd males or females.

Genotypes	6PGD activity in % of normal flies	CRM material
l13 su_2 Pgd	15 - 35	complete identity with the wild type antigen
l50 su_2 Pgd	12	
l109 su_2 Pgd	20	
l35 su_2 Pgd	<3	
l45 su_2 Pgd	<2.5	
l71 su_2 Pgd	<1	partial identity with the wild type antigen
l93 su_5 Pgd	<1	
l94 su_5 Pgd	<0.4	
l100 su_5 Pgd	<1.6	
l39 su_2 Pgd	<2.0	
l111 su_2 Pgd	<1.5	

Suggestion of a monocistronic type of the Pgd locus is also supported by the observation of a suppressor effect of the Zw^- mutations. In order to explain the biochemical mechanism of suppression it is necessary to suppose that all the Pgd^- mutations altered only the 6PGD function without effect on the essential adjacent genes in a hypothetical operon-like structure.

References: 1) Gvozdev, V.A. et al. 1973, DIS 50:34; 2) Gvozdev et al. 1975, Genetika (Russ.) 11:73; 3) Lindsley, D.L. and E.H. Grell 1968, Genetic Variations of D.m., Carnegie Inst. Wash. Bull. 627.

Triantaphyllidis, C.D., Z.G. Scouras and J.N. Panourgias. Aristotelian University of Thessaloniki, Greece. Genetics of six allozyme loci of some species of the melanogaster species group.

Starch gel electrophoresis was used to examine the esterase (Est-C and Est-6), acid phosphatase (Acph), alkaline phosphatase (Aph), leucine aminopeptidase (Lap-D), and α-glycerophosphate dehydrogenase (α-GPDH) patterns of single individuals of D. yacuba (melanogaster subgroup), D. prostipennis (takahashii subgroup), D. eugracilis (eugracilis subgroup), D. elegans (elegans subgroup), D. rajasekari (suzukii subgroup), D. bipectinata and D. malercotliana (ananassae subgroup). A D. melanogaster (Oregon R) stock was used as reference material. The Est-6^S, Est-C^F, Acph-1^F, 1-Aph^S, Lap-D^F and α-GPDH-1 of D. melanogaster are designated here as Est-$6^{1.0}$, Est-$C^{1.0}$, Acph-$1^{1.0}$, $Aph^{1.0}$, Lap-$D^{1.0}$ and α-$GPDH^{1.0}$ respectively (Figure 1).

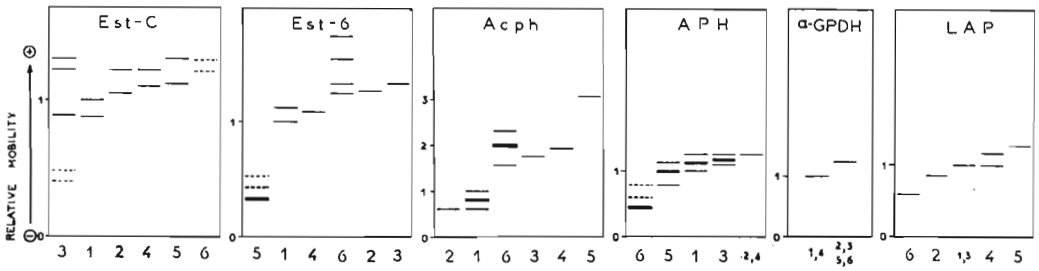


Figure 1. Schematic representation of the relative positions of Est-6, Est-C, Acph, Aph, Lap-D and α-GPDH of six species of the melanogaster species group. No. 1 = D. melanogaster, No. 2 = D. yacuba, No. 3 = D. prostipennis, No. 4 = D. elegans, No. 5 = D. eugracilis and No. 6 = D. rajasekari.

1. Est-C: In D. yacuba, D. elegans, D. eugracilis and D. rajasekari two Est-C allozyme bands were found, which are coded by two codominant alleles at one locus (Figure 1). Five allozyme bands of Est-C activity were found in D. prostipennis. The data from different crosses indicate that three of these bands are coded by three codominant alleles at one locus. The other two Est-C bands in the same species are faint and they appear in all individuals.

2. Est-6: In D. elegans, D. yacuba and D. prostipennis one allozyme band of Est-6 activity was found (Figure 1), while in D. rajasekari, four bands of Est-6 activity were detected. In a single fly there were always two or three Est-6 bands. The mode of inheritance of these Est-6 zones does not resemble simple mendelian inheritance at one locus.

Three Est-6 zones were found always in individuals of D. eugracilis. The intensity of these bands is different and the most slowly moving band has the highest activity, the intermediate band has lower activity than the previous band, while the faster moving band is only slightly active. This stable phenotype may represent a series of polymeres.

3. Acph: In D. yacuba, D. prostipennis, D. elegans and D. eugracilis one Acph band was observed. In D. rajasekari three different Acph phenotypes were found, which are coded by two co-dominant alleles at one locus. The mode of inheritance of Acph of D. rajasekari is similar to that of D. melanogaster.

4. Aph: The results from different crosses indicated that in D. eugracilis and D. prostipennis there are two codominant Aph alleles at one locus (Figure 1). The homozygotes for these alleles have one Aph band while the heterozygotes have three Aph bands. Third instar larvae from D. yacuba and D. elegans have one Aph band, while third instar larvae from D. rajasekari always have three Aph bands (Figure 1).

5. α-GPDH: Two distinct regions of α-GPDH activity were found in the α-GPDH zymograms. D. melanogaster and D. elegans have one α-GPDH zone with the same electrophoretic mobility, while the α-GPDH zone of the other species migrate more anodically.

6. Lap-D: Polymorphism was observed only in D. elegans, and this appears to be controlled by a pair of codominant alleles at one locus.

The mode of inheritance of the six allozymic loci of D. malercotliana and D. bipectinata is similar to that of D. melanogaster and according to the isozyme patterns given by Yang et al. (1972).

From Figure 1 it is evident that there are some common allozyme bands between the species of the melanogaster groups studied. D. yacuba and D. melanogaster share only 11.8% of their allozyme bands. Thus, our results support the observations of F. Lemeunier and Ashburner (1976) that D. melanogaster and D. yacuba are not very closely related species, although they belong to the same subgroup.

The average % of the common bands (similarity) among the studied species is about 11.5%, which approximates the estimates of average genetic similarity (18%) between distantly related species (Hubby and Throckmorton, 1968).

Acknowledgements: We would like to thank Dr. C.D. Kastritsis, Head of the Lab, for his helpful suggestions. Thanks are also due to Dr. R.H. Richardson for supplying the different Drosophila stocks.

References: Hubby, J.L. and L.H. Throckmorton 1968, Am. Natur. 102:193-205; Lemeunier, F. and M.A. Ashburner 1976, Proc. R. Soc. London 193:275-294; Yang, S.Y., L.L. Wheeler and I.R. Bock 1972, Univ. Texas Publ. 7213:213-227.

Mulley, J.C. University of Sydney, Australia. The electrophoretic detection of mannosephosphate isomerase in Drosophila.

This report communicates a method for the electrophoretic detection of another biochemical locus in Drosophila. Mannosephosphate isomerase (MPI, E.C.5.3.1.8) catalyses the reversible conversion of mannose-6-phosphate to fructose-6-phosphate and has been detected from mice by coupling with glucose-6-phosphate dehydrogenase and glucosephosphate isomerase (Nichols et al. 1973). This method was successfully applied to adult Drosophila after starch gel electrophoresis at 200 volts for 4 hours on the buffer system described by Poulik (1957).

The staining mixture consisted of 20 mg mannose-6-phosphate, 20 mg $MgCl_2$, 25 units GPI, 25 units G-6-PD, 3.75 mg NADP, 12.5 mg MTT and 1 mg PMS in 0.1M Tris-HCl buffer at pH 8.0. No activity was detected when mannose-6-phosphate was omitted from the staining mixture. MPI occurred as a single zone slightly cathodal to the borate front.

Table 1. Wild Drosophila populations examined for MPI

Drosophila	Location	Sample size
melanogaster	Leeton	100
	Camden	63
	Hunter Valley	100
	*Canberra	40
simulans	Camden	41
	Canberra	49
immigrans	Camden	24
	Canberra	45
hydei	Camden	4
	Canberra	13
lativittata	Camden	23
	Canberra	3

*Laboratory population recently collected from nature

No heterozygosity was observed from the five species tested (Table 1). Low effective population size is an unlikely cause of homozygosity since all Drosophila examined are prevalent near household garbage and all except D. lativittata are cosmopolitan (Bock, 1976). The lack of electrophoretically detectable variation is in contrast to the high level of polymorphism found in species of penaeid prawns (Mulley and Latter - unpubl. data).

MPI in Drosophila is a useful addition to the staining procedures now available for estimating average heterozygosity and genetic distance. However, the heterozygote banding pattern cannot be described, the inheritance cannot be validated and MPI cannot be mapped in D. melanogaster until variation is discovered. Such variation will undoubtedly arise if MPI is included in future electrophoretic surveys from other geographical regions.

Acknowledgement: This work was supported by a grant from the Australian Research Grants Committee to Professor B.D.H. Latter as part of a program on the relative fitness of Drosophila.

References: Bock, I.R. 1976, Aust. J. Zool. Suppl. Ser. No. 40:1-105; Nichols, E.A., V.M. Chapman and F.H. Ruddle 1973, Biochem. Genet. 8:47-53; Poulik, M.D. 1957, Nature 180: 1477-1479.

Kazatschenko, B.N. and V.A. Mglinetz. Institute of Medical Genetics, USSR Academy of Medical Sciences, Moscow.

Expression of mutant genes Ns, Pc and Xa in triploid females and intersexes of D. melanogaster.

In crosses $\overline{XX}$, z/FM7;+/+/+ ♀♀ x FM7/Y;Ns/+ ♂♂ triploid females $\overline{XX}$, z/FM7;Ns/+/+ and intersexes $\overline{XX}$, z/Y;Ns/+/+ were obtained. In respective crosses of $\overline{XX}$, z/FM7;+/+/+ ♀♀ x FM7/Y;Pc/Xa ♂♂ (involving either Pc^1 or Pc^2 mutants) triploid females $\overline{XX}$, z/FM7;Pc/+/+ and $\overline{XX}$, z/FM7;Xa/+/+, as well as intersexes $\overline{XX}$, z/Y;Pc/+/+ and $\overline{XX}$, z/Y;Xa/+/+ were obtained.

Mutant phenotype Ns or Xa was observed in flies having three sets of autosomes and a single dose of the respective mutant gene. No effects of either of the Pc alleles were observed in flies of similar genotypic constitution.

One of the most likely interpretations of the dominant action of mutant genes involves the suggestion that the active product of the corresponding locus is multimeric, and that the activity of the product is lost in case a defective mutant polypeptide participates in the multimeric complex (Garen, A and S. Garen 1963, J. Mol. Biol. 7:13; Bernstein, H. and K.M. Fisher 1968, Genetics 58:307). Then, expression or non-expression of a single dose of a mutant gene in triploids may be helpful in distinguishing between dimeric and tetrameric composition of the gene product. If it is dimeric, then in cells of diploid heterozygotes a quarter of the dimeric molecules would consist of normal polypeptides only, and this quantity could be insufficient to suppress the dominant mutant phenotype. But, in cells of triploids with a single dose of mutant allele half of the dimeric molecules would be normally homomeric, and this could suffice to restore the distorted function. This is probably true for Pc. If the gene product is tetrameric, then the fraction of normal homomeres would comprise 0.062 in diploid heterozygotes and 0.197 in triploids with a single dose of the mutant allele. Either amount of the normal tetrameric product could be insufficient to suppress the mutant phenotype. This is probably true for Ns and Xa. It seems worth noting that still lower fractions of normal gene products are expected to be present in cells in cases of higher degrees of multimerity.

Ochando, D. Universidad Complutense de Madrid, Spain.* Annual distribution of four species of Drosophila.

During three years and in different seasons, Drosophilas were captured in a hitherto unstudied area, 11 km. from Vallecas (Madrid) in the center of the Iberian Peninsula. The site was an unpopulated zone, quite a distance from the city and had a plateau-like climate with little rain, hot summers and cold winters. The area - quite isolated - is a riverbed whose humidity creates favorable conditions for the development of an elm grove, where we collected our material.

Our process of classification of the specimens followed that of Monclus (1964), based on the code used by Burla in studying Swiss species of Drosophila, and adapted by her to Spanish species.

Observing the 13 samples obtained (see Table, next page) it can be seen that individuals belonging to 11 different species were captured; only five of these species appeared with relative frequency: phalerata, busckii, melanogaster, simulans and subobscura. Phalerata, which is never very numerous, remains at a relatively constant level throughout the year, whereas the remaining four more abundant species show that their relative predominance varies according to the time of year, i.e., show a seasonal distribution: subobscura predominates in late autumn, winter and early spring; busckii is present above all in the spring, melanogaster is present at the end of spring, in summer and fall, and simulans can be found a bit in summer but mostly in the fall; in the latter season it is more numerous than melanogaster.

Data from captures 8 and 9, apparently anomolous, are in fact due to the intense rains and lower temperatures of that spring, which caused the "climatological" spring to arrive later than the "astronomical" spring, which in its turn "delayed" the distribution pattern of the species.

Briefly, we can say that busckii assumes a preponderance in the spring, surpassing subobscura (present in the winter), that melanogaster assumes a similar preponderance over busckii in summer, that simulans does so over melanogaster in the fall, and, finally, subobscura does so over similans in the winter, thus closing the annual cycle.

Reference: Monclús, M. 1964, Genetica Ibérica 16:143-165.

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Table: Date of capture; number and percentage of individuals of the different species.

Date	12-69	3-70	4-70	6-70	7-70	10-70	3-71	6-71	7-71	4-72	6-72	7-72	8-72	Total
busckii			55 (28.64)	27 (10.84)		3 (0.23)	1 (2.70)	253 (99.22)	36 (18.00)	238 (57.91)	19 (9.95)		1 (1.72)	633
cameraria	9 (1.94)	2 (0.85)												11
confusa										1 (0.24)				1
funebris		1 (0.43)								10 (2.43)			1 (1.72)	12
hydei	2 (0.43)	1 (1.43)			1 (1.96)	5 (0.38)		2 (0.78)					1 (1.72)	14
melanogaster			4 (2.08)	219 (87.95)	40 (78.43)	445 (33.84)			84 (42.00)	3 (0.73)	155 (81.15)	365 (88.59)	45 (77.59)	1,360
phalerata	30 (6.48)	7 (2.99)	12 (6.24)	1 (0.40)		4 (0.30)	1 (2.70)		9 (4.50)	3 (0.73)				67
repleta			1 (0.52)											1
simulans	94 (20.30)		2 (1.04)		(19.61)	856 (65.09)			17 (8.50)	2 (0.49)		44 (10.68)	10 (17.24)	1,035
subobscura	322 (69.55)	217 (92.74)	115 (59.89)	2 (0.80)		2 (0.15)	35 (94.54)		54 (27.00)	153 (37.23)	17 (8.90)	3 (0.73)		920
transversa	6 (1.30)	4 (1.71)	3 (1.56)											13
Total	463	234	192	249	51	1,315	37	255	200	410	191	412	58	4,068

Alonso, C., E. Barbera* and M. Pages.
Instituto de Biología Molecular, Universidad Autónoma de Madrid, Spain. *Instituto de Investigaciones Citologicas, Valencia. Absolute DNA content of polytene nuclei of D. hydei.

The total DNA content of diploid and of the largest salivary gland nuclei of D. hydei have been previously determined by Mulder et al. (1968) using as a standard the Feulgen absorbtion of chicken erythrocyte nuclei. It was found that the average DNA content of the largest salivary gland nuclei was 330 ± 2 pgrs. In order to have a more direct measurement of DNA content of polytene nuclei we have estimated the amount of DNA of fixed non-stained polytene chromosomes from the distal part of late third instar salivary glands by calculation of the integrated absorbance at 265, 280 and 313 nm wave length, using the expression N.A. = $0.92\int_{A265} - 0.75\int_{A280} - 0.25\int_{A313}$ (10^{-12} g.), proposed by Sandritter (1958). The measurements were made in a Zeiss SMP 05 cytophotometer connected on line with a digital PDP 12 computer. The glands were fixed at 4°C in alcohol-acetic acid (3:1) for 10 min., squashed in 45% acetic acid on quartz slides and dehydrated through 70% and 100% ethanol. Afterwards the chromosomes were treated with 1 mg/ml pancreatic RNase A and 100 units/ml of RNase T_1 (DNase free) in 2 x SSC at 37°C for 2 hrs.

Table 1 shows the results of 5 different measurements. The average DNA content of each chromosomal set was 365 ±6 pgrs for male larvae and 418 ± 7 pgrs for female larvae. These

Table 1. Absolute DNA content in polytene nuclei of Drosophila hydei.

Source	Exp. No.	DNA content (pgrs)
Male chromosomal sets	1	360
	2	365
	3	371
	4	368
	5	359
Female chromosomal sets	1	419
	2	412
	3	411
	4	425
	5	420
Chromosome region 4-73D	1	2.2
	2	2.1
	3	2.4
Chromosome region 4-92B	1	0.22
	2	0.24
	3	0.20

values are in fair agreement with those of Mulder (see above) and with biochemical DNA determinations of late third instar salivary glands using the modified method of Giles and Myers (1965). We found a DNA value of 0.55 ± 0.02 µgrs DNA for each gland. As the mean number of nuclei per gland is 130, we estimated that each polytene nuclei has an average DNA content of 422 ±16 pgrs. On this basis the number of chromatids forming each chromosome pair at the stage measured should be 2048.

Table 1 also shows the absolute amount of DNA of a single region (73 D) in the proximal part of the 4th chromosome. The DNA amount was 2.2 ± 0.2 pgrs. This region contains at the E.M. four thick bands of about equal size meaning that the amount of DNA of each chromomere at the haploid level is 2.6×10^{-4} pgrs (not taking into account the DNA on interbands). The method we present here will allow the direct measures of absolute amounts of DNA of thick bands and with the aid of the E.M. to have a good approximation of the DNA of any thin band.

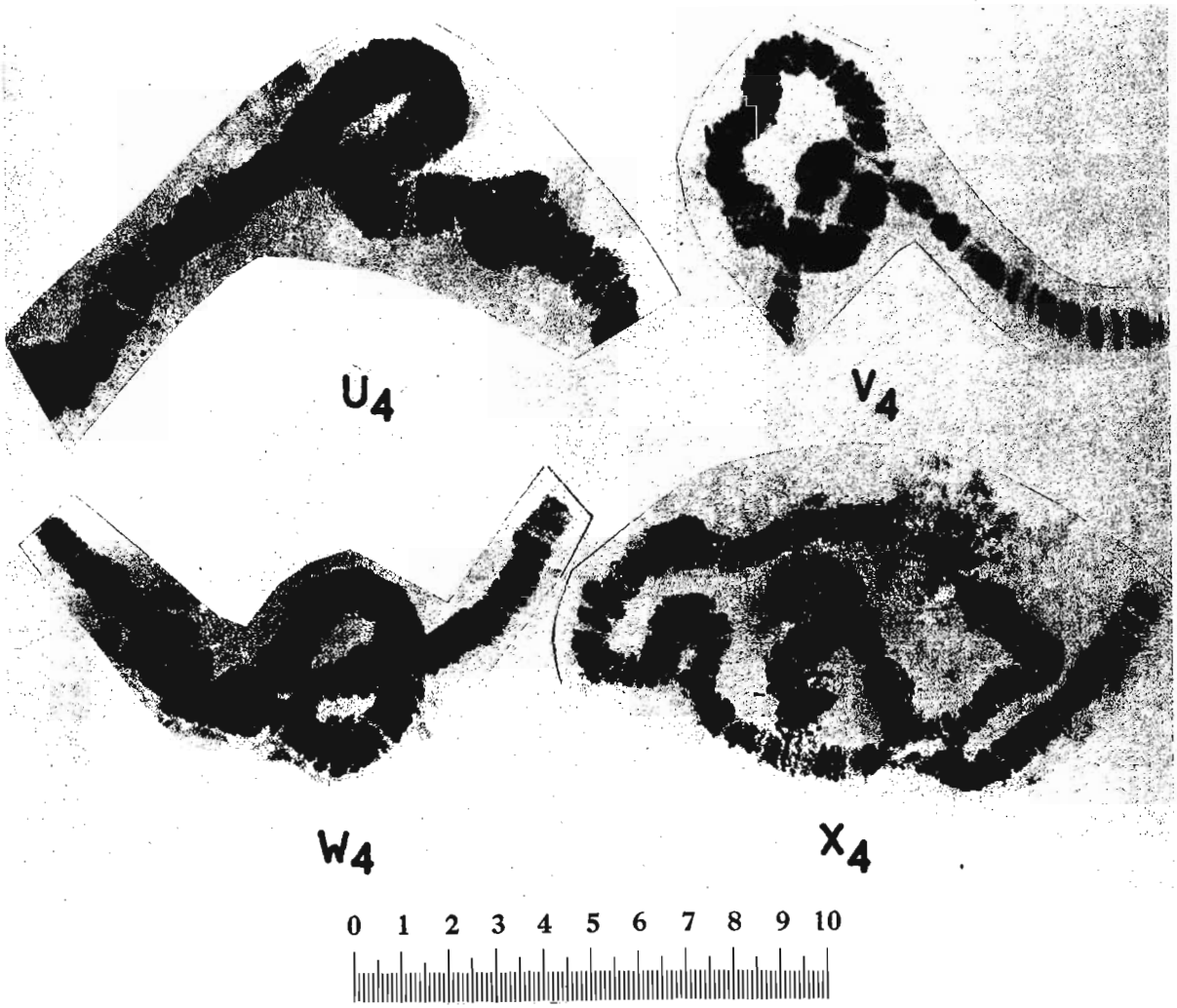
References: Mulder, M.P., P. Van Duijn and H.J. Gloor 1968, Genetica 39:385-428; Sandritter, W. 1958, (Histochemie Graumann Wand Neumann) (Edit). U.V. Mikrospektophotometric Handbuchder; Giles, K.W. and A. Myers 1965, Nature 206:93 (London).

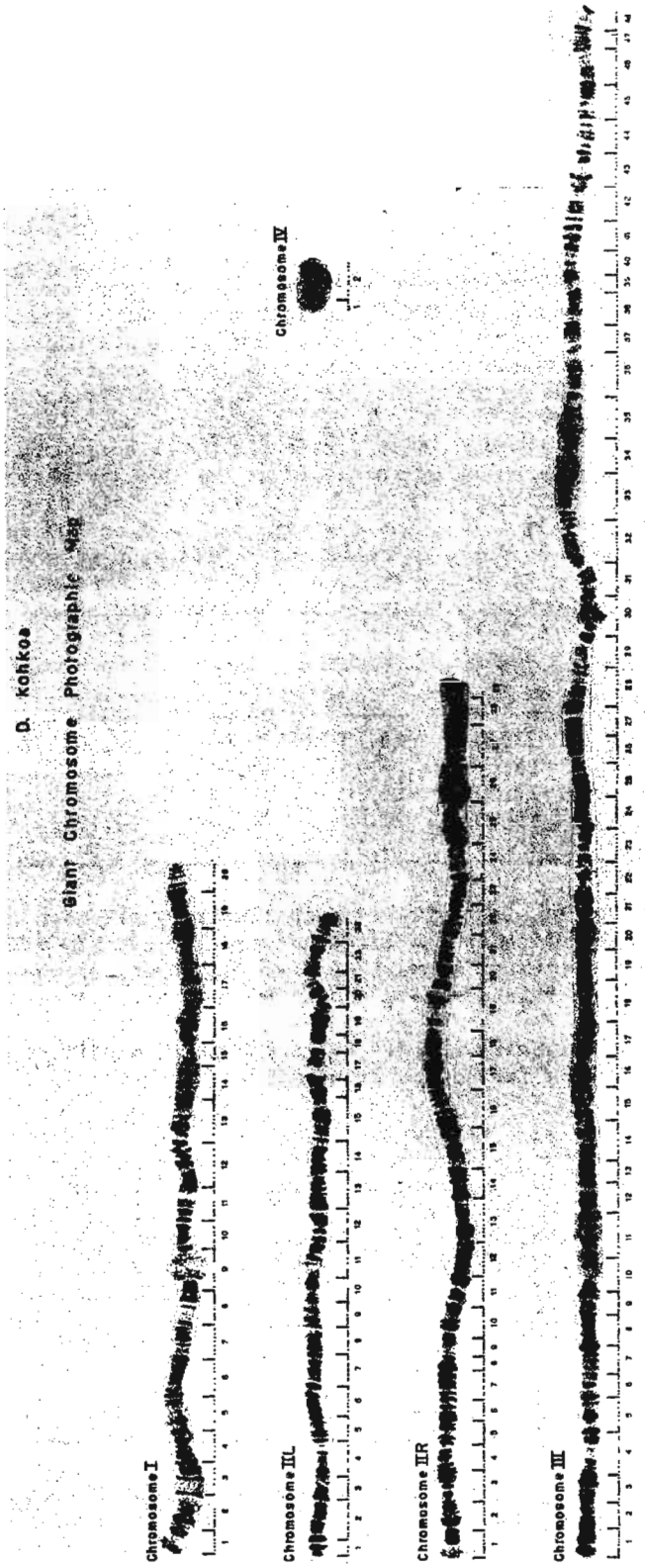
Mather, W.B. and P. Thongmeearkom.
University of Queensland, Brisbane, Australia. Inversions in D. kohkoa from Phuket, Thailand.

From a collection of Drosophila at Phuket in southern Thailand (December 1975) a single iso-line of the comparatively rare species D. kohkoa was established. This line proved to be of considerable interest in that it contains four new simple inversions. Photographs of these four inversions are presented and their breakpoints recorded with reference to a standard photographic map. This map was constructed from an inversion-free strain which was developed from an isoline established from Luzon, Philippines. (See photographs on this and the following pages.)

Inversion	Chromosome	Breakpoints
U_4	III	24.8 - 28.1
V_4	III	30.5 - 38.0
W_4	III	37.1 - 44.3
X_4	I	6.0 - 17.8

The material was collected and the iso-lines established by W.B.M. The laboratory work was carried out by P.T.




D. kohkoa
Giant Chromosome Photographic Map
Chromosome I
Chromosome IIL
Chromosome IIR
Chromosome III
Chromosome IV

Narise, T. Josai Dental University, Sakado, Saitama, Japan. Mass dispersive activity in a genetically heterogeneous condition.

Sakai et al. (1958) found that there were two kinds of dispersion. One of them is mass dispersion which is related to population density, and the other is random dispersion being due to random movement of individual.

The purpose of this experiment is to study mass dispersion under a genetically heterogeneous condition.

The strains used in this experiment were a wild strain (MS-1) and four mutant strains (ss, ss^a, cn bw, and vg) of Drosophila melanogaster. The six kinds of population density were utilized, and they were 20, 40, 60, 80, 100 and 150 flies. In the single condition, a certain number of flies of a strain were introduced into a migration tube, and kept for 24 hours in it. Then, three new tubes were connected to the tube, and dispersion was allowed for 6 hours. The dispersed flies to the three connected tubes were counted. For mixed condition, the equal number of flies of two strains were introduced simultaneously into a migration tube, and dispersed flies of each strain were counted separately. The other procedures were the same as those in the single condition. Experiment was conducted in a dark room at $25^{\circ} \pm 0.5^{\circ}C$.

Table 1 shows the dispersive activities of each strain in different population densities under the single condition. As seen in Table 1, the dispersive activities of MS-1 are about 23%, and not different among densities. In ss strain the activities were about 14% except 80 flies. However, the dispersive activity increased at the density of 40 flies in ss^a, 80 flies in cn bw and 60 flies in vg strain. From the result, the mass dispersive activity of MS-1 is considered as more than 150 flies, and the same was held in the ss strain. However, the activity is 40 flies in ss^a, 80 flies in cn bw and 60 flies in vg strain respectively.

Table 1. Dispersive activities of the five strains and mass dispersive activity in single condition.

	20	40	60	80	100	150	Mass dispersive activity
MS-1	20.00	25.00	27.33	24.10	21.00	21.10	> 150
ss	15.00	12.00	15.66	26.75	15.40	14.50	> 150
ss^a	10.00	23.00	32.00	26.30	16.10	22.70	40
cn bw	13.00	11.50	15.00	28.80	24.60	26.40	80
vg	5.00	1.50	11.00	14.25	12.80	26.50	60

Table 2 presents the dispersive activity in each mixture. As shown in Table 2, in MS-1+ss, MS-1+cn bw and ss+cn bw the activity increases between 100 and 150 flies, and at the density of 80 flies in MS-1+ss^a mixture as well as MS-1+vg. However, the activity increases at the density of 60 flies in se+vg, ss^a+cn bw and cn bw+vg mixture.

Table 2. Dispersive activity in the mixed condition and mass dispersive activity in each mixture.

	40	60	80	100	150	Mass dispersive activity
MS-1+ss	12.00	17.68	17.50	17.20	31.00	100 ~ 150
MS-1+ss^a	7.00	10.00	37.25	15.40	21.10	80
MS-1+cn bw	15.00	14.66	16.25	20.40	28.60	100 ~ 150
MS-1+vg	16.00	12.00	27.00	23.20	32.40	80
ss+ss^a	23.00	27.67	33.00	9.70	18.70	---
ss+ cn bw	16.00	14.33	17.75	15.20	28.90	100 ~ 150
ss+vg	2.50	27.00	27.15	22.20	20.70	60
ss^a+cn bw	5.00	27.00	24.25	16.20	36.10	60
ss^a+vg	30.00	26.00	23.50	15.60	18.30	---
cn bw+vg	20.00	34.00	32.00	32.10	26.00	60

The activity of ss^a+vg mixture decreases with the increase of the population density, and it is considered that there is the maximum activity at the density of 80 flies in the ss+ss^a mixture.

From these experimental results, it can be said that in general mass dispersive activity is affected by a strain having higher activity when two strains co-exist in a population.

Jaenike, J.[1], D.D. Miller[2], and R.K. Selander[1]. [1]University of Rochester, Rochester, New York, and [2]University of Nebraska, Lincoln, Nebraska. Electrophoretic differences among semispecies of Drosophila athabasca.

Three semispecies of Drosophila athabasca, "eastern A," "eastern B," and "western-northern," manifest cytological and behavioral differences (see for example, Miller and Voelker, 1972; Patty, 1975) and are distinguishable by male courtship sounds (Miller et al., 1975). Here we report electrophoretically detectable differences among these semispecies at loci encoding enzymes. The methods used are generally those of Selander et al. (1971). Pgm, Me, Mdh, and Acph were assayed on their gel-buffer type 4; and Lap, Adh, Hk, and αGpdh on buffer type 5. Acph was stained in 50 mg Na α naphthyl acid phosphate and 20 mg Black K salt dissolved in 50 ml .05 M Na acetate buffer (pH 5.0). Hk was stained in 20.3 mg $MgCl_2$, 45 mg D-glucose, 12 mg ATP, 17.5 mg NADP, 20 units G-6-Pdh, 2 mg PMS, and 3.2 mg MTT dissolved in 50 ml .2 M Tris-HCl buffer (pH 8.0).

Semispecies were identified by male courtship sounds. The origin and number of strains of each semispecies are as follows: "Eastern A": Rochester, New York (3); Lake Shamineau, Minnesota (1); Netcong, New Jersey (1); mixed Orland, Maine - South Williamstown, Massachusetts, "early bright" (eyes) (1); West Mills, Maine (3); and Mt. Desert Island, Maine (2). "Eastern B": Lincoln, Nebraska (1); Bloomington, Illinois (1); Philadelphia, Pennsylvania (1); and Princeton, New Jersey (2). "Western-northern": Gothic, Colorado (3); Eugene, Oregon (1); Deer Isle, Maine (1); and Mt. Desert Island, Maine (2).

Electromorph frequencies at eight loci in semispecies of Drosophila athabasca.

		Frequency		
Locus	Electromorph	"Eastern A" (N=22)	"Eastern B" (N=10)	"Western-northern" (N=14)
Pgm	120			.07
	110	.09		.07
	100	.91	1.00	.79
	95			.07
Me	110		.10	
	105		.50	
	100	1.00	.40	1.00
Mdh	100	1.00	1.00	1.00
Acph	105			.36
	100		.30	.57
	95			.07
	90	1.00	.70	
Lap	110	.05		
	105	.09	.20	
	100	.86	.80	.79
	95			.21
Adh	100			1.00
	95	1.00	1.00	
αGpdh	100	1.00	1.00	.86
	90			.14
Hk	105			1.00
	100	1.00	1.00	

The frequencies of electromorphs (which we provisionally equate with alleles) at the eight loci in each of the semispecies are shown in the accompanying Table. Adh and Hk appear to be monomorphic within each semispecies. At these loci, "eastern A" and "eastern B" are electrophoretically identical, while "western-northern" has unique, diagnostic allozymes. These two loci, then, may be useful for identifying individuals as "western-northern." But because there are no loci at which "eastern A" and "eastern B" have non-overlapping sets of

electromorphs, individuals of these semispecies cannot be distinguished by electrophoretic analysis. The number of loci and strains studied admittedly is small, but the data suggest that "eastern A" and "eastern B" are the most closely related of the three semispecies.

References: Miller, D.D., R.B. Goldstein and R.A. Patty 1975, Evol. 29:531; Miller, D.D. and R.A. Voelker 1972, J. Hered. 63:2; Patty, R.A. 1975, Anim. Behav. 23:344; Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson and J.B. Gentry 1971, Studies in Genetics 6:49.

Korochkin, L. Laboratory of Developmental Genetics, Institute of Cytology and Genetics, Novosibirsk-90, USSR. New data about esterase isozymes of ejaculatory bulbs in Drosophila of the virilis group.

Organospacific S-esterases were observed in ejaculatory bulbs of males in Drosophila littoralis Maigen and in some stocks of D. virilis. The structural gene of esterase-S was located on the second chromosome (192.1 ± m.u.). The activity of this esterase isozyme is controlled by modifier genes which were located on the X- and 5th chromosomes (Korochkin et al., 1976).

Additionally new stocks of D. littoralis were selected. Some of them were characterized by a high activity of α_2-esterase (Figure 1) which can be seen after homogenization of samples using 0.5% Triton X-100.

S-esterase can exist in two states: free and membrane-bound. The ratio, free/membrane-bound, differs in different stocks (Figure 1).

In genitals of females it was observed that two specific substances occur: one of them activates α_2-esterase like Triton X-100; the other activates the S-esterase. Probably these substances break the binding of isozymes with the membrane.

