

DROSOPHILA INFORMATION SERVICE

72

July 1993

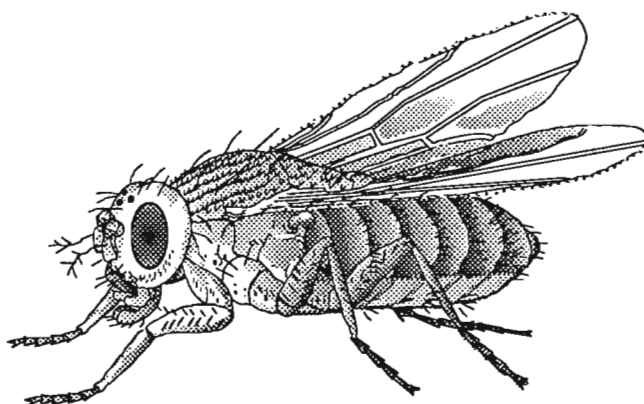
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Drosophila Information Service



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Preface

Two new additions to this issue will hopefully increase the access to useful information for *Drosophila* researchers. First, an author/title and key word index to back issues of DIS (volumes 50 - 71) has been prepared. Photocopies of individual out-of-print research and technique articles can be obtained from the editor for \$1.00 each, including postage. A key word index to the most recent issue will be included in future volumes. Second, we will begin printing contributions at regional and national *Drosophila* meetings as space allows. This will help researchers identify others with similar interests and will increase the visibility of presentations at smaller meetings world-wide. Publicity of these conferences will, however, only be possible if you bring them to our attention.

To help identify special materials to include in future issues, a DIS Advisory Group is being formed. Current members include Michael Ashburner (Cambridge University), Daniel Hartl (Harvard University), Kathleen Matthews (Indiana University), and R.C. Woodruff (Bowling Green State University). Others will be added during the coming months, and recommendations are invited.

The production of DIS 72 could not have been completed without the generous efforts of many people. Jean Ware, Caroline Tawes, Coral McCallister, Christine LaFon, Stanton Gray, William Dutton, and Mingull Jeung in the Department of Zoology have assisted in the preparation and correction of typescripts, maintenance of computer records, shipping, and correspondence. Melva Christian at the University of Oklahoma Printing Services oversaw the printing of this issue.

We hope that you find a lot of useful information here, and we invite you to let us know what can be done to improve DIS as an informal source of communication among *Drosophila* researchers. The publication of *Drosophila* Information Service is supported in part by a grant from the National Science Foundation to R.C. Woodruff for the Mid-America *Drosophila* Stock Center, Bowling Green, Ohio.

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Announcements

New Books

Models for Embryonic Periodicity

"Models for Embryonic Periodicity" by Lewis I. Held, Jr. (1992) presents the principal models of pattern formation and extensive supporting literature citations. It covers mechanisms proposed for positional information, prepatter, and determination waves. Darwinian, rearrangement, and cell-lineage mechanisms are also presented. The final section covers the role of computers in developmental biology. Clear diagrams illustrate the key features of alternative models, and an extensive bibliography completes the volume. It is published by Karger Publishers, Inc., 26 West Avon Road, P.O. Box 529, Farmington, CT 06085 (120 pages, 11 figures, 2 tables, hardcover; ISBN: 3-8055-5598-9; list price \$156.00).

Using the Author/Title and Key Word Index DIS 50 - 71
and
Obtaining Copies of Out-of-Print DIS Articles

Only a limited number of copies of DIS back issues are available (see page 172 for ordering information). Yet, much valuable information is to be found in the research and technique articles in out-of-print issues. In order to make this material available, we have prepared an author/title index and a key word index for DIS volumes 50 - 71. These entries were scanned into our computer files from the printed copies, and type faces and styles varied somewhat during this period (1973 - 1992). While we have tried to make the index as uniform and accurate as possible, there are probably still some errors that originated in the scanning process. We plan to continue preparing a complete author/title index with the goal of making it available on computer diskette that can be searched by standard word processing software.

The first part of this index is an alphabetical listing of research and technique articles in DIS volumes 50 to 71. The second part is a key word index, with numbers that correspond to the page of the author/title index on which the item is located. Although this entails some searching on a page to find the entry, it should be relatively easy to locate them.

Photocopies of individual technical and research articles can be obtained from the editor for \$1.00 each (mailing included). Costs for copies of stock lists and other long material can be obtained by writing the editor.

Cumulative Titles and Key Word Index Volumes 50 - 71

The following cumulative title and key word index covers all research and technical notes in DIS volumes 50 through 71, except for special issues.

- AARON, C.S. Homosexual behavior induced in *Drosophila* by sodium tungstate. (1977, 52: 57).
- AARON, C.S. and W.R. LEE. Rejection of ethyl methanesulfonate feeding solution by *D. melanogaster* adult males. (1977, 52: 64).
- AARON, C.S., H.E. NARDIN and W.R. LEE. Glass filter supports for treatment of adult *D. melanogaster* with chemical mutagens. (1977, 52:166).
- AARON, C.S., W.R. LEE, P.M. SEAMSTER and F. JANCA. A non-aqueous gas exposure technique for microliter quantities of chemical mutagens. (1977, 52:174).
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- ABDELHAY, E., T.M. CASTRO and H. ROCHA. A method to isolate segments of *D. melanogaster* embryos in great quantities. (1987, 66:155).
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- ACHARY, P.M.R. and P.K. DUTTA. Apparatus for mutagenizing *Drosophila*. (1984, 60:209).
- ACHARY, P.M.R., P.K. DUTTA and A.K. DUTTAGUPTA. Replicon properties of *Drosophila* genome. 1. DNA fibre autoradiography of salivary gland cells held at the mid-part of S-phase. (1984, 60:43).
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- ADELL, J.C. and L.M. BOTELLA. Effect of long-term competition on development of *Drosophila melanogaster*. (1986, 63:13).
- ADELL, J.C. and L.M. BOTELLA. Selection for rate of pupation in crowded cultures of *Drosophila melanogaster*. (1987, 66:1).
- AGNEW, J.D. Morphological differences in larval *D. melanogaster* and *D. simulans*. (1973, 50:51).
- AIZENZON, M.G. and E.S. BELYAEVA. Genetic loci in the X-chromosome region 2A-B. (1982, 58:3).
- AIZENZON, M.G. and M.R. STOLINA. Sequences of adeno-associated virus genome are non-mutagenic for *Drosophila*. (1991, 70:14).
- AL-TAWEEL, A.A. The frequency of spontaneous and induced recessive lethals in the sex chromosome of the *Drosophila melanogaster* wild type from Iraq. (1986, 63:23).
- ALAHOTIS, S. α -Glycerophosphate dehydrogenase and the locomotor activity in *Drosophila melanogaster*. (1977, 52:145).
- ALAHOTIS, S., A. ZACHAROPOULOU and M. PELECANOS. The effect of two ecological factors upon the inversion frequencies in *D. melanogaster* cage populations. (1977, 52:106).
- ALAHOTIS, S.N. and G.N. GOULIELMOS. Fertile F₁ males and females from crosses between *Drosophila mauritiana* females and *D. melanogaster* or *D. simulans* males. (1986, 63:14).
- ALATORTSEV, V.E. and E.V. TOLCHKOV. Complementation analysis of rearrangements affecting the Sd-F region of the *D. melanogaster* X-chromosome. (1985, 61:24).
- ALBERS, K.B.M. and R. BIJLSMA. Selection for increase in tolerance with respect to xenobiotics in *Drosophila melanogaster*. (1986, 63:15).
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- ALBORNOZ, J. and J. RUBIO. Location of a region controlling the suppression of normal bristles in *D. melanogaster*. (1980, 55:11).
- ALCORTA, E., A. DOMINGUEZ and J. ALBORNOZ. Egg retention in *Drosophila melanogaster* females. (1987, 66:5).
- ALCORTA-AZCUE, E., E. GARCIA-VAZQUEZ and F. SANCHEZ-REFUSTA. Influence of the altitude in Asturian communities of *Drosophilidae*. (1986, 63:17).
- ALEXANDROV, I.D. and M.V. ALEXANDROVA. A new nw allele and interallelic complementation at the vg locus of *Drosophila melanogaster*. (1987, 66:11).
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- ALEXANDROV, I.D. and M.V. ALEXANDROVA. The distribution patterns of roo and copia retrotransposons under reduction of fitness. (1992, 71:215).

- ALEXANDROV, I.D. and M.V. ALEXANDROVA. The genetic and cytogenetic boundaries of the radiation-induced chromosome rearrangements scored as lethal black mutations in *D. melanogaster*. (1991, 70:16).
- ALEXANDROV, I.D. and Yu.A. SIOMIN. Cyto- and genotoxicity of formaldehyde and products of its interaction with lysin and ATP for different genotypes of *D. melanogaster*. (1982, 58:14).
- ALEXANDROV, I.D. Comparative frequency and spectrum of w mutations induced by gamma-rays and neutrons in *D. melanogaster*. (1982, 58:9).
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- ALEXANDROV, I.D. Different patterns of spontaneous mutability in two wild-type sib stocks of *D. melanogaster* with long-term laboratory history. (1992, 71:213).
- ALEXANDROV, I.D. Enigmas of radiation mutagenesis in c(3)G mutant of *Drosophila melanogaster*. (1987, 66:7).
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Mutation Notes - *Drosophila melanogaster*

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vg^{s2}: *vestigial-strap2*

vestigial-strap2 is one of the *vestigial* alleles (*vg*: 2-67.0). It was found by Williams (1956) but only the phenotype of *vg^{s2}/vg* flies from the stock *ta net vg^{s2} sp/af² Cy pr Bl cn² L⁴ vg sp²* was described (see also Lindsley and Grell, 1968).

The mutant is maintained by Williams as *ta net vg^{s2} sp/dp^{tdl} Cy pr Bl cn² L⁴ sp²*

Using the same stock provided by the Bowling Green stock Center (coded as g396), we noted that homozygous segregants could not be recovered, meaning that *vg^{s2}* is lethal or there is a linked lethal on the same chromosome. After crossing with an Oregon stock, various single recombinant chromosomes were replicated and some lines of homozygous *vg^{s2}* flies were obtained.

The mutant shows excellent viability, wings slightly open, turned down, and distally notched (Figure 1a). It also shows few trichomes in the triple, double, and posterior rows (Figure 1b), and ectopic bristles (3 or 4) on L3 and L4 veins (Figure 1c).

Complementation tests with various *vg* alleles show that it is a hypomorph allele (Figure 2). Like most *vg* alleles it complements with *vg^{83b27}*.

The *vestigial* gene has been cloned and its transcripts have been characterized (Williams et al., 1988, 1990), so we have attempted molecular characterization of the *vg-strap2* mutant. Molecular analysis done by genomic hybridization (Maniatis et al., 1989) shows that there is an insert of about 5 kb in the distal part of the gene, between *EcoRI* (+13) and *HindIII* (+15) on *vg* map (Figure 3).

Figure 1 (see next page). Wings are distally notched (a), wings show few trichomes in the triple, double, and posterior rows (arrowheads, b), and ectopic bristles on L3 and L4 veins (arrows, c).

Figure 2. Complementation tests with various *vg* alleles. (a) *vg^{s2}/vg^{s2}*, (b) *vg^{s2}/vg^B*, (c) *vg^{s2}/vg^{nw}*, (d) *vg^{s2}/vg¹*, (e) *vg^{s2}/vg^{np}*, (f) *vg^{s2}/vgⁿⁱ*.

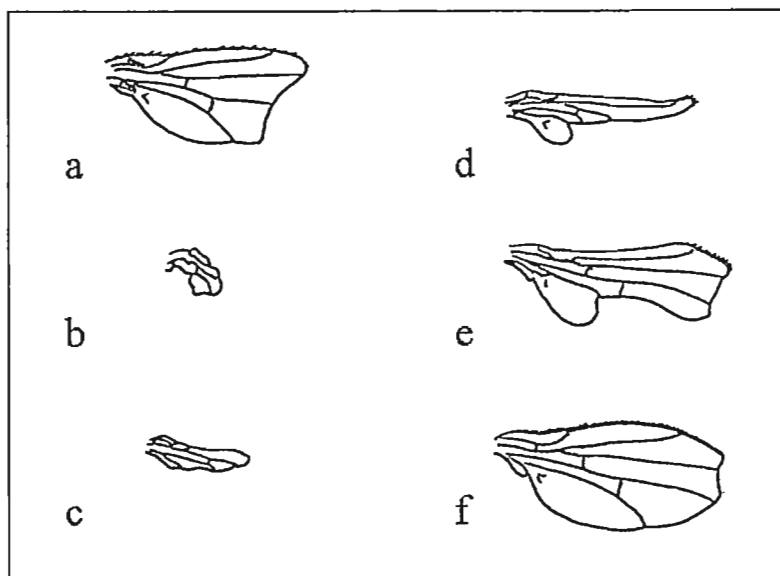
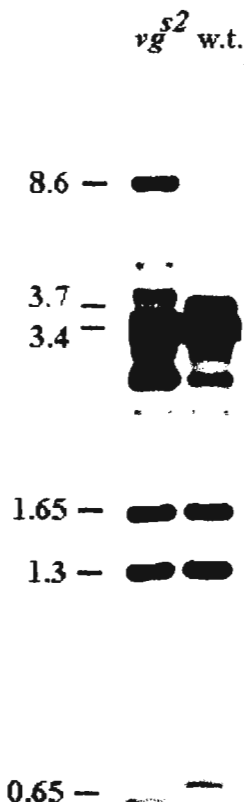
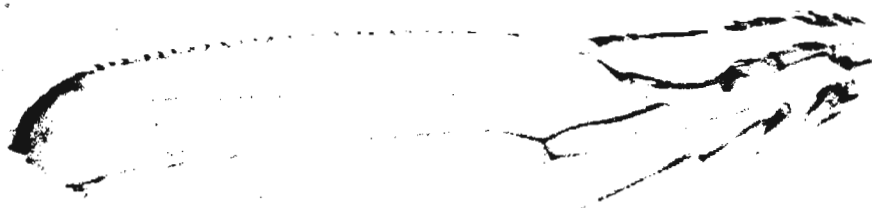


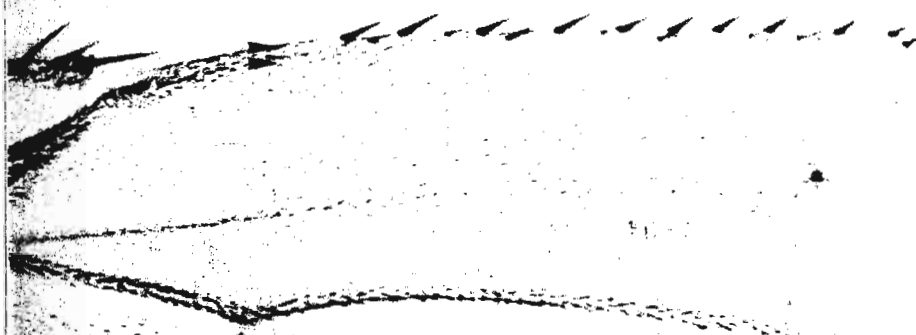
Figure 3 (at the left). Genomic hybridization of DNA digested with *EcoRI* and hybridized with *SstI-BamHI* probe shows the presence of an insert of about 5 kb (8.6 minus 3.7) in the distal part of the gene, between *EcoRI* (+13) and *HindIII* (+15) on the *vg* map (Williams et al., 1988).

Reference: Lindsley, D.L. and E.H. Grell 1968, Carnegie Inst. Wash. Publ. 627; Sambrook, J., E.F. Fritsch, and T. Maniatis 1989, Cold Spring Harbor Laboratory Press, New York; Williams, 1956, Dros. Inf. Serv. 30: 80; Williams, J. and J.B. Bell 1988, EMBO J. 7: 1355-1363; Williams, J., A.L. Atkin and J.B. Bell 1990, Mol. Gen. Genet. 221: 8-16.

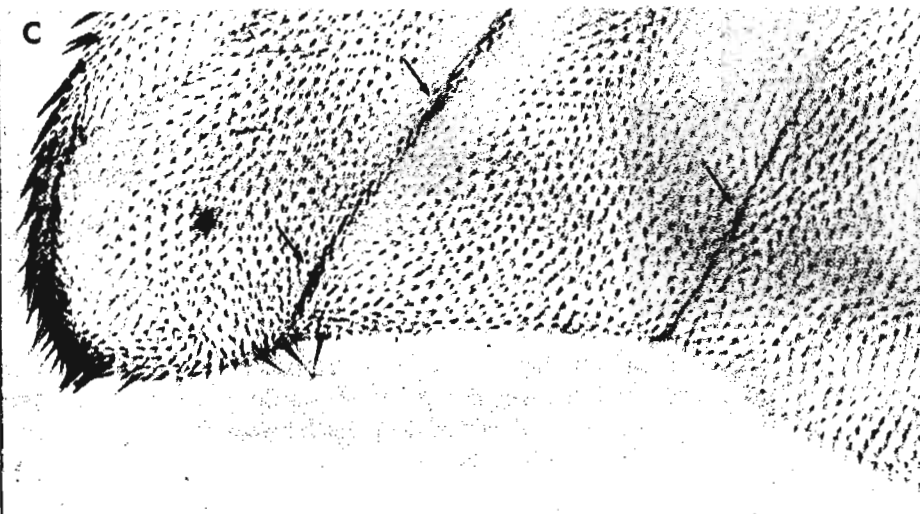
a



b



c



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app^s: approximated spider, a new strong allele of *app*: 3-37.5

The mutant was recovered after exposure to a magnetic field. Mutant flies show reduced viability. *apps* seems to be mainly involved in leg development. All the legs are affected and show reduction of coxa, femur, tibia and tarsal segments. Body size is smaller than in wild type. Wing size is reduced and wings are slightly open. Flying ability is strongly reduced and flies walk in a typical way.

Cytology: it is placed in 69A1-3 on the basis of its inclusion in *Df(3L)BK9/TM3 = Df(3L)068E;069A01* but not in *In(3L)274/TM6B = In(3L)069A03-B03;081F*.

Reference: Lindsley, D.L. and G.G. Zimm 1992, Academic Press, Inc., San Diego, California; Akam M.E., D.B. Roberts, G.P. Richards and M. Ashburner 1978, Cell 13: 215-225.

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psb: post scutellar bristles, a new mutant on the X chromosome (1-13.9).

The mutant was recovered after exposure to a magnetic field. The mutant is recessive viable. *psb* mutant flies show short and thin post scutellar bristles.

Cytology: placed in 5C2-5 on the basis of its inclusion in *Df(1)C149/FM6 = Df(1)005A08-09;005C05-06* and *In(1)N73/FM6 = In(1)005C02;005D05-06*.

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New lethal mutations.

A sex-linked recessive lethal test done to determine the mutagenicity of ellipticine in the early germ cells of *D. melanogaster* gave negative results. Several lethals, presumably of spontaneous origin were isolated during this study and balanced with chromosome *FM6* or *FM7*. Three of them, *l(1)rp1*, *l(1)rp2* and *l(1)rp3* were localized in the genetic map of the procedure described by Strickberger (1962). Virgin females, *l/FM6* or *l/FM7*, were crossed to *y cv v f* males; the F_1 *l/y cv v f* daughters were allowed to mate freely with their brothers but only their male progeny were scored. From 1,500 to 2,000 F_2 males were classified for each lethal. The mutations were first localized between two markers. Then, the frequency of crossing over between the lethal and the left marker was calculated and multiplied by the standard map distance for the region. In addition, we determined the approximate stage of development of the hemizygous lethal genotype at the time of death. Eggs were collected for periods of four hours from the experimental crosses: *l/FM6 X FM6/Y* or *l/FM7 X FM7/Y*, and from the control crosses: *+ /FM6 X FM6/Y* or *+ /FM7 X FM7/Y*. The number of larvae, pupae and adults obtained from the experimental eggs was compared to the number obtained from the control eggs.

All flies were cultured in banana medium and tests were done in an incubator at 25°C. Results are given in Table 1.

Table 1. Results obtained with the four-point test and approximate time of death for the lethal genotype.

| Lethal | Crossover region | Crossovers Between Lethal and Left Marker | Total Crossovers in Region | Standard Map Distance for Region | Locus of Lethal | Approximate Stage of Development at Death |
|----------------|------------------|---|----------------------------|----------------------------------|-----------------|---|
| <i>l(1)rp1</i> | cv-v | 244 | 568 | 19.3 | 22.0 | Embryos and Larvae |
| <i>l(1)rp2</i> | v-l | 234 | 422 | 23.7 | 46.1 | All stages, but mainly pupae |
| <i>l(1)rp3</i> | cv-v | 247 | 498 | 19.3 | 23.3 | Larvae and pupae |

Reference: Strickberger, M.W. 1962, Experiments in Genetics with *Drosophila*, John Wiley and Sons, Inc., New York.

Mutation Notes - Other Species

Etges, William J. Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701 U.S.A.
New and undescribed mutants of *Drosophila mojavensis*.

A number of previously undescribed and newly arisen spontaneous, recessive mutations from natural and laboratory populations of cactophilic *Drosophila mojavensis* are described herein. Geographical locations mentioned are listed in Etges (1990). Because of the paucity of morphological genetic markers in this species, the locations for the autosomal mutants remain to be determined. The only morphological mutants so far assigned to particular chromosomes are either X linked or have been previously assigned through linkage analysis using allozyme markers described by Pantazidis and Zouros (1988; recessive mutant *brown eye*, *br*, located on the fourth chromosome). All mutants are available from the author and will be deposited in the Bowling Green Stock Center.

X chromosome:

yellow body, *y*, absence of most dark body pigmentation, abdominal tergites lightly banded, and most significantly, all spots at the base of the hairs on the thorax, the key character for the entire *repleta* species group, are missing. A *y* allele with similar effects is also known from *D. hydei* (Spencer, 1947). Derived from an inbred line from Punta Onah, Sonora, collected in 1988.

tan body, *t*, slight reduction in dark pigmentation. Derived from a laboratory population from Punta Onah, Sonora, collected in 1991.

white eye, *we*, no eye color pigment, does not effect male testes coloration. This mutant was isolated from separate made collections in 1988 and 1989 from Punta Prieta, Baja California Norte. Therefore, this allele must be segregating in low frequency in nature.

vermillion eye, *v*, bright red-orange eyes. This strain has been in culture in W.B. Heed's lab for years. He obtained it from L.E. Mettler, and it originated from wild flies collected in the Chocolate Mountains in southern California, U.S.A.

Autosomes:

adobe, *ad*, dark brick red eyes. This was isolated by Richard H. Thomas from a stock collected in the Cerro Colorado region near Desemboque, Sonora. It is not the same as *br* described by Pantazidis and Zouros (1988). F2 progeny from *ad* X *br* crosses contained both phenotypes and a very dark brown eye phenotype, but it has not been determined whether these two are independently assorting.

ruby, *ru*, dark red eyes. Derived from a stock collected from the Santa Rosa Mountains near Tucson in 1988.

Several compound mutants have also been constructed:

vermillion eye-ruby eye, *v-ru*. These flies have an apricot eye color (duplicate recessive epistasis).

yellow body-adobe eye, *y-ad*.

References: Etges, W.J. 1990, *Ecological and Evolutionary Genetics of Drosophila* (J.S.F. Barker and W.T. Starmer, eds.), Plenum Press, pp. 37-56; A.C. Pantazidis and E. Zouros 1988, *Heredity* 60:299; Spencer, 1947, *Adv. Genet.* 1:359.

Mestres, F. Dept. de Genetica, Universitat de Barcelona, Spain.
A wing mutation in *D. subobscura*.

To study the viability in homozygous condition of O chromosomes of *D. subobscura* from Bellingham (Washington), appropriate crosses using the lethal balanced strain *Va/Ba* were performed (Sperlich et al., 1977; Mestres et al., 1990). One of the chromosomal lines obtained in homozygous condition presented a wing mutation. Veins L4

and L5 did not reach wing margin (Figures 1 and 2). All individuals analyzed presented this phenotype. Furthermore, a small number of flies had the same alteration also in vein L2 (Figure 3). The mutation is recessive and located in the O chromosome. Probably, this mutation is similar to *shv* described by Burla (1968), although they differ in some aspects. The mutation could be qualified as rank RK 1.

References: Sperlich, D., H. Feuerbach-Mravlag, P. Lange, A. Michaelidis and A. Pantzos-Daponte 1977, Genetics 86: 835-848; Mestres, F., G. Pegueroles, A. Prevosti and L. Serra 1990, Evolution 44: 1823-1836; Burla, H. 1968, Dros. Inf. Serv. 43: 76-78.

Figure 1. Aspect of the vein L4.

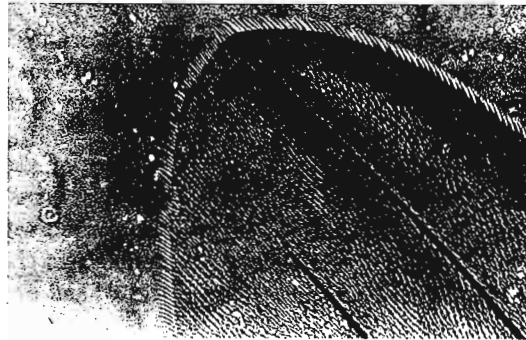


Figure 2. Aspect of the vein L5.

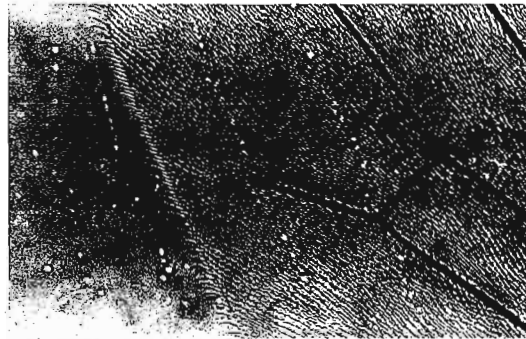
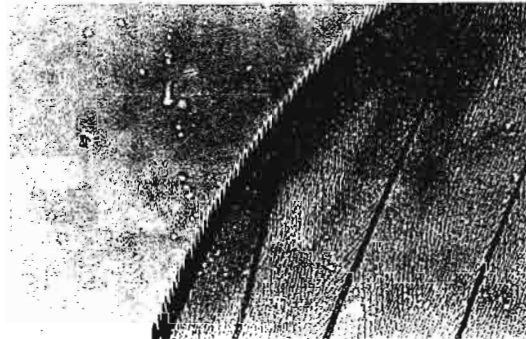


Figure 3. Small number of flies had the alteration in vein L2, too.



Standing Orders

Several years ago, formal standing orders were discontinued, due to the need to obtain prepayment for issues. "Standing Orders" are now handled through a mailing list of active subscribers. All individuals on the active subscriber list will receive notices for forthcoming regular and special issues and a Standing Order Invoice to facilitate prepayment. If you would like to be added to the *Drosophila* Information Mailing list, please write to the editor, Jim Thompson, at the address on the front.

Technique Notes

Fukui, H.H. and A.W. Kirscher. University of Minnesota, St. Paul MN U.S.A. Thanatometer II: A chamber designed for large mixed-sex populations of *Drosophila melanogaster*.

A chamber specifically designed for longevity measurement of *Drosophila melanogaster* (Thanatometer) has been reported (DIS 71, p180). Here we expand the same basic concept: 1) To enlarge the capacity of the chamber to enable us to test larger sample sizes, and 2) To measure mixed-sex populations.

The first goal is accomplished by using a larger chamber. The second goal is accomplished by connecting two chambers to make a single unit. Then, application of the food source can be alternated to each subunit to restrict the development of eggs. The results from an experiment using these new chambers (Thanatometer II) indicated that: 1) We were able to test approximately 1000 flies per chamber, and 2) Egg development was suppressed enough to eliminate development of progeny.

A complete chamber is shown in Figure 1. The main part of these chambers is a one gallon (3.79 liter) Polyethylene Terephthalate jar manufactured by Jareen Co. (3355 East Slauson Avenue, Los Angeles, CA). First a 2.25" x 7" ventilation hole was cut out of the handle area of the jar and covered with mesh screen. Next, a 4" in diameter hole was cut out of the opposite side and about 0.5" from the top. To make one chamber, two of these jars were put together with a 1.75" long tube of acrylic tubing with an outer diameter of 4" and an inner diameter of 3.25". The approximate space between the two jars in one chamber is 1.5".

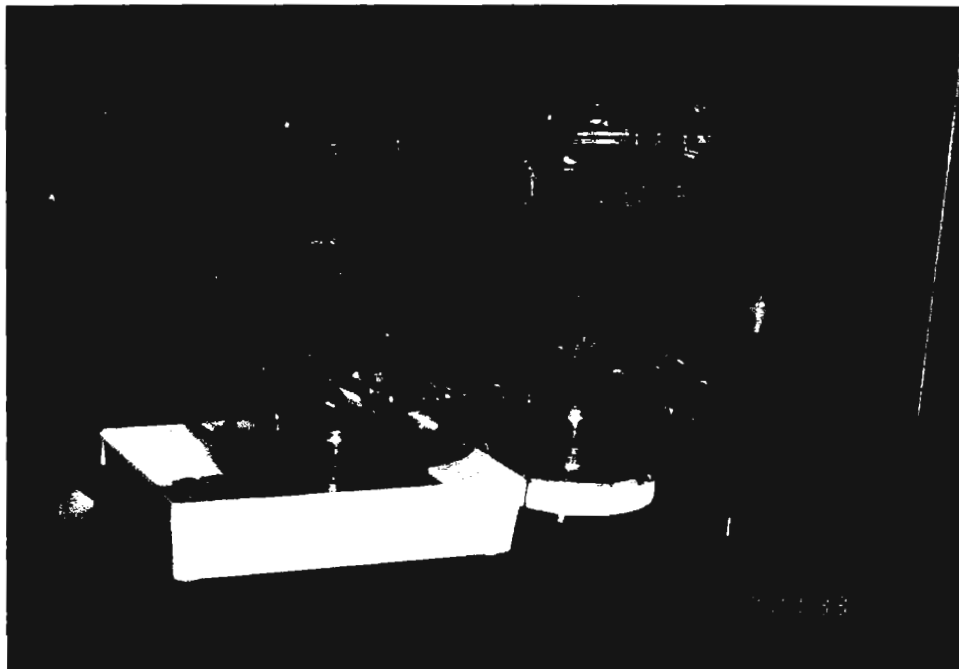


Figure 1.

Then one hole approximately $1\frac{1}{32}$ " in diameter was cut into one side of each jar near the bottom. The hole was covered with three layers of rubber sheet with cross-hatched incisions. A glass tube connected to a vacuum pump was inserted to collect dead flies daily. One additional hole was drilled in the side of one of the two chambers and was used to transfer flies into the chamber at the beginning of the experiment.

Finally, a piece of mesh screen large enough to cover the 4" mouth was held on and held taut with a hose clamp. Glue (Shoe Goo II; Eclectic Products, Inc. Carson, CA) was used to fasten together all parts of these chambers.

The standard yeasted-cornmeal-molasses media was placed in the screw top lids of the jars. The lid was placed on one side of the chamber and a sponge sitting in water was placed under the other opening. In this way the flies could get food only from one side of the chamber and moisture could be kept high in the other side. The culture media were replaced every day. To prevent egg development, we altered the side of the cage with media so that eggs on the mesh screen would not develop. With some of the lines of flies it was necessary for the bottom of the mesh screen to be scraped to remove eggs to help ensure they would not develop to the pupa stage.

The flies in our experiment lasted 16 weeks and we started out with as many as 1200 flies in a single chamber. The labor involved to maintain 21 chambers at once never became more than one person could handle in a day (approximately 4-5 hours a day was spent at a peak time of fly deaths).

Acknowledgment: This work was supported by NIH grants "Oldest-old Mortality" to J.W. Curtsinger and J. Vaupel (PO1AG08761).

Lim, John K. Department of Biology, University of Wisconsin-Eau Claire, Eau Claire, Wisconsin. *In situ* hybridization with biotinylated DNA.

This detailed protocol outlines *in situ* hybridization with biotinylated DNA. Direct questions and suggestions to: John Lim, at (715)-836-3860; FAX, (715)-836-2380; or Internet, LIMJK@UWEC.EDU.

PREPARATION OF SLIDES

1. Prepare unstained polytene chromosome slides.

A recommended practice:

- Dissect a well-fed larva in 45% acetic acid on a siliconed slide. Use stainless steel needles for dissection. Creamy-white colored glands with dense cytoplasm yield the best results in preparing the chromosome squashes. Do not proceed further if the glands are translucent in appearance and deflate and shrivel in the acid.
- Cut away salivary ducts and anterior part of the glands with smaller gland cells.
- Transfer the dissected salivary glands to a new drop of 45% acetic acid. Frequently, the membrane surrounding the gland cells and the fat body cells attached to the membrane will detach from the gland cells within a minute or two.
- Transfer the salivary glands, free of attached fat body cells and membrane, into a small drop (ca. 15-20 ul) of fixative composed of 1:2:3 = lactic acid (86%):water:glacial acetic acid on a clean siliconed coverslip. Fix the gland cells for about three to five minutes.
- Pick up the coverslip with the fixed gland cells on it with a clean slide* (not treated with silicone). Squash the cells and spread the chromosomes. This can be accomplished by gently tapping the coverslip with the eraser end of a pencil, or gently streaking over the coverslip with a needle, or a combination of both. Be patient. You will be able to make superb squashes in no time!

*I use a specially painted slide ProbeOn Plus microscope slide from Fisher Scientific, Catalog number 15-188-52, directly from the box. This slide is convenient for treating the slides with Vectastain (see 10 below).

- Keep the slides with chromosome squashes at room temperature for several hours, but no more than ten hours. After several hours, chromosomes and cytoplasmic materials will reach an equilibrium. Freeze the slides in 95% ethanol chilled to -70°C with dry ice for about an hour. Pry off the coverslip with a razor blade. Then make sure to return the slide without a coverslip into 95% ethanol chilled to -70°C. Unlike stained chromosomes, unstained chromosomes lose their banding details when they are placed in ethanol at room temperature. Apparently, a temperature jump of 100 degrees causes a disruption of chromatin organization. If you are curious about this recommendation, try a small experiment with ethanol at room temperature and at -70°C. You will never again want to subject unstained chromosomes to such a drastic shift in temperature. Let the slides return to room temperature gradually (it takes about three hours).

Most people cannot make 100 slides of polytene chromosome squashes a day. You may want to store slides until you have collected a large number of slides for processing. The slides with coverslips can be stored for about a week in 95% ethanol at -75°C in an ultralow freezer set at this temperature. However, it is best not to keep them longer than a week.

- Heat treat the chromosomes by placing the slides in 2 x SSC and incubate at 65°C for 55 to 60 minutes. After the incubation, leave the slides in 2 x SSC at room temperature for about 45 minutes. During this time, the temperature of 2 x SSC will approach 35°C. Transfer the slides into 2 x SSC at room temperature and leave them in it for about 5 minutes. Now, you are ready for the next step.

- Denature the chromosomal DNA in 0.14 M NaOH in 2 x SSC (pH should be ca. 11.7) for 3 minutes.

A recommended practice:

Prepare the solution by diluting 7M NaOH solution in 2 x SSC.

4. Rinse in fresh 2 x SSC twice, 5 minutes each. (Start step 7 for hybridization.)
5. Dehydrate the slides by passing through 70% ethanol twice and 95% ethanol once or twice, ten minutes each, and let the slides air dry for at least ten minutes.

HYBRIDIZATION

6. Nick translation of the probe DNA.

A recommended practice:

Prepare the following solutions and agents:

- (1) DNase I stock solution. Prepare 1 ug/ul solution of DNase I (Worthington, DPFF grade) in autoclaved 50% glycerol. Keep the solution in -20°C freezer.
- (2) 10x Nick translation buffer. Can be stored at -20°C in small aliquots.
 - 0.5 M Tris-HCl, pH 7.5
 - 0.1 M MgSO₄
 - 1 mM dithiothreitol (DTT)
 - 500 ug/ml bovine serum albumin (BSA)
- (3) Hybridization buffer (to make 1 ml). Can be stored at -20°C in small aliquots.
 - 100 ul 20 x SSC
 - 200 ul 50% dextran sulfate
 - 500 ul formamide
 - 200 ul deionized water
 - 1000 ul total
- (4) GAC mix, a mixture of dGTP, dATP, and dCTP (0.3 mM each). This mixture can be stored at -20°C in small aliquots.
- (5) Just before nick translation, prepare 1/400 dilution of 1 ug/ul solution of DNase I by mixing 400 ul of TM10 (10 mM Tris, pH 7.5; 10 mM MgCl₂) and 1 ul of DNase I stock solution in (1) above.

Nick translate the probe DNA by mixing:

| | |
|--|---|
| 10 x Nick translation buffer | 2.5 ul |
| GAC mix | 2.5 ul |
| Bio-16-dUTP (Boehringer Mannheim Cat. No. 1093 070) | 2.0 ul |
| probe DNA | ca. 0.5 ug |
| DNase I (1:400 dilution) | 1.5 ul |
| distilled deionized water to | 25 ul (Remember to adjust for DNA polymerase I) |
| DNA polymerase I | 10 units |

Leave the mixture at room temperature for 60 minutes. Stop the reaction by adding 25 ul of 50 mM EDTA, pH 8.0.

Add about 20 ug of carrier DNA (Herring sperm or salmon sperm DNA or equivalent) and 5 ul of 3 M sodium acetate and gently tap the tube. Precipitate the DNA by adding 250 to 300 ul of ice-cold 100% ethanol. Incubate at -20°C for a few hours at least.

Recover the DNA by centrifugation and dry under vacuum for about 10 minutes. Resuspend the pellet in 75 ul of hybridization buffer. The probe in the hybridization buffer is stable for at least one year.

7. Denature the probe at 65°C for 5 minutes (or 95°C for 10 minutes if the probe does not contain formamide), and quench on ice for at least several minutes before use. Apply 10 ul of denatured probe to each slide, cover with a coverslip, and seal the edges of the coverslip with rubber cement.
8. Hybridize for about 20 hours at 37°C.

WASHES AND DETECTION

9. Carefully remove rubber cement and place the slides in 2 x SSC for a minute or two. The coverslip will fall off in 2 x SSC. Wash slides as follows:
 - 2 times 10 minutes each in 2 x SSC pre-warmed at 37°C.
 - 2 times 5 minutes each in 1 x PBS at room temperature.
 - Wash for 2 minutes in 1 x PBS with 0.1% Triton-X-100.
 - Rinse for 5 minutes in 1 x PBS at room temperature.

A recommended practice:

10 x PBS:

1.3 M NaCl
0.07 M Na₂HPO₄
0.03 M NaH₂PO₄

DO NOT LET SLIDES DRY OUT AT ANY TIME AFTER THIS STEP OR YOU WILL HAVE A LOT OF BACKGROUND; KEEP IN PBS IF NECESSARY.

11. Mix 10 ul each of reagent A and B in Vectastain ABC Kit** to 1.25 ml of 50 mM Tris, pH 7.6, 4% BSA. Let stand at least 5 minutes at room temperature before use. (It is convenient to freeze 1.25 ml aliquots of the buffer/BSA solution).

** Vectastain ABC Kit can be purchased from:

Vector Laboratories, Inc., 30 Ingold Road, Burlingame, CA 94010. The one I am using is *Elite* Standard, Cat. No. PK6100.

11. Tap remaining PBS solution off slides and add 200 ul of Vectastain complex to a slide. Cover the first slide with second slide with chromosomes on the second slide facing those on the first slide. The result is a two-slide sandwich with Vectastain filling with chromosomes in each slide sharing a common Vectastain. Incubate the "slide sandwiches" at 37°C in a moist chamber for 30 minutes.
12. Separate slides in PBS and wash in PBS twice, for 5 minutes each.
13. Make up a fresh solution of DAB (3,3' diaminobenzidine tetrahydrochloride grade II, available from Sigma, Cat. No. D-5637) at a concentration of 0.7 mg/ml in 0.05 M Tris pH 7.6 that has been pre-warmed to 37°C. Immediately before use add 10 ul of 30% hydrogen peroxide for each 5 ml of reagent. (CAUTION: DAB IS A CARCINOGEN - WEAR GLOVES. Also, it is an unstable compound at room temperature, and it is photo-sensitive in base form. Take necessary precautions to avoid exposing the solution to direct light, especially sun light -- we dissolve it in dark room with constant stirring for about a minute, and stir additional several seconds while hydrogen peroxide is added.)

A recommended practice:

Dissolve about 0.21 to 0.22 g of DAB in 300 ml of 50 mM Tris, pH 7.6 in a staining dish with magnetic stirrer. Make sure that DAB is completely dissolved. Then add 600 ul hydrogen peroxide. We freeze hydrogen peroxide in 1 ml aliquots at -70°C.

DAB and contaminated glassware can be deactivated by bleach.

14. Place slides in DAB solution and incubate for 15 to 20 minutes at 37°C.
15. Wash slides in PBS for 5 minutes and rinse well in distilled water and air dry.

16. Stain with Giemsa stain (1% solution in 50 mM phosphate buffer, pH 7.0***) for 5 minutes. Rinse well in distilled water and dry in vacuum for about 30 minutes. Clear the slides by dipping them in xylene for a second or two and mount a coverslip with euparal. Make the slides permanent by warming them at 50°C for several hours to overnight. Study with a phase contrast microscope. Chromosome bands will show up as light sky blue color, strongly labeled bands will show up as brown-black color while weak labels show up as faint brown-colored bands. If the slides are to be studied with brightfield optics, stain the slide in 5% Giemsa for 10 minutes, then proceed with washing and mounting as recommended above.

A recommended practice:

*** 50 mM phosphate buffer, pH 7.0

To make 1,000 ml, mix 850 ml distilled water, 125 ml of 0.2 M Na_2HPO_4 , and 25 ml of 1 M NaH_2PO_4 . Then adjust pH, if necessary.

Additional Comments and Recommendations

- 1, a. Stainless steel needles for dissection:

A surgical needle fastened into the end of a 1/4-inch-diameter dowel about 5 inches in length serves as an excellent needle for dissection.

- 1, d. A method for transferring the gland cells without membrane:

Gently push the glands without membranes onto the tip of a dissecting needle with another needle. Bring the needle tip with the glands to the surface of the 45% acetic acid with a quick motion, remove the needle with the gland cells from the acid. Then transfer them to the fixative. Transfer the glands to the fixative soon after (in a minute or two) the membrane has been removed because the cells start to separate from each other quickly in many of the glands. In other glands, cells do not separate.

- 1, f. The slides can be stored in ethanol at -70°C for more than a month. However, slides kept for a long time (more than about two weeks) do not hybridize as well as those kept for only a few days. It is best to use (hybridize) them as soon as you can. The chromosomes for hybridization are like eggs -- the fresher the better.

2. The purpose of heat treatment:

The heat treatment of the chromosomes is an important step in stabilizing the structure of bands (chromomeres). A prolonged heat treatment yields chromosomes with excellent banding pattern, but difficult to hybridize with biotinylated-DNA probes. Chromosomes that have been heat treated for two hours retained an excellent banding pattern, but failed to show good hybridization signals in two tries.

11. A method for tapping PBS:

Firmly hold a corner of the slide where there are no chromosomes and shake it three or four times in the air. You will still have a thin film on the slide, especially in the area where the chromosomes are present, but this small amount will not affect the outcome.

13. A method for preparing DAB solution:

Place 300 ml of 50 mM Tris that has been pre-warmed to 37°C in a staining dish, and place it on a magnetic stirrer. While the Tris solution is being stirred, add 0.21 to 0.22 g of DAB. Continue stirring for about one minute, add hydrogen peroxide, stir for a few minutes, then place your slides for incubation. The solution should be almost colorless. A deteriorated DAB will yield a solution with a light salmon color.

16. The source of Giemsa stain and euparal:

Bio/medical Specialties, P.O. Box 1687, Santa Monica, CA 90406. Tel: (310) 454-1995

Giemsa stain Gurr's improved R66 Giemsa's stain. It comes in 100 ml, 500 ml, or in 1,000 ml bottle.

Euparal It comes in 250 ml bottle.

Xylene treatment: Xylene removes Giemsa stain. It is best not to keep the slides in xylene for more than a few seconds. You may, of course, destain your over-stained slides with xylene.

Acknowledgments: I thank Bibba Goode of the Laboratory of Genetics, NIEHS, for her protocol, which served as the beginning material to arrive at this version. I also thank Todd Laverty and Dena Johnson-Schlitz for useful suggestions and discussions.

Manock-Wehrman, R., R.D. Seager and V.S. Berg.
Department of Biology, University of Northern Iowa,
Cedar Falls, Iowa 50614. A behavioral apparatus with
low humidity for determining temperature preferences
of *Drosophila*.

We describe an apparatus which allows the determination of behavioral responses of *Drosophila* to a temperature gradient when the relative humidity is low but ecologically reasonable (17-21%). It is a modification of the apparatus described by Hostert et al. (1987). The temperature gradient (Barbour and Racine,

1967) is created along an aluminum bar (110 cm long, 48.5 cm wide, and 0.6 cm thick) by placing its ends in cold (-2°C with antifreeze) and hot (40°C) water reservoirs. The behavioral apparatus is placed directly on the temperature gradient bar.

The behavioral apparatus is made of sealed 1/4 inch acrylic, is 65 cm long, 11.5 cm wide and 8.5 cm high and is without a bottom (Figure 1; the vertical dimension in this figure is exaggerated in order to allow detail to be seen). All internal acrylic surfaces are covered with clear Teflon film to prevent water from diffusing from the acrylic into the apparatus. The apparatus is divided into six compartments (internal dimensions of each are 10 cm long, 10 cm wide, 1 cm high). The compartments can be separated by 1/8 inch acrylic partitions (10 cm wide, 6 cm high with additional 4 cm handles). The handles extend through slits in the top of the apparatus to the outside, thus allowing the partitions to be raised or lowered from the outside.

Lids (2 cm high, 9.3 cm wide, 9.5 cm long) of each compartment have acrylic sides and nylon mesh bottoms. Insulating window seal foam on the sides of the lids seals them in place. Lids are supported by small acrylic blocks in the corners of the compartments (Figure 1).

Teflon coated trays (9.3 cm, 6.3 cm, 2.0 cm high) of 1/4 inch acrylic sides and 1/8 inch acrylic bottoms are filled with desiccant and placed on top of the lids. Indicating Drierite is used as the desiccant and maintains a relative humidity of 17-21% RH throughout the apparatus. Mini-hygrometers (available from museum supply companies) are placed in the apparatus at each end and in the middle and are used to measure relative humidity. Thermocouples are placed at each end of the apparatus and between the compartments to measure temperature. They are shielded from direct radiation by aluminum foil. We use a temperature range of 14°C to 32°C in the apparatus. Difference in temperature from the top of each compartment to the gradient bar is less than one degree.

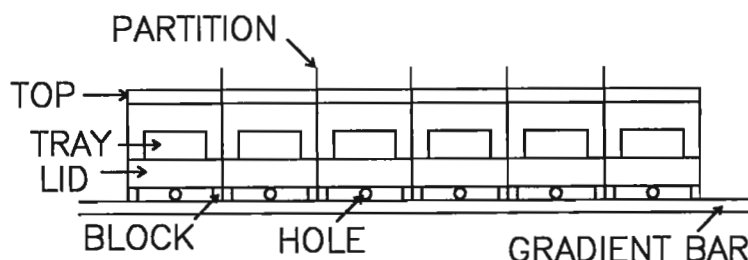
The top of the apparatus is a sheet of 1/4 inch acrylic (11.5 cm long, 65 cm wide) with insulating window seal foam to seal it to the acrylic frame using clear weights on the top. Space above the desiccant trays below the top allows for free air circulation.

We introduce twenty flies into each closed compartment through a 0.6 cm diameter hole drilled into the outside frame in the center of each compartment, below the compartment lids. After introducing the flies into the closed compartments, the holes are plugged with rubber stoppers (size 000). The partitions are raised and the flies are permitted to move freely within the apparatus for two hours. The partitions are then lowered, separating the flies based on their temperature preferences. The top of the apparatus and the desiccant trays are removed and the lid to each chamber is carefully removed so flies can be aspirated and counted.