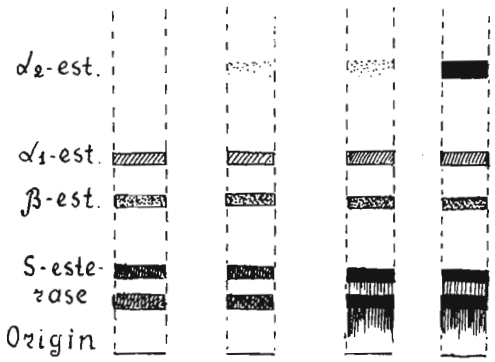


Figure 1. Results of microelectrophoresis of single ejaculatory bulbs of D. littoralis.

According to our preliminary data the success of crosses between different stocks of D. littoralis or D. virilis depends upon the peculiarities of esterase pattern of ejaculatory bulbs in males and the specificity of esterase activating substances in the corresponding females. It can be a possible molecular mechanism of sexual isolation in fly populations.

References: Korochkin, L., N. Matveeva, E. Belayeva and B. Kuzin 1976, 5th Europ. Dros. Res. Conf. Louvain-La-Neuve, Belgium. Abstracts.

Marsh, J.L. University of Virginia, Charlottesville. Construction of a third chromosome balancer bearing a dominant temperature sensitive lethal.

It is useful to be able to kill heterozygous flies in large populations by means of dominant conditional lethality. In doing so the lethal should be in a balanced chromosome. Earlier attempts to induce a dominant temperature-sensitive lethal (DTS) in the available third chromosome balancers have failed (Falke & Wright, 1972) presumably because of the very low rate of induction of DTS in the third chromosome, (Suzuki, 1970). I have constructed a balanced DTS-bearing third chromosome by introducing additional alterations into an already partially rearranged DTS chromosome.

The scheme was to irradiate a chromosome bearing both In(3L)C90 (into which DTS4 (Holden and Suzuki, 1973) had been introduced) and In(3R)C. The complete constitution of the starting chromosome was In(3L)C90 62B;80C, DTS4 th st In(3R)C 92D;100F, Sb e. Since in this chromosome the rate of crossover between DTS4 and Sb is about 16% one additional rearrangement could reduce this figure to near zero. 45 0-2 day old males heterozygous for this chromosome and a wild type third chromosome were irradiated with 4000 rads (10mA 300kv 45 min), mated en masse to +/+ females and removed after 4 days.

Scheme for detecting balanced DTS bearing third chromosome:

$\frac{+}{+}$ ♀♀ x $\frac{\text{In(3L)C90, DTS4 th st In(3R)C Sb e (X-ray)}}{+}$ ♂

900 single $\frac{\text{In(3L)C9 In(3R)C}}{+}$ ♀♀ x $\frac{5}{+}$ or 5 rucuca ♂♂

vial 1 → vial (1) 18° until 2nd Instar larvae visible then shift up to 29° and check for Sb flies

vial 2 → vial (2) keep at 18° to recover stock

vial 3 → subsequent vials 18° for 4 days then shifted to 29°

As the vials that were shifted to 29° hatched, they were scored for the presence of Sb that should appear with a frequency of about 16% as a result of crossover between Sb and DTS4. If an event occurred which suppressed crossing over, few or no Sb flies would appear. If 3 or more Sb flies were observed the line was discarded. If a line produced less than 3 Sb flies, it was rechecked by selecting 3 Sb ♀♀ from the 18° vial, raising their progeny individually at 29°, and again checking for Sb progeny. If one or more of the females produced many Sb flies or if all 3 produced 5% or more Sb flies, the line was discarded. The initial screen identified 33 chromosomes which produced few or no Sb flies. On retesting many of these were eliminated because of low fecundity, leaving six chromosomes that produced fewer than 5% Sb flies. Of these 6 only three produced no Sb flies at 29°. Two of these, designated TM8 and TM9, were tested further by selecting a single male to establish a stock and by performing the following tests:

Table 1. Testing of TM8 and TM9 isolates.

			TM8		TM9	
A.	DTS4 th st Sb e/rucuca ♀ x rucuca ♂					
	22°	rucuca	1558		955	
		Sb th st e	1444		1012	
		Crossovers	2	(.067%)	2	(.1%)
		Total	3004		1969	
B.	DTS4 th st Sb e/+ ♀ x rucuca ♂					
	22°	+++	1480		1263	
		th st Sb e	929		952	
		Crossovers	1	(.04%)	5	(.23%)
		Total	2411		2220	

(Table continued next page)

			TM8		TM9	
C.	DTS4 th st Sb e/rucuca ♀ x rucuca ♂					
	22°	rucuca	1220		646	
		th st Sb e	0		0	
		Crossovers	0	(<.08%)	2	(.3%)
		Total	1220		648	
D.	DTS4 th st Sb e/rucuca ♀ x +/+ ♂					
	30°	+++	3177		441	
		Sb "escapers"	82	(2.5%)	58	(11.6%)
		Total	3259		499	

The results, presented in Table 1, show that total crossovers at 22° were .05% (3/5415) for TM8 and .17% (7/4189) for TM9. At 30° crossing over between DTS4 and Sb apparently occurred at a frequency of less than .08% in TM8 and .3% in TM9 when Bal/rucuca females were mated to rucuca males. However if the same females were crossed to wild type males many Sb flies were recovered (i.e., 2.5% for TM8 and 12% for TM9). Using rucuca for subsequent testing only one bona fide crossover was found among 59 of these "Sb flies", two others were fertile but were shown to have the balancer intact and the rest were sterile and died within two days. Moreover the majority appeared to be "sick", with drooping unexpanded wings, bloated abdomens, etc. It seems that these Sb flies were escapers from the DTS phenotype and not crossovers.

Thus it appears that TM8 and TM9 are relatively well-balanced chromosomes which may be useful in selecting against heterozygotes. Events separating Sb from the DTS occur in less than .08% of the progeny of TM8 and in less than .3% of the progeny in TM9. The chromosomes are available over TM3, Ser or over Gl.

Thanks are due to Dr. J. Holden who had put DTS4 into In(3L)C90 and Dr. Ish-Horowitz for making the inversion stocks available to me. This work was supported in part by a USPHS National Institute of Health Research Fellowship No. 1F32HD05053-01. I am indebted to Dr. T.R.F. Wright and Dr. W.J. Gehring in whose laboratories this work was carried out.

References: Falke, E.V. and T.R.F. Wright 1972, DIS 48:89; Holden, J.J. and D.T. Suzuki 1973, Genetics 73:445-458; Suzuki, D.T. 1970, Sci. 170:695-706.

Parkash, R. and K.S. Gill. Panjab Agricultural University, Ludhiana, India.

Isozyme polymorphism in Drosophila.

Genetic variability has been studied at acid phosphatase and esterase loci in local populations of six species of Drosophila (malerkotliana, nepalensis, takahashii, jambulina, punjabiensis, immigrans) and Zaprionus paravittiger using starch gel electrophoresis. Acid phosphatase is coded by a single autosomal gene, Acph-1, in all the species studied. This locus is monoallelic in jambulina, immigrans, and Z. paravittiger but triallelic in punjabiensis and tetra-allelic in malerkotliana, takahashii and nepalensis. There is more than one esterase-determining gene in each species studied, all of which are autosomal. Each gene determines a distinct esterase. There are two such genes in punjabiensis, three in nepalensis, takahashii, jambulina, and Z. paravittiger, four in malerkotliana and immigrans. Most of the genes are monoallelic. Est-2 in immigrans and Est-3 in nepalensis, takahashii, jambulina and Z. paravittiger are diallelic and Est-1 in the latter species is triallelic. Alleles at the variable Acph-1 and Est loci are codominant. Acid phosphatase in all species showing genic variability and Est-1 in Z. paravittiger are dimeric enzymes, while all other variable esterases are monomeric enzymes.

With regard to Acph-1 locus, four alleles (three in nepalensis and one in malerkotliana) have a low frequency (<0.06), ten alleles in three different species have a moderate frequency (0.10 - 0.48) and one allele in nepalensis has a very high frequency (0.91). With respect to variable esterases, allelic distribution within each species is nearly symmetrical. Natural populations show considerable heterogeneity. They are also extensively heterozygous; combined heterozygosity in takahashii is 0.89. Mean heterozygosity per locus is 0.12, 0.26, 0.15, 0.125, 0.20, 0.098 and 0.28 in malerkotliana, takahashii, nepalensis, jambulina, punjabiensis, immigrans and Z. paravittiger, respectively. The mean heterozygosity over all loci in different species studied is 0.17.

Three acid phosphatase allozymes of malerkotliana were characterised biochemically on the

basis of specific activities, pH optima, effect of organic and metalic ions, inhibitor sensitivity, and Vma and Km values. Kinetic differences in the allozymes have been interpreted to signify adaptive value differences among the alleles controlling these three allozymes, thus supporting the selectionist's hypothesis for the maintenance of genetic polymorphism. The use of allozymes as diagnostic tools has been demonstrated. On the basis of allozymes, takahashii and nepalensis comprise one group and jambulina and punjabiensis another group. Allozyme data also indicated that within Drosophila species, reorganization at the Acph-1 locus involved eight mutational (including mutation-like) events. Similar data was obtained for some esterase loci.

Kirschbaum, W.F. Dpto de Radiobiología, Comisión Nacional de Energía Atómica, Buenos Aires, Argentina. Chromosomal rearrangements of visible mutations induced at specific loci in motile sperm.

Chromosomal analysis of 54 visible mutations induced by 3000 rads at specific loci in motile sperm, was carried out in order to detect chromosomal rearrangements. All the mutations were obtained at this laboratory by G.D. Maroni (DIS 43:133) irradiating 3163 "Binsc" males. These males were afterwards mated to females bearing the "maple" chromosome that carries 13 recessive marker genes.

The 54 mutations at specific loci, were obtained over 10,132 mated females.

The "Binsc" chromosome, that was used to produce mutations by irradiation, carries a portion of heterochromatin near the locus of y. Therefore all the slides showed the anterior part of the X chromosome, attached to the chromocenter, making this zone unclear for chromosome analysis. Consequently all the 18 mutations of the y, ac, sc zone were discarded for further analysis.

Considering the 36 remaining mutations, the analysis of the polytene chromosomes showed that 24 were point mutations and 12 were chromosomal rearrangements (Table 1).

No. of mutations.						Av. mut. freq. locus/r x 10^{-7}
10 loci	Point mutations			24	.78	1.18
36	Chromosome rearr.	Deficiencies	2	12	.40	
		Inversions	4			
		Translocations	6			
Eye colour mutations 8 loci.	Point mutations			20	.82	1.07
26	Chromosome rearr.	Deficiencies	1	6	.25	
		Inversions	2			
		Translocations	3			
ct locus	Point mutations			2	.66	2.63
8	Chromosome rearr.	Deficiencies	1	6	1.97	
		Inversiones	2			
		Translocations	3			

This means that for a mean overall mutation frequency of the 10 remaining loci of 1.18×10^{-7}/locus/r, the resulting average mutation frequency of the point mutations is $.78 \times 10^{-7}$/locus/r, and an average mutation frequency of $.4 \times 10^{-7}$/locus/r for the chromosomal rearrangements.

The eye colour mutations had similar results concerning the number of chromosomal rearrangements detected on them. On the other hand, the ct mutations showed a high proportion of chromosomal rearrangements. Analysing comparatively the eye colour mutations, considering the 8 loci together with the ct locus, the results shown in the table were obtained.

The mean overall mutation frequency for the eye colour mutations is 1.07×10^{-7}/locus/r, with an average mutation frequency of $.82 \times 10^{-7}$ for the point mutations and $.25 \times 10^{-7}$/locus/r corresponding to the chromosomal rearrangements. On the other hand, the mean mutation frequency of the ct mutations reached 2.63×10^{-7}/locus/r, with an average mutation frequency of $.66 \times 10^{-7}$/locus/r, for the point mutations and 1.97×10^{-7}/locus/r, due to the chromosomal rearrangements.

Therefore we can assume that in this case the high rate of ct mutations is caused by the high chromosomal rearrangements, especially translocations associated with the ct locus.

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Fertility components and maternal effects.

The maternal genotype appears to be an important component in determining fertility traits, e.g., egg production, hatchability, larval survival, etc. (Sturtevant and Beadle, Genetics 21:554; Parsons, Genetics 44:1325; Bateman, Heredity 17: 107; Kearsey and Kojima, Genetics 56:23; Barnes, Heredity 23:563 and Caligari and Mather, Proc. R. Soc. Lond. 191:387). Some of these authors consider that non-maternal effects are also contributory. In this note, the hypothesis that maternal effects are more pronounced during the earlier stages of development than in the later stages is examined.

Diallel crosses among six isogenic lines (I Oregon-R, Canton S, Chicago, M Oregon-R, Formosa and Urbana - labelled as strains A, C, Ch, D, F and U) were performed in two blocks. The traits determined for singly-mated females were egg production (E), (on days 4 and 5 post-eclosion), egg hatchability (L/E) and larva-to-adult survival (A/L). The results as mean values over blocks are presented in Table 1. Also determined (from the same material) but not

Table 1. Egg production (per female per day), % hatchability and % larva-to-adult survival.

	A	C	Ch	D	F	U
	48.0	55.6	45.4	57.0	38.8	53.4
A	82.6	87.8	89.6	31.7	84.5	92.7
	85.2	86.6	79.7	58.4	70.7	83.0
	47.9	55.0	38.9	40.9	32.5	50.1
C	90.1	89.8	95.1	47.4	84.2	95.4
	87.1	82.2	85.3	38.7	72.7	79.8
	58.8	49.9	44.9	53.6	37.5	44.6
Ch	90.7	89.4	91.3	76.9	80.9	93.4
	86.8	87.5	82.7	67.7	77.4	83.8
	56.6	53.8	38.5	47.7	40.1	51.1
D	83.9	86.1	93.4	66.4	84.4	91.3
	85.1	87.6	86.0	62.8	56.8	85.5
	48.1	53.6	44.4	44.2	32.7	53.0
F	72.3	84.7	82.5	33.9	78.5	84.8
	75.5	83.3	77.7	60.7	52.5	63.8
	51.5	50.5	41.4	47.9	33.1	48.5
U	93.4	90.7	95.5	77.9	80.6	89.9
	85.8	82.8	86.3	62.1	75.5	64.0

recorded here were egg-to-adult survival (A/E) and adult yield (A), two traits frequently encountered in the literature. Each diallel was examined in terms of the following model:

$$Y_{ijk} = M + g_i + g_j + s_{ij} + m_j + r_{ij} + b_k + e_{ijk}$$

where:

- M = the common mean
- g_i = the common genetic contribution of the ith paternal line
- g_j = the common genetic contribution of the jth maternal line
- s_{ij} = the interaction between the genetic contributions of the ith and jth lines
- m_j = the maternal contribution of the jth line
- r_{ij} = the additional effect of using the ith line as male parent and the jth line as female parent
- b_k = the effect of the kth replicate
- e_{ijk} = random error

This model is a combination of Wearden's (Heredity 19:669) maternal and non-maternal effects models. Assuming random effects, corresponding variance components were estimated within the framework of the analysis of variance of diallel tables provided by Hayman (Biometrics 10:235). The results are set out in Table 2. An examination of the EMS column reveals what each Hayman term reflects. The significances of the b and d components were tested by the error mean

Table 2. Analysis of variance of fertility traits (mean squares) and variance components (%)

		E	L/E†	A/L†	A/E†	A	EMS
a	5	1143.0	0.1389	0.0952	0.2187	2822.9	$s_e^2 + 6g_m^2 + 4s_s^2 + 24s_g^2$
b	15	108.6	0.0132**	0.0088**	0.0178**	275.5**	$s_e^2 + 3.33\ s_s^2$
c	5	1178.7**	0.1205**	0.0630**	0.1740**	2412.9**	$s_e^2 + 4s_r^2 + 6s_m^{2*}$
d	10	119.5	0.0143**	0.0120**	0.0089**	153.4*	$s_e^2 + 4s_r^2$
Blocks	1	409.8	0.0346	0.0123	0.0055	5.0	-
Error	35	132.4	0.0025	0.0023	0.0020	66.5	s_e^2
* $P < 0.05$; ** $P < 0.01$; † performed on original ratios.							
s_g^2		0	2.7	16.6	3.6	2.4	
s_s^2		0	11.8	10.6	12.7	11.6	
s_r^2		0	10.9	13.3	4.6	4.4	
s_m^2		56.9	65.4	46.7	73.6	69.7	
s_e^2		43.1	9.2	12.8	5.4	12.3	
s_m^2		100.0	72.0	53.6	77.9	79.4	

squares, while the c components were compared with the d's. There is no valid variance ratio test of the a's. Negative variance component estimates were set equal to zero. From an examination of the testable mean squares and variance components, the following conclusions can be made: (a) Egg production, apart from a large error variance, is almost entirely maternally inherited, (b) Non-maternal effects, although relatively small, are nevertheless significant for all other traits and (c) As development proceeds, maternal effects are less pronounced, i.e. E < L/E < A/L for s_m^{2*} (expressed as a percentage excluding error variance).

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Resistance to radiation induced chromosomal rearrangements in Drosophila simulans.

Although most natural populations of Drosophila are characterized by chromosomal polymorphisms, a very small minority of species have proved to be consistently chromosomally monomorphic; that is, very few or no gene arrangements other than the standard sequence have ever been described for these species[1]. Notable among the monomorphic species is Drosophila simulans, a sibling species of the geneticist's workhorse, D. melanogaster. Adults of these two species are virtually indistinguishable morphologically and differ cytologically by only a single major inverted sequence in the banding patterns of the salivary gland chromosomes[2]. Both are cosmopolitan species, in the sense that they have been recorded from all six biogeographic regions of the world[3] and both are often found together in areas of human habitation. D. melanogaster however, is a highly polymorphic species with more than fifty inversions having been described from natural populations[4], whereas D. simulans is virtually chromosomally monomorphic throughout its range[1]. Only one chromosomal aberration has ever been described from a natural population of this species, and that was seen in but a single individual[5].

Why two such similar species should differ so significantly with respect to degree of chromosomal polymorphism is a question that has not yet been satisfactorily answered. The purpose of this study has been to approach the problem from a new direction, that is, to determine if a quantitative difference exists in the susceptibility of the chromosomes of the these two species to ionizing radiation. Although the manner of origin of chromosomal aberrations in natural populations remains unknown, it has long been known that aberrations can be artificially induced by X-irradiation[6].

The flies utilized in this experiment were a strain of D. simulans supplied by the Department of Biology at Amherst College, Amherst, Massachusetts, and the Oregon-R strain of D. melanogaster. Males of each species were given 4000 rads of X-irradiation at a dose rate of 250

rads per minute, using a Varian Clinac-4 linear accelerator. Each male was subsequently mated to non-irradiated females of his respective strain. Third instar larvae were removed and aceto-orcein squashes of their salivary gland chromosomes were prepared. Chromosomal aberrations induced in the germ tissue of the males appear as structural heterozygotes in these larval cells. Only inversions and translocations were scored; deficiencies occurred in both species but were not scored due to the difficulty of detection and confirmation.

There is an appreciable difference in the susceptibility of the chromosomes of these two species to damage by X-irradiation. Among 480 slides, each representing a single F_1 individual of D. melanogaster, 24.8% showed different chromosomal aberrations. Eighty-five carried inversions and 34 translocations. In D. simulans, however, only 0.8% of 650 slides demonstrated aberrations in the F_1. Three were translocations and two were inversions.

The results of the present study indicate a marked difference between two species having very similar genomes. As previously mentioned, the banding patterns of the salivary gland chromosomes of these two species are virtually identical[2]. Furthermore, the haploid genome of both species contains the same amount of DNA with similar base compositions although DNA-DNA hybridization studies indicate that the DNA's of these two species show differences in base sequence[7]. Since there appears to be no substantial difference between the DNA's of D. simulans and D. melanogaster, perhaps the basis for their differential radiosensitivity is to be found in the other major component of their chromosomes, the protein. Alternatively, the capacity for repair of chromosomes broken by X-irradiation may be markedly different in each of these two species.

The latter hypothesis was tested by measuring radiation-indiced dominant lethality in zygotes. The results are presented in Table 1. The average percentage of eggs hatching among the non-irradiated D. melanogaster controls was 80.9% and among the D. simulans 83.4%. The radiation induced dominant lethality, or the additional percentage of eggs failing to hatch beyond the control values due to irradiation of males was very similar for the two species, simulans averaging 47.1% and melanogaster 50.8%. Thus simulans shows no increased sperm viability after irradiation.

Many studies have repeatedly shown that inversion polymorphisms are maintained in a population because they confer some type of selective advantage on their bearers[8]. In a series of comparisons between monomorphic and polymorphic populations of D. pseudoobscura, a number of researchers have succeeded in demonstrating that the polymorphic populations are consistently superior in fitness[9,10].

D. melanogaster is distributed throughout the world, usually being found in close association with human habitations[11]. Perhaps the half dozen or so polymorphisms present in natural

Table 1. Radiation-induced zygotic mortality. One to three day old males were irradiated and mated to virgin females of the same age. For D. simulans, five pairs were placed in each vial and for D. melanogaster, three pairs. Every 24 hours for 7 days the media plates of banana agar were removed from the vials and replaced with new plates. The number of eggs laid on each plate were counted, retained for 24 hours in an incubator and then scored for the number of eggs hatched.

Exp. no.	Species	No. Replicates	Dose (rads)	Total No. eggs counted	% hatched	% radiation-induced zygotic mortality*
1a	simulans	20	control	1486	76.9	--
1b	simulans	20	4000	1482	29.8	47.1
2a	simulans	12	control	828	82.2	--
2b	simulans	12	4000	1008	35.2	47.0
3a	simulans	12	control	938	83.5	--
3b	simulans	12	4000	1339	36.4	47.1
4a	melanogaster	12	control	1184	82.3	--
4b	melanogaster	12	4000	1384	34.4	47.9
5a	melanogaster	12	control	1202	84.3	--
5b	melanogaster	12	4000	1186	30.6	53.7

*Difference between means of two species is insignificant at 1% level, $z = 0.15$.

populations of this species throughout the world are responsible for adapting it to some specific niche that is closely associated with man. Drosophila simulans, on the other hand, while being widely distributed and often closely associated with civilization, tends to be

more common in warmer temperate regions[12] and in tropical Brazil is found in regions far removed from the human habitation[11]. The genetic system of simulans does not appear to be open to the formation of inversions. Instead, the species must generate variability through genetic recombination, a process whose outcome is restricted with the occurrence of inversions and necessary for the colonizing behavior of the species. The genic heterozygosity of simulans, in terms of the proportion of polymorphic loci per population, has been shown to be greater than in melanogaster[13] by some investigators but not by others[14,15].

A good deal of further study will be required to elucidate the exact mechanisms by which inversions are suppressed in Drosophila simulans, and to determine whether or not these mechanisms are indeed under genetic control. Answers to these questions will bring us closer to an understanding of the evolutionary strategies of at least two species of Drosophila.

References: 1. Carson, H.L. 1965, in The Genetics of Colonizing Species, 503-521 (Acad. Press, N.Y.); 2. Horton, I.H. 1939, Genetics 24:234-243; 3. Patterson, J.T. and W.S. Stone 1952, in Evolution in the Genus Drosophila, 45-111 (Macmillan, N.Y.); 4. Stalker, H.D. 1976, Genetics 82:323-247; 5. Dobzhansky, Th. 1939, Biol. Rev. 14:339-368; 6. Catcheside, D.G. 1938, J. Genetics 36:307-320; 7. Laird, C.D. and B.J. McCarthy 1968, Genetics 60:302-322; 8. da Cunha, A.B. 1960, Ann. Rev. Entomol. 5:85-110; 9. Beardmore, J.A., Th. Dobzhansky and O. Pavlovsky 1960, Hered. 14:19-33; 10. Dobzhansky, Th., R.C. Lewontin and O. Pavlovsky 1964 Hered. 19:597-614; 11. Dobzhansky, Th. 1965, in The Genetics of Colonizing Species, 533-546 (Acad. Press, N.Y.); 12. Parsons, P.A. 1975, Quart. Rev. Biol. 50:151-169; 13. Kojima, K., J. Gillespie and Y.N. Tobari 1970, Biochem. Genet. 4:627-637; 14. MacIntyre, R. 1966, Genetics 53:461-474; 15. Berger, E.M. 1970, Genetics 66:677-683.

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Golubovsky, M.D. Institute of Cytology and Genetics, Novosibirsk, USSR. The comparison of direct and back mutations in germinal and somatic cells for three pairs of unstable singed alleles in D. melanogaster.

Three pairs of unstable singed alleles (mutant and normal on phenotype) were isolated from original unstable mutant sn77-27: sn^{+IIm} - sn^{IIm}; sn^{+SRI} - sn^{SRI}; sn^{+SR2} - sn^{SR2}. When mutating, they mainly "switch" into each other. All three pairs are mutable both in germinal and somatic cells, but the rate of mutations is allele-specific. The number of reversions in crosses like I ♂ sn x ♀ $\overline{XX}$/Y or I ♂ sn^{+} x ♀ $\overline{XX}$/Y can be a definite measure of mutation rate in germinal cells. The percentage of mosaic males in the same crosses may be the measure of mutability in somatic cells. It is hard to compare directly these two values. But it is possible to compare the ratio of direct and back mutations in the same type of cells for each pair of alleles. The data are given in Table I. The number of analysed males in each type of cross was about 3-5 thousand. It appears that for +IIm - IIm pair the rate of change in sn → + direction is by 23 times more in germinal and by 5 times more in somatic cells than for mutations + → sn. The supermutable or strongly reversible "SR" alleles are highly unstable in germinal cells. But in the somatic cells, on the contrary, their normal derivatives "+SRI" and "+SR2" are quite unstable and give enormous numbers of mosaic flies, about 13 and 33%. This comparison shows that the rate of mutation in germinal and somatic cells for the same allele may be quite different and can be regulated in both directions.

Table I. Comparison of direct and back mutations in somatic and germinal cells for three pairs of unstable sn alleles.

Pairs of alleles	Tissue, direction and frequency of mutations, %					
	germinal cells			somatic cells		
	+ → sn	sn → +	ratio	+ → sn	sn → +	ratio
sn^{+IIm} - sn^{IIm}	0.90	21.6	1:23	0.19	0.94	1:5
sn^{+SRI} - sn^{SRI}	0.74	42.4	1:57	12.9	0.56	23:1
sn^{+SR2} - sn^{SR2}	0.35	27.8	1:79	33.3	2.1	16:1

Mariani, C. and E. Boncinelli. International Institute of Genetics and Biophysics, CNR, Naples, Italy. Ring R(1)2,y B chromosome bears no ribosomal RNA cistron.

It has been claimed (1) that bobbed loci located on D. melanogaster ring X chromosomes cannot undergo magnification, a phenomenon implying an increase in rDNA cistrons redundancy of bobbed deletions in suitable genetic conditions (2). Since the start we have worked on the stock R(1)2,y B & C(1)DX,y f originally obtained from Oak Ridge. Following the standard magnification schedule, we crossed R(1)2,y B males with C(1)RM,y v f/Ybb$^-$ females. We were unable to recover any single R(1)2,y B/O male out of a progeny of 3,000 flies. We then mated our stock males to C(1)RM,y w/O females to test the viability of R(1)2,y B/O males. Again no male was recovered, not even from crosses with C(1)RM,y w/Dp(1;f) 1337 females. This duplication has been shown (4) to suppress, at least partially, position effect lethality in scute inversions. We moved on to the bobbed lethal magnification procedure (2) which implies the use of a "helper" Ybb chromosome carrying 50-60 rDNA cistrons on its locus. The stock used was C(1)RM,y v f/B^{S}Ybb y$^+$. The phenotype of R(1)2,y B/B^{S}Ybb y$^+$ males was strong bobbed and did not show any significant improvement throughout 20 generations of back crossing these males to C(1)RM,y v f/B^{S}Ybb y$^+$ females. Beginning from the seventh generation, some males were tested against a Ybb$^-$ chromosome showing no viability recover.

The R(1)2,y B chromosome has been reported (3) to be not viable either as an XO male or as a homozygous female and to show a bobbed phenotype in females heterozygous for the ring and a In(1)B^{M1}, bb chromosome. From this report and our own results we wondered what rDNA content the chromosome should have. We verified first its present circular nature performing appropriate recombination tests with both a normal order and an inverted rod X chromosome. We then measured its rDNA level with a series of rRNA/DNA hybridization experiments (2), using both males and females from the original stock. Because it is known (2) that C(1)DX,y f chromosome carries no rDNA, values obtained from our females are directly the values contributed by the Y chromosome. Deducting this value from that of the males we can derive the R(1)2,y B chromosome contribution. Four series of parallel experiments were performed as shown in Table 1.

Table 1. rRNA/DNA hydridization saturation values from R(1)2,y B/Y males and C(1)DX,y f/Y females.

Experiment	XY males	$\overline{XX}$Y females	determinations
A	166 ± 4*	159 ± 4	10
B	169 ± 3.2	164 ± 3.3	15
C	164 ± 3	159 ± 4	12
D	164 ± 2.7	160 ± 4	12

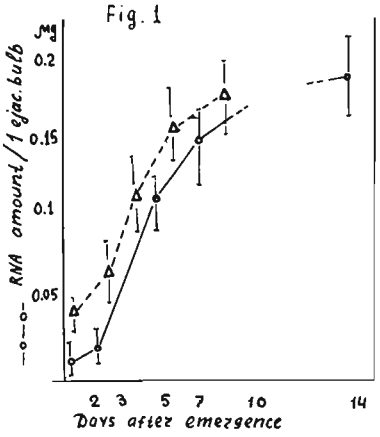
* Data are given as (mean ± S.E.) x 100. Approximately 40 µg of DNA were loaded per filter. Filters were incubated in a solution of 3 µg/ml of ^{3}H-rRNA with a specific activity of about 150,000 cpm/µg.

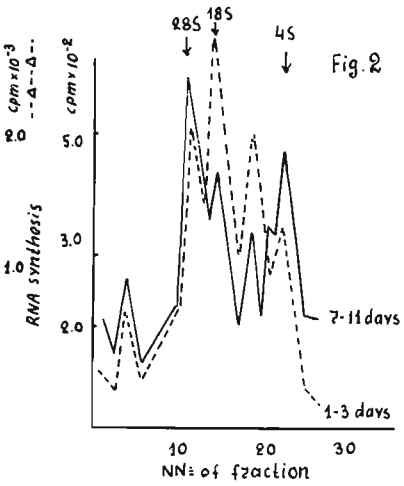
One should note that female saturation levels have been multiplied by 1.1 to take into account the extra DNA pertaining to the XXY asset.

Considering the resolution power of the methodology we can conclude that this ring chromosome carries no more than 3-4 genes coding for 28S + 18S ribosomal RNA. Of course from the conceptual point of view it makes a great difference between carrying a very few genes and no gene at all, but our method cannot go further. Probably accurate cytological observations could assess whether or not nucleolar association occurs. However we are confident that from now on the R(1)2,y B chromosome can be used in genetic crosses as an actual Xbb^o or X$_{NO}$ much the same as the sc^4 sc^8 X chromosome is used.

References: (1) Tartof, K.D. 1974, Proc. Nat. Acad. Sci. USA 71:1272-1276; (2) Ritossa, F. 1976, The Genetics and Biology of Drosophila (Ashburner, M. & E. Novitski, eds., Academic Press) Vol Ib:801-846; (3) Leigh, B. 1976, ibid. 505-528; (4) Baker, W.K. 1971, Proc. Nat. Acad. Sci. USA 68:2472-2476.

Karasik, G., L. Korochkin, L. Maximovsky, and B. Kuzin. Institute of Cytology and Genetics, Novosibirsk, USSR. The RNA synthesis in the ejaculatory bulb in Drosophila imeretensis.





The amount of RNA and RNA synthesis were investigated in the ejaculatory bulb of Drosophila imeretensis at different periods after emergence. The synthesized RNA was characterized by the help of the electrophoretic technique according to Daneholt et al. (1969). ^{3}H-uridin ("Amersham", England, 41 Ci/mM) was injected (dose - 0.2 mCi) into the abdomen of males at the different stages after emergence. The RNA amount increases in ejaculatory bulbs after emergence (Figure 1). The similar increase of RNA synthesis takes place especially during 2-9th days after emergence (Figure 1). The electrophoretic profiles of labeled RNA extracted from ejaculatory bulbs are depicted in Figure 2. It can be concluded that the synthesis of 28S, 18S and 4S RNA take place. The incorporation of ^{3}H-uridin into heterodisperse RNA can be seen too. Some differences in these profiles are obtained between flies on the 1-3rd and 7-11th days after emergence.

Reference: Daneholt, B., J.-E. Edstrom, E. Egyhazi, B. Lambert, U. Ringborg 1969, Chromosoma (Berl.) 28:379.

Spillmann, E. and R. Nöthiger. University of Zürich, Switzerland. Cytological localization of "red" (3-53.6).

Wildtype males of strain "Sevelen" were irradiated with 4000 r (50 kV, 25 mA, 0.3 mm Al, 1000 r/min) and then mated to cu red sbd sr e virgins. The progeny was scored for red phenotypes. All treated chromosomes with newly induced mutations were then introduced into balanced stocks (TM2, In(3LR) Ubx^{130}, Ubx^{130} e^{s}) and later subjected to a cytological analysis. One lethal red mutant (red-21) proved to be a very small deficiency of 2-3 bands (Figure 1).

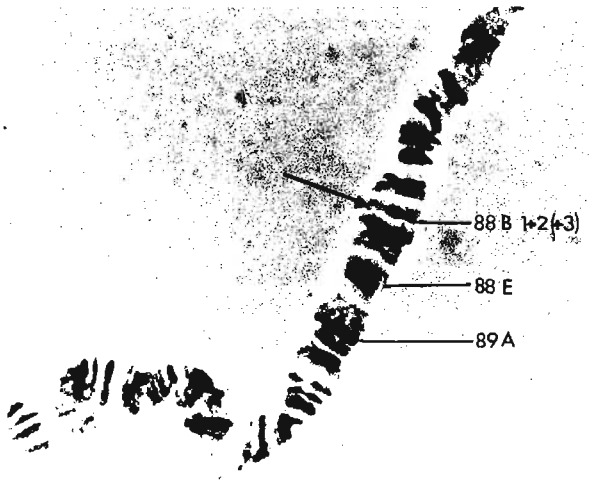


Figure 1. Df(3R)red-21. Arrow points to a heterozygous deficiency for bands 88B1-2 (or perhaps 88B1-3).

In view of the already existing information (1), this deficiency places the locus for red into 88B1-3.

We want to thank Dr. E.B. Lewis for valuable help in identifying the chromosome rearrangements.

Reference: (1) Lindsley, D.L. and E.H. Grell 1968, Carnegie Inst. Publ. 627.

Spillmann, E. and R. Nöthiger.
University of Zürich, Switzerland.

Cytology, genetics and lethality patterns of homozygous lethal mutations in the sbd region.

As part of a larger project we have isolated a number of lethal sbd mutants (sbd: 3-58.2). Wildtype or mwh e males were irradiated with 4000 r (50 kV, 25 mA, 0.3 mm Al, 1000 r/min) and crossed to virgins cu red sbd sr e. Among a total of 64,762 offspring, 54 displayed the sbd phenotype. Among these, 13 were homozygous lethal, and 11 could finally be preserved in balanced stocks. The cytology, complementation patterns, and the lethality phases of these 11 sbd mutants were investigated.

The cytological analysis revealed 3 inversions, 2 deficiencies, 1 translocation and 5 chromosomes without any visible cytological aberration (see Table 1). The complementation

Table 1. Cytological analysis of X-ray induced homozygous lethal sbd mutations.

mutation	cytological data
sbd-12	In(3R) BP: 88B2-C1; 89B3-16
sbd-13	-
sbd-17	In(3R) BP: 80; 89B10-12 sbd near heterochromatin
sbd-18	-
sbd-21	In(3R) BP: 86D2-E1; 89B3-12
sbd-26	Df(3R) BP: 89B9-10; 89C7-D1
sbd-32	-
sbd-35	-
sbd-43	-
sbd-45	Df(3R): missing are the bands 89B4-89B10, maybe B13
sbd-47	T(2;3) BP: 41; 89B10-12 sbd near heterochromatin
sbd^{105}*	Df(3R) sbd^{105} BP: 88F9-89A1; 89B4-5 (after Lewis 1948) (after Bridges' revised map: right BP is 89B9-10)

- no detectable aberration
* obtained from the Pasadena stock collection

Table 2: Complementation experiments.

	sbd 12	sbd 13	sbd 17	sbd 18	sbd 21	sbd 26	sbd 32	sbd 35	sbd 43	sbd 45	sbd 47	sbd 105	sbd^1
sbd-12	L												
sbd-13	L	L											
sbd-17	L*	V	L										
sbd-18	L	L	V	L									
sbd-21	L	L	V	L	L								
sbd-26	L*	L*	V	L*	L*	L							
sbd-32	L*	V	V	V	V	L*	L						
sbd-35	L	L	V	L	L	L	L*	L					
sbd-43	L	L	V	L	L	L	L*	L	L				
sbd-45	L	L	V	L	L	L	L*	L	L	L			
sbd-47	L	L	L*	L	L	L	L	L	L	L	L		
sbd^{105}	L	L	V	L	L*	L*	L*	L	L	L	L	L	
sbd^1	L*	L*	V	L	-	L	V	L	L	L*	-	L	L

L = lethal; V = viable and sbd in phenotype; L* = lethal, with adult escapers of sbd phenotype
- cross not performed

pattern of all possible combinations is given in Table 2. The results, in summary, show that sbd-17 and sbd-32 yield viable offspring with most other sbd mutants while 9 of the newly induced sbd mutants as well as sbd^{105} and sbd^{1} did not complement, or produced a few escapers at best. All surviving heterozygotes, including the escapers, exhibited the sbd phenotype. Thus whereas complementation did occur for lethality, this was never the case for the phenotype of the bristles.

The lethality phases were determined by counting the number of eggs, larval stages, pupae and adults produced by crossing F_1 heterozygotes (sbd-m/+) inter se. The mutants sbd-32, sbd-35, sbd-43 are embryonic lethals, while sbd-12, sbd-13, sbd-17, sbd-18, sbd-21 kill their carriers shortly after hatching as first instar larvae. For the three deficiencies (see Table 1), the lethal phase begins during embryonic development and continues for 24 hours after hatching. - In summary, the sbd lethals may be considered as "embryonic-larval boundary lethals" (Hadorn, 1961). It is possible that small differences in genetic background or environment may shift the time of death to just prior to or after hatching.

Research supported by Julius Klaus-Stiftung, Zürich.

Reference: Hadorn, E. 1961, Developmental Genetics and Lethal Factors (John Wiley).

Kearney, J. University of Leeds, England.

A note on the breeding sites of D. obscura Fallen and D. subobscura Collin.

Two woodlands (Adel Dam and Wike), both situated north of Leeds, were used as study areas. In Adel Dam there are seven species of tree or shrub which produce fruit: the common hawthorn (Crataegus monogyna Jacq.), rowan (Sorbus aucuparia L.), woody nightshade (Solanum dulcamara L.), dog rose (Rosa canina agg.), blackberry (Rubus fructicosus L.), common elder (Sambucus nigra L.) and the snowberry (Symphoricarpus rivularis Suksdorf). In Wike there are only three species: rowan, blackberry and the common elder.

Throughout the autumn of 1975 collections of these fruits were made in three different ways: 1) Fruits were picked directly from the tree or shrub and about 50 were returned to the laboratory. 2) A further 50 berries were placed in each of two sterile, quarter-pint bottles, which were left at two different sites within the woodland. The bottles were returned to the laboratory after a period of two weeks. 3) Fallen fruits (or leaf litter plus fruits when the latter were very decayed) were collected and returned to the laboratory.

When returned to the laboratory, the fruits were put into jars containing moist peat, and kept in near-natural conditions of light and temperature in an outdoor insectory. The numbers and species of Drosophila that emerged from each sample of fruit were noted.

Results: Three collections were made at Wike (10 and 22 September and 15 October). No Drosophila emerged from any of the fruit. However, the following emergence data were obtained for Adel Dam.

Date of collection	Method of collection	Site	Species of fruit	Species of Drosophila	Details
29 Aug.	3	-	rowan	subobscura	5♂ 15♀
13 Sept.	2	1	rowan	subobscura	15♂ 15♀
	2	1	blackberry	subobscura	37♂ 47♀
	2	1	snowberry	subobscura	6♂ 13♀
	2	1	woody n-s	subobscura	8♂ 6♀
	2	2	blackberry	obscura*	19♂ 25♀
	3	-	rowan	subobscura	10♂ 12♀
28 Sept.	2	1	snowberry	subobscura	6♂ 2♀
	2	1	woody n-s	subobscura	9♂ 14♀

*These flies all eclosed over a period of two days suggesting that they were possibly the progeny of a single female.

Two further collections at Adel Dam (18 October and 14 November) produced no Drosophila. It is important to note that only rowanberries produced flies directly from natural sites.

Tsakas, S.C. Agricultural College of Athens, Greece. Ethanol as a probable selective factor for aldehydeoxidase (A.O) locus polymorphism in D. subobscura.

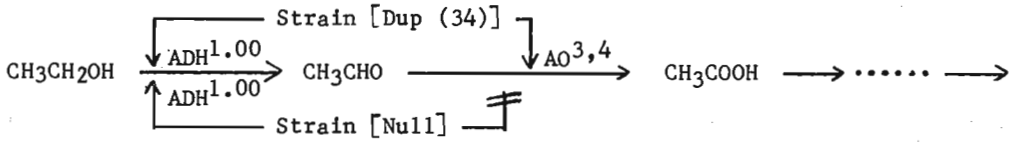
Two isogenic strains for O Chromosome from the natural population Crete (Greece) have been found to be the first homozygous for not active (Null) variants of the genes X.D.H and A.O, with $^{OST}/_{OST}$ chromosomal structure, and the other probably homozygous for duplicated variants $A.O^3$ and $A.O^4$ and with chromosomal structure O_{3+4+8}/O_{3+4+8}.

The map position for X.D.A. and A.O genes are 87.3 and 55.9 respectively. Both strains are homozygous for the common variant $A.D.H^{1.00}$ of the ADH locus (U-chromosome), and for the locus O.D.H. variant $O.D.H^{1.00}$ (O-chromosome, 50.6 map position).

After that, we decided to see the comparative viability among the strains on food with different concentrations of ethanol (5, 7, 5 and 10%).

The main idea was that, because of the absence of any electrophoretically detectable aldehyde oxidase zone in the strain Null/Null, we could have an increase of the toxic compound (aldehyde) in the body of the insect, perhaps causing its death.

A single enzymatic reaction pathway can be as follows:



In a series with pairs of vials (a vial for each strain) with the above concentrations and four replicates for every concentration, we put in each vial four pregnant females laying eggs for five days (approximately two hundred eggs per vial).

The results are given in the table as arithmetic mean of offspring for each concentration per vial. The experiments are in progress and publication will follow.

	Ethanol concentration		
Strains	5%	7.5%	10%
Null/Null	0^F	0^F	0^F
Dup/Dup	60(fertile)	35(fertile)	12(fertile)

F = death at the first larval stage

Ethanol as a selective factor is of course a specific one for the biochemical pathway, but may have a strong relationship to the food needs of the insect (mainly larval stages) even under natural conditions (yeast).

The same experiments with D. melanogaster didn't give us any clear screening ethanol concentration.

The reason for that was perhaps that both strains, the null and duplicated one, in this case were homozygous for active variant for the "low-aldehyde-oxidase locus" (Frond zone on gels).

Parkash, R. and A.K. Sharma. Punjab Agricultural University, Ludhiana, India. Colour dimorphism in Drosophila jambulina.

Wild-caught as well as laboratory reared individuals of Drosophila jambulina (Parshad and Paika, 1964) comprise males which are all alike phenotypically while in females only the last abdominal tergite is either heavily pigmented (dark colour) or no pigmentation (light yellowish colour). On this basis, the females can be phenotypically grouped as dark type and light type.

A series of single pair matings were set up to get homozygous stocks for dark and light types. Reciprocal crosses were made between flies of two homozygous stocks. In all such cases, the resulting F_1 individuals depicted dark type phenotype. The F_1 flies were mated inter se to get F_2 population. The dark and light type phenotypes showed a good fit to the expected ratio of 3:1 respectively, although with insignificant deviations. These results indicate that the trait under consideration is under control of one gene and it is represented by two alleles in natural population of D. jambulina. The allele controlling dark type is dominant over the allele responsible for light type. The wild caught flies of D. jambulina included 152 light type and 138 dark type females, showing thereby that both the alleles are quite frequent in the natural population of this species.

Götz, K.G. Max-Planck-Institut für biologische Kybernetik, Tübingen, Germany. The effect of VLF-magnetic fields on progeny yield and sex ratio in Drosophila melanogaster.

Attempts to substantiate irreversible actions of a variety of magnetic fields on the fruitfly have been successful and unsuccessful in about equal numbers. The most conspicuous mutagenic effects apparently induced by pulsed HF-fields (Heller and Mickey, 1961; Mickey, 1963) failed to appear under continuous electromagnetic irradiation (Mittler, 1973). This seems to correlate the observed damage with the VLF-components of the pulsed fields. The present investigation is motivated by the occurrence of these components both in the atmosphere and in the vicinity of electrical appliances. A strain of normally viable wild type males and subnormally viable attached-X y w females was used in which a decrease in the yield and in the male-to-female ratio of the progeny indicate, respectively, the extent of developmental damage and of sex-linked recessive lethal mutation induced by the exposure to detrimental conditions. Evaluation of 73,800 flies from subsequent generations of a control group and two test groups raised in steady, or rotating, homogeneous 9.6 kHz magnetic fields of about 2.5 Gauss showed only an insignificant increase in the yield (+4%) and in the sex ratio (+0.3%) of the progeny in the test groups as compared to the controls. There is less than 5% probability for the existence of developmental or hereditary defects sufficient to decrease the yield by more than 9% or the sex ratio by more than 7%. Although there remains considerable latitude for undetected effects of the selected fields, the results safely exclude genetic hazard of the order of a 1:8 sex ratio which was said to occur in a VLF-modulated HF-field.

Bock, I.R. and P.A. Parsons. Dept. of Genetics and Human Variation, La Trobe University, Bundoora, Victoria, Australia. Scaptomyza pallida in Australia.

There are nine cosmopolitan species of Drosophilidae. Eight of them are Drosophila ananassae, busckii, funebris, hydei, immigrans, melanogaster, repleta, and simulans; the ninth is Scaptomyza pallida.

S. pallida is widespread within Australia (but has not been recorded from Tasmania), specimens ranging from far northern Queensland to Victoria and southwestern Western Australia. The species, however, appeared to be rather rare, few specimens having been collected in any one locality. The total number of specimens in the Australian National Insect Collection (ANIC) from extensive sweeping over several decades is 7♂♂ and 13♀♀, compared with 83♂♂ and 185♀♀ for S. australis, the only other Scaptomyza known in Australia (Bock 1977). Additionally, out of over 150 drosophilid collections carried out by the present authors in the last two years, in excess of 90 S. australis and only 1♂ S. pallida have been found.

On August 8, 1976 a drosophilid collection was attempted on the summit of Mt. Bellenden Ker, the second highest mountain in Queensland (5,220'), which is covered in dense rain forest from base to summit. Collecting at the summit was attempted by sweeping, fruit baiting and mushroom baiting. The temperature was 11°C rising later in the day to 14°C and the humidity near to 100% since cloud enveloped the peak most of the day (annual rainfall is about 360").

Baiting was entirely unsuccessful indicating that few or no Drosophila were likely to be present, since elsewhere in Australia Drosophila come to baits at 12°C and above. However, large numbers of S. pallida (in excess of 50) were obtained in a short period by sweeping sedges in a previously cleared area bordering the rain forest. Sweeping within the rain forest was entirely unsuccessful except for one S. pallida collected over a period of several hours. Hence S. pallida was abundant in this mountain habitat where no other drosophilids and few other insects were collected. There was no evidence that the flies were utilizing any environmental resources other than the vegetation itself.

It therefore appears that S. pallida is found under more extreme environmental conditions than Drosophila, many species of which are found at lower altitudes of the Bellenden Ker range. Recent collections in arid regions of Australia indicate the existence of S. australis under much more extreme high temperature/desiccation stresses than Drosophila. The occurrence of these two Scaptomyza species in extreme habitats confirms observations in the literature that Scaptomyza species exist in more extreme physical environments than Drosophila; two previous observations on this are from Hawaii (Throckmorton 1975) and southern Chile (Brncic and Dobzhansky 1957).

Acknowledgments to Telecom Australia for transport to the summit of Mt. Bellenden Ker.

References: Bock, I.R. 1977, Aust. J. Zool. (in press); Brncic, D. and Th. Dobzhansky 1957, Am Nat. 91:127-8; Throckmorton, L.H. 1975, in Handbook of Genetics, R.C. Kind, Ed., 421-69, Plenum Press.

Collier, G.E. University of Texas Health Science Center, San Antonio. Brief ecological notes on Drosophila collections from Ithaca, New York.

From July, 1972, to September, 1975, Drosophila were collected from the Ithaca area, primarily for the purpose of studying the inter- and intraspecific variation of the enzyme α-glycerophosphate dehydrogenase. The collection data are presented here as a matter of record.

The vast majority of these collections were made on the side of a hill overlooking Fall Creek, 0.7 miles east of the Cornell University campus. While most of the flies were caught in traps baited with ripe banana, many of the fungus-feeding species were aspirated directly off fungi found in the area.

Of the 24 species collected (Table 1), three species of the affinis subgroup of the obscura group, D. affinis, athabasca, and algonquin, were by far the most commonly collected species, accounting for 46% of the total catch. A fourth species of the obscura group, D. narrangansett, reported from the northeastern United States (Patterson and Stone, 1952) was not detected in these collections by either morphological characters (Sulerud and Miller, 1966) or electrophoretic characters (Lakovaara et al., 1972). The second most common component of the Drosophilid fauna was D. robusta, which accounted for 18% of the total catch.

Table 1. Drosophila collected in Ithaca 1972 - 1975

Species	Female	Male	Not sexed	Total
D. robusta	512	468	125	1105
D. athabasca	453	699	-	1152
D. affinis	251	323	-	572
D. algonquin	69	226	-	295
ath.-aff.-alg.(♀♀)	735	-	-	735
D. falleni	248	184	63	495
D. melanogaster	55	177	-	
D. simulans	5	25	-	389
mel.+sim.(♀♀)	127	-	-	
D. immigrans	84	67	189	340
D. paramelanica	186	81	1	269
D. putrida	84	63	-	148
D. busckii	43	71	-	125
D. tripunctata	19	24	44	87
Chymomyza amoena	44	8	1	53
D. funebris	22	27	-	49
D. nigromelanica	26	16	-	42
D. duncani	14	20	-	34
Scaptomyza graminum	17	8	-	25
D. quinaria	11	0	-	11
D. testaceae	1	10	-	11
D. hydei	3	3	-	6
D. nigrohalterata	3	1	-	4
D. colorata	3	0	-	3
D. palustris	2	0	-	2
Grand total				5984

These dark-bodied species, particularly athabasca, algonquin, and robusta, seemed the best cold-adapted species as they were among the first and last species collected each year. During the winter months, adults of these species could be collected almost everytime the temperature was above 50°F two or more days in succession. Also, each spring, the first flies of these species that were caught were quite "ragged", i.e., their wings frequently had frayed margins, they lacked one or more bristles, and occasionally had damaged or missing aristae. Some individuals of robusta were missing all of the major bristles of the head and thorax, both aristae, and had wings so badly torn and frayed they could barely fly. These defects were seldom seen later in the season. This suggests that these individuals were quite old and probably over-wintered as adults.

The collections for the latter half of 1972, and all of 1973 and 1975, were made continuously throughout the season. Since temperature could be a major factor affecting the temporal distribution of species in a temperate climate, correlation coefficients were calculated for the arcsin transformation of the relative frequencies of each commonly trapped species and the average daily high temperature for each two-week collecting period. These results are presented in Table 2. Only paramelanica, nigromelanica, and robusta were found to be significantly correlated with temperature. Although the affinis subgroup species as a whole were not correlated with temperature, interesting relationships exist between these species. Compared to the total number of affinis subgroup species, affinis is positively correlated with high temperature, algonquin is strongly, negatively correlated, while athabasca doesn't appear to be correlated. Although these figures are informative, the interesting interrelationships among these three species are seen better in a graphical presentation of the data (Figure 1).

There are differences in the patterns of relative frequencies for 1973 and 1975, but these can be explained in terms of the different temperature profiles for these years. For both years, the highest relative frequencies of affinis occur during the warmest months.

Table 2. Correlation coefficients for species frequency with temperature.

For frequencies relative to total flies

Species	N		
D. nigromelanica	31	+0.537	***
D. robusta	31	+0.350	**
D. paramelanica	31	+0.315	*
D. immigrans	31	+0.180	n.s.
affinis subgroup	31	-0.120	n.s.
D. melanogaster and simulans	31	+0.046	n.s.

For frequencies relative to total affinis subgroup species

D. affinis	22	+0.74	****
D. algonquin	24	-0.53	***
D. athabasca	22	-0.16	n.s.

n.s. - not significant; significance: * - 0.1 level, ** - 0.05 level, *** - 0.01 level, **** - 0.001 level.

While athabasca was more abundant in the cooler parts of both years and in the fall of 1972, the abundance of athabasca that occurred in the spring of 1973 did not occur in 1975. This could be because the spring of 1973 was mild from the end of April to the beginning of June. In contrast, the temperature in the spring of 1975 rose to near the maximum for the year over a much shorter period of time. If temperature is indeed a major factor affecting the abundance of affinis in the area, then the prolonged cool weather at the beginning of the 1973 season may have prevented the increase in relative frequency of affinis until the warmer weather of June, July and August. Likewise, the very rapid rise in temperature at the beginning of the 1975 season may have allowed the concomitant rapid increase in the relative frequency of affinis.

The apparent dependence of the relative frequencies of these species on temperature is also illustrated by their response to the cool spell of the first half of June 1975. The frequency of affinis decreased markedly while the frequency of both athabasca and algonquin increased. These trends reversed when the average temperature again rose in the last half of June 1975.

These temporal changes in relative frequencies of athabasca, affinis, and algonquin in the Ithaca area agree well with the tentative comclusions reached by Miller (1958) regarding the distribution of these species.

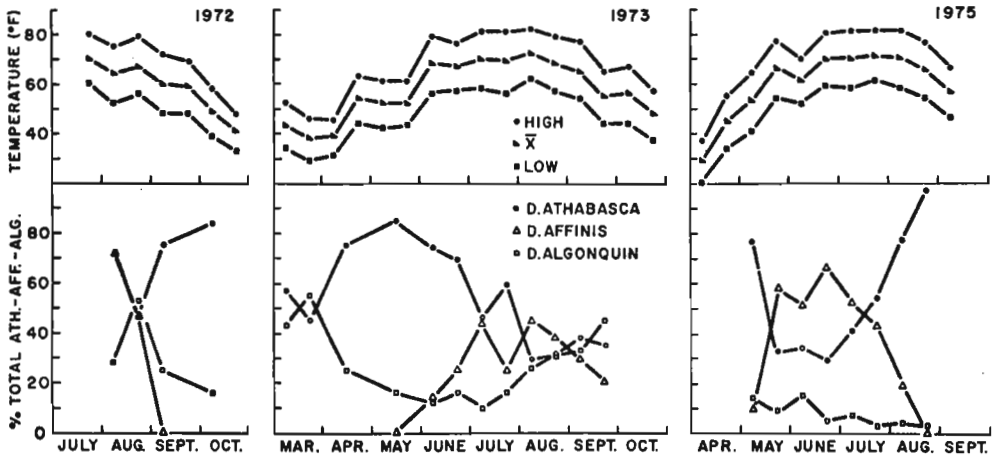


Figure 1. Temporal change in temperature and the relative frequencies of affinis subgroup species.

A final observation regarding one of the fungus-feeding species is worthy of mention. The four specimens of nigrohalterata were aspirated off a large bracket fungi, Polysporus squamosa. It was not found near any of the other species of fungi in the area. In the lab, this species will not live on any of the common Drosophila foods, including one similar to that of Speith (1974). However, this species was maintained in the lab for several generations in quart jars with a layer of moist sand in the bottom on which 2 inch cubes of P. squamosa were placed. D. nigrohalterata would not live on a solid agar media made with a homogenate of this fungi.

References: Sulerud and Miller 1966, Am. Midl. Nat. 75:446; Lakovaara et al. 1972, Proc. 8th Int. Cong. Zool., Monte Carlo; Miller 1958, Am. Midl. Nat. 60:52; Speith 1974, DIS 51:146.

Keltner, L. and J. Puro. University of Oregon, Eugene, Oregon and University of Turku, Finland. Induction of inversions in 2R carrying the gene ix.

Triploid lines carrying C(2)EN have been maintained by selection in each generation. When the recessive mutant ix was introduced to sterilize the diploid female progeny with two free second chromosomes, there was sufficient crossing over between ix on the normal second and the centromere of the compound to make this scheme ineffective. To circumvent this problem, it was decided to induce inversions in 2R carrying ix to eliminate crossing over between the compound and the free second in the triploid.

Males from a stock with the second chromosome mutants pr, cn, and ix balanced with a Cy inversion were irradiated and subsequently mated to females homozygous for bw and st. Virgin non-Cy females from this cross were then pair mated to males homozygous for b, cn, and bw. Cultures producing no progeny with either white or wild type eyes were retained.

From 430 pair matings, three such cultures were found. It was ascertained that the suppression in all three cases was not due to a 2:3 translocation (by crossing back to the stock with st). For the most promising stock, of 1390 total pair matings in the genetic tests, one incidence of recombination between cn and bw was noted. (The remaining two stocks were retained for later testing.) Verification of the integrity of the mutants on the chromosome was made straightforward when it was found that inversion homozygotes were viable, with males fertile and females consistently displaying the phenotypic characteristics of ix.

Cytological observation verified the existence of a paracentric inversion in 2R, and the break points proved to be at 41A-B and 57F2-58A1. The most fully tested of the three, In(2R) LK1, is now being introduced into the 3N stock, and is kept as a regular stock balanced over Curly. Testing the other two inversions is in progress.

Khlebodarova, T., N. Matveeva, D. Tscherbakov and L. Korochkin. Institute of Cytology and Genetics, Novosibirsk, USSR. The comparison of β- and S-esterases in Drosophila of the virilis group.

Organospecific slow (S) esterase was described in bulbus ejaculatorius of Drosophila of the virilis group (Korochkin et al., 1976). Some properties of this esterase similar to unspecific β-esterase can be seen. Therefore it is necessary to investigate whether S-esterase is an independent fraction of enzyme or belongs to conformers of β-esterase. The homology of β- and S-esterases was estimated by the method of the inhibition of the immunoadsorbtion of I^{125}-S-esterase on the immunosorbent with fixed antibodies against S-esterase. The purified antibodies against S-esterase were fixed on the diasocellulose according to Rokhlin et al. (1971). The purified β- and S-esterases were iodinated according to Bale et al. (1966). We used 20 μg β- and S-esterase in each inhibition's reaction. The immunoadsorbtion of I^{125}-S-esterase was decreased by 37% on the immunosorbent. The reaction of immunosorbent with I^{125}-β-esterase was absent. Therefore S-esterase is not conformere of β-esterase, but the independent isozyme. Recently this conclusion was confirmed by our genetic experiments. It occurs that the structural gene coding S-esterase is located on the second chromosome on the position 192.1 map units. Gene Est-β is located on the position 209.3 m.u. on the second chromosome too (Korochkin et al., 1973).

Acknowledgments: We are grateful to O. Rokhlin (Moscow) for the help in this investigation.

References: Bale, W., K. Helkamp, T. Davis, R. Izzo, R. Goodland, M. Contreras and I. Spar 1966, Proc. Soc. Exp. Biol. 122:407; Korochkin, L., N. Matveeva, M. Evgeniev, and M. Golubovsky 1973, Biochem. Genetics 10:363; Korochkin, L., E. Belayeva, N. Matveeva, B. Kuzin and O. Serov 1976, Biochem. Genetics 14:161; Rokhlin, O., T. Vengerova and R. Nezlin 1971, Immunochem. 8:525.

Golubovsky, M.D. Institute of Cytology and Genetics, Novosibirsk, USSR.

Two types of instability of singed alleles isolated from populations of D. melanogaster during mutation outburst in 1973.

In 1973 the outburst of mutability of sex linked singed locus was discovered in natural populations of D. melanogaster[1,2]. Two phenotypically different and unstable alleles sn77-27 and sn63-15 were isolated from Tashkent population (Middle Asia) and briefly described[2]. In a previous report[3] were presented detailed data about the character of mutability of the sn77-27 mutant and its derivatives which change according to the "all or none" rule. Table 1 gives data on the other mutant, sn63-15. It originally had an intermediate mutant phenotype - hooked bristle ends and slightly curved hairs on the thorax. In the progeny of individual matings, 1 ♂ sn63-15 x ♀ $\overline{XX}$/Y, it was possible to find males with weak mutant expression (waved bristles), wild or almost wild type and also males with extreme mutant expression (twisted, gnarled, diminished bristles, curved hairs - some derivatives are female sterile and some are female fertile). The phenotypically normal and weakly mutant allelic derivatives were also unstable, and only extreme mutant derivatives were

Table 1. Instability of sn63-15 mutant and its phenotypically distinct allelic derivatives.

Bristle phenotype of males in cross ♀♀ $\overline{XX}$/Y x 1 ♂	Bristle phenotype of progeny (%)				
	hooked	waved	normal	extreme mutant	Total
x ♂ hooked	6169	72 - 1.2%	18 - 0.3%	11 - 0.2%	6270
x ♂ waved	7 - 0.4%	1699	48 - 2.7%	2 - 0.1%	1755
x ♂ normal	2 - 0.2%	56 - 5.4%	1041	1 - 0.1%	1100
x ♂ extreme mutant	0	0	0	2153	2153

relatively stable (Table 1). On the basis of detailed investigation of the mode of inheritance of the supermutability feature during a series of successive generations, we can establish two main types of instability: Type A - when mutation occurs in accordance with the "all or none" rule, from extremely mutant condition to phenotypically normal and vice versa. Intermediate and weak mutant conditions do not appear. The alleles sn77-27 and sn49-5 mutate so; Type B - when mutations go in different directions and phenotypically distinct allelic derivatives can be isolated in the progeny of single individual mating (sn63-15). Within each type of instability it is possible to find different "classes" of alleles distinguished on the basis of their mutation rate - relatively stable and super-mutable or strongly reversible alleles. The character of mutations of the two alleles is shown in Figure 1.

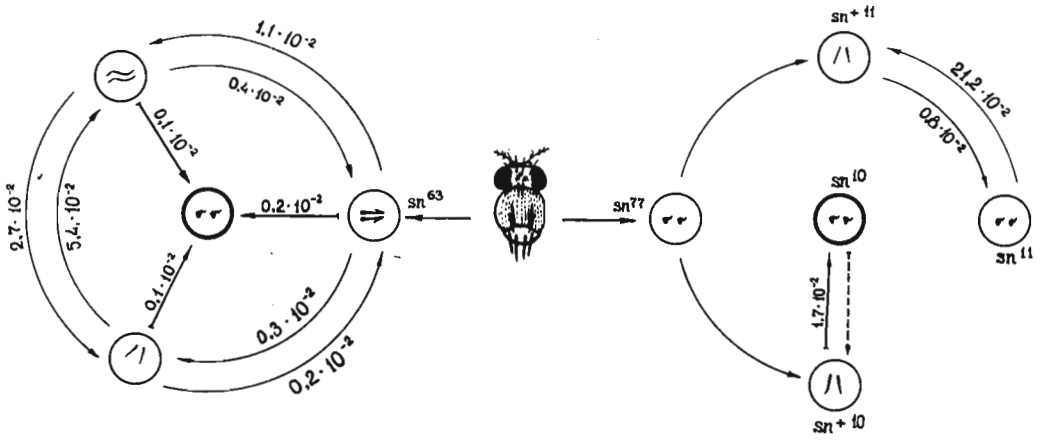


Figure 1. Two types of instability of singed alleles; on the right - Type A, left - Type B.
⇌ extreme bristles
⇉ hooked ends
≈ waved
≃ normal

References: [1]Berg, R.L. 1974, DIS 51:100; [2]Ivanov, Yu.N. 1974, DIS 51:74; [3]Golubovsky, M.D. 1976, DIS (this issue, DIS).

Vigue, C.L. Saint Joseph College, West Hartford, Connecticut. A system to demonstrate the differential selectivity of the octanol dehydrogenase isoalleles.

The evolutionary and physiological significance of isozymic variation in natural populations has remained elusive since its discovery in the 1960s. The system which has been used most extensively to elucidate the significance of isozymic variation is the alcohol dehydrogenase (ADH) isoalleles of Drosophila melanogaster. We thought it would be desirable to develop a second scheme to study another allelic isozymic system. The system chosen was octanol dehydrogenase (ODH). Like ADH, ODH is controlled by two allelic genes, $Odh^{4(Fast)}$ and $Odh^{6(Slow)}$. The system developed may be used to study selective differences between the two Odh isoalleles.

Materials and Methods: For the present studies derivatives of a wild-type population of D. melanogaster collected in Maine in August 1976 were used. The gene frequencies of the population are approximately 0.15 Odh^6 and 0.85 Odh^4. In addition an Adh^{n2}, b, pr, cn strain which has no detectable ADH activity was used.

Studies with adults were performed by placing approximately 100 four day old adults in one-half pint milk bottles in which was suspended a triple size Johnson and Johnson absorbent cotton ball saturated with 4 ml of 5% sucrose. Various amounts of octanol were placed on the cotton. The bottles were plugged with a foam stopper, covered with parafilm to prevent evaporation, and placed in a 25°C incubator for 24 hours. The number of flies alive was then determined.

Studies using embryos and larvae were conducted by placing approximately 100 embryos in a 110.5 mm x 3.5 mm glass culture vial containing approximately 3 gms of Carolina Biological Supply Co. Instant Drosophila Medium Formula 424, reconstituted with distilled water. Various amounts of octanol were placed on top of the food. The vials were plugged with rubber stoppers and placed in a 25°C incubator for three days. The rubber stoppers were then removed and replaced with foam stoppers. The cultures were maintained at 25°C. Distilled water was added periodically to prevent drying. The number of flies emerging was determined.

Considerable variability was encountered during these experiments which we were unable to overcome. The methods described above gave us the most consistent results.

Results and Discussion: Inspection of the Tables reveals that adults are more sensitive to high amounts of octanol than are larvae. The LD_{50}s, however, are approximately the same (9 microliters). A significant number of larvae are able to tolerate octanol amounts greater than 20 microliters whereas adults have difficulty tolerating more than 16 microliters.

Table 1
The effect of octanol on Drosophila larvae.

Amount of octanol (microliters)	% emerging
0	82
4	73
5	63
6	59
8	75
10	35
20	21
30	13
40	1
50	2
60	0

Table 2
The effect of octanol on Drosophila adults.

Amount of octanol (microliters)	% alive
0	100
1	99
2	98.5
3	99
4	91.5
6	67.5
10	46.5
13	15
16	2
20	0

When Adn^{n2}, b, pr, cn adults and larvae were subjected to 6 and 10 microliters of octanol respectively, only 20% of the adults were alive after 24 hours and only 0.3% of the larvae emerged as adults. This observation supports the data of others which suggests that ADH metabolizes octanol in vivo.

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The effect of magnetic fields on crossing-over in D. melanogaster females.

It has been well established that recombination in Drosophila melanogaster is affected by a number of chemical agents and high energy radiation including X-ray. There have been a limited number of studies concerning the mutagenic effects of low energy fields such as electrostatic and magnetic fields on Drosophila (Diebolt, 1978; Mittler, 1971, 1972), but few studies concerning the effects of magnetic fields on recombination.

In this experiment the influence of strong and medium strength magnetic fields on recombination in the distal tip and proximal end of the X-chromosome and the region around the centromere and in the right arm of chromosome 2 in D. melanogaster females was studied. Isogenic stocks of y w^a spl rb, m f car and b cn c bw were used to study recombination in the afore respectively mentioned chromosome regions.

Virgin females were collected from homozygous mutant stocks and mated on cornmeal, agar, molasses medium to isogenic wild type males. Upon eclosion of the F_1 progeny, virgin females and males were collected, separated, and aged for 3 days on yeasted medium. In the studies dealing with the sex linked markers, single, aged, F_1 virgin females were mated in vials with 2-3 of their aged F_1 brothers. The aged F_1 virgin heterozygous b cn c bw/+ + + + females were individually mated to 2-3 homozygous b cn c bw males 3 days of age.

After placing the F_1 parents in the initial mating vials, the vials were divided into two groups. One group served as the controls and the second group consisting of 3 vials was placed in the magnetic field. The controls were placed on top of the metal frame which surported the electromagnet.