Only flies of the same sex and age are tested at one time to minimize behavioral differences. To minimize physiological differences, flies are kept in controlled temperature (20°C) and humidity (35% RH) growth chambers set on 24 hour light cycles. All testing is done at the same time of day to minimize the effects of circadian cycles.

Acknowledgments: We wish to thank J. Cunningham, J. Trainer and P. Whitson for help with designing this apparatus.

References: Barbour, M.G. and C.H. Racine 1967, *Ecology* 48:861-863; Hostert, E.E., R.D. Seager and V.S. Berg 1987, *DIS* 66:161-162.



Reuter, G.¹, G. Hoffmann², R. Dorn¹, J. Gausz² and H. Saumweber³. ¹Institut für Genetik, Martin-Luther-Universität, Halle, Germany; ²Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary; ³Institut für Entwicklungsphysiologie, Universität Köln, Germany. Construction and characterization of a *TM3* balancer carrying *P[(ry⁺)delta2-3]* as a stable transposase source.

In order to have a third chromosomal efficient balancer carrying a genetically stable P element transposase source for genetic manipulation of P transposons, the *P[(ry⁺)delta2-3](99B)* element (Robertson *et al.*, 1988) has been introduced into a *TM3,ry^{rk}Sb e* chromosome. The *ry^{rk}* mutation (X ray induced onto *TM3* by B. Wakimoto, unpublished) has been used to select for a rearrangement of *P[(ry⁺)delta2-3]* into this balancer chromosome after X ray treatment.

Females of the constitution $+/TM3,ry^{rk}Sb e/ry^{506}P[(ry^{+})delta2-3](99B)$ were irradiated with 4,000R X rays and crossed to *ry⁵⁰⁶/ry⁵⁰⁶* males. In 34,335 *TM3,ry^{rk}Sb e/ry⁵⁰⁶* (*ry Sb*) offspring (hatched from eggs laid after the fifth day post irradiation) two *ry⁺Sb* exceptional flies were isolated and stocks heterozygous with multiply marked third chromosomes were established. The *Sb ry⁺* exceptional chromosomes carry the recessive markers *ri* and *e*. They balance effectively the third chromosome. Cytological examination of both exceptional strains proved that the *TM3* balancer chromosome was present which had no new visible rearrangements. The *ry⁺* function is absolutely linked to *Sb*. No differences were found in the frequency of losses or putative jumps of *ry⁺* compared to the original *ry⁵⁰⁶Sb P[(ry⁺)delta2-3](99B)* chromosome (Table 1). In the stocks constructed $+/TM3,ry^{rk}Sb e P[(ry^{+})delta2-3](99B)-2/ry^{506}Df(3R)C7$ and $w^{m4h}; TM3,ry^{rk}Sb e P[(ry^{+})delta2-3](99B)-2/Ly$ the *P[(ry⁺)delta2-3]* element proved to be stably inherited with the *TM3,ry^{rk}Sb e* chromosome and no loss was found.

Table 1. Frequency of losses and jumps of *ry⁺* in the *TM3,ry^{rk}Sb e P[(ry⁺)delta2-3]* chromosomes. Results of crosses of *TM3,ry^{rk}Sb e P[(ry⁺)delta2-3]/ry⁵⁰⁶ x *ry⁵⁰⁶/ry⁵⁰⁶**

| Experiment | Offspring | | Jumps | Losses |
|--|--------------------------|--------------------------------------|--------------------------------------|-----------------------------|
| | <i>Sb ry⁺</i> | <i>Sb⁺ ry⁻</i> | <i>Sb⁺ ry⁺</i> | <i>Sb ry⁻</i> |
| <i>ry⁵⁰⁶/ry⁵⁰⁶ x ry⁵⁰⁶Sb P[(ry⁺)delta2-3]/ry⁵⁰⁶ (control)</i> | 1,738 | 978 | 5 (1.8 x 10 ⁻³) | — |
| <i>ry⁵⁰⁶/ry⁵⁰⁶ x TM3,ry^{rk}Sb e P[(ry⁺)delta2-3](99B) - 1/ry⁵⁰⁶</i> | 231 | 263 | — | — |
| <i>TM3,ry^{rk}Sb e P[(ry⁺)delta2-3](99B) - 1/ry⁵⁰⁶ x ry⁵⁰⁶/ry⁵⁰⁶</i> | 152 | 162 | — | — |
| <i>ry⁵⁰⁶/ry⁵⁰⁶ x TM3,ry^{rk}Sb e P[(ry⁺)delta2-3](99B) - 2/ry⁵⁰⁶</i> | 665 | 605 | — | 1 (7.9 x 10 ⁻⁴) |
| <i>TM3,ry^{rk}Sb e P[(ry⁺)delta2-3](99B) - 2/ry⁵⁰⁶ x ry⁵⁰⁶/ry⁵⁰⁶</i> | 231 | 263 | 1 (2.0 x 10 ⁻³) | — |

Table 2. Transposase activity in somatic cells of a the genotype $w/Y; P(w:ry^{A-R})038/TM3,ry^{rk}Sb e P[(ry^{+})delta2-3]$ (Control: $w/Y; P(w:ry^{A-R})038/ry^{506}Sb P[(ry^{+})delta2-3]$).

| Experimental Cross | Offspring males | | | |
|---|-----------------|-----------------|------------|--------------|
| | Control | Sb ⁺ | Sb | |
| | no mosaics | with mosaics | no mosaics | with mosaics |
| $w, P(w:ry^{A-R})038 / P(w:ry^{A-R})038 x ry^{506}Sb P[(ry^{+})delta2-3] / TM6$ (Control) | 505 | — | — | 621 (55.1%) |
| $w, P(w:ry^{A-R})038 / P(w:ry^{A-R})038 x +/Y; TM3,ry^{rk}Sb e P[(ry^{+})delta2-3](99B) - 1/ry^{506}$ | 106 | — | — | 69 (39.4%) |
| $w, P(w:ry^{A-R})038 / P(w:ry^{A-R})038 x +/Y; TM3,ry^{rk}Sb e P[(ry^{+})delta2-3](99B) - 2/ry^{506}$ | 107 | — | — | 97 (47.5%) |

The transposase activity of the *P[(ry⁺)delta2-3]* element in the *TM3,ry^{rk}Sb e* chromosome was tested using the *w⁺* P transposons *P(w:ry^{A-R})038* and *P[(w)A^R]923* (Hazelrigg *et al.*, 1984) or with the P insertional mutations *sn^w* (Engels, 1984). *P(w:ry^{A-R})038* is inserted into euchromatin whereas *P[(w)A^R]923* is inserted into 2R heterochromatin and shows a strong white position-effect variegation.

The results presented in Tables 2-4 clearly demonstrate that the two *TM3,ry^{rk}Sb e P[(ry⁺)delta2-3]* chromosomes carry an efficient P transposase source.

In situ hybridization with *ry* probes revealed in both *TM3,ry^{rk}Sb e P[(ry⁺)delta2-3]* chromosomes signals in 88D and 99B (Figure 1). Therefore, we concluded that the *P[(ry⁺)delta2-3]* element in both chromosomes is at the same site

as it was originally in the γ^{506} *Sb P[(γ^+)delta2-3]* chromosome at 99B. On Southern blots (*Bam*HI digested DNA) using *p 25.1* as a probe, only a single fragment with P element homology is found indicating that the strain is free of any other P elements. Plots (after *Xho* digestion of DNA) with a 7.3 *Hind*III γ^+ DNA fragment showed no differences in flanking sequences between the *TM3, γ^{RK} Sb e P[(γ^+)delta2-3]* chromosomes. Therefore, it is concluded that the *P[(γ^+)delta2-3]* element in both *TM3, γ^{RK} Sb e P[(γ^+)delta2-3]* chromosomes probably was recombined into the *TM3* chromosome by an X ray induced reciprocal exchange.

The chromosomes already have been successfully used for a genetic analysis of third chromosomal P transposon induced mutations (suppressors and enhancers of position-effect variegation) as well as for mobilization of enhancer trap elements in both females and males (Dorn *et al.*, in preparation).

A sample of the $+$; *TM3, γ^{RK} Sb e P[(γ^+)delta2-3](99B)-2/Df(3-R)C7* and w^{m4h} ; *TM3, γ^{RK} Sb e P[(γ^+)delta2-3](99B)-2/Ly* the *P[(γ^+)delta2-3]* was sent to the Bloomington *Drosophila* Stock Center.

References: Engels, W.R. 1984, *Science* 226: 1194-1196; Hazelrigg, T., R. Levis and G.M. Rubin 1984, *Cell* 36: 469-481; Robertson, H.M., C.R. Preston, R.W. Phillis, D. Jonson-Schlitz, W.K. Benz and W.R. Engels 1988, *Genetics* 118: 461-470.

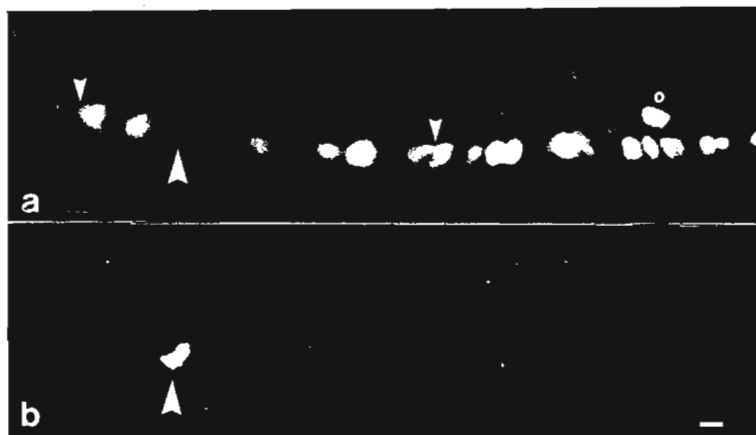
Table 3. Test of transposase activity in w/Y ; *P[(w) Δ^R] 923 / *TM3, γ^{RK} Sb e P[(γ^+) $\Delta 2-3$]* males*

| Experimental Cross | Offspring males | | | |
|--|-----------------|--------------|------------|--------------|
| | Control Sb+ | | Sb | |
| | no mosaics | with mosaics | no mosaics | with mosaics |
| Control | 613 | — | 572 | 47 (7.6%) |
| $w, P[(w)\Delta^R] 923 / P[(w)\Delta^R] 923 \times \gamma^{506} Sb P[(\gamma^+) \Delta 2-3] / TM6$ | 130 | — | 100 | 12 (1.2%) |
| $w, P[(w)\Delta^R] 923 / P[(w)\Delta^R] 923 \times +/Y; TM3, \gamma^{RK} Sb e P[(\gamma^+) \Delta 2-3] (99B) - 1 / \gamma^{506}$ | 133 | — | 111 | 14 (1.1%) |

Table 4. Test of transposase activity in males of the genotype $\gamma^{snW}/Y; \gamma^{506}/TM3, \gamma^{RK} Sb e P[(\gamma^+) \Delta 2-3]$ originated by a cross of $\gamma^{snW}; \gamma^{506}$ females with $+/Y; TM3, \gamma^{RK} Sb e P[(\gamma^+) \Delta 2-3] / \gamma^{506}$ males.

| Experimental Cross | Offspring males | | | |
|--|-----------------|--------------|------------|--------------|
| | Control Sb+ | | Sb | |
| | no mosaics | with mosaics | no mosaics | with mosaics |
| Control: $\gamma^{snW}/Y; \gamma^{506}/\gamma^{506} \times \gamma^{506} Sb P[(\gamma^+) \Delta 2-3] / TM6$ | 296 | — | 2 | 201 |
| $\gamma^{snW}/Y; \gamma^{506}/\gamma^{506} \times \gamma^{506} Sb P[(\gamma^+) \Delta 2-3] / \gamma^{506}$ | 63 | — | — | 54 |

Figure 1. In situ localization of *P[(γ^+)delta2-3]* on polytene chromosomes. A, DNA staining with Hoechst dye-. 3R telomeric and indicated by a circle-. The limits of numbered subdivisions 90 and 100 are shown by small arrow heads. B, Hybridization signal visualized by rhodamine-labeled streptavidin-. Bar 2 μ m.



Simmons, M.I. and E.A. Drier*. Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108-1095, USA. *Present address: Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA. An apparatus for the microinjection of *Drosophila* embryos.

their progeny are examined for genetic transformants.

Many researchers perform the injections with a micromanipulator and an inverted microscope in a room kept at 18°C. We use a different set up that is less costly and more comfortable for the researcher, but that still gives excellent results.

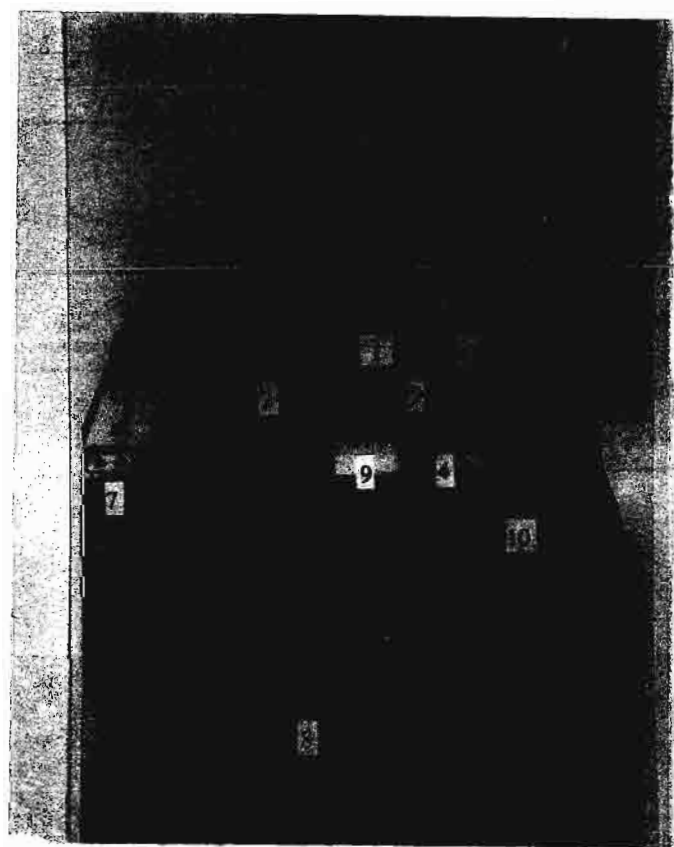


Figure 1. Injection apparatus. (1) stereozoom microscope, (2) fiber optic illuminator, (3) power stand, (4) micromanipulator, (5) microvise, (6) microinjector, (7) control box for power stand and illuminator, (8) foot pedal focus controls, (9) stage (metal block on top of wooden platform), (10) metal plate set in table, (11) column stand with magnetic chuck.

Genetic transformation with transposable element vectors has become a standard technique in *Drosophila* research. To date, two transposons, *P* and *hobo*, have been developed for use as transformation vectors. In a typical experiment, the transposon DNA is injected through a fine capillary needle into the posterior pole of pre-blastoderm embryos. Injected animals that survive to the adult stage are mated to a standard stock, and

Our apparatus (Figure 1) is set up on a sturdy wooden table in a room kept at 20-21°C. A standard stereo-dissecting microscope with 20X eye pieces and a 1-4X zoom objective has been attached to a vertical power stand so that the microscope can be focused by foot pedal controls. This convenient feature frees the researcher's hands for micromanipulations with the embryos. The microscope stage is a simple platform constructed from several layers of plywood (4 in x 4 in x 3/8 in) that have been glued together and fastened to the table by stout screws. The top of the stage is a removable metal block with a smooth, painted (yellow) surface. It is illuminated by a cool, fiber optic lamp fixed to the end of a flexible goose-neck.

The micromanipulator is attached to a column that is fastened by a magnetic chuck to a metal plate (10 in x 12 in x 1 in) that has been screwed to the underside of a window cut in the table top. This arrangement offers flexibility in the placement of the micromanipulator relative to the microscope stage. The injection needle is set in the front end of a microvise mounted in the micromanipulator. The back end of the microvise is connected by a luer-link to plastic tubing, which is, in turn, connected by another luer-link to a glass barrel syringe partially filled with water. The syringe is held in a microinjector with a set screw that allows fine control over the position of the plunger. Depressing the plunger transmits pressure through the tubing to the microvise and into the injection needle, causing fluid to flow out the tip.

Table 1. Items in injection apparatus.

| | |
|---|---------|
| Wooden table | donated |
| Stereozoom microscope | \$ 850 |
| 20X eyepieces | \$ 280 |
| Bonding arm | \$ 190 |
| Power stand with power box and double foot switch | \$ 1790 |
| Fiber optic illuminator | \$ 269 |
| Micromanipulator | \$ 650 |
| Column stand on magnetic chuck | \$ 110 |
| Microvise | \$ 28 |
| Microinjector with tubing, syringe and two luer connections | \$ 145 |
| Metal plate | \$ 20 |
| Wooden stage | \$ 10 |
| Metal block | \$ 5 |
| Capillary tubes | \$ 42 |
| Total | \$ 4389 |

Needles are pulled from omega dot capillary tubes (1.0 mm OD x 0.75 mm ID) that have been cut in half and are loaded with DNA solution from the back. The inner fiber in these capillary tubes facilitates movement of the solution to the needle's tip. We add sterile blue food coloring to the solution to make it easier to see. After loading, the back end of each needle is closed with dab of halocarbon oil to prevent water from the syringe from entering and diluting the DNA. A loaded needle is set in the microvise and locked in place by screwing down the collar around it. Water is drawn up into the plastic tubing from a beaker by depressing the plunger in the syringe all the way, and then allowing the plunger to return to its resting position. The free end of the tubing is then attached to the back end of the microvise, bringing the standing pressure in the syringe to bear on the DNA solution in the tip of the needle.

We break the tip of the needle by gently pushing it into the side of the tape on the microscope slide that carries the embryos prepared for injection. Usually only a tiny piece breaks off and fluid flows immediately from the tip. The flow rate can be determined by injecting a few embryos; if it is too slow, more pressure can be applied by turning the set screw on the syringe barrel. If it is too fast, the aperture of the needle is too wide and a new needle with a better tip must be installed.

Embryos are collected on fruit juice/agar/live yeast petri plates during 15-30 min egg-laying sessions. After collecting, we chill the plates at 4°C for 20-30 min to make the embryos more rigid; embryos treated in this way are easier to handle, especially during the dechorionation process. The embryos are transferred to a thin band of double-stick tape that is slightly indented from the long edge of half a microscope slide. We use water and a paint brush to wash the embryos from the collecting plate into a black cloth sieve, and then pick the embryos off the sieve and onto the microscope slide with a metal dissecting needle. The posterior of each embryo points to the edge of the slide. The embryos are dechorionized by rolling them over the tape on the slide. Some researchers have found that this step is unnecessary as long as the injection needle is sturdy enough to puncture the chorion. Dechorionated embryos are desiccated in a box with drying chips at 4°C for about 5 min, or until the vitelline membrane just begins to wrinkle, and then covered with a thin film of halocarbon oil. Injection follows immediately. Because the stage of our apparatus is not equipped with a caliper, we position the embryos for injection by moving the slide manually. This requires a steady left hand. The micromanipulator is adjusted with the right hand. It is usually not necessary to change the height of the injection needle, which we keep essentially horizontal, so the main adjustment is to move the needle in and out as the embryos come into alignment. The stereomicroscope provides excellent depth perception, so the entire process is viewed in three dimensions. A magnification of 70-80X is quite adequate.

After injecting, a thin strip of culture medium is set alongside the embryos to provide food for the hatching larvae and to soak up excess halocarbon oil. Each slide is put on moistened blotting paper in the bottom of a large plastic petri dish, which is, in turn, put in a sealed tupperware container to maintain high humidity. The container is incubated at 25°C and hatched larvae are picked from the slides into vials at daily intervals. We put 10-20 larvae into a vial and collect hatched adults from the vial as virgins. Survival depends very much on the injection technique, but typically 10-30% of the injected embryos reach the adult stage, and the vast majority of these are fertile.

Table 1 summarizes the items in our apparatus, along with estimated costs. The total is less than what one would pay for most inverted microscope systems, so researchers on limited budgets might find our set up very attractive. It is simple and comfortable to use and gives excellent results.

Research Notes

Coyne, J.A. Department of Ecology and Evolution, The University of Chicago, 1101 E. 57th St., Chicago, IL 60637. Absence of a substantial Y-chromosome effect on male sterility in the *D. simulans*/*D. sechellia* hybridization.

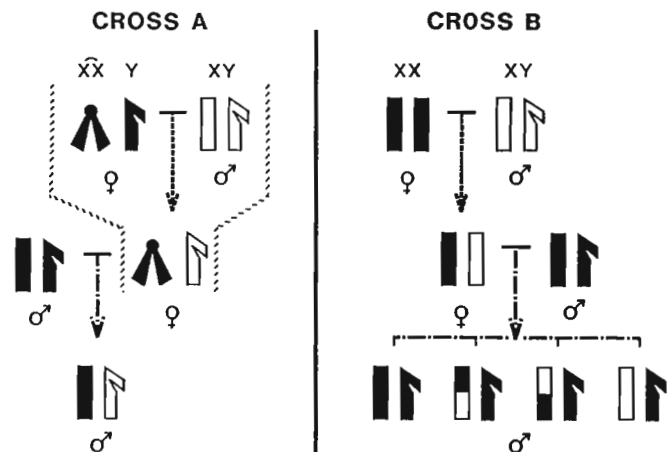
(F_1 females have one X chromosome and one autosome from each species, but F_1 males have one set of autosomes lacking a corresponding X); and interspecific X-Y interaction (sterility may be caused by deleterious interactions between X chromosomes from one species and autosomes from another).

In the hybridization between *D. mauritiana* and *D. simulans*, Coyne (1985) showed that sterility of males was not due to X-autosome imbalance: F_1 hybrid females having the same X-autosome imbalance as sterile F_1 males were nevertheless perfectly fertile. Using a backcross of F_1 females to *D. simulans*, Coyne showed that males having heterospecific X and Y chromosomes were far less fertile than those with homospecific chromosomes, even though the latter class had a greater discrepancy between the X chromosome and the autosomes. In this hybridization, then, male sterility was apparently due largely to X-Y interactions.

Here we repeat these experiments using *D. sechellia* instead of *D. mauritiana*. *D. sechellia*, like *D. mauritiana*, is a sibling species of *D. simulans*, differing from these species only by the shape of the male genital arch. It is found only on the Seychelles Islands in the Indian Ocean. Like *D. mauritiana*, its hybrids with *D. simulans* are sterile as males and fertile as females. We performed the two backcrosses shown in Figure 1, using a *D. simulans* strain from Oxnard, California, the attached-X strain of *D. simulans* (these two strains are identical to those used by Coyne, 1985) and the *D. sechellia* isofemale strain described by Tsacas and Bächli (1981).

Cross A produces males with an intact *D. simulans* X chromosome, a *D. sechellia* Y chromosome, a mixture of autosomes from each species (on average, 3/4 from *D. simulans* and 1/4 from *D. sechellia*), and cytoplasm from F_1 hybrid mothers. Cross B produces males with autosomes and cytoplasm identical to those in cross A, but with a mixture of X chromosomes from each species (on average, 50% *D. simulans* and 50% *D. sechellia*), and a Y chromosome from *D. simulans*. The male offspring from cross B are known to be highly sterile, as the *D. sechellia* X chromosome carries at least 3 loci causing male sterility in this backcross (Coyne and Charlesworth, 1989). Because of this, class B is expected to show at least twice the sterility of class A. If, however, X/Y interaction makes a large contribution to male sterility, the proportion of sterility in cross A is expected to be more than half that seen in class B. (This was observed in the cross of *D. simulans* X *D. mauritiana* backcross, with the proportion of sterile males 0.99 and 0.90 respectively [Coyne, 1985]).

Figure 1. Crosses to ascertain the effect of X-Y interaction on male sterility. Cross A: attached-X *D. simulans* females (with sex chromosomes shown in black) are crossed to *D. sechellia* males (sex chromosomes in white), and the F_1 hybrid females backcrossed to *D. sechellia* males. Cross B: *D. simulans* females (sex chromosomes shown in black) are crossed to *D. sechellia* males (sex chromosomes in white), and the F_1 females backcrossed to *D. sechellia* males. Offspring from both backcrosses are shown at the bottom. All males from cross A have heterospecific X and Y chromosomes, those from cross B have greater compatibility between the sex chromosomes.



Both crosses were made at 23°C; backcross males were stored for four days and their testes examined for motile sperm as described by Coyne (1985).

Of 317 backcross males examined from cross A, 97 lacked any motile sperm, giving a proportional sterility of 0.305. Of 649 males examined in cross B, 403 lacked motile sperm, a sterility of 0.621. The higher sterility of males from cross B, approximately twice that of cross A, is what is expected if sterility is due to deleterious interactions between the X chromosome and the autosomes. Unlike the *D. simulans*/*D. mauritiana* cross, however, there is no obvious effect of the Y chromosome.

The lack of Y-effects on male fertility has also been reported by Orr (1989) for the *D. pseudoobscura* USA/*D. pseudoobscura* Bogotana hybridization, and by Orr and Coyne (1989) in *D. virilis*/*D. novamexicana* hybrids. Y-effects have, however, been seen in *D. pseudoobscura*/*persimilis* (Orr 1987) and *D. virilis*/*D. texana* hybrids (Orr and Coyne, 1989). Clearly, there is no consistent effect of the Y chromosome across all hybridizations that produce sterile males, so, as noted by Coyne and Orr (1989) the X/Y interaction hypothesis for Haldane's rule is probably not a general explanation. The lack of consistent Y-effects also militates against meiotic-drive hypotheses for male sterility, which propose that postzygotic isolation results from divergence of meiotic drive genes between the sex chromosomes in related species (Frank, 1991; Hurst and Pomiankowski, 1991).

References: Coyne, J. A. 1985, Nature 314:736-738; Coyne, J.A. 1992, Nature 335: 511-515; Coyne, J.A. and B. Charlesworth 1989, Heredity 62:97-106; Coyne, J.A. and M. Kreitman 1986, Evolution 40:673-691; Frank, S. 1991, Evolution 45:262-267; Hurst, L.D. and A. Pomiankowski 1991, Genetics 128:841-858; Orr, H.A. 1987, Genetics 116:555-563; Orr, H.A. 1989, Evolution 43:180-189; Orr, H.A. and J.A. Coyne 1989, Genetics 121: 527-537; Tsacas, L. and G. Bächli 1981, Rev. Fr. Entomol. 3:146-150.

Kim, Dae Il. Department of Biology, Mok-won University, Taejon 301-729, South Korea. P factor activity for flies with *In(2L)t* in natural populations of *Drosophila melanogaster*.

The distribution of P elements on the chromosomes and the cytotype polymorphism from hybrid dysgenesis resulting by the interaction of P - M system has been investigated by many workers in natural populations of *Drosophila melanogaster*. The results of this study were to observe the degree of P factor activity

of flies with *In(2L)t* from hybrid dysgenesis. These populations were mainly M' and Q strains, but true M strain was at a very low frequency, P (moderate) strain was nearly absent. The M' and Q strains of flies with *In(2L)t* were at slightly higher frequencies than flies without *In(2L)t*.

Materials and Methods: The collection of wild strains of *Drosophila melanogaster* was performed at two Taejon locations in Korea: Pan-am dong (vineyard) and Yu-sung (apple orchards) between early September and mid-November, 1990 and 1991. Two employed strains, the standard M strain Canton-S and a strong P strain π_2 , were used to investigate GD sterility of the wild strains. Wild collected males were used immediately for crosses with the M strain females. The females inseminated in the wild to make isofemale lines were transferred to vials containing medium. These lines were kept at room temperature $25 \pm 1^\circ\text{C}$ and humidity $65 \pm 1\%$.

The salivary gland cells of an F_1 larva from each isofemale line were selected randomly. The salivary chromosomal preparations were made by the Lactic-Acetic-Orcein methods using siliconized slides and then cover slides were sealed with nail polish. The salivary chromosomes were observed with a BH₂ Olympus microscope for the presence of heterozygous inversions. The standard chromosomal map of Bridges (1935) and revised map of Lefevre (1976) were employed to identify the break points of the chromosomal inversion.

Reciprocal crosses with P or M strains were used to assay GD sterility of unknown wild strains: Cross A, M (Canton-S) females x U (unknown) male; Cross A', U female x P(π_2) males. These crosses were cultured at 29°C. Each cross was made at 29°C for the seventh day in a vial with medium and parents were discarded. All F_1 flies emerging by the eleventh day were transferred to fresh vials with medium at 25°C. After it matured for four additional days, 24 F_1 females per line to assay gonadal sterility were dissected to detect whether rudimentary ovaries have one or two. The females with two dysgenic ovaries were classified as sterile. According to Kidwell's criteria (1983, 1986), the strains were identified as P, Q, M' and M limiting a cutoff point at 10% (Table 1).

Results and Discussion: The overall mean frequency of gonadal sterility of F_1 daughter following the crosses of wild male with Canton-S females was 0.6% among 14205 from four Taejon populations (428 wild males). Results from wild males of this study were similar to reports of Paik *et al.* (1989) and Kim (1990). The GD sterility frequency of Japanese populations was 0.46% and P factors were absent (Ohishi *et al.*, 1982). In cross A, wild F_1 males tested from 230 TP and 221 TY isofemale lines were 1.03% and 0.94% from sterility frequencies of both populations. All F_1 males tested in both populations were completely absent from GD sterility except for one TY population. Paik *et al.* (1989)

reported that GD sterility of Taegoo and Cheju populations was 0.18% and 0.17%, respectively. In cross A*, 230 TP and 221 TY isofemale lines were tested and the mean sterility frequencies were 38.84% and 38.63%. In Taegoo and Cheju populations, Paik *et al.* (1989) reported that average sterility in both populations was 31.98 ± 2.59 and 32.84 ± 3.39 , respectively.

Table 1. Characteristic values for GD sterility of various categories of strains according to Kidwell's criteria (1983, 1986)

| Strain type | Cross A % GD sterility | Cross A* % GD sterility |
|--------------|---------------------------|----------------------------|
| M (true) | 0 | 100 |
| M' (pseud-M) | 0 - ? | 0 - 100 |
| Q (weak P) | 0 - 10 | 0 - 10 |
| P (moderate) | 11 - 80 | 0 - 10 |
| P (strong) | 81 - 100 | 0 - 10 |

Table 2. Frequency (%) of GD sterility strain tested from two local populations

| Strains Tested | Cross A No. ovaries per female | | | | Cross B No. ovaries per female | | | |
|-------------------|-----------------------------------|------|------|------|-----------------------------------|------|-------|-------|
| | 2 | 1 | 0 | GD | 2 | 1 | 0 | GD |
| TP (90) | 99.15 | 0.25 | 0.60 | 0.96 | 57.70 | 1.64 | 40.66 | 41.52 |
| TP' (91) | 98.90 | 0.24 | 0.86 | 1.10 | 63.84 | 0.90 | 35.25 | 36.16 |
| TY (90) | 98.80 | 0.14 | 1.06 | 1.08 | 59.40 | 2.00 | 38.60 | 39.76 |
| TY' (91) | 99.20 | 0.05 | 0.75 | 0.80 | 62.50 | 0.40 | 37.10 | 37.50 |
| means | 99.01 | 0.17 | 0.82 | 0.99 | 60.86 | 1.24 | 37.90 | 38.74 |

Table 3. Strain identified with GD frequencies in strains tested from two local populations of *D. melanogaster*

| Strains tested | Strains of functional properties for GD sterility | | | | | | (X ²) Chi Square test |
|-------------------|---|--------|--------|--------|--------|------|---|
| | N | M | M' | Q | P(M) | P(S) | |
| TP (90) | 134 | 0.0149 | 0.4179 | 0.5821 | 0.0224 | 0.0 | 4.2423 |
| TP' (91) | 96 | 0.0208 | 0.4167 | 0.5521 | 0.0104 | 0.0 | |
| TY (90) | 108 | 0.0370 | 0.4074 | 0.5370 | 0.0185 | 0.0 | |
| TY' (91) | 113 | 0.0177 | 0.4159 | 0.5664 | 0.0000 | 0.0 | |
| means | 451 | 0.0222 | 0.4145 | 0.5499 | 0.0133 | 0.0 | |

Table 4. Sterility frequencies of flies with *In(2L)t* of hybrid dysgenesis of P - M system

| Site | <i>In(2L)t</i> | | | | | | X ² |
|----------|----------------|-----|--------|--------|-----|--------|----------------|
| | N | M | M' | Q | P | total | |
| TP (90) | 134 | 0.0 | 0.0448 | 0.0672 | 0.0 | 0.1119 | 0.1875 |
| TP' (91) | 96 | 0.0 | 0.0625 | 0.0833 | 0.0 | 0.1458 | |
| TY (90) | 108 | 0.0 | 0.1204 | 0.1389 | 0.0 | 0.2593 | |
| TY' (91) | 113 | 0.0 | 0.0442 | 0.0708 | 0.0 | 0.1150 | |
| means | 451 | 0.0 | 0.0687 | 0.0887 | 0.0 | 0.1574 | |

Table 5. The relative frequencies of strains for GD sterility of flies without *In(2L)t* and flies with *In(2L)t*

| Site | N | | M | | M' | | Q | | P | |
|----------|-----|-------|--------|-----|--------|--------|--------|--------|--------|-----|
| | N | N(ln) | n | ln | n | ln | n | ln | n | ln |
| TP (90) | 134 | 15 | 0.0149 | 0.0 | 0.4179 | 0.4000 | 0.5821 | 0.6000 | 0.0224 | 0.0 |
| TP' (91) | 96 | 14 | 0.0208 | 0.0 | 0.4167 | 0.4286 | 0.5521 | 0.5714 | 0.0104 | 0.0 |
| TY (90) | 108 | 28 | 0.0370 | 0.0 | 0.4074 | 0.4643 | 0.5370 | 0.5357 | 0.0185 | 0.0 |
| TY' (91) | 113 | 14 | 0.0177 | 0.0 | 0.4159 | 0.4286 | 0.5664 | 0.5714 | 0.0000 | 0.0 |
| means | 451 | 71 | 0.0222 | 0.0 | 0.4146 | 0.4366 | 0.5499 | 0.5634 | 0.0133 | 0.0 |

N = tested individuals; ln = frequencies of flies with *In(2L)t*; n = frequencies of flies without *In(2L)t*; TP = Taejon Pan-am dong populations; TY = Taejon Yu-sung populations

Each strain following crosses of cross A* and cross A was classified according to Kidwell's criteria (1983, 1986). Most strains in these populations were determined with M' and Q strains, but the Q strain was always higher in frequency than the M' strain in four populations (Table 3). True M strain was at very low frequencies in four populations and P (moderate) strain was nearly absent (Table 3). Chi-square test (X²) of each strain between four populations was not significantly different (X² = 4.2423, P > 0.05). Boussy and Kidwell (1987) reported that tests of recent collections of *Drosophila melanogaster* from the southeastern coast define the previously described cline as comprising three discrete, apparently contiguous

regions of P, Q and M phenotypes, respectively. Sterility frequency of flies with *In(2L)t* was only M' and Q strains. True M and P strains were not completely observed on flies with *In(2L)t* in these populations is given in Table 4. Relative GD sterility frequency of two in these populations (Table 4). Relative GD sterility frequency of two strains was higher in flies with *In(2L)t* than in flies without *In(2L)t* (Table 5).

References: Bridges, C.B. 1935, J. Heredity 26:60-64; Boussy, I.A. and M.G. Kidwell 1987, Genetics 115:737-745; Kidwell, M.G. 1983, Proc. Natl. Acad. Sci., USA 80:1655-1659; Kidwell, M.G. 1986, In: *Drosophila* (Roberts, D.B., ed.) IRL Press, Oxford, Washington, DC, pp. 70; Kim, D.I. 1990, Korean J. Genetics 12-3:272-280; Lefevre, G. 1976, In: The Genetics and Biology of *Drosophila* (Ashburner, M. and E. Novitski, eds.) Academic Press, London, pp. 31-66; Ohishi, K., E. Takanashi and S.I. Chigusa 1982, Jpn. J. Genet. 57:423-428; Paik, Y.K., M.S. Lyu and C.G. Lee 1991, D.I.S. 70:163-66.

Goles-Fainé, Pedro and Raúl Godoy-Herrera.

Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Casilla 70061, Santiago, Chile. Multigenic control of a phenotype of *Drosophila hydei* similar to *Curly* monogenic mutant of *Drosophila melanogaster*.

Comparisons between mutant phenotypes of *Drosophila* offer an opportunity to establish correspondence between the genomes (Spencer, 1949). It is of interest to compare specific allelic forms in different *Drosophila* species because this could help to understand their phylogenetic origins. Comparisons between some mutants of *Drosophila* help also to know the effects of mutations on the development of each

species, and which organ(s) they act upon. Here we report the phenotype and genetics of a mutation that spontaneously appeared in a culture of *Drosophila hydei*. The strain was started with flies collected in 1988 in Chillán, Chile, Latitude 30°. The phenotype of the mutation reminds us of the *Curly* mutant described by Ward (1920) in *D. melanogaster*.

Description of the mutant. The morphological characteristics of the specimens belonging to this phenotype are as follows (see also Figures 1 and 2): wings curled upward and slightly divergent increasing from proximal to distal. Adult fly size is slightly less than wild type. The phenotype is expressed equally well in both sexes. Crosses between mutants produce an offspring conformed by: i) wild type individuals; ii) mutants similar to parentals; iii) mutants showing a phenotype intermediate between wild type and mutant flies; and iv) more extreme phenotypes characterized because they have rudiments of wings (Figure 2 and Table 1). Viability and fertility reduced, particularly in the more extreme phenotypes which usually die one or two hours post-hatching. Mutants grow up well at 24°C but we were unable to establish cultures at 18°. The cultures show a propensity to infestations by fungus and bacteria. Mutant developmental period is over 10 days longer than that of wild type flies born in the same stock.

Table 1. Percentage of wild type, mutant and intermediate mutant phenotypes of *D. hydei* in different crosses

| Crosses | | | Percentage of each phenotype | | | | | | Total number of flies counted |
|--------------------------|----------------------------|-----------|------------------------------|--------|--------|------------------------|--------|--------|-------------------------------|
| male | female | wild type | | mutant | | intermediate phenotype | | | |
| | | male | female | male | female | male | female | | |
| wild type | x wild type | 49.04 | 50.96 | — | — | — | — | 994 | |
| mutant | x mutant | 6.71 | 6.13 | 25.10 | 18.95 | 21.89 | 21.20 | 3740 | |
| mutant | x wild type* | 48.64 | 42.92 | 1.69 | 1.22 | 2.56 | 2.95 | 2537 | |
| wild type* | x mutant | 49.58 | 42.23 | 1.39 | 1.39 | 2.36 | 3.04 | 2370 | |
| F ₁ mutant | x F ₁ mutant | 2.01 | 1.34 | 36.39 | 28.56 | 15.73 | 15.96 | 1341 | |
| F ₁ wild type | x F ₁ wild type | 5.41 | 3.74 | 38.79 | 44.00 | 3.08 | 4.25 | 1552 | |
| F ₁ mutant | x F ₁ wild type | 33.40 | 32.50 | 11.80 | 8.50 | 6.00 | 7.70 | 1042 | |
| F ₁ wild type | x F ₁ mutant | 27.20 | 24.40 | 13.30 | 9.10 | 14.00 | 11.90 | 143 | |
| wild type | x F ₁ wild type | 49.50 | 46.70 | 0.10 | 0.00 | 2.00 | 1.60 | 880 | |
| F ₁ wild type | x wild type | 48.10 | 43.70 | 0.70 | 0.30 | 3.70 | 3.40 | 952 | |
| mutant | x F ₁ wild type | 29.40 | 30.60 | 12.40 | 11.20 | 8.90 | 7.40 | 938 | |
| F ₁ wild type | x mutant | 28.90 | 25.10 | 13.30 | 10.10 | 11.20 | 11.30 | 457 | |
| Total | | | | | | | | 15,917 | |

* Wild type individuals originated from crosses between wild type flies.

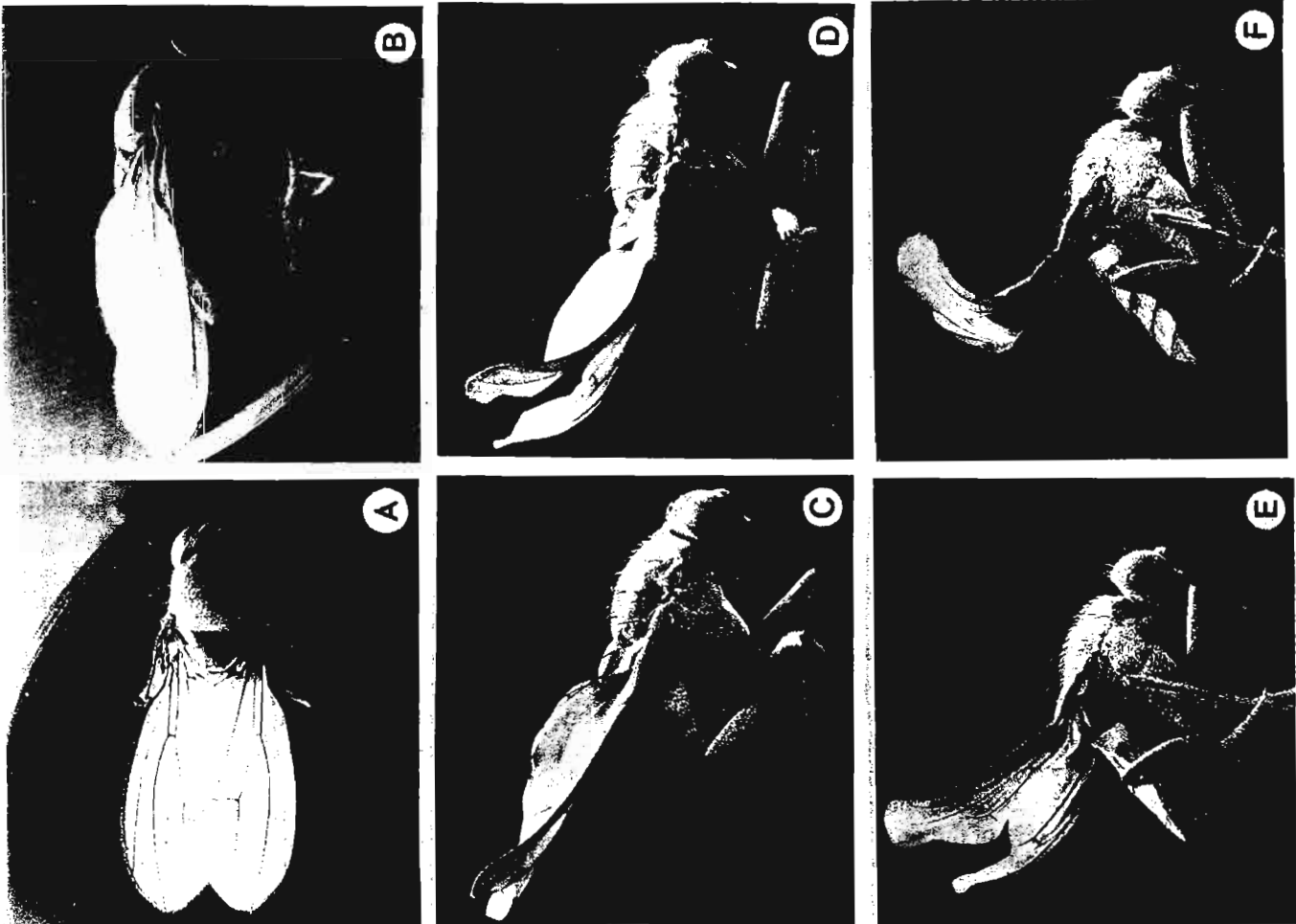
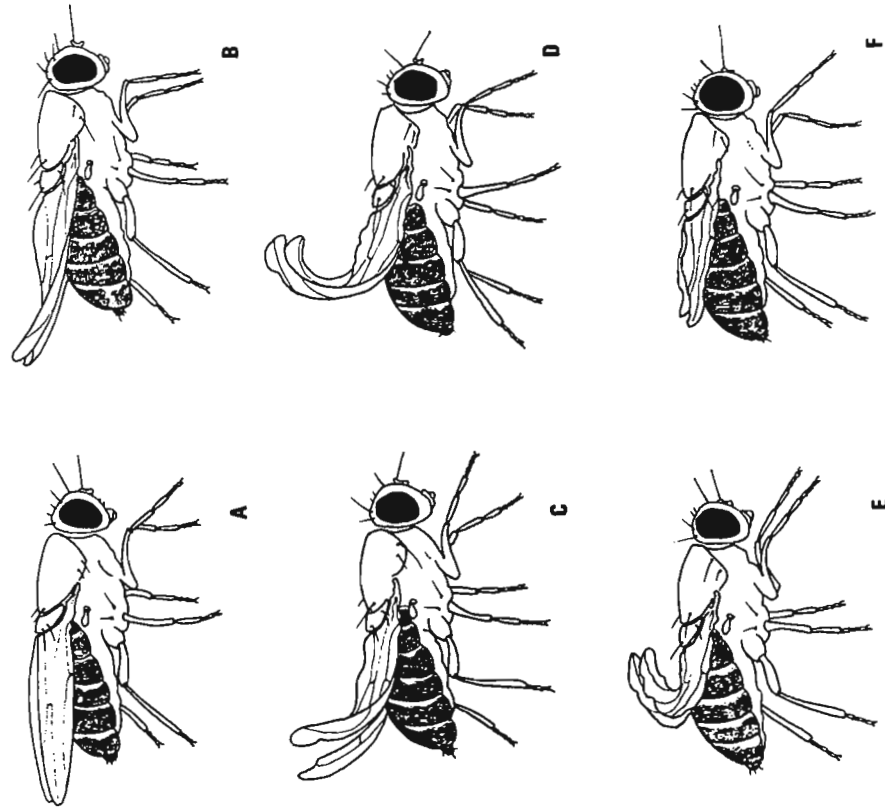
Crosses. Number and kind of crosses done to study the heredity of the mutation are given in Table 1. Two kinds of wild type flies were used as parents: i) wild type flies originated to cross wild type males and females of the original stock (those marked with an asterisk in Table 1); ii) wild type flies obtained in the F₁ generation of crosses between mutant flies (those wild type flies marked F₁ in Table 1).

With the exception of the progeny originated to cross "pure" wild type flies (the first cross in Table 1), the rest of the crosses made show some new kinds of mutants with an intermediate phenotype between that of the primitively found mutants and wild type flies (see Figures 1 and 2 for further details); those mutants are included in Table 1 under the column named "intermediate phenotypes". A fourth group of mutants was also obtained. These showed absence of wings or a primordium of this structure dying one or two hours after eclosion (see Figures 2E and F); they presumably are produced by genetic segregation of more balanced mutant phenotypes. That sort of mutant is not in Table 1 because very few were occasionally found. They emerged about 10 days after the rest of the flies had enclosed, that is, when the culture bottles were very contaminated and the culture medium polluted by excreta of the flies.

As expected, crossing between "pure" wild type flies produce 100% wild type flies (the first cross in Table 1). To cross mutant flies about 75% of the progeny exhibit mutant and intermediate phenotypes, the rest of the flies being wild type (the second cross in Table 1). Crossing between mutant and "pure" wild type flies originates an offspring formed by about 92% of wild type, mutants were about 3% and intermediate phenotypes about 5%. By contrast, to cross the F₁

Figure 1. Scanning electron micrographs illustrating some wing phenotypes in *D. hydei*: A, wild type; B through F, wings showing different degrees of curling. The mutation here reported was initially discovered after finding and crossing flies with wings like those of Figure 1F. Magnification 35x.