The magnetic field was produced by a Harvey-Wells, model HS-1365B, water cooled electromagnet with 7.7 cm diameter pole faces. The interpole face distance was 3.8 cm. The electromagnet operated at 0-65 D.C. amperes and 0-135 D.C. volts. A piece of styrofoam with three holes large enough to hold the mating vials was placed between the magnet pole faces.

The F_1 parents were pre-treated in the magnetic field for two days and then dump transferred to fresh food vials and immediately placed back in the magnetic field. Subsequently the parents were transferred every two days for a total of three transfers. The vials were numbered so that the parents were placed back in the field in the same position after the transfer. After the parents had been transferred to fresh vials the used vials were incubated at 25 $\pm 1^{\circ}$C. Only the progeny from the three transfer vials were classified and counted. The progeny eclosing from the original pre-treatment vial were not included in the count because the pre-meiotic stages of oogenesis would not have occurred while the females were being subjected to the treatment field. The temperature in the treatment and control fields was monitored and was always equal to room temperature (24 $\pm$ 1°C) during the treatment period.

The results of the experiments are summarized in Tables 1, 2, and 3. From crosses of y w^a spl rb/+ + + + ♀♀ x y w^a spl rb/Y ♂♂ a total of 2037 F_2 females and males were recovered from the parents subjected to a 0.7366 tesla (T) magnetic field (1 T = 10^4 gauss) and 6648 F_2 females and males from the control flies. The recombination frequency between y - w^a, w^a - spl, and spl - rb was not significantly different from the control. The recombination frequency in the f - car region in crosses of m f car/+ + + females subjected to a 1.1366 T magnetic field was significantly lower than in the control. A total of 1086 F_2 females and males were recovered from the treated m f car/+ + + females and 6777 F_2 progeny from the control females.

The recombination frequency between cn - c on chromosome 2 was somewhat lower in the b cn c bw/+ + + + females subjected to a 1.24 T magnetic field than in the control females but the

Table 1. Recombination frequencies (with standard error) from y w^a spl rb/+ + + + females subjected to a 0.7366 T magnetic field.

		Region		
Treatment	N	y - w^a	w^a - spl	spl - rb
B field	6	1.72 ± 0.43	1.27 ± 0.25	3.88 ± 0.30
Control	18	1.80 ± 0.18	1.32 ± 0.25	3.25 ± 0.23
	P	-	-	-

- indicates nonsignificance
N = number of females tested

Table 2. Recombination frequencies (with standard error) from m f car/+ + + females subjected to a 1.1366 T magnetic field.

		Region	
Treatment	N	m - f	f - car
B field	5	22.29 ± 2.11	3.67 ± 1.04
Control	26	22.02 ± 0.64	5.72 ± 0.25
	P	-	*

* indicates significance at .05 level
N = number of females tested

Table 3. Recombination frequencies (with standard error) from b cn c bw/+ + + + females subjected to a 1.24 T magnetic field

		Region		
Treatment	N	b - cn	cn - c	c - bw
B field	6	5.78 ± 0.68	17.22 ± 1.83	29.10 ± 1.89
Control	42	5.84 ± 0.30	19.01 ± 0.52	29.33 ± 0.55
	P	-	-	-

- indicates nonsignificance
N = number of females tested

difference was not significant. A total of 1157 progeny were recovered from the magnetic field treated females and 11115 F_2 females and males from the control females.

The results suggest that a magnetic field may have an inhibiting effect on recombination in the proximal region of the X-chromosome near the centromere. There was no indication, however, that recombination in the most proximal region to the left or right of the centromere of chromosome 2 was affected by the magnetic field. However, there was an indication that crossing-over in the right arm proximal region may be slightly inhibited as evidenced by the cn - c recombination frequency. The mechanisms of the effects of magnetic fields on biological material are not understood. The force exerted on biological material in a magnetic field would be quite small, however, it might be possible that a magnetic field may inhibit exchange pairing of chromosomes as described by Grell (1962).

References: Diebolt, J.R. 1978, Mut. Res. 57:169-174; _____ The influence of electrostatic fields on nondisjunction in Drosophila melanogaster, (In review); Grell, R.F. 1962, Proc. Natl. Acad. Sci. U.S., 48:165-172; Mittler, S. 1971, Mut. Res. 13:283-287; _____ 1972, NTIS:1-13.

Metakovsky, E.V. and V.A. Gvozdev.
Kurchatov Institute of Atomic Energy, Moscow, USSR. Changes of cell surface glycoprotein pattern in the established cell line of D. melanogaster as a result of ecdysterone action.

Ecdysterone effects in the diploid cell line of D. melanogaster established in 1969 (Kakpakov, Gvozdev, Platova and Polukarova, 1969) were studied. Preliminary results were obtained (Metakovsky, Kakpakov and Gvozdev, 1975) showing alterations of cell membrane properties during 24 - 48 hours after hormone addition: 1) cells detached from glass surface and began to flow in aggregates in culture medium, and 2) ability of cells to agglutinate in the presence of Concanavalin A was drastically decreased. Ecdysterone influence on the biosynthesis of cellular glycoproteins was investigated because of a well known role of glycoproteins in determination of cell surface properties.

Cells were cultured in Schneider medium supplemented with 10% foetal calf serum. Three days after addition of hormone (1 μg/ml), cells were labeled 48 hours by ^{14}C-N-acetylglucosamine, ^{14}C-galactose or ^{3}H-N-acetylmannosamine. Control cultures contain no ecdysterone.

Cell extracts were prepared in Laemmli's buffer (Laemmli, 1970), electrophoresis was performed in 9% polyacrylamide-1% SDS gel. Distribution of radioactivity (newly formed glycoproteins) was determined along the gel columns. Two glycoprotein fractions were revealed in control cells labeled with sugars, major fraction having an apparent molecular weight of approximately 120,000 contains 70% of incorporated label, and minor fraction having an apparent molecular weight which exceeds 200,000. Ecdysterone changes a pattern of radioactivity of both fractions. The 85,000 - 120,000 molecular weight fractions appeared instead of the major peak (Figure 1). The minor peak of glycoprotein radioactivity has now a molecular weight of 200,000. The observed decrease of an apparent molecular weight of both fractions was reproduced in experiments where cells were labeled with N-acetylglucosamine, N-acetylmannosamine or

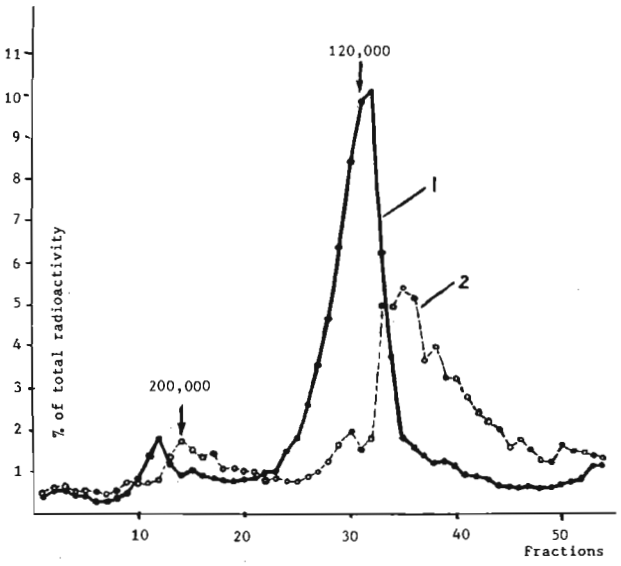

Figure 1. Changes of glycoprotein pattern induced by ecdysterone. 1 - control; 2 - ecdysterone. Cells were labeled with ^{14}C-N-acetylglucosamine.

galactose. Ecdysterone-induced intensification of Coomassie stained band of molecular weight 200,000 and increase of incorporation of labeled amino acids in the same region of the gel were observed earlier (Metakovsky, Kakpakov and Gvozdev, 1975). Hence we suppose that ecdysterone causes accumulation of both carbohydrate and protein moieties of a specific high molecular weight glycoprotein. Incorporation of ^{14}C-galactose in acid-insoluble fraction calculated per cell increased by 1.5 - 2.0 times although incorporation of ^{14}C-N-acetylglucosamine has been reduced by 30% as a result of ecdysterone treatment.

The results show that hormone induces a biosynthesis of glycoproteins having diminished molecular weights and possibly changed ratio of galactose to N-acetylglucosamine. Protein moieties in these glycoproteins may remain possibly unchanged or, contrarily, are represented by different polypeptides.

Mild pronase treatment of cells in control cultures without hormone resulted in partial degradation of the major and minor fractions of glycoproteins showing their surface localization. Neuraminidase treatment eliminates only minor glycoprotein(s) of higher molecular weight (more than 200,000). We suppose that both the inability of cells to agglutinate by Concanavalin A and to attach to glass surface (Metakovsky, Kakpakov and Gvozdev, 1975) is due to the observed changes of cell surface glycoproteins induced by hormone.

Similar alteration of glycoprotein biosynthesis (accumulation of the newly formed glycoprotein(s) with molecular weight of 200,000) was observed during ecdysterone-induced in vitro evagination of imaginal discs (in preparation). We suggest that ecdysterone-induced changes of cell surface glycoproteins may play an essential role affecting cell interactions during a process of imaginal disc differentiation.

References: Kakpakov, V.T., V.A. Gvozdev, T.P. Platova and L.G. Polukarova, 1969, Genetika (USSR) 5(12):67 Laemmli, U.K. 1970, Nature 227:680 Metakovsky, E.V., V.T. Kakpakov and V.A. Gvozdev 1975, Compt. Rend. Acad. Sci. de l'URSS 221:960.

Hames, B.D. and M. Bownes. University of Essex, Colchester, England. Analysis of yolk proteins in D.m.

Previous studies by Gelti-Douka et al. (1974) and Gavin and Williamson (1976) have indicated the presence of one or two yolk proteins in Drosophila melanogaster respectively. However, using gradient polyacrylamide gel electrophoresis, we have recently shown that three major yolk proteins with molecular weights of 44,700 (YP-1), 45,700 (YP-2), and 47,000 (YP-3) are present in eggs and ovaries of this species (Bownes and Hames, 1977). Further studies using gradient polyacrylamide gel electrophoresis have suggested that fat body from male or female 3rd instar larvae contain a major protein with molecular weight similar, if not identical, to YP-1. Similarly, a protein with molecular weight similar to YP-3 was detected in adult female gut, female malphigian tubules, male fat body, male accessory gland and male testis. Major proteins with identical molecular weights to YP-1, YP-2 and YP-3 were present in female fat body and haemolymph.

In order to determine whether the proteins observed in tissues other than eggs and ovaries were indeed yolk proteins or unrelated proteins of similar molecular weight, we raised antisera against YP-1, YP-2 and YP-3 as prepared by preparative gel electrophoresis of whole, dechorionated eggs. Purified anti-yolk protein antibody was then reacted against either egg or ovary extracts, the resulting immunoprecipitate washed, denatured in Laemmli buffer (Laemmli, 1970) and run on gradient polyacrylamide gels. In addition to antibody chains, only three other proteins were visible after gel staining and these corresponded to YP-1, YP-2 and YP-3, showing that the antibody was reactive against all three yolk proteins. Next, ^{3}H leucine was injected into 3rd instar larvae and adult females and after 6 hours these were used to prepare extracts of larvae, female gut and female ovary.

The extracts were challenged with anti-yolk antibody, in the presence of authentic yolk protein carrier, and the resulting immunoprecipitate analysed by polyacrylamide gel electrophoresis as previously described. In all cases, examination of the stained gel revealed that only three proteins had been precipitated and these correlated exactly with YP-1, YP-2, and YP-3, yet after slicing and counting the gels only female ovary contained any label and this was in yolk protein.

Therefore, during a 6 hour labelling period, yolk protein is accumulated by ovary but is absent from larvae and female gut. This was confirmed by immunodiffusion studies with anti-yolk antibody which reacted positively against egg, female fat body and female haemolymph extracts but not against larvae or female gut. We conclude that although larvae and female gut contain major proteins of similar molecular weight to yolk proteins they are unrelated.

Interestingly, when anti-yolk antibody (purified IgG) is reacted against egg extracts in immunodiffusion studies, two precipitin lines are observed, similar to those reported previously by Gavin and Williamson (1976). These are also observed in female haemolymph and fat body. We are currently attempting to analyse the relationship between the three yolk proteins observed by polyacrylamide gel electrophoresis and the two observed by immunodiffusion.

References: Bownes, M. and B.D. Hames 1977, J. Exp. Zool., In press; Gavin, J.A. and J.H. Williamson 1976, J. Insect Physiol. 22:1457-1464; Gelti-Douka, H., Gingeras, T.R. and M.P. Kambysellis 1974, J. Exp. Zool. 187:167-172; Laemmli, U.K. 1970, Nature 227:680-685.

Bicudo, H.E.M. de C. Universidade Estadual Paulista, São José do Rio Preto, Brasil. On the occurence of sex-ratio females in D. prosaltans.

Sex-ratio females in Drosophila prosaltans from Bertioga were reported by Cavalcanti (1950, Abstract presented to the Academia Brasileira de Ciências): in a collection of 82 females, 11 yielded exclusively female progeny. In a series of papers, Cavalcanti and his coworkers studied the inheritance of this characteristic in the species. In short they presented evidence that the characteristic was due to an interaction between nuclear genes and cytoplasmic factors. Aiming at a contribution to the knowledge of sex-ratio female distribution in natural populations, the present note reports the finding of a D. prosaltans sex-ratio female in Mirassol, another locality of the State of São Paulo, about 500 km from Bertioga. In a collection of 5 D. prosaltans females (this species is considered rare; only exceptionally have a large number of the flies been captured) one of them yielded 44 females and 0 males. Five of these F_1 females singly crossed to males from the same locality also yielded exclusively female progeny. Mass cultures of these females were maintained for several generations (more than one year) by adding males from the same locality. During this time the progeny consisted exclusively of females.

Carrasco de Maldonado, C., Y. Perez-Chiesa and D. Bruck. University of Puerto Rico, Rio Piedras, P.R. Anodal esterases of Drosophila dunni dunni.

Townsend and Wheeler (1955), Heed and Krishnamurthy (1957), and Heed (personal communication) have previously established the existence of Drosophila dunni as a polytypic species found in Puerto Rico, St. Thomas and Tobago (V.I.) and belonging to the dunni subgroup (cardini group). We have been interested in investigating further the differences between D. dunni dunni, found only in Puerto Rico, D. dunni thomensis, found in St. Thomas and Tobago (V.I.), and other species of the subgroup, including polymorphism for various isozymes. This work is a preliminary analysis of some of the anodal esterases of D. dunni dunni and their frequencies in various body parts, as well as their presence in various stages of development, as revealed by starch slab gel electrophoresis. Our methods are described in Bush and Huettel (1972).

Five esterase systems were characterized, which we have tentatively called loci, and a sixth, esterase E, remains difficult to establish, but probably exists as a separate locus. Table 1 (next page) and Figure 1 list the results. As the first column of Table 1 indicates, esterase F was found in males only. It is apparently not Y-linked since two male parents exhibited no activity, yet some of the male progeny did in each case. In other crosses, male parents exhibited an F band while their progeny did not.

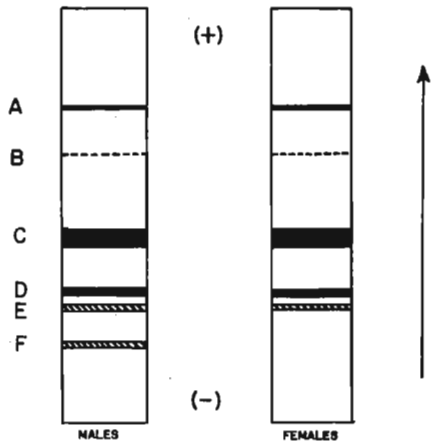

Figure 1. Typical esterase band pattern of mature whole male and female flies of Drosophila dunni dunni. Variations in electrophoretic mobilities of the polymorphic regions are ignored. Width of bands indicates relative staining intensity. Solid bands indicate alpha-specificity; dashed bands, beta-specificity. Dotted band is ambiguous for substrate specificity.

All the loci were found to be polymorphic except D, all were found to have null alleles except D, and no active esterase was detected in the pupal stage.

Locus B and what we believe to be locus E posed some problems. Esterase B gave positive reactions with both α and β naphthyl acetate and is thus uncharaterized in that respect. In locus E, the heat lability test was the primary means of distinguishing between that locus and locus D.

On the other hand, locus C appeared quite clearly to comprise a single locus with three active and one null allele, according to genetic tests. Three allozymes were never observed together in any single individual assayed, and no hybrid or intermediate bands occurred in the progeny of crosses between phenotypically different flies, which rules out a dimer molecule. Crosses with double banded males and females (each with two active alleles) yielded results completely consistent with the one-locus hypothesis. We feel it is unlikely that the system involves two loci in strong linkage disequilibrium, where one locus has two active and one null allele, and the other has one active and one null allele, although this remains as a possibility.

The behavior of the nulls themselves is worthy of comment. Aside from the pure homozygous lines that were constructed (including the C^{null} and $C^{1.08}$ where no segregants were observed) a strain with $C^{1.00}$ was observed to consistently produce adults which were classified as $C^{1.00}/C^{null}$ heterozygous. We believe that a lower adaptive value of the homozygous null, or the presence of a tightly linked deleterious allele may explain this result. Moreover, this strain, as well as the pure C^{null} strain were observed to reproduce more slowly and yield less progeny than the other strains.

Finally, it is of some interest to us that esterase A, which appears mainly in the abdomen, was found only in flies raised on breadfruit (Artocarpus altilis) or jackfruit (Artocarpus heterophyllus), but not on flies raised on cornmeal. More specifically, the esterase was found in 214 out of 535 flies raised on breadfruit or jackfruit, but never in 253 flies raised on cornmeal. The fact that a small percentage of the enzyme was found in the head and

Table 1

SYSTEM	SEXES	TYPE OF SYSTEM	ADULT BODY PARTS (percentages observed among samples of each part examined) Flies aged at least 8 days				LARVAL THIRD INSTAR	HEAT LABILITY TESTS 5 minutes at 60°C	ESSERINE SULPHATE INHIBITION (65 mg in 5 mls propanol + 95 mls of stain-buffer solution)
			Head	Thorax	Abdomen	Gonads			
A	present in both	α-esterase diallelic null/active system	8% (6/78 heads)	8% (6/76 thor.)	32% (20/62 abd.)	-	-	denatured	not tested
B	present in both	α/β test gave ambiguous results diallelic null/active system	46% (36/78 heads)	-	-	-	-	results ambiguous	not affected
C	present in both	α-esterase triallelic 1 null/2 active system	-	-	100% (62/62 abd.)	-	present in small amounts in sample studied	denatured	not affected
D	present in both	α-esterase no variation - found in all flies	100% (78/78 heads)	100% (76/76 thor.)	-	-	strongly present	denatured	slightly inhibited
E Probable see text	present in both	β-esterase probably tetra-allelic 1 null/3 active system	21% (16/78 heads)	78% (59/76 thor.)	-	-	strongly present	not denatured	slightly inhibited
F	found in male only	β-esterase diallelic null/active system	-	-	-	61% (14/23 ♂♂ gonads)	-	not denatured	slightly inhibited

thorax (see Table 1) suggests the possibility that the enzyme is really present in the ingested material - perhaps the yeast found in the breadfruit and/or jackfruit. We hope to produce zymograms of samples of the food medium shortly.

This work was partially supported by NSF Institutional Grant 00-204-12-19-0.

References: Bush, G.L. and R.N. Huettel 1972, Starch-gel Electrophoresis of Tephridid Proteins. A Manual of Techniques. Internat. Bio. Prog.; Heed, W.B. and N.B. Krishnamurthy 1957, Univ. Texas Pub. 5914: 155; Townsend, J.L. and M.R. Wheeler 1955, J. Agric. U. of Puerto Rico 39: 57.

Golubovsky, M.D. Institute of Cytology and Genetics, Novosibirsk 90, U.S.S.R.

The "lethal giant larvae" - the most frequent second chromosome lethal in natural populations of D. melanogaster.

The frequency of second chromosome lethals and rate of allelism have been studied continually since 1963 in geographically isolated populations of the USSR. About 40,000 diallelic crosses were made. It was established that each population is characterized by a certain definite level of concentration of lethal mutation which can serve as one of the parameters of the genetic structure of a definite population. In samples of lethals isolated simultaneously from neighboring populations, alleles of some genes are frequent, alleles of others are rare. In the course of time, however, both frequent and rare lethals are considerably changed. Gene sets of lethal mutations isolated from the same population in different years differed from one another just as widely as those of geographically isolated populations. The persistence of the same total concentration of lethals (15 - 20% on the average), their allelic sets being permanently renewed, was named by Dr. Raisa L. Berg as the "quasi stationary state". Against a background of this constant gene change, alleles of one locus, namely 1(2)gl - "lethal giant larvae" - 2-0.3, are permanently present in most of the studied populations. Their concentration was relatively high - about 1-2% from all second chromosomes. Here are the data on the widespread distribution of 1(2)gl:

Region and years		Isolated chromosomes with lethals studied	Number of 1(2)gl alleles among them
Ukraine	1963-1970	606	40
Crimea	1963-1971	208	11
Caucasus	1964-1972	273	22
Middle Asia	1969	63	0
Far East	1971	60	5
Total		1210	78

The deficiency for "net" and 1(2)gl genes was experimentally obtained and it was then possible to study the concentration of 1(2)gl by direct test, crossing wild males with ♀♀ Cy/L and then F_1 "Cy" ♂♂ with ♀♀ Cy/Df. The absence of non-Cy flies in the F_2 indicates the presence of 1(2)gl mutation in wild chromosomes. I found in such manner this lethal in a Birsk population (South Ural) in 1974 and L.S. Korochkina isolated it in 1975 from Uman (Ukraine), Sudak (Crimea) and Krasnodar (Caucasus). On the average, one fly in 50 - 70 carried 1(2)gl allele. It was reported earlier[1] that the ubiquitous appearance of 1(2)gl was connected not with diffusion of the only mutant allele, but with the independent occurrence of different alleles which are genetically not identical. It was also discovered that under normal laboratory conditions (25°), heterozygotes on all alleles of 1(2)gl reduce the viability of their carriers but have considerable advantage in low temperatures. The net and 1(2)gl genes are very closely linked. So we obtained the net/1(2)gl heterozygotes and founded some cage populations from 50 pairs of these flies. The populations were kept in normal and low temperatures so it was possible to compare the theoretical rates of elimination of lethals in each generation with experimental data. It was observed that in cage populations kept at 18° the 1(2)gl remained in higher concentration. Resistance to low temperature may be the main selective factor favoring it in nature.

Reference: [1]Golubovsky, M.D. and K.B. Sokolova 1973, DIS 50:124.

Pieragostini, E.*, M.L. Vanelli **, S. Sangiorgi ** and D. Palenzona **.
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Glucose 6-phosphate dehydrogenase in Drosophila melanogaster: autosomal determination and relationship to vg marker.

Ever increasing experimental evidence indicates that very often genetic determination of isozymes does not fit the simple molecular equation one gene - one enzyme - one catalytic reaction and rather suggests the existence of more complex relationships between isozyme structural genes and the genetic background.

Such is the case of glucose 6-phosphate dehydrogenase (G6PD): according to the model originally proposed by Young et al.[1] for Drosophila melanogaster, the structural gene (Zw) was located on chromosome X, two codominant alleles controlling the electrophoretic variants A (fast) and B (slow). Later, Komma[2], Steele et al.[3], Seecof et al.[4] observed effects unexplainable in the frame of Young's model and postulated the existence of several autosomal factors. In a recent study, Giesel[5] redefined the function of Zw locus as a modifier of G6PD activity, its structural genes being autosomal.

The present experiments investigate the problem of autosomal determination of G6PD and its relationship to the vg marker in D. melanogaster.

Examined flies belonged to Canton strain, to an unrelated strain carrying the vg marker and to six populations PMvg$^+$, PFvg$^+$, PKvg$^+$, PMvg, PFvg, PKvg having a Canton x vg cross as their common origin, all maintained in mass culture and overlapping generations.

The last six populations derived from laboratory lines M, F, K (plateaued after ≈ 70 generations of directional selection for short wing length, see Palenzona et al.[6] and Alicchio et al.[7] for detailed procedures), where both wild-type and vestigial flies were segregating; in particular PMvg$^+$, PFvg$^+$, PKvg$^+$ were obtained crossing ♀♀+/vg x ♂♂+/vg and PMvg, PFvg, PKvg crossing ♀♀vg/vg x ♂♂vg/vg.

In order to characterize G6PD banding patterns, electrophoretic analysis was performed on cellulose acetate. A buffer containing 0.105 M TRIS, 0.75 M boric acid, 0.002 M EDTA and 2 mg per 100 ml NADP (pH 8.6) was used to soak strips and to homogenate samples. Homogenates contained single individuals ground in 5 µl buffer or random samples of 100 flies in 20, 30 µl.

Bridge buffer as above, except for the omission of EDTA and the addition of 2 mg per 100 ml NADP on the cathodal side[8]. Runs were for 2 h at a constant I of 2 mA per cm.

Staining mixture as in Young et al.[1] Readings were taken after 2 h incubation at 25°C in a moist chamber.

Electrophoresis of G6PD revealed the presence of three variants, called "a", "b", "c" on the basis of their mobilities; they show up in single or double banding patterns in individual homogenates, in double or triple banding patterns in mixed ones (Figure 1).

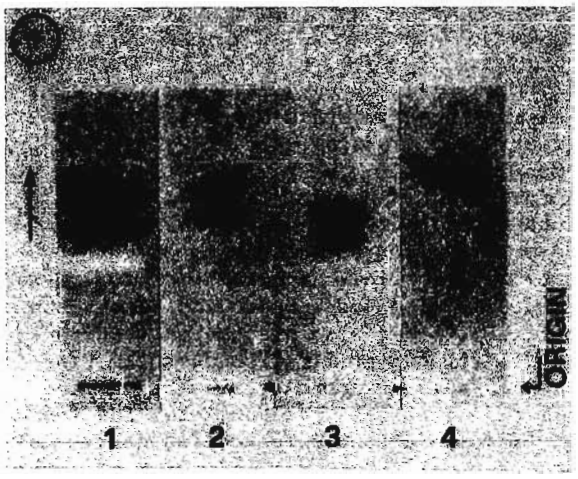


Figure 1. Some G6PD electropherograms of D. melanogaster: 1) 100 ♀♀ vg mixed homogenate. 2) Single ♂ vg. 3) Single ♂ wild-type. 4) Single ♀ vg.

In particular, winged populations (Canton strain and PMvg$^+$, PFvg$^+$ and Pkvg$^+$ populations, sampled after the loss of the vg marker) exhibit electrophoretic patterns of the "b+c" type in both sexes, as combination of individual patterns with two bands (b+c) or single band (b or c) (Figure 2). On the other side, individuals of both sexes in vestigial populations (vg original strain and PMvg, PFvg, PKvg populations) exhibit a single mobility variant (a or b or c) or double banding patterns: in multiple homogenates they sum up to three banded patterns (Figure 2).

As parallel genetic analysis to make homozygous chromosome X and thus isolate any allelic

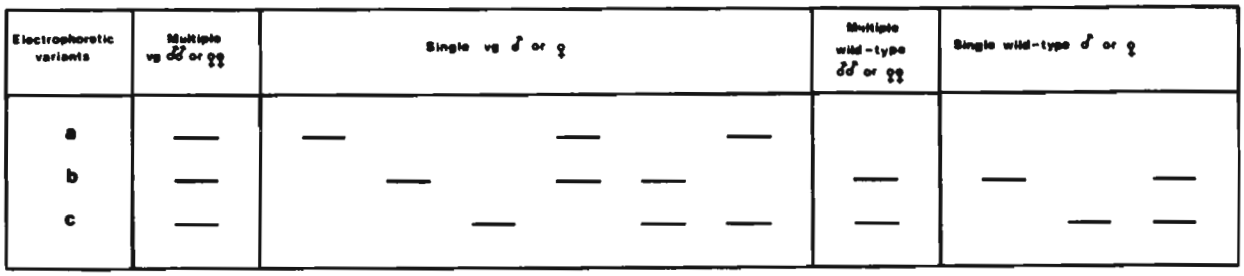


Figure 2. G6PD electrophoretic patterns of wild-type and vg flies.

variant at Zw locus, strains and lines M, F and K were crossed to ClB strain. Despite the chromosome X identity obtained by this method, heterogeneity of G6PD phenotype of the populations was maintained in their progenies; in particular single males exhibit two bands of equal intensity. These results would suggest an hypothesis of autosomal determination of G6PD, as recently proposed by Giesel[5].

Three alleles at one locus represent the simplest model to interpret the appearance of one or two bands only in individual homogenates and three in the mixed ones.

The association of the "a" mobility variant to the vg marker also deserves attention. If this remarkable difference between Canton and vg strains were only due to their genetic unrelatedness (lack or loss of the allele for variant "a" in Canton strain) we would not expect it to be maintained in vestigial and wild type flies belonging to the same population. In fact after many generations of vg x wild type crossings, the genotype would be substantially homogeneous within the same population, with the only exception of the vg locus region; therefore, any differences of the genetic origin should be looked for in this region. The expression of the allele responsible for the mobility variant "a", postulated by our model of autosomal determination, could be strictly dependent on the vg marker (or a linked gene).

As electrophoretic examination of a Canton x vg F_1 individuals uncovers mobility patterns typical of vg populations, we could conclude that the allele for variant "a" can be expressed, even with the vg marker in the heterozygous condition.

G6PD "a" could then be associated with vestigial phenotype through an epistatic effect of the vg marker or through a regulatory action of vg region gene on the allele responsible for the fastest mobility variant.

References: 1) Young, W.J., J.E. Porter and B. Childs 1964, Science 143:140-141; 2) Komma, D.J. 1966, Genetics 54:497-503; 2) Komma, D.J. 1968, Biochem. Genet. 1:229-237; 2) Komma, D.J. 1968, Biochem. Genet. 1:337-346; 3) Steele, M.W., W.J. Young and B. Childs 1969, Biochem. Genet. 3:359-367; 4) Seecof, R.L., W.D. Kaplan and D.G. Futch 1969, Proc. Nat. Acad. Sci. USA 62:528-535; 5) Giesel, J.T. 1976, Biochem. Genet. 14:823-833; 6) Palenzona, D.L. and R. Alicchio 1973, Genetics 74:533-542; 7) Alicchio, R. and D.L. Palenzona 1974, Theoret. Appl. Genet. 45:122-125; 8) Brewer, G.J. 1970, An introduction to isozyme techniques. pp. 72-73. New York and London, Academic Press.

Bélo, M. and M.V.F. Lemos. Fac. de Med. Vet., Agro. e Zoot. de Jaboticabal da Universidade Estadual Paulista "Julio de Mesquita Filho", Jaboticabal, Brazil.

Domestic Drosophila species. IV. Males and females collected at different hours of the day.

Data were obtained through collections carried out in Olímpia, São Paulo State, Brazil, using naturally yeasted banana baits placed on the ground, always at the same place. The collecting period covered from May 24th, 1970, to May 23rd, 1971. The time interval between two successive collecting days was 13 days. In each day, collections were carried out at 9, 10 and 11 o'clock in the morning and 2, 4 and 6 o'clock in the afternoon.

Table 1 shows the number of males and females collected at each hour, during the experimental period, the number of individuals of each sex that were captured and the values of χ^2 that were calculated for the equal sex proportions (1:1).

Table 1. Number of males and females captured at different collection hours throughout the experimental period and values of χ^2 for equal proportions 1:1 (for one degree of freedom, $\chi^2 = 3.84$, $P = 0.05$).

Species		Times of Collection						
		9	10	11 AM	2	4	6 PM	Total
simulans	F	2289	996	841	1007	760	1638	7531
	M	5504	1241	634	645	514	1722	10260
	χ^2	1326.35	26.83	29.05	79.32	47.50	2.10	418.61
latifasciaeformis	F	1363	571	394	939	1222	2039	6528
	M	3801	762	427	1440	1710	2320	10461
	χ^2	1151.02	27.63	1.33	105.51	81.22	18.11	910.40
ananassae	F	1316	631	563	574	414	288	3786
	M	2964	855	496	535	210	120	5180
	χ^2	634.56	33.76	4.24	1.37	66.69	69.18	216.73
kikkawai	F	498	378	203	236	207	281	1803
	M	1047	412	183	186	120	198	2146
	χ^2	195.08	1.46	1.04	5.92	23.15	14.38	29.79
willistoni group	F	503	171	97	84	48	111	1014
	M	507	138	47	35	24	76	827
	χ^2	0.02	3.52	17.36	20.18	8.00	6.55	18.99
repleta group	F	95	41	23	49	52	200	460
	M	301	57	15	24	50	407	854
	χ^2	107.16	2.61	1.68	8.56	0.04	70.59	118.14
polymorpha	F	230	112	50	82	39	88	601
	M	393	120	51	34	30	47	675
	χ^2	42.65	0.28	0.01	19.86	1.17	12.45	4.29
nebulosa	F	178	67	32	61	70	80	488
	M	379	84	31	47	35	46	622
	χ^2	72.53	1.91	0.02	1.81	11.67	9.17	16.18
cardinoides	F	108	62	47	24	32	87	360
	M	270	58	34	23	26	91	502
	χ^2	69.43	0.13	2.09	0.02	0.62	0.09	23.39
sturtevanti	F	72	29	21	29	26	60	267
	M	245	35	37	41	22	95	475
	χ^2	94.41	0.46	4.41	2.06	0.33	7.90	58.31

A common characteristic presented was that of a higher number of males captured, excepting the willistoni group. Considering the collection times the flies presented: a) higher numbers of males at one (9 AM) or two times (9 and 10 AM) and a higher number or equal proportions of the sexes at the other hours for D. simulans, ananassae, kikkawai, polymorpha, cardinoides and nebulosa; b) higher numbers of males or equal numbers of the sexes for D. latifasciaeformis and sturtevanti; c) equal numbers of the two sexes at two times of collection (9 and 10 AM) and a higher number of females at the other times for the willistoni group; d) equal numbers of the two sexes or female superiority at four times of collection (10, 11 AM and 2, 4 PM) and higher numbers of males at the extreme hours of collection (9 AM and 6 PM). These represented by the repleta group which also presented a higher number of males and females at 6 o'clock than 9.

If we consider the sexual proportions of the flies as a measure of the degree of their mobility in the direction of the baits, at different times and places of collection, the data presented here suggest different behavior among males and females and also within species.

This work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil).

Carton, Y. C.N.R.S., Gif-sur-Yvette, France. Olfactory responses of Cothonaspis sp. (parasitic Hymenoptera, Cynipidae), to the food-habit of its host (Drosophila melanogaster).

Entomologists have faithfully observed and reported the attraction of insects to certain foods, extract of plants, animals or chemicals. There is still little information on the exact nature of these insect attractants. For an entomophagous parasite selecting its host, it initially and fundamentally seeks a certain environment (host-habitat finding), and it does this irrespective of the presence or absence of hosts. In this host-habitat finding process, the parasite may be attracted by the food supply of its host.

The present study is an exploration of the sequential steps in the behavioural process of a Guadalupe stock of the entomophagous parasite Cothonaspis sp. (Hymenoptera, Cynipidae) in encountering and finally parasitizing its host: Drosophila melanogaster. This Cothonaspis strain has been collected in Drosophila traps deposited among banana cultures in Guadalupe.

The female Cothonaspis lays an egg inside a Drosophila larva, especially in second instar larvae. The egg hatches within about 48 hours; the young wasp larva develops inside the Drosophila larva, which continues its development and finally pupates. The parasitic wasp larva completes its host development in the host puparium and emerges as an adult after 18 days (at 25° C).

This species appears to parasitize specifically Drosophila melanogaster; of the six other Guadalupe species of Drosophila (D. nebulosa, D. sturtevanti, D. willistoni, D. latifasciaeformis, D. ananassae, D. cardini) (David, 1973) only D. ananassae are utilized as host, at low frequency.

An air conditioned olfactometer was constructed for measuring the responses of adult Cothonaspis to chemical attractants.

The olfactometer consists of three major parts: a starting, a choice Y-tube and two trap-tubes. Compressed pure air flow is divided by a Y-connector and led into two separate lines; the flow rate in each line is regulated, using a flowmeter to provide 500 cm^3/mn into each arm of the choice Y-tube; each air flow then bubbled into a gas washing bottle containing 100

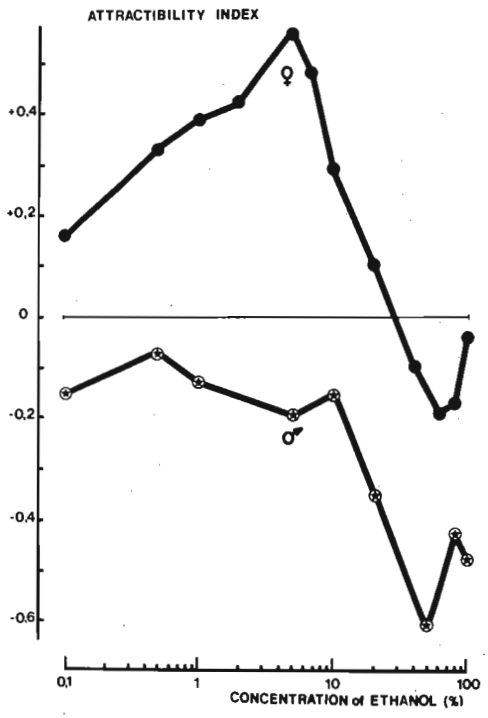


Figure 1

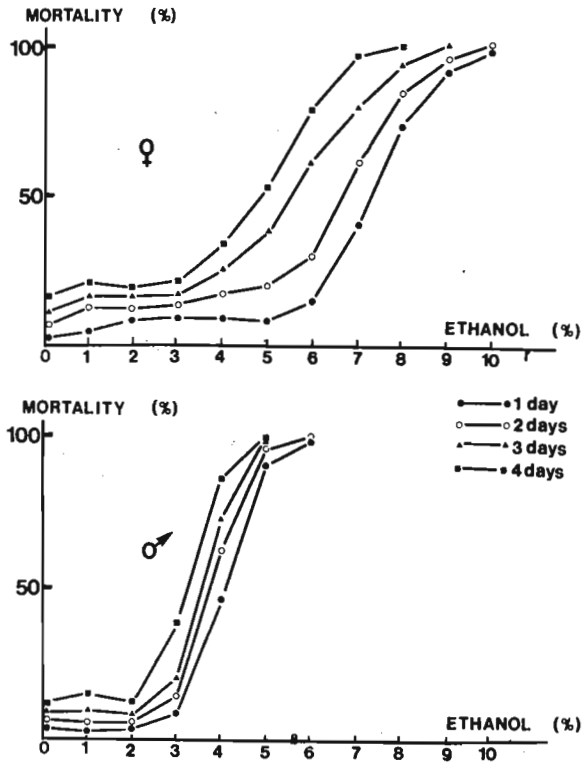


Figure 2

cm^3 of distilled water or ethyl alcohol, kept at 25°C where it attains a constant vapor pressure. An attractibility index (A.I.) was calculated: A.I. = (number of parasites which entered the odor trap minus number of parasites which entered the control trap) divided by the number of parasites started.

In this study, it has been demonstrated that females of Cothonaspis were attracted (IA = + 0.73) by odiferous substance (s) issued from synthetic medium in laboratory, on which D. melanogaster larvae are breeding. The same result was obtained with medium with live yeast (Saccharomyces cerevisiae) (IA = + 0.85).

Begon (1975) has shown that flies are instrumental in producing a very rich yeast and microbial flora in jars where fermentic process develops with production of ethanol.

The response of parasitic wasps to various concentrations of ethyl alcohol were assessed. As observed, females are attracted (+ 0.27 < A.I.< + 0.56) to this chemical over certain ranges of concentration (between 0.5% and 10%); above 50%, ethyl alcohol was slightly repellent (I.A. = -0.9). With Cothonaspis males, results are very different. For dilutions between 0.1% and 10%, attractibility index varies between -0.1 and -0.2; beyond 10% alcohol we noted a high repulsion. Consequently males are never attracted by ethyl alcohol (Figure 1).

Some authors (Fuyama, 1974, 1976) have shown that either fermentation fruits or solutions containing ethyl alcohol exert an attraction for D. melanogaster; it is clear that an environment in which D. melanogaster occurs is one of an alcohol associated resource. This fly has a tendency to lay eggs preferentially on these media. Consequently larvae of D. melanogaster in which females of Cothonaspis oviposit live also in these media. Cothonaspis is attracted to medium with ethyl alcohol (fermenting fruits in natural conditions) where live its host.

We decided to study ethanol tolerance of Cothonaspis: adult parasites are put in closed vials containing various quantities of ethanol and dead are counted every day. With this method, it has been shown that females of Cothonaspis were tolerant to ethanol and not the males (Figure 2). Consequently, females are able to live in contact with highly alcoholic substances like Drosophila melanogaster (David et al., 1974); the other species of Drosophila are more susceptible to ethanol.

References: Begon, M. 1974, DIS 51:106; David, J., P.Fouillet and M.F. Arens 1974, Arch. Zool. Exp. Gen. 115:401; Fuyama, Y. 1974, DIS 51:142; Fuyama, Y. 1976, Beh. Gen. 6: (in press).

Detwiler, C.R. and J. Tonzetich. Bucknell University, Lewisburg, Pennsylvania. The effect of two Minute mutations on meiotic crossing-over in D. melanogaster.

Females heterozygous for M(2)z and ed dp or M(2)S7 and c wt were crossed to males homozygous for the recessive marker genes. Each pair of markers represents loci on either side of the Minute locus being considered. Crosses using the corresponding marker systems but without the Minute mutants were used as controls. Females were mated 6 hours after eclosion and maintained at 25°C ±0.5°C over a 12 hr light-dark cycle. The results are presented in Table 1.

Table 1. Effect of Minute mutations on the percentage recombination in chromosomal segments containing a Minute locus.

Mutant	% recombination	Total count
+/ed dp	2.6	4000
M(2)z/ed dp	1.5	3000
Difference	1.1	
+/c wt	7.1	2600
M(2)S7/c wt	5.3	2500
Difference	1.8	

In both Minute heterozygotes, a sizable reduction occurs between markers on either side of the Minute locus. In the controls, the average values for percent recombination agree approximately with values given in Lindsley and Grell (1968). Two hypotheses may account for the Minute effect. The Minute phenotype has been postulated to result from a deletion (Ritossa, Atwood and Spiegelman, 1965). M(2)z is included in a very small deficiency but the salivary gland chromosomes of M(2)S7 appear normal. Alternately, the Minute mutation may modify some aspect of the recombination mechanism and hence should generally affect recombination throughout the genome. Although this hypothesis has not been tested, Kaplan (1953) has found that mitotic crossing over is increased in these two mutants.

References: Kaplan, W.D. 1953, Genetics 38:630-51; Lindsley, D.L. and E.H. Grell 1968, Gen. Var. in D.m. 156-157; Ritossa, F.M., K.C. Atwood and S. Spiegelman 1966, Genetics 54: 633-676.

Angus, D.S. and D.J. Colgan. University of Newcastle, N.S.W., Australia. Hybrid sterility in geographic races of D.m.

A strain of D. melanogaster was established from a single wild inseminated female from Para Wirra, South Australia, and maintained in the laboratory since 1972. This strain, PW, differs from standard Canton S in sternopleural chaeta number, PW = 17.3 ± .2 and Canton S = 24.6 ± 0.2 chaetae respectively.

Pair matings on semolina agar medium in 25 x 100 mm vials yielded the following results:

	Matings ♂ x ♀	Vials with Progeny	Vials without Progeny	% Successful
Parental Crosses	PW x PW	12	0	100
	CS x CS	12	0	100
	PW x CS	5	0	100
	CS x CS	6	0	100
F_1 Crosses	(CS x PW) x (CS x PW)	12	0	100
	(PW x CS) x (PW x CS)	1	11	8
Backcrosses to PW	PW x (PW x CS)	4	8	33
	PW x (CS x PW)	9	3	75
	(CS x PW) x PW	12	0	100
	(PW x CS) x PW	3	9	25
Backcrosses to CS	CS x (PW x CS)	2	10	17
	CS x (CS x PW)	11	1	92
	(PW x CS) x CS	3	9	25
	(CS x PW) x CS	10	2	83

From these data it is apparent that only the cross PW ♂ x CS ♀ produces F_1 progeny which are sterile. From the backcrosses it is apparent that both F_1 ♂♂ and ♀♀ are affected.

These results suggest a cytoplasmic interaction between the Canton S ♀ and the Para Wirra spermatozoa.

Voelker, R.A. and C.H. Langley. NIEHS, Research Triangle Park, North Carolina. Cytological localization of Roi (Rough eye).

During the course of experiments designed for other purposes, the following cross was performed at 25°C:

SM1, Roi/+ ♀♀ x Df(2L)X_i/CyO ♂♂

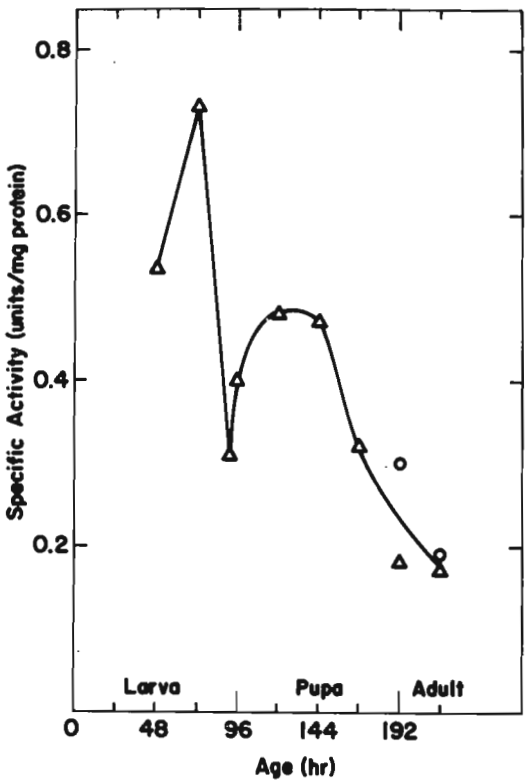
where X_i represents any of 15 different deficiencies in the pr region. The specific deficiencies (X_i's) used were: Df(2L)137, m cn bw; Df(2L)50, cn; Df(2L)E71, rdo hk pr; Df(2L)137, M cn bw; Df(2L)158, cn bw; Df(2L)130, cn bw; Df(2L)E55, rd hk pr; Df(2L)2, Tft 1(2)74i; Df(2L)9, Tft cn; Df(2L)12, Tft 1(2) 74i; Df(2L)84, Tft 1(2)74i; Df(2L)150, cn bw; Df(2L)65, Tft 1(2)74i; Df(2L), Tft 1(2) 74i; and Df(2L)161, M cn bw. Normally four classes of progeny would be expected in approximately equal frequencies from the above generalized cross: 1) +/CyO 2) SM1, Roi/CyO 3) +/Df(2L)X_i and 4) SM1, Roi/Df(2L)X_i. The expected progeny classes were obtained in all crosses, with the exception that the last class was rare or absent in four crosses. The frequencies of the last two progeny classes for these four crosses were as follows:

	Number of Progeny	
Deficiency	+/Df(2L)X_i	SM1, Roi/Df(2L)X_i
Df(2L)3, 1(2)74i	126	0
Df(2L)50, cn	103	6
Df(2L)E71, rdo hk pr	116	3
Df(2L)137, M cn bw	117	0

Since Roi/Roi homozygotes are reported to be lethal, the absence or near absence of the Roi/def progeny class suggests that the region common to these four deficiencies contains the Roi locus. The smallest of the four deficiencies, Df(2L)3, 1(2) 74i, is wholly contained within the other three and has the following breakspoints: 36F7-37A1 and 37B2-B8. The Roi locus is, therefore, very likely located within that interval.

Ali, Z.I. Northwestern University, Evanston, Illinois. Specific activity of acid phosphatase in the larval body of Drosophila melanogaster.

The larval fat body of Drosophila melanogaster is present during the larval and pupal period and for the first two days of adult life (Butterworth et al., 1965). Since the tissue undergoes histolysis during development, it is of interest to determine developmental changes in degradative enzymes. Although the natural substrate(s) of acid phosphatase (Acph) is unknown, it is reasonable to assume that this enzyme plays a role in tissue histolysis. A study of the developmental changes of Acph may indicate the importance of this enzyme.



MacIntyre (1966) with Drosophila melanogaster did not find any enzymatic variation during development. Pasteur and Kastritsis (1971) found no activity in larval fat body, but I have found measurable activity. The present study is a description of quantitative changes of Acph in the fat body of flies at different stages.

Eggs of Oregon-R flies were collected and larvae were isolated 0-1 hours after hatching to provide staged larvae. For staged pupae white pupae were isolated (96 hr > hatching) and analyzed at various time periods. For determination of Acph activity, the fat body from individuals of known age were dissected in Drosophila Ephrussi and Beadle's Ringer solution, homogenized, and assayed by the method of Postlethwait and Gray (1975) with minor changes. One enzymatic unit is defined as 1 A_{400} unit of product formed per 30 min period. Specific activity is units Acph/mg protein.

For protein determination the fat body from known aged flies were homogenized in .85 ml of ice cold .01 M buffer. The homogenate was centrifuged for 1 min at 5500 x g, and supernatant used for determination of protein by the Schaffner and Weissmann (1973) method, modified in that the supernatant aliquots were spotted and dried on analytical filter paper disks. Assays were performed in duplicate and the values averaged.

Changes in the specific activity during development is shown in Figure 1. The specific activity is highest in early 3rd instar larvae, then it decreases during the latter half. This drop in specific activity may be attributable to protein granule deposition (Butterworth et al., 1965). No increase in specific activity was noted during the first 24 hr of adult life. This suggests that if in situ Acph plays a role in tissue histolysis, the endogenous titer is sufficient. Thus, the enzyme does not seem to be inducible by juvenile hormone in this tissue in contrast to Acph in ovary tissue (Postlethwait and Gray, 1975).

References: Butterworth, F.M., D. Bodenstein and R.C. King 1965, J. Exp. Zool. 158, 141-154; MacIntyre, R.J. 1966, Genetics 53:451-474; Pasteur, N. and C.D. Kastritsis 1971, Devel. Biol. 26:525-536; Postlethwait, J.H. and P. Gray 1975, Devel. Biol. 47:196-205; Schaffner, W. and C. Weissmann 1973, Analyt. Biochem. 56:502-514.

Felten, T.L.* Oregon State University, Corvallis. Observations on the influence of cAMP in puff induction along the salivary gland chromosomes of D. melanogaster (Canton S).

Rensing and Hardeland (1972) reported DBcAMP influenced temporal differences in puff response and concentration-dependent puff induction of selected regions of the X and 3L chromosomes in D. melanogaster (Meigen). In this study puff induction, size, and distribution, influenced by cAMP concentration, were observed for all chromosome arms of salivary gland cells of 3rd instar larvae. One salivary gland of the paired gland was incubated 1 hr. in 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, or 10^{-6}M cAMP in Ephrussi and Beadle Ringers (Demerec, 1950). The other salivary gland, incubated 1 hr. in Ephrussi and Beadle Ringers, served as a control. Regions determined susceptible to cAMP influence at this developmental stage were: X(3C-D); 2L(32C-D & 34A); 2R(47A); 3L(61B & 61C-D); and 3R (88F, 98F, 100C, & 100E). Comparison of puff ratios for regions X(3C-D), 3R(98F) and 3R(100E), as seen with varying concentrations of cAMP, indicated that the response of these regions required a cAMP level of 10^{-3}M for any response to occur and this response was maximal. The other responsive regions showed concentration-dependent puff ratios. Chromosomes were minimally responsive (37% maximal response or less) or unresponsive with 10^{-6}M cAMP, suggesting that this might be the threshold concentration. However, region 2L(32C-D) responded with a puff ratio 86% of maximal response with 10^{-6}M. This preliminary evidence suggests that cAMP may influence gene activity of Drosophila salivary glands by more than one mechanism. It also appears that region 2L(32C-D) is significantly more sensitive to cAMP influence than the other regions which respond only at higher concentrations.

References: Rensing, L. and R. Hardeland 1972, Exp. Cell Res. 73:311-318; Demerec, M. 1950, in: Biology of Drosophila, John Wiley & Sons, Inc.

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Kekić, V. and D. Marinković. Faculty of Science, University of Belgrade, and Institute for Biological Research, Belgrade, Yugoslavia. The list of Drosophila species in Yugoslavia.

The first information about the species of Drosophila which inhabit Yugoslavia was provided by Strobl (1898), who described 5 species (after Basden, 1961). Coe (1958) made an additional analysis, adding 8 new species to this list. In the period between 1971 and 1977 we collected Drosophila species with the purpose of making genetic analyses of D. malanogaster and D. subobscura. Our traps contained mixed fruits, e.g. bananas, peaches, watermelons, pears and apples, and they were located at the places where the two species could be most frequently found. Among the species of Diptera, we found 11 species of Drosophila which have not been described by previously mentioned authors. Consequently the list of species described so far in Yugoslavia amounts to a total of 24 species:

Scaptodrosophila
- D. deflexa Duda
- D. rufifrons Loew

Dorsilopha
- D. busckii Couquillett

Lordiphosa
- D. andalusiaca Strobl
- D. fenestrarum Fallen

Sophophora
- D. melanogaster Meigen
- D. simulans Sturtevant
- D. ambiqua Pomini
- D. limbata von Roser
- D. subobscura Collin
- D. obscura Fallen
- D. tristis Fallen

Hirtodrosophila
- D. cameraria Haliday
- D. trivittata Strobl

Drosophila
- D. funebris Fabricius
- D. littoralis Meigen
- D. hydei Sturtevant
- D. immigrans Sturtevant
- D. kuntzei Duda
- D. phalerata Meigen
- D. transversa Fallen
- D. testacea von Roser
- D. confusa Staeger
- D. histrio Meigen

The authors wish to acknowledge the help of Dr. O. Kitagawa, from Tokyo Metropolitan University, and of Dr. G. Bächli, from the University of Zürich, in determining the species of Drosophila.

References: Basden, E.B. 1961, Beiträge zur Entomologie 11, 1/2, 160:224; Coe, R. 1968 Glasn. Prir. Muz. B. 12:187-207; Strobl, G. 1898, Glasn. Zem. Mus. Bosn. Herc. 10:561-616.

Hägele, K. and W.-E. Kalisch. Ruhr-Universität, Bochum, Germany.

^{3}H-thymidine labeling over homologous asynapsed loci in polytene chromosomes.

In a study concerning localized DNA synthesis in polytene chromosomes, Plaut and Nash[1] found by means of ^{3}H-thymidine autoradiography that homologous asynapsed loci in Drosophila synthesize DNA not only synchronously according to the number of silver grains, but also in equivalent amounts. These results refer to about twenty observations. On the basis of these findings, autoradiographic data concerning replication participation of homologous chromosomes and replicative organization have been discussed in a number of investigations[2,3,4,5]. Our reinvestigation of the results of Plaut and Nash[1], however, reveal that the statement of these authors cannot be maintained absolutely. Consequently, on the basis of our results, a different interpretation[6] was possible from that of Bender, Barr and Ostrowski[2] concerning labeling behavior of the X chromosomes of heterozygous Dp(1;f)AM female larvae of Drosophila.

The autoradiographs in Figure 1a and b show that homologous asynapsed loci are not always in synchrony or labeled in equivalent amounts during DNA synthesis. We have scored silver grain distribution over ninety homologous asynapsed loci. The absolute differences in the number of silver grains between these loci and their number of observations are given in Figure 2a. Chi-square test (for the range of 0-7 silver grain difference) reveals sufficient correspondence with the Poisson distribution: $\chi^2(7) = 11.09$; $p > 0.12$.

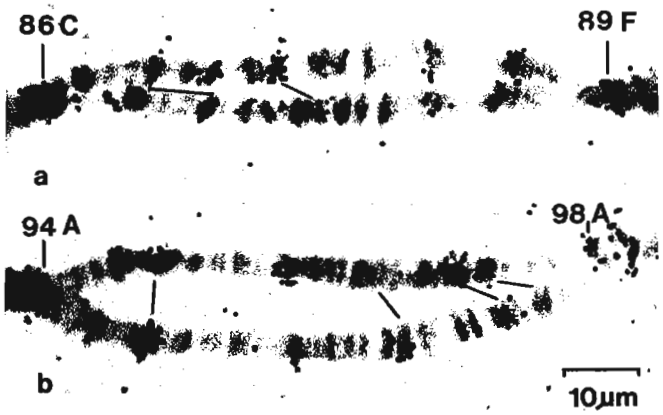


Figure 1a and b. Examples of unequal silver grain labeling over homologous asynapsed chromosome loci. ^{3}H-thymidine autoradiographs of salivary gland chromosome 3R from late 3rd instar larvae of the wild type (Berlin) stock in Drosophila melanogaster. ^{3}H-thymidine incubation (20 µ Ci/ml; spec. activity 15.1 Ci/m mol), arithmetic mean of background silver grains in all autoradiographs analyzed [M(50) = 26.5; s = 15.3]. Autoradiographic and other methods used have already been published[7,8,9].

Figure 2b shows that differences in the number of silver grains between two homologous loci do not depend upon the labeling intensity (i.e. total silver grain numbers) of both homologues. For the range of 0-7 silver grain difference the arithmetic means of silver grain classes 0-10, 11-20, 21-30, 31-40 and 41-50 are 1.98, 1.53, 2.83, 1.75, 3.67, respectively.

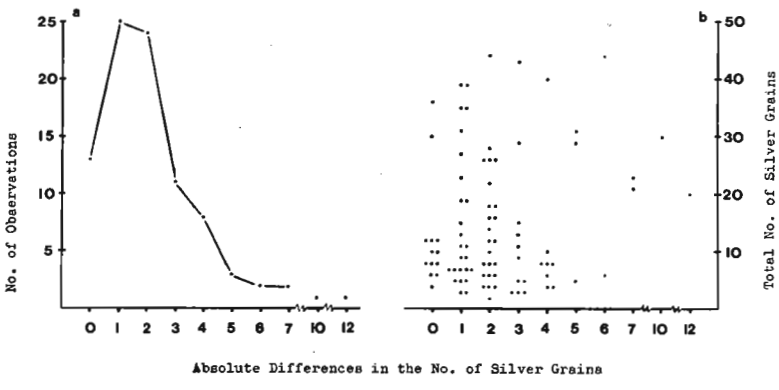


Figure 2a and b. Deviations in labeling intensity between ninety homologous loci in thirty-one different asynapsed polytene chromosome regions. The regions were found in twenty-six nuclei with discontinuous labeling at the endphase of replication in ten autoradiographs. Background determination was done according to Kalisch and Hägele[9]. (a) Frequency of differences in the number of silver grains between homologous loci. (b) Correlation between differences in (a) and the total number of silver grains over the homologous chromosome loci.

Background determinations[9] indicate that deviations between homologous loci (Figure 2a) cannot exclusively be due to background labeling.

These results demonstrate that labeling in homologous loci of normally structured asynasped polytene chromosomes shows only a high degree of correspondence, but not the absolute correspondence of Plaut and Nash[1].

References: 1) Plaut, W. and D. Nash 1964, In: The role of chromosomes in development, 113 (Academic Press, New York); 2) Bender, H.A., H.J. Barr and R.S. Ostrowski 1971, Nature New Biol. 231:217; 3) Yang, D.-P. and E.O. Dodson 1970, Chromosoma 31:309; 4) Mulder, M.P. P. van Duijn and H.J. Gloor 1968, Genetics 39:385; 5) Plaut, W., D. Nash and T. Fanning 1966 J. Mol. Biol. 16:85; 6) Kalisch, W.-E. and K. Hägele 1974, Nature New Biol. 249:862; 7) Kalisch, W.-E. and K. Hägele 1973, Chromosoma 44:265; 8) Hägele, K. and W.-E. Kalisch 1974, Chromosoma 47:403; 9) Kalisch, W.-E. and K. Hägele 1976, DIS 52.

Gajardo, G. and M. Budnik. University of Chile, Santiago, Chile. Low temperature resistance in Drosophila pavani[1].

Drosophila pavani is an endemic species very abundant in the central zone of Chile. The population size changes seasonally; it increases in summer and decreases in winter (Brncic 1970).

Previous laboratory observations have shown that D. pavani preadults are not able to resist temperatures of -30°C (Budnik and Brncic 1974, 1976). However at higher temperatures (-24°C), certain numbers of individuals survive and develop to imagoes when culture media temperature rises to normal (unpublished data). The present communication describes survival under less drastic temperatures in order to postulate possible temperature dependent seasonal population changes.

Series of 75 vials each containing 20 D. pavani preadults (eggs, third instar larvae or pupae), taken from stock laboratory population, were submitted to a temperature of -24°C (the experimentals) or + 25°C (the controls) for 4 hours. Afterwards, all vials were maintained at + 25°C.

Table I compares viabilities under the above described conditions. The observed viability after a -24°C thermic shock was: eggs > third instar larvae > pupae.

Table I. Effect of low thermic shock upon D. pavani preadult viability.

Development state	Temp. of treatment	Number of replicas	Total number of adults emerged	$\bar{X}$ viability per vial ± S.E.	t
Egg	+ 25°C	75	831	11.03 ± 0.43	8.348*
	- 24°C	75	695	9.24 ± 0.55	
Larvae	+ 25°C	75	831	11.03 ± 0.43	26.667*
	- 24°C	75	405	5.38 ± 0.49	
Pupae	+ 25°C	75	873	11.64 ± 0.65	11.840*
	- 24°C	75	36	0.48 ± 0.22	

* $P < 0.001$ (148 D.F.)

Similar experiments were performed with D. pavani adults, and results were expressed as the percentage of individuals surviving after the low temperature shock. Adult survival was 62.6%.

The results show that after the above described thermic shock, some individuals can survive. This suggests that temperature may be one of the physical environmental conditions controlling the abundance of D. pavani populations in nature. Further studies are under way to elucidate this point.

[1]Research supported by grants from "Servicio de Desarrollo Cientifico y Creación Artística", U de Chile, Proyect No. 4013-R, Proyect No. 5 PNUD/UNESCO RLA 76/006 and Program Multinational of Genetics (O.E.A.).

References: Brncic, D. 1957, Las especies chilenas de Drosophilidae, Col. Monografias Biol. Univ. Chile. Imp. Stanley, Santiago, Chile; Brncic, D. 1970, Studies on the evolutionary biology of chilean species of Drosophila. In Essays in Evolution and Genetics in honor of Theodosius Dobzhansky (A Supplement to Evolutionary Biology). Edited by M.K. Hecht and W.C. Steere; Budnik, M. and D. Brncic 1974, Ecology 55:657-661; _____ 1976, Evolution 29:777-780.

Bournias-Vardiabasis, N. and M. Bownes. University of Essex, Colchester, England.
Genetic analysis of the tumorous head mutation of D. melanogaster.

Tumorous head (tuh) is a recessive homeotic mutation affecting the eye-antennal disc of D. melanogaster. It has a two gene action, tuh-1 located on the X chromosome at approximately 65.8 (Pyati, 1976) and a third chromosome tuh-3 gene located at approximately 58.5 (Gardner, 1970). Tumorous head stocks differ in the presence of a third chromosome dimorphism (3A/3B, 3B containing a Payne inversion in the left arm which is responsible for a high male to female ratio, Kuhn 1970). In our stock we found an approximate 1:1 male to female ratio and cytological analysis of the third chromosome showed no Payne inversion. In a cross of tuh flies to st stock (the Payne inversion contains a st marker) there were no st progeny; thus our stock is 3A/3A.

We have used a new method for mapping the tuh-3 gene, taking into account the variable penetrance of this mutation. Tumorous head females were crossed with $sbd^2bx^3pbx/TM1$ males (sbd^2 mapping to the left of tuh-3 and pbx to its right). Female tuh/sbd^2bx^3pbx progeny were backcrossed to their fathers and recombinants of sbd^2++/sbd^2bx^3pbx were crossed to tuh females. We mapped the tuh-3 gene as being between 58.44 and 58.56. When mutations are placed over deficiencies for that region of the chromosome the penetrance of the phenotype would vary according to the way in which the mutant gene acts. To date all homeotic genes placed over a deficiency for that region have exhibited an increased penetrance of their homeotic phenotype. Df bxd^{100} (DF (3R) 89B5-6; 89E 2-3) flies were crossed to tuh flies, the F_1 generation showed a 1.7% penetrance while controls had a 55% penetrance. Thus tuh-3 over the deficiency showed a decreased penetrance; a phenomenon which has not, to our knowledge, been observed before.

We have also found an enhancer of the tuh-1 gene. When females carrying y mwh jv were crossed to tuh males the progeny showed a 52% penetrance of the tuh phenotype, while tuh-1 as a maternal gene has a 30% penetrance. Thus the X chromosome carrying y mwh jv has a better maternal effect than original chromosome carrying tuh-1.

References: Gardner, E. 1970, Advances in Genetics 15:115-146; Kuhn, D. 1970, Evolution 24:181-190; Pyati, J. 1976, Molec. gen. Genet. 146:189-190.

Zuchowski-Berg, C.I. SUNY at Buffalo, New York.
Unintegrated ribosomal genes in Drosophila melanogaster: Inversion (1)yellow - 3 of Patterson.

Previous studies by Zuchowski and Harford (1977) and Harford and Zuchowski (1977) have shown that a rearrangement of the X heterochromatin resulting in the transposition of the nucleolus organizer to the distal end of the chromosome induces the formation of low molecular weight ribosomal genes ($\sim 3 \times 10^8$ daltons) which are not integrated into the bulk of the DNA. These unintegrated ribosomal genes were present only in D. melanogaster females heterozygous for the X chromosome inversion. In addition, specific X chromosome inversions (e.g. $In(1)sc^{L8}$ and $In(1)sc^{S1}$) have been reported by Baker (1971) to cause variegated suppression of rDNA when present as $In(1)sc^{L8}/0$ and $In(1)sc^{S1}/0$ males. It has also been shown that these specific male genotypes undergoing position effect variegation exhibit unintegrated ribosomal genes and that the extent of unintegrated ribosomal gene formation for specific X chromosome inversions can be regulated by using the suppressor of variegation (Su(var)) locus (Zuchowski-Berg, 1978). In(1)yellow-3 of Patterson has its genetic breakpoints between 1(1)J1 and y and between bobbed (bb) and the centromere (Lindsley and Grell, 1968). The purpose of this investigation was to determine whether $In(1)y^{3P}$ is yet another inversion which can induce unintegrated ribosomal gene formation. Spofford (1976) has also reported that Su(var) increases the yellow variegation of $In(1)y^{3P}$. Therefore, $In(1)y^{3P}$ also lends itself to the study of unintegrated ribosomal gene formation during the phenomenon of position effect variegation.

In the first series of experiments, D. melanogaster females either homozygous or heterozygous for $In(1)y^{3P}$ were analyzed. DNA of high molecular weight ($\sim 5 \times 10^9$ daltons) was extracted and subjected to sucrose gradient sedimentation. All experimental procedures are described in Zuchowski and Harford (1976) and Zuchowski-Berg (1978). Hybridization of ^3H-Drosophila rRNA across the gradient fractions showed that homozygous female adults have no unintegrated ribosomal genes whereas heterozygous females exhibit $\sim$ 35% ribosomal genes of low molecular weight (Table I). An analysis of the diploid brain and imaginal discs also showed a similar pattern of ribosomal gene distribution. The pattern of ribosomal gene inte-

gration or unintegration in adult flies is always the same for the diploid tissue. The polytene salivary glands in both homozygous and heterozygous females exhibit 42% of their ribosomal genes as unintegrated (Table I) perhaps due to ineffective pairing in polytene tissue.

Table I. Unintegrated ribosomal genes in $In(1)y^{3p}$ genotypes.

Genotype	Origin of DNA	% rDNA unintegrated	Gradients analyzed
$In(1)y^{3p}/In(1)y^{3p}$ ♀	adult	0	3
$In(1)y^{3p}/In(1)y^{3p}$ ♀	diploid tissue	0	3
$In(1)y^{3p}/In(1)y^{3p}$ ♀	polytene tissue	41.2	3
$In(1)y^{3p}$/Ore-R X ♀	adult	35.1	3
$In(1)y^{3p}$/Ore-R X ♀	diploid tissue	36.2	3
$In(1)y^{3p}$/Ore-R X ♀	polytene tissue	42.3	3
$In(1)y^{3p}/Y$ ♂	adult	0	3
$In(1)y^{3p}/Y$ ♂	diploid tissue	0	3
$In(1)y^{3p}/Y$ ♂	polytene tissue	41.4	3
$In(1)y^{3p}/0$ ♂	diploid tissue	14.8	3
$In(1)y^{3p}/0$ ♂	polytene tissue	42.0	3

When male flies carrying $In(1)y^{3p}$ were studied, no unintegrated ribosomal genes were found (Table I). Similarly, diploid tissue from the same male genotype showed no low molecular weight ribosomal genes. A recent exception to the general lack of unintegrated genes in males has been the finding of low molecular weight ribosomal genes of varying amounts in males undergoing position effect variegation (Zuchowski-Berg, 1978). When high molecular weight DNA from diploid tissue of $In(1)sc^{V2}/0$, $sc^{8}/0$, $sc^{L8}/0$, and $sc^{S1}/0$ was analyzed by sucrose gradient sedimentation, 5.2%, 9.6%, 39.1%, and 46.2% respectively of the ribosomal genes were found to be unintegrated. This led to the hypothesis that unintegrated ribosomal genes and position effect variegation are at least related, but the type of causal relationship could not be inferred. Similarly, in this report, diploid and polytene tissues from $In(1)y^{3p}/0$ males were analyzed. As given in Table I, ~ 15% of the ribosomal genes are unintegrated in male diploid tissue. Polytene tissue displayed its usual 42% unintegrated genes. In addition, a decrease in the viability of $In(1)y^{3p}/0$ male genotype was evident compared to female survival.