Figure 2. Drawings showing wild type and mutant wings in *D. hydei*. Drawings were made on the basis of flies observed under a Zeiss stereomicroscope. A, wild type; B through F, as in Figure 1; E and F, wing phenotypes are exhibited by a very small number of flies which rise out several days after the rest of wing phenotypes A through D (see also text). Magnification 16x.



mutants most of the flies (97%) are mutant or show an intermediate phenotype between the wild type and mutant flies; about 3% of the progeny exhibits a wild type phenotype (Table 1). Similar results were obtained to cross F_1 wild type flies, and F_1 mutant and wild type flies, that is, the progenies show again wild type, mutant and intermediate mutant phenotypes; percentage in the offspring of each phenotype depends on the kind of crossing (Table 1). When F_1 wild type flies are backcrossed with "pure" wild type males and females about 1% of the progeny exhibit a mutant phenotype, 95% are wild type flies and about 4% show an intermediate mutant phenotype. However, offspring obtained to backcross F_1 wild type flies with mutant flies includes about 58% wild type flies, 23% of mutants and over 16% of intermediate phenotypes.

By contrast with the *Curly* mutant of *D. melanogaster* the mutation here described in *D. hydei* seems to be under a polygenic control. For instance, crosses between mutant flies produced some wild type individuals, and crosses between F_1 wild type flies originated some mutant imagines. The mutation here reported is difficult to maintain in the laboratory. The few wild type flies obtained to cross mutants, like those shown in Figures 1E and F, start to increase their frequency per generation up to totally replace the mutant phenotypes. Thus, in each generation it is necessary to separate some mutants which rise the next generation.

In summary, our findings suggest that the mutant phenotypes here described in *D. hydei* do not have a genetic homology with the *Curly* mutant of *D. melanogaster*. This is in contrast with other mutations affecting other organs in *D. hydei* which show genetic homology with *D. melanogaster* (see Spencer, 1949). Thus our results suggest that the genetic program that leads to the development of wings in *D. hydei* is substantially different from that of *D. melanogaster*.

Acknowledgments: We wish to thank L. Milla for her assistance in the preparation of the manuscript. We are also indebted to H. Martínez for his help and advice in the preparation of the figures. This work was supported by the University of Chile (Grant B-2309-8645) and FONDECYT 91-1275.

References: Spencer, W.P. 1949, In: Genetics, Paleontology, and Evolution (Jepsen, G.L., G.G. Simpson and E. Mayr, eds.) Princeton University Press, Princeton, pp. 23-44; Ward, L. 1923, Genetics, 8:276-300 (see also 1985 D.I.S. 62:75-76, for a short description of *Curly* mutant in *D. melanogaster*).

Gupta, J.P. Department of Zoology, Banaras Hindu University, Varanasi 221 005, India. A consolidated list of the Indian species of Drosophilidae.

In this report attempts have been made to include all the species described and recorded from India. However, the present data in no way furnish a complete picture of Indian Drosophilidae since a vast area of the Indian subcontinent still awaits exploration.

A copy of this 12-page list containing 254 species of Drosophilidae can be obtained at no charge by writing to the Editor, Drosophila Information Service, at the address in the front. Please request DIS 72 manuscript file number 1993-14. Copies can also be obtained from the author.

Ivannikov, Andrey B., Ilya K. Zakharov, and Eugeny E. Skibitskiy. Institute of Cytology and Genetics of the Russian Academy of Sciences, Siberian Department, Novosibirsk, 630090, Russia. Synantropic *Drosophila* species in the Ukrain and Moldova.

Drosophila flies were collected in 1990 in the Ukrain. The flies were captured in the factories of wine productions, the conversion of fruits and vegetables, and private gardens. Geographically, the captures were restricted to the cities of Yalta, Zaporozhye, Uman and their vicinities. In the previous years, data had been gathered on the *Drosophila* species residing in the cities

of Kishinev and Odessa.

In the area mentioned we have found eleven species of the genus *Drosophila* from four subgenera:

1. *Drosophila* (*Sophophora*) *obscura*
2. *Dros.* (*Soph.*) *melanogaster*
3. *Dros.* (*Soph.*) *simulans*
4. *Dros.* (*Scaptodrosophila*) *lebanonensis*
5. *Dros.* (*Dorsilopa*) *busckii*
6. *Dros.* (*Drosophila*) *funnebris*
7. *Dros.* (*Dros.*) *immigrans*
8. *Dros.* (*Dros.*) *virilis*
9. *Dros.* (*Dros.*) *hydei*
10. *Dros.* (*Dros.*) *mercatorum*
11. *Dros.* (*Dros.*) *repleta*

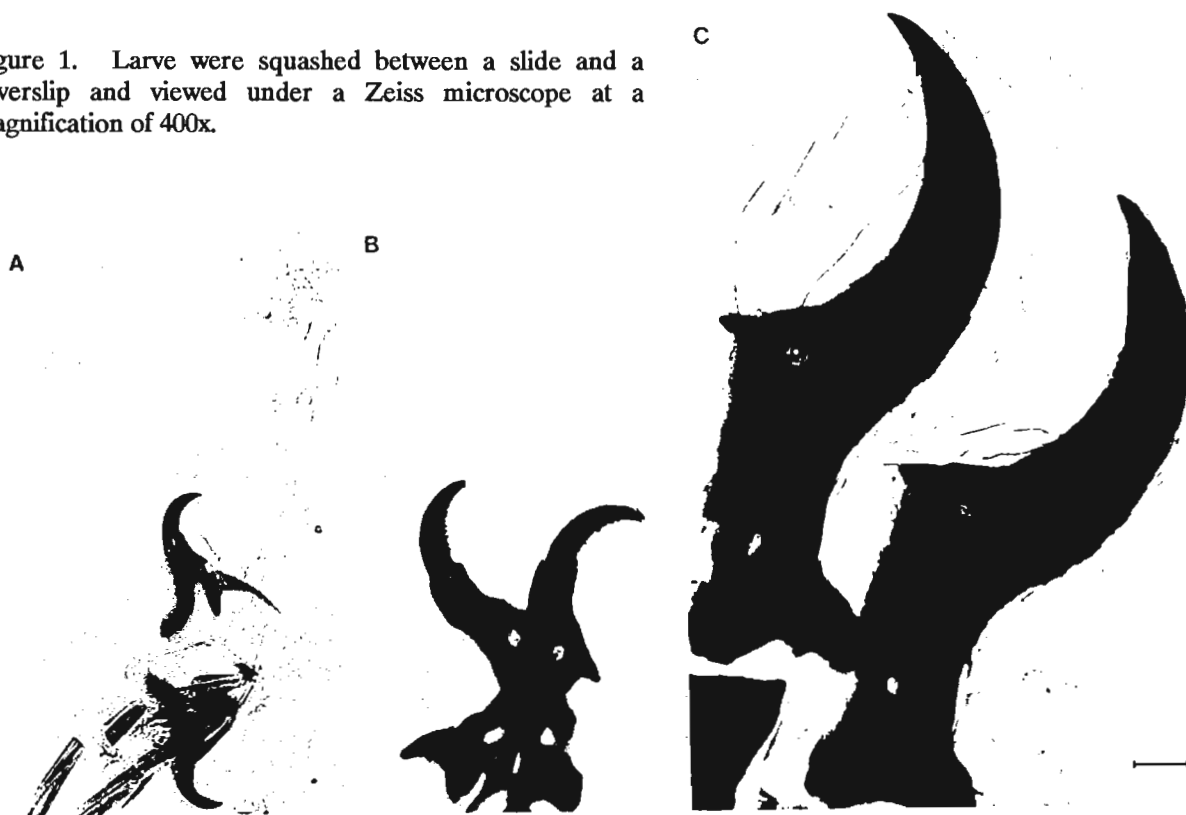
Both generally spread and numerous species: *D. melanogaster*, *D. funnebris*, *D. immigrans*, *D. busckii*, and *D. hydei*. Generally spread but scarcely numerous *D. obscura*. Permanently observed *D. virilis*. Out of the 11 listed, each of the following were found but once in groups by several specimens: *D. simulans* (Kishinev); *D. mercatorum*, *D. repleta* (Uman); *D. lebanonensis* (a Magarach wine factory near Yalta).

Quintana, A. and E. Juan. Department de Genetica, Universitat de Barcelona, Diagonal 645, 08071 Barcelona, Spain. Morphology of mouth hooks and anterior spiracles through the larval development of *D. lebanonensis*.

The two characteristics used to stage larvae in *D. melanogaster* are the change in the morphology of mouth hooks and spiracles through larval development. Studies of temporal gene regulation in *D. lebanonensis* require the staging of larvae and make it necessary to confirm the changes in morphology along the larval development.

Flies were allowed to lay eggs on ethanol-acetic acid-agar medium (2.5%, 1%, 2%) seeded with live yeast for 12 hours. Adult flies were removed and 10 drops of a 10% glucose solution were added daily. The temperature was 23°C. Larvae appear 48 hours after the eggs have been laid. The morphology of the mouth hooks is shown in Figure 1A. They have no teeth at all. Five days after hatching, larvae which have changed the mouth hook morphology begin to appear. Hooks have increased in size and a higher number of teeth than in *D. melanogaster* are observed (Figure 1B). At this time spiracles are apparent but have no papillae. This structure does not change until two days later. Then the size has nearly doubled and no teeth are visible (Figure 1C). At this time spiracles show papillae. Ten days after hatching begins pupariation, and six days later begins eclosion. The shortest life cycle from egg to adult is 18 days at 23°C.

Figure 1. Larve were squashed between a slide and a coverslip and viewed under a Zeiss microscope at a magnification of 400x.



Dernoncourt-Sterpin, C., J. Lechien, E. Depiereux, E. Feytmans and A. Elens. FUNDP, Namur, Belgium. Direct observation of rare-type advantage in *Drosophila melanogaster*.

When two types of *Drosophila* are in competition, the frequency dependence of mating success is routinely measured in our laboratory by direct observation of mating pairs in "Elens-Wattiaux" observation chambers. Observations are carried out "with food" in observation chambers, as well as "without food", nine relative fre-

quencies are assayed, and the chambers are monitored from 8 a.m. until the end of the 5 hour test period (copulation is recorded every 15 min). In the present experiments, the method is exactly the same as previously described (Depiereux *et al.*, 1989), except that only *white* mutant (B) and wild-type CS (A) are used and that 10 replications are carried out.

For the first analysis of variance, applied to the matings recorded at the end of the five hour observation period, the

method is also the same. However, three criteria only are considered: 4 levels of matching - AA, BB, AB, BA -, 2 levels of treatment (food, no food), and 9 levels corresponding to the relative frequency of A and B. The results are also expressed as a deviation between the observed and expected number of matings.

As shown in Table 1, the difference in matching types is significant, the mating success is frequency dependent, but the treatment (food or no food) has no significant influence. From Figure 1, the rare-type advantage is evident: for a relative frequency of 0.1 (03/27) the deviation is well above expectation. Above this threshold it is below the expected value. The greater activity of wild-type male accounts for the differences in matching (Figure 2).

A second analysis of variance is applied to the matings recorded at the first (a), the third (b), and the fifth (c) hours. As shown in Table 2, the difference is great between a and c and between a and b, but not between b and c, - in presence of food as well as without food. A three-hour observation period is quite sufficient. A "rare-male mating advantage" has been described, for a long time, for the same strains (Petit, 1954). This rare-male advantage has been confirmed by many authors (Knoppin, 1985). Later, a "rare-female mating advantage" has been observed (Lichtenberger *et al.*, 1988). The "rare-type advantage" seems more the rule than the exception in such competition experiments.

A conclusion of the present observation is that, although the presence of food enhances and extends the sexual activity (Lichtenberger *et al.*, 1988), the rare-type advantage does not depend on the presence or the absence of food. Another conclusion is that a three-hour observation period is quite enough to detect a rare-type advantage. However, if the purpose of experiments is a comparison between the level and the duration of sexual activity of two strains, a five-hour observation period would of course be better.

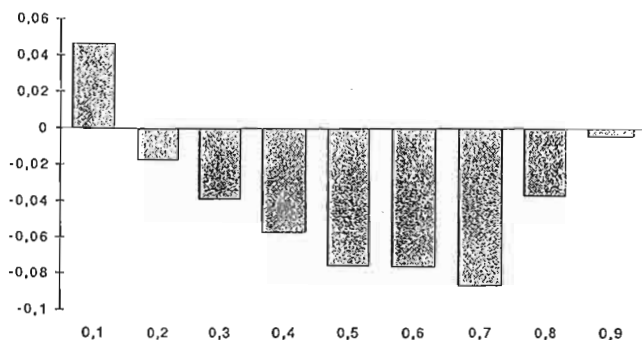


Figure 1. Rare-type mating advantage. Relative frequency: 0.1 (03/27), 0.2 (06/24), 0.3 (09/21), 0.4 (12/18), 0.5 (15/15), 0.6 (18/12), 0.7 (21/09), 0.8 (24/06), 0.9 (27/09). Deviation from expected values: +, above, -, below. For the B flies, the relative frequency is always the reciprocal of the corresponding A flies relative frequency (e.g., if the relative frequency of A is 0.1 (03/27), it is 0.9 (27/03) for B); in Figure 1 the order of the B strain relative frequencies has been inverted.

Table 1. Analysis of variance (matings recorded at the fifth hour).

| Source of variation ^a | | Error term | df | Mean square | F |
|----------------------------------|------|------------|-----|-------------|-----------|
| Treatment | F | R(Fp) | 1 | 0.0080 | 0.16 |
| Matching | M | MR(Fr) | 3 | 3.3333 | 84.54(**) |
| ratio A/B | r | R(Fr) | 8 | 0.1441 | 2.89(**) |
| Replication | R | R(Fr) | 162 | 0.0498 | |
| Interactions | | | | | |
| | FM | MR(Fr) | 3 | 0.1004 | 2.55 |
| | Fr | R(Fr) | 8 | 0.0077 | 0.15 |
| | Mr | MR(Fr) | 24 | 0.1002 | 2.54 |
| | FMr | MR(Fr) | 24 | 0.0226 | 0.57 |
| | mRFR | | 486 | 0.0394 | |

^aF, treatment (food, no food); M, mating type (AA, AB, BA, BB); r, ratio; R, replication. Factor R is nested to M, F, and r; M, F, and r factors are crossed, interaction between crossed factors. ** $P < 0.01$.

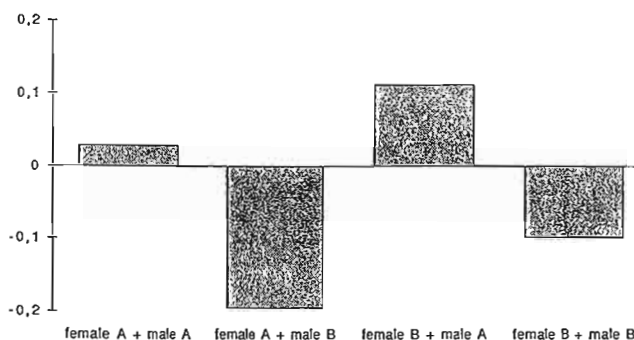


Figure 2. Comparison of four mating types. From left to right: fAmA (AA), fAmB (AB), fBmA (BA), fBmB (BB). Deviation from expected values: +, above; -, below. A, wild type; B, white.

Table 2. Analysis of variance (matings recorded at the first, the third, and the fifth hour).

| Source of variation | Sum of squares | df | Mean square | F |
|---------------------|----------------|-----|-------------|----------|
| Food | | | | |
| between a & c | 326.70 | 1 | 326.70 | |
| int. | 8981.68 | 718 | 12.50 | 26.11** |
| between a & b | 160.55 | 1 | 160.55 | |
| int. | 8233.42 | 718 | 11.46 | 13.99*** |
| between b & c | 29.20 | 1 | 29.20 | |
| int. | 10281.46 | 718 | 14.31 | 2.03 ns |
| No food | | | | |
| between a & c | 157.73 | 1 | 157.73 | |
| int. | 10716.36 | 718 | 14.92 | 10.56*** |
| between a & b | 155.86 | 1 | 155.86 | |
| int. | 10716.30 | 718 | 14.92 | 10.45*** |
| between b & c | 0.00 | 1 | 0.00 | |
| int. | 11859.79 | 718 | 16.51 | 0.00 ns |

*** $P < 0.001$

References: Depiereux, E., C. Dernoncourt-Sterpin, J. Lechien, E. Feytmans, and A. Elens 1990, Behav. Gen. 20:511; Dernoncourt-Sterpin, C. J. Lechien, and A. Elens 1991, Behav. Gen. 21:471; Knoppin, P. 1987, Biol. Rev. 60:81; Lichtenberger M., J. Lechien, and A. Elens 1988, Genetica 77:25; Lichtenberger M., J. Lechien, and A. Elens 1989, Behav. Gen. 19:575; Petit, C. 1954, Bull. Biol. 88:435.

Parkash, R. and M. Vashist. M.D. University, Rohtak, India. Cryptic allozymic variability in *Drosophila immigrans* populations.

genetically differentiated on the basis of cryptic variation analysis (Spiess, 1989). Thus, the present studies were carried out to examine the extent of genic differentiation in *D. immigrans* populations on the basis of electrophoresis as well as cryptic variation analysis. The population samples of this species were collected from different Indian geographical sites. The homogenates of single individuals were subjected to 12% starch gel and were run electrophoretically at 250 V and 30 mA at 4°C for 3.5 hours and the gel slices were stained for acid phosphatase and esterase activity. The application of a heat denaturation technique (Trippa *et al.*, 1978, 1980) involved post-electrophoretic heating of the enzyme *in situ* in starch gel at 57°C for 15 ± 1 minutes.

The *Acph* locus revealed one most frequent allele (*Acph*^S, 0.92 to 1.0) and one rare allele (*Acph*^F, 0.02 to 0.08). However, another rare allele, *Acph*^F, was observed only in the Hasimara population (Table 1). Thus, on the basis of electrophoresis analysis, all the ten populations revealed fit to Hardy-Weinberg expectations at the *Acph* locus, and the allelic frequency patterns were found to be uniform in all the populations. The application of the heat denaturation

Table 1. Data on *Acph* allelic frequencies, heterozygosity and effective number of alleles (n_e) on the basis of electrophoresis plus post electrophoretic heat denaturation test in twelve natural populations of *Drosophila immigrans*.

| Populations | Frequencies of thermostability | | | | | | Total allele | | | | | | |
|--------------|--------------------------------|-----|----|-----|-----|-----|--------------|-----|------|-----|----------------|------------------|----------------------------------|
| | F | | F | | S | | frequency | | Het. | | n _e | n _e ' | n _e /n _e ' |
| | tr | ts | tr | ts | tr | ts | tr | ts | H | H' | | | |
| Jammu | — | — | — | .04 | .73 | .23 | .73 | .27 | .08 | .41 | 1.08 | 1.70 | 1.57 |
| Chandigarh | — | — | — | .04 | .74 | .22 | .74 | .26 | .11 | .40 | 1.12 | 1.67 | 1.49 |
| Dehradun | — | — | — | .06 | .68 | .26 | .68 | .32 | .11 | .47 | 1.12 | 1.89 | 1.68 |
| Saharanpur | — | — | — | .07 | .74 | .19 | .74 | .26 | .13 | .41 | 1.15 | 1.69 | 1.47 |
| Risikesh | — | — | — | .08 | .74 | .18 | .74 | .26 | .15 | .41 | 1.18 | 1.69 | 1.43 |
| Roorkee | — | .02 | — | — | .72 | .26 | .72 | .28 | .04 | .41 | 1.04 | 1.70 | 1.64 |
| Rohtak | — | — | — | .02 | .83 | .15 | .83 | .17 | .04 | .39 | 1.04 | 1.41 | 1.35 |
| Darjeeling | — | — | — | .07 | .69 | .24 | .69 | .31 | .13 | .46 | 1.15 | 1.86 | 1.62 |
| Dhulabari | — | — | — | — | .75 | .25 | .75 | .25 | 0 | .37 | 1.00 | 1.60 | 1.60 |
| Phuntsholing | — | — | — | .05 | .73 | .22 | .73 | .27 | .09 | .42 | 1.10 | 1.72 | 1.56 |
| Bagdogra | — | .06 | — | .05 | .68 | .21 | .68 | .32 | .20 | .49 | 1.25 | 1.95 | 1.56 |
| Hasimara | — | .04 | — | .03 | .70 | .23 | .70 | .30 | .13 | .45 | 1.15 | 1.83 | 1.59 |

tr (thermorensistant) and ts (thermosusceptible) isoelectrophoretic alleles; H and n_e (heterozygosity and effective number of alleles) under electrophoresis alone; H' and n_e' refer to such indices under electrophoresis plus heat stability test.

The occurrence of additional cryptic genic variation has helped in differentiating species populations which were considered similar on the basis of standard gel electrophoresis, e.g. an isolated population of *D. pseudoobscura* from Bogota, Columbia, was found to be comprised of two isoelectrophoretic alleles (tr and ts) which occurred with polymorphic frequencies, and thus resulted in a significant increase in heterozygosity in this locus in all the populations (Table 1). The rare allelic variants (*Acph*^F and *Acph*^F) did not reveal any isoelectrophoretic or cryptic variation. The patterns of isoelectrophoretic thermostability variants, heterozygosity (H') and effective number of alleles (n_e') at the *Acph*, *Est-2* and *Est-6* loci were found

Table 2. Patterns of distribution of allelic frequencies, heterozygosity and effective number of alleles (n_e) on the basis of electrophoresis plus heat stability test at *Est-2* and *Est-6* loci in seven natural populations of *Drosophila immigrans*

| Locus | Esterase electromorphs | Chandigarh | | Dehradun | | Saharanpur | | Risikesh | | Rohtak | | Darjeeling | | Hasimara | |
|--------------|---------------------------|------------|------|----------|------|------------|------|----------|------|--------|------|------------|------|----------|------|
| | | tr | ts | tr | ts | tr | ts | tr | ts | tr | ts | tr | ts | tr | ts |
| <i>Est-2</i> | F | .566 | .188 | .61 | .20 | .704 | .166 | .72 | .13 | .69 | .14 | .60 | .20 | .56 | .16 |
| | S | .184 | .062 | .15 | .04 | .10 | .03 | .11 | .04 | .14 | .03 | .15 | .05 | .21 | .07 |
| | Total tr & ts | .750 | .250 | .76 | .24 | .804 | .196 | .83 | .17 | .83 | .17 | .75 | .25 | .77 | .23 |
| | H & H' | .37 | .61 | .31 | .56 | .23 | .47 | .25 | .43 | .28 | .48 | .32 | .58 | .40 | .61 |
| | n_e & n_e' | 1.59 | 2.54 | 1.45 | 2.29 | 1.30 | 1.89 | 1.34 | 1.75 | 1.39 | 1.94 | 1.47 | 2.35 | 1.66 | 2.56 |
| | n_e' / n_e | 1.59 | | 1.58 | | 1.45 | | 1.31 | | 1.38 | | 1.60 | | 1.54 | |
| <i>Est-6</i> | F | .80 | .20 | .80 | .20 | .82 | .18 | .78 | .18 | .81 | .19 | .76 | .24 | .76 | .19 |
| | S | — | — | — | — | — | — | — | .04 | — | — | — | — | — | .05 |
| | Total tr & ts | .80 | .20 | .80 | .20 | .82 | .18 | .78 | .22 | .81 | .19 | .76 | .24 | .76 | .24 |
| | H & H' | 0 | .32 | 0 | .32 | 0 | .30 | .08 | .36 | 0 | .31 | 0 | .37 | .09 | .38 |
| | n_e & n_e' | 1.0 | 1.47 | 1.0 | 1.47 | 1.0 | 1.43 | 1.08 | 1.56 | 1.0 | 1.45 | 1.0 | 1.58 | 1.10 | 1.61 |
| | n_e' / n_e | 1.47 | | 1.47 | | 1.43 | | 1.44 | | 1.45 | | 1.58 | | 1.46 | |

tr (thermorensistant) and ts (thermosusceptible) isoelectrophoretic alleles; H and n_e (heterozygosity and effective number of alleles) under electrophoresis alone; H' and n_e' refer to such indices under electrophoresis plus heat stability test.

to be nearly uniform in all the populations of *D. immigrans* (Table 2). The *Est-6* locus, which was monomorphic on the basis of electrophoresis (in most of the populations), was found to be polymorphic according to thermostability tests. Since the standard electrophoretic techniques can overestimate electrophoretic similarity, the combination of electrophoretic and heat denaturation techniques have revealed considerable molecular variation within electromorphs, and thus helped in revealing genetic differentiation at enzymatic loci (Ayala, 1983; Coyne, 1982; Chyurlia, 1985; and Hernandez, 1986). The distribution patterns of thermoresistant (tr) and thermosensitive (ts) alleles were found to be uniform in all the populations of *D. immigrans*. The heat denaturation technique increased the extent of genic variation but did not alter the pattern of genetic variability at three loci in *D. immigrans* populations. Thus, Indian populations of *D. immigrans* did not reveal any genetic differentiation on the basis of electrophoretic as well as cryptic variation analysis and this could be due to its narrow niche-width (Steiner, 1980).

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Vashist, M. and R. Parkash. M.D. University, Rohtak, India. Ethanol tolerance in *Zaprionus indianus* populations.

Zaprionus indianus constitutes one of the most successful colonizing species of the Indian subcontinent. Many sympatric species of *Zaprionus* are endemic to Africa (Okada and Carson, 1983). It had been argued that the genus *Zaprionus* evolved from close to the *D.*

immigrans species group radiation (Throckmorton, 1974) and that various *Zaprionus* species might have originated in the Afrotropical continent and later on migrated to other tropical continents such as India (Tsacas *et al.*, 1981). *Zaprionus indianus* was found to exploit a variety of fermenting fruits in nature, and larvae were found to be physically immersed in fermented media. Thus, *Zaprionus indianus* closely resembled *Drosophila melanogaster* in its habitat as well as potential to utilize environmental ethanol resources. Since ADH is known to be involved in the utilization and detoxification of exogenous alcohol, the present studies were made to analyze the extent of ethanol utilization as well as ethanol tolerance potential in geographical populations of *Z. indianus* from India.

Zaprionus indianus, a member of the family Drosophilidae, is a successful colonizing species throughout the Indian subcontinent. Isofemale lines were established from population samples of *Z. indianus* from ten Indian geographical sites (Cochin to Chandigarh; 10°N to 30°43'N). Homogenates of single individuals were subjected to electrophoresis at 250 V and 25 mA at 4°C for 4 hours. Genetic control of ADH banding patterns was interpreted from the segregation patterns of enzyme electromorphs of parents, F₁, and F₂ progeny of several single-pair

Table 1. Distribution of genotypes, allelic frequencies, heterozygosities, effective number of alleles (n_e), Wright's co-efficients (f) and G-values for log-likelihood χ^2 test for fit to Hardy-Weinberg expectations at *Adh* locus in ten Indian natural populations of *Zaprionus indianus*

| Populations | Latitude | Genotypes | | | Sample size | Allelic freq. | | Het. obs/exp. | f | n_e | G-values |
|-------------|-----------|-----------|----|----|-------------|---------------|-----|---------------|------|-------|----------|
| | | FF | SS | FS | | F | S | | | | |
| Cochin | 10° N | 4 | 30 | 18 | 52 | .25 | .75 | .35 / .37 | .05 | 1.59 | 0.30 |
| Bangalore | 12° .58'N | 24 | 66 | 42 | 132 | .34 | .66 | .32 / .45 | .29 | 1.82 | 11.04* |
| Tirumala | 13° .40'N | 8 | 26 | 30 | 64 | .36 | .64 | .47 / .46 | -.02 | 1.85 | 0.04 |
| Hyderabad | 17° .20'N | 17 | 35 | 36 | 88 | .40 | .60 | .41 / .48 | .15 | 1.92 | 1.87 |
| Nagpur | 21° .16'N | 18 | 30 | 38 | 86 | .43 | .57 | .44 / .49 | .10 | 1.96 | 0.84 |
| Bhopal | 23° .16'N | 14 | 20 | 29 | 63 | .45 | .55 | .46 / .49 | .06 | 1.96 | 0.32 |
| Jaipur | 26° .55'N | 12 | 21 | 39 | 72 | .44 | .56 | .54 / .49 | -.10 | 1.96 | 0.73 |
| Rohtak | 28° .94'N | 23 | 25 | 47 | 95 | .49 | .51 | .49 / .50 | .02 | 2.0 | 0.01 |
| Dehradun | 30° .19'N | 20 | 24 | 48 | 92 | .48 | .52 | .52 / .50 | -.04 | 2.0 | 0.88 |
| Chandigarh | 30° .43'N | 21 | 18 | 42 | 81 | .52 | .48 | .52 / .50 | -.04 | 2.0 | 0.12 |

* Significant at 5% level.

Table 2. Data on the comparison of ethanol tolerance indices (increase in longevity - LD₅₀ hrs, LD₅₀ max / LD₅₀ control values at 4% ethanol), ethanol threshold concentrations in adults as well as larvae and LC₅₀ ethanol concentration in ten geographical Indian populations of *Zaprionus indianus*

| Populations | Latitude | Increase in longevity at 4% ethanol | | Ethanol threshold conc. | | Ethanol conc. for LC ₅₀ |
|----------------|-----------|-------------------------------------|---|-------------------------|-------|------------------------------------|
| | | LD ₅₀ (hrs) | LD ₅₀ max / LD ₅₀ control | Larvae | Adult | |
| 1. Cochin | 10° N | 89 | 0.91 | 3.8 | 3.8 | 4.55 |
| 2. Bangalore | 12° .58'N | 35 | 0.94 | 4.0 | 3.9 | 4.00 |
| 3. Tirumala | 13° .40'N | 67 | 1.01 | 5.0 | 4.2 | 4.2 |
| 4. Hyderabad | 17° .20'N | 85 | 1.25 | 6.5 | 4.4 | 4.6 |
| 5. Nagpur | 21° .16'N | 151 | 1.40 | 7.0 | 4.6 | 4.9 |
| 6. Bhopal | 23° .16'N | 109 | 1.50 | 8.0 | 6.1 | 7.3 |
| 7. Jaipur | 26° .55'N | 185 | 3.8 | 8.5 | 6.7 | 6.0 |
| 8. Rohtak | 28° .94'N | 225 | 4.5 | 9.5 | 7.0 | 6.9 |
| 9. Dehradun | 30° .19'N | 210 | 5.01 | 11.0 | 8.4 | 8.0 |
| 10. Chandigarh | 30° .43'N | 270 | 5.0 | 12.0 | 8.1 | 8.0 |

matings. The adult ethanol tolerance was assessed following the procedure of Starmer *et al.* (1977) and David and Van Herrewege (1983). The larval ethanol tolerance behavior of geographical populations of *Z. indianus* was analyzed by following the method of Gelfaud and McDonald (1983). Five replicates were tested at each ethanol concentration at 20°C for each of the *Z. indianus* populations. The data on observed ADH genotypes, sample size, allelic frequencies, observed and expected heterozygosity, Wright's coefficient (*f*), effective number of alleles (n_e) and application of G-test for fit to Hardy-Weinberg expectations at the polymorphic *Adh* locus in the ten Indian populations of *Z. indianus* are given in Table 1. The range of high heterozygosity values observed at the *Adh* locus were in agreement with allelic frequency distribution patterns. The population sample from Bangalore revealed deviation from Hardy-Weinberg expectation. The *Adh*^F frequency increased significantly with increasing latitude (> 1% with 1° latitude). The longevity periods were found to increase significantly in the range of 1% to 4% ethanol in south Indian populations while 1% to 9% ethanol revealed enhanced longevity in the north Indian populations of *Z. indianus*. The longevity data revealed that south Indian populations of Cochin represented minimum increase (80 hours) as compared with the highest increase (270 hours) in the north Indian populations of Chandigarh. However, the other eight geographical populations revealed intermediate response. The adult ethanol threshold values were found to vary clinally in the range of 3.8% to 8.0% among the ten populations of *Z. indianus* from north to south localities of the Indian subcontinent (Table 2). Thus, ethanol concentrations in the range of 6% to 8.4% served as resources for north Indian populations while significantly lower ethanol concentrations (3.8% to 4.6%) could be utilized by south Indian populations of *Z. indianus*. The LC₅₀ ethanol concentrations were calculated from mortality data of adults after four days of ethanol treatment. These LC₅₀ values revealed clinal variation in the range of 4% to 8%, i.e. southern populations of *Z. indianus* depicted significantly lower ethanol tolerance as compared with north Indian populations. The data on larval ethanol preference behavior towards a range of ethanol concentrations (1% to 15%) have been represented in Table 2. The larval ethanol threshold values varied from 3.8% in Cochin populations to 12% in Chandigarh populations. The ranking order of populations include Chandigarh > Dehradun > Rohtak > Jaipur > Bhopal > Nagpur > Hyderabad > Trimula > Bangalore > Cochin. Except Cochin and Bangalore populations, the larval individuals of eight populations of *Z. indianus* had revealed significantly higher ethanol tolerance than those of adults, but the pattern of clinal variation was found to be similar for both the adult as well as larval stages (Table 2). The observed data on *Zaprionus indianus* could be explained on the basis of the niche-width variation hypothesis, i.e. the amount of variation in a species was proportional to its niche-width (Wills, 1981; Hedrick, 1983). It had been argued that a species characterized by utilization of diverse food resources and/or climatic adaptations should possess significantly higher amounts of genic divergence as compared to the narrow niche-width species (Spiess, 1989). Since *Drosophila* species are fruit-niche species, they are known to utilize ethanol as a resource in nature (Parsons and Spence, 1980; David, 1988). Interspecific divergence for ethanol tolerance levels was found for most of the *Drosophila* species, i.e. only three species (*D. melanogaster*, *D. lebanonensis* and *D. virilis*) were found to be highly ethanol tolerant while other *Drosophila* species revealed either lower levels of ethanol tolerance or ethanol sensitivity (David, 1988). Such observations concurred with suggested relationships between ethanol tolerance and larval habitat in several *Drosophila* species (David and Van Herrewege, 1980). In the present studies, *Z. indianus* populations were found to be significantly ethanol tolerant. Thus, the various ethanol tolerant drosophilid species suggest a lack of correlation between ethanol tolerance and phylogeny. The two ethanol tolerant species (*D. melanogaster* and *Z. indianus*) are only distantly related and belong to different genera, and thus, their adaptations could be a case of convergence.

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Sharma, S. and R. Parkash. M.D. University, Rohtak, India. Ethanol tolerance in some Indian *Drosophila* species.

Colonizing *Drosophila* species constitute excellent materials for experimental population genetics as well as evolutionary studies (Endler, 1986). The *melanogaster* species group had undergone extensive speciation events, and adaptive radiations occurred largely in the

oriental region. Even the colonizing populations of *D. melanogaster* from different continents vary in their evolutionary history (David and Tsacas, 1981). The Indian subcontinent represents a diverse array of climatically variable habitats,

and there is little information on *Adh* polymorphism and ethanol tolerance analysis in various drosophilid populations from India. The present studies report genetic variability at the *Adh* locus as well as ethanol tolerance in seven Indian natural populations of *D. melanogaster* and in three other *Drosophila* species.

Isofemale lines were established from population samples of *D. melanogaster* from seven Indian geographical sites (Cochin to Dehradun; 9°58'N to 30°18'N) as well as local populations of some other *Drosophila* species. Homogenates of single individuals were subjected to electrophoresis at 250 V and 25 mA at 4°C for 4 hours. Genetic control of ADH banding patterns was interpreted from the segregation patterns of ADH electromorphs of parents, F₁ and F₂ progeny of several species specific single-pair matings. The adult ethanol tolerance was assessed following the procedure of Starmer *et al.* (1977) and Van Herrewege and David (1978). The larval ethanol tolerance behaviour of *D. melanogaster*, *D. ananassae*, *D. takahashii* and *D. nepalensis* was analyzed by following the method of Parsons and Spence (1980).

The data on the mean number of larvae of each of the four *Drosophila* species choosing agar plus various concentrations of ethanol for experimental duration of 30 minutes are given in Table 1. The larval ethanol tolerance response revealed significant variation (2.4% to 11%) between four different *Drosophila* species, i.e. *D. melanogaster*, *D. ananassae*, *D. takahashii* and *D. nepalensis*. Thus, *D. melanogaster* revealed

Table 1. *Adh* gene frequencies, ethanol threshold concentrations, relative longevities in adults and larval attraction behaviour in four *Drosophila* species from Rohtak

| Species | Adh allelic frequency | | Adult | | Larva | |
|------------------------|-----------------------|--------------|---------------------------|--|----------------------------------|---------------------------|
| | Fast Adh - F | Slow Adh - S | % Ethanol threshold value | LT ₅₀ Max/ LT ₅₀ control | Mean no. attracted at 4% ethanol | % ethanol threshold value |
| <i>D. melanogaster</i> | 0.74 | 0.26 | 12.0 | 2.95 | 8.0 | 11.0 |
| <i>D. ananassae</i> | 0.68 | 0.32 | 3.5 | 2.00 | 5.0 | 4.0 |
| <i>D. takahashii</i> | 0.98 | 0.02 | 2.0 | 1.50 | 4.0 | 2.8 |
| <i>D. nepalensis</i> | 0.97 | 0.03 | 2.0 | 1.50 | 3.0 | 2.4 |

Table 2. Data on *Adh* allelic frequencies, percent ethanol tolerance and ethanol utilisation (LT₅₀ max / LT₅₀ control), adult LC₅₀ ethanol concentration and larval ethanol threshold values among adult individuals of seven latitudinally varying Indian populations of *Drosophila melanogaster*.

| Parameter | Cochin (9° .58'N) | Hyderabad (17° .20'N) | Nagpur (21° .09'N) | Bhopal (23° .16'N) | Rohtak (28° .54'N) | Saharanpur (29° .58'N) | Dehradun (30° .19'N) |
|--|-------------------|-----------------------|--------------------|--------------------|--------------------|------------------------|----------------------|
| Allelic frequency | | | | | | | |
| Adh ^F | .11 | .21 | .30 | .56 | .74 | .78 | .80 |
| Adh ^S | .89 | .79 | .70 | .44 | .26 | .22 | .20 |
| Adult Ethanol tolerance (threshold values) | 9.0 | 9.4 | 11.2 | 11.4 | 12.4 | 13.0 | 13.2 |
| Ethanol utilisation | 1.75 | 2.0 | 2.3 | 2.6 | 2.75 | 3.0 | 3.10 |
| Adult LC ₅₀ | 9.25 | 10.8 | 11.8 | 12.0 | 12.0 | 13.5 | 14.0 |
| Larval ethanol threshold | 6.0 | 8.0 | 9.0 | 9.5 | 11.0 | 13.0 | 13.0 |

Latitudes are given in parenthesis.

the highest ethanol tolerance levels as compared with the other three sympatric *Drosophila* species (2.4% to 4%). The adult ethanol threshold values were found to follow the similar ranking order as observed for species specific larval individuals. The ethanol threshold values among adults were found to be differential but low in the range of 2.0% to 3.5% in three *Drosophila* species, i.e. *D. takahashii* and *D. nepalensis* (2.0%) and *D. ananassae* (3.5%) as compared with *D. melanogaster* (12%). Thus, the present observations revealed interspecies differences in ethanol utilization as well as ethanol tolerance in larval and adult stages.

The allelic frequency patterns at *Adh* revealed significant clinal variation, i.e. *Adh*^F frequency increased linearly with increasing latitude (about 3% increase with 1° latitude) and revealed significant positive correlations (+0.96) while *Adh*^S allelic frequency was found to be negatively correlated with latitude ($r = -0.96$) among seven geographical populations of *D. melanogaster*. The intraspecific variation for adult as well as larval ethanol tolerance between different populations of *D. melanogaster* was found to be significantly different among various populations along the north-south axis of the Indian subcontinent (Table 2). The adult individuals of different populations of *D. melanogaster* were analyzed for their potential to utilize ethanol vapors (ethanol utilization) and to detoxify ethanol (ethanol tolerance) in a closed system, and such data were found to agree with larval ethanol tolerance analysis. The data on LT₅₀ (hrs) and LT₅₀ max/LT₅₀ control (which are the measures of resource versus stress) have been shown in Table 2. The longevity periods were found to increase significantly at 1% to 9% ethanol concentrations in all the populations of *D. melanogaster*. However, the adult ethanol threshold values were found to vary clinally in the range of 9.0% to 13.2% among seven geographical populations of *D. melanogaster*. The adult individuals of *D. melanogaster* from south Indian populations revealed lower ethanol concentrations up to 9% as compared with northern populations (12.4% to 13.2%). However, the larval ethanol threshold values were found to vary from 6% in Cochin populations to 13% in Dehradun

populations. The ethanol utilization indices as well as ethanol tolerance threshold values were found to vary latitudinally in different populations of *D. melanogaster*.

Studies on biogeographic, phylogenetic, ecological and adaptive physiological traits in global populations of *D. melanogaster* revealed that Afrotropical populations constitute the ancestral populations which later colonized Eurasia and more recently America and Australia (David and Capi, 1988). Thus, the *D. melanogaster* populations inhabiting different continents have very different evolutionary histories. The present studies on Indian populations of *D. melanogaster* have revealed symmetrical clinal variations at the *Adh* locus, along 10°N to 32°N latitude, and thus, further validate and support the hypothesis that such clinal variations are being maintained by natural selection mechanisms (Parsons, 1983; David *et al.*, 1989; and Spiess, 1989).

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Parkash, R. and S. Sharma. M.D. University, Rohtak, India. Cryptic genic variability in *Zaprionus indianus* populations.

Colonizing species offer suitable material for microevolutionary studies (Endler, 1986). Electromorphs (allozymes) constitute molecular markers because electrophoretic mobility variants for different enzyme loci can be genetically interpreted and

have been used to characterize the genetic structure of natural populations of diverse taxa (Wills, 1981; Spiess, 1989). However, standard gel electrophoresis does not distinguish all allelic variants, and hence, it can underestimate the level of genic variability occurring in natural populations (Hedrick, 1983). The levels of genetic diversity have been compared in the cosmopolitan and colonizing sibling species pair, *D. melanogaster* and *D. simulans*, and such studies revealed extensive clinal as well as geographical genetic divergence among *D. melanogaster* populations as compared to genetic uniformity among *D. simulans* populations (Watada *et al.*, 1986; Singh and Rhomberg, 1987). However, such studies have not been attempted so far on colonizing drosophilids of the Indian subcontinent. Thus, it was considered worthwhile to examine electrophoretic and cryptic patterns of allozymic variation in populations of *Z. indianus*.

The population samples of *Zaprionus indianus* were bait-trapped from eight latitudinally varying sites (10°N to 32°19'N). Electrophoretic allozymic variants as well as isoelectrophoretic thermostability variants of *alpha-Gpdh* and *Est* loci were analyzed following the standard methods (Harris and Hopkinson, 1976; Trippa *et al.*, 1980). Homogenates of single individuals were applied to 12% starch slab gels and were analyzed electrophoretically at 250 V and 30 mA at 4°C for 4 hours. The isoelectrophoretic thermoresistant (tr) and thermosusceptible (ts) variants were examined in individuals by heat treating the enzyme *in situ* in the starch gel slices at 56°C for 12 minutes in case of esterase and *alpha-GPDH*. The electromorph patterns in control as well as treated gel slices were compared so as to identify isoelectrophoretic variants (tr & ts).

The data on allelic frequencies at three polymorphic loci in populations of *Z. indianus* along the north-south transect of the Indian subcontinent were given in Table 1. These loci have shown diallelic variation patterns represented by three genotypes (FF, SS and FS) while genotypic distribution patterns at *Est-2* are largely due to the occurrence of

Table 1. Data on the distribution of allelic frequencies and application of log-likelihood χ^2 test (G-test) for Hardy-Weinberg expectations at three polymorphic loci in eight natural populations of *Zaprionus indianus*.

| Locus | Alleles | Allelic frequency data in populations | | | | | | | |
|----------------|----------|---------------------------------------|-----------|----------|-----------|--------|--------|--------|----------|
| | | Ernakulam | Bangalore | Tirumala | Hyderabad | Nagpur | Bhopal | Rohtak | Dehradun |
| α -Gpdh | F | 0 | .03 | .04 | .06 | .04 | .03 | .12 | .15 |
| | S | 1.0 | .97 | .96 | .94 | .96 | .97 | .88 | .85 |
| | N | 60 | 85 | 70 | 80 | 98 | 92 | 68 | 102 |
| <i>Est-1</i> | G-values | 0 | 0.14 | 0.29 | 0.69 | 0.34 | 0.15 | 2.47 | 0.06 |
| | F | .71 | .77 | .79 | .76 | .74 | .78 | .90 | .93 |
| | S | .29 | .23 | .21 | .24 | .26 | .22 | .10 | .07 |
| <i>Est-2</i> | N | 57 | 55 | 63 | 72 | 96 | 72 | 68 | 86 |
| | G-values | 0.63 | 0.45 | 2.28 | 0.47 | 0.61 | 0.11 | 0.14 | .90 |
| | F | — | — | — | — | — | — | .02 | .03 |
| | F | .61 | .57 | .63 | .67 | .64 | .66 | .48 | .49 |
| | M | — | .07 | .11 | .04 | .05 | — | .06 | .05 |
| | S | .39 | .36 | .26 | .29 | .31 | .34 | .42 | .43 |
| | S' | — | — | — | — | — | — | .02 | — |
| | N | 57 | 86 | 69 | 76 | 90 | 57 | 113 | 86 |
| | G-values | 0.02 | 2.07 | 26.96* | 12.55* | 10.48* | 0.16 | 37.26* | 41.86* |

F, F', M, S and S' represent faster, fast, medium, slow and slower electromorphs respectively. N = sample size.

* Significant at 5% level

rare alleles. The *Est-2* locus has revealed two common alleles in all the populations, but some populations have shown rare alleles. However, two rare alleles were found in Dehradun populations and three rare alleles in Rohtak populations. The changes at three loci were significant and indicated regular trends of increase or decrease in allelic frequency in latitudinally varying populations of *Z. indianus* (Table 1). The data on distribution of thermoresistant (tr) and thermosensitive (ts) allelic frequencies at *Est-1* and *Est-2* loci in the five populations of *Z. indianus* are shown in Table 2. The application of the heat denaturation technique revealed that the most common electromorphs at *Est-1* and *Est-2* loci were comprised of tr and ts variants which occurred with polymorphic frequencies in all of the populations. The cryptic variants have resulted in a significant increase in heterozygosity at such loci. However, the patterns of allelic frequencies of isoelectrophoretic thermostability variants have shown clinal variations at both the loci in all the eight populations of this species (Table 2). The present study has revealed the occurrence of heterogeneous patterns of heat stability polymorphism (cryptic variations) in addition to electrophoretic variability at *Est-1* and *Est-2* loci in natural populations of *Z. indianus*. However, no additional cryptic genic variation was observed at the *alpha-Gpdh* locus through the heat denaturation technique.