As a further test for a general variegation association, $In(1)y^{3p}$ was analyzed in the presence of Su(var) or the $Su(var)^{+}$ allele. Su(var) was found to enhance yellow variegation and as shown in Table II, ~ 15% unintegrated ribosomal genes were present in adult males. When $Su(var)^{+}$, which is a suppressor of $In(1)y^{3p}$ variegation, was present, no unintegrated ribosomal genes were found (Table II). Similar results have been reported using the Su(var) locus in association with $In(1)sc^{4}$, rst^{3}, and w^{m4} (Zuchowski-Berg, 1978).

Table II. Effect of Su(var) on unintegrated ribosomal genes in $In(1)y^{3p}$ genotypes.

Inversion	Mother	Progeny adult males	% rDNA unintegrated	Gradients analyzed
y^{3p}	Su(var)/Su(var)	Su(var)/Su(var)	15.4	3
y^{3p}	$Su(var)^{+}/Su(var)^{+}$	$Su(var)^{+}/Su(var)^{+}$	0	3

The relationship of unintegrated ribosomal genes to position effect variegation remains clouded in mystery. Whether the production of unintegrated ribosomal genes is part of the mechanism of variegation gene inactivation or whether it is an additional consequence of the variegation mechanism is unknown. In any case, $In(1)y^{3p}$ is another X chromosome inversion which is not only associated with unintegrated ribosomal gene formation in heterozygous females, but also seems to be correlated with unintegrated gene occurrence during position effect variegation.

References: Baker, W.K. 1971, Proc. Nat. Acad. Sci. USA 68:2472; Harford, A.G. & C.I. Zuchowski 1977, Cell 11:389; Lindsley, D.L. and E.H. Grell 1968, Carnegie Inst. Wash. Publ. No. 627; Zuchowski, C.I. & A.G. Harford 1976, Chromosoma 58:219; 235; Zuchowski, C.I. & A.G. Harford 1977, Cell 11:383; Zuchowski-Berg, C.I. 1978, Nature 271:60.

Soliman, M.H. University of New England, Armidale, N.S.W., Australia. Olfaction of phenylthiocarbamide by various mutants of Drosophila melanogaster.

Four mutants and a wild type strain of Drosophila melanogaster were tested for their preference for normal medium and medium supplemented with 0.5% phenylthiocarbamide (PTC) in a Y-shaped maze made of glass (Soliman, 1975). Males and females (3-5 days old) were observed separately at room temperature and in the presence of light for a 24 hour period.

There was a distinctive difference among genotypes and between sexes in their PTC perception (Tables 1 and 2). The greatest and most consistent preference for normal medium was displayed by both sexes of the white mutant followed by, but less consistently, the vestigial. Both wild and yellow females preferred normal, while their males showed no general preference for either medium. The ebony mutant was at the other end of the spectrum. Neither sex of this genotype could sense the presence of PTC.

Table 1. Choice between normal and PTC supplemented medium by D. melanogaster adults.

Genotype	Males					Females					Sex by medium interaction
	Normal	PTC	$\chi^2_{1:1}$	Replication	χ^2_h	Normal	PTC	$\chi^2_{1:1}$	Replication	χ^2_h	χ^2_1
wild	204	191	0.53	5	7.41	277	109	73.12[b]	5	3.85	33.40[b]
yellow	186	184	0.01	5	19.29[b]	258	121	49.52[b]	5	7.92	24.58[b]
ebony	192	214	1.91	5	31.06[b]	140	149	0.28	4	20.88[b]	0.09
vestigial	242	163	15.44[b]	5	40.60[b]	232	159	13.63[b]	5	13.59[b]	0.03
white	285	55	155.59[b]	4	5.24	288	55	158.28[b]	4	7.66	0.00
Total	1109	807	47.60[b]			1195	593	202.69[b]			31.53[a]

[a] $p < 0.01$
[b] $p < 0.005$

Table 2. Analysis of variance for PTC-perception by Drosophila melanogaster adults.

PTC vs Normal				PTC			
Source of variation	df	Mean square[a]	Percent variation	Source of variation	df	Mean square	Percent variation
Medium	1	4855.3[f]	32.00	Replicate	4	196.4[e]	15.57
Replicate x medium	4	454.1[d]	11.97	Sex	1	261.7[d]	5.19
Sex x medium	1	520.6[c]	3.43	Genotype	4	531.6[f]	42.14
Genotype x medium	4	903.3[f]	23.81	Replicate x sex	4	38.5[ns]	3.05
Error[b]	50	87.4	28.78	Replicate x genotype	16	56.4[ns]	17.89
				Sex x genotype	4	111.1[c]	8.81
				Replicate x sex x genotype	13	28.5	4.34

[a] Only significant sources of variation are presented
[b] Includes second order interactions which were not significant
[c] $p < 0.05$
[d] $p < 0.01$
[e] $p < 0.005$
[f] $p < 0.001$

The activity of the flies as influenced by chemoperception and measured as the number of flies that left the source, indicated some differences among genotypes and between sexes (Table 3). The activity of both sexes was similar except for the ebony mutant where the females of this genotype were less active than the males. The general activity of the vestigial, wild and yellow were similar. An activity index (the active number of flies divided by the inactive number) could rank the different genotypes in the following order: white > ebony males > vestigial > wild $\geq$ yellow $\geq$ ebony females.

Table 3. Activity of Drosophila melanogaster as influenced by chemoperception of adults.

Sex	Genotype	Active	Inactive	Replication	χ^2_h	Activity index[c]
Male	wild	395	105	5	10.00[a]	3.8
	yellow	370	130	5	2.80	2.8
	ebony	406	94	5	33.15[b]	4.3
	vestigial	405	95	5	24.80[b]	4.3
	white	340	60	4	23.76[b]	5.7
	Total	1916	484			
Female	wild	386	114	5	40.83[b]	3.4
	yellow	379	121	5	33.87[b]	3.1
	ebony	289	111	4	43.79[b]	2.6
	vestigial	391	109	5	6.51	3.6
	white	343	57	4	7.55	6.0
	Total	1788	512			

The difference between active and inactive adults was significant at $P < 0.005$ for all genotypes.

[a] $P < 0.05$

[b] $P < 0.005$

[c] The active number divided by the inactive number of flies.

The observed response to PTC in the present study may even be less than what natural populations may possess, since it is well known that strains and inbred lines of Drosophila which have been kept in laboratory for years show less activity than recently captured natural populations.

The pleiotropic effects of single point mutations (expressed in a morphological trait) on the behaviour of carriers are well documented for Drosophila sexual behaviour. Recently, Burnett et al. (1973), fed larvae of D. melanogaster on α-dimethyltryosine to produce yellow mutant phenocopy. This treatment resulted in changes in the courtship behaviour of the males similar to that of the yellow mutant. This experiment and others using body colour mutants indicate that the normal brown pigment of the body is related to the normal behaviour. This relation may not be direct, since intermediates in the pathway of melanin synthesis, such as nonadrenalin, are known to have important functions in the central nervous system and also interact with other neurotransmitters (Laverty, 1974).

Since isogenic lines were not used the findings cannot be attributed to either the mutant loci or the genetic background (petit, 1958).

References: Burnet, B., K. Connolly and B. Harrison 1973, Science 181:1059-1060; Laverty, R. 1974, Progress Neurobiol. 3:31-70; Petit, C. 1958, C.R. Acad. Sci. 248:3484-3485; Soliman, M.H. 1975, J. Stored Prod. Res. 11:203-209.

Geurts v. Kessel, A. and L. Douglas. Erasmus University, Rotterdam, The Netherlands and 11121 Erich Lane, Balch Springs, Texas (75180). Evidence that black pigment spots, induced in eyes of spa^{pol}/spa^{pol} (=pol/pol) D. melanogaster by outcrossing, are heritable.

In testing the effects of outcrossing on pol/pol phenotypes (Douglas et al., 1977), we showed that backcrosses of pol/pol^+ F_1's resulted in pol/pol offspring, many of which had black pigment spots in the eyes. However, those outcrossing experiments did not establish that the changes observed were heritable - the black spots in the F_2's could conceivable have been caused by an infectious agent introduced into the wild type (and other) stocks used for obtaining the F_1's.

Therefore selection for - and against - blackening was carried out on a pol/pol stock derived from the F_2's; 5 to 10 flies with the darkest eyes in each of six successive generations were used to establish a "high" line, whereas the "low" line was obtained by selecting flies with only a few spots (but not zero) over the same number of generations. After this, the two lines were transferred to a stock room and maintained without further selection for more than

a year, allowing them to stabilize.

Examination of a sample from each of the two lines revealed impressive differences. Even though both still produced flies with, ("positives"), and without, ("negatives"), black spots, the extent of blackening was distinctly greater in positive flies from the high line than in lows - large sections of the eye were blackened, whereas only a small number of black spots were observed in positive flies of the low line.

Also the negative-to-positive ratio was significantly greater among flies in the high line than in the lows (Tables 1 and 2), with the overall $F_{8,2}$ ratio suggesting a significance level of $P < 0.005$ (see the Appendix and Table 2).

Table 1. ♂♂ offspring without, and with, dark spots in high and low lines (four bottles were examined in the highs and two in the lows)

high		low	
neg	pos	neg	pos
78	181	39	15
73	147	125	24
123	113	$\chi_1^2 \sim 3.29$	
42	38		
$\chi_3^2 \sim 34.46$			

	combined	
	neg	pos
high	316	479
low	164	39
$\chi_1^2 \sim 114.98$		

$F_{4,1}(37.75/4,114.98/1) \sim 12.18$, $P < 0.025$

Table 2. ♀♀ offspring (see the caption of Table 1)

high		low	
neg	pos	neg	pos
23	209	31	20
47	151	110	26
33	142	$\chi_1^2 \sim 7.62$	
19	48		
$\chi_3^2 \sim 20.36$			

	combined	
	neg	pos
high	122	550
low	141	46
$\chi_1^2 \sim 212.95$		

$F_{4,1}(27.99/4,212.95/1) \sim 22.83$, $P < 0.01$

$F_{8,2}(65.74/8,328.03/2) \sim 19.96$, $P < 0.005$

Although a definitive explanation of the results cannot be made with the information available, the suppression of blackening known to occur in pol/pol stocks with relatively small amounts of heterochromatin suggests a tentative hypothesis: that outcrossing could have removed small blocks of heterochromatin, or genes responsible for heterochromatin formation, from our pol/pol stock, causing an increase in proportion of positives - especially after selection. (For evidence bearing on the effect of heterochromatin, compare Tables 1 and 2, remembering that the Y is heterochromatic. See also Douglas et al., 1977, and Morgan, 1947.)

Appendix. The χ_1^2 of 114.98 in Table 1 comparing negative to positive ratios in high and low lines is deceptively high, probably because of a source of variation within the two lines (penetrance?); a similar statement could be made for the 212.95 in Table 2. In an extreme hypothetical case, the χ^2 measuring difference between two lines could have fallen within limits observed (between bottles) within lines, in which case a χ_1^2 even larger than 212.95 might not have been taken as suggestive of a heritability difference between highs and lows.

Although rarely seen, a more suitable statistic for testing difference between high and low lines in data like these is the F ratio. Since it is, by definition, a ratio between two χ^2's divided by their respective degrees of freedom (Mood et al., 1974), it can reveal whether one set of data is more variable than another, independently of the degree of heterogeneity in either set (for an analogous approach, using arcsine transforms, see Fisher and Yates, 1963, pg. 17). Thus the $F_{8,2}(65.74/8,328.03/2)$ of approximately 19.96 strongly suggests that the two lines are genetically distinct (see the bottom line of Table 2), because the difference between lines, measured by 328.03/2 is very much larger than variation within lines.

References: Douglas, L, A. Geurts v. Kessel, R. Douglas and A. Sinnege 1977, DIS 52:103; Fisher, R. and F. Yates 1963, Statistical Tables. Oliver and Boyd (London); Mood, A, F. Graybill and D. Boes 1974, Introduction to the theory of statistics. McGraw-Hill; Morgan, L. 1947, Genetics 32:200-219.

Sokolova, K. and M.D. Golubovsky.
Institute of Cytology and Genetics, Novosibirsk 90, USSR. Temperature-sensitive alleles at the 1(2)gl locus.

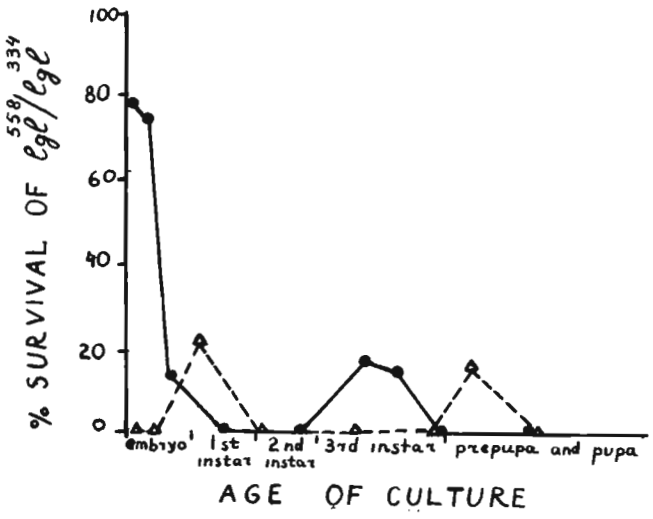
The lethal giant larvae locus has attracted the attention of geneticists for many years and for many reasons. Mutations of the 1(2) gl locus block ontogenesis before metamorphosis is completed. In natural populations 1(2)gl alleles are constantly present in relatively high concentrations. It was established that the main factor favourable to over-all occurrence of the 1(2)gl mutations was resistance to extreme temperature conditions (Golubovsky and Sokolova, 1973). The 1(2)gl locus is represented by a series of multiple alleles which are differentiated by their effective lethal stages and by their capacity to induce neoplastic growth (Gateff, Golubovsky and Sokolova, in press). We have tried to obtain some viable 1(2)gl/1(2)gl homozygotes and compounds under variable temperature conditions. Experiments were conducted both under continuous exposure to high (29°) and low (17°) temperature, and under the temperature-shift conditions. We have studied 5 homoallelic and 8 heteroallelic lethal combinations under continuous exposure. Among homoallelic combinations only one (namely l^{119}/l^{119}) gave some viable adults; among heteroallelic combinations we found viable compounds in four combinations (Table 1).

Table 1. Viability of 1(2)gl/1(2)gl individuals (adults) in offspring from Cross: ♀ l Bl/Cy x ♀ l/BiL under continuous exposure to different temperature.

Combinations of lethals	t°C	Number of progeny	Number of viable adults lgl/lgl	
Homoallelic (5 combinations)	25°C	2081	0	
	29°C	627	4 (l^{119}/l^{119})	1.1%
	17°C	1040	0	
Heteroallelic (8 combinations)	25°C	4184	4*	0.7%
	29°C	1784	8**	3.8%
	17°C	1368	10***	7.7%

l - some lethal allele 1(2)gl
Bl - gene-marker Bristle.
Viable compounds were obtained in such combinations:
* l^{558}/l^{D150}
** l^{558}/l^{D150}, l^{558}/l^{334}
*** l^{558}/l^{334}, l^{334}/l^{309}, l^{119}/l^{D150}

Temperature-shift experiments were conducted for two combinations of 1(2)gl lethals: l^{558}/l^{334} and l^{D150}/l^{119}. We used shift-up and shift-down methods ($25° \rightleftarrows 17°$ and $25° \rightleftarrows 29°$) and shift-pulse as well. The results of shift experiments were of great interest: the temperature action on definite developmental stages for short time lead to emergence of a considerable number of compounds (especially during the early embryonic stage). The presence of both lethals in viable compounds was confirmed by crossing to Cy/Df (net, lgl). Temperature-sensitive period for l^{558}/l^{334} under the influence of 17° was found to be diphasic: the first phase was the early embryonic stage (Figure 1), and the second was the third larval stage. Tsp under the influence of 29° was also diphasic: the first phase was the first larval stage and the second, the prepupal stage. Temperature sensitive period for l^{D150}/l^{119} combinations was monophasic: only the third larval stage proved to be sensitive to low temperature (about 20% of compounds were viable). Thus,



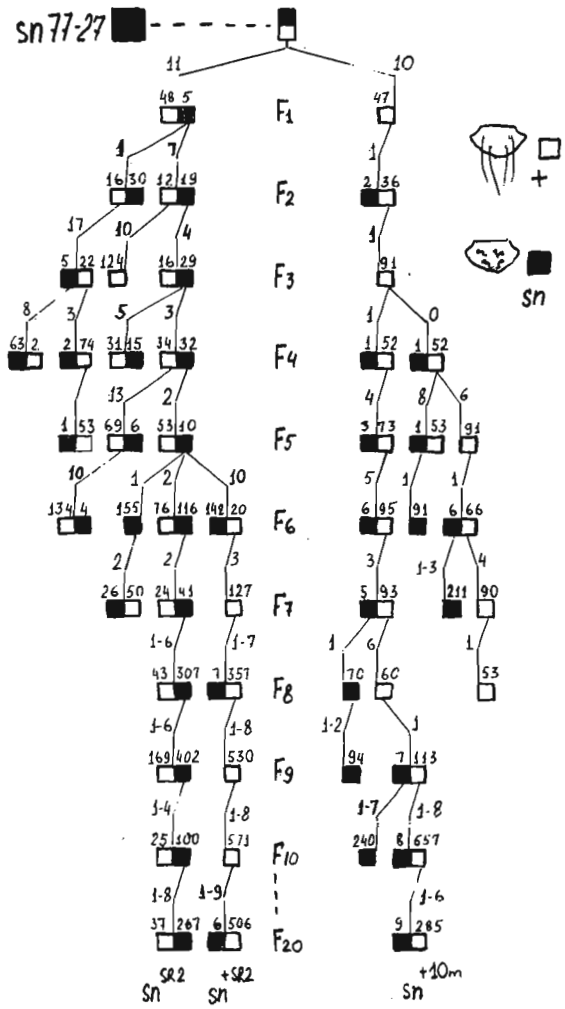
according to these data, the l(2)gl action had begun considerably earlier than lethality of the locus. It corresponds to Gateff's data concerning neoplastic growth of l(2)gl mutants which was found in the embryonic stage. The fact that the viability of homozygotes is increased so strongly under the temperature - shift conditions remains to be resolved.

Reference: Golubovsky, M.D. and K.B. Sokolova 973, DIS 50:124.

Golubovsky, M.D. Institute of Cytology and Genetics, Novosibirsk 90, USSR.

Unstable allele of singed locus and its derivatives mutating according to the rule "all or none"; investigation during 20 successive generations.

Mutant sn77-27 was isolated by the Muller-5 test from the X-chromosome of Tashkent population in 1973 when an outburst of mutability in the sn locus was discovered[1,2]. It mutated to the normal condition and the normal derivatives were also unstable. The mutant remained unstable after one year of maintenance in mass cultures without selection. Then I investigated its instability character in detail for 20 successive generations. In all cases the males were individually crossed with $\overline{XX}$/Y unrelated females. A small fragment of pedigree for lines 11 and 10 is shown in Figure 1. It is possible to see a continuous row of mutational transitions in line 11: sn → + → sn and so on. Both mutant and normal derivatives of line 11 were quite unstable. The percent of gonadal reversions from sn^{11} to sn^{+11} was about 20% and reversions from sn^{+11} to sn^{11} occurred in 1% of gametes. Another situation was in line 10. Here normal phenotypic conditions were quite unstable but their mutant derivatives were relatively stable. The difference in the instability character between the two pairs of alleles and the rate of direct and back mutations are schematically presented in Figure 2. The complete data are given in Table 1. The main conclusions are as follows:



← Figure 1. The small fragment of pedigree tree showing the mode of instability inheritance during 20 successive generations in two different lines N11 and N10 originated from the unstable mutant sn77-27. The results of individual crosses of 1 ♂ sn x ♀ $\overline{XX}$/Y or 1 ♂ "+" x ♀ $\overline{XX}$/Y are given above the squares.

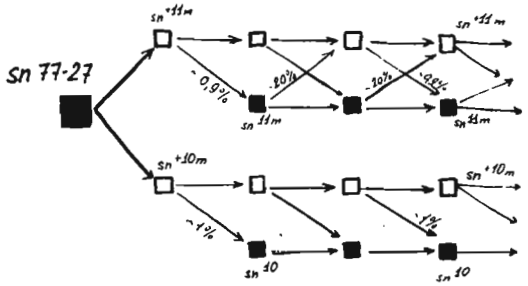


Figure 2. The different character of instability of two derivatives of originally unstable allele sn77-27. Black squares - mutant phenotype; white squares - phenotypically normal condition. The rates of mutation are given above the arrows.

1) The sn77-27 and its derivatives, as mutant as normal in phenotype, are unstable in both germinal and somatic cells. The mutations in somatic cells give origin to the mosaics of two types: mos(sn) - when most of the bristles are singed, but some are normal and mos(+) - when most of the bristles are normal but some are mutant.

2) In all cases direct and back mutations occurred according to the "all or none" rule: from the strongly mutant phenotype of sn77-27 (twisted, gnarled, diminished bristles, curved hairs, female sterile) to the absolutely normal phenotypic condition without any intermediate expressions (for instance, hooked at ends or waved bristles).

Table 1. The phenotype of progeny of mutant, normal or mosaic flies - carriers of unstable signed alleles.

Phenotype of males in cross ♀♀ $\overline{XX}$/Y x	Phenotype of progeny					Direction and frequency of mutations (%)
	sn	mos(sn)	+	mos(+)	Total	
	Alleles sn^{11m} and sn^{+11m}					
x ♂ sn	8833	84	2409	46	11372	sn → + 21.6
x ♂ mos(sn)	472	16	133	25	646	sn → + 24.3
x ♂ "+"	31	0	3352	58	3441	+ → sn 0.9
	Alleles sn^{10} and sn^{+10m}					
x ♂ sn	2385	0	0	1	2386	sn → + 0.0
x ♂ "+"	91	0	6936	220	7247	"+" → sn 1.2
x ♂ mos(+)	5	0	370	17	392	"+" → sn 1.3

3) The character of mutational transitions is "pair allelic specific". It means that some pairs of alleles mainly mutate in to each other. We isolated such pairs:

$$sn^{+11m} \rightleftarrows sn^{11m}; \quad sn^{+10m} \rightarrow sn^{10}; \quad sn^{+SR2} \rightleftarrows sn^{SR1}; \quad sn^{+SR2} \rightleftarrows sn^{SR2}$$

(here the letter "m" means "mutable", letters "SR2" - "strongly reversible").

4) The percentage of gonadal reverses in the progeny of somatic mosaic and non-mosaic flies is similar; mutational switch from mutant to normal condition takes place at early stages of development.

5) The frequency of reversions of mutable sn alleles in males is twice as high as in females (in one X-chromosome). So the peculiar process of dosage compensation takes place as Demerec first found for a similar situation in D. virilis[3].

References: [1]Berg, R.L. 1974, DIS 51:100; [2]Ivanov, Yu.N. 1974, DIS 51:71; [3]Demerec, M. 1932, Proc. Nat. Acad. Sci. USA 18:656.

Korochkina, L.S. and M.D. Golubovsky. Institute of Cytology and Genetics, Novosibirsk, USSR. Cytogenetic analysis of induced mutations on the left end of the second chromosome of D. melanogaster.

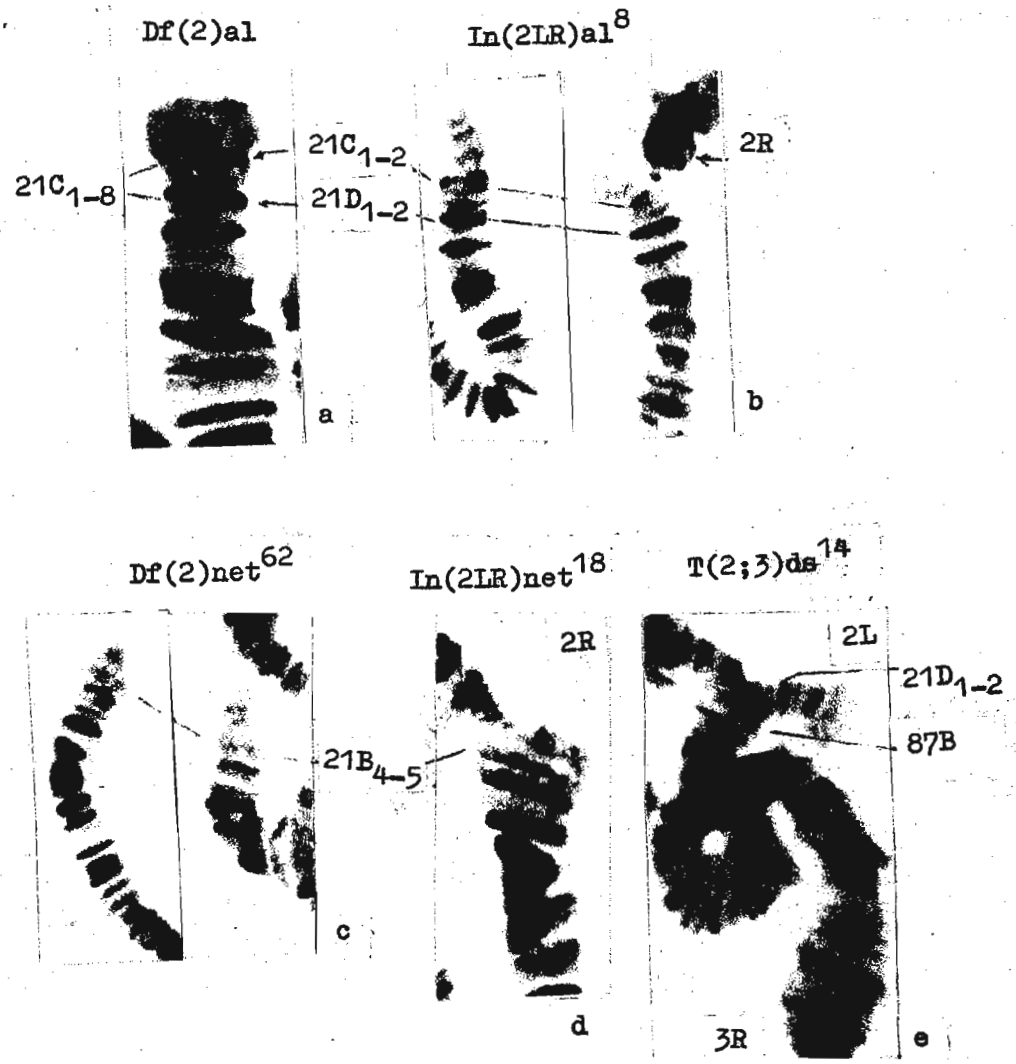
The extreme left end of the second chromosome in D. melanogaster has a special interest: 1) here are located genes net and l(2)gl, widespread in natural populations; 2) the mutations of l(2)gl locus are connected to neoplastic growth of imaginal discs and a malignant neuroblastoma in the presumptive adult optic centers of the larval brain[1]; and 3) the block of highly repeated DNA sequences is located in the region of 21C-D[2]. So we decided to study this region carefully.

Normal males were irradiated and crossed with females of the marker strain net al ex ds S/SM1 in order to obtain mutations and deficiencies in the left end. In F_1 flies having the phenotype of at least one of the characters net, al, ex, ds were selected and analyzed genetically. Those cases were picked out when the irradiated chromosome involved one of the mentioned genes and was lethal in the homozygote. After that it was checked as to whether the lethality was related to the l(2)gl gene. The chromosomes in which deficiencies could be expected on the basis of genetical data were analyzed cytologically. Only one deficiency is known in this region, Df(2)al, which involves the genes al, ex, ds, but not l(2)gl and net. It was found by Lewis in 1945[3]. The data obtained by us in the region 21AD are presented in Table 1 and Figure 1. The deficiency obtained by Lewis involves the discs 21C1-8 (Figure 1a).

Table 1. Comparison of genetical data and cytological analysis of some mutations in the extreme left end of chromosome 2.

Strain	Phenotype of heterozygote with markers net al ex ds	Phenotype of homozygote for this strain	Conclusions from genetic data	Conclusions from cytological analysis
Df(2)al	"al ex ds" and Minute	lethal	deficient for genes al ex ds but not for net and 1(2)gl	Df(2L)21C1-8
8	"al"	lethal	heterozygous 8/Df(2)al are poorly viable (1%) and have a phenotype: reduced aristae, very broad thorax, arched wings, incomplete veins, eye size increased	$In(2LR)al^{8}$ cytology: In(2LR)21C1-2;41C new order: 21-21B/41C-21C1/41D-60
62	"net"	lethal	deficient for genes net and 1(2)gl but not for al	$Df(2)net^{62}$ deficient for 21A-B3
18	"net" and Minute	lethal	deficient for genes net and 1(2)gl but not for al	$In(2LR)net^{18}$ cytology: In(2LR)21B3;42C new order: 21-21B3/42C-21B4-5/42D1-60
81A;12	"ds" and weak "al"	extreme expression of three genes: al, ex, ds poorly viable (1-3%)	heterozygotes on 81A/+, 12/1(2)gl absolutely normal; heterozygotes 81A/$Df(2)net^{62}$ and 81A/In $(2LR)al^{8}$ are also normal	Region 21A-D is normal
14	"ds" and weak "al"	lethal	In heterozygotes 81A/14, 12/14 is extreme expression of three closely linked genes: "al, ex, ds", poor viability	$T(2;3)ds^{14}$ cytology: T(2;3)21D3;50B1-2;87B new order: 21-21D/87B-80;60-50B1-2/21D3-50A/87B-100

Figure 1



The description of some mutations obtained by us follows:

1) In(2LR)al^{8}. The irradiated chromosome 8 in heterozygote with net, al, ex, ds had an "al" phenotype and the 8/8 homozygotes were lethal. In cytological preparations of 8/+ heterozygote one can see that the breakage goes through the disc 21C1-2 and that the region 21AB is attached to 41C (Figure 1b). Maybe this inversion is similar to In(2LR)al^{V}, detected by Lewis, which had aristaless-variegated effect suppressed by an extra Y chromosome (in the presence of the extra Y al^{V}/al was wild type). It turned out that a small percentage of Df(2)al/In(2LR)al^{8} heterozygotes survive and have an abnormal phenotype (Table 1). Cytologically these two rearrangements can overlap only in the region 21B8-C1 where gene al must be located. The semilethal effect and abnormal phenotype of the heterozygote may point to the existence of other vital genes in the region 21B8-C1.

2) The chromosomes 62 and 18 genetically are deficient for net and 1(2)gl genes. In heterozygotes 62/+ it is possible to see the absence of 21A-B4 region, so chromosome 62 maybe has a terminal deficiency, Df(2)net^{62}. The chromosome 18 cytologically is an inversion, with breakage points 21B3 and 42C, the small terminal region 21A-B3 may be lost (Figure 1c and 1d). The designation of the last rearrangement is In(2LR)net^{18}. The cytogenetic data on chromosomes 18 and 62 show that genes net and 1(2)gl are located to the left of the discs 21B4-5.

3) Of special interest was also the analysis of the chromosomes 81, 12 and 14 which show

a "ds" and weak "al" effect in heterozygotes 81/net al ex ds, 12/net al ex ds, 14/net al ex ds but homozygotes 81/81, 12/12 and compounds 12/81, 14/81, 12/14 are poor viable and have a strong mutant phenotype on three closely linked genes al, ex, ds. The existence of some regulator locus was suggested for explanation of this effect[4]. No special cytological abnormalties were found in phenotypically normal 81/+ and 12/+ heterozygotes. The strain 14 contains the translocation T(2,3), with one of the breakages in the 21D region (Figure 1e). So the suggested regulator gene which is mutant in the chromosomes 81, 12 and 14, seems to be located in the region 21D.

Thus the data of analysis: a) delineate the boundaries of cytological localization of the gene net and 1(2)gl by 21A-B4-5 region; b) localize the gene al in the region 21B8-C1; c) show that in the region 21B8-C1 there are some other vital genes; d) suggest the existence in the region 21D of the regulator locus which influences the expression of three closely linked genes al, ex, ds.

The influence of heterozygous rearrangement on the expression of the puff in the region 21AB was also studied. In the case of T(2,3) translocation, when 2L end is attached to 3R in the region 87B, the expression of 21AB puff is considerably increased in comparison with the normal condition.

References: [1]Gateff, E. and H.A. Schneiderman 1969, Nat. Cancer Inst. Monogr. 31:365-397; [2]Peacock, W.J., D. Bretlag, E. Geldring, R. Appels, C.W. Hinton and D.L. Lindsley 1973, Cold Spring Harb. Symp. Quant. Biol. 38:405-416; [3]Lewis, E.B. 1945, Genetics 30:137-166; [4]Golubovsky, M.D. and L.A. Kulakov 1976, DIS (this issue DIS).

Voelker, R.A., C.H. Langley, A.J. Leigh-Brown and S. Ohnishi. NIEHS, Research Triangle Park, North Carolina.

New data on allozyme loci in D. m.

The following table presents data on allozyme loci in D. melanogaster. For most of the loci, no previous information exists. For others our findings modify previously reported map locations or add information on the cytogenetic locations. The cytogenetic breakpoints and nomenclature are those of Lindsley and Grell (1968) or the listing of "Stocks obtained from George Lefevre and now maintained at the Caltech Stock Center;" no attempt was made to verify the previously reported breakpoints of the deficiencies and duplication. Further details will be published elsewhere.

Enzyme	Symbol	Genetic locus	Cytogenetic localization	Included in:
Hexokinase A,B	Hex-A,B	1-29.2	to the left of 9B1	?
Glutamate pyruvate transaminase	Gpt	1-42.6	11F1-2;12A1-2	Df(1)C246 but not Df(1)N12, ras v nor Df(1)JA26
NAD-Malate dehydrogenase (cytoplasmic)	Mdh-1	2-37.2	31B;31F	Df(2L)J-der-27
Dipeptidase A	Dip-A	2-55.2	41A-B;42A2-3	$Df(2R)bw^{VDe2L}Cy^{R}$ $Df(2R)M-S2^{4}$
Phosphoglucose isomerase	Pgi	2-58.7	?	?
Adenylate kinase C	Ak-C	2-ca. 100	?	?
NADP-Malate dehydrogenase (malic enzyme)	Me	3-ca. 51.7	87C(left of Xdh)	$Df(3R)kar^{31}$
Aldehyde oxidase	Ao	3-56.7	88F9-89A1;89B4-5	$Df(3R)sbd^{105}$
NAD-Malate dehydrogenase (mitochondrial)	Mdh-2	3-62.8	90C2-D1;91A2-3	Df(3R)P14
Aldolase	Ald	3R	?	?
Triose phosphate isomerase	Tpi	3-101.3	99B-E	Dp(3R)L127

Triantaphyllidis, C.D. and R.H. Richardson. Aristotelian University of Thessaloniki, Greece, and The University of Texas at Austin, Texas. Acid phosphatases isozyme patterns of twenty-five species from Drosophila montium subgroup.

Triantaphyllidis and Kastritsis (1976) observed that in D. auraria, a species which belongs to the Drosophila montium subgroup, there are three Acph bands in homozygous and five Acph bands in heterozygous individuals. Because this species has a peculiar Acph pattern, we decided to investigate the electrophoretic pattern of Acph isozymes from several species of the montium subgroup. For this investigation we used 25 species that were collected in nature and have been maintained in the laboratory of the Genetics Foundation at the University of Texas at Austin. The names of the species used are listed in Figure 1. D. melanogaster (Oregon R) was used as reference material.

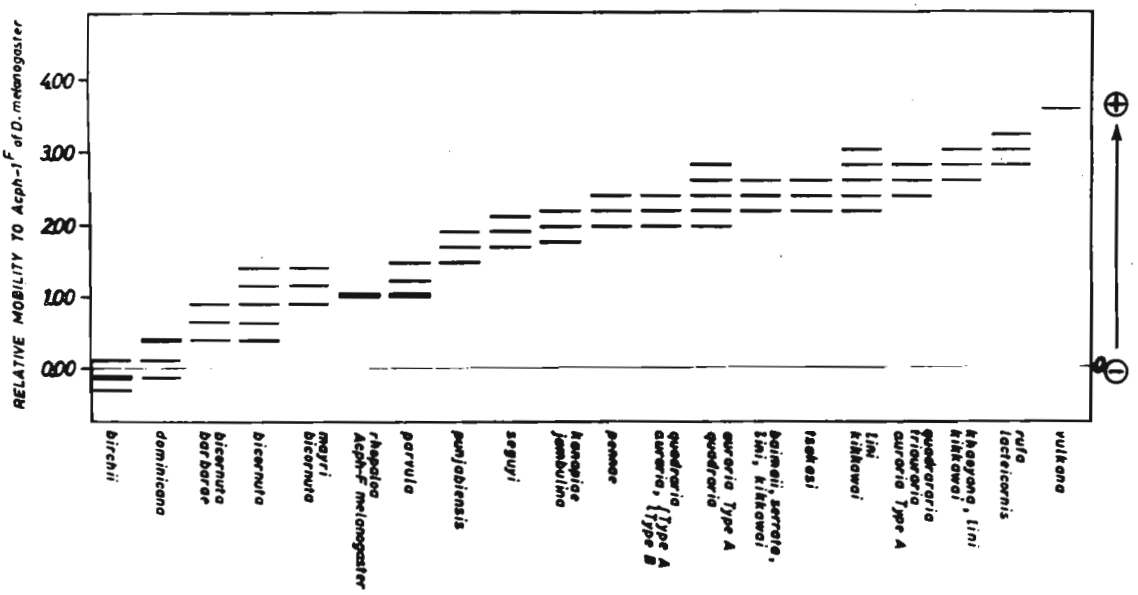


Figure 1. Schematic representation of the relative positions of acid phosphatases in 25 species of the montium subgroup and the $Acph^F$ band of D. melanogaster. The lines represent only the position of each Acph band and not their activity variation, except D. birchii, D. dominicana, D. parvula and $Acph^F$ of D. melanogaster.

A diagram of the observed Acph isozyme patterns for each species is shown in Figure 1. In D. birchii two Acph bands while in D. bicornuta one Acph band from the three migrate toward the cathode, while in the other species, all the Acph bands migrate toward the anode. Two of the studied species have only one Acph band (i.e., D. rhopaloa and D. vulkana). It is interesting that the Acph band of D. rhopaloa has the same mobility with the $Acph^F$ band of D. melanogaster. Eighteen of the studied species have only three Acph zones, while five (i.e., D. bicornuta, D. auraria Type A, D. quadraria, D. kikkawai and D. lini) have three or five Acph bands. In some species, the relative amount of Acph activity in adult individuals is greater in the intermediate zone (i.e., D. birchii); in other species (i.e., D. parvula) it is the slowest migrating band, and even in some other species (i.e., D. dominicana) it is the most anodal band. Although the studied species have different patterns of Acph, they share one or more bands with one or more species of the same species group.

D. auraria Type A and B, D. triauraria and D. quadraria are very closely related species (Bock and Wheeler, 1972) and have similar Acph patterns. Also, the very relative species of D. kikkawai and D. lini share five Acph bands. D. pennae share two Acph bands with the previous two species, while D. barbarae do not share any Acph bands with the relative species D. kikkawai and D. pennae.

D. birchii, D. dominicana and D. serrata are sibling species (Ayala, 1965). The two preceding species share two Acph bands, while there is no common Acph band with D. serrata.

The fact that 23 of the 25 studied species have three or five Acph bands supports the

hypothesis (Triantaphyllidis and Kastritsis, 1976) that the Acphs of the montium subgroup are polymeric consisting of at least two subunits.

Research supported by NSF Grant G B 2270 to Dr. R. Richardson.

References: Ayala, F.J. 1965, Evol. 19:538-545; Bock, I.R. and M.R. Wheeler 1972, In: Studies in Genetics Vol VII. Univ. of Texas Publ. No 7213:1-102; Triantaphyllidis, C.D. and C.D. Kastritsis 1976, Experientia 32:1277-1278.

Diamantopoulou, E. and S. Tsakas.
Agricultural College of Athens, Greece.

Non-active (Null) allele of the Xanthine dehydrogenase (XDH) locus in D. subobscura and some activity properties.

One isogenic strain for O chromosome with OST/OST structure from a natural population of Crete (Greece), has been found to be homozygous for non-active allele of the locus X.D.H. The same strain was also homozygous for non-active allele for aldehyde oxidase (A.O.) locus.

The electrophoretic phenotype of the strain appeared as a very faint zone at the same position with the zone of the common allele $XDH^{1.00}$.

The strain has eye colour a little bit lighter than the wild type. This is considered as a positive indication that flies homozygous for the non-active allele of XDH in D. subobscura can be "rosy" as it happens at the correspondent situation in D. melanogaster (Glassman and Mitchell, 1959, Genetics 44:153).

We did not find any kind of activation of the null isozyme (Duke, Rusing and Glassman, 1975, Biochem. Genetics 13:53) in vivo (food) or in vitro (heads extract) by using the same molybdenum concentrations with them. On the contrary, activation appeared for active alleles in both cases. The difference in eye colour among the two strains becomes more clear after molybdenum treatment.

Benedik, J.K., J. Poruba and M. Svobodová.
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The estimation of fitness components of lethal heterozygotes in D. melanogaster.

The nature of all processes acting on the change of frequencies are biological factors that we can cluster under the term fitness. On the selection as the main process affecting the frequency of the mutation in the population, these biological factors act complexly, but their shares can be different. Until this time, all studies dealing with this problem studied the single components of fitness separately. Such approach towards the study of this problem has not provided us with knowledge of the importance of single components of fitness. To make it possible, a model was done (Benedík and Franek, in press) in which the frequency of lethal heterozygotes in generation n+1 is a function of genotypic frequency of lethal heterozygotes in generation n and that of five components of fitness. In this model, we suppose such components of fitness:

- m - defined as relative mating ability of Aa males, that of AA being 1;
- f - defined as relative fecundity of Aa females, that of AA being 1;
- s - defined as relative competitive ability of a sperms, that of A being 1;
- w - defined as relative ability of fertilization of a eggs, that of A being 1, and
- v - defined as relative zygotic viability of Aa individuals, that of AA being 1 and aa being lethal.

Solution of such a model is possible only with help of a computer. In our case, we utilize the minimalizing method Letagrop (Sillen, 1968) adjusted to the solution of population-genetics problems.

In the experiment we took three different lethals in the second chromosome of D. melanogaster. We started with the genotypic frequency of lethal heterozygotes equaling 1 and in the 1st, 2nd, 3rd, 4th and 7th generations we tested the changes of frequency of lethal heterozygotes by the modification of the Cy method. The estimated values of studied parameters are in the Table.

Components of fitness	lethals lp 97	lp 99	lp 115
f	1.8630	1.1000	1.4990
m	1.8654	1.0999	1.1999
v	1.1406	.9526	.9026
s	.3157	.6821	.7702
w	.3101	.9000	1.0000

From the table we can see that the highest value for all three lethals showed parameters f and m (always higher than 1) and the lowest value parameter s. It means that factor s (relative competitive ability of lethal sperm) tends to the elimination of

these lethals from the population, while factors f and m (fecundity of heterozygous females and mating ability of heterozygous males) act to the persistency of lethals. Relative fast decrease of heterozygote frequency during initial generations witness on the superiority of factors tending to the elimination of these lethals.

References: Sillen, L.G. 1968, Pure Appl. Chem. 17:57-78; Benedík, J.K. and J. Franek (in press).

Racine, R., T. Beck and F.E. Würgler.
Swiss Federal Institute of Technology, Zürich, Switzerland. Heterogeneity of a larval population of Drosophila melanogaster with respect to MMS sensitivity.

In connection with studies on the genetic control of mutagen sensitivity we determined the MMS sensitivity of larvae of a balancer stock. This stock contains an X chromosome from a Hikone-R stock and balancer autosomes: + ; In(2LR)SM5, al^2Cy $lt^v cn^2 sp^2$/ In(2LR)bw^{VI}; In(3LR)Ubx^{130}/ In(3R)Sb; spa^{pol}. Eggs were collected from 1.5 day old flies during 4 hours using the egg collection units described by Büchi and Bürki (1975). The eggs were kept in a large petri dish in a humid environment until the larvae hatched. A first group of larvae was collected 16-22 hours after the end of the egg collection period. These larvae - which had a comparatively fast embryonic development - were counted and transfered into treatment tubes (Mollet and Weilenmann, 1977). We used about 150 larvae per tube. A second group of larvae was collected 22-42 hours after the end of the egg collection period. From every group of larvae one half of the tubes were treated for 6 hours with a 5.88×10^{-3}M aqueous MMS solution (Mollet and Weilenmann, 1977). The other half of the tubes were kept as controls. Treatment started immediately after transfering the larvae into the tubes. At the end of the treatment the larvae were carefully washed and cultivated further with living yeast and standard corn meal medium. Finally the number of surviving adults was determined for each tube.

	lethality in the first group	lethality in the second group
control	213 / 386 = 55%	484 / 572 = 85%
MMS-treated	317 / 384 = 83%	678 / 691 = 98%
(induced rate)	(61%)	(88%)

The data of the table show: (i) In the nontreated controls the spontaneous lethality among the late hatching larvae is higher, and (ii) the MMS induced lethality is higher in the late hatching larvae than in the early hatching ones. The different spontaneous lethality in the two groups might result from some of the postembryonic lethal karyotypes (e.g. those homozygous for Cy) which have a prolonged embryonic development. They hatch late and therefore contribute markedly to the postembryonic lethality in the late hatching group. This explanation does not hold for the increased MMS-induced lethality in the late group. By applying Abbott's correction the MMS-induced lethality in only those individuals which would survive in the control is determined. All these individuals have the same genotype (+ ; SM5/bw^{VI}; Ubx/Sb ; spa^{pol}). In our experiment the MMS treatment of the first group of larvae started when the average age of the larvae was 3 ± 3 h. The second group was treated at an average age of 10 ± 10 h. Based on the observations of Graf and Würgler (1975) it is expected that the older larvae are less sensitive than the younger ones. But in contrast to this expectation we found an increased sensitivity. This result indicates that individuals having a prolonged embryonic development exhibit an increased MMS sensitivity.

We suggest that on a fraction of the balancer chromosomes "subvital" factors may have accumulated. Such factors might, by some pleiotropic effect, be responsible for the slower rate of development as well as for the increased MMS sensitivity. With respect to the studies on genetic control of mutagen sensitivity one has to consider such a correlation between the rate of development and the mutagen sensitivity. This will be a factor to be studied in cases where an unexpected heterogeneity of the response of larval populations to a mutagen treatment is observed.

References: Büchi, R. and K. Bürki 1975, Archiv Genetik 48:59-67; Graf, U. and F.E. Würgler 1975, Archiv Genetik 48:112-115; Mollet, P. and W. Weilenmann, DIS, In press.

Acknowledgement: Work supported by Swiss National Foundation for Scientific Research, grant 3.3620.74.

Spofford, J.B. and R. DeSalle. University of Chicago, Illinois. In(1)w^{m4} is deficient for heterochromatin distal to NOR.

In examining the recombinant inversion chromosomes In(1)$w^{m4}L_{w}{}^{m51bR}$ and In(1)$w^{m51b}L_{w}{}^{m4R}$, we noticed that the w^{m51b} heterochromatic element was larger than that of the w^{m4} in both chromosomes. The recombinant chromosomes bore markers derived from the In(1)w^{m4}, y cv m f parent chromosome. The heterochromatic break point of w^{m4} is to the left of the nucleolus organizer (Cooper 1959) and that of w^{m51b} is cited as being to the right of the nucleolus organizer (Lindsley and Grell 1968). Neither has been stated to have an unusual amount of heterochromatin; Cooper drew without comment a w^{m4} chromosome with an inverted heterochromatin segment similar to sc^{4} in length but with a proximal segment similar in size to that of rst^{3}.

We measured the lengths of the X heterochromatic segments, the euchromatin, and the 4th chromosomes on enlarged micrographs of neuroblast cells from six or more individuals of each class. The chromosomes were in various stages of colchine-metaphase or pro-metaphase, prepared by a modification of the Holmquist (1975) and Gatti et al. (1974) methods. Ganglia were cultured 2 hours in 10^{-4}M colchine in .7% saline, then transferred to 1% sodium citrate for 10 minutes before fixing 3 minutes in 1:1 methanol: glacial acetic acid. They were then transferred to a drop of 60% acetic acid on a slide prewarmed to 38°C. After 30 seconds, the drop was rolled spirally across the slide, leaving a spiral trail of cells. The slide was then air dried before aceto-orcein staining.

With this procedure, the nucleolus is not reliably detectable as a secondary constriction. However, the distal end of the inverted X is distinguishable by the presence of the splayed euchromatic chromatid tips. Only cells in which the chromatids were separated in euchromatic but not heterochromatic regions were used. Since only linear measurements were taken, with no attempt to estimate chromosome volumes or relative DNA amounts, the accompanying tabulation gives only a first approximation to the chromosome differences. However, there were no appreciable systematic differences in the relative thicknesses of the heterochromatic regions of the various chromosome types. Lengths are given as multiples of the average length of chromosome 4 in the same cell, mean ± standard error of the mean.

	heterochromatin lengths						
X chromosome	w^{m51bL}	w^{m51bR}	w^{4L}	w^{4R}	total XH	XE	XH/XE
w^{m4}(unmarked)	-	-	.67 ± .05	1.58 ± .16	2.25	2.30 ± .06	.98
w^{m4}, y cv m f	-	-	.73 ± .07	1.54 ± .12	2.27	2.17 ± .22	1.05
$w^{m4}L_{w}{}^{m51bR}$, y	-	1.23 ± .06	.77 ± .06	-	2.00	2.63 ± .15	.76
$w^{m51bL}{}_{w}{}^{m4R}$	2.36 ± .11	-	-	1.76 ± .11	4.12	2.92 ± .09	1.41
w^{m51b}	2.38 ± .11	1.23 ± .05	-	-	3.60	2.57 ± .06	1.40
Oregon-R	-	-	-	-	3.58 ± .10	2.63 ± .33	1.36

Some cell-to-cell variation in chromosome condensation is inadequately randomized between chromosome types, so that one should not overdraw conclusions. The w^{m51b} was probably the result of X-raying Oregon-R chromosomes and retains essentially the same amount of heterochromatin. The wild ancestor of the w^{m4} chromosome may not be easy to ascertain (Muller 1930). It may in fact have had less heterochromatin than Oregon-R before the inversion was induced. At least, differences in numbers of rDNA cistrons from different wild stocks have been noted (Spear and Gall 1973). However, it is also possible that a deficiency for some of the hC region was induced simultaneously with the inversion, or has since appeared. At least, two contemporary stocks of w^{m4}, as indicated in the table, agree in heterochromatin amount.

Because of its relative deficit of hC, we urge caution in the interpretation of experiments with w^{m4}. (Work supported by NIH Grant GM 22040)

References: Cooper, K. 1959, Chromosoma 10:535-588; Gatti, M., et al. 1974, Genetics 77:701-719; Holmquist, G. 1975, Chromosoma 49:333-356; Lindsley, D. and E. Grell 1968; Muller, H.J. 1930, Jour. Gen. 22:299-334; Spear, B. and J. Gall 1973, PNAS 70:1359-1363.

Novitski, E. and J. Puro. University of Oregon, Eugene, Oregon, and University of Turku, Finland. The genetics and cytology of some new chromosome types.

1. The ring XY chromosome. The synthesis of this chromosome has been described in Volume I of Ashburner and Novitski, the photograph presented here (Figure 1) is somewhat more detailed than the one printed in that book. Like other rings containing Y material, in certain genetic backgrounds this chromosome results in the death of almost all progeny carrying it (filicidal) and its successful use may depend upon a non-filicidal genetic background. It is not obviously unstable. The identification EN (entire) refers to the fact that neither X nor Y in the ring lack any essential viability or fertility genes.

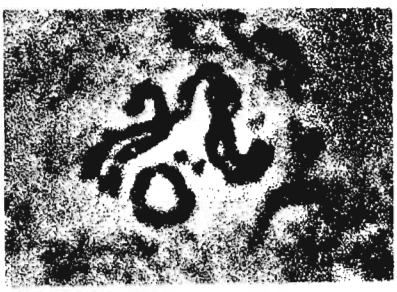


Figure 1

Figure 2

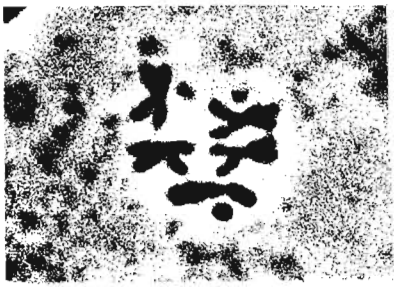


Figure 3

2. Derivatives of C(2)EN. Since males with normal chromosomes mated to C(2)EN females will yield no progeny (except for rare cases of loss or triploidy) it is a relatively simple matter to detach the compound by irradiating one sex carrying the compound and mating them to flies with normal chromosomes. A number of detachments have been found and are kept in stock. These chromosomes show a somewhat reduced transmission through the male, although not as extreme as the reduction found in the progeny of males carrying the complete compound. This chromosome is most readily identified in anaphase preparations where its unusual length is evident (Figure 2).

3. Small duplications for the centromere region of the second chromosome can be picked up by irradiating normal males and mating to females carrying C(2)EN. Such duplications can most easily be formed by the rejoining of segments which lie on either side of the centromere forming a small ring. One such duplication is Dp(2;F)Bl which was obtained after irradiation of Bristle-bearing males. It appears to be reasonably stable, although the appearance of occasional non-Bl scutellars suggests some somatic loss. This chromosome has been used in segregation studies with the fourth chromosome by Puro and Nokkala (Chromosoma 63). In metaphase preparations, the chromosome appears to be considerably larger than the fourth chromosomes and its dense roundish configuration strongly suggests a small compact ring, although the ring is too small to be seen as such in these preparations (Figure 3). In salivary gland preparations however (Figure 4) where this is present as a duplication, it can be seen that the basal region is triplicated with one segment joining the chromocenter. The left break appears to be in 38C, the right in the chromocenter presumably in 2R. The duplication does not cover pr.

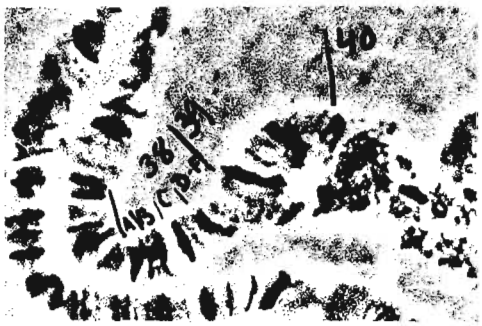


Figure 4

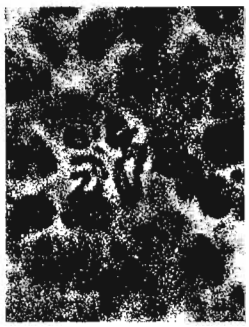


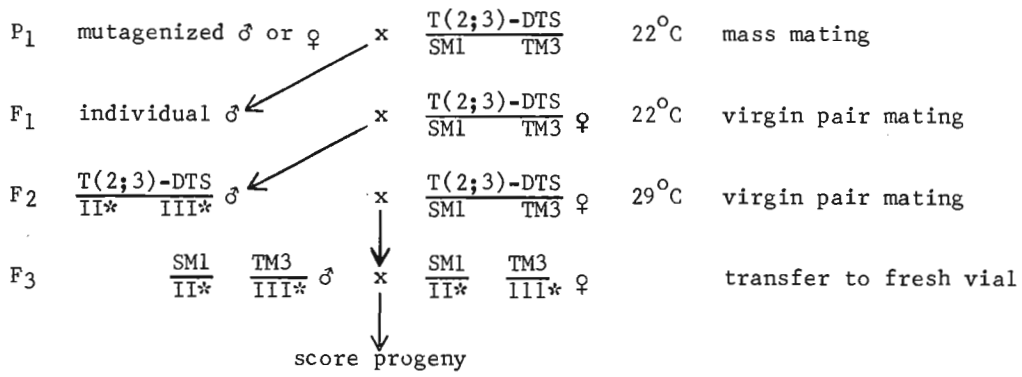
Figure 5

4. To make up a complete compound of the third chromosome which includes the entire essential complement of third chromosome genes (corresponding to C(2)EN for the second), it is essential to attach the two third chromosome arms in tandem. This has been accomplished in a chromosome which has the composition 3R 3L·3R. Although the entire compound (3R 3L·3L 3R) has not yet been put together, its manufacture should present no great problem. The long arm including both 3R and 3L has been identified in mitotic anaphases, where its length is similar to that of Figure 2. As one of the byproducts of this synthesis, three cases of ring formation involving all of 3L have been found (Figure 5).

Fryberg, E. and J.J. Donady. Wesleyan University, Middletown, Connecticut.

An improved screen for autosomal recessive lethals.

Screens for autosomal recessive lethal mutations have always been tedious, but by far the most laborious step is the collecting of virgin females, heterozygous for the putitive lethal, and their subsequent mating. This step usually occurs in the F_3 (assuming a mosaic elimination step has been incorporated into the screen). To avoid this time consuming step we have recently placed the dominant temperature sensitive mutation #4 (Suzuki and Procunier, 1969. PNAS 62:369) onto the second chromosome of the T(2;3)Pu^{Gr}. The translocation is maintained in a stock with the second and third chromosome balancers, SM1 and TM3. Using this stock the following scheme is possible:



Due to the restrictive temperature in the F_2 cross, the F_3 will contain only SM1/II*; TM3/III* individuals and therefore need only be transferred to a fresh vial to score for the presence of a lethal in the next generation. When using this scheme the option of screening the major autosomes either simultaneously or separately is available. Approximately 80% of the euchromatic genome is covered by the screen. The T(2;3)DTS stock is available upon request. Research supported by National Science Foundation grant GN43474 and National Institutes of Health grant HD08286.

ANNOUNCEMENTS I

European Drosophila Population Biology Group report from Dr. Bryan Shorrocks, University of Leeds, England: At a Drosophila Research Meeting held at Leeds in April, 1975, a cooperative European project was initiated.

Initially this project will involve the collection of data on the following:

1. Species distribution;
2. Reproductive condition throughout the year, so that the number of generations/year can be estimated for different species in different parts of their geographic range;
3. Emergence from breeding sites.

It is hoped that this will lead to a more systematic comparison by both ecologists and population geneticists of marginal and central populations of Drosophila.

In order to help solve the problem of organisation, six centres have been nominated, through which the projects can be coordinated. Information for a particular species or geographical region can be obtained by writing to the Leeds laboratory, to which all collection data should be sent. The six laboratories are:

Dr. D. Marinkovic
Institute for Biological Research
University of Belgrade
29, Novembra 142
Belgrade, YUGOSLAVIA

Professor S. Lakovaara
Department of Genetics
University of Oulu
SF 90100 Oulu 10, FINLAND

Dr. G. Bächli
Zoologisches Museum der Universität Zürich
8006 Zürich
Künstlergasse 16
SWITZERLAND

Professor A. Prevosti
Departamento de Genetica
Facultad de Ciencias
Universidad de Barcelona
Av. Jose Antonio 585
Barcelona 7, SPAIN

Professor L. Tsacas
Laboratoire de Biologie et Genetique Evolutives
Centre National de la Recherche Scientifique
91190 Gif-sur-Yvette, FRANCE

Dr. B. Shorrocks
Department of Pure and Applied Zoology
The University
Leeds LS2 9JT, ENGLAND

Species distribution: It would be most helpful if the details of each collection could be written out on separate data sheets, along with the relevant climatic and botanical data if available.

Bait: The initial meeting at Leeds decided that in addition to the existing baits used by various laboratories in Europe, it would be good to have a standard bait for making collections in the future. The malt bait used by Professor Lakovaara's group in Finland was chosen.

> Malt bait: In collecting drosophilids we have used traps, which have been processed in the following way: The bait is made by swelling rye or barley malt (broken grains) in hot water, after which the malt mush has been allowed to cool. The water-malt ratio in the mush has been 2:1 (1 litre water and 500 gms malt). After cooling baker's yeast suspended in water is added. A 5 cm layer of the bait is poured into wide two-litre plastic jars and the jars are then carefully closed. The baits are then allowed to ferment in closed jars at room temperature for about a week. During this time some of the alcohol formed as the result of fermentation was further fermented into acetic acid. Thereafter the opened jars are ready for trapping. A bait can be used in the Finnish climate for about 2-8 weeks. If the traps are open all that time and become dry, it is practical to add water.

Reproductive condition: This is most easily recorded by placing the flies into one of a number of predetermined classes. A set of standard classes (with descriptions) for a number of European species are available from the Leeds laboratory. For D. subobscura and D. obscura see M. Begon (1976) Oecologia 23.

Emergence from breeding sites: Once again it would be most convenient if the details could be written out on a standard sheet. Information on the various fungus-breeding species should be easily obtained.

However, all kinds of records, including negative ones, are needed. In the woodlands around Leeds we find that the major breeding site of D. subobscura is the fallen fruit of the Rowan tree, Sorbus aucuparia. It would therefore be interesting to examine these fruits in other parts of the species range, along with other fruits of the genus Sorbus.

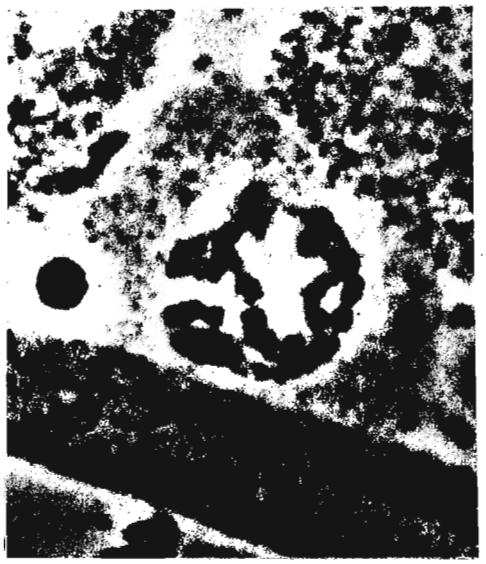
The initial response to this plea for more cooperation between European workers has been most encouraging. By the time this report appears in DIS 53 a second meeting of the group will have been held. The details of this meeting will be reported in the next issue of DIS.

Tsakas, S.C.* University of Edinburgh, Scotland. Simultaneous analysis of a single larva of D. melanogaster for inversions, mitotic chromosomes and enzymatic loci.

It is often useful in population studies of Drosophila species to analyse the same larva for inversions, mitotic chromosomes and enzymatic loci or any combination of these.

This is not possible for D. melanogaster with the present techniques. We can overcome this difficulty by modifying as follows the dissecting solution in the protocol described by Strickberger (1962): 1) 1 gr trichloroacetic acid; 2) 50 ml 5N hydrochloric acid; 3) 25 ml glacial acetic acid; 4) 25 ml 88% lactic acid; 5) 25 ml triton x 100 (Sigma).

This colourless dissecting solution permits the orceine of the staining solution to perfuse the material unhindered by fats (HCl), phosphorolipid membranes (triton x 100) and proteins (T.C.A.). The staining solution is composed of 2 g of synthetic orcein (Edward Gurr,



Ltd., 42 Upper Richmond Road, West London) dissolved into 50 ml hot glacial acetic acid, plus 50 ml of 85% lactic acid after removing from heat.

The dissection is done dry on a slide; the rear part is removed quickly for electrophoresis, a drop of dissecting solution is added to the front part and the salivary glands and cerebral ganglion after a fer seconds are spearated onto two slides with a drop of staining solution. After five minutes each slide is pressed as described by Strickberger.

The rear part of the larva is homogenised and two samples are taken for electrophoresis; the first one for the enzymatic loci A.D.H., a.Gl.D.H., T.O. and Est 6 and the second one for M.D.H., M.E., O.D.H. and A.O. This technique, up to now, has been successfully used to type single larvae for inversions, mitotic chromosomes and eight loci. The technique is illustrated by the accompanying two photographs which show salivary chromosomes of an heterozygous animal for reciprocal translocation between the 2L and 3R arms (Figure 1) and the characteristic ring of its mitotic chromosomes (Figure 2).

This technique has also been used with D. subobscura larvae.

Reference: Strickberger, M.W. (1962), Experiments in Genetics with Drosophila, John Wiley & Sons, Inc., New York - London.

*Permanent address: Department of Genetics, Agricultural College of Athens, Greece.

Dapkus, D. Winona State University, Winona, Minnesota. A convenient method for maintaining small continuous Drosophila populations.

Reed and Reed (1948) introduced the use of "population units" for the continuous maintenance of Drosophila populations. Each population unit consisted of two half pint milk bottles joined together. The bottles were changed alternately to keep the population going. Such units are more convenient, less costly and take up less space than the various types of population cages, and replications are easier to run.

The population size achieved in these units is on the order of a several hundred flies at the maximum. Large scale fluctuations in population size can be induced by lengthening the period between bottle changes.

Ludwin (1951) introduced a useful modification by joining the bottles by a piece of rubber radiator hose. Over the years Dr. D.J. Merrell has found these units convenient for a number of population studies including mutant competition, species competition, and DDT selection experiments.

However, changing the bottles is slow and difficult in that each bottle must be worked into the elastic radiator hose. Great care must be exercised to insure a tight seal between the hose and the bottle. The possibility of chips in the bottle edge or cracks in the radiator hose must be excluded. The population units must be carefully handled when they are moved because they are not locked together.

These difficulties have been overcome in a variation of the basic design that uses screw top plastic milk bottles. (Pacific Plastic Bottle Corp., 305 E. Home St., Rialto, California 92376.) These bottles can be quickly and easily changed by screwing them into the holder shown in Figures 1 and 2. The seal between the bottle and the bottle cap in the holder is very secure.

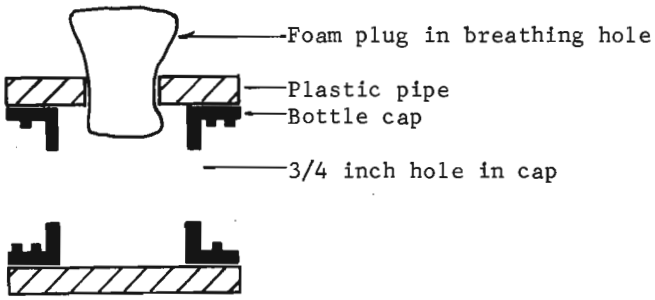


Figure 1
Cross section of a bottle connector

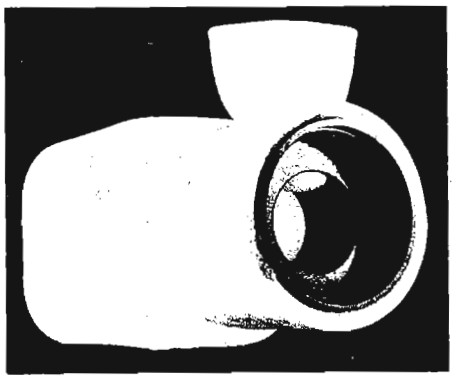


Figure 2
Population unit with one bottle removed

As the figure shows, the bottle holder is made up of a two inch section of 1 1/2 in. I.D. plastic pipe, into the ends of which two 38 mm bottle caps (Curtin Matheson Scientific, 2218 University Avenue S.E., Minneapolis, Minnesota 55414 - Size 38; Catalog #032-433) have been glued. A 3/4 in. hole drilled through the plastic pipe is plugged with a foam plug (Mogul-Ed, 1222 West South Park Avenue, Oshkosh, Wisconsin 54901 - Catalog #L0915). This hole is necessary as a breather and allows easy sampling of the population unit. Each bottle cap has to have its outer edge ground down slightly to fit the plastic pipe. A 3/4 in. hole is also drilled through each bottle cap with a hole saw. If epoxy glue is used to join the caps to the plastic pipe, the holder and the bottles are autoclavable.

All the caps should be oriented in the same direction when they are glued in place so that the media in the square bottles will be on the bottom. This can be done by screwing the caps onto a bottle before gluing them. Each bottle can then be oriented with the same side up (such as the "Half" of "One Half Pint"). Slants can then be prepared by pouring media into the bottles and tipping them over so that "Half" is up.

These population units should be useful for a variety of studies with Drosophila populations because of their ease of handling and the number of replicas that can be run.