The present data on clinal variations at *Est-1* and *Est-2* polymorphic loci in Indian populations of *Z. indianus* further support and validate the hypothesis that the occurrence of latitudinal clines across different drosophilid populations provides strong evidence of natural selection maintaining clinal allozymic variation (Anderson *et al.*, 1984; David *et al.*, 1989). Different population samples of *Z. indianus* have revealed

Table 2. Distribution patterns of allelic frequencies at two polymorphic loci (*Est-1* and *Est-2*) on the basis of standard gel electrophoresis plus post-electrophoretic heat denaturation technique in five population samples of *Zaprionus indianus*.

| Locus | Alleles | Ernakulam | | Bangalore | | Hyderabad | | Rohtak | | Dehradun | |
|--------------|-----------------------------------|-----------|------|-----------|------|-----------|------|--------|------|----------|------|
| | | tr | ts | tr | ts | tr | ts | tr | ts | tr | ts |
| <i>Est-1</i> | F | .69 | .02 | .73 | .04 | .70 | .06 | .85 | .05 | .86 | .07 |
| | S | .26 | .03 | .20 | .03 | .20 | .04 | .08 | .02 | .06 | .003 |
| | Total tr & ts | .95 | .05 | .93 | .07 | .90 | .10 | .93 | .07 | .927 | .073 |
| | H & H' | .41 | .45 | .35 | .42 | .36 | .46 | .18 | .27 | .13 | .25 |
| | n _e & n _e ' | 1.69 | 1.82 | 1.54 | 1.72 | 1.56 | 1.85 | 1.22 | 1.37 | 1.15 | 1.33 |
| | n _e ' / n _e | 1.08 | | 1.12 | | 1.19 | | 1.12 | | 1.16 | |
| <i>Est-2</i> | F | — | — | — | — | — | — | — | .02 | — | .03 |
| | F | .56 | .05 | .51 | .06 | .60 | .07 | .39 | .09 | .40 | .09 |
| | M | — | — | .07 | — | .04 | — | .06 | — | .045 | .005 |
| | S | .34 | .05 | .32 | .04 | .22 | .07 | .34 | .08 | .33 | .10 |
| | S' | — | — | — | — | — | — | — | .02 | — | — |
| | Total tr & ts | .90 | .10 | .90 | .10 | .86 | .14 | .79 | .21 | .775 | .225 |
| | H & H' | .48 | .57 | .54 | .63 | .47 | .58 | .59 | .71 | .57 | .71 |
| | n _e & n _e ' | 1.92 | 2.32 | 2.17 | 2.70 | 1.89 | 2.38 | 2.44 | 3.45 | 2.32 | 3.45 |
| | n _e ' / n _e | 1.21 | | 1.24 | | 1.26 | | 1.41 | | 1.49 | |

F, F', M, S and S' represent faster, fast, medium slow and slower electromorphs respectively. H and n_e = heterozygosity and effective number of alleles on the basis of electrophoresis alone; and H' & n_e' = heterozygosity and effective number of alleles on the basis of post-electrophoretic heat denaturation technique; n_e' / n_e = increase in effective number of alleles; tr and ts refer to temperature resistant and temperature sensitive variant allozyme respectively.

almost clinal patterns of thermoresistant (tr) and thermosensitive (ts) isoelectrophoretic variants of *Est-1* and *Est-2* loci. The cryptic isoelectrophoretic variation significantly increased the heterozygosity and effective number of alleles at three out of four polymorphic loci. However, the common occurrence of heat stability polymorphism in all the populations suggests that natural selection might be responsible for the maintenance of such cryptic genic variation. Temperature constitutes an important component of the environment, and empirical data exists on the adaptive correlation of biochemical properties of allozymic variants (allelic isozymes) with habitat temperatures in some organisms (Wills, 1981; Hedrick, 1983). Thus, it can be suggested that in clinally varying heterogeneous environments of the Indian subcontinent, tr and ts variants might confer adaptive advantages to *Z. indianus* individuals which occur in its natural habitats during all seasons of the year and that the species has colonized all of the Indian subcontinent. Present observations on the widespread occurrence of heat stability polymorphism at two loci in *Z. indianus* concur with other reports in *D. melanogaster* (Bewley, 1978; Trippa *et al.*, 1978).

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Parkash, R. and Shamina. M.D. University, Rohtak, India. Temporal patterns of allozymic variation in two colonizing drosophilids.

Allozymes (genetic variants of enzymes) revealed by gel electrophoretic techniques constitute useful markers to examine the role played by micro-evolutionary processes in modifying the genetic architecture of species populations (Hedrick, 1983;

Hartl, 1980; Milkman, 1983; Wallace, 1981). However, there is a lack of information on temporal genic variation patterns in the Indian natural populations of the various colonizing drosophilids. The present paper reports the temporal patterns of electrophoretic and cryptic variation in natural population samples of *D. immigrans* from Hasimara (W.B.) and of *Z. indianus* from Dehradun. The individuals of *D. immigrans* were bait-trapped from Hasimara (W.B.) in the month of January, 1988, as well as in January, 1990, and were maintained as isofemale lines. However, *Z. indianus* individuals were bait-trapped from Dehradun (U.P.). Homogenates of single individuals from isofemale lines were applied to 12% starch gels and electrophoresed at 250 V and 30 mA at 4°C for 4 hours. The application of a heat denaturation technique (Trippa *et al.*, 1978) involved post electrophoretic thermal treatment of the enzyme *in situ* in the starch gel slice (at 52°C for 12 ± 1 min in case of acid phosphatase; at 56°C for 15 ± 1 min for esterase; and at 48°C for 15 ± 1 min for alcohol dehydrogenase).

The data on distribution patterns of genotypes, allelic frequencies, observed and expected heterozygosity and G-values for log-likelihood χ^2 test for Hardy-Weinberg equilibrium for the five polymorphic loci are given in Table 1. The patterns of genic variation include occurrence of a common allele and few rare alleles at the *AcpH* locus, while four loci (*Est-2*, *Mdh-1*, *AO* and *OdH*) are represented by two frequent alleles. Thus, the range of heterozygosities observed at various polymorphic loci correlates well with the number of alleles and allelic frequencies. The patterns of allelic frequencies at various loci (samples collected in 1990) have been found to be very similar to those of the population sampled in 1988. The yearly data on allelic frequency patterns revealed no deviation on the basis of Student's *t*-test. The yearly population samples revealed homogeneity patterns in allelic frequencies and heterozygosity (Table 1). The yearly

Table 1. Data on temporal distribution patterns of allelic frequencies, observed and expected heterozygosity and G-values for log-likelihood χ^2 test for fit to Hardy-Weinberg equilibrium in yearly population samples of *Drosophila immigrans* for Hasimara (W.B.) and *Zaprionus indianus* from Dehradun (U.P.).

| <i>Drosophila immigrans</i> | | | | | | | <i>Zaprionus indianus</i> | | | | | | | | |
|-----------------------------|------|-------------------|------|-----|-----|------------------|---------------------------|---------------|------|-------------------|-----|-----|-----|-------------------|----------|
| Locus | Year | Allelic Frequency | | | N | Het. Obs/exp. | G-values | Locus | year | Allelic Frequency | | | N | Het. Obs. Exp. | G-values |
| | | F | F | S | | | | | | F | S | ** | | | |
| <i>ACPH</i> | 1988 | .04 | .03 | .93 | 111 | .15 / .13 | 0.93 | <i>ACPH-1</i> | 1989 | .47 | .48 | .05 | 82 | .66 / .55 | 21.33* |
| | 1990 | .035 | .075 | .89 | 102 | .21 / .20 | 2.68 | | 1990 | .49 | .46 | .05 | 108 | .59 / .55 | 27.62* |
| <i>EST-2</i> | 1988 | | F | S | | | | <i>ACPH-2</i> | 1989 | F | S | | 66 | .27 / .32 | 1.16 |
| | 1990 | | .72 | .28 | 115 | .36 / .40 | 0.92 | | 1990 | .80 | .20 | | 102 | .23 / .34 | 10.72* |
| <i>MDH-1</i> | 1988 | | .72 | .28 | 115 | .36 / .40 | 0.92 | <i>α-GPDH</i> | 1989 | .13 | .87 | | 116 | .16 / .23 | 8.45* |
| | 1990 | | .67 | .33 | 88 | .30 / .44 | 9.46* | | 1990 | .78 | .22 | | 102 | .23 / .34 | 10.72* |
| <i>MDH-1</i> | 1988 | | .18 | .82 | 102 | .31 / .30 | 4.66* | <i>α-GPDH</i> | 1989 | .13 | .87 | | 116 | .16 / .23 | 8.45* |
| | 1990 | | .16 | .84 | 95 | .26 / .27 | 0.14 | | 1990 | .15 | .85 | | 102 | .25 / .25 | 0.06 |
| <i>AO</i> | 1988 | | .59 | .41 | 119 | .57 / .49 | 3.90* | <i>ADH</i> | 1989 | .51 | .49 | | 80 | .47 / .50 | 0.20 |
| | 1990 | | .61 | .39 | 87 | .44 / .48 | 0.61 | | 1990 | .49 | .51 | | 95 | .49 / .50 | 0.01 |
| <i>ODH</i> | 1988 | | .75 | .25 | 107 | .41 / .38 | 0.90 | <i>MDH-1</i> | 1989 | .83 | .17 | | 160 | .24 / .28 | 3.36 |
| | 1990 | | .80 | .20 | 84 | .32 / .32 | 0.40 | | 1990 | .84 | .16 | | 107 | .28 / .27 | 0.28 |

* Significant at 5% level; other G-values are non-significant. ** Cumulative genic frequency of three rare alleles (F = .02, M = .01 & S = .02). F, F, M, S and S' represent faster, fast, medium, slow and slower electromorphs respectively. N = sample size.

Table 2. Data on temporal patterns of cryptic / isoelectrophoretic thermostability polymorphism on the basis of post-electrophoretic heat denaturation test, at two polymorphic loci in yearly population samples of *D. immigrans* from Hasimara (W.B.) and *Z. indianus* from Dehradun (U.P.).

| <i>Drosophila immigrans</i> | | | | | | | | <i>Zaprionus indianus</i> | | | | | | | |
|----------------------------------|------|------|------|-------|------|------|------|---------------------------|------|------|------|------|------|------|------|
| ACPH | | | | EST-2 | | | | EST-1 | | | | ADH | | | |
| 1988 | | 1990 | | 1988 | | 1990 | | 1989 | | 1990 | | 1989 | | 1990 | |
| tr | ts | tr | ts | tr | ts | tr | ts | tr | ts | tr | ts | tr | ts | tr | ts |
| F | — | .04 | — | .035 | — | — | — | — | — | — | — | — | — | — | — |
| F | — | .03 | — | .75 | .54 | .17 | .52 | .85 | .05 | .86 | .07 | .40 | .09 | .33 | .15 |
| S | .70 | .23 | .67 | .22 | .22 | .07 | .25 | .08 | .02 | .067 | .003 | — | .51 | — | .52 |
| Total freq. | .70 | .30 | .67 | .33 | .76 | .24 | .77 | .93 | .07 | .927 | .073 | .40 | .60 | .33 | .67 |
| H & H' | .13 | .45 | .20 | .49 | .40 | .63 | .44 | .18 | .27 | .13 | .25 | .50 | .57 | .50 | .60 |
| n _e & n' _e | 1.15 | 1.83 | 1.24 | 1.96 | 1.66 | 2.70 | 1.79 | 1.22 | 1.37 | 1.15 | 1.33 | 2.0 | 2.33 | 2.0 | 2.50 |
| n _e / n' _e | 1.59 | | 1.58 | | 1.62 | | 1.55 | 1.12 | | 1.16 | | 1.17 | | 1.25 | |

H & n_e = heterozygosity and effective number of alleles on the basis of electrophoresis alone; H' & n'_e = heterozygosity and effective number of alleles on the basis of post electrophoretic heat denaturation technique; n'_e / n_e = increase in effective number of alleles. tr & ts refer to temperature resistant and temperature sensitive variant allozyme

population samples have revealed deficiencies of heterozygotes at the *Est-2* locus and excess of heterozygotes at the *AO* locus. However, three loci (*Est-2*, *Mdh-1* and *AO*) have revealed significant deviations from the Hardy-Weinberg equilibrium.

The patterns of distribution of allelic frequency in *Z. indianus* include the occurrence of two common alleles and two rare alleles at the *Acph-1* locus, two common alleles at the *Adh* locus and one frequent and one less frequent alleles at the other three loci (*Acph-2*, *alpha-Gpdh*, *Mdh-1*). The electrophoretic analysis at such five polymorphic loci in natural population samples, taken in two consecutive years from two different sites, have revealed persistence of common, less frequent, as well as rare alleles in the natural populations sampled in two different years. However, the temporally analyzed population samples at two loci (*Acph-2* and *alpha-Gpdh*) have shown differential patterns in terms of deviation from Hardy-Weinberg expectations (Table 2). Thus, the yearly population samples of *Z. indianus* have revealed patterns of genetic homogeneity.

The data on distribution of thermoresistant (tr) and thermosensitive (ts) alleles, allelic frequencies, heterozygosity and effective number of alleles (n_e) at two polymorphic loci in the yearly population samples of *D. immigrans* as well as *Z. indianus* are given in Table 2. The single frequent allele at *Acph* and two frequent alleles at the *Est-2* locus have revealed two isoelectrophoretic variants (tr and ts) which occur with polymorphic frequencies in the yearly population samples. The occurrence of cryptic variation has resulted in a significant increase in heterozygosity as well as effective numbers of alleles (Table 2). However, there is no differentiation with respect to distribution patterns of tr and ts alleles in the yearly population samples. The present investigation has revealed temporal stability of allelic frequencies at five polymorphic loci in *D. immigrans* and *Z. indianus* populations. Our results concur with earlier reports of temporal constancy at phosphoglucumutase (*Pgm*) locus in *D. melanogaster* (Berger, 1971; Dobzhansky and Ayala, 1973; Lamooza *et al.*, 1985). Present observations on the temporal similarity of thermostability variants at *Acph* and *Est-2* loci in *D. immigrans* and at *Est-1* and *Adh* loci in *Z. indianus* are in agreement with earlier reports on persistence of alcohol dehydrogenase ADH cryptic variation in *D. melanogaster* (Hernandez *et al.*, 1986).

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Shamina and R. Parkash. M.D. University, Rohtak, India. *Adh* allozymic variation in *D. melanogaster* populations from India.

Evolutionary potential of a species is a function of the amount of genetic variation occurring in it (Wills, 1981; Hedrick, 1983; Sperlich, 1988). Populations of colonizing species offer excellent material for microevolutionary studies (Endler, 1977, 1986). Studies

on biogeography, ecology and adaptive physiological traits in global populations of *D. melanogaster* revealed that Afrotropical populations constitute the ancestral populations which later colonized Eurasia and more recently America and Australia (David and Capi, 1988). Most studies on allozymic polymorphism had been made on U.S. and Australian populations of *D. melanogaster* while Asian populations remain unexplored (David, 1982; Anderson *et al.*, 1987). Gel electrophoretic analysis has helped in elucidating the genetic structure of geographical populations of diverse taxa; therefore, it was considered to characterize the extent of genic divergence at the *Adh* locus in latitudinally varying (9°58'N to 33°0'N) Indian natural populations of *D. melanogaster*.

The data on observed and expected genotypes, sample size, allelic frequencies, heterozygosity values and application of a G-test for fit to Hardy-Weinberg expectations at the polymorphic *Adh* locus in *D. melanogaster* populations are given in Table 1. The allelic frequency patterns at the *Adh* locus revealed significant clinal variation (along the north-south axis) among Indian populations of *D. melanogaster*. The extent of clinal variation at the *Adh* locus was found to be significantly higher (3% with 1° latitude; $r = 0.96$; $b = 0.036$) and revealed significant deviations from the Hardy-Weinberg equilibrium at the *Adh* locus in Indian populations of *D. melanogaster* (Table 1). The genotypic as well as allelic frequency patterns at the *Adh* locus revealed significant interpopulation heterogeneity ($X^2 = 75.82$) and allelic frequency heterogeneity ($X^2 = 378.46$) on the basis of contingency chi-square tests among Indian populations of *D. melanogaster*. The data on Wright's fixation index ($F_{ST} = 0.25$) revealed significant genic divergence at the *Adh* locus in Indian *D. melanogaster* populations.

The statistical comparison of *Adh* allelic frequency data in Indian populations of *D. melanogaster* with those of

other allopatric populations (Afrotropical, Chinese, Japanese and European) revealed: a) consistency of the direction of latitudinal clines on the different continents; b) the extent of the latitudinally related range of allelic frequencies differed significantly at the *Adh* locus among Indian versus Afrotropical populations as well as Indian versus European populations (Table 2). Thus, the direction of the latitudinal cline was found to be similar among different allopatric populations, but the allelic frequencies differed significantly on the basis of student's *t*-test (Table 2).

Table 1. Data on alcohol dehydrogenase (*Adh*) observed and expected genotypes, allelic frequencies, heterozygosities (obs/exp.), Wright's coefficients (*f*), effective number of alleles (*n_e*) and G-values for log-likelihood χ^2 test for fit to Hardy-Weinberg equilibrium in ten Indian geographical populations of *D. melanogaster*.

| Populations | Latitude | Obs. and exp. genotypes | | | Sample size | Allelic freq. | | Het. Obs/exp. | <i>f</i> | <i>n_e</i> | G-values |
|-------------|----------|-------------------------|--------------|------------|-------------|---------------|-----|---------------|----------|----------------------|----------|
| | | FF | SS | FS | | F | S | | | | |
| Cochin | 9° 58'N | 5 / 1.90 | 129 / 124.36 | 23 / 30.74 | 157 | .11 | .89 | .15 / .20 | .25 | 1.25 | 5.78* |
| Madras | 13° 04'N | 8 / 2.73 | 128 / 122.61 | 26 / 36.64 | 162 | .13 | .87 | .16 / .23 | .30 | 1.43 | 10.40* |
| Tirumala | 13° 40'N | 12 / 3.79 | 113 / 104.43 | 23 / 39.78 | 148 | .16 | .84 | .15 / .26 | .42 | 1.36 | 20.30* |
| Hyderabad | 17° 20'N | 10 / 3.83 | 60 / 54.29 | 17 / 28.87 | 87 | .21 | .79 | .20 / .33 | .39 | 1.19 | 13.20* |
| Nagpur | 21° 09'N | 16 / 7.38 | 48 / 40.18 | 18 / 34.44 | 82 | .30 | .70 | .21 / .42 | .47 | 1.72 | 18.51* |
| Bhopal | 23° 16'N | 21 / 16.93 | 15 / 10.45 | 18 / 26.61 | 54 | .56 | .44 | .33 / .49 | .32 | 1.97 | 5.83* |
| Rohtak | 28° 94'N | 62 / 56.40 | 13 / 6.96 | 28 / 39.63 | 103 | .74 | .26 | .27 / .38 | .29 | 1.62 | 8.54* |
| Saharanpur | 29° 58'N | 78 / 70.57 | 12 / 5.61 | 26 / 39.81 | 116 | .78 | .22 | .22 / .34 | .34 | 1.52 | 11.72* |
| Dehradun | 30° 19'N | 80 / 74.24 | 10 / 4.64 | 26 / 37.12 | 116 | .80 | .20 | .22 / .32 | .31 | 1.47 | 8.75* |
| Dalhousie | 33° N | 90 / 84.05 | 10 / 4.05 | 25 / 36.90 | 125 | .82 | .18 | .20 / .29 | .32 | 1.42 | 10.92* |

* Significant at 5% level; F and S represent fast and slow electromorphs respectively.

Table 2. Statistical comparison of *Adh* allelic frequencies of Indian versus other continental populations of *D. melanogaster*.

| | India | Japan | China | Europe | Afrotropical |
|-----------|---------------------------------|-----------------------|----------------------|------------------|------------------|
| N | 6 | 6 | 6 | 6 | 6 |
| F | (Range) .13 - .82 (Mean) .49 | .50 - .93 .73 | .57 - .80 .75 | .92 - .94 .93 | .01 - .38 .09 |
| S | (Range) .18 - .87 (Mean) .51 | .07 - .50 .27 | .20 - .43 .25 | .06 - .08 .07 | .62 - .99 .91 |
| t | — | 1.43 | 1.62 | 3.33* | 3.73* |
| Reference | Present study | (Watada et al., 1986) | (Jiang et al., 1989) | (David, 1982) | (David, 1982) |

N = number of populations analysed; allelic frequencies range include minimum and maximum values; F and S denote electromorphs; t = Student *t*-test; * Significant at 5% level.

Latitudinal clines have been reported in *D. melanogaster* in U.S. populations (Marks et al., 1980), Australian populations (Oakeshott et al., 1982), Afrotropical populations (David et al., 1986, 1989), Japanese populations (Watada et al., 1986), and Chinese populations (Jiang et al., 1989). The present data on clinal variation at the *Adh* locus in Indian populations of *D. melanogaster* further supports and validates the hypothesis that occurrence of parallel or complementary latitudinal clines across different continental populations provide strong evidence of natural selection maintaining *Adh* clinal allozymic variation (Nagy-

laki, 1975; Endler, 1977; Van Delden, 1982). The occurrence of clinal variation across diverse biogeographical regions can not be explained on the basis of stochastic process (genetic drift) and/or gene flow, since the continental populations of *D. melanogaster* differ significantly in their evolutionary history as well as ecogeographical conditions. The existence of parallel clinal allelic frequency changes at the *Adh* locus provides strong evidence for the action of latitudinally related environmental gradients.

ADH catalyzes the oxidation of primary and secondary alcohols to aldehydes and ketones, respectively. Secondary alcohols are more toxic than primary alcohols, because secondary alcohols are oxidized to ketones rather than to less toxic aldehydes (Geer et al., 1989). The tropical region (southern Indian localities) are characterized by greater plant diversity as compared with the northern region, and hence, result in production of secondary alcohols through fermentation of diverse sweet plant resources. Thus, it is suggested that the abundance of secondary alcohols in the southern tropical environment of the Indian subcontinent might exert selective pressure favoring higher frequency of *Adh^S* alleles. On the contrary, the relative absence of secondary alcohols in the fly habitat in the north Indian localities might have favored the *Adh^F* allele. Thus, the observed clinal variation at the *Adh* locus in Indian populations of *D. melanogaster* seems to be maintained by balancing natural selection, varying spatially along the north-south axis of the Indian subcontinent.

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62:11-16; Watada, M., Y.N. Tobari and S. Ohba 1986, Jpn. J. Genet. 61:253-269; Jiang, C., J.G. Gibson and H. Chen 1989, Heredity 62:193-198; Nagylaki, T. 1975, Genetics 80:595-615; Van Delden, W. 1982, Evol. Biol. 15:187-222; Geer, B.W., P.W.H. Heinstra, A.M. Kapoun and A. Van Der Zel 1989, In: *Ecological and Evolutionary Genetics of Drosophila*, Chapter 13, Barker, J.S.F. et al. (eds.), Plenum Press, New York, pp:231-252.

Sundaran, A.K. and J.P. Gupta. Department of Zoology, Banaras Hindu University, Varanasi 221 005, India. Species spectrum of drosophilids at three localities in the western ghats, South India.

The western ghats extending from river Tapi in the north to Kanniyakumari in the south possess a natural cover of various types of tropical forests: evergreen, semi-evergreen, moist and dry deciduous. Exploratory studies carried out in this region have uncovered the occurrence of several interesting species (Prakash and

Reddy, 1984). The present report embodies the results of intensive field studies conducted at three different localities in this region during June and September, 1988. Altogether 53 species including two subspecies were collected; out of them, 14 species are detected new to science, while 11 others are recorded for the first time from India. The taxonomic descriptions of all new species have been published elsewhere (Gupta and Sundaran, 1990a, b; Sundaran and Gupta, 1990, 1991a, b). The details pertaining to the geographic distribution of species are given in Table 1.

Table 1. Distribution of drosophilid species at three localities in western ghats.

| Species | Collection Localities | | | Total |
|-------------------------------------|-----------------------|-------------|-----------|-------|
| | Virajpet | Elenthikara | Moozhayar | |
| Genus: <i>Drosophila</i> | | | | |
| Subgenus: <i>Drosophila</i> | | | | |
| <i>D. metasetigera</i> | 7 | — | — | 7 |
| <i>D. nasuta</i> | 30 | 26 | 7 | 63 |
| <i>D. neomigrans</i> | 57 | — | — | 57 |
| <i>D. neonasuta</i> | 25 | 7 | 8 | 40 |
| <i>D. neosignata</i> | 20 | — | — | 20 |
| <i>D. pentafusca</i> | 11 | — | — | 11 |
| <i>D. purpurea*</i> | 10 | — | — | 10 |
| <i>D. sternopleuralis</i> | 11 | 9 | 2 | 22 |
| <i>D. synpanishi</i> | 77 | 27 | 8 | 112 |
| <i>D. tongpua</i> | 19 | — | — | 19 |
| Subgenus: <i>Sophophora</i> | | | | |
| <i>D. anomelani</i> | 305 | — | — | 305 |
| <i>D. bipectinata</i> | 27 | 11 | 24 | 62 |
| <i>D. eugracilis</i> | 119 | 114 | 136 | 369 |
| <i>D. jagni</i> | 76 | — | — | 76 |
| <i>D. jambulina</i> | 17 | 13 | 5 | 35 |
| <i>D. kikkawai</i> | 146 | 111 | 60 | 317 |
| <i>D. maggulae*</i> | 109 | — | — | 109 |
| <i>D. malerkotliana</i> | 14 | 4 | 9 | 27 |
| <i>D. pseudoananassae</i> | 28 | — | — | 28 |
| <i>D. sampagensis</i> | 22 | — | 8 | 30 |
| <i>D. suzukii indus</i> | 9 | — | — | 9 |
| <i>D. takahashii</i> | 27 | — | — | 27 |
| Subgenus: <i>Hirtodrosophila</i> | | | | |
| <i>D. chandleri</i> ** | — | — | 9 | 9 |
| <i>D. latifrontata</i> ** | 10 | — | — | 10 |
| <i>D. latifrontata yakuensis</i> ** | 37 | — | — | 37 |
| <i>D. longiphallus*</i> | 50 | — | — | 50 |
| <i>D. parafatfrontata</i> ** | 31 | — | — | 31 |
| Subgenus: <i>Scaptodrosophila</i> | | | | |
| <i>D. bansadharae</i> | 7 | — | — | 7 |
| <i>D. elenthensis*</i> | — | 57 | — | 57 |
| <i>D. ovidenticulata*</i> | — | 54 | — | 54 |
| <i>D. oviminiatus*</i> | 12 | — | — | 12 |
| <i>D. paracubata*</i> | — | 7 | — | 7 |
| <i>D. paratriangulifer*</i> | 14 | — | — | 14 |
| <i>D. peniquadrata*</i> | 5 | — | — | 5 |
| <i>D. pressobrunnea</i> ** | 39 | — | 13 | 52 |
| <i>D. puriensis</i> | 32 | — | 15 | 47 |
| Subgenus: <i>Dudaica</i> | | | | |
| <i>D. senilis</i> ** | 34 | — | — | 34 |

| Species | Collection Localities | | | Total |
|-------------------------------|-----------------------|-------------|-----------|-------|
| | Virajpet | Elenthikara | Moozhayar | |
| Genus: <i>Colocasiomyia</i> | | | | |
| <i>C. colocasiae</i> ** | 581 | — | — | 581 |
| Genus: <i>Hypselothyrea</i> | | | | |
| <i>H. guttata</i> | 5 | — | — | 5 |
| Genus: <i>Liodrosophila</i> | | | | |
| <i>L. ceylonica</i> ** | 20 | — | — | 20 |
| <i>L. globosa</i> | 119 | — | — | 119 |
| Genus: <i>Microdrosophila</i> | | | | |
| <i>M. elangata</i> ** | 7 | — | 6 | 13 |
| <i>M. neodistincta</i> * | 25 | — | — | 25 |
| <i>M. pleurolineata</i> | 22 | — | 12 | 24 |
| <i>M. virajpetiensis</i> * | 31 | — | — | 31 |
| Genus: <i>Mulgravea</i> | | | | |
| <i>M. parasitica</i> | 6 | — | — | 6 |
| Genus: <i>Mycodrosophila</i> | | | | |
| <i>M. gordonii</i> ** | 13 | — | — | 13 |
| <i>M. melanopleura</i> * | 9 | — | — | 9 |
| <i>M. parallelinervia</i> ** | 16 | — | 8 | 24 |
| <i>M. penihispidus</i> * | 12 | — | — | 12 |
| <i>M. xanthopleura</i> * | — | — | 20 | 20 |
| Genus: <i>Zaprionus</i> | | | | |
| <i>Z. multistriatus</i> | 5 | — | — | 5 |
| <i>Z. pyinoolwinensis</i> | 15 | — | — | 15 |
| | | | | 3103 |

* New species; ** Newly recorded from India

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Band, H. T. Michigan State University, E. Lansing, MI 48824 and UVA's Mt. Lake Biological Station, Pembroke, VA 24136. A breeding site for *Chymomyza procnemoides*.

Chymomyza procnemoides has been found to be breeding under bark of damaged white oak trees in Giles Co., VA. Heavy equipment used in dormitory construction in summer 1992 damaged a number of trees fronting a road on the Biological Station grounds. *Chymomyza procnemoides* adults were captured

on damaged white oaks in early July, singly and in mating pairs. Most were released after identification in the laboratory. Bark pulled off from one tree in mid-July where a number of mating pairs were seen had hatched and unhatched *Chymomyza* eggs, early instar *Chymomyza* larvae, 2 third instars of a *C. procnemoides* size species and a third instar of a larger *Chymomyza* species. Also present were frass and a lepidopteran larva. A region of sap flow on the tree was visible after the bark was pulled off. The larger *Chymomyza* larva had posterior black spiracles, so probably was not a *Chymomyza caudatula* larva which is shown in Ferrar (1987). A *Chymomyza caudatula* male and another larger *Chymomyza* male were captured on a different damaged white oak also in July, as in past years (Band 1988a). Pictures were taken of the *Chymomyza* eggs and *Chymomyza* larvae at VPI & SU in Blacksburg. Attempts to grow the larvae in the laboratory failed. However, in early October bark pulled from another white oak again had frass, 7 empty drosophilid pupae cases, 3 empty *Chymomyza* pupae cases and two *Chymomyza* pupae (1 dried). A *C. procnemoides*

male emerged after return to East Lansing.

Chymomyza aldrichii were also captured on the damaged oaks along with *C. procnemoides* in the latter part of July. Where *C. aldrichii* breeds in the Mt. Lake area remains unknown. This species has been found under bark of injured aspens in Minnesota (Spieth, 1957) and reared from logs of white spruce, trembling aspen and a white birch stump in Canada (Teskey, 1976).

The occurrence of *C. procnemoides* larvae in association with other insect larvae and frass (insect excreta) in damaged oaks suggests that the occurrence of *C. amoena* in parasitized fruits and nuts, also containing frass from the exiting curculio or lepidopteran larva (Band, 1988b), is not unique among species of this genus.

Chymomyza procnemoides is also the most recent Nearctic member of this genus to be found in Europe. It has now been captured in Budapest, Hungary, on freshly cut poplar trunks (Papp, 1992).

Acknowledgements: Thanks are gratefully extended to Dr. Gerhard Bächli for alerting me to the existence of *Chymomyza procnemoides* in Europe.

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Band, H.T. Michigan State University, E. Lansing, MI 48824 and UVA's Mt. Lake Biological Station, Pembroke, VA 24136. Just the right size.

The discovery that *Chymomyza amoena* in Switzerland is breeding in nuts of the European chestnut *Castanea sativa* (Band, 1991; Burla and Bächli, 1991, 1992) raises the question, Is this a new niche for the species? There were no reports of drosophilids

breeding in chestnuts *Castanea dentata* in this country (Williams, 1989) in contrast to its secondary invader status in acorns (Dorsey et al., 1962). Nineteenth century references to *C. (D). amoena* are to its breeding in apples along with *D. (ampelophila) melanogaster* (Comstock, 1882; Lintner, 1883), or to the two "Drosophilas" at cider mills (Comstock, 1882) or as household pests (Howard, 1896).

Table 1. Location of *C. amoena* eggs on parasitized oriental chestnuts, 24 hour egg laying period.

| Location | Nut | | | | total |
|----------------------|-----|----|-----|-----|-------|
| | 1 | 2 | 3 | 4 | |
| Stylar hairs at apex | 6 | 49 | 43 | 13 | 111 |
| hilum (base) | 4 | 2 | 6 | 7 | 19 |
| hole(s) | 0 | 1 | 5 | fly | 6+ |
| fly in hole | yes | | | yes | |
| fly probably in hole | | | yes | | |
| total no. of eggs | 10 | 52 | 54 | 20 | 136+ |

However Downing (1869) reported that the European chestnut was the most common of the non-native species in this country, while the nuts identified to carry the chestnut blight fungus *Endothia parasitica* here were Spanish or European chestnuts (Collins, 1913, 1915). Since Chinese chestnuts *Castanea mollissima* are present at the Rt. 700 farm in Virginia, collections of nuts were made in Fall 1992. Nuts from which weevil larvae had exited (Payne, 1978) were old. Weevil larvae had not exited from the 1992 crop of fallen nuts, the parasitized nuts denoted by a "black sting area" or an occasional tiny hole. Therefore 36 "old"

nuts with exit holes and 35 new nuts lacking exit holes were collected. Eight empty pupal cases and two *Chymomyza* larvae were present in 8 of the old nuts. The larvae successfully pupated and one *C. amoena* emerged. Among 24 new nuts, 12 showed evidence of parasitism upon dissection. The rest were left entire and saved after weevil larvae emerged later in October.

To determine if *C. amoena* females would oviposit in/on the parasitized oriental chestnuts, nuts were first soaked in distilled water a week to restore moisture (Spieth 1988). On an initial group of 3 chestnuts, females especially oviposited around the hilum at the base of the nut, but also in exit holes in a 24 hour period. A total of 104 eggs were counted. On a second group of 4 nuts, oviposition in a 24 hour period was predominantly in the stylar hairs at the apex of the nut. Two larvae were later seen to enter the nut through the style at the apex. Eggs were also laid at the base in the hilum area. However, one fly had entered an exit hole and was stuck via her wings inside the nut, a second fly emerged on a nut while eggs were being counted. On a third nut, two red eyes peered out of a weevil larval exit hole and the fly emerged, indicating chestnut weevil larval exit holes are "just the right size" for *C. amoena* females to enter, oviposit, and exit.

Table 1 shows the distribution of eggs on the second group of chestnuts. No dissection was made to count egg numbers inside, though the likelihood is that more eggs are outside than in, and *C. amoena* larvae gain entrance either via the exit holes or "peak." Both groups of nuts were placed with moistened tissue and regularly sprayed with distilled water to prevent drying. However, experiments were carried out in February, nuts were quite dry so a week's soaking may not have been sufficient to restore moisture enabling successful development of *C. amoena* larvae. No adults had

emerged after 6 weeks from hatching dates.

That "blue mold" (*Penicillium* sp.) developed in/on the weeviled chestnuts is expected. Payne (1978) notes that *Penicillium* was the most frequent mold isolated from weevil-damaged chestnuts. Winston (1956) notes that in decaying acorns *C. amoena* larvae fed on *Penicillium* sp. and *Fusarium* sp. which Payne (1978) also notes may be among the decay fungi found in oriental and European chestnuts.

The fact that *C. amoena* in Switzerland emerged from parasitized chestnuts collected in November and again March (Burla and Bächli, 1992), the fact that this species can continue to breed in this country through fall (Malloch and McAtee, 1924) and the parasitized nut offers better protection to the overwintering larvae than a disintegrating apple where the 3rd instar stage seems the most successful overwinterer (Band and Band, 1984), the fact that European chestnuts were present also in this country makes it likely that *C. amoena* was breeding in chestnuts when this was the dominant North American hardwood tree. Hence the occurrence of European *C. amoena* in nuts of the European chestnut is probably an old niche rediscovered. The ability of *C. amoena* to invade Chinese chestnuts also creates the possibility that this species may at some future date achieve a world wide Northern Hemispheric distribution.

Whether or not *C. amoena* might also have been a vector for the chestnut blight fungus in this country is still under investigation, since nuts of both European chestnuts (Collins, 1915) and American chestnuts (DePalma and Jaynes, 1982; Jaynes and DePalma, 1984) can carry the fungus and the Swiss noted that fresh tree wounds were one means by which the blight fungus was spread (Roane et al., 1986). *Chymomyza amoena* is among the *Chymomyza* attracted to freshly damaged trees. However oriental chestnuts are immune to chestnut blight fungus and imported nuts from the "cured" European chestnuts were free of the fungus (Jaynes and DePalma, 1984).

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van't Land, Jan, Wilke van Delden, and Albert Kamping. Department of Population Genetics, University of Groningen, P.O. Box 14, 9750 AA Haren, the Netherlands. Variation in *In(2L)t* frequency in relation to the *Adh* and *alpha-Gpdh* polymorphisms in tropical populations of *Drosophila melanogaster*.

Although a lot of research has been done on the worldwide latitudinal cline for the S(low) and F(ast) alleles of the *Adh* (II, 50.1) and *alpha-Gpdh* (II, 20.5) loci, and on the cline for the cosmopolitan inversion *In(2L)t* (22D3-E1;34A8.9) in *D. melanogaster* (Mettler et al., 1977; Knibb, 1982; Oakeshott et al., 1982; Singh et al., 1982; Inoue et al., 1984; Anderson et al., 1987; and see Lemeunier and Aulard, 1992 for a review), little

attention has been paid to the presence of these clines on the Latin American continent (Pipkin et al., 1976; Capy et al., 1986). In this study we present some data on wild populations of *D. melanogaster* from the Republic of Panama.

Between June and November 1991 wild *D. melanogaster* flies were collected on 5 locations in Panama: Panama Ciudad (ST), Las Tablas (LT), Isla de Barro Colorado (BC), Chiriqui Grande (CG) and Bocas del Toro (BT). All these locations are situated at an altitude below 100 meter. A total of 597 flies were collected (286 males and 311 females).

Individual females were allowed to lay eggs, and weight and wing length were measured. Horizontal polyacrylamide gel electrophoresis was carried out to determine the *Adh* and *alpha-Gpdh* genotypes for each individual. Populations were maintained in mass cultures at 25°C and 40-50% RH on standard food (sucrose/dead yeast/agar medium) on a 2-week schedule. The frequency of *In(2L)t* was determined by carrying out 90 single-pair crosses per population between wild type males and virgin females with second chromosome markers *dumpy* (*dp*: II, 13.0) and *black* (*b*: II, 48.5). Eight F1-females from each cross were backcrossed to *dp b* males. The presence of flies with *dp* or *b* phenotype in the F2 indicated the occurrence of recombination of the second chromosome and consequently this meant the absence of *In(2L)t*. This procedure enabled us to detect the exact proportion of flies which were heterozygous and homozygous for *In(2L)t* in each population. The average percentage of recombinants within F2-progenies where *In(2L)t* was absent, varied between 31.1% and 33.2% for the 5 populations. These percentages are less than expected (35.5%). This is probably due to a mixture of chromosomes with regular recombination (no inversions) and some chromosomes with reduced recombination (e.g., small inversions) (van Delden and Kamping, 1989). *In(2L)t* appeared to be always associated with a *Adh^S/alpha-Gpdh^F* allele combination. The observed *In(2L)t*, *Adh^S* and *alpha-Gpdh^F* frequencies are shown in Table 1, together with mean female wing length and mean female fresh weight.

Table 1. Number of collected flies, *Adh* and *alpha-Gpdh* allele frequencies, frequencies of chromosomes (with *Adh^S/alpha-Gpdh^F* allele combination) containing *In(2L)t*, mean wing length (mm) and mean fresh weight (mg) of 5 Panamanian populations of *D. melanogaster*, together with the annual rainfall at the respective locations. Data from other populations are taken from Knibb *et al.*, 1981 (*), Oakeshott *et al.*, 1982 (**), David, 1982 (†) and Capy *et al.*, 1986 (†).

| Pop. code | latitude | annual rainfall (mm) | # collect. | | Allele freq. | | mean ♀ wing length (mm) | mean ♂ fresh wght. (mg) | <i>In(2L)t</i> freq. in | |
|---------------|----------|----------------------|------------|-------------------|------------------------|-------------------------------|-------------------------|-------------------------|--|------------|
| | | | ♂♂ | ♀♀ | <i>Adh^S</i> | <i>alpha-Gpdh^F</i> | | | <i>Adh^S/alpha-Gpdh^F</i> chrom. | all chrom. |
| Burundi† | 3° S | | | >100 ^a | .62 | 1.00 | | | | |
| Congo† | 4° S | | | >100 ^a | .96 | .98 | | | | |
| Bénin† | 6° N | | | >100 ^a | .98 | .99 | | | | |
| Cameroon† | 7° N | | | >100 ^a | 1.0 | .99 | | | | |
| Sogerit*,** | 8° 8' S | | | 22 | .91 | .78 | | | | 0.46 |
| LT | 7° 45' N | 1150 | 43 | 38 | .91 | .90 | 1.28 | .86 | 0.22 | 0.18 |
| CG | 8° 56' N | 3750 | 13 | 18 | .81 | .92 | 1.30 | .99 | 0.34 | 0.30 |
| ST | 9° 00' N | 1750 | 62 | 60 | .85 | .86 | 1.29 | .88 | 0.17 | 0.13 |
| BC | 9° 08' N | 2250 | 159 | 186 | .96 | .93 | 1.29 | .92 | 0.20 | 0.18 |
| BT | 9° 19' N | 3300 | 8 | 9 | .85 | .97 | 1.24 | .85 | 0.54 | 0.44 |
| Snake Bay*,** | 11° 4' S | | | 11 | .92 | .92 | | | | 0.55 |
| Darwin*,** | 12° 5' S | | | 12 | .91 | .92 | | | | 0.25 |
| Martinique† | 14° 5' N | | | 32 | 1.00 | .91 | | | | |
| Guadeloupe† | 16° 1' N | | | 124 | .99 | .92 | | | | |

^aNumber of collected females estimated from number of genes sampled.

The observed *Adh^S*, *alpha-Gpdh^F* and *In(2L)t* frequencies are compatible with the frequencies found at similar latitudes in Australasia (Knibb *et al.*, 1981; Oakeshott *et al.*, 1982), in Africa (David, 1982) and in the Caribbean (Capy *et al.*, 1986). These comparative data are summarised in Table 1. The *Adh* and *alpha-Gpdh* loci showed Hardy-Weinberg equilibria for all 5 populations although significant deviations ($p < 0.025$) were found when *In(2L)t* karyotypes were tested. Populations "BC" and "ST" both showed a lack of flies heterozygous for this inversion. The reason for this is unclear. It is noteworthy that although there was no significant difference between the 5 populations in frequency of chromosomes with an *Adh^S/alpha-Gpdh^F* combination, we found significantly different *In(2L)t* frequencies (varying from 16.67% to 53.73% of all *Adh^S/alpha-Gpdh^F* chromosomes). Though the number of populations is small, these *In(2L)t* frequencies appear to be positively correlated with the annual rainfall on the respective locations ($r = 0.724$, $0.10 > p > 0.05$). No correlations have been found between *In(2L)t* frequencies and local temperature (mean, max. and min.), local humidity (mean, max. and min.), mean fresh weight or mean wing length. Similar results on the relation between rainfall and the frequency of *Adh^S* and cosmopolitan inversions were reported earlier (Knibb, 1982; Oakeshott *et al.*, 1982), although these associations disappeared when a larger data set was used (Anderson *et al.*, 1987). Obviously, more populations have to be sampled to give conclusive answers on the question whether *D. melanogaster* populations in South and Central America fit in the worldwide latitudinal cline. A combination of field and experimental work should provide a better understanding of the possible factors influencing and maintaining the *Adh/alpha-Gpdh* and *In(2L)t* polymorphisms.

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Offenberger, M. and A.J. Klarenberg. Zoologisches Institut der Universität, München, Germany. Electrophoretic alpha-amylase variants in the *quinaria* section of *Drosophila*.

procedures. Electrophoretic alpha-amylase variants are useful in population genetic studies of *Drosophila* (De Jong et al., 1972). Doane (1969) listed the electrophoretically analysed alpha-amylases of 42 *Drosophila* species, covering 18 species groups, and the closely related *Zaprionus vittiger* (now: *Zaprionus indianus*; Tsacas, 1985). Some of the species studied (*D. funebris*, *D. quinaria*, *D. tripunctata* of the *quinaria* section, *D. repleta* of the *repleta* group and *D. bifasciata* of the *obscura* group) showed alpha-amylases that were "not resolved by the applied buffer system and remained at the cathode" (Doane, 1969).

For detection of alpha-amylase enzyme variants in these species, agarose and cellulose acetate gel electrophoresis (Hillis and Moritz, 1990) can be performed on homogenates of flies and larvae. For homogenization, Ringer's solution or electrophoresis buffer was used; addition of the non-ionic detergents Triton X-100 or Digitonin to the homogenates in a final concentration (w/v) of 0.6% resulted in more distinct electrophoretic bands. Electrophoretic mobility of alpha-amylases of all examined species was not influenced by these detergents. Flies and larvae were grown on media with 2% dead baker's yeast to yield high alpha-amylase amounts (Klarenberg et al., 1988). Electrophoresis with agarose gels was run for five hours at 4°C, 100 mA, 200 V (De Jong et al., 1972). Gels were made of 0.9 g agarose in 100 ml of 41 mM Veronal (5,5-diethyl barbituric acid sodium salt) buffer pH 8.4. The gel buffer was identical to the electrophoresis buffer. Cellulose acetate electrophoresis was performed with Tris Glycine buffer pH 8.5 for one hour at 4°C, 10 mA, 250 V. After electrophoresis the gel was stained for alpha-amylase activity on recording clean spots in the dark blue field of the starch-iodine complex.

Five members of the *quinaria* group of the Genus *Drosophila* (*D. limbata*, *D. kuntzei*, *D. phalerata*, *D. transversa*, *D. falleni*), three further species of the *quinaria* section (*D. funebris*, *D. putrida* and *D. immigrans*), four species of the *virilis-repleta* section (*D. hydei*, *D. littoralis* and *D. repleta*) and *Zaprionus indianus* were analysed with respect to electrophoretic variants of their alpha-amylases. Table 1 lists the results for various iso-female strains on the nomenclature used by Doane (1969). All members of the *quinaria* group of the sub-

Table 1. α -Amylase enzyme variants detected with agarose and cellulose acetate electrophoresis of homogenates of third-instar larvae and adults of the *quinaria* section of the genus *Drosophila*.

| <i>Drosophila</i> section | <i>Drosophila</i> species | origin of strains | No. of strains | No. of Adults (A) and Larvae (L) | Amy-Variants |
|---------------------------|---------------------------|-------------------|----------------|----------------------------------|------------------|
| <i>quinaria</i> | <i>D. limbata</i> | Germany | 8 | 71 L + 57 A | Amy -6, Amy -7 |
| | <i>D. phalerata</i> | Germany | 3 | 15 L + 55 A | Amy -4 |
| | <i>D. kuntzei</i> | Germany | 9 | 117 L + 33 A | Amy -3, Amy -4 |
| | <i>D. transversa</i> | Finland | 2 | 15 L + 6 A | Amy -4 |
| | <i>D. falleni</i> | USA | 1 | 25 L | Amy -2 |
| | <i>D. funebris</i> | Germany | 1 | 15 L | Amy -7 |
| | <i>D. putrida</i> | USA | 1 | 15 L | Amy -10 |
| | <i>D. immigrans</i> | Germany | 10 | 46 L + 124 A | Amy +10, Amy +11 |
| | | Spain | 2 | 20 A | Amy +10 |
| | <i>D. repleta</i> | Germany | 1 | 15 A | Amy +10 |
| <i>virilis-repleta</i> | <i>D. hydei</i> | Germany | 1 | 15 A | Amy +7 |
| | <i>D. littoralis</i> | Germany | 1 | 15 A | Amy +4 |
| <i>Zaprionus</i> | <i>Z. indianus</i> | Zaire | 1 | 15 L | Amy +6 |

genus *Drosophila* and two further species of the *quinaria* section (*D. funebris* and *D. putrida*) showed alpha-amylases moving to the cathode. Homogenates of third instar larvae and adult flies of the same strain yielded identical iso-amylase bands. The isoenzymes of *D. immigrans* -- a member of the *quinaria* section, too -- and of all other species tested behaved like those studied by Doane (1969) in moving to the anode. With the exception of the enzyme of *D. repleta*, the mobilities of the Amy bands were the same as noted by Doane (1969). Confirming her observation, *D. immigrans* alpha-amylase is moving very slowly, but mobility can be increased on cellulose acetate gels by using a more alkaline buffer system (Tris Glycin pH 9.6).

The existence of positively charged alpha-amylases in all of the examined species of the *quinaria* group and in two further species of the *quinaria* section is strengthening the assumption that electrophoretic mobilities of alpha-amylases reflect the phylogenetic relationship of the various species of this taxon (Table 2). This list is based on data of several authors (Doane 1969; Daniöu et al., 1987; Da Lage et al., 1989). The genus *Drosophila* comprises at least 20 different mobility variants. If Amy-variants with intermediate mobilities (e.g., Amy +3.4 in *D. melanogaster*; Daniöu et al., 1987) are included (not listed in Table 2), then the genus *Drosophila* has at least 27 different mobility variants. However, the

Table 2. A compilation of the number of α -amylase electrophoretic bands and their mobilities in different species groups in the subgenera of the genus *Drosophila*. In parentheses the number of species are given. * Denotes molecular evidence of a Amy gene-duplication, #multiple Amy genes on different chromosomes.

| SUB/GENERA and SPECIES GROUPS | α -AMYLASE BANDS | | | | | | | | | | | | | | | | | |
|----------------------------------|-------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|-----|
| | -10 | -7 | -6 | -4 | -3 | -2 | -1 | +1 | +2 | +3 | +4 | +5 | +6 | +7 | +8 | +9 | +10 | +11 |
| SG <i>Sordophila</i> (1) | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - |
| SG <i>Sophophora</i> | | | | | | | | | | | | | | | | | | |
| <i>obscura</i> (5)* | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | + |
| <i>melanogaster</i> (21)*# | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | - | - |
| <i>willistoni</i> (4) | - | - | - | - | - | - | - | - | - | + | + | + | - | - | - | - | - | - |
| <i>saltans</i> (1) | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - |
| SG <i>Hirtodrosophila</i> (1) | - | - | - | - | - | - | - | - | - | - | - | + | - | + | + | - | - | - |
| SG <i>Pholadon</i> | | | | | | | | | | | | | | | | | | |
| <i>victoria</i> (1) | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + |
| <i>latifasciaeformis</i> (1) | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |
| SG <i>Drosophila</i> | | | | | | | | | | | | | | | | | | |
| <i>quinaria</i> section | | | | | | | | | | | | | | | | | | |
| <i>immigrans</i> (1) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + |
| <i>funbris</i> (1) | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>quinaria</i> (5) | - | + | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>testacea</i> (1) | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>virilis-repleta</i> section | | | | | | | | | | | | | | | | | | |
| <i>virilis</i> (9) | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - |
| <i>robusta</i> (1) | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - |
| <i>melanica</i> (3) | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - |
| <i>annulimana</i> (1) | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - |
| <i>repleta</i> (3)* | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - |
| G <i>Zaprionus</i> (1) | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - |

actual number of electrophoretic alpha-amylase variants may increase further by the use of special techniques like sequential electrophoresis (Keith, 1983) and investigation of more species.

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Goerick, S. and W.-E. Kalisch. Institut für Genetik, Ruhr-Universität Bochum, FR Germany. Is dosage compensation female-regulated by a chromosome 2 factor in the *D. nasuta* subgroup?

There are several advantages to analyse the regulation of dosage compensation of secretion proteins in the *D. nasuta* subgroup towards *D. melanogaster*. (1) The *D. nasuta* subgroup consists of strong related species of which several are totally cross fertile (Wilson et al., 1969). (2) At least 4 X-chromosomal major

fractions are found in SDS-PAGE of each subgroup species, compared with one fraction in *D. melanogaster* (Goerick and Kalisch, 1993a). (3) Total amount of individual X-chromosomal fractions in third instar larvae is twice the amount found in *D. melanogaster*. This enables densitometric measurements in the SDS-PAGE from individual salivary glands. (4) Each species has a characteristic pattern of protein fractions by the number, the amount, and the mobility in SDS-PAGE (Ramesh and Kalisch, 1989). By this, it is possible to identify and to quantify parental fractions in heterozygotes as well as in hybrid larvae (Goerick and Kalisch, 1993b).

In the *D. nasuta* subgroup, dosage compensation of larval secretion protein fractions is female-regulated by hypoactivity of both X chromosomes. This is indicated by F1 *D. nasuta* / *D. n. albomicans* female hybrids (as well as other female hybrids in the *D. nasuta* subgroup) which show a dosage effect (Goerick and Kalisch, 1993a,b). To prove whether or not the hybrid combination of the female X-chromosomes is exclusively responsible for the dosage effect (i.e., the failure to compensate gene expression in females), we measured the X-chromosomal secretion proteins of F2 hybrid females from reciprocal *D. n. nasuta* X *D. n. albomicans* crosses (Table 1).

Our results were not concordant with our assumptions. We expected, based on the data of the F1 female hybrids, that F2 females with a hybrid combination of X chromosomes (N/A; A/N) exclusively should express dosage effect, whereas F2 female hybrids with homozygous X-chromosomes exclusively should express dosage compensation based on

data of the P-generations. We found that F2 females with a hybrid combination of X chromosomes show dosage effect and dosage compensation, respectively. In a comparable way, dosage compensation and dosage effect are respectively found in F2 females with a homozygous combination of X chromosomes.

These data indicate that the combination of X chromosomes (as well as any hybridity effect) are not exclusively responsible for dosage compensation or dosage effect. There must be at least one autosomal factor regulating dosage compensation in the females which has to be different in both species involved. In Table 1, autosomal differences exist between homozygous females of the P-generations, F1 hybrid females and F2 females, which are homozygous or hybrid for each pair of autosomes. Any autosomal factor involved in the regulation could, for example, initiate dosage compensation by homozygosity as well as dosage effect by hybrid combination.

Table 1. Dosage compensation (comp.) and dosage effect (effect) of X-chromosomal secretion protein fractions in individual larvae of different X-chromosomal genotypes. Protein amounts are indicated by densitometric measurements (for methods see Goerick and Kalisch, 1993a,b). Data of homozygous females (N/N and A/A in the P-generation) have been compared with those of homozygous males of the same species; data of hybrid F1 and F2 females as well as males have been compared with those of homozygous females and males of both species involved. Data presented are based on more than two hundred individual larvae in each generation.

| | | | | | | |
|------------|-------|--------|-------|--------|-----|--|
| P | N/N | | x | | A/Y | |
| | comp. | effect | | | | |
| | + | | | | | |
| | A/A | | x | | N/Y | |
| | comp. | effect | | | | |
| | + | | | | | |
| F1 | N/A | | x | | N/Y | |
| | comp. | effect | | | | |
| | | + | | | | |
| | A/N | | x | | A/Y | |
| | comp. | effect | | | | |
| | | + | | | | |
| F2 females | N/N | | | | N/A | |
| | comp. | effect | comp. | effect | | |
| expected | + | | | + | | |
| found | + | + | + | + | | |
| | A/A | | | | A/N | |
| | comp. | effect | comp. | effect | | |
| | + | | | + | | |
| | + | + | + | + | | |

N: *D. n. nasuta*; A: *D. n. albomicans*; Y: Y-chromosome

We suggest that the autosomal factor involved in the regulation of dosage compensation is a transcription factor which normally acts in XX genotypes by hypoactive transcription of secretion protein genes. The factor (or its target sites) has to be species-specific. By this, hybrid genotypes fail to compensate the dosage effect by hypoactivity of the female X chromosomes. We know that the *D. nasuta* subgroup is characterised by a large variety of evolution genetic differences concerning regulation of larval secretion proteins (Goerick and Kalisch, 1993a). By this, even transcription factors responsible for the dosage compensation of secretion proteins could have been changed. At least we know that females indicating a dosage effect of secretion proteins are normally viable and fertile. Therefore, differences even in transcription factors (and their target sites) basically cannot be excluded in the *D. nasuta* subgroup.

So far, the formal genetics of the whole *D. nasuta* subgroup is insufficient for any detailed chromosomal localisation. However, fusion of chromosome 3 with the gonosomes in *D. albomicans* indicates that regulation of dosage compensation in the F2 females of Table 1 cannot be involved by an autosomal factor in chromosome 3. Additionally, the relation of chromosome length between chromosome 2 and chromosome 4 makes it very unlikely that the transcription factor is localised in the dot-like chromosome 4 (Ranganath and Hägele, 1982). By this, chromosome 2 remains a candidate for further investigations.

Using two *D. n. nasuta* mutations, we could establish a series of X chromosomal homozygous and hybrid genotypes with homozygous and hybrid combinations of chromosome 2, respectively (Kalisch and Goerick, 1993). By this, we can switch on and off dosage compensation according to the autosomal background used. This system could favour future analysis of any transcription factor in regulation of dosage compensation in the *D. nasuta* subgroup.

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Chatterjee, R.N. Department of Zoology, University of Calcutta, India. Investigation of transcriptional activity of the X chromosome of *Drosophila hydei* carrying a male lethal mutation.

synthetic activity pattern of the X chromosome was estimated by autoradiographic processing. The procedure used in this laboratory has been followed (Chatterjee and Mukherjee, 1981).

The configuration and ^3H -uridine labeling pattern of the X chromosome of the mutant male (l_3) is shown in Figure 1. As it appears from the photomicrograph, the diameter of the male X chromosome was 1.5 times that of the diploid autosomes. In fact, the whole X chromosome represents a generalised puff. The width of the X chromosome is also consistent with the level of ^3H -uridine incorporation, which is greater than that of the diploid autosomes. It is possible that the male lethal effect of the progeny may be due to elevated levels of transcription in the single X chromosome of the male.

Acknowledgment: This work is supported by a UGC grant.

Reference: Chatterjee, R.N. and A.S. Mukherjee 1981, J. Cell Sci. 47: 295-309.

A number of lethal mutants were isolated during a sex linked lethal test performed in *Drosophila hydei*. Most of the mutant males die as embryos. In one case (l_3), the mutant males died as larvae or early pre-pupae. Salivary gland chromosome preparations from the larvae that would eventually die were made, and the level of



Figure 1. Photomicrograph showing ^3H -uridine labeling over the X chromosome and autosomes in a nucleus from male larval gland of *Drosophila hydei* carrying a male lethal mutation. X = X chromosome; A = autosome; n = nucleolus. Bar represents 10 μm .

Shirolkar, Seema M. and R. Naresh Singh. Molecular Biology Unit, Tata Institute of Fundamental Research, Homi Bhabha Road, Navy Nagar, Colaba, Bombay 400 005, India. Development of synapses in the embryo and larva of *Drosophila melanogaster*.

In *Drosophila* a wealth of information is available on the formation of the peripheral and central nervous system (CNS) during the embryonic development (Poulson, 1937, 1950; Sonnenblick, 1950; Kankel *et al.*, 1978; Campos-Ortega and Hartenstein, 1985; Canal and Ferrus, 1986; Hartenstein *et al.*, 1987). Except a few studies at the ultrastructural level (Bastiani *et al.*,

1984; Raper *et al.* 1984), most of the studies have used light microscopy for following the progress of embryonic development. Bastiani *et al.* (1984) and Raper *et al.* (1984) carried out ultrastructural analyses of the interactions between the growth cone filopodia of the G axon with the fascicle of A/P axons. They showed that filopodia of the G axon have selective affinity for the P axons as compared to the A axons or other fascicles.

The larval development in *Drosophila* was investigated through light microscopy by White and Kankel (1978), Thomas *et al.* (1984), Truman and Bate (1988), Hartenstein (1988), Prokop and Technau (1991), and both through electron and light microscopy by Singh *et al.* (1989). Studies with respect to the changes occurring in the neuropils of larva, such as development of synapses have not received sufficient attention. Since synapses are important sites through

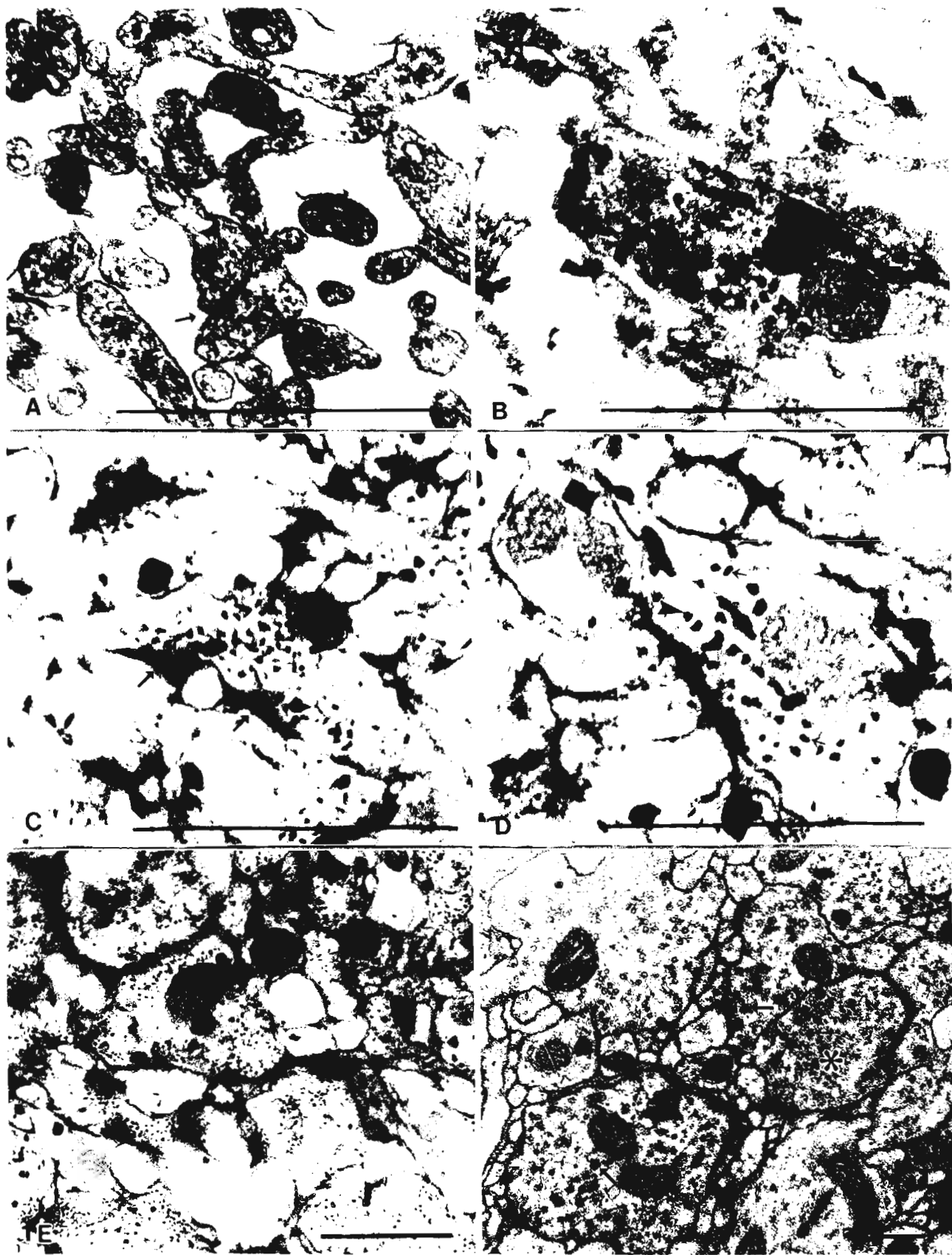
which the neurons communicate with each other, we decided to study the development of synapses during the formation of CNS in the embryo and larva of *Drosophila*. Synapses are mainly of two types - chemical and electrical. As chemical synapses can be identified with confidence by the presence of synaptic vesicles in the presynaptic region, we preferred to concentrate our study on chemical synapses. We have used transmission electron microscope (TEM) for following the course of development of synapses in *Drosophila* embryos.

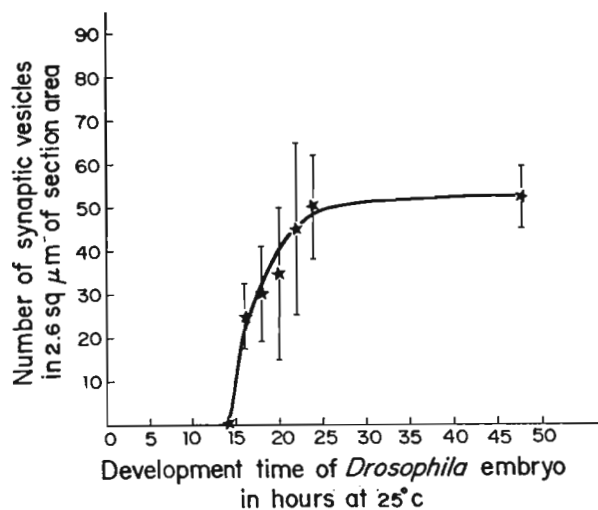
Three to five day old *D. melanogaster* wild type Canton S flies were used for collection of eggs at 25°C. The initial two lots of eggs laid during the first two hours were discarded. Subsequent batches of eggs were collected at hourly intervals and were allowed to grow at 25±1°C separately on fresh food surfaces containing yeast. At the end of the required period of growth, the chorion and vitelline membrane of the eggs were removed by placing them on a double sticky tape and rolling them individually with a tooth pick in a drop of fixative (Karnovsky, 1965). Such eggs were fixed with 2-3 ml Karnovsky's fixative in a glass cavity for 4-6 hours at room temperature (RT) (Karnovsky, 1965). Post fixation was done with Dalton's K₂Cr₂O₇ and OsO₄ mixture for 2 hours on ice and 1 hour at RT (Dalton, 1955). Although this fixation method gave good tissue preservation for embryos older than 24 h in age, for embryos of this or younger ages these fixatives did not give optimal fixation. Therefore, we have used zinc iodide-osmium tetroxide as a fixative (Akert and Sandry, 1968; Schurman, 1980). For both regimens of fixatives, specimens were washed with 0.1 M phosphate buffer of pH 7.4, dehydrated with increasing concentrations of ethyl alcohol followed by propylene oxide, infiltrated with Durcupan ACM (Fluka), oriented and embedded in the same resin mixture in BEEM capsules. The polymerisation was done by an overnight incubation at 60°C. Specimens were sectioned with glass knives using LKB Ultratome III. Sections were picked up on Formvar film made from 0.4% polyvinyl formal solution in 1-2, dichloroethane, held on copper slots, stained with saturated aqueous uranyl acetate at 60°C for 10 minutes washed with water and stained with Reynolds' lead citrate at RT for 5 min (Reynolds, 1963). Subsequent examination was done with a Jeol JEM 100 S electron microscope.

Drosophila embryos were examined at different ages. Up to 13 h of development in the embryo, neither synaptic junctions nor vesicles are observed. This is in agreement with earlier observations of Thomas *et al.* (1984). Neuropil at this stage contains loosely packed neuronal profiles and the intervening space is filled with extracellular fluid. The neuronal profiles of 14 h old embryo contain growth cones at their tips (Figure 1A, arrow). However, they do not contain either synaptic vesicles nor show any synaptic specializations. Later at about 16 h, these profiles show further enlargement at the growth cone region and clusters of synaptic vesicles are found within them (Figure 1B). Most of these vesicles are either round, 30-50 nm in diameter or flattened vesicles of 20-30 nm in size (Figure 1D). But, the junctional specializations like pre- and post-synaptic membrane densities are not formed at this stage. At 18 h, the neuropil becomes more compact and enhanced staining of synaptic vesicles can be visualized in the neuronal profiles which show pre- and post-synaptic specialization (Figure 1C).

In the CNS of vertebrates quite a few types of synaptic vesicles are known to exist: i) electron lucent spherical 40-60 nm diameter vesicles containing acetylcholine or amino acids; ii) electron lucent flattened vesicles 30-60 nm diameter, having gamma-amino butyric acid or glycine; iii) electron-dense or dense core 40-100 nm diameter vesicles containing catecholamines; and iv) large electron-dense vesicles of diameter 100-160 nm known to be neurosecretory granules (Shepherd, 1979). We have used a mixture of zinc and osmium tetroxide as a fixative, and hence all the synaptic vesicles found within the neuronal profiles become electron-dense. Because of this, we have been able to classify the synaptic vesicles present in the CNS of *Drosophila* embryo of various stages into two types. They are either spherical vesicles or the flattened type vesicles (Figure 1B-D). We have also counted the average number of synaptic vesicles per synapse in different developmental stages. The average number of synaptic vesicles per unit area of ultrathin sections or for that matter per synapse rises rapidly from zero value at 16 h until 24 h and subsequently it stabilizes (Figure 2). For stages later than 48 h of development, we were able to characterize 3 types of synaptic vesicles: electron lucent, electron dense and dense-core (Figure 1F) using Karnovsky's and Dalton's fixatives successively. The number of synapses, however, increase sharply between 15 h and 22 h and then decrease with similar rapidity by 24 h to

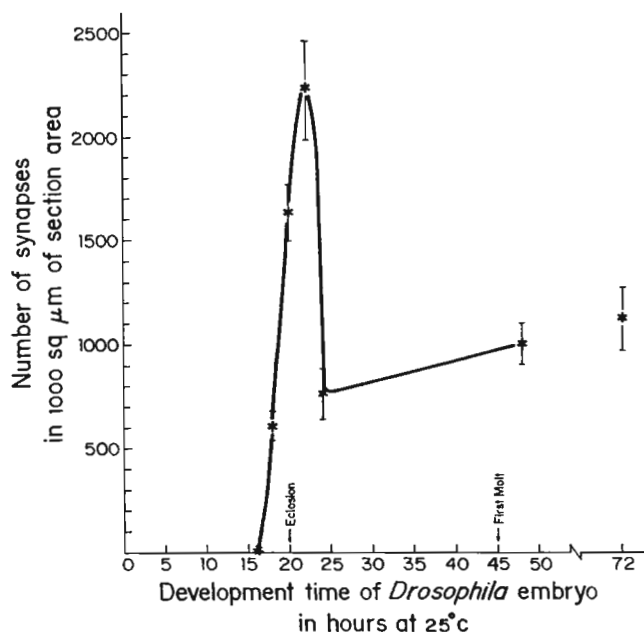
Figure 1. Electron micrographs of the developing neuropil of *D. melanogaster* embryos and larvae grown at 25°C. A, loosely packed neuronal profiles at 14 h. Some growth cones make contact with each other (arrow) as a prelude to fascicle formation. B, synaptic vesicles are prominently visible at 16 h (white arrow). C, neuronal profiles compactly fill the neuropil at 18 h. Synaptic junctions are formed (arrow). D, synapses become mature by 20 h. Flat (arrow) and round (arrowhead) vesicles are identifiable. E, population of synapses and synaptic vesicles in a brain hemisphere neuropil at 48 h, stained with zinc iodide-osmium tetroxide. F, neuropil of the brain hemisphere of third instar larva treated with Karnovsky's fixative followed by Dalton's chrome-osmium tetroxide fixative, showing: electron lucent (small arrow), electron dense (arrowhead) and dense-core (thick arrow) vesicles. Glycogen (asterisk), typical of larval neuronal profiles (Singh *et al.*, 1991) in *Drosophila* neuropil. Magnification bar = 1 µm.





2

Figure 2. Increase in the average number of synaptic vesicles with the progress of development of *D. melanogaster* embryo and larva.



3

Figure 3. Formation and selective degradation of synapses in the embryo and larva of *D. melanogaster*.

an approximately stable value (Figure 3). The net loss of synapses from the peak value to stable value is approximately 70%.

The loss in total number of synapses in *Drosophila* may be viewed as a sum total of their formation and breakdown. The very processes of eclosion, motor activity or sensory experience may play some role in the loss of number of synapses. It is plausible that the reserved pool of *pro-synaptic* molecules is utilized between the time of eclosion (20 h) to the peak of number of synapses (22 h). A similar increase and decrease in the number of synapses was also observed during the development of the visual system of *Musca domestica*, but the net loss of synapses was found to cease around eclosion (Frolich and Meinertzhagen, 1983).

Acknowledgments: We thank Dr. Rajashekhar Patil, Mrs. Shubha Shanbhag and Mrs. Kusum Singh for helpful suggestions and help on various occasions.

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McNamee, S. *Drosophila* Population Biology Unit, University of Leeds, Leeds, LS2 9JT, UK. Collection of *Drosophila ambigua* from an alcoholic resource in Scotland.

Similar species, particularly the ethanol tolerant *D. melanogaster* and *D. virilis*, would have been expected from collections made in the alcoholic habitat of a 'bodega' (sherry maturation cellar). In July 1992 *Drosophila* larvae and pupae were observed in the seepage from leaking sherry casks in a bodega attached to a distillery in central Scotland. Some of these larvae and pupae were collected so they could be identified as emerging adults at a later date. Baits of apple, onion, rowanberry and lemon were chopped and pre-rotted for a week before being placed on 2-3 cm agar in 1" x 3" glass tubes. These were left in the bodega for a week to allow the *Drosophila* time to lay eggs. After collection, the tubes were closed with foam bungs and placed in an 18°C incubator. All the emerging adults (>200 individuals) from the baits and the sherry seepage were of one species, *D. ambigua* ('obscura' group). Despite the close proximity of a brewery (<800 m) no *D. melanogaster* or *D. virilis* were collected. Many individuals of the parasitoid *Asobara tabida* were also recorded and this may be the first record of this parasitoid from *D. ambigua*. A possible explanation for the presence of *D. ambigua* in the bodega, but not other alcoholic sites in Britain, is that it may have been transported directly from Spain with the original sherry casks. It is unclear why other species from the nearby brewery have not invaded the bodega since *Drosophila* are known to disperse over these distances.

References: Newbury, S.F. 1984, *PhD Thesis, Univ. of Leeds*; McNamee, S. unpubl. data.

Dytham, C. and S. McNamee. *Drosophila* Population Biology Unit, University of Leeds, Leeds, LS2 9JT, UK. Identifying hybrids of *D. melanogaster* and *D. simulans*.

(Sperlich, 1962). Males of the two species can be separated by their genitalia. Females of the two species are usually deemed indistinguishable but may be reliably separated by taking linear head and size measurements and performing a canonical variate analysis (Dytham and McNamee, 1992; McNamee and Dytham, 1993). This method of female separation has been extended to differentiate between *D. melanogaster*, *D. simulans*, and their hybrids. The *D. melanogaster* and *D. simulans* stocks used were initiated from flies collected at a wholesale fruit market in Leeds in summer 1991. Hybridization of these species is asymmetric with the cross of *D. melanogaster* females with *D. simulans* males occurring much more readily, and this cross was used to produce the hybrids for measurement. Since only sterile female adults are produced from this cross, 20 female hybrids were compared with 20 females from each of the parent stocks. Thorax length, tibia width, eye height, eye width and five eye margin measurements were taken (see McNamee and Dytham, 1993, for accurate description of measurements taken). A canonical variate analysis was carried out using all nine measurements.

The results show that hybrids are intermediate between the two parent

Breweries are a 'domestic' habitat known to have several *Drosophila* species associated with them. Collections made in breweries in Yorkshire recorded *D. melanogaster*, *D. virilis*, *D. subobscura*, *D. buskii*, *D. funebris*, *D. immigrans*, *D. hydei* and from one brewery *D. obscura* (Newbury, 1984; McNamee, unpubl. data).

Drosophila melanogaster and *D. simulans* are both domestic, cosmopolitan species and often coexist in the wild. The two species are closely related and will produce sterile hybrids intermediate in their morphology in the laboratory (Sturtevant, 1920) and in the field

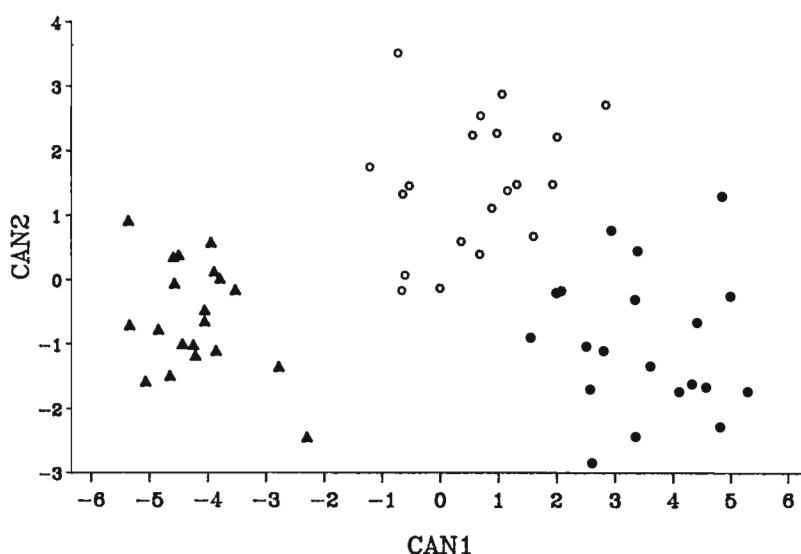


Figure 1. Position of individual flies on first two canonical axes. Filled circles, *D. melanogaster*; triangles, *D. simulans*; open circles, hybrids.

species (Figure 1), suggesting the characters measured are heritable. All three groups are highly significantly different from one another ($p < 0.0001$ in all cases), although the hybrids were more similar to *D. melanogaster* females. There is no overlap in the distribution of individuals (Figure 1). The first canonical axis explains 89.7% of the variation between groups and uses eye margin characters (especially cheek width and forehead width) as the main distinguishing features and is not a measure of size. The second canonical axis uses the size measure, thorax length, and eye height to separate the groups. If samples of both pure species were available, this technique could be used to monitor the incidence of hybrids of *D. melanogaster* and *D. simulans* in mixed populations in the laboratory or the field.

References: Sturtevant, A.H. 1920, *Genetics* 5:488-500; Sperlich, D. 1962, *Dros. Inf. Serv.* 36:118; Dytham, C. and S. McNamee 1992, *Dros. Inf. Serv.* 71:253-254; McNamee, S. and C. Dytham 1993, *Sys. Ent.*, in press.

McNamee, S. and C. Dytham. *Drosophila* Population Biology Unit, University of Leeds, Leeds, LS2 9JT, UK. Morphology of *Drosophila melanogaster* in populations sympatric with and allopatric to *D. simulans*.

Two populations of *D. melanogaster* from different habitats and a single population of *D. simulans* are compared using the methods described by Dytham and McNamee (1992) and more fully in McNamee and Dytham (1993).

Strains of both *D. simulans* and *D. melanogaster* were initiated with flies collected from the Pontefract Lane wholesale fruit market, Leeds, in summer 1991, where the two species have been shown to coexist over a number of years (Atkinson, 1977; Rosewell, 1986; McNamee, unpubl. data). A second (allopatric) *D. melanogaster* strain was initiated with flies collected from four breweries in Yorkshire, UK in summer 1991. Breweries have similar species of *Drosophila* to those present in fruit markets, except *D. simulans* is absent (McNamee, unpubl. data; Newbury, 1984). The two market populations have been analysed previously, showing that accurate identification of females of the two species is possible (Dytham and McNamee, 1992; McNamee and Dytham, 1993).

Canonical variate analysis using the linear head and size measurements places the brewery (allopatric) *D. melanogaster* between the market (sympatric) *D. melanogaster* and *D. simulans* (Figure 1), though closer to the market *D. melanogaster*. The difference observed is greater for females than males of the species though all groups (species and sexes) are significantly different from one another ($p < 0.0001$ in all cases). The principal measurements important in discrimination on the first canonical axis (which explains 72% of the variation between groups) are eye margin measures; cheek width and forehead width. The second canonical axis (explaining 22% of the variation) gives most weight to the 'size' measure, thorax length. The first axis separates the groups by strain, whereas the second axis is largely a 'size' measure and thus separates the relatively small males from large females in all the three strains.

The sympatric, market popu-

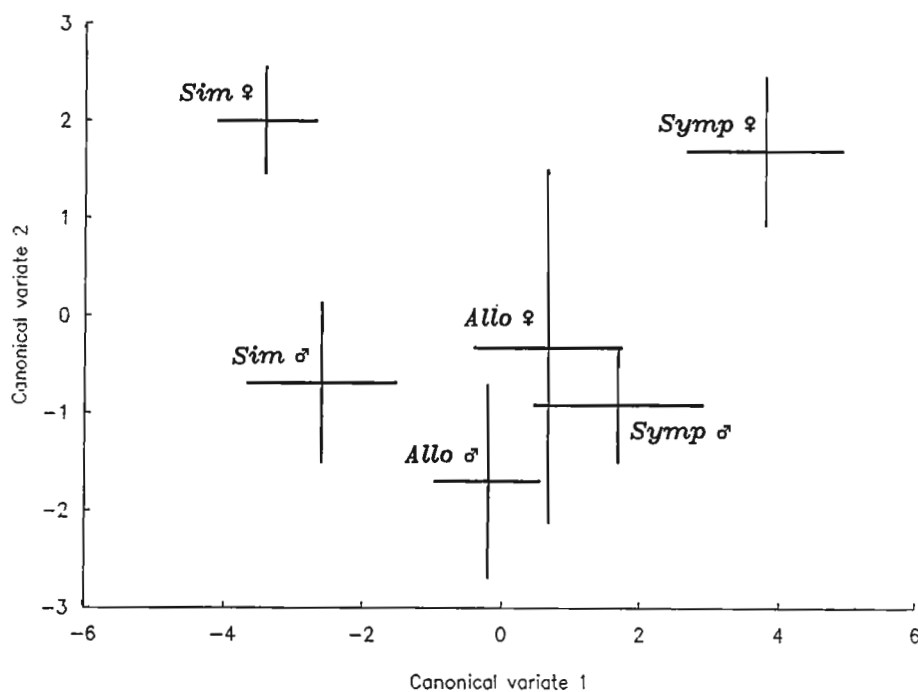


Figure 1. Position of centroids of groups on the first canonical axes calculated using canonical variate analysis. Bars indicate ± 1 standard deviation. Each group contains 20 individuals and is significantly different from all others ($p < 0.0001$). Key: allo - allopatric *D. melanogaster*, symp - sympatric *D. melanogaster*, sim - *D. simulans*.

lation of *D. melanogaster* is more different from *D. simulans* than is the allopatric, brewery population. There are three possible explanations for this:

(1) This could simply be a chance effect, with genetic drift causing these two, perhaps isolated, populations of *D. melanogaster* to differ morphologically.

(2) The narrower eye margins of the brewery *D. melanogaster* might also represent an adaptation to the alcoholic brewery environment and be a selected trait.

(3) The difference between the sympatric and allopatric populations could also be interpreted as an example of character displacement, where the market population has evolved in the presence of *D. simulans* to become less like its competitor.

References: Dytham, C. and S. McNamee 1992, *Dros. Inf. Serv.* 71:253-254; McNamee, S. and C. Dytham 1993, *Sys. Ent.*, in press; Atkinson, W. 1977, PhD Thesis, Univ. of Leeds; Rosewell, J. 1986, PhD Thesis, Univ. of Leeds; McNamee, S., unpubl. data; Newbury, S.F. 1984, PhD Thesis, Univ. of Leeds.

McNamee, S. and C. Dytham. *Drosophila* Population Biology Unit, University of Leeds, Leeds, LS2 9JT, UK. Morphological discrimination of four species of the *melanogaster* species sub-group.

The '*melanogaster*' species sub-group comprises of eight species. Four of these species; *D. melanogaster*, *D. simulans*, *D. mauritiana* and *D. sechellia* are very closely related and form the '*melanogaster*' species complex (Lachaise, 1988). Males may be distinguished by the processes on the genital arches (Sturtevant, 1920;

Tsacas and David, 1974; Tsacas and Bächli, 1981), while females are usually distinguished only by their male progeny. Females of *D. melanogaster* and *D. simulans* have previously been separated reliably using a series of linear head measurements (Dytham and McNamee, 1992). Here we extend the analysis to include *D. mauritiana* and *D. sechellia*. The method used is identical to that described in McNamee and Dytham (1993). Samples of 20 males and 20 females of each of the four species were used. Two general size measurements (thorax length and tibia width) and seven head measurements (eye height, eye width and five measurements of the eye margin) were taken.

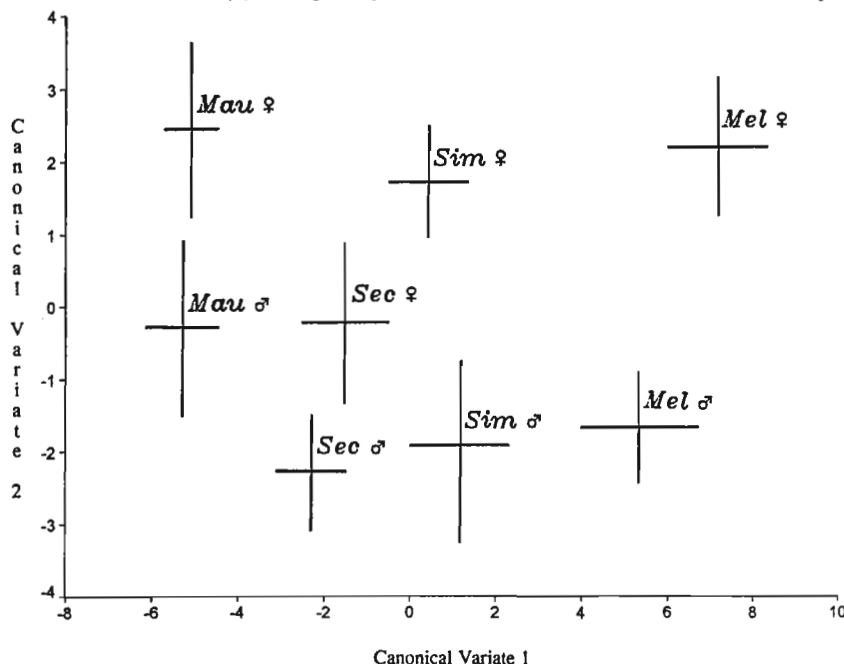


Figure 1. Position of centroids of groups on the first two canonical axes calculated using canonical variate analysis. Bars indicate ± 1 standard deviation. Each group contains 20 individuals and is significantly different from all others ($p < 0.0001$). Mau - *D. mauritiana*, Mel - *D. melanogaster*, Sec - *D. sechellia*, Sim - *D. simulans*.

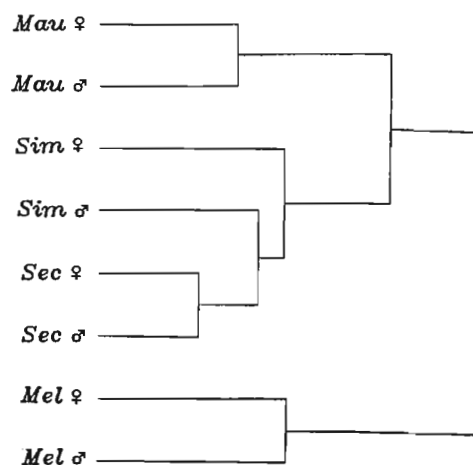


Figure 2. Dendrogram based on the Mahalanobis distances between groups calculated from the canonical variate analysis. Key as Figure 1.

The results of a canonical variate analysis of these data show that each group is highly significantly different from all other groups (Figure 1). As previously noted (Dytham and McNamee, 1992; McNamee and Dytham, 1993) the first canonical variate separates the species and the second is a measure of size, separating smaller males from females. A dendrogram based on the Mahalanobis distances between the groups after the canonical variate analysis shows that *D. sechellia* and *D. mauritiana* are very much more similar to *D. simulans* than they are to *D. melanogaster* (Figure 2). This observation concords very well with dendrograms calculated from allozyme data (Lachaise, 1988) and a range of morphological and genetic characters (David and Capi, 1988).

This technique could be used to identify females of these four species reliably.

References: Lachaise, D. *et al.* 1988, *Evol. Biol.* 22:159-226; Sturtevant, A.H. 1920, *Genetics* 5:488-500; Tsacas, L. and J.R. David 1974, *Bull. Soc. Entomol. Fr.* 79:42-46; Tsacas, L. and G. Bächli 1981, *Rev. Fr. Entomol.* 3:146-150; Dytham, C. and S. McNamee 1992, *Dros. Inf. Serv.* 71:253-254; McNamee, S. and C. Dytham 1993, *Systematic Entomology*, in press; David, J.R. and P. Capi 1988, *Tr. Gen.* 4:106-111.

Dytham, Calvin and Lynne O'Brien. *Drosophila* Population Biology Unit, Department of Biology, University of Leeds, Leeds, LS2 9JT, U.K. Transmission of an inserted sequence from *Drosophila melanogaster* to hybrids with *D. simulans*.

The sibling species *D. melanogaster* and *D. simulans* will hybridize freely in laboratory conditions (Sturtevant, 1920). The offspring of such hybridizations are always sterile females, the males dying at the third instar. The cross is much more likely to produce offspring if female *D. melanogaster* are used (Eoff, 1973). This suggests there is the possibility of horizontal

transmission of foreign DNA from one species to another if the female *D. melanogaster* was placed in a food tube with two, 2-3 day old, male *D. simulans*. This was repeated 50 times. The *D. melanogaster* were taken from a population which had a high frequency of a DNA sequence inserted by P-element mediated transformation. Two genes were inserted in tandem, an Hsp 70-NPTII fusion gene which confers neomycin resistance and the bacterial beta-galactosidase gene regulated by a composite *Hsp 27/Hairy* promoter. The population was not homozygous for the inserted DNA. (Transformed flies were provided by Dr. D. Ish-Horowicz, ICRF Developmental Biology Unit, Oxford).

The tubes were monitored every other day and the parents were removed once the larvae had emerged. Eight of the 50 tubes (16%) produced adult female hybrids. The parents (where available) and 6-8 of the progeny from six of these tubes were frozen at -70°C and then analyzed by PCR, using primers complementary to the NPTII coding region. The results, shown in Table 1, indicate that none of the male *D. simulans* have the inserted DNA, but all the female *D. melanogaster* are transgenic. In three of the six sets of hybrid progeny the inserted sequence is present in all of the samples while in the other three there are some which have the foreign DNA and some which do not. This is consistent with what is known of the original *D. melanogaster* population where some of the population would be homozygous for the inserted sequence while others would be heterozygotes. In this case it appears that three of the original female *D. melanogaster* were homozygous transgenics and three were heterozygous. Thus the inserted sequence appears to be inherited in the hybrids in normal Mendelian fashion.

Table 1. Presence (+) or absence (-) of an inserted sequence as determined by PCR in male *D. simulans*, female *D. melanogaster* and their hybrid progeny. Empty cells were not tested

| Male | Female | Hybrid Progeny | | | | | | | |
|------|--------|----------------|---|---|---|---|---|---|---|
| - | + | + | + | + | + | + | + | + | + |
| - | + | + | + | + | + | + | + | + | + |
| - | + | + | - | + | + | - | - | + | + |
| | | + | - | + | - | - | + | - | + |
| | | + | + | + | - | - | + | - | + |
| | | + | + | + | + | + | + | + | |

If all hybrids are sterile, then there is no possibility of horizontal transmission of the inserted sequence from *D. melanogaster* to *D. simulans*. However there are two routes available for successful horizontal transmission. The first is through viable hybrids of these two species. The second is through an intermediate species which produces viable hybrids with both species considered here. Another member of the species sub-group, *D. mauritiana* (Goulielmos and Alahiotis, 1989), may provide such a route, although the possibility of fertile hybrids between *D. mauritiana* and *D. simulans* has been fiercely contested (Naveira, 1992).

Acknowledgments: We would like to thank Emmanuelle Berthier for excellent technical assistance. This work is supported by the U.K. Dept. of Environment.

References: Eoff, M. 1973, *Am. Nat.* 107: 247-255; Goulielmos, G.N. and S.N. Alahiotis 1989, *Genome* 32: 146-154; Naveira, H.F. 1992, *Heredity* 211-217; Sturtevant, H.T. 1920, *Genetics* 5: 488-500.

Eid-Dib, C.¹, B. Moreteau¹, G. Vannier², Y. Carton¹, and J.R. David¹. ¹CNRS, Laboratoire de Biologie et Génétique Evolutives, 91198 Gif-sur-Yvette Cedex, France, and ²Laboratoire d'Ecologie Générale, URA 689 CNRS, Museum National d'Histoire Naturelle, 4 avenue du Petit chateau, 91800 Brunoy, France. Tolerance to a progressive desiccation stress in *Drosophila melanogaster* and *D. buzzatii* populations from a desert oasis.

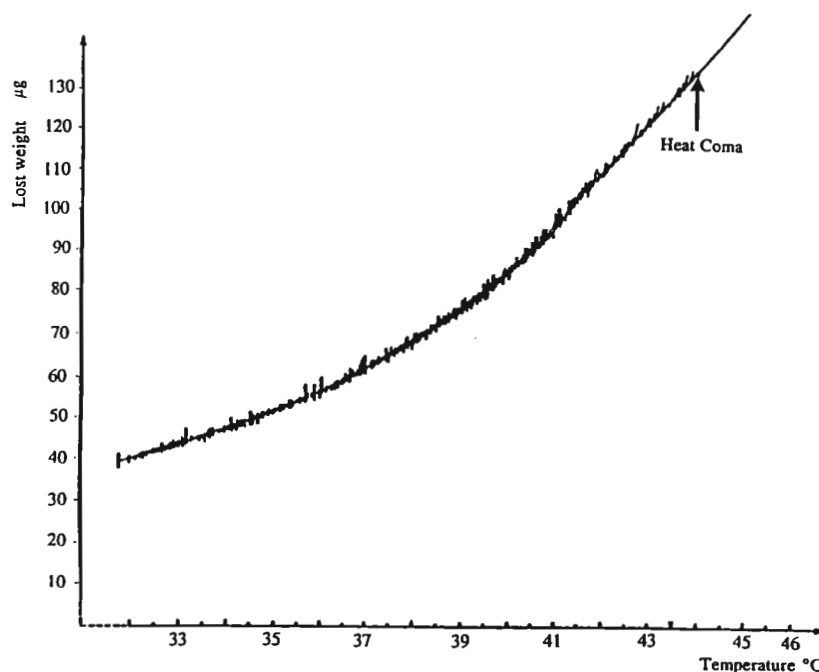
Drosophila adults are very sensitive to heat desiccation stress and adult survival in a dry and hot environment is a major ecological problem for understanding their geographic and microhabitat distribution (David *et al.*, 1983; Hoffmann and Parsons, 1991). Numerous investigations deal with this problem, comparing either different species or geographic populations, and a diversity of techniques have been worked out. However, temperature was generally kept constant, and two kinds of methods have been used.

a short duration (a few minutes or hours) and then measuring the percent survival, generally after a recovery of 24 hours.

2) Chronic stress: adults are submitted to desiccating conditions without food at a given temperature, and survival duration is measured; if the norm of reaction of the species is analysed, aliquot samples of flies are studied at different temperatures (e.g., Da Lage *et al.*, 1989)

1) Acute stress (i.e., high temperature) applied for

Figure 1. Example of a weight-loss curve of an adult *D. melanogaster* male, when submitted to a progressively increasing temperature under desiccating conditions. A regular curve without oscillations indicates the temperature at which the fly enters heat coma (H.C.) (in this case, 43.6°C).



Such experimental studies, although providing interesting results, fail to reproduce stress conditions existing in nature. Natural conditions are characterized by progressive variations and also by daily periodicity. For example, under a dry hot climate during day time, temperature will increase from 25°C to 40°C while relative humidity (RH) may simultaneously decrease from 70% to 20%. For an adult fly there are two possible survival strategies: either being able to tolerate for a while such adverse conditions, or to escape by finding a favorable microhabitat, e.g., a close vicinity of water (Parsons, 1983).

In the present paper, we have tried to compare two populations of two species living in a dry environment, i.e. a Tunisian oasis. These species are the widespread cosmopolitan *D. melanogaster* and a cactophilic species of American origin, *D. buzzatii*. In the case of *D. melanogaster*, it was already shown that using a different technique, the Tunisian population was especially tolerant to desiccation, in absence of any heat effect (Hoffmann and Parsons, 1991). For

Table 1. Biological parameters of flies submitted to a flux of dry air when entering heat coma (both sexes pooled).

| | | <i>D. melanogaster</i> | <i>D. buzzatii</i> | Comparison (t) |
|--------------------|-----------|------------------------|--------------------|----------------|
| Body water | \bar{X} | 166.30 \pm 4.90 | 181.20 \pm 3.75 | 2.45* |
| | CV | 12.50 | 10.14 | |
| Transpiration flux | \bar{X} | 207.61 \pm 9.54 | 172.79 \pm 7.92 | 2.81* |
| | CV | 19.49 | 22.45 | |
| Heat coma (°C) | \bar{X} | 43.80 \pm 0.25 | 48.80 \pm 0.24 | 13.82*** |
| | CV | 2.42 | 2.41 | |
| n | | 18 | 24 | |

Body water and transpiration flux are given for the heat coma. \bar{X} : mean value with SEM, CV: coefficient of variation (in %). n : number of flies

comparing the two species, we have used a method already applied to numerous insect species (Vannier, 1987) and in which temperature is progressively increased under desiccating conditions.

Individual flies are put in a small perforated cage of aluminum foil and suspended to the balance pan in a chamber 3 cm in diameter. The recording microbalance is itself within an incubator and a programmed regulator increases the air temperature from 20°C to 70°C at a regular speed of 0.5°C per minute. Silica gel in the incubator ensures zero RH throughout the experiment.

An example of a weight recording curve is shown in Figure 1. Weight loss is mainly due to transpiration and water loss. During the thermal increase, movements of the fly are registered as small oscillations on the curve, which is thus an actogram. At a given temperature, these oscillations disappear since the fly stops its movements and enters a heat, but reversible, coma. On Figure 1, heat coma (or thermostupor point) occurred at 43.6°C.

Analysis of weight loss curves allows the calculation of the transpiration curves, i.e. their derivative. The average curves for both species are shown in Figure 2. The two curves have similar shapes: transpiration flux increases with temperature, reaches a maximum, then decreases back to zero up to a complete desiccation of the body. Maximum (water loss) transpiration occurs at 56°C for *D. melanogaster* and 61°C for *D. buzzatii* which is thus more tolerant to desiccation.

At the beginning of the experiment, the body water was 206% of the dry weight in *D. melanogaster*, and 215% for *D. buzzatii*. A critical stage is the heat coma point, whose average position is shown in Figure 2.

Some characteristic parameters at the heat coma are given in Table 1. The remaining body water was significantly higher in *D. buzzatii* than in *D. melanogaster* and the transpiration flux lower. But the differences are not very pronounced ($p < 0.05$) and the between-individuals variability is quite high, with coefficients of variation ranging between 10% and 22%. The temperature of heat coma provides, however, a

better contrast between the two species ($p < 0.001$) and also the variability is much less (CV of 2.4%). We see that *D. melanogaster* enters heat coma at 43.8°C while *D. buzzatii* stops moving at 48.8°C. Heat coma temperatures have been measured in numerous other insects and, as a rule the values exhibit a low variability between individuals of the same species (Vannier, 1987). On the other hand, greater variations are observed between taxa: low values (23°C) were found in cave Collembola; a very high tolerance was observed in a stored products beetle (*Oryzaephilus*) with a temperature of 54.4°C. More numerous *Drosophila* species, living in very different environments, should be investigated with this technique.

References: Da Lage, J.L., P. Capy, and J.R. David 1989, *J. Insect Physiol.* 35(6):453-457; Da Lage, J.L., P. Capy, and J.R. David 1990, *Genet. Sel. Evol.* 22:384-391; David, J.R., R. Allemand, J. Van Herrewege, and Y. Cohet 1983, in: *The Genetics and Biology of Drosophila* (M. Ashburner, H.L. Carson, and J.N. Thompson, jr., eds.) Vol. 3D, Academic Press, London, 105-170; Hoffmann, A.A. and P.A. Parsons 1991, in: *Evolutionary Genetics and Environmental Stress*, Oxford University Press; Parsons, P.A. 1983, *The Evolutionary Biology of Colonizing Species*, Cambridge Univ. Press, New York; Vannier, G. 1987, *Bull. Soc. Ecophysiol., France* 12(2):165-186.

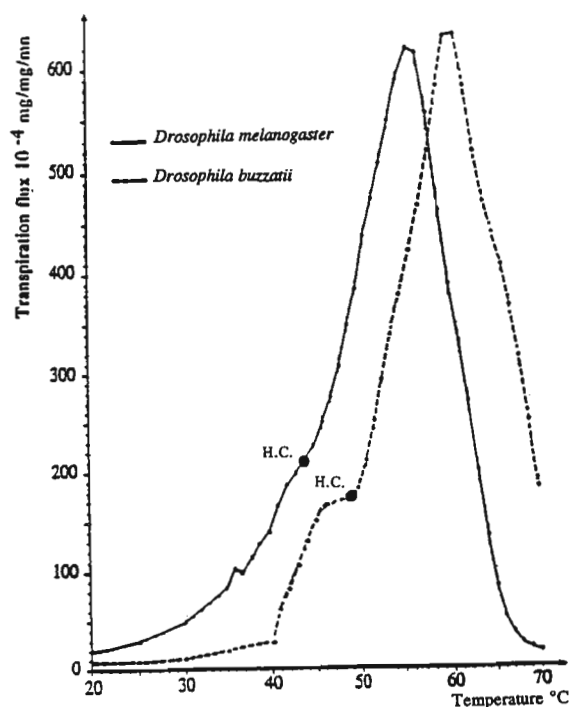


Figure 2. Variation of the transpiration flux (loss of weight per minute) according to increasing temperature in dry air. Average points of heat coma (H.C.) are indicated on each curve. For *D. melanogaster*, 18 flies were studied, and 24 for *D. buzzatii*.

Prout, Timothy. Department of Genetics, University of California, Davis, California 95616. Failure of P element transfer from *Drosophila* to parasitic wasp.

Drosophila larva and finally consumes the *Drosophila* pupa to emerge from the *Drosophila* pupa case.

Wasps without P elements were allowed to parasitize the larvae of the *Drosophila* strain, Nakhodka, from Siberia (provided by M.M. Green), which has many P elements (>30). DNA from the emerging wasps was probed by Southern analysis with labeled P DNA and none was found.

This experiment is similar to Houck et al.'s (1991) famous experiment with the mite *Proctolaelaps regalis*. These authors proposed a study of "small arthropods" associated with *Drosophila* in order to find a "categorical subset of biological agents for a systematic attack of (sic) the problem". *Leptolina boulandi* is an obvious member of their category, since it is one of the very common, highly specific parasitoid wasps of *Drosophila* (Carton et al., 1986).

The primary objective of this wasp experiment was to simply demonstrate the lateral transfer of P elements from *Drosophila* to wasp. However, it was speculated at the time that the wasp might possibly be an agent for *Drosophila*-to-*Drosophila* lateral transfer since infected *Drosophila* larvae occasionally "overcome" the wasp larva and survive as adults (Carton et al., 1986). An analogous recovery phenomenon is required of *Drosophila* embryos when attacked by P element-bearing mites as noted by Houck et al. (1991). However, it was also conjectured at the time of this failed experiment that there was already evidence against the wasp being a lateral transfer agent because the wasp regularly parasitizes *D. simulans*. This species, which it has been shown experimentally can accommodate P elements, is intimately sympatric with *D. melanogaster* world wide but has received no P elements from it. This same kind of three-way sympatry of these two *Drosophila* species and mites is evidence against Houck et al.'s (1991) hypothesis that mites are lateral transfer agents.

References: Carton, Y., M. Mouletreau, J.C.M. Van Alphen and J.C. van Lenteren 1986, The *Drosophila* parasitic wasps in *Genetics and Biology of Drosophila* (eds. M. Ashburner, H. Carson and J. Thompson) vol. 3e. pp 347-394. Academic Press, London and Orlando; Houck, M.A., J.B. Clark, K.R. Peterson and M.G. Kidwell 1991, Science 253: 1125-1128.

Parkash, Ravi. Biosciences Department, M.D. University, Rohtak, India. Biochemical differences among three acid phosphatase allozymes in *Drosophila malerkotliana*.

enzyme systems in certain taxa. The present studies reveal catalytic functional differences among three common allozymes in *D. malerkotliana* (Figure 1).

The data on biochemical properties of three ACPH homozygous allozymic strains ($Acph^{FF}$, $Acph^{MM}$, $Acph^{SS}$) with respect to specific activities, pH dependent activity profiles, V_{max} values, K_m values (at 15°C, 25°C, 35°C, and 45°C) and K_i values have been represented in Table 1. The total protein content did not differ significantly in the three allozymic homogeneities. A linear increase in the enzyme activities of the allozymes was obtained with an increase in

enzyme concentration or incubation time (Table 1). The ACPH specific activity was found to be highest for the SS genotype and lowest for the FF genotype, e.g. the slope values for reaction rates includes $Y = 0.43X$, $Y = 0.63X$, and $Y = 0.71X$ for SS, MM and FF respectively, revealing catalytic activity differences (SS > MM > FF). The data percentages on maximum activities for pH values (3.0 to 7.0) are given in Table 2. The activity profile differences among the homozygous genotypes were found to be consistent at the pH range examined. The allozymic homogeneities MM and SS displayed maximum activity at pH 4.2, while FF revealed maximum activity at pH 5.0. However, all the allozymes performed optimally in a narrow range of pH values (Table 2).

The ACPH catalyzed reactions displayed only one Michaelis

Table 1. Biochemical characteristics of allelic acid phosphatases (FF, MM and SS) in *D. malerkotliana*.

| | FF | MM | SS |
|---|-------|-------|-----------|
| Sp. activity | 60 | 89 | 100 |
| pH optimum | 5.0 | 4.2 | 4.2 |
| Inhibitor constants (K_i values $\times 10^{-5}M$) | 10.0 | 14.0 | 54.0 |
| V_{max} at: | | | |
| 15°C | 7.86 | 10.98 | 15.43 |
| 25°C | 13.11 | 17.18 | 19.81 |
| 35°C | 23.09 | 32.89 | 38.91 |
| 45°C | 41.32 | 49.85 | 63.69 |
| K_m (mM) at: | | | |
| 15°C | 0.49 | 0.41 | 0.42 |
| 25°C | 0.69 | 0.66 | 0.50 |
| 35°C | 0.32 | 0.37 | 0.34 |
| 45°C | 0.34 | 0.32 | 0.34 |
| Effect of Mg^{2+} or Mn^{2+} | — | — | promotory |

* Units of activity = μ -mole of α -naphthol released / min / mg protein

Table 2. Data on percent maximum activity on the basis of pH in three acid phosphatase allozymes in *D. malerkotliana*

| pH (Values) | 3.0 | 3.5 | 4.0 | 4.2 | 4.4 | 4.6 | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 |
|-----------------------|------|------|------|-----|------|------|------|------|-----|------|-----|
| Percent Max. Activity | | | | | | | | | | | |
| FF | 24 | 27 | 50 | 58 | 76.2 | 84.3 | 100 | 72.4 | 60 | 37.4 | 25 |
| MM | 19.3 | 36.7 | 82.6 | 100 | 92 | 89.2 | 75.6 | 67.5 | 34 | 32 | 27 |
| SS | 18.6 | 38.6 | 78.6 | 100 | 89 | 80 | 73.3 | 66.6 | 58 | 27 | 18 |

FF, MM, SS refer to homogenates of allozymic strains

Table 3. Data on acid phosphatase activity as a function of substrate concentrations at four different temperatures in three allozymic homogenates of *D. malerkotliana*

| | | Enzyme activity (v /1 _N) | | | | | | | | |
|----|------|---------------------------------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| | | s | 0.2 | 0.4 | 0.8 | 1.0 | 2.0 | 5.0 | 8.0 | 10.0 |
| | | 1/s | 5.0 | 2.5 | 1.25 | 1.0 | 0.5 | 0.2 | 0.125 | 0.10 |
| FF | 15°C | | 2.3 / 0.4 | 3.7 / 0.7 | 4.6 / 0.2 | 5.1 / 0.19 | 5.7 / 0.17 | 7.2 / 0.14 | 8.0 / 0.12 | 8.0 / 0.12 |
| | 25°C | | 2.9 / 0.34 | 4.6 / 0.21 | 6.3 / 0.16 | 8.0 / 0.12 | 9.2 / 0.11 | 10.9 / 0.09 | 12.6 / 0.08 | 14.3 / 0.07 |
| | 35°C | | 9.2 / 0.11 | 12.9 / 0.08 | 16.0 / 0.06 | 17.0 / 0.06 | 19.5 / 0.05 | 21.7 / 0.046 | 23.5 / 0.04 | 23.5 / 0.04 |
| | 45°C | | 1.5 / 0.06 | 22.0 / 0.04 | 28.0 / 0.03 | 31.0 / 0.03 | 35.0 / 0.028 | 39.0 / 0.026 | 40.0 / 0.025 | 40.0 / 0.025 |
| MM | 15°C | | 3.5 / 0.28 | 5.7 / 0.17 | 6.9 / 0.14 | 7.7 / 0.13 | 8.6 / 0.12 | 9.5 / 0.10 | 11.2 / 0.09 | 11.2 / 0.09 |
| | 25°C | | 4.1 / 0.24 | 6.3 / 0.16 | 8.0 / 0.12 | 10.3 / 0.10 | 13.7 / 0.07 | 15.2 / 0.066 | 16.0 / 0.06 | 18.3 / 0.05 |
| | 35°C | | 11.2 / 0.09 | 16.0 / 0.06 | 21.0 / 0.05 | 23.5 / 0.04 | 26.4 / 0.038 | 28.0 / 0.035 | 31.0 / 0.031 | 38.0 / 0.026 |
| | 45°C | | 19.5 / 0.05 | 27.0 / 0.036 | 35.0 / 0.03 | 39.0 / 0.026 | 40.0 / 0.025 | 42.0 / 0.023 | 46.0 / 0.021 | 46.0 / 0.021 |
| SS | 15°C | | 5.2 / 0.19 | 6.9 / 0.14 | 10.0 / 0.10 | 11.2 / 0.09 | 12.3 / 0.08 | 13.3 / 0.07 | 15.2 / 0.06 | 15.2 / 0.06 |
| | 25°C | | 5.7 / 0.17 | 8.6 / 0.11 | 11.5 / 0.08 | 12.9 / 0.07 | 16.0 / 0.06 | 17.5 / 0.057 | 19.0 / 0.055 | 20.0 / 0.05 |
| | 35°C | | 14.3 / 0.07 | 21.8 / 0.046 | 24.0 / 0.041 | 27.0 / 0.037 | 34.0 / 0.029 | 36.0 / 0.027 | 38.0 / 0.026 | 43.0 / 0.023 |
| | 45°C | | 24.1 / 0.04 | 33.0 / 0.03 | 42.0 / 0.023 | 46.0 / 0.021 | 56.0 / 0.018 | 60.0 / 0.016 | 62.0 / 0.016 | 62.0 / 0.016 |

Data on $1/v$ are given in oblique.Table 4. Data on the values of slope ($1/v$ Vs $1/s$), V_{max} , $1/V_{max}$ and K_m at four different temperatures for three acid phosphatase allozymes in *D. malerkotliana*

| Allozymes | | 15°C | 25°C | 35°C | 45°C |
|-----------|-----------------|-------|-------|-------|-------|
| FF | (a) Slope | 0.06 | 0.05 | 0.014 | 0.008 |
| | (b) V_{max} | 7.86 | 13.11 | 23.09 | 41.32 |
| | (c) $1/V_{max}$ | 0.13 | 0.08 | 0.04 | 0.02 |
| | (d) K_m | 0.49 | 0.69 | 0.31 | 0.34 |
| MM | (a) Slope | 0.037 | 0.038 | 0.011 | 0.006 |
| | (b) V_{max} | 10.98 | 17.18 | 32.89 | 49.85 |
| | (c) $1/V_{max}$ | 0.09 | 0.06 | 0.03 | 0.02 |
| | (d) K_m | 0.41 | 0.66 | 0.36 | 0.32 |
| SS | (a) Slope | 0.027 | 0.025 | 0.009 | 0.005 |
| | (b) V_{max} | 15.43 | 19.81 | 38.91 | 63.69 |
| | (c) $1/V_{max}$ | 0.065 | 0.05 | 0.026 | 0.016 |
| | (d) K_m | 0.42 | 0.50 | 0.34 | 0.34 |

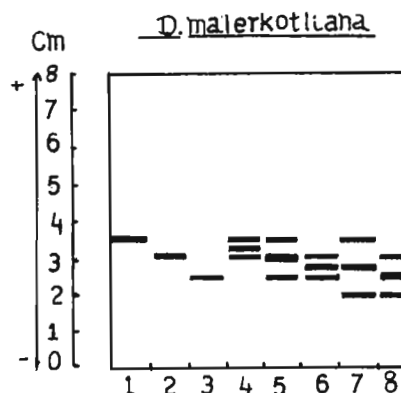


Figure 1. Starch gel electrophoresis as well as schematic representation of acid phosphatase electrophoretic phenotypes in single individuals of *D. malerkotliana*. The single-band variants and triple-band patterns reveal homozygous and heterozygous genotypes. The triple-banded patterns in samples 7 and 8 have resulted due to a rare (cathodal) allele or allozyme. The genotypes of samples 1 to 8 include FF, MM, SS, FM, FS, MS, FS', and MS'.

constant (K_m), which indicates affinity of an enzyme for its substrate. The data on the temperature related K_m profiles of the allozymic homogeneities are given in Table 3. The data are expressed in terms of regression lines between $1/v$ and $1/s$ (V_{max} and K_m values) (Table 4). The V_{max} for SS was found to be the highest and FF the lowest at all temperatures. The differences in the slope values ($1/v$ vs. $1/s$) were found to be greater at the lower temperatures (15°C and 25°C) than the higher temperatures (35°C and 45°C) (Table 4). All the allozymes depicted consistent and significant differences in K_m values at 25°C, while the differences do not seem significant at the other temperatures. Data on the inhibitor constant (K_i) are given in Table 5. The K_i values for FF and MM did not differ much, i.e. 10×10^{-5} M for FF; 14×10^{-5} M for MM, while the SS allozyme revealed significant differences (54×10^{-5} M). Since lower K_i values indicate higher affinity of the enzyme for the inhibitor (sodium fluoride), the inhibitor specificity for the SS allozymic strain, seems significantly lower (Table 5).

Since the allozymes often differ in their amino acid sequence, they may reveal biochemical or functional differences

(Fersht, 1977). Selectionists argue that biochemical characterization of allozymes demonstrates their adaptive significance. The present observations reveal catalytic efficiency divergence among common ACPH allozymes in *D. malerkotliana* (SS > MM > FF).

Since acid phosphatases are believed to act on heterogeneous substrates, the natural selection might discriminate between ACPH allozymes and result in the maintenance of genic polymorphism at this locus in *D. malerkotliana*. The present observations concur with earlier reports on loci coding for alcohol dehydrogenases, alpha-glycerophosphate dehydrogenase and alkaline phosphatase allozymes in *D. melanogaster* (McDonald *et al.*, 1980; Miller *et al.*, 1980; Harper and Armstrong, 1973). The ADH allozymes reveal activity differences ($Adh^{FF} > Adh^{FS} > Adh^{SS}$) and thermostability differences ($Adh^{SS} > Adh^{FF}$) (Day *et al.*, 1974). The alkaline phosphatase allozymes in *D. melanogaster* reveal higher thermal stability, but least inhibition constant, for common alleles as compared with the rare alleles, at the polymorphic alkaline phosphatase locus (Harper and Armstrong, 1973). The glycerophosphate allozymes (fast and slow) in *D. melanogaster* have been shown to differ in their K_m and K_i values (Miller *et al.*, 1975). Likewise, glucose-6-phosphate dehydrogenase allozymes in man are shown to differ in K_m as well as K_i values (Yoshida, 1973). It may be emphasized that the observed biochemical differences between allozymes contradict the Neutralist hypothesis, which maintains that allozymes are physiologically relevant, and are in agreement with the Selectionist hypothesis (Wills, 1981).

References: McDonald, J.F., S.M. Anderson and M. Santos 1980, *Genetics* 95:1013; Miller, S., R.W. Pearcy and E. Berger 1975, *Biochem. Genet.* 13:175; Narise, S. 1980, *Biochem. Biophys. Acta* 615:289; Fersht, A.R. 1977, *Enzyme Structure and Function*, San Francisco: W.H. Freeman and Co.; Harper, R.A. and F.B. Armstrong 1973, *Biochem. Genet.* 10:29; Yoshida, A. 1973, *Science* 179:532; Day, T.H., P.C. Hillier and B. Clarke 1974a, *Biochem. Genet.* 11:141; Day, T.H., P.C. Hillier and B. Clarke 1974b, *Biochem. Genet.* 11:155; Wills, C. 1981, *Genetic Variability*, Oxford: Clarendon Press.

Parkash, Ravi. Biosciences Department, M.D. University, Rohtak, India. Biochemical analysis of acid phosphatase allozymes in *Drosophila takahashii*.

of genetic variation. This paper reports acid phosphatase allozyme variation in *D. takahashii*. The homozygous and heterozygous strains for the two most common *Acph* alleles ($Acph^{100}$ and $Acph^{95}$) in this species were examined with respect to specific activity and thermal inactivation profiles.

Drosophila takahashii Sturtevant is a subspecies of the *melanogaster* species (Subgenus *Sophophora*; Genus *Drosophila*) and its distribution range includes India, Japan and Southeast Asia. The flies were individually homogenized in 0.01 ml of gel buffer and the homogeneities were loaded in a 12% starch gel and were run electrophoretically for 4 hours at 250 V and 25 mA. The gels were sliced and stained for ACPH activity. Two homozygous stocks and their heterozygous stock ($Acph^{FF}$, $Acph^{SS}$ and $Acph^{FS}$) were established for the electrophoretic variants $Acph^{100}$ and $Acph^{95}$ and mass cultured. Homogeneities (100 mg/8 ml) for the three stocks were prepared and subjected to a refrigerated centrifuge, and the supernatants were used for the enzyme assays. Three different enzyme preparations were made for each of the three stocks of *D. takahashii*. ACPH activity was estimated in triplicate at 25°C. The protein content for each enzyme preparation was then determined. The enzyme preparations were compared on the basis of specific activity, pH profile, effect of reaction temperature, thermal inactivation and effect of GUHCl, a protein denaturing agent.

A total of 15 ACPH electrophoretic phenotypes were observed in four population samples of *D. takahashii*. The ACPH patterns are identical for both sexes and the homogeneities of single individuals depict either a single-band

Table 5. Data on the determination of inhibitor's constant (K_i) by Dixon's method for three allelic acid phosphatases in *D. malerkotliana*.

| Conc. (mM NaF) | Enzyme activity (α -naphthol released μ M / min / mg protein) | | | | | | | | | | | |
|-------------------|---|------|----------------|------|------------------------------|------|----------------|------|------------------------------|------|----------------|------|
| | FF | | | | MM | | | | SS | | | |
| | S ₁ | | S ₂ | | S ₁ | | S ₂ | | S ₁ | | S ₂ | |
| | V | 1/V | V | 1/V | V | 1/V | V | 1/V | V | 1/V | V | 1/V |
| Control | 9.17 | 0.11 | 14.32 | 0.07 | 13.75 | 0.07 | 18.34 | 0.05 | 16.04 | 0.06 | 20.63 | 0.05 |
| 0.1 | 1.72 | 0.58 | 2.29 | 0.43 | 4.01 | 0.02 | 8.02 | 0.12 | 5.16 | 0.19 | 11.46 | 0.09 |
| 0.2 | 1.14 | 0.87 | 2.0 | 0.5 | 2.86 | 0.35 | 5.16 | 0.19 | 3.43 | 0.29 | 8.02 | 0.12 |
| 0.5 | 0.74 | 1.35 | 0.97 | 1.03 | 1.72 | 0.85 | 3.44 | 0.29 | 1.72 | 0.58 | 4.01 | 0.25 |
| 1.0 | 0.40 | 2.5 | 0.57 | 1.75 | 1.15 | 0.87 | 1.14 | 0.68 | 1.42 | 0.71 | 2.29 | 0.44 |
| 2.0 | — | — | 0.34 | 2.94 | 0.57 | 1.75 | — | — | 0.86 | 1.16 | 1.72 | 0.58 |
| K_i value | $A_1 = 10.0 \times 10^{-5}M$ | | | | $A_2 = 14.0 \times 10^{-5}M$ | | | | $A_3 = 54.0 \times 10^{-5}M$ | | | |

(S₁ = 2.0 mM; S₂ = 10.0 mM of Na - α -naphthyl phosphate).

variant or a triple-band pattern with particular mobility values. The segregating patterns of *Acph* bands were in accord with monogenic inheritance. Single-band variants were allelic isozymes (allozymes) and represented homozygous genotypes and triple-band patterns represented heterozygous genotypes. The occurrence of a hybrid band of intermediate mobility in heterozygotes implied that ACPH in *D. takahashii* is a dimeric enzyme. The observed ACPH phenotypes were governed by 5 autosomal codominant alleles (*Acph*⁸⁵, *Acph*⁹⁰, *Acph*⁹⁵, *Acph*¹⁰⁰, and *Acph*¹⁰⁵) in order of increasing electrophoretic mobility.

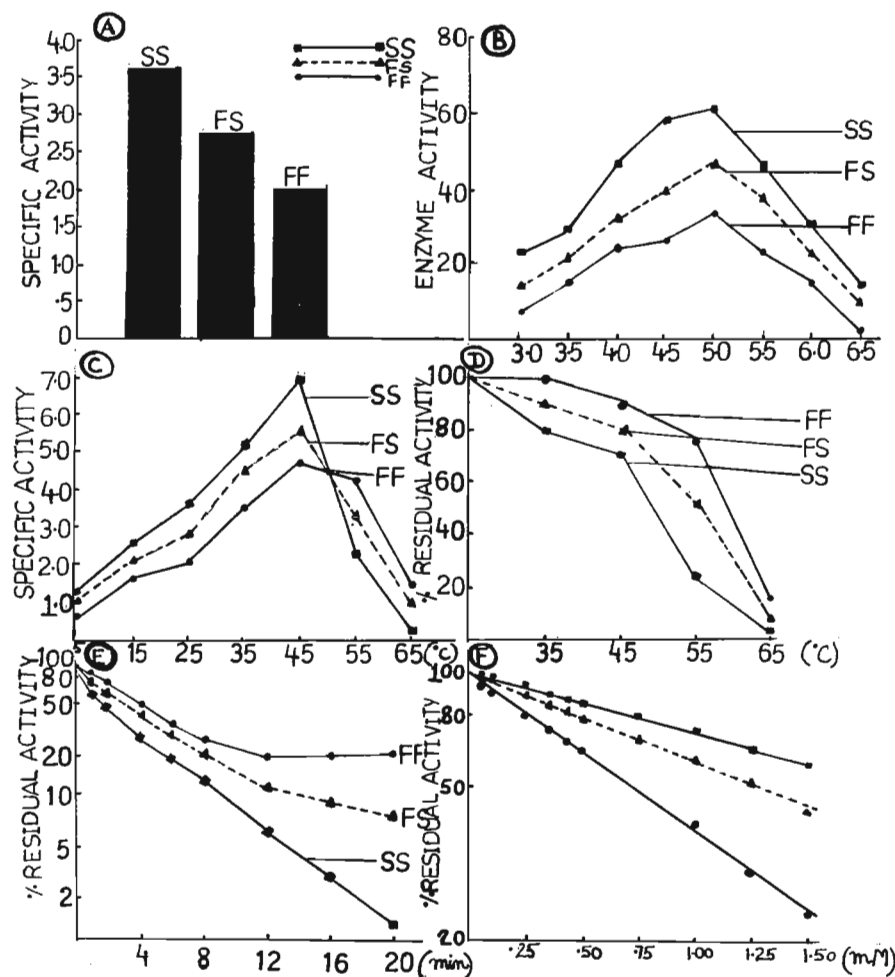


Figure 1. Biochemical differences among *Acph* allozymic genotypes (*Acph*¹⁰⁰ = FF; *Acph*⁹⁵ = SS; *Acph*^{100/95} = FS); specific activity profiles (A); pH dependent (B); temperature dependent activity profiles (C); thermal inactivation patterns (D); semilog of temporal thermal inactivation (E); and effect of GUDCl (F) in enzyme preparations of homozygous (FF & SS) and heterozygous (FS) stocks.

The biochemical properties of two homozygous *Acph*^{SS} and *Acph*^{FF} and their heterozygous *Acph*^{FS} strains have been analyzed with respect to specific activity, pH dependent as well as temperature dependent activity profiles, thermal inactivation as well as response to GUDCl, and the data has been presented in Figure 1. The total protein content (580 ± 8 ug per assay condition) did not differ significantly in the enzyme preparations of the three genotypes (*Acph*^{100/100} = FF; *Acph*^{95/95} = SS; *Acph*^{100/95} = FS). ACPH activity (A: u mol. p-nitrophenol released/30 min) and specific activity (SA) were found to be highest for the SS genotype (A = 61.87; SA = 3.55) and lowest for the FF genotype (A = 36.30; SA = 2.05), while the FS genotype revealed intermediate values (A = 47.85; SA = 2.75; Figure 1A). No differences have been observed among the three genotypes with regard to pH optimum (5.0). However, the activity profile differences (SS FS FF) between the three genotypes were found to be consistent at the pH range examined (Figure 1B). The effect of reaction temperatures in the range of 5°C to 45°C revealed a proportionate increase in enzyme activity for all three genotypes, while temperatures at 55°C and above the enzyme preparation of the FF genotype was found to be stable compared to that of the SS genotype. However, the FS enzyme preparation showed an intermediate response (Figure 1C). All three enzyme preparations depicted 45°C as the optimum temperature. The enzyme preparations of the three genotypes were incubated at 35°C, 45°C, 55°C, and 65°C for 15 minutes each, and a comparison of residual activity determined at 25°C is represented in Figure 1D. The SS genotype has been found to be more heat sensitive than the FF genotype, while the FS genotype is intermediate in response. The semilog plot illustrating the thermal

inactivation of the ACPH activity of the enzyme preparations of 3 genotypes have been represented in Figure 1E. The SS enzyme extract lost almost all activity up to 20 minutes at 60°C while the FF enzyme preparation revealed 25% residual activity after 10 minutes at 60°C. The SS enzyme preparation was found to be more stable towards the effect of a protein denaturing agent (0.25 to 1.5 M GUHCl) as compared to the enzyme preparation of the FF genotype (Figure 1F). However, the enzyme preparation of the FS genotype revealed a non-intermediate response. Thus, the homozygous and heterozygous strains for the two most common *Acph* alleles (*Acph*¹⁰⁰ and *Acph*⁹⁵) have been found to differ in specific activity and thermal inactivation patterns. The homozygous *Acph*⁹⁵ strain revealed higher specific activity but lower thermostability, while *Acph*¹⁰⁰ showed lower specific activity but higher thermostability. The maintenance of two common alleles at higher frequencies in natural populations can be argued on the basis of the potential biochemical differences which could be subject to selection.

Parkash, Ravi. Biosciences Department, M.D. University, Rohtak, India. *Adh* and *alpha-Gpdh* genic variation in some *Drosophila* species.

The present investigation was undertaken to examine the extent of electrophoretic and cryptic genic variation at loci coding for alcohol dehydrogenase (*ADH*) and alpha-glycerophosphate dehydrogenase (*alpha-GPDH*) in eleven natural populations of drosophilids.

Population samples of eleven drosophilids (*Drosophila melanogaster*, *D. takahashii*, *D. nepalensis*, *D. malerkotliana*, *D. bipectinata*, *D. ananassae*, *D. jambulina*, *D. punjabiensis*, *D. immigrans*, *D. busckii* and *Zaprionus indianus*) were bait-trapped from gardens and horticultural farms at Delhi, Rohtak, Pinjore, Jammu, Roorkee, Hasimara and Bagdogra (West Bengal) and Dhulabari (Nepal). Homogeneities of single individuals were applied to 12% starch gel slabs and were run electrophoretically at 250 V and 30 mA at 4°C for 4 hours and the gel slices were stained for alcohol dehydrogenase (*ADH*) and alpha-glycerophosphate dehydrogenase (*alpha-GPDH*) isozyme patterns (Brewer, 1970; Harris and Hopkinson, 1976). The application of a heat denaturation technique (Trippa *et al.*, 1978) involved heating the *ADH* and *alpha-GPDH* enzymes *in situ* in the starch gel slices at 50°C for 15 ± 1 min after electrophoretic separation. The genetic basis of *ADH* and *alpha-GPDH* electromorphs was interpreted from the segregation patterns of enzyme phenotypes as well as F₁ and F₂ progeny of several species-specific genetic crosses.

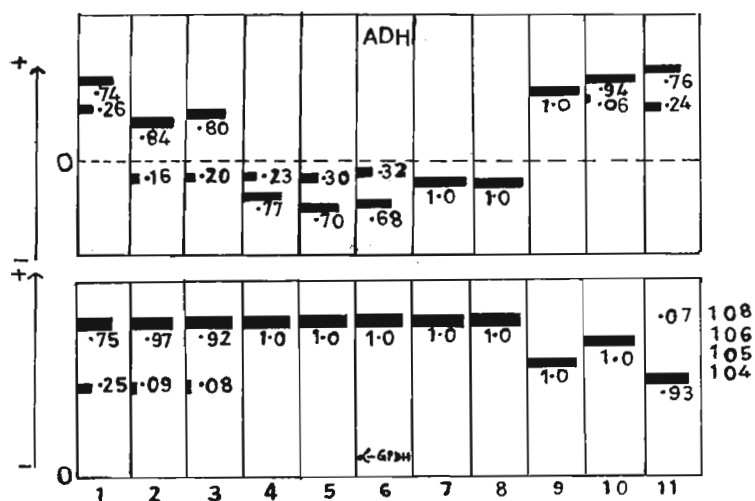


Figure 1. Schematic representation of species specific electrophoretic variants (allozymes) of alcohol dehydrogenase (*ADH*) and alpha-glycerophosphate dehydrogenase (*alpha-GPDH*). The bars represent electrophoretic mobilities and allelic frequencies of allelic variants. "O" represents the origin and the arrow indicates the direction of current flow. Species include: 1. *Drosophila melanogaster*; 2. *D. takahashii*; 3. *D. nepalensis*; 4. *D. malerkotliana*; 5. *D. bipectinata*; 6. *D. ananassae*; 7. *D. jambulina*; 8. *D. punjabiensis*; 9. *D. immigrans*; 10. *D. busckii*; and 11. *Zaprionus indianus*.

Drosophila species (*D. malerkotliana*, *D. bipectinata*, *D. ananassae* and *D. punjabiensis*) have displayed monomorphic and similar *alpha-GPDH* isozyme patterns, while enzyme phenotypes of *D. immigrans* and *D. busckii* are species specific (Figure 1). Polymorphic *alpha-GPDH* patterns have been observed in *D. melanogaster*, *D. takahashii*, *D. nepalensis* and *Zaprionus indianus*. Except for *D. jambulina*, *D. punjabiensis* and *D. immigrans*, all other drosophilids have depicted *ADH* polymorphism (Figure 2). Species specific genetic crosses involving segregating two-banded enzyme patterns resulted in four-banded phenotypes in F₁ and a 1:2:1 ratio of alternating two-banded and four-banded phenotypes in the F₂ progeny. These results were in agreement with monogenic control of segregating *alpha-GPDH* zone as well as the *ADH* zone. The present observations on *ADH* and *alpha-GPDH* concurred with earlier reports on *D. melanogaster* that in NAD-requiring dehydrogenases, more than one electromorph (conformational isozymes) may

arise due to post-translational differential binding of co-enzyme NAD. Thus, the two-banded and four-banded enzyme phenotypes represented homozygous and heterozygous genotypes, respectively. The *ADH* and *alpha-GPDH* enzyme phenotypes did not vary with the sex and are thus governed by autosomal loci.

Figure 2. Alcohol dehydrogenase electrophoretic phenotypes in homogenates of single individuals of drosophilids: a. *Drosophila melanogaster*; b. *D. takahashii*; c. *D. nepalensis*; d. *D. malerkotliana*; e. *D. bipectinata*; f. *D. ananassae*; g. *D. jambulina*; h. *D. punjabensis*; i. *D. immigrans*; j. *D. busckii*; k. *Zaprionus indianus*; l. *Z. sepscides*; and m. *Z. tuberculatus* (g through i are monomorphic and l through m are laboratory strains). Differential binding of coenzyme (NAD) caused two-banded patterns in homozygotes.

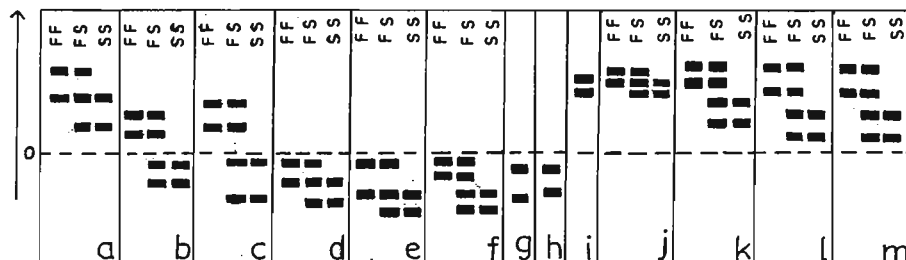


Table 1. Data on observed *ADH* and α -*GPDH* genotypes, allelic frequencies, heterozygosities, Wright's inbreeding coefficients (F) and application of log likelihood G-test at *ADH* and α -*GPDH* loci in different drosophilids.

| Species | Sample size (N) | ADH genotypes | | | | | | | Allelic frequencies | | | | Heterozygosity | F-value | G-Value |
|-------------------------|-----------------|--------------------------|-----|-----|-----|---------|-----|-----|---------------------|------|------|------|----------------|---------|---------|
| | | 1.1 | 2.2 | 3.3 | 4.4 | 1.2 | 2.3 | 3.4 | 1 | 2 | 3 | 4 | Obs. / Exp. | | |
| <i>D. melanogaster</i> | 108 | 8 | 60 | — | — | 40 | — | — | 0.26 | 0.74 | — | — | 0.37 / 0.38 | 0.026 | 0.138 |
| <i>D. takahashii</i> | 114 | — | 84 | 6 | — | — | 24 | — | — | 0.84 | 0.16 | — | 0.28 / 0.27 | -0.037 | 2.85 |
| <i>D. nepalensis</i> | 176 | — | 116 | 12 | — | — | 48 | — | — | 0.80 | 0.20 | — | 0.27 / 0.32 | 0.156 | 4.30 |
| <i>D. malerkotliana</i> | 114 | — | — | 8 | 70 | — | — | 36 | — | — | 0.23 | 0.77 | 0.31 / 0.36 | 0.138 | 1.10 |
| <i>D. bipectinata</i> | 106 | — | — | 12 | 54 | — | — | 40 | — | — | 0.30 | 0.70 | 0.38 / 0.42 | 0.095 | 1.19 |
| <i>D. ananassae</i> | 124 | — | — | 16 | 60 | — | — | 48 | — | — | 0.32 | 0.68 | 0.38 / 0.44 | 0.136 | 1.61 |
| <i>D. busckii</i> | 100 | 4 | 92 | — | — | 4 | — | — | 0.06 | 0.94 | — | — | 0.04 / 0.11 | 0.636 | 18.40* |
| <i>Z. indianus</i> | 205 | 6 | 113 | — | — | 86 | — | — | 0.24 | 0.76 | — | — | 0.41 / 0.38 | -0.079 | 3.91 |
| | | α -GPDH genotypes | | | | | | | Allelic frequencies | | | | | | |
| | | 104 | | 108 | | 104/108 | | | 104 | | 108 | | | | |
| <i>D. melanogaster</i> | 130 | 10 | | 75 | | 45 | | | 0.25 | | 0.75 | | 0.34 / 0.37 | 0.081 | 0.83 |
| <i>D. takahashii</i> | 164 | 5 | | 141 | | 18 | | | 0.09 | | 0.91 | | 0.11 / 0.16 | 0.312 | 9.46* |
| <i>D. nepalensis</i> | 120 | 3 | | 103 | | 14 | | | 0.08 | | 0.92 | | 0.11 / 0.15 | 0.266 | -0.09 |
| <i>Z. indianus</i> | 174 | 153 | | 3 | | 18 | | | 0.93 | | 0.07 | | 0.10 / 0.13 | 0.230 | 2.48 |

* Significant at 5% level

The data on the distribution of *ADH* and *alpha-GPDH* genotypes, allelic frequencies, observed and expected heterozygosity, Wright's inbreeding coefficient (F) and log-likelihood X^2 test for fit to Hardy-Weinberg expectations in species polymorphic for these two gene-enzyme systems are given in Table 1. The range of heterozygosities observed at *ADH* and *alpha-GPDH* loci correlates with the allelic frequency distribution patterns (Table 1). The lower F-values in most of the species analyzed (except the *ADH* locus in *D. busckii*) indicate that populations are randomly mating. Data in Table 1 revealed that there are deviations from Hardy-Weinberg equilibrium as well as occurrences of excess homozygotes at the *ADH* locus in *D. busckii* and the *alpha-GPDH* locus in *D. takahashii*. Except *D. melanogaster*, the *alpha-GPDH* locus was represented by a most common allele (0.90) and one rare allele. However, both the *ADH* alleles occur with high frequencies in most of the species under study, except *D. busckii* (Table 1). The *D. melanogaster* population was characterized by occurrence of two common alleles, high heterozygosities and lack of deviations from Hardy-Weinberg expectations at the *ADH* and *alpha-GPDH* loci.

The application of the heat denaturation technique did not reveal occurrence of cryptic isoelectrophoretic thermoresistant and thermosensitive variants in population samples of the drosophilids monomorphic or polymorphic at *ADH* and *alpha-GPDH* loci. The observed low level of genic polymorphism at the *alpha-GPDH* locus concurred with the functional constraint hypothesis, which suggests that loci coding for enzymes, such as *ADH*, acting on nonspecific substrates were more polymorphic as compared with those involved in essential energy producing metabolic pathways, such as *alpha-GPDH*.

References: Brewer, G.J. 1970, *An Introduction to Isozyme Techniques*, New York: Academic Press; Ferguson, A. 1980, *Biochemical Systematics and Evolution*, New York: Wiley; Harris, H. and D.A. Hopkinson 1976, *Handbook of Enzyme Electrophoresis in Human Genetics*, Amsterdam: North Holland; Niesel, D.W., Y.C.E. Pand, G.C. Bewley, F.B. Armstrong and S.S.L. Li 1982, *J. Biol. Chem.*, 257:979-983; Trippa, G., A. Catamo, A. Lombardozzi and R. Cicchetti 1978, *Biochem. Genet.* 16:299-305.

Parkash, R. and J.P. Yadav. M.D. University, Rohtak, India. Analysis of ethanol tolerance in Indian populations of *D. immigrans*.

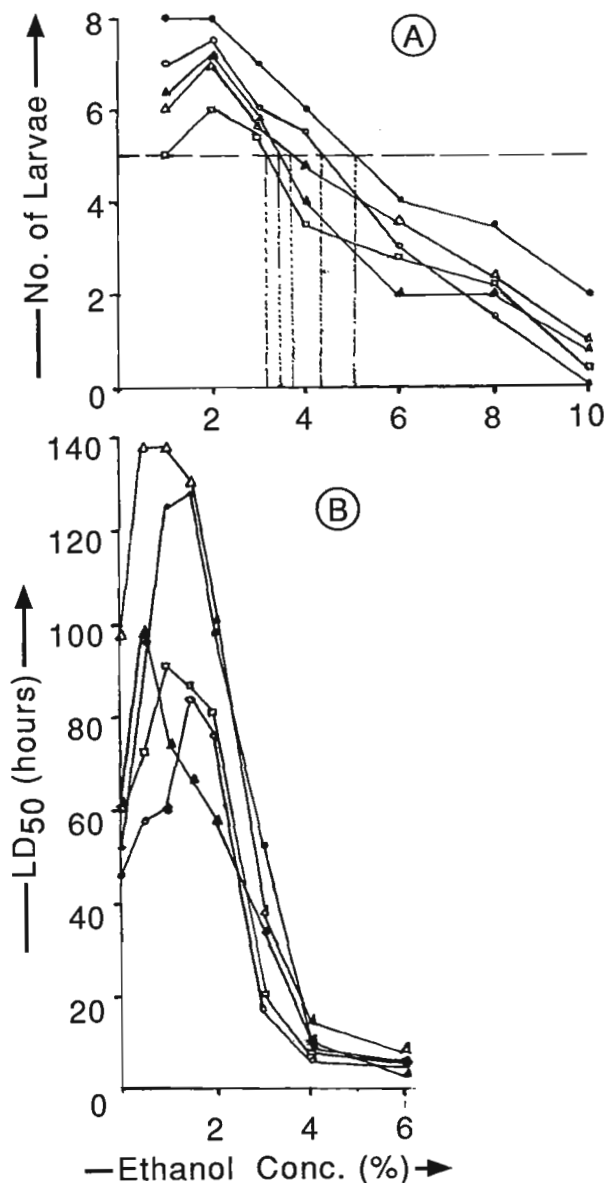
Colonizing populations of *Drosophila* species constitute excellent material for experimental population genetics as well as evolutionary studies (Parsons, 1983a). The Indian subcontinent represents a diverse array of climatically variable habitats and there is little information on the electrophoretic and ethanol tolerance analysis in various drosophilids. The present studies were undertaken so as to analyze the nature and extent of genetic variability at the *Adh* locus in some Indian natural populations of *D. immigrans*. *D. immigrans* occurs abundantly in northern parts of India during winter months. Isofemale lines were established from population samples of *D. immigrans* from five sites (Darjeeling to Chandigarh). Homogenates of single individuals were subjected to electrophoresis at 250 V and 30 mA at 4°C for 4 hours and the slices of each gel were stained for ADH gene-enzyme system (Harris and Hopkinson, 1976).

Figure 1. Mean number of larvae preferring different ethanol concentrations up to ten percent in different populations of *D. immigrans* (A). Adult survivorship expressed as LD₅₀ hours at ethanol concentrations up to six percent. The populations include: open squares, Chandigarh; open triangles, Dehradun; closed circles, Saharanpur; closed triangles, Rohtak; open circles, Darjeeling.

The adult ethanol tolerance was assessed following standard procedures (Starmer *et al.*, 1977; David and Van Herreweghe, 1983). Adult survivorship was expressed as the number of adults alive after various time intervals. The ethanol resource utilization values were represented as LT₅₀ maximum/LT₅₀ control, i.e. if this ratio was more than 1, the ethanol vapors were utilized as a resource, and if this value was less than 1, it represented stress. The ethanol threshold concentration was obtained at LT₅₀ maximum/LT₅₀ control = 1. The larval ethanol tolerance behavior of the geographical populations of *D. immigrans* was analyzed by following the standard method (Gelfaud and McDonald, 1983).

The *Adh* locus was found to be monomorphic in all the populations of *D. immigrans*. Ethanol tolerance levels were analyzed for the larvae as well as adults of *D. immigrans* (Table 1, Figure 1). The data on the mean numbers of *D. immigrans* larvae choosing agar plus various concentrations of ethanol for experimental durations of 30 minutes are given in Figure 1A. The response of larvae did not differ much for different time durations such as 15 min, 30 min and 40 min. However, the larval ethanol tolerance response revealed significant variation between the different populations of *D. immigrans*. The ranking of *D. immigrans* populations on the basis of larval ethanol tolerance is: sugarmill populations of *D. immigrans* from Saharanpur > Darjeeling > Dehradun > Chandigarh > Rohtak. The sugarmill populations of *D. immigrans* from Saharanpur revealed the greatest ethanol response. The larval threshold values were found to follow almost similar ranking order (Table 1).

The adult individuals of five different populations of *D. immigrans* were analyzed for their potential to utilize ethanol vapors in a closed system (Figure 1B). The LC₅₀ ethanol concentrations for adult individuals of different populations were found to vary in the range of 2.8 to 3.5%. The data on LT₅₀ hrs and LT₅₀ max./LT₅₀ control are given



in Table 1. The longevity periods at various ethanol concentrations were found to increase at 0.5% and 1% ethanol in sugarmill populations of *D. immigrans* from Saharanpur. The threshold values were found to vary in the range of 1.6 to 3.0%. Thus, in adult individuals of *D. immigrans*, ethanol concentrations up to 2% served as resource, while higher ethanol concentrations served as stress. Both the larval as well as adult stages of *D. immigrans* revealed lower levels of ethanol tolerance.

Drosophila species are fruit-niche species and they are known to utilize ethanol as a resource in nature (David *et al.*, 1986). *D. immigrans* populations showed ethanol sensitivity. Such observations concurred with suggested relationships between ethanol tolerance and larval habitat in several *Drosophila* species (David and Van Herrewege, 1983; Parsons, 1983b). Indian populations of *D. immigrans* did not reveal any geographical differentiation with respect to LC₅₀ ethanol and to ethanol threshold levels of adults and larval forms. Thus, the occurrence of monomorphism at the *Adh* locus and the lower levels of ethanol tolerance in *D. immigrans* suggest that natural selection might not be operating at the *Adh* locus.

Table 1. Data on ethanol tolerance indices (adult and larval ethanol threshold concentrations), LC₅₀ ethanol concentration, increase in longevity (LD₅₀ hours and LT₅₀ maximum / LT₅₀ control) in five natural populations of *D. immigrans*.

| Populations | Ethanol conc. threshold values (in %) | | LC ₅₀ ethanol conc. (in %) | Increase in longevity at 2% ethanol conc. | |
|-----------------------|---------------------------------------|--------|---------------------------------------|---|---|
| | Adult | Larvae | | LD ₅₀ hrs | LT ₅₀ max / LT ₅₀ control |
| Chandigarh (30° 43'N) | 2.3 | 3.2 | 3.0 | 80 | 1.332 |
| Dehradun (30° 19'N) | 2.0 | 3.7 | 3.5 | 100 | 1.02 |
| Saharanpur (29° 58'N) | 3.0 | 5.0 | 3.2 | 98 | 1.88 |
| Rohtak (28° 94'N) | 1.6 | 3.5 | 3.35 | 58 | 0.94 |
| Darjeeling (27° 03'N) | 2.5 | 4.4 | 2.8 | 76 | 1.65 |

References: David, J.R. and J. Van Herrewege 1983, *Comparative Biochem. Physiol.* 74:283; David, J.R., H. Mercot, P. Capi, S.F. McEvey and J. Van Herrewege 1986, *Genet. Sel. Evol.* 18:405; Gelfaud, J.L. and J.F. McDonald 1983, *Behaviour Genetics* 13:281; Harris, H. and D.A. Hopkinson 1976, *Handbook of Enzyme Electrophoresis in Human Genetics*, Amsterdam: North Holland; Parsons, P.A. 1983a, *The Evolutionary Biology of Colonizing Species*, London: Cambridge University Press; Parsons, P.A. 1983b, *Annu. Rev. Ecol. Syst.* 14:35; Starmer, W.T., W.B. Heed and E.S. Rockwood-Sluss 1977, *Proc. Natl. Acad. Sci. USA* 74:387.

Hellstén, M. and E. Hauschteck-Jungen. Zool. Inst. der Univ. Zurich, Switzerland. Fertility of *Drosophila subobscura* males with the sex ratio chromosome $A_{2+3+5+7}$ depends on autosomal gene arrangements.

The gene arrangement of the sex ratio chromosome of *D. subobscura*, the $A_{2+3+5+7}$ exhibits four inversions, which cover nearly the whole chromosome. It is endemic for North America and the Canary Islands (Jungen, 1968 a,b; Prevosti, 1974), where males with this gene arrangement are predominantly

fertile as shown by Hauschteck-Jungen *et al.* (1987) for Tunisian males and by Hellstén (unpublished data) for males from the Canary Islands.

It was recently found that the Tunisian $A_{2+3+5+7}$ combined with autosomal standard gene arrangements was incompatible with male fertility (Hauschteck-Jungen, 1990), while other gene arrangements from the same populations had no effect on male fertility. The result indicates the presence of coadapted supergenes on the $A_{2+3+5+7}$ (Prevosti *et al.*, 1975). The sterility inducing character of the $A_{2+3+5+7}$ could be special for this gene arrangement originating from Tunisia. If this character would be common to the $A_{2+3+5+7}$ we can assume that the gene arrangement occurred only once and spread thereafter.

The $A_{2+3+5+7}$ from Tunisia and the Canary Islands were introduced into karyotypes which consisted out of one set of autosomal standard gene arrangements and one set of chromosomes from the Canary Islands or one set of predominantly Tunisian chromosomes, respectively. In a second experiment the standard gene arrangement of the O chromosome was replaced by the O_{3+4} . Males with these two sets of autosomes and the $A_{2+3+5+7}$ or as a control the A_{st} were tested for fertility by backcrossing them to the standard strain or the strain with the O_{3+4} .

All backcrossed $A_{2+3+5+7}$ males had reduced number of offspring compared to control males with the A_{st} and with the same autosomal gene arrangements as the $A_{2+3+5+7}$ males (Figure 1). No offspring was produced by 23% and 33% of $A_{2+3+5+7}$ males from the Canary Islands and from Tunisia, respectively. The difference in percentage of sterile males with the $A_{2+3+5+7}$ from Tunisia and the Canary Islands could be a genetical difference between the A chromosomes from the two localities. But more probable is another explanation, namely the presence of Middle European E_{st} gene arrangements in the Tunisian karyotypes originating from former matings. Standard gene arrangements disrupt male fertility in $A_{2+3+5+7}$ males (Hauschteck-Jungen, 1990).

The number of offspring was between 1 and 31, with the exception of one male which had 64 offspring. The average number of offspring of control males with the A_{st} was between 100 and 200 offspring per male.

When $A_{2+3+5+7}$ males carried instead of one O_{st} the O_{3+4} , more males had no offspring (47% instead of 23% [Canary Islands] and 67% instead of 33% [Tunisia]). Our findings were unexpected because the O_{3+4} is the O gene arrangement which is found at the Canary Islands with a frequency higher than 98% (Prevosti, 1974), and there it is together with the $A_{2+3+5+7}$ no fertility barrier. The O_{3+4} we used did neither originate from the Canary Islands nor from Tunisia but presumably from a Middle Eastern population. Thus the O_{3+4} in this experiment and that from the Canary Islands are supposed to be genetically different. Differences between O_{3+4} gene arrangements can occur at the non-inverted segment II (Pinsker and Sperlich, 1981). That the O_{3+4} is not a stable unity was shown by Rozas and Aguadé (1990). It can for instance recombine with the O_{st} as well as with the O_{3+4+8} , and has a high level of nucleotide variation.

It could be that the decreased fertility is not only induced by the O gene arrangement alone. But also the standard gene arrangement of the chromosomes I, E and U could have an effect on progeny production in this experiment. The standard chromosomes which were introduced together with the O_{3+4} came from another line than in the upper experiment, and could be genetically different from the former line.

The incompatibility of the $A_{2+3+5+7}$ together with standard autosomes and also with at least one specific O_{3+4} for male fertility is a general feature of this A gene arrangement, regardless of its origin. Therefore we assume 1) that the origin of the $A_{2+3+5+7}$ is monophyletic, and 2) that the $A_{2+3+5+7}$ arrived at the Canary Islands from North Africa ev. only once (Larruga and Pinsker, 1984) from Morocco (Prevosti, 1974) with a set of autosomes compatible with male fertility.

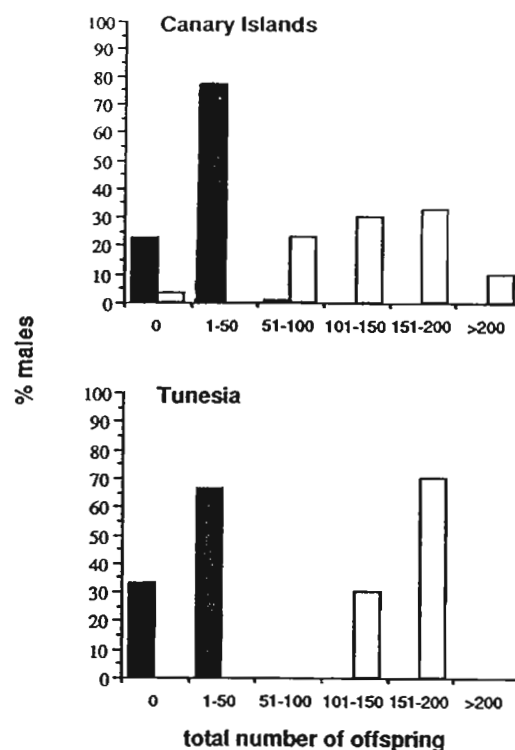


Fig. 1
top: Canary Islands
■ 64 males with the $A_{2+3+5+7}$
□ 29 males with the A_{st} (control)
bottom: Tunisia
■ 20 males with the $A_{2+3+5+7}$
□ 10 males with the A_{st} (control)

Figure 1. Number of offspring from males with different karyotypes. All males in Figure 1 had one set of standard autosomes. The second set of autosomes is different in males which have their $A_{2+3+5+7}$ from the Canary Islands or from Tunisia, respectively. But $A_{2+3+5+7}$ males and A_{st} males in the same graph derived the second set of autosomes from a common pool of gene arrangements.

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Bevan, Charlotte, John Roote, Steven Russell, and Michael Ashburner. Department of Genetics, Downing Street, Cambridge, England. On the allelism of *killer-of-male* and *male-specific-lethal* mutations.

In 1972 Pierre (Dros. Inf. Serv. 48:16) described two EMS-induced autosomal male-specific lethals, *kmA* and *kmB*. The former mutation mapped to 2-45, and the latter to 2-20; both were said to be "early" embryonic lethals. For many years we, and others, assumed that these mutations were lost. This is a pity, because their

map positions suggested that they were mutations at otherwise unknown genes. The three genes on chromosome 2

known to have male-specific lethal alleles are *msl-1* (2-53.3; 37F7-37F8), *msl-2* (2-9.0; 23E1-23F6) and *mle* (2-56.8; 42A2-42A8). Mutations of all of these genes are late larval lethals. We recently discovered the availability of *kmA* and *kmB* from the Laboratoire de Genetique des Virus at Gif-sur-Yvette. Through the kindness of Didier Contamine *kmA/CyO* and *kmB/SM5* stocks were obtained. These were crossed to *msl-1*²¹⁶, *msl-2*²²⁷ and *mle*². *kmA/msl-2*²²⁷ males died (n = 809) as did *kmB/msl-1*²¹⁶ males (n = 545). All other genotypes survived.

It is possible that these data result from a dominant interaction between the pairs of non-complementing alleles, rather than of allelism. To check this, *msl-1*²¹⁶/*kmB* females were crossed to *msl-1*²¹⁶/*CyO*, *msl-2*²²⁷/*CyO* and *kmB/CyO* males. Were *msl-1*²¹⁶ and *kmB* to be alleles then no adult wild-type male progeny would be expected from the first or last of these crosses. None were found (n = 1245 and n = 2323, respectively). Similarly *msl-2*²²⁷/*kmA* females were crossed to *msl-1*²¹⁶/*CyO*, *msl-2*²²⁷/*CyO* or *kmA/CyO* males. Were *msl-2*²²⁷ and *kmA* to be alleles then the last two crosses would not be expected to yield adult wild-type sons. None were found in total progenies of n = 1345 and n = 559, respectively. The simplest interpretation of these data is that the original map positions reported for *kmA* and *kmB* are in error and that *kmA* is an allele of *msl-2* and *kmB* is an allele of *msl-1*. *kmB* is included within both *Df(2L)TW50* and *Df(2L)TW137*, giving a cytogenetic position of 36E4-37C1, consistent with that of *msl-1* (36F7-37B8). Males heterozygous for *kmA* and *msl-2*²²⁷ or for *kmB* and *msl-1*²¹⁶ die as larvae and not embryos, suggesting that the "early" embryonic lethality of the *kmA* and *kmB* chromosomes may be due to mutations at other loci.

We thank Didier Contamine and John Lucchesi for stocks.

Joly, D.^{1,2} and D. Lachaise². ¹Department of Biology, MacMaster University, Hamilton, Ontario, Canada, L8S 4K1; ²Laboratoire de Biologie et Génétique Evolutive, CNRS, 91198, Gif-sur-Yvette Cedex, France. Multiple mating frequency differs in two different geographic strains of *D. teissieri*.

Capacity of multiple mating for each sex has been extensively reported in literature for Drosophilidae species. Males of some of them are consistently polygynous with new virgin females; for example, *D. melanogaster* males copulate up to 10 times (Duncan, 1930; Stenveld and Bijlsma, 1988), *D. latifasciaeformis* males up to 8 times, *D. affinis* males up to 9 times, and *D. littoralis* males up to 11 times in an 8-hour period

(Bressac *et al.*, 1991). If in the first species the size of the progeny sired by the male decreases significantly in successive matings (Vianen and Bijlsma, 1991) this is not true for the fourth species, *D. littoralis* (Bressac *et al.*, 1991).

In females, the "mating pattern" (Rosenqvist and Berglund, 1992) shifts from "high recurrence polyandry" like in *D. littoralis* or *D. latifasciaeformis* (up to 3 mates in an 8-hour period) to "low recurrence polyandry" like in *D. affinis*, the females of which have only 2 rematings every 7 days (Bressac *et al.*, 1991). Here is reported a significant difference in the mating patterns for both males and females of two strains of one single species, *D. teissieri* of the *melanogaster* subgroup. Of interest in this species is the existence of a within-ejaculate sperm polymorphism presumably associated with "female facultative polygamy" (Joly *et al.*, 1991).

The two strains used in these experiments originated from the most distant populations of the geographic range of *D. teissieri* in Africa, that is Mount Silinda, Zimbabwe (1970), Gif stock 128-2, and Tai forest, Ivory Coast (1981), Gif stock 231-2. Tests were performed with a long confinement technics (8 hours per day) and enriched food medium (Harshman *et al.*, 1988). The first mating was obtained with single pairs, each comprised of one virgin 3-day-

Table 1. Cumulative number of matings and rematings in 24, 48 and 72 hour-periods between individuals from two different populations of *D. teissieri*. In each combination the first line represents the cumulative number of successful pairs on the total number tested while cumulative percentages are given on the second line (T= Tai; S= Silinda).

| Pairs | Mating number | Remating with S ♂ | | | Remating with T ♂ | | | Total |
|---------|---------------|-------------------|-------|-------|-------------------|-------|-------|-------|
| | | 24h | 48h | 72h | 24h | 48h | 72h | |
| T♀ x T♂ | 38/39 | 5/20 | 8/20 | 10/20 | 1/18 | 2/18 | 3/18 | 13/38 |
| | 97.4 | 25.0 | 40.0 | 50.0 | 5.6 | 11.1 | 16.7 | 34.2 |
| S♀ x S♂ | 29/30 | 5/14 | 13/14 | 14/14 | 7/15 | 13/15 | 15/15 | 29/29 |
| | 96.7 | 35.7 | 92.9 | 100.0 | 46.7 | 86.7 | 100.0 | 100.0 |
| T♀ x S♂ | 81/81 | 1/40 | 6/40 | 13/40 | 4/39 | 12/39 | 16/39 | 29/79 |
| | 100.0 | 2.5 | 15.0 | 32.5 | 10.3 | 30.8 | 41.0 | 36.7 |
| S♀ x T♂ | 77/82 | 8/38 | 30/38 | 34/38 | 7/35 | 24/35 | 29/35 | 63/73 |
| | 93.9 | 21.1 | 78.9 | 89.5 | 20.0 | 68.5 | 82.9 | 86.3 |

old female and one single male reared on standard cornmeal medium at 21°C. Males were discarded when copulation terminated. The inseminated females were then given an opportunity to remate after a 24, 48 or 72 hour-delay with two virgin 3-day-old males. Eight combinations were performed: four consisting in homogamic versus heterogamic pairs of Tai and Silinda flies and in each case rematings involved these two types of males.

Results show that the percentages of successful matings are very high (more than 93.9%) in homogamic as well as in heterogamic pairs (Table 1). However, great differences are expressed in remating tests between the two populations. Considering homogamic multiple matings, all Silinda females remate while only 16.7% of the Tai females remate. In

heterogamic pairs, *Silinda* females mate and remate with Tai males two and a half times as much as Tai females with S males (82.9 and 32.5%, respectively). Moreover, previously inseminated Tai females accept more readily *Silinda* than Tai males (50 and 32.5 versus 16.7 and 41%, respectively), and multiple mating with Tai females occurred twice as often with two successive *Silinda* than two successive Tai males (32.5 versus 16.7%, respectively). Consistent with this is the fact that first and second T males copulate longer than the respective first and second *Silinda* males (Joly *et al.*, 1991).

These results suggest that *Silinda* females have greater receptivity for remating than Tai females, and *Silinda* males mate more easily with previously inseminated *Silinda* than Tai females. Paradoxically, the same result is obtained with Tai males. From all these considerations it can be concluded that, with this method, mating patterns differ strongly between Tai and *Silinda* individuals although both are conspecific. Unlike the other species of *Drosophilidae* cited above, multiple matings are however more difficult and occur within a more prolonged period. Copulation duration could partly explain these results, because they are particularly long in *D. teissieri* lasting generally more than half an hour in first mating and more than one hour in remating (Joly *et al.*, 1991). Although not definitely conclusive, these results, particularly those for the Tai population, support the "female facultative polygamy" hypothesis.

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Campos, H.A. and H.F. Hoenigsberg. Instituto de Genética, Universidad de los Andes, Santafé de Bogotá, D.C., Colombia. Survival at -5°C and diapause in *Drosophila pseudoobscura* from the Altiplano Cundiboyacense of Colombia.

Drosophila pseudoobscura from Oicatá, Colombia, going into larval diapause at room temperature (18-22°C) for at least 30 days have been reported in studies done by Manuel Guillem in 1990, 1991 (not published). Since then, we have had some more spectacular results with eggs, larvae, pupae, and adults resulting from eggs oviposited at 22°C by *Drosophila*

pseudoobscura from Potosi. These have survived at -5°C for 120 days and are still going. Potosi is an artificial orchard at an altitude of 2,740 m in the province of Cundinamarca, Colombia, where daily temperatures fluctuate from 14-18°C and night temperatures are from 5-10°C during most of the year. In October and in January, temperatures can go down to -5°C at 4-5:00 am. In January, such low temperatures occur during dry weather conditions; the rainy season usually ends in December, about four weeks previously. However, fruiting orchards and artificially cultivated flowers are generally maintained all year around. Our experimental stations in the altiplano consist of banana and yeast placed on the ground and left there to ferment in order to produce local demes from which adults are sampled for our population and molecular studies.

For a long time, temperature has been known to be an important environmental factor that inflicts considerable penalty on *Drosophila* survival (Loeb and Northrop, 1917; Alpatov and Pearl, 1929; Maynard Smith, 1958). An increase in temperature results in an increased metabolism and a decreased longevity (Pearl, 1928).

It is a well established fact that, in *Drosophila*, developmental stages and adult life are well separated. Egg, larva, and pupa "produce" an adult fly; but these early developmental stages do not characterize the adult. Since several components of fitness depend on adult physiological changes, it is of obvious importance to determine what happens to longevity under below freezing conditions. Moreover, it is also important to measure the individual time of death following larval dormancy which has permitted adults to avoid stressing conditions.

We do not have knowledge of any complete study over a set of temperatures in any *Drosophila* species. In this short research note we want to report that *Drosophila pseudoobscura* adult females from Potosi, that were previously fertilized by their own males, lived far longer at -5°C than average adults. If one examines the figure showing the relationship between ambient temperature and average longevity in *D. melanogaster* in the paper by David (1988), one finds that above 32°C high temperatures produce very definite deleterious effects. Probably thresholds, in the physiology of catabolic reactions, that accumulate noxious effects and rapid aging, are responsible for this reaction.

On the other hand, below 10-12°C there are also negative effects on survival. At 0-2°C, survival of *D. melanogaster* varies (Johnson and Powell, 1974; Parsons, 1983). At -5°C survival time is about 2 hr; at -10°C it is about 20 min. From other work (Tucic and Krunic, 1975) we know that death does not occur as a consequence of internal freezing since hemolymph freezing temperature is about -18°C.

Thus, our results with highly consanguineous F₂ adults (F_{IS} 0.800 in Hoenigsberg *et al.*, 1992) of *Drosophila pseudoobscura* from Potosi were that the fertilized females survived for approximately four months, and her eggs and pupae give us an entirely new panorama of an overwintering population structure with a surviving strategy that may

operate even under (the most) extreme conditions.

By way of perspective, it should be said that studies by Cohet and David (1978) which related developmental temperature and several egg production characteristics in *D. melanogaster* (see Figure 3.5 of David, 1988), point out that egg production is permanently affected by the developmental temperature. Also, such studies revealed that the developmental temperature greatly affects the number of ovarioles (David and Clavel, 1967).

Almost all investigations have been done either with *D. subobscura* or with *D. melanogaster*. Since *D. melanogaster*'s tropical origin makes it rather cold-sensitive, it is important that future work be done with temperate species such as *D. pseudoobscura*.

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Das, A., N. Mahapatra, and B.B. Parida. Genetics Laboratory, Department of Zoology, Utkal University, Bhubaneswar- 751 004, India. Growth temperature and abdominal pigmentation in females of Indian *Drosophila melanogaster*.

Studies on the genetic variability of the reaction norms to temperature in *Drosophila* have been performed for several traits including bristle numbers, wing length, duration of development and viability (Gupta and Lewontin, 1982; Coyne and Beecham, 1987; Scheiner and Lyman, 1989). In females of *Drosophila melanogaster* the extension of black pigment on the

abdomen is a trait which exhibits a wide range of variations in response to growth temperature and is expected to have a genetic effect (Zucker, 1958; Robertson and Riviera, 1972; Robertson *et al.*, 1977). Adaptive significance in variation of the body color in natural populations of *Drosophila* has been suggested in several observations (David *et al.*, 1983, 1985; Payant, 1986; Capy *et al.*, 1988).

India is a tropical country and experiences a drastic change in temperature at different seasons of a year at different places. The population and quantitative genetical work on this species has been initiated in India recently (Das, 1991). In this preliminary survey, we report the results on the effect of different growth temperature on the pigmentation of the last three abdominal segments of females of *D. melanogaster*.

Table 1. Average value of pigmentation score in 12 isofemale lines subjected to different growth temperature in three abdominal segments of *D. melanogaster* females.

| Isofemale lines | Growth Temperature / Abdominal Segments | | | | | | | | | | | | | | |
|-----------------|---|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | 15°C | | | 20°C | | | 25°C | | | 27°C | | | 30°C | | |
| | 5th | 6th | 7th | 5th | 6th | 7th | 5th | 6th | 7th | 5th | 6th | 7th | 5th | 6th | 7th |
| 1 | 2.0 | 5.0 | 1.5 | 3.67 | 9.0 | 3.0 | 2.2 | 4.8 | 1.8 | 1.2 | 2.6 | 1.2 | 1.5 | 2.3 | 1.0 |
| 2 | 3.38 | 3.0 | 1.7 | 3.0 | 10.0 | 2.0 | 3.0 | 5.38 | 1.13 | 2.2 | 4.4 | 1.5 | 2.0 | 4.0 | 0.7 |
| 3 | 1.83 | 2.67 | 1.0 | 2.5 | 7.0 | 4.0 | 1.8 | 4.6 | 1.3 | 2.1 | 4.0 | 1.0 | 1.6 | 2.7 | 0.6 |
| 4 | 7.29 | 7.2 | 5.2 | 2.83 | 9.0 | 3.17 | 1.4 | 3.8 | 1.4 | 1.9 | 3.5 | 1.3 | 1.5 | 2.6 | 1.0 |
| 5 | 6.5 | 3.92 | 4.2 | 3.33 | 9.0 | 3.0 | 2.3 | 3.3 | 1.3 | 1.86 | 3.29 | 1.14 | 2.1 | 3.5 | 0.4 |
| 6 | 2.0 | 4.0 | 1.0 | 3.0 | 9.25 | 2.0 | 2.2 | 5.7 | 1.0 | 2.17 | 3.29 | 1.0 | 2.0 | 3.4 | 0.7 |
| 7 | 2.22 | 5.22 | 1.44 | 2.9 | 8.5 | 3.3 | 2.1 | 4.4 | 1.8 | 2.0 | 3.0 | 1.0 | 1.7 | 2.9 | 0.4 |
| 8 | 3.8 | 8.6 | 1.45 | 3.67 | 9.67 | 3.67 | 2.2 | 5.0 | 1.1 | 2.18 | 3.5 | 1.2 | 2.0 | 3.4 | 0.4 |
| 9 | 2.5 | 6.5 | 1.0 | 2.6 | 7.6 | 2.8 | 2.2 | 4.0 | 1.5 | 1.88 | 3.75 | 1.0 | 1.9 | 2.9 | 0.8 |
| 10 | 4.0 | 9.0 | 3.0 | 2.7 | 8.1 | 2.9 | 1.9 | 4.0 | 1.5 | 1.88 | 2.5 | 1.3 | 1.6 | 2.2 | 0.9 |
| 11 | 1.0 | 2.0 | 0.0 | 4.67 | 10.0 | 2.17 | 1.8 | 5.3 | 1.4 | 1.5 | 4.0 | 1.0 | 1.7 | 2.7 | 0.9 |
| 12 | 5.0 | 10.0 | 2.0 | 3.5 | 9.0 | 2.0 | 2.0 | 4.8 | 1.2 | 2.29 | 3.57 | 1.0 | 1.88 | 3.57 | 0.86 |

A population sample of *D. melanogaster* was collected locally with banana bait, and the females were isolated in culture vials with fresh food at 25°C to initiate isofemale lines. Twelve lines are chosen at random, and a group of ten pairs of each sex were used to produce the next generation. After a few days, each group of every isofemale line was allowed to oviposit for a few hours at 25°C in successive culture vials (5 in this case). One vial of each isofemale line was kept in one of the five experimental temperature (15, 20, 25, 28 and 30°C). On emergence, adult females were transferred to fresh food in the room temperature (25 ± 1°C) and examined a few days later. As the extension of black pigment is far more variable on the posterior tergites than on the anterior ones, we have only considered the last three tergites of the female abdomen which corresponds to abdominal segments 5, 6 and 7. For each segment, 11 classes were established which range from 0 (no pigment) to 10 (completely pigmented). The estimation of pigmentation was done

from a lateral view of the female abdomen. About ten females were studied for each isofemale line and for each temperature treatment.

The average values of pigmentation scored in twelve isofemale lines in segments 5, 6 and 7 are shown in Table 1. The data of all the five temperature treatments on the abdominal pigmentation in female *D. melanogaster* are also shown in the Table. It is evident from the table that the growth temperature is inversely proportional to the occurrence of pigmentation in *D. melanogaster*, i.e. when the growth temperature is low, the pigmentation is high. Although this trend was well evident at 20-30°C, at 15°C, however, the pigmentation was not as high as expected. The highest pigmentation was recorded on all the three abdominal segments at 20°C except in some isofemale lines for the 5th and 7th segment. However, the 6th segment is found to be more variable than the other two abdominal segments and the highest pigmentation was recorded to 10 in two incidences, i.e. at 15°C and 20°C in the isofemale line 12 and 11, respectively.

In insects like *Drosophila*, the isofemale line technique is generally preferred in order to know phenotypic plasticity and to analyze a reaction norm (Gupta and Lewontin, 1982; Coyne and Beecham, 1987; Gebhardt and Stearns, 1988). In the present study concerning the effect of growth temperature on the rate of pigmentation in three abdominal segments of female *D. melanogaster*, we detect that a lower temperature increases the area of black pigment in all the three segments. However, at 15°C of growth temperature the occurrence of pigmentation was not as high as expected from the general trend. In a similar study conducted by David *et al.* (1990) in a French population of *D. melanogaster*, the general trend was observed. From our results, if we delete the data at 15°C, the general trend is confirmed.

It is known that several insect species exhibit a darkening of their cuticle at low temperature and this reaction is generally related to thermal balance; darker individuals better absorb energy from light and infrared radiations. However, this property may be beneficial in the cold, but deleterious at high temperature (David *et al.*, 1985). This argument is very well supported by the observation of latitudinal clines in natural populations of *D. melanogaster* for thoracic trident pigmentation polymorphism (David *et al.*, 1985; Capy *et al.*, 1988). Therefore, it is expected, under this hypothesis, that the overall pigmentation, either dark or light, is the phenotype related to fitness. As India is a tropical country and Bhubaneswar, the collection site of *D. melanogaster*, experience a humid and high temperature climate, temperature below 20°C is rarely encountered. Due to this fact, it may be assumed that the trait is maintained by natural selection. This assumption is supported by the fact that the phenotypic plasticity for abdominal pigmentation in females of *D. melanogaster* was maximum at intermediate temperatures which were most likely to be encountered during breeding season in temperate countries (David *et al.*, 1983; Jones *et al.*, 1987).

Acknowledgments: The financial support in the form of a Research Associateship of the CSIR, New Delhi, to the senior author is thankfully acknowledged.

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Gundacker, D., B. Phannavong,¹ O. Vef, E. Gateff, and U. Kurzik-Dumke*. Institute of Genetics, Johannes Gutenberg-University, Mainz, F.R.G. ¹ZMNH, Universität Hamburg, UKE, Martinstr. 52, 2000 Hamburg 20. *corresponding author. Genetic and molecular characterization of breakpoints of five deficiencies in the genomic region 59F-60A.

The cloning of the *Drosophila melanogaster* tumor suppressor gene *lethal(2)tumorous imaginal discs (l(2)tid)* (Kurzik-Dumke *et al.*, 1992, 1993) required the isolation and characterization of deficiency stocks for the chromosome region 59F-60A and the performance of an extensive genomic walk in this region. Five deletions, propagated as *CyObw*, *CyO* or *SM5* balanced stocks, were generated in an X-ray and P-element mutagenesis.

In Table 1, designation, genetic background, cytology, lethal phase, complementation with the *l(2)tid* gene and the mode of induction of the deficiency stocks are present. In Figure 1, the five deficiencies are aligned to the schematic drawing of the salivary gland chromosomal region 59F-60A. Black boxes represent deficiencies which do not complement the *l(2)tid* gene, while white boxes complement this gene. The *l(2)tid* gene could at first be delimited to 70 kb of genomic DNA between the telomeric breakpoint of *Df(2R)tid* and the centromeric breakpoint of *Df(2R)x32* (Kurzik-Dumke *et al.*, 1992). With the help of the *l(2)tid* allelic *Df(2R)OVI*

Table 1.

| Designation and genetic background of the balanced deficiencies | Cytology | lethal phase | <i>l(2)tid</i> allele yes / no | mode of induction |
|---|-------------|--------------|--------------------------------|-------------------|
| <i>Df(2R) 106 / SM5</i> | 59F6-60A7 | embryo | no | x-ray |
| <i>Df(2R) b23, b lf / CyO, bw</i> | 59F8-60A3 | embryo | no | x-ray |
| <i>Df(2R) x32, b lf / CyO, bw</i> | 59F4-60A2 | embryo | yes | x-ray |
| <i>Df(2R) tid, b lf / CyO, bw</i> | 59F4-5 | embryo | yes | x-ray |
| <i>Df(2R)OV1, cn pr / CyO</i> | 59F5,6-60A1 | embryo | yes | P-Element |

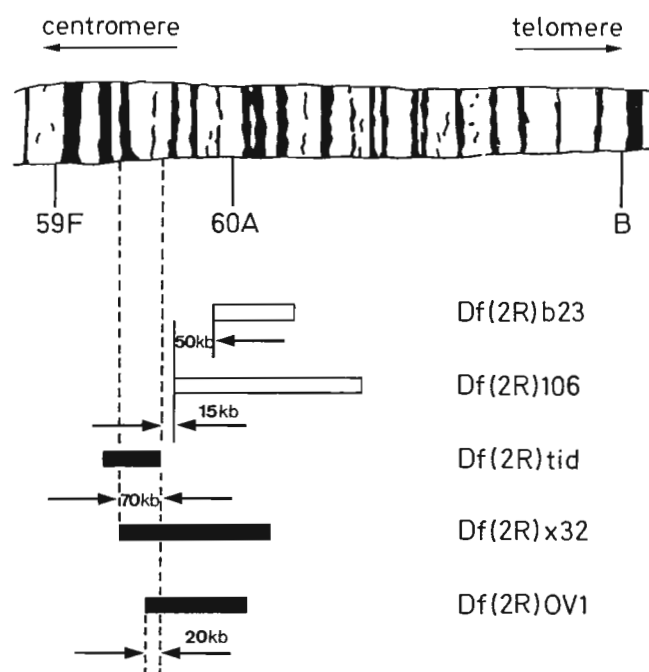
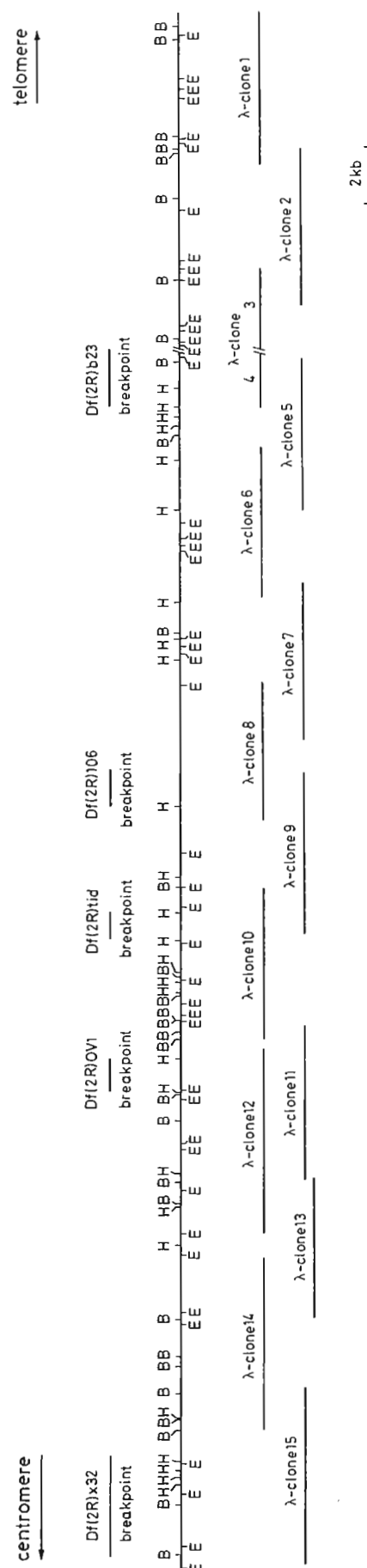


Figure 1. Genetic map of the 59F-60A genomic region. White boxes indicate deficiencies which complement, and black boxes deficiencies which do not complement the *l(2)tid* gene, identified in the 20 kb region between the telomeric breakpoint of *Df(2R)tid* and the centromeric breakpoint of *Df(2R)OV1*.

Figure 2 (at the right). Molecular map of the cloned genomic region.

(Vef, 1991), first available to us recently, the *l(2)tid* gene region could be further restricted to approximately 20 kb between the telomeric breakpoint of *Df(2R)tid* and the centromeric breakpoint of *Df(2R)OV1* (Figure 2, Kurzik-Dumke *et al.*, submitted). In order to clone the *l(2)tid* gene, a genomic walk was started with a 10 kb BamHI-fragment isolated from cosmid clone *P(wvar):L8-1* (Blondeau, 1990) hybridizing to the giant chromosomes at position 60A4 in a genomic library isolated from *Df(2R)b23* flies (Gundacker, 1989) (lambda-clones 1-3). After reaching the *Df(2R)b23* break-



points with lambda-clone 3, the genomic walk was continued in the Oregon R library (Bittler, 1988) (lambda-clones 4-15). Figure 2 shows the molecular map of the investigated genomic region, the extent of the isolated overlapping lambda-clones and the determined centromeric breakpoints of *Df(2R)b23*, *Df(2R)106*, *Df(2R)OV1* and *Df(2R)x32* and the telomeric breakpoints of deficiencies *Df(2R)b23* and *Df(2R)tid*. The breakpoints were determined via *in situ* hybridization of lambda-clones 1-15 to the giant chromosomes of the balanced heterozygous deficiencies and by genomic Southern-analysis. The distances in kb between the breakpoints are shown in Figures 1 and 2.

The aim of this report is to present five deficient stocks we have isolated in the 59F-60A genomic region. Since six breakpoints of these deficiencies are genetically and molecularly determined, the whole cloned genomic region of approximately 180 kb can be subdivided into different complementing groups. This information should prove useful for the identification of mutants belonging to the described genomic region.

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Sheen, Fang-miin. Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108-1095, USA. Present address: Fred Hutchinson Cancer Research Center, Seattle, Washington, 98104-2092, USA. Mutations caused by the mobilization of gypsy transposons in *Drosophila melanogaster*.

In *D. melanogaster*, an X chromosome called *Uc* is genetically unstable, frequently acquiring lethal mutations and accumulating conspicuous chromosome rearrangements (Lim, 1979, 1981; Lavery and Lim, 1982). The rearrangements and their associated mutations are apparently caused by *hobo* transposable elements (Lim, 1988; Sheen, Lim and Simmons, 1993). Careful analysis of several of these *hobo*-induced aberrations suggests that they are formed by recombination between *hobo* elements inserted at different positions in the *Uc* X chromosome. However, another transposon seems to be responsible for the mutations that are not associated with chromosome rearrangements. Unlike the *hobo*-induced mutations, these mutations are not confined to *Uc*, but may also occur on an X chromosome that has been paired with *Uc* for one generation (Lim *et al.*, 1983). Mapping and complementation experiments have indicated that many of these mutations are new lethal alleles of the *cut* locus, and molecular analyses of a sample of these *cut*-lethals have revealed insertions of a *gypsy* retrotransposon (Jack, 1985). I have analyzed eight additional lethal mutations that arose on *Uc*, or on a chromosome that had been associated with *Uc*, and have found that in all cases, a *gypsy* insertion has occurred in the mutant locus. Seven of the mutations were lethal alleles of *cut* (cytological position 7B1-2) and one was a lethal allele of the *nullo* locus, which is situated nearby; none of these mutations was associated with a cytologically visible chromosome rearrangement.

The lethal mutations were all independently derived from a screen conducted in 1981 (Lim *et al.*, 1983). In each case, the lethal had been localized by duplication/deficiency mapping to the interval between polytene bands 6E2; 7C4-6. Complementation tests with alleles of loci in this interval established the identity of the *cut*-lethals. The *nullo* lethal was positioned in the 6F1-2 bands by additional deficiency mapping, but its identity as an allele of *nullo* was established only later, when the 6F1-2 region was cloned (Sheen, Lim and Simmons, 1993) and the *nullo* locus was functionally defined (Simpson and Wieschaus, 1990; Rose and Wieschaus, 1992). Curiously, this allele is lethal over a deficiency, but viable in hemizygotes and homozygotes, indicating that it does not completely abolish the function of the *nullo* gene.

Molecular probes from the 6F1-2 and 7B1-2 regions were used in Southern blot analyses to determine if any of these mutations were associated with transposon insertions. Genomic DNA from heterozygous carriers was digested separately with different restriction enzymes, fractionated in agarose gels, blotted to a nylon membrane and hybridized with the probes. DNA from two nonlethal parental chromosomes (*Uc*, *H7* and a chromosome marked with *m*) was analyzed simultaneously to permit recognition of differences associated with the lethal mutations. The results are summarized in Figure 1. In each case, a *gypsy* element was inserted in the mutant gene, suggesting that it was responsible for the lethal effect.

Gypsy is abundant in the genomes of some *D. melanogaster* stocks (Peifer and Bender, 1988), and in some of these

it has been implicated in genetic instabilities (Mevel-Ninio, Mariol and Gans, 1989; Kim, Belyaeva and Aslanian, 1990; Lyubonmirskaya *et al.*, 1990). In no case, however, is it clear what is responsible for the high level of *gypsy* activity. A mutant element with a transpositional advantage might have arisen and subsequently been amplified, or a system for controlling *gypsy* activity might have broken down, leading to an elevated frequency of transposition. On either hypothesis, however, there is no need to invoke "hybrid dysgenesis" (see Gerasimova *et al.*, 1984) as the cause of *gypsy* transposition since *gypsy* elements have accumulated in these stocks in the absence of outcrossing. Of course, *gypsy*-induced mutations have been detected in the progeny of hybrids between *gypsy*-rich and *gypsy*-poor stocks - in fact, all the mutations analyzed here had this origin - but this probably reflects an aspect of genetic methodology rather than a precondition for mobilization itself.

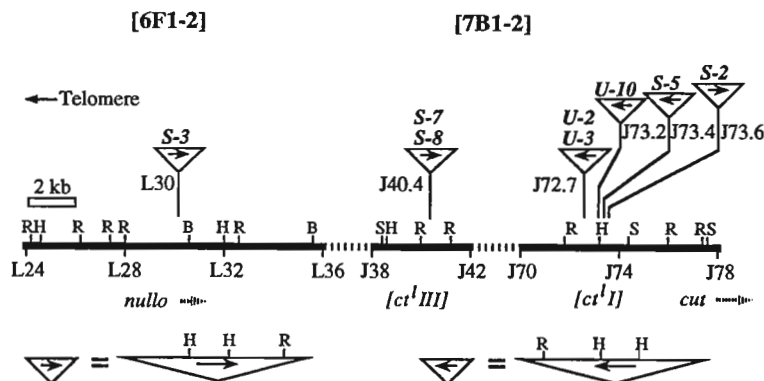


Figure 1. Restriction maps of portions of polytene bands 6F1-2 and 7B1-2 showing *gypsy* insertions in X chromosomes with recessive lethal mutations. Each insertion is named for the chromosome that carries it (U = *Uc*, H7 and S = *m*) and the orientations of the insertions are indicated by arrows. The S-3 mutant chromosome was also studied by Simpson and Wieschaus (1990) and Rose and Wieschaus (1992), who referred to it as *L-II-27*. The coordinate systems are based on the analyses of Lim (letter L; see Sheen, Lim and Simmons, 1993) and Jack

(letter J; see Jack, 1985), with distances in kilobases from the origin indicated by the numbers following the letters. The cleavage sites of the enzymes *Hind*III (H), *Eco*RI (R), *Bam*HI (B) and *Sal*I (S) and the transcripts of the *nullo* and *cut* genes (striped bars) are shown; the *cut* transcript extends approximately 60 Kb to the right of the map. The sequences to the left of this transcript comprise two lethal complementation groups, denoted *ct*^I and *ct*^{III}; see Liu, McLeod and Jack (1991) for details.

Gypsy seems to have a tendency to insert in some loci (Jack, 1985; Mevel-Ninio, Mariol and Gans, 1989). In the present study, the frequent recovery of *gypsy* insertion mutations in *cut*, compared to other loci in the 6E; 7C region, suggests that *cut* was a preferred target. Five of the *gypsy* insertions in *cut* mapped to a one kilobase region around coordinate J73 and two mapped in a one kilobase region around coordinate J40. Jack (1985) also observed a clustering of *gypsy* insertions around these coordinates.

What might explain the relatively high frequency of *gypsy* insertions in the *cut* locus, and what might explain the clustering of these insertions in relatively small regions within this locus? One possibility is that certain regions of the *cut* DNA contain many copies of the preferred target sequences for *gypsy* insertion, TAYAYA, where Y is either pyrimidine (Freund and Meselson, 1984; Marlors, Parkhurst and Corces, 1986). However, this would not explain the almost complete absence of *gypsy* insertion mutations in the other genes of the 6E; 7C region, which would be expected to contain at least some copies of this target sequence. This suggests that some other factor, such as chromatin accessibility during *gypsy* transposition, is responsible for the preferential and clustered pattern of insertion.

Acknowledgments: I thank J.K. Lim, T. Cline, T. Watanabe, W. Bender and J. Jack for DNA clones and M.J. Simmons for guidance. This work was supported by NIH grants ESO1960 and GM40263.

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Dunker, B.P., J.A. Hermans, P.L. Davies and V.K. Walker. Queen's University, Kingston, Ontario, Canada. Biological containment of transgenic fruit flies.

Certain transgenes have the potential to confer a selective advantage to host flies while others might prove deleterious if introduced into natural *Drosophila* populations. Such concerns have prompted calls for effective biological containment of transformants (Walker, 1989; Ashburner, 1990).

Our laboratory is using *Drosophila* as a model organism for the expression of fish antifreeze proteins (AFP) (Rancourt et al., 1987, 1990). Because flies that synthesize AFP are potentially more freeze-resistant than those in the wild, we feel it is essential to keep them confined to the laboratory. To this end strains with the flight muscle mutation *Ifm(3)3* (Tansey et al., 1987) that are suitable as hosts for P element-mediated transformation have been developed. Flies homozygous for this mutation are unable to fly due to a deficiency of indirect flight muscle tropomyosin. Heterozygotes, unlike homozygotes, are able to jump short distances, but are also incapable of sustained flight.

The first flightless host strain generated, *b cn; ry⁸; Ifm(3)3* (Rancourt et al., 1991) has been used to establish a variety of transgenic lines. The combination of homozygous *cn* and *ry⁸* mutations results in orange colored eyes (Lindsley and Zimm, 1992). Carnegie 20 (Rubin and Spradling, 1983), the P element vector used with this line, includes the wild-type xanthine dehydrogenase gene (*ry⁸*) which restores the natural eye color.

Many researchers prefer to use *white (w)* as a marker gene. The product of this gene is required for eye pigmentation, thus mutants have colorless eyes (Lindsley and Zimm, 1992). P element vectors that incorporate a wild-type *white* gene include the pCaSpeR (Pirotta, 1988) and pW (Klemenz et al., 1987) series. These plasmids have several advantages over their *ry* system counterparts, including a smaller size which facilitates genetic manipulation, and a polylinker region with a greater number of restriction enzyme sites. Also, the intensity of the eye color change can, in some cases, be used to distinguish between homozygous and heterozygous individuals.

We have introduced *Ifm(3)3* into a strain of flies carrying *white (w⁶⁷)* and *yellow* body color (*y*) mutations through a series of genetic crosses. The resulting line of flies *y, w⁶⁷; Ifm(3)3* is incapable of flight, but appears as vigorous as *y, w⁶⁷* flies in all other respects. A fish AFP gene expression cassette has been cloned into pW8 and corresponding transgenics have been generated through microinjection of *y, w⁶⁷; Ifm(3)3* embryos (unpublished results). It is our hope that other laboratories conducting transgenic studies will find this fly useful. We intend to donate this stock to the Mid-America *Drosophila* Stock Center, Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio.

Acknowledgments: We would like to thank John Bell (University of Alberta), Ross MacIntyre (Cornell University) and Robert Storti (University of Chicago) for contributing the stocks *y, w⁶⁷; T(2,3) ap^{Xa}/CyO; TM2* and *Ifm(3)3*, respectively. This work was supported by NSERC and MRC grants to V.K.W. and P.L.D., respectively, as well as a NSERC postgraduate scholarship to B.P.D.

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Thörig, George E.W. University of Utrecht, Department of Plant Ecology and Evolutionary Biology, Section of Evolutionary Genetics, Padualaan 8, 3584 CH Utrecht, The Netherlands. The female rescue capacity of *Drosophila simulans* stocks using hybridisations with *Drosophila melanogaster* stocks.

Earlier (Thörig, 1992), I reported sterility breakdowns during hybridisations between *D. melanogaster* and *D. simulans*. We also reported that the *simulans* stocks used in our hybridisation experiments carry a trait. It rescues hybrid females in the cross of *D. simulans* females x *D. melanogaster* males, this contrasts with the *Lhr* stock of Watanabe (1979). In Table 1, one is shown an experiment that

revealed two issues:

1. The temperature effect on female hybrid rescue depending on the background of the stock. The stock *s22^C* has the best expression at 18°C. All stocks rescue hybrid females at 18°, 25°, and > 25° (> 25° not shown). The morphology (Coyne, 1983) of the posterior process of the genital tergite of the *D. simulans* males is represented by Figure 1a and that of the males from the *D. melanogaster* stocks by Figure 1b.

2. The reverse crosses reveal normal frequencies of hybrid males.

References: Thörig, G.E.W. 1992, DIS 71: 198; Watanabe, T.K. 1979, Genetics 54: 325-331; Coyne, J.A. 1983, Evolution 37: 1101-1118; Sturtevant, A.H. 1921, Genetics 5: 488-500.

Table 1. Cross-results 1990 - 1991

| Type | Parent | | Offspring ♂♂ | Offspring ♀♀ |
|----------------------------|--------|------|-----------------|-----------------|
| | ♀♀ | ♂♂ | | |
| Breeding at 18° | | | | |
| s22 ♀ x mel ♂ | 415 | 621 | 209 | 277 |
| s22 ^B ♀ x mel ♂ | 85 | 115 | 42 | 72* |
| s22 ^C ♀ x mel ♂ | 301 | 277 | 105 | 389* |
| s8 ♀ x mel ♂ | 133 | 202 | 220 | 203 |
| Total | 934 | 1215 | 576 | 891* |
| Breeding at 25° | | | | |
| s22 ♀ x mel ♂ | 1250 | 670 | 787 | 726 |
| s22 ^B ♀ x mel ♂ | 22 | 27 | 2 | 0 |
| s22 ^C ♀ x mel ♂ | 830 | 481 | 254 | 213 |
| s8 ♀ x mel ♂ | 45 | 67 | 46 | 38 |
| Total | 2147 | 1245 | 1089* | 977 |
| Breeding at 18° | | | | |
| mB ♀ x s22 ♂ | 282 | 395 | 6 | 2775 |
| mB ♀ x s22 ^C ♂ | 349 | 323 | 9 | 2969 |
| Breeding at 25° | | | | |
| mB ♀ x s22 ♂ | 247 | 222 | 1 | 448 |
| mB ♀ x s22 ^C ♂ | 994 | 819 | 16 | 7745 |

mel = mB or mIV^A; mb = st⁺ melanogaster; mIV^A = st⁺ melanogaster; s8 = st simulans; s22 = st simulans; s22^B = st simulans; s22^C = st simulans. Hybrids see Coyne (1983) and Sturtevant (1921). * = significant $\chi^2 > 3.86$

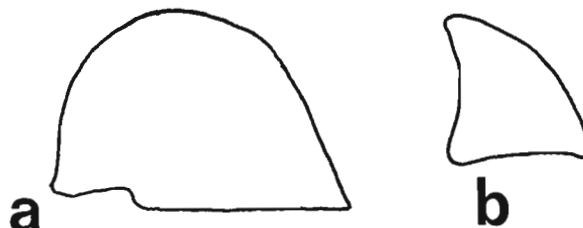


Figure 1. Morphology of the posterior process of the male genital tergite: a, *D. simulans*; b, *D. melanogaster*.

Bruins B.G., W. Scharloo and G.E.W. Thörig.
University of Utrecht, Section of Evolutionary Genetics,
Padualaan 8, 3518 CH Utrecht, The Netherlands. Effect
of illumination level on the appearance of aberrant
phenotypes.

appearance of several mutant phenotypes in a wild type strain.

For many generations we have reared a part of our wild type control strain under low and high light intensities, respectively. While testing the survival from egg to adulthood of these two selection lines under different light intensities, we found several phenotypes, which resemble some well known mutants. Under low light intensities flies of both selection lines mostly showed the wild type phenotype, except for *Curly*-like and *Wrinkle*-like phenotypes, which appeared in a low frequency (Table 1). Under high illumination there appeared several phenotypes, which resemble the mutants *singed* wing and *Notch* (Table 1). When these selection lines were tested on media with 20 g yeast/l, we got similar results. However, under these conditions we found less aberrant phenotypes than on the 16G medium, and a few (Bruins et al., 1991b) flies with a brown eye colour.

Table 1. Effect of illumination level on the appearance of mutant phenotypes (expressed as % of number of examined flies) on 16G media (16 g yeast/l). Illumination was continuous (24h). The light sources were Philips 58W/33 fluorescent tubes. n = number of examined flies. Six vials were tested at each condition.

| Selection line: | L-line | | H-line | |
|------------------|--------|------|--------|------|
| | low | high | low | high |
| phenotype: | | | | |
| Curly-like | 1.3 | 3.6 | 3.1 | 5.6 |
| Wrinkle-like | 0.3 | 0.8 | 1.0 | 5.1 |
| dumpy-like | 0.3 | 1.2 | 0 | 0 |
| singed wing-like | 0 | 0.4 | 0 | 1.1 |
| Notch-like | 0 | 20.1 | 0 | 14.0 |
| n | 399 | 249 | 393 | 173 |

Table 2. Effect of illumination level on the appearance of Notch-like phenotypes in a wildtype strain on 16G food media. n = number of examined flies. Number of examined vials in parenthesis.

| illumination | Notch-like phenotypes (%) | n |
|--------------|------------------------------|---------|
| 60 - 80 lx | 0 | 224 (3) |
| 120 - 230 lx | 0.5 | 221 (3) |
| 135 - 240 lx | 0.9 | 231 (3) |
| 150 - 245 lx | 21.4 | 131 (3) |
| 175 - 300 lx | 21.7 | 69 (2) |

Besides, we have tested the survival from egg to adulthood of the wild type control strain under a range of different light intensities and we have counted the number *Notch*-like phenotypes. Table 2 shows that under relatively low light intensities (150-300 lx) a rather high percentage *Notch*-like phenotypes do appear.

A 16G food medium supplemented with vitamin C (2 g/l) reduces the appearance of *Notch*-like phenotypes. However, under higher light intensities (500 lx), we noticed that some 8% of the flies do have notched wings (not shown in Table 2). In addition we also found the dark trident phenotype (on both media).

On media supplemented with vitamin C, we found some (3.1%) vermilion eye coloured flies (at 150 lx) and some (2.8%) flies with *Ski*-like wings (at 400 lx). We have not observed these phenotypes on the unsupplemented 16G medium. The *Curly*- and *Wrinkle*-like phenotypes also appeared on food medium with vitamin C.

References: Bruins, B.G., W. Scharloo and G.E.W. Thörig 1991a, *Insect Biochemistry* 21: 535-539; Bruins, B.G., W. Scharloo and G.E.W. Thörig 1991b, *Insect Biochemistry* 21: 541-544.

Thörig, George E.W. University of Utrecht, Department of Plant Ecology and Evolutionary Biology, Section of Evolutionary Genetics, Padualaan 8, 3584 CH Utrecht, The Netherlands. The frequency of rescuing both lethal and sterile hybrids from crosses between *Drosophila simulans* and *Drosophila melanogaster*.

In September 1980 we caught two *D. simulans* females at the fruit auction of Utrecht. They produced an offspring with 100% hybrid males at the breeding condition 25°C. This conclusion was based on their morphology and electropherograms of the enzyme alcohol dehydrogenase. One female generated, after continued breeding at 18°C, a partial transformed offspring: next to hybrid males the offspring consisted of

fertile *D. melanogaster* males and females and also suppressing the expression of the *D. simulans Adh* gene.

Conclusion:

1. Offspring has been transformed at 18°C into the paternal phenotype "melanogaster".
2. If females from wild type populations can produce such an offspring after hybridization, the trait is likely wide spread over the species. The impact of carrying it may be far reaching in terms of adaptation.
3. The result suggests that environmental circumstances such as temperature modify the suppression of this phenomenon by rescuing embryos with the females sex, which normally died, and moreover by maintaining fertility on the imago stage on which they normally are sterile, plus replacement of the hybrid morphology by that of the male parent. The ultimate result is a fertile offspring of both sexes, phenocopying the male parent species morphology.

Definitions of *D. melanogaster* and *D. simulans* morphology were based on descriptions of others and myself (see under "The female rescue capacity of *D. simulans* stocks during hybridizations with *D. melanogaster* stocks" in this issue of DIS).

To test this conclusion we cross with laboratory stocks under different circumstances and at different seasons to investigate the suppression of normally lethal and sterile hybrids:

In the offspring of the cross *D. melanogaster* females X *D. simulans* males, the male sex dies as a larva and the female sex develops into sterile imagoes with the morphology of hybrids.

In that of the reverse cross the female sex dies as an embryo, while the male sex develops into sterile imagoes with the morphology of hybrids.

During the period 1981-1992 the phenomenon of transformation frequently took place in hybrid crosses. In 1990-1991 large scale crosses were performed and in several batches transformed offspring were found. They are shown in the Tables 1,2,3 and 4.

Table 1: One cross gave an offspring with 6% transformed males and females. They all phenocopied the species morphology of the parental male "simulans" and were fertile.

Table 2: Two crosses had a transformed offspring. The offspring from one cross contained one transformed male, again phenocopying the species morphology of the parental male "melanogaster", the male produced no offspring with his sisters. A second cross produced 14 males and 14 females all phenocopying the species morphology of the parental males "melanogaster". Only four males and four females were fertile.

Table 3: One cross produced an offspring at 18°C which produced two eggs after isolation. Another cross produced at 25°C an offspring with one male, phenocopying the species morphology of the parental males "simulans" except for the scarlet eye colour. The male produced no offspring with *D. simulans* virgins.

Table 4: One cross produced both at 18°C and 25°C males and females phenocopying the species morphology of the parental males "melanogaster" next to hybrid phenotypes. Also females with the hybrid phenotype were fertile, however males with the hybrid phenotype were sterile and males with the phenotype st^+ melanogaster were predominantly fertile.

Table 1. A batch with one transformation phenomenon

| mB ♀♀ | x | s22 ^c ♂♂ | F ₁ inspection | 25° ♀♀ | 18° ♀♀ |
|-------|---|---------------------|---------------------------|--------|---------|
| 23 | | 11 | +(24/12) | 133 | 246 |
| 24 | | 15 | +(24/12) | 39 | 178 |
| 5 | | 6 | +(24/12) | 18 | 141 |
| 4 | | 5 | -(24/12) | 0 | 0 |
| 37 | | 21 | +(24/12) | 27 | 202/1 ♂ |
| 19 | | 15 | +(24/12) | 10 | 95 |
| 30 | | 20 | +(24/12) | 18 | 203 |
| 19 | | 15 | +(24/12) | 7 | 127 |
| 20 | | 20 | +(24/12) | 115 | 130 |
| 34 | | 12 | +(24/12) | 145 | 242 |
| 23 | | 15 | +(24/12) | 21 | 148 |
| 45 | | 20 | +(24/12) | 47 | 163 |
| | | | | | 17 ♂♂ * |
| | | | | | 13 ♀♀ * |
| 43 | | 20 | +(24/12) | 117 | 197 |
| 57 | | 24 | +(24/12) | 27 | 168 |

The flies were bred at 18°, while hybridization (13/12/90) was started at 25° after fertilization, breeding was continued at 18° (24/12/90). mB = melanogaster stock; s22^c = scarlet simulans stock (see also figure 1). * = phenotypically scarlet simulans. Other progeny phenotypically st⁺ hybrid.

Table 2. A batch with two transformation phenomena

| s22 | x | mB | F ₁ ♀♀ 25° | F ₁ ♂♂ 25° | F ₁ ♀♀ 18° | F ₁ ♂♂ 18° |
|-----|---|----|-----------------------|-----------------------|-----------------------|-----------------------|
| 45 | | 20 | 0 | 0 | 5 | 11 |
| 39 | | 20 | 0 | 0 | 16 | 21 |
| 24 | | 15 | 5 | 8 | 9 | 4* |
| 23 | | 15 | 0 | 3 | 0 | 1 |
| 51 | | 23 | 7 | 3 | 14** | 14*** |
| 50 | | 23 | 0 | 0 | 0 | 0 |
| 50 | | 26 | 3 | 13 | 27 | 19 |

s22 = scarlet simulans; mB = st⁺ melanogaster; * = 1 male st⁺ melanogaster sterile; ** = st⁺ melanogaster (4 flies fertile); *** = st⁺ melanogaster (4 flies fertile). Other progeny phenotypically st⁺ hybrids

Table 3. A batch with one transformation phenomenon

| s22 ♀♀ | x | mB ♂♂ | Offspring ♀♀ | Offspring ♂♂ | Offspring ♀♀ | Offspring ♂♂ |
|--------|---|-------|--------------|--------------|--------------|--------------|
| 31 | | 20 | 353 | 1 | 124 | 0 |
| 46 | | 27 | 444 | 1 | 195* | 2 |
| 42 | | 20 | 239 | 1 | 112 | 0 |
| 44 | | 25 | 308 | 3** | 119 | 1 |

s22 = scarlet simulans; mB = st⁺ melanogaster; * = after isolation two eggs were found; ** = 1 male was simulans with st⁺ eyes, all other offspring were phenotypically hybrid.

Table 4. A batch with one transformation phenomena

| s22-♀♀ | x | mB ♂♂ | F ₁ ♀♀ 25° | F ₁ ♂♂ 25° | F ₁ ♀♀ 18° | F ₁ ♂♂ 18° |
|--------|---|-------|-----------------------|-----------------------|-----------------------|-----------------------|
| 30 | | 18 | 0 | 0 | 0 | 0 |
| 30 | | 25 | 1 | 1 | 10 | 6 |
| 29 | | 21 | 15 | 30 | 15 | 24 |
| 48 | | 20 | 30 | 43 | 18 | 24 |
| 60 | | 25 | 9 | 6 | 9 | 13 |
| 33 | | 20 | 37* | 59** | 40*** | 42**** |
| 32 | | 20 | 4 | 8 | 16 | 12 |
| 33 | | 20 | 1 | 0 | 0 | 0 |

s22 = scarlet simulans; mB = st⁺ melanogaster; * = all females were fertile single pair tested, phenotype hybrid up to melanogaster; ** = 43 st⁺ melanogaster (fertile) and 16 hybrid (sterile); *** = 27 melanogaster type and 13 hybrid (most fertile); **** = 22 melanogaster type (fertile) and 13 hybrids (sterile). Other progeny phenotypically st⁺ hybrids.

Conclusion:

The transformation phenomenon found in the offspring from a *D. simulans* female caught at the fruit auction in Utrecht has been repeated under laboratory circumstances with *D. simulans* stocks present in our laboratory. Moreover transformation takes place into both directions "male and female parent" and with both cross types: *D. melanogaster* females X *D. simulans* males and the reverse cross. From all the hits during 1980-1992 only a fraction is shown in this note. Obviously, the trait is wide spread because the stocks used are not related to the auction female. The role of the lethal female rescue trait is not clear. Certainly stocks carrying this trait *Lfr* can transform the offspring from hybrid crosses, however other stocks absent for that trait also transform (e.g., the auction female and her offspring transform after hybridization). Further it has been found that stocks with the potency to transform, crossed with wild type stocks with the same morphology (e.g., s22 X wild type *D. simulans*) produced an offspring with the capacity to transform more frequently than the female parent stock.

The temperature effect on transformation seems to be overruled by the conditions of the season. Particularly in the month December, the frequency of hits increases; this lasted up to and including May. One maternal transformation was found in August 1990 between s22 females X mB males at 18°C. Further it is found that increased humidity favours transformation.

It is clear that certain environmental circumstances affect the suppression of deleterious effect in hybrids during the ontogeny of juvenile hybrids. Rescuing lethality and maintaining fertility have a cost, because the hybrid phenotype has been overruled by that of either the paternal or maternal parent. It seems impossible to sustain the pure hybrid stage during transformation. One genome overruled the other, because both are present in transformed F₁. Only imprinting can explain such a construction, and if so we must conclude that both genomes united in a hybrid have a different species specific print. If parts of the genome are inactivated, it can configure a phenotype absent for essential functions or even being lethal. Close related species will decrease their print specificity, which suppres-

ses inactivation by imprinting. Exponents of this mechanism are genes such as *mhr* (Hutter and Ashburner, 1987), *lhr* (Watanabe, 1979) and *lfr*. They overrule essential imprinting (Sapienza, 1990) which suppress lethality. So the sex ratio will be restored.

References: Hutter, P. and M. Ashburner 1987, Nature 327: 331-333; Watanabe, T.K. 1979, Japan J. Genetics 54: 325-331; Sapienza, C. 1990, Scientific American, October: 26-32.

Thörig, George E.W. University of Utrecht, Department of Plant Ecology and Evolutionary Biology, Section of Evolutionary Genetics, Padualaan 8, 3584 CH Utrecht, The Netherlands. Morphological transformation in crosses between *Drosophila melanogaster* and *Drosophila simulans* a spin-off from a cold adaptation?

During the past 14 years sampling in the fruit auction of Utrecht shows that both *D. melanogaster* and *D. simulans* females can mate with males of the other species. These matings result in a hybrid offspring. One *D. simulans* female gives next to a sterile hybrid male offspring, a fertile offspring of males and females phenocopying the *D. melanogaster* morphology. Earlier reports refer to these phenotypic transformation phenomena (Thorig, 1992). We also repeated this result

under laboratory circumstances with laboratory stocks. Obviously these results suggest that transformation is favoured during December up to and until May: the months with the lowest temperature in the Netherlands.

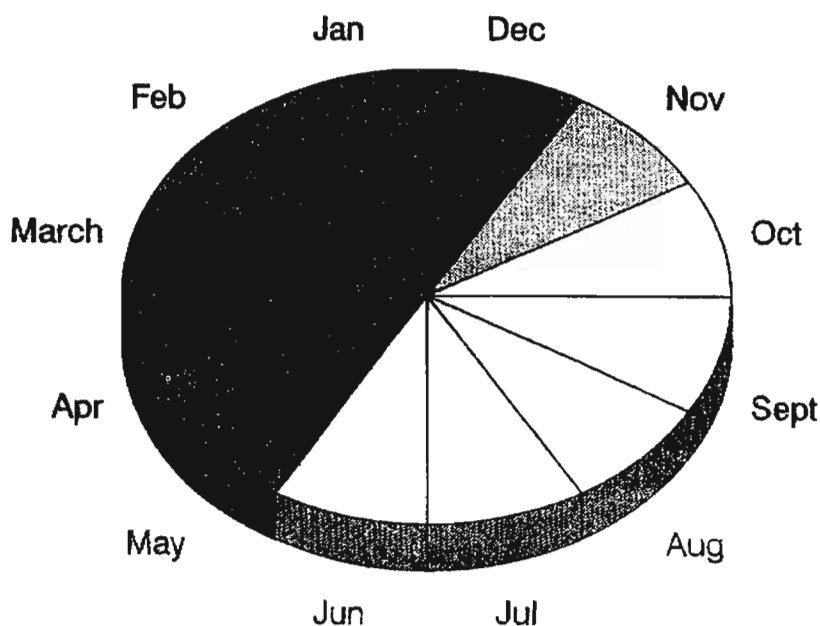
Conclusion: season circumstances affect the events of transformation under laboratory circumstances.

The species *Drosophila simulans* and *Drosophila melanogaster* overwinter by a state of dormancy (Izquierdo, 1991). Males normally become sterile at these circumstances. It means that those females, which have been mated before that period will produce offspring. The embryos are exposed during the winter period by cold circumstances. Perhaps these circumstances induced the event of transformation in our lab. Figure 1 shows that both species can be sampled during the period June up to and until October-November (the light segments). It shows that both species are immobile during the period December up to and until May (the dark segments).

Transformation as a trait can be explained if we consider that the two species evolved an adaptation to cold circumstances. The species *Drosophila melanogaster*, which arrived earlier in these regions than the species *Drosophila simulans* (Lachaise *et al.*, 1988) is more cold resistant. *Drosophila simulans* perhaps introduced those cold-resistance genes by transformation during a period (Lewontin and Birch, 1966). This process has led to this trait. It is present, but its frequency suggests that it

is normally suppressed and accidentally expressed. If this trait is wide spread among the species, it will have its impact during hybridisations, because it can break down the species barrier and favour gene transport.

References: Thörig, G.E.W. 1992, Dros. Inf. Serv. 71: 198; Izquierdo, J.I. 1991, Entomol. exp. appl. 59: 51-58; Lachaise, D., M-L. Cariou, J.R. David, F. Lemeunier, L. Tsacas and M. Ashburner 1988, Historical biography of the *Drosophila melanogaster* Species Subgroup; Lewontin, R.C. and L.C. Birch 1966, Evolution 20: 315-336.



Komma, D.J. and K.C. Atwood¹. Duke University Medical Center, Durham, NC. ¹Deceased. Tritiated thymidine enables ring chromosomes to magnify in *D. melanogaster*.

suggest that chromosome topology is important for magnification. This would be true if the mechanism of magnification involved sister chromatid recombination, since sister chromatid exchange in a ring chromosome would lead to the formation of inviable dicentric ring chromosomes. The observation (Endow et al., 1984) of elevated frequencies of dicentric rings in meiosis II of rDNA-deficient ring X males, compared with non-deficient males, is consistent with this hypothesis. An essentially complete inhibition of magnification was observed for ring X chromosomes kept under magnifying conditions for many successive generations (Tartof, 1974; Endow et al., 1984). This inhibition suggests that the magnifying exchanges are probably single exchanges, since multiple even-numbered exchanges would permit some of the magnified rings to separate and be recovered. The observation that loss of a X^{bb^o} ring (that carries no detectable rDNA) is not increased during meiosis in rDNA-deficient males compared with non-deficient males, whereas loss of a X^{bb} ring is increased (Endow et al., 1984), suggests that the magnifying sister chromatid exchanges are confined to the rDNA. Gibson and Prescott (1972) demonstrated that 3H -thymidine incorporated into DNA is an inducer of sister chromatid exchanges. We therefore conjectured that 3H -thymidine might enable ring chromosomes to magnify by inducing additional exchanges outside the rDNA.

The phenotype produced by rDNA deficiency, *bobbed* (*bb*), is characterized by short thin thoracic bristles and incomplete pigmentation of abdominal tergites. We found that a *R(1)2* chromosome, obtained from the Bowling Green Stock Center and marked with *yellow* and *cut*, also carried a strong *bb* allele (Endow et al., 1984). Males carrying this chromosome together with the Y^{bb^-} chromosome were grown on media containing 3H -thymidine. As a control, males carrying Y^{bb^-} and a rod X chromosome, *uco*₃, with a *bb* allele comparable in severity to the ring *bb* allele, were grown on media containing the same concentrations of 3H -thymidine. The resulting *bb* males were mated en masse to *X/X/Y* females carrying the *In(1)sc^{4L}sc^{8R}* chromosome and a *B^SY* chromosome. The *In(1)sc^{4L}sc^{8R}* chromosome is deficient for the entire heterochromatic region, including the rDNA. The relevant offspring were round-eyed females that carried an *In(1)sc^{4L}sc^{8R}* chromosome together with a potentially magnified ring or rod X^{bb} chromosome. These females were scored for magnification by inspection of thoracic bristle length and abdominal pigmentation.

Results of these experiments are shown in Table 1. No *bobbed* magnified (bb^m) offspring were recovered from ring-bearing males grown on medium without 3H -thymidine, whereas 3H -thymidine medium allowed recovery of up to 30-40% bb^m offspring. Recombination tests of 14 of the bb^m males indicated that the rings had not opened. This demonstration that ring X^{bb} chromosomes do not magnify in *bb* males grown on normal media, but do magnify if the *bb* males are grown on medium containing 3H -thymidine, is expected if the mechanism of magnification involves sister chromatid exchange, but is not predicted by the extracopy hypothesis proposed by Ritossa (1972). These data therefore provide additional evidence that the basis of rDNA increase during magnification is unequal sister chromatid exchange.

In contrast, the rod X chromosome showed a concentration-dependent inhibition of magnification, with the highest proportion of bb^m in the offspring from the control medium without 3H -thymidine. We did not test other rod X^{bb} alleles or medium containing cold thymidine. A possible explanation for the inhibition of rod X^{bb} magnification with increasing 3H -thymidine concentration is that it is caused by saturation of an exchange-prone repair system. According

Magnification is an increase in the number of ribosomal RNA cistrons (rDNA) in gametes produced by rDNA-deficient flies. Magnification occurs readily in males carrying rod X chromosomes, but does not normally occur in males carrying ring X chromosomes (Tartof, 1974; Endow et al., 1984). These observations

Table 1. Tritium-induced recovery of bb^m in a ring X chromosome and inhibition of magnification in a rod X chromosome.

| Progeny of mating treated males to $X^{bb^o}/X^{bb^o}/B^SY$ females * | | | | | | | |
|---|----------------|-----|--------|--------|-----------|-------|----------|
| 3H -thymidine | Females | | | | | Males | % bb^m |
| mCi / ml | B ^S | bb | bb^m | bb^+ | bb mosaic | | |
| A. Ring X^{bb} chromosome | | | | | | | |
| Control | 487 | 684 | 0 | 0 | 0 | 962 | 0 |
| 0.0005 | 159 | 76 | 34 | 0 | 4 | 231 | 31 |
| 0.0015 | 332 | 149 | 90 | 0 | 2 | 367 | 38 |
| 0.0035 | 12 | 6 | 3 | 0 | 0 | 0 | — |
| 0.0050 | 171 | 64 | 39 | 2 | 9 | 231 | 39 |
| B. Rod X^{bb} chromosome | | | | | | | |
| Control | 159 | 26 | 106 | 0 | 0 | 144 | 80 |
| 0.0005 | 159 | 97 | 61 | 0 | 0 | 160 | 39 |
| 0.0015 | 242 | 138 | 17 | 0 | 0 | 193 | 11 |
| 0.0035 | 183 | 203 | 7 | 1 | 0 | 244 | 4 |
| 0.0050 | 190 | 140 | 8 | 0 | 0 | 215 | 5 |

*The X^{bb^o} chromosome carried by the tester females was *In(1)sc^{4L}sc^{8R}*.

to this hypothesis, the initial lesions - perhaps single-strand nicks - are converted into sister-strand exchanges during repair. If ^3H -thymidine induces nicks at random throughout the length of the X chromosome, most of the nicks would be outside the rDNA segment. When the number of lesions is large, the repair component that converts them into sister chromatid exchanges might become saturated. The frequency of magnification would then be reduced if the nicks in the rDNA compete with those outside the rDNA for components of the repair system. In closed chromosomes, loss of rings with unmagnified bb counterbalances the decline in frequency of magnifying events, so that the concentration-dependent inhibition is not observed.

The data are therefore consistent with a model (Endow and Atwood, 1988) in which the basis of magnification is unequal sister chromatid exchange in the rDNA, mediated by an exchange-prone repair system.

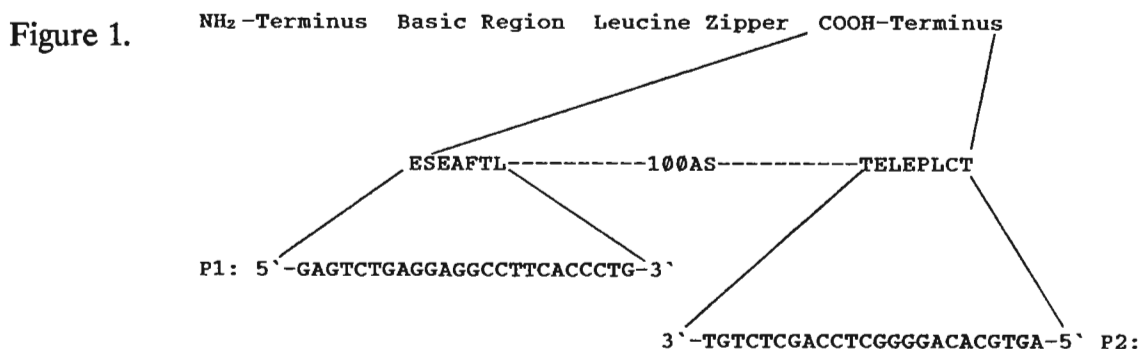
Acknowledgments: This work was carried out at Columbia University.

References: Endow, S.A. and K.C. Atwood 1988, Trends in Genetics 4:348-351; Endow, S.A., D.J. Komma and K.C. Atwood 1984, Genetics 108:969-983; Gibson, D.A. and D.M. Prescott 1972, Exp. Cell Res. 74:397-402; Ritossa, F. 1972, Nature New Biol. 240:109-111; Tartof, K.D. 1974, Proc. Nat. Acad. Sci. USA 71:1272-1276.

Kurzik-Dumke, Ursula¹, Roman Mullenbach², Karlheinz Holzmann², Elisabeth Gateff¹, Nikolaus Blin² and Steven Dooley³. ¹Institut für Genetik, Johannes Gutenberg-Universität Mainz; ²Institut für Anthropologie und Humangenetik, Universität Tübingen; ³Institut für Humangenetik, Universität des Saarlandes, Gebäude 68, D-6650 Homburg/Saar, F.R.G. Genetic mapping of a new *Drosophila fos*-related sequence isolated via PCR technology using mammalian *c-fos* specific primers.

A number of mammalian proto-oncogene homologs have been described in *Drosophila*. Among the early response oncogenes, both *jun*- and *fos*-related were characterized via purification of the encoded proteins from *Drosophila* nuclear extracts using AP1 sequence specific DNA affinity chromatography or by means of low stringency cross-species-hybridization to *Drosophila* genomic libraries (Zhang *et al.*, 1990; Perkins *et al.*, 1990). A computer aided sequence comparison of human, mouse and rat *fos* oncogene sequences revealed several blocks of 20 bp with 100% nucleic acid conservation between these species. These sequences

code for important functional domains of the protein and seem to be subject to a high selection pressure. We used two sequence blocks derived from a part of the human *c-fos* gene coding for the protein domain which is responsible for gene activation via phosphorylation (Barber and Verma, 1987; Wilson and Treisman, 1988; Gins *et al.*, 1990; Ofir *et al.*,

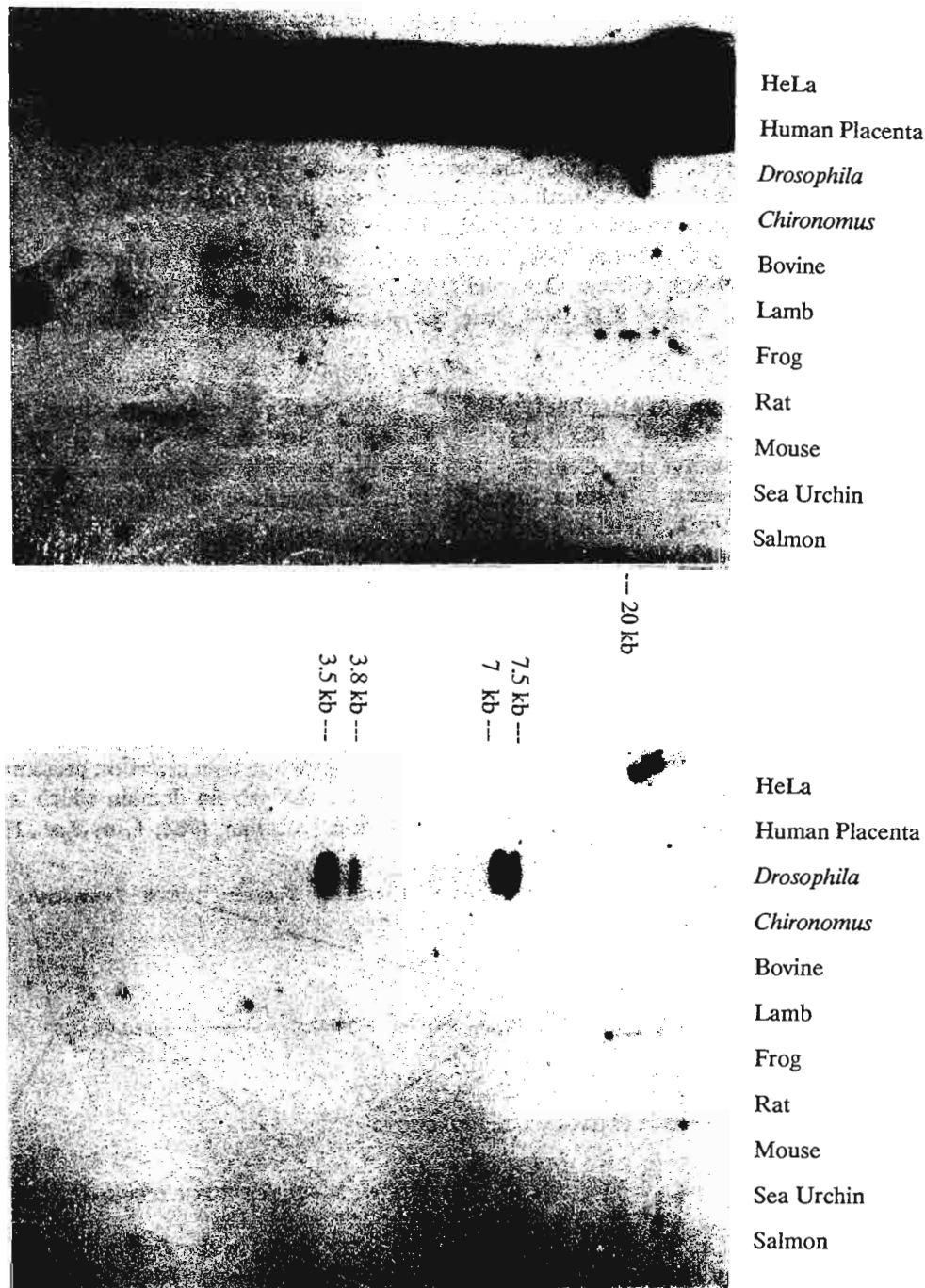


1990) as PCR primers to search for additional members of the *fos*-gene family in the *Drosophila* genome. Figure 1 shows the structure of the mammalian *Fos* protein and the localization of both primers (P1 and P2). Two distinct DNA fragments, 500 bp and 800 bp, were isolated. Figure 2 shows the hybridization at high stringency of the two cloned genomic fragments to a Southern blot of EcoRI-digested DNA from various species. Only *Drosophila*-specific signals in the molecular range indicated in the figure were obtained with both PCR fragments. The 800 bp probe recognized one fragment suggesting a single copy. However, *in situ* hybridization of this DNA fragment to polytene chromosomes, presented in Figure 3, resulted in two signals, at positions 84E and 93F1-2 on the right arm of the third chromosome.

The 500 bp fragment hybridized on a genomic Southern blot to four bands, indicating that this sequence motif is present in more than one genomic region, but clear signals could not be obtained by *in situ* hybridization to squash preparations of giant chromosomes. This can be explained by the restricted capacity of the *in situ* hybridization technique in the case of short DNA sequences.

In order to determine the transcription pattern of the putative genes, RNA from several developmental stages was

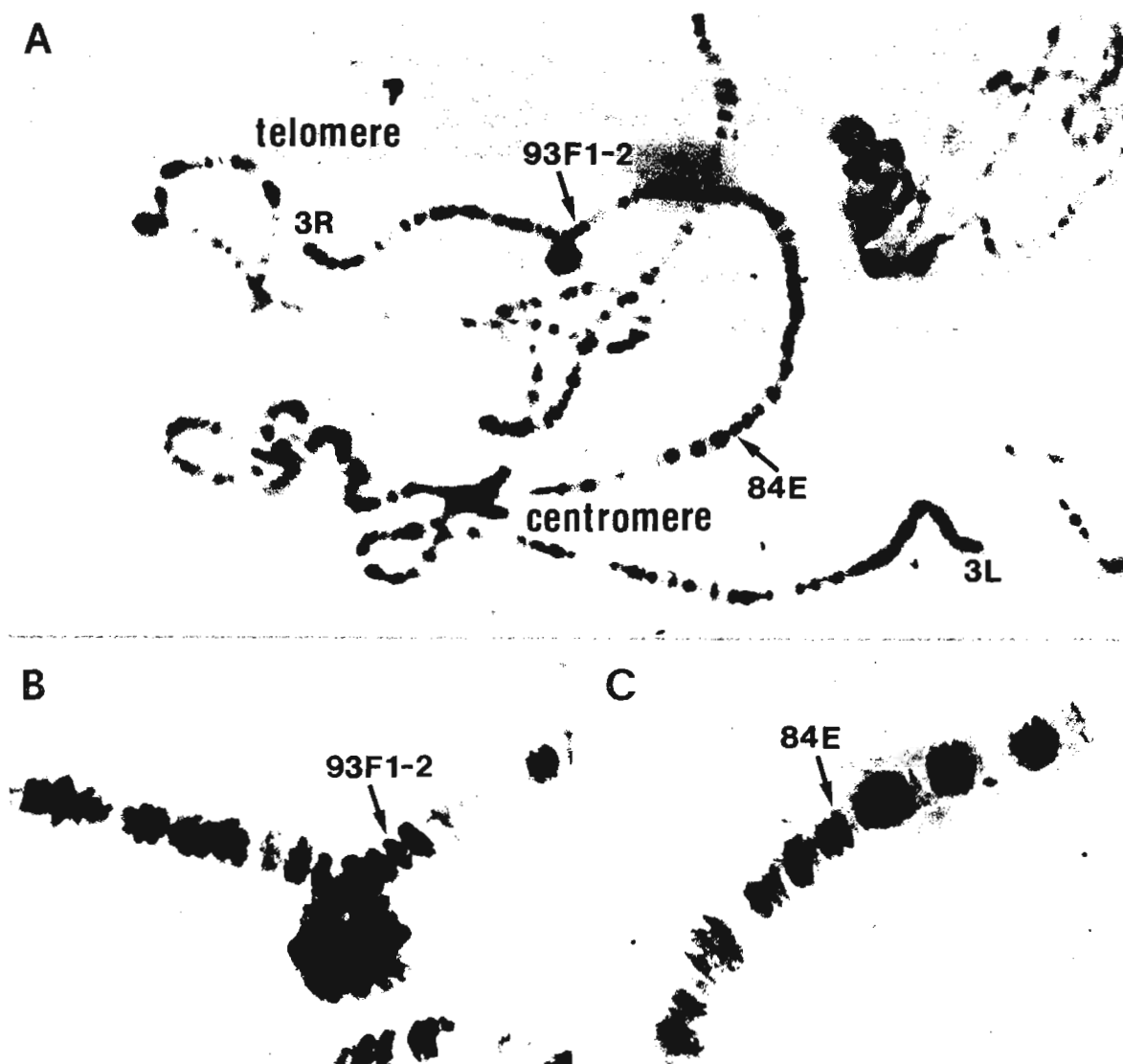
Figure 2.



isolated and hybridized to both isolated DNA fragments. The results of the Northern analysis were negative. Sequence data, however, indicate in the case of the 500 bp probe an open reading frame. The predicted protein is characterized by putative phosphorylation sites for cAMP-dependent protein kinase, protein kinase C (PKC) and casein kinase II (CKII), Asu glycosylation sites and several myristylation sites. So we performed a more sensitive RNA detection analysis using RNA-PCR to find out if there is any basal transcription. Surprisingly, we found a correct sized amplification product in all developmental stages. These data suggest a ubiquitous basal expression of this new gene, whose function remains to be determined.

In both isolated *Drosophila*-specific sequences no homology to the known *fos*-related gene recently characterized by Perkins and co-workers (1990) could be shown.

Figure 3.



References: Barber and Verma 1987, Mol. Cell. Biol. 7(6): 2201-2211; Guis et al., 1990, Mol. Cell. Biol. 10(8): 4243-4255; Ofir et al., 1990, Nature 348: 80-82; Perkins, K.K., A. Admon, N. Patel and R. Tjian 1990, Genes Dev. 4: 822-834; Wilson and Treisman 1988, EMBO J. 7(13): 4193-4202; Zhang K., J.R. Chaillet, L.A. Perkins, T.D. Halazonetis and N. Perrimon 1990, Proc. Natl. Acad. Sci. USA 87: 6281-6285.

Mardahl, Michelle, Richard M. Cripps, Robert R. Rinehart, Sanford I. Bernstein, and Greg L. Harris.
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Introduction of y^+ onto a *CyO* chromosome.

We were interested in introducing a marker visible in the embryo or larva onto a second chromosome balancer, so that we could identify lethal homozygotes. We chose y^+ as the marker to be carried on a *CyO* balanced chromosome. Six hundred virgin females (aged 2-4 days) of the genotype $y; Dp(1;2)E1, pr\ cn/CyO, Cy\ dp^{lv1} pr$ (this *CyO* chromosome does not carry

cn^2) were collected and X-irradiated for a total exposure of 3000 R, then crossed with y males. The progeny were screened for curly-winged individuals with wild-type body color phenotype. Fourteen lines were isolated and backcrossed with y . Two fertile lines maintained y^+ and *CyO*. To confirm that the y^+ marker is stably associated with the balancer chromosome, we crossed $y; CyO, Cy\ dp^{lv1} pr\ y^+ / +$ to y males and scored for independent segregation of

the *Cy* and *y*⁺ phenotypes. We found very few recombinants (1 recombinant in 3,113 total progeny for the line 2-1, and 2 recombinants in 1,818 for line 17). To ensure that the third chromosome does not contain any unintentional genetic lesions, the irradiated third chromosome was removed by standard techniques using *TM3*, and a stable stock was generated with the genotype *y w; In(2LR)O + Dp(1;2)E1, Cy dp^{lv1} pr y⁺/Bc Elp*.

Acknowledgments: We are grateful to Bloomington Stock Center for providing the stock: *y; Dp(1;2)E1, pr cn/CyO, Cy dp^{lv1} pr* and Dr. John Sparrow for his critical comments. Recently another *y*⁺ *CyO* stock has been added to the Bloomington Stock Center.

Khazaeli, A.A., H.H. Fukui, and J.W. Curtsinger.
University of Minnesota, St. Paul, MN U.S.A. Egg and larval densities and survival rates in an inbred line of *Drosophila melanogaster*.

A preliminary experiment in our laboratory using the *Drosophila melanogaster* inbred line JWC.1 showed that the number of flies emerging from different densities of larvae per vial was approximately constant. However, the number of flies emerging from a higher density of eggs was less than that at a lower density. The

aim of this work was to quantify these observations.

The inbred line JWC.1 was derived from a large random-mating laboratory population, "LF350" (Weber and Diggins, 1990). The experiment was carried out in two parts. In the first experiment, eight small cylindrical chambers (100 x 150 mm) were used for egg and larva collections. Petri dishes (100 x 15 mm) containing food were attached to the bottom of the chambers. An equal number of male and female flies (200 + 10) were transferred into each chamber. After 12 hr, flies were removed from the chambers. Eggs were transferred from four petri dishes into 8-dram vials with densities of 20, 40, 60, 80, and 100 (five vials for each density). Four other petri dishes were kept in the laboratory for a period of 24 hr. The first instar larvae were transferred from these petri dishes into the same vials as the egg density classes. In order to compare the performance of the inbred line to those in outbreeding *D. melanogaster*, a similar experiment for was performed using LF350.

A second experiment was done to test for effects of excreted materials which might be toxic to eggs and larvae. Five vials each with 100 eggs (high density) and five other vials each with 20 eggs (low density) were used. After 48 hr, 30 more eggs were transferred into each vials. Due to the gap between the two transfers, primary flies and added flies were distinguishable at eclosion. In both experiments, flies, larvae and eggs were reared and kept on standard yeast-molasses-cornmeal-agar-medium in a dark room at 24°C.

For the first experiment, a model I three-way ANOVA was used to test the effects of density (5 levels) and treatment (2 levels - egg or larvae stages). A block (*n* = 5) design was used to test macro-environmental effects and a nested design (vials were nested within density) was used to test micro-environmental effects. For the second experiment, a model I block design ANOVA was used to test the effect of initial egg density on the survival of added eggs. The proportion of flies emerging was arcsine-square-root-transformed. Means were compared by using the Tukey multiple comparison test at *P* = 0.05 level (Zar, 1984, p 186). The SAS version 5.0 ANOVA procedure (SAS Institute, 1985) was used for actual calculations.

For the first experiment, the result of ANOVA indicated no significant difference between blocks. Thus, data were pooled (Figure 1). However, vial effect was significant (Table 1). In general, the proportion of flies emerging was higher when they were transferred as larvae than as eggs (Figure 1 and Table 1). Densities of larvae did not affect the

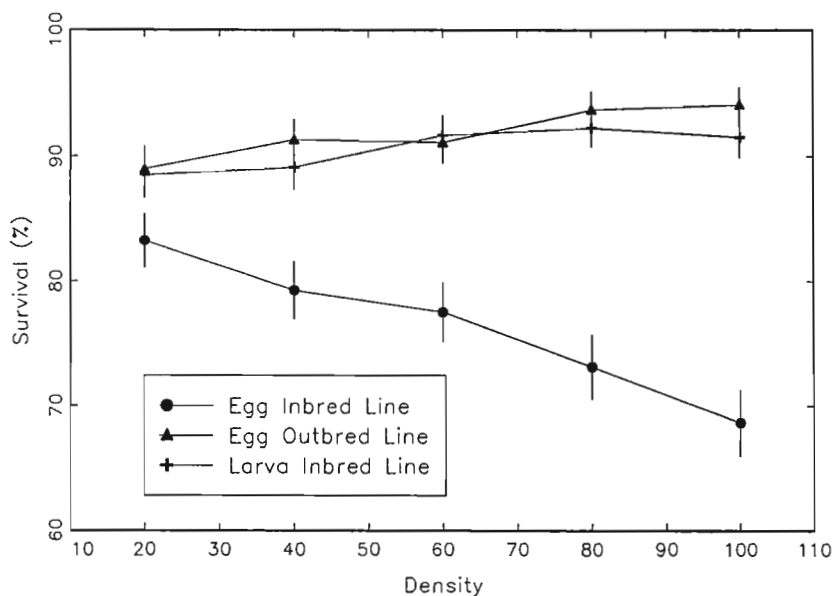


Figure 1. The survival rate of egg and larva at different densities.

proportion of flies emerging from the inbred line ($92.2 \pm 1.5\%$ to $88.5 \pm 1.4\%$; Figure 1). On the other hand, the proportion of flies emerging decreased with density when they were transferred as eggs ($83.3 \pm 2.1\%$ at 20 eggs/vial to $68.8 \pm 1.7\%$ at 100 eggs/vial; Figure 1). Outbred *D. melanogaster*, unlike the inbred line, shows no such variations (Figure 1). This result in the outbred line is in agreement with the finding of Fukui (1990).

Table 1. The result of ANOVA to evaluate the effect of density and treatment

| Source | df | MS | F |
|-----------------------|-----|---------|-----------|
| Model | 55 | 204.24 | 4.18*** |
| Block | 4 | 27.99 | 0.66 |
| Density | 4 | 97.28 | 2.29 |
| Treatment | 1 | 7260.39 | 171.13*** |
| Block*Density | 16 | 33.66 | 0.79 |
| Block*Treatment | 4 | 56.86 | 1.34 |
| Density*Treatment | 4 | 277.67 | 6.54*** |
| Vials(Treatment) | 6 | 191.57 | 4.52*** |
| Three-way-interaction | 16 | 27.88 | 0.66 |
| Error | 114 | 42.43 | |
| Total | 199 | | |
| $R^2 = 0.65$ | | | |

***p < 0.001.

Table 2. An ANOVA testing for effects of initial density on the added eggs.

| Source | df | MS | F |
|---------------|----|--------|----------|
| Model | 3 | 401.36 | 9.16*** |
| Block | 1 | 904.83 | 20.65*** |
| Density | 1 | 202.50 | 5.03* |
| Block*Density | 1 | 78.73 | 1.80 |
| Error | 16 | 43.82 | |
| Total | 19 | | |
| $R^2 = 0.63$ | | | |

* p < 0.05; *** p < 0.001.

Kar, Anita and J.K. Pal. Department of Zoology, University of Poona, Pune 411 007, India. A mutation controlling the decondensation of the male X chromosome.

male X chromosome compensates for the difference in sex-linked gene dosage. Dosage compensation operates through enhanced transcriptive activity of the genes on the haploid X chromosome (Mukherjee and Beermann, 1965). A number of strains have been reported where the male X chromosome becomes abnormally decondensed (see Ashburner, 1972). In male larvae of the strains, *l(3)tl* (Zhimulev *et al.*, 1976), *In(1)B^{m2}* reared at 10°C (Lakhotia and Mishra, 1982) and *In(1)B^{m2}* (reverted) (BM2) (Majumdar *et al.*, 1978), the haploid X chromosome appears more puffy than the female X chromosomes and the paired autosomes. The latter two strains are distinct from *l(3)tl*, since in these strains, the decondensation is sex- and chromosome-specific, affecting only the structure of the male X chromosome (Mukherjee and Ghosh, 1986). Furthermore, these X chromosomes (termed "pompoms"), although abnormally decondensed, show no increase in transcriptive activity which remains at the level observed in normal males (Zhimulev *et al.*, 1976; Lakhotia and Mishra, 1982; Bose and Duttaroy, 1986; Kar and Pal, communicated). Thus, at least in these three instances, no correlation seems to exist between the rate of transcription and the decondensed state of the X chromosome.

We are interested in determining whether there are specific genes that control the decondensation of the X chromosome in *Drosophila* males. In BM2, a recombinationally distinct locus at the 16A region of the X chromosome is responsible