References: Ludwin, I. 1951, Evol. 5:231-242; Reed, S.C. and E.W. Reed 1948, Evol. 2: 176-186.

Kiss, I. Institute of Genetics, Biological Research Centre, Szeged, Hungary.

A "split" vial for collecting larval-puparial and adult gynander mosaics of Drosophila melanogaster.

A study of non-pupariating and late-pupariating X-chromosome lethals made in our laboratory needed the collection of larval-puparial and adult gynanders at the same time, from the same cross (Kiss et al., 1976). A special "split" vial was devised for this purpose (Figure 1) consisting of two glass parts. The lower part was filled up with the medium to the edge and attached to the upper part by the aid of two rubber rings fixed onto the glass cams on both sides. The glass was ground on the surface of attachment.

The flies were left to lay eggs for 1-2 days and then removed from the vial. From the third day on after hatching, several drops of water were added daily onto the surface of the medium to keep it wet. Mature larvae left the wet food and crawled up to the wall of the upper part to form puparium. The upper parts of the vials were routinely changed each day for a new one. Puparia on the removed upper parts were checked for larval-puparial gynanders while larvae were put back into the vials. The upper parts were then plugged at both ends with cotton and kept in a humid atmosphere at 25°C. When the adults started to emerge they were collected once a day and checked for mosaicism. As we recovered adult gynanders with both the lethal and the balancer chromosome as well as larval-puparial gynanders from the same cross, it was possible to use the number of balancer mosaics as an internal standard and to eliminate, in this way, the variations caused by the different frequencies of ring-X loss in the different experiments (Bryant and Zornetzer, 1973).

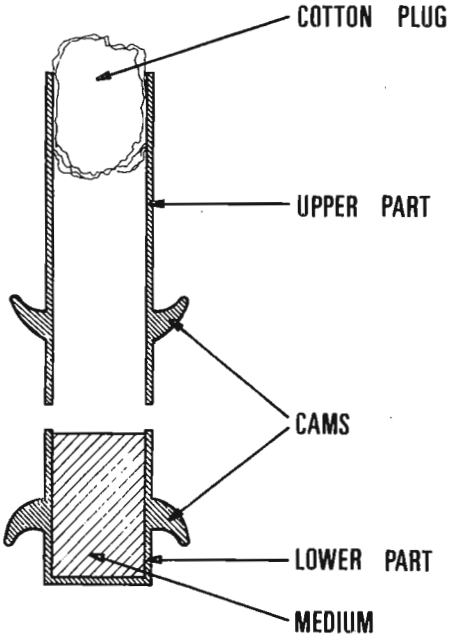


Figure 1. A "split" vial for collecting larval-puparial and adult gynander mosaics.

References: Kiss, I. et al., 1976, Nature 262:136-138; Bryant, P.J. and M. Zornetzer 1973, Genetics 75:623-637.

Mittler, S. Northern Illinois University, DeKalb, Illinois.

An agarless media for Drosophila.

With the rapidly rising cost of agar (approximately $11.00 per lb.) a search was made for an agar substitute. The National Starch and Chemical Corp., 3641 S. Washtenaw, Chicago, IL 60632, manufactures "Clear Jel" which is used to thicken food for humans. "Clear Jel Regular" requires boiling and sells for $7.10 for 25 lbs. Rolled oats is also added to help solidify the media.

1000 ml water
51 gm of "Clear Jel"
40 gm of yellow cornmeal
40 gm of brewers yeast
33 gm of rolled oats
40 gm of Brer Rabbit Molasses
40 ml of Karo Dark Corn Syrup
5 ml of propionic acid

The water is brought to boil, the ingredients are stirred in one at a time and brought to a boil. If cooked in an autoclave for 15 min. at 15 lbs. this media will assume the solidness of the regular agar cornmeal molasses media.

Tompkins, L.*, J.A. Fleischman and T.G. Sanders. Princeton University, Princeton, New Jersey. A new countercurrent device.

We have designed a countercurrent device, similar in principle to Benzer's (1967), for fractionating Drosophila populations. This apparatus, which has been used to isolate and analyze contact chemosensory mutants (Cardosa et al., 1974; Tompkins et al., ms. in preparation), could be used, with appropriate modification of the stimulus, to study behaviors such as chemotaxis with olfactory stimuli, oviposition, phototaxis, and geotaxis. The apparatus can be constructed quickly and cheaply from readily available materials by persons who are not machinists. An additional advantage is that at least 250 flies can be tested at one time, which facilitates use of the device for selecting mutants and for teaching demonstrations.

The apparatus, shown in Figure 1, is made from Styrofoam blocks, 2' x 6' x 1", cut from 2' x 8' x 1" sheets available at builders' supply companies. Holes are made in the Styrofoam with a number 15 cork borer. The blocks are supported on a base of scrap lumber by two rows of metal brackets, sold as tablecloth clips. If these are unavailable, strips of wood or any material which firmly supports the blocks in an upright position could be substituted.

To begin an experiment, standard glass Drosophila vials, 25 x 95 mm, are inserted into the holes so that their open ends touch, and an index card is placed between the starting vial and its mate. After flies have been introduced into the starting vial, the card is removed. At appropriate intervals, the device is manipulated essentially as described by Benzer (1967). At the end of the experiment, flies are conveniently anesthetized for scoring by inserting a syringe needle, connected via flexible tubing to a source of carbon dioxide, between the blocks at the interface between members of pairs of vials.

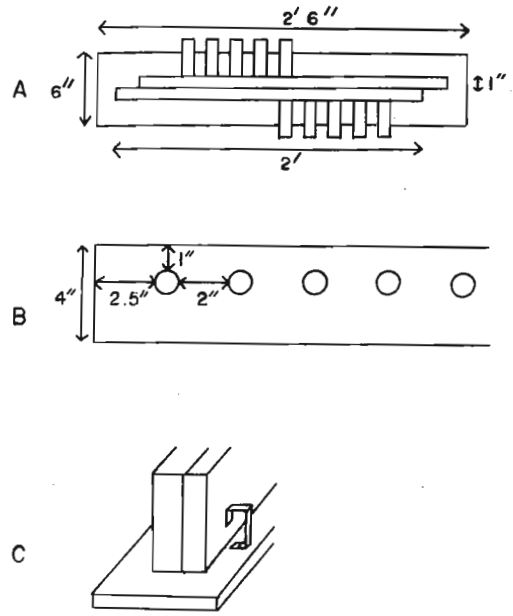


Figure 1. The countercurrent device.
A. Top view, with vials inserted
B. Side view of Styrofoam block without vials
C. End view, showing position of tablecloth clip

To obtain reproducible results, the vials must be clean and free from detergent residue, and the Styrofoam blocks should be examined for cracks, which can be repaired with tape, before use. In addition, males and females should be tested separately to avoid the distractions of courtship and mating.

References: Benzer, S. 1967, Proc. Nat. Acad. Sci. 58:1112; Cardosa, M.J., J.A. Fleischman and T.G. Sanders 1974, Genetics 77:s9.

* Present address: Biology Dept., Brandeis University, Waltham, Massachusetts 02154.

PERSONAL AND LABORATORY NEWS

Dr. E.S. Gersh, School of Veterinary Medicine, University of Pennsylvania, will discontinue maintenance of all Drosophila stocks after June 30, 1978. This will include all stocks listed in DIS 51:16 except nos. 3,4,5,6,11,17 & 18 which have already been discontinued.

Fogleman, J. Cornell University, Ithaca, New York. A thermal gradient bar for the study of Drosophila.

Thermal gradient bars of various designs have been used in experiments ranging from investigations of temperature optima for seed germination (Wagner, 1967) to studies of thermotaxis in nematodes (Hedgecock and Russell, 1975) and habitat selection in flour beetles (Langer and Young, 1976). I have taken the basic design of Wagner and modified it for use in studies on Drosophila. The gradient bar is a 3/4 inch thick sheet of aluminum with the dimensions as shown (see Figure 1). The gradient effect is produced by having one end sit on a very precisely controlled hot plate and the other end sit in a cold water reservoir. The water temperature in the reservoir is kept constant by the periodic action of a small immersion cooler. A timing mechanism attached to the cooler is set so that some ice is maintained in the reservoir. Ninety holes (1 inch diameter), drilled through the bar are just large enough to hold a standard shell vial snugly. Most of the aluminum is covered by styrofoam insulation which is one inch thick. This is done in such a manner as to create a box over the upper, horizontal surface. The water trough in the back of

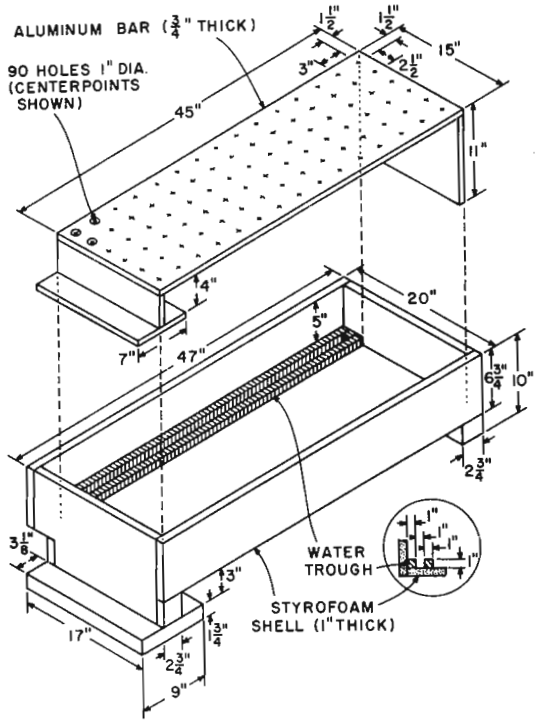


Figure 1. Thermal gradient bar.

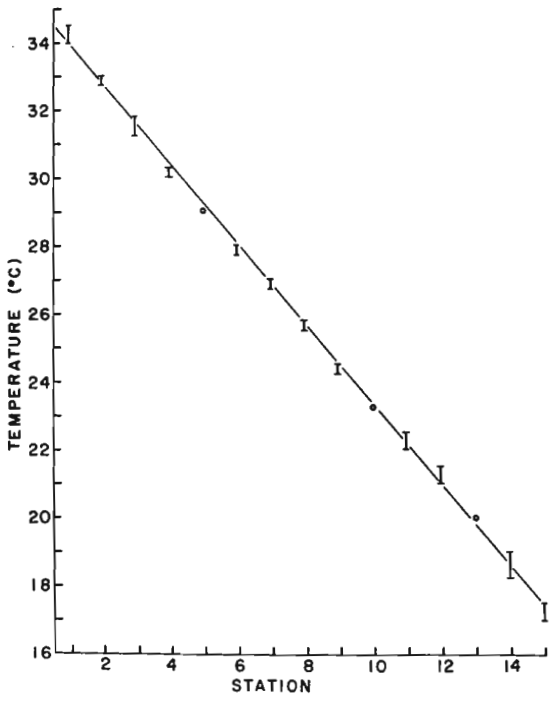


Figure 2. A typical temperature profile. Vertical bars indicate ranges.

the box and the plexi-glass cover (20" x 47" x 1/8") allow the maintenance of the relative humidity at 60% within the enclosed area. The upper, horizontal surface of the aluminum bar is covered by a 1/8 inch layer of rubber to reduce convective currents.

Food vials placed in the holes are held at a specific, constant temperature. There are 15 stations ranging from approximately 17°C to 34°C with 6 replicates of each temperature. The range, of course, is easily adjusted. Figure 2 is a typical temperature profile generated by measuring the temperature of the food in the vials on two occasions separated in time by three weeks. The line, then, is a least squares fit to a set of points, each of which is an average of twelve temperature measurements. The thermal gradient has been in continuous operation for a year and a half and has not deviated significantly from this profile. Variation between replicate vials expressed in terms of average standard deviation is generally less than 0.1°C. As can be seen from Figure 2, the measurements at three of the fifteen stations did not vary at all.

The stations of this device represent discrete points along a stable, linear temperature gradient. I have also designed an accessory which makes use of the continuous nature of the gradient effect. Collapsible, plexiglass boxes (internal dimensions, 1" x 1" x 40", see Figure 3) containing a standardized thickness of food may be placed directly on top of the aluminum bar between the rows of vials. The food itself then, represents a continuous temperature gradient. The usefulness of these boxes, however, is restricted to short term experiments because the food at the warm end shrinks and dries up within a period of several days.

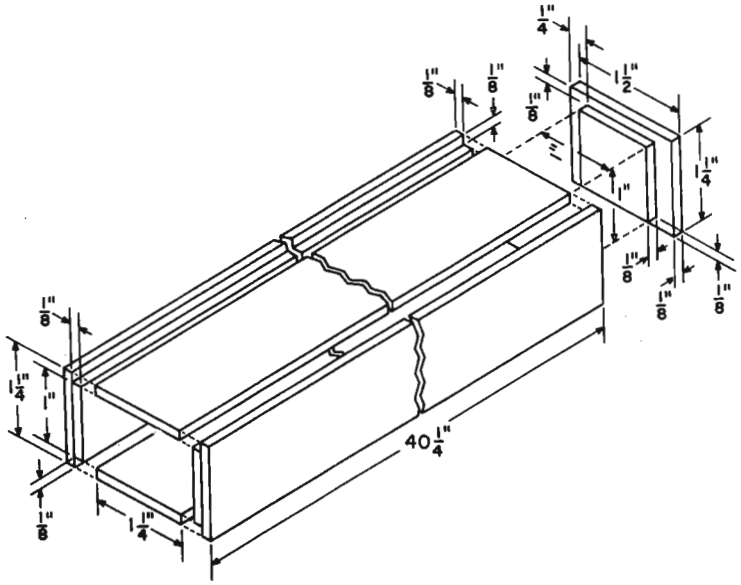


Figure 3. Collapsible food box for use on thermal gradient bar. The box is held together with masking tape.

This research was supported by Grant GM01035 from NIH.

References: Hedgecock, E.M. and R.L. Russell 1975, Proc. Natl. Acad. Sci. 72:4061-4065; Langer, G.S. and A.M. Young 1976, Am. Midl. Nat. 95:131-143; Wagner, R.A. 1967, Am. Midl. Nat. 77:86-92.

Williamson, J.H., L. Browder and A. Thompson. University of Calgary, Alberta, Canada. A method of collecting Drosophila embryos of known age.

Determination of when during early development activity of a particular enzyme can be detected requires rather precise aging of embryos and existing methods of embryo collection, usually based on the method of Mitchell and Mitchell (DIS 39:135-136, 1964), are simply inadequate. In our experience of using this method developmental stages varied from newly oviposited eggs to newly hatched larvae. The latter obviously resulted from extended retention of a fertilized egg in the uterus. When one is studying late larval or pupal enzymes and using gram quantities of organisms this variation may not be of major concern. However, when analyzing events during early embryogenesis and when using only 10-25 embryos per sample such variation is disastrous. We have found the following method very successful. Males and females of the desired genotypes are isolated within 12 hours of eclosion and aged 4-5 days on standard culture medium sprinkled with live dry yeast as a food source. The egg collection medium is 2% agar, 5% dextrose, 3% sucrose, 40% grape juice concentrate adjusted to pH 6 before autoclaving and is prepared the day before it is used. A layer approximately 1.5 cm deep is poured in plastic trays approximately 13 x 17 x 6.5 cm, allowed to cool and tightly covered to prevent drying. A hole 3 cm in diameter was cut into the tray cover to allow introduction of the adult flies without anaesthetiziation; the hole was plugged with a styrofoam plug. The flies and the egg collection medium were prewarmed at 25°C prior to introduction of the flies. Optimal egg collections were obtained when the flies were maintained in full illumination at 25°C for 12 hours before mating and in darkness during mating and oviposition. (We simply cover the tray with a black cloth). The adults were discarded after 3 hours in the tray and the tray returned to the 25°C incubator. The age of embryos (and larvae) were calculated from 2 hours after introduction of the flies into the egg collection tray. At routine intervals the required number of embryos were picked off the surface of the grape food medium with a needle without disturbing the other embryos. Use of this method produced a very homogeneous sample of embryos. At 4.5 hours more than 90% of the embryos were at gastrulation; the remainder were at pre-gastrulation stages.

Grace, D. and R. Sederoff. University of Oregon, Eugene, Oregon. Vial washing without brushing using alkali.

Cleaning shell vials containing old fly medium is an unpleasant task, usually relegated to lab assistants. Most labs autoclave old vials to soften the food and then wash out the food while it is still soft. In practice, food and pupae cases stick to the sides of the vials and bottles, so that each container must be scrubbed with a brush. Whether brushing is done by hand or with a motor driven brush, the cleaning of large numbers of vials or bottles is a slow and onerous task.

We found that a dilute solution of sodium hydroxide (0.1M), added to vials before autoclaving, dissolved the food so that it would not harden upon cooling (after autoclaving) and that no brushing was needed.

METHOD: Food vials (or bottles) with dead flies[1] are filled (2/3 full) with dilute NaOH and then autoclaved[2]. The food and residue dissolves and the pupae cases detach from the walls during heating. After autoclaving, the vials can be rinsed with a fast stream of water while still in the trays. An empty tray can be placed over the vials and the other rinses are done (without removing the vials) by dunking the whole tray in cold water five times. If rinsing is incomplete, a white insoluble but innocuous film will form on the glass surface. Many trays of vials (15) can be cleaned in 2 hours or less. The limiting factor is the size and timing of the autoclave.

Precautions should be followed in making up the concentrated stock solutions. A well-ventilated room or hood should be used and gloves and goggles worn[3]. The vials should not be cleaned in aluminum trays.

STOCK SOLUTION: 1) A cup of NaOH crystals (150 mls) is added dry to a one gallon glass jug which is filled with water without stirring or shaking. The jug should be left to stand overnight to allow the crystals to dissolve and the solution to cool.

2) One gallon of stock solution is diluted to 50 liters in a polyethylene carbuoy and dispensed into vials with a pipetting device, or by placing the carbuoy on a pedestal for gravity flow.

FOOTNOTES: 1. In our kitchen, flies are killed by keeping discarded trays in a cold room (4°C) until they are washed, or by autoclaving. 2. Autoclaving is not required for cleaning the medium and pupae cases from the vials, but is used mainly to help keep down excessive contamination by mold. If no autoclaving is done, plastic culture containers, as well as glass, may be cleaned with this technique. 3. Goggles should be worn at all times while working with the alkali solution.

MATERIALS REQUESTED OR AVAILABLE

Charles L. Vigue is looking for stocks of D. melanogaster homozygous for Odh^S and Odh^F. Please send stocks or information to him at Saint Joseph College, Department of Biology, West Hartford, Connecticut 06117.

Professor A.R. Cordeiro has moved to the new Departamento de Genética, Universidade Federal do Rio de Janeiro, Caixa Postal 68.011, Cidade Universitária, 20.000, Rio de Janeiro, RJ, Brazil (from Porto Alegre, Brazil). He would like to receive available reprints to organize the library on Drosophila genetics and Evolution.

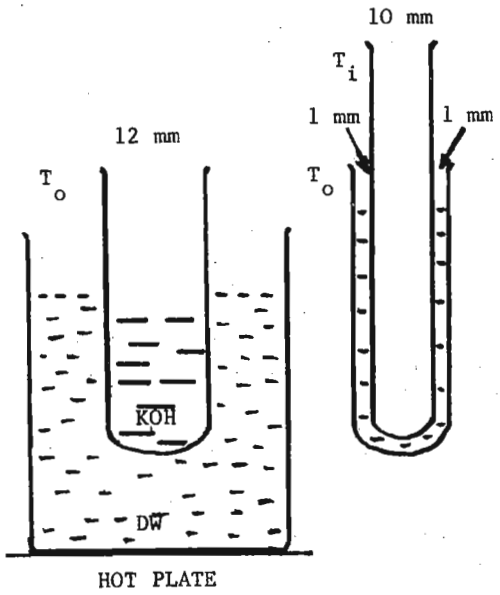
Availability of University of Texas Publications, 1938-1972, from M.R. Wheeler, University of Texas, Austin. Of the 19 publications with the general title "The University of Texas Publications" edited by Patterson and later by Wheeler, 12 are available, upon request. I maintain clean copies of the others and will loan them out. They are listed here by publication number only; note that the first two digits represent the year of publication.

Volumes available by loan only: UTP 3825; 4032; 4228; 4213; 4313; 4445; 5422.

Volumes available as long as the supply lasts: UTP 4720; 4920; 5204; 5721; 5914; 6014; 6205; 6615; 6818; 6918; 7103; 7213.

Mail requests to me at the Department of Zoology, University of Texas, Austin TX 78712. Enclose gummed, self-addressed mailing labels, please. There is no charge for these publications. However, if you would include some postage with the request, it would be appreciated.

Szabad, J. Biocentrum, Szeged, Hungary.
Quick preparation of Drosophila for microscopic analysis.

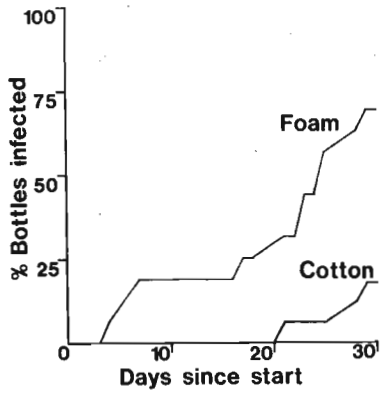


Put anaesthetized or preserved flies into a test-tube (T_o, see Figure) containing 10% alkali, preheated in a water bath. It is unnecessary to open the body cavity of the flies. Cook flies until eye color starts to fade. Suck off alkali and wash the cooked flies twice with hot distilled water (DW) taken from the water bath. Add hot DW again and introduce a test-tube of smaller diameter (T_i) into T_o. Press T_i against T_o to squash the flies. Avoid rotating! By this treatment the residue of the soft parts of the flies will be separated from the cuticle. Wash the cuticles by introducing DW down the wall of T_o. Repeat the pressing and washing procedure if necessary. No part of the flies, not even bristles are lost during the treatment described. No air bubbles are formed inside the flies. The cuticle is completely devoid of the soft parts. Flies treated in this way are ready to be mounted, e.g. in Faure's.

With the above apparatus about 50 flies can be prepared in one run. The procedure has also been successfully applied to larvae and embryos.

Chambers, G.K., T.S. Fletcher and F.J. Ayala. University of California, Davis.
Exclusion of "media mites" from bottle cultures of Drosophila by means of cotton (rayon) plugs.

Various Drosophila stock collections in our department have suffered from infestations by large white mites, which we believe belong to the genus Proctolaelaps, species unknown. We have named them "media mites" to distinguish them from the more commonly encountered fly mites Histiosoma julorum (Koch). While we have no evidence that media mites harm the flies directly they are an undesirable variable in experiments in population genetics.



Experiences with outbreaks of mites suggested that vials and bottle cultures with cotton or rayon plugs were more resistant to infestation than cultures with reusable foam plugs. A test of this hypothesis was, therefore, devised. On day 0 a tray was set up holding 1/2 pint milk bottles with either foam (total 16 bottles) or cotton plugs (total 17) arranged in an alternating pattern. Five wild-type D. melanogaster females from uninfested stock were introduced to each bottle and the experiment positioned on a shelf beneath some infected population cages. Each bottle was examined for the presence or absence of mites each day over a period of 30 days. A progress curve is shown in Figure 1. After a single observation of one mite in a bottle, that bottle was declared contaminated and scored positive on all subsequent days.

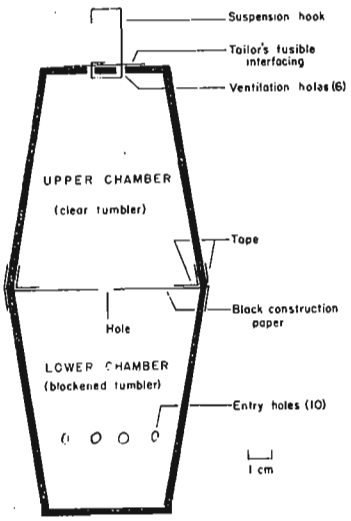
The cotton-plugged bottles were much more resistant to infection. In fact, in the three cotton-plugged bottles infected, only one mite was observed, whereas all the foam-plugged bottles contained several mites (average 3-4/bottle). Close examination of infected bottles with foam plugs revealed that the "media mites" tended to reside inside the foam plugs making occasional forays into the bottle.

In conclusion, cotton plugs were found to afford protection from media mites for periods of up to 21 days, and are now used widely in the department. They are, however, no substitute for clean work and regular transfer of stocks.

Heim, W.G. Colorado College, Colorado Springs. An inexpensive, reusable fly trap.

During a recent project it became necessary to collect flies at a number of sites at considerable distances from each other and over a period of several days. Consequently, many sturdy, inexpensive, easily made and reasonably weatherproof traps, capable of holding flies for several days, were needed. I developed and field tested such a trap, based partly on the design reported by Lower, Drobney and Rose (DIS 52: 182-184, 1977).

The main features of the design are shown in the cross-sectional drawing. The body of the trap is constructed of two 10 ounce "disposable" plastic tumblers (Amoco "Crystal Host"). The inside of one tumbler of each pair is sprayed three times with flat black spray paint, while using a fume hood and wearing plastic gloves. About ten holes are then made around the periphery of this tumbler, approximately 3 cm above the bottom. About six ventilation holes are also made in the bottom of the clear tumbler. All holes are about 3-4 mm diameter and may be easily produced by passing a heated, pointed piece of metal, such as a center punch, through the plastic. The ventilation holes in the clear tumbler are then covered by gluing a piece of fusible interfacing, as used in tailoring, over them. This material has two principal advantages over the usual screening materials: First, it can be fastened in place securely with a few drops of household cement and, second, it is dense enough to keep out all but the most severe rain. A suspension hook, made from a paper clip, is passed through two of the ventilation holes.



A piece of bait, consisting of the usual slice of aged banana impregnated with baker's yeast, is placed in both tumblers. Then a disk of black construction paper, perforated by a 3-4 mm hole near its center, is fastened with pressure sensitive tape over the mouth of one of the tumblers and the two tumblers are affixed to each other, mouth to mouth, with more pressure sensitive tape.

The traps are hung from trees or bushes at one or two meters above the ground and may be left out for three to five days. Flies will enter the lower, blackened tumbler through the entrance holes, attracted by the banana bait within. Then they will move through the hole in the black paper disk into the upper tumbler due to their combined positive phototropic and negative geotropic tendencies. In the upper chamber they can feed on the second piece of banana.

The flies in a trap can be harvested by pipetting either ether or carbon dioxide into the upper chamber through the fusible interfacing. Blocking the entrance holes during anesthesia is usually not required. Traps may be cleaned and re-baited by removing the tapes and, if necessary, replacing the paper disk.

QUOTABILITY OF NOTES

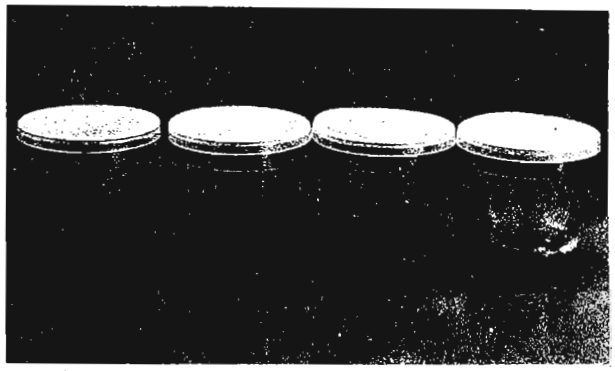
Note: It is not the policy of DIS to enter permissions to quote in this section for material appearing in the same issue.

Bates, D., L. Ehrman & I. Perelle 51:94-95
Gersh, E.S. All notes quotable.
Arthur, C.G. & E.S. Goldstein 52:48
Lambert, D.M. 52:82-83
Mather, W.B. & M. Clyde 52:62-63
Mather, W.B. & M. Clyde 52:147
Petri, W.H., A.R. Wyman & S. Heinkoff 52:80
Szabo, P. & V. Donifrio 52:146-147
Thongmeearkom, P. 52:154-157
Thongmeearkom, P. 52:117
Thongmeearkom, P., M. Clyde & W.B. Mather 52:123
Traut, H. 52:168
Williamson, R. 52:150-151

Magnusson, J., B. Sjögren and M. Zimdahl.
Wallenberg Laboratory, Stockholm, Sweden.

Disposable containers for recessive lethal tests.

The large number of vials required for recessive lethal tests is a serious drawback both from a practical and an economic point of view. In our laboratory we have solved this problem to a considerable extent by using small disposable plastic containers, which are made for hospitals for distributing medicines to patients. These containers are made of polystyrene and have a slightly conical shape with a bottom diameter of 26 mm and a top diameter of 32 mm. The containers have polyetene lids. In order to secure air circulation in the containers, holes are made with a hot needle in the lids, before using them. This system has turned out to work excellently for breeding a limited number of flies per container. The polystyrene of the containers is glass clear and enables a rapid and direct inspection of the flies for the presence of wild type males in a Muller 5 (Basc) test. The disadvantage of the containers is that the shallow shape makes them difficult to use when transferring flies and therefore they are most suitable for the last step in a recessive lethal test, which otherwise is the most vial-consuming part of the test.



The conical shape of the containers makes them easy to store - about 90,000 containers per m^3 and about the same space for the lids. The containers are only used once but the lids can easily be washed and used again. The cost of the containers is about 1 cent of U.S. currency and the same amount for the lids. Plastic trays for these containers are available. The firm, which manufactures these items is Plastlemeco, address: Strandvägen 7B, 11456 Stockholm, Sweden.

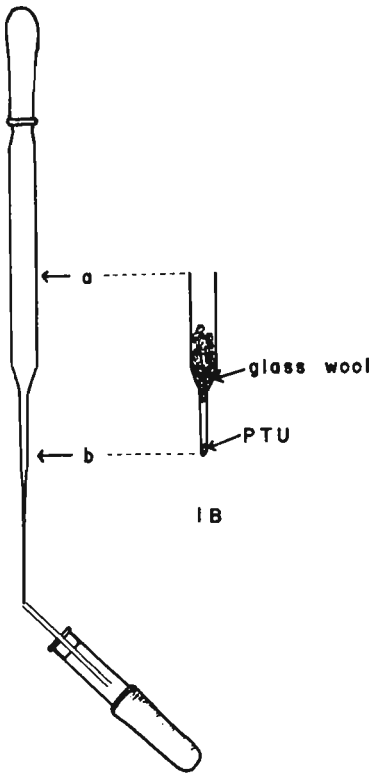
ANNOUNCEMENTS II

S.J. Counce, Duke University, speaking for the Waddington Memorial Fund Committee (A. Robertson, C. Auerbach, G.H. Beale, H.G. Callan, D.S. Falconer, A. McLaren and M. Birnstiel), writes: C.H. Waddington, who died in September of 1975, had an important impact on the development of both genetics and embryology in the mid-years of this century, not only through his own research and writings, but through his Directorship of the Institute of Animal Genetics at the University of Edinburgh. Through the efforts of friends and colleagues, a memorial fund is being established in his name at the University to support attendance of junior members of the Institute at international meetings. This seems a most appropriate memorial, for Wad himself was an inveterate traveller, and cherished his worldwide circle of friends and acquaintances in the scientific community. Many Drosophila workers who enjoyed the hospitality of the Institute for varying periods of time during Wad's tenure as Director, may wish to contribute to this memorial fund. This can be done by sending contributions marked for the Waddington Memorial Fund to: The Secretary, Department of Genetics, West Mains Road, Edinburgh EH9 3JN, Scotland. Checks should be made payable to the University of Edinburgh.

Thomas Hunt Morgan: Pioneer of Genetics, a biography by Ian Shine and Sylvia Wrobel, with a forward by George W. Beadle, has been published by The University Press of Kentucky, Lexington. The illustrated book has 176 pages. The first author is director and medical geneticist at the Thomas Hunt Morgan Institute of Genetics in Lexington, and the second author, a writer, was an assistant at the Institute.

Kambysellis, M.P. New York University, New York. A simple technique for collecting hemolymph from adult Drosophila.

In our studies with yolk proteins during the last few years, we encountered two troublesome problems—how to collect and estimate the quantity of hemolymph from individual Drosophila, and how to collect, from a group of individuals, sufficient quantities of hemolymph to determine the presence of vitellogenin electrophoretically or immunochemically. We have found it very convenient to use Pasteur pipettes in both cases. In the first instance, a pipette is drawn to a very fine capillary, the fly is anaesthetized, and with fine forceps a small opening is made in the middle line of the thorax, exactly above the cardia. The capillary is then inserted in the opening and the hemolymph is withdrawn by capillary action. Light pressure on the abdomen and proboscis facilitates the operation. Once the hemolymph is collected, the space occupied in the pipette is marked with a fine point marking pen, the hemolymph is released and quickly bioassayed to prevent darkening due to tyrosinase activity. The volume of hemolymph is then estimated by refilling the capillary up to the mark with water, and subsequently releasing it into a 1 µl "microcap" or "Yankee micropet" (Fig. 1A). The fraction of the "microcap" occupied by water is measured with a micrometer under a dissecting microscope. With this method we were able to collect most of the hemolymph from flies of various ages and measure quantities up to 0.2 µl from newly emerged D. melanogaster and 0.05 µl from females 24 hours old. Young flies always have more hemolymph than older ones and females more than males. The variability in the size of flies should be considered in the analysis.



1B

1A

To collect larger quantities of hemolymph from a group of flies, we devise a simple centrifuge tube, by cutting the two ends of a Pasteur pipette (points a and b in Figure 1A) and sealing the narrow end in an alcohol flame. A few crystals of repurified PTU (phenylthiourea) are placed in the tube (to prevent tyrosinase activity) and a small quantity of glass wool is inserted and packed in the tube (Figure 1B). Anaesthetized flies are punctured with extrafine forceps or fine pointed wire or glass rods and then inserted into the prepared centrifuge tube. When an adequate number of flies are injured (30-40 females for D. melanogaster), they are centrifuged at low speeds. We use a Sorvall SR-2B centrifuge and spin at 5,000 rpm for 7 minutes. From 30-40 four day old female flies we can collect about 2 µl of hemolymph. It is advisable to use ice-chilled centrifuge tubes and a refrigerated centrifuge to prevent protein denaturation.

If very few flies are available and some contamination can be tolerated, more hemolymph can be collected by cutting the proboscis and rupturing the last abdominal tergite of the fly. In this case lower centrifugation speeds should be used to avoid rupturing of the gut and release of the fat body cells.

This method has been successfully applied to other small insects, such as mosquitoes. Speed in the operation, and care not to rupture the various organs of the fly, are the secrets of success with this technique.

Supported by NSF Grants GB-34168 and PCM75-18172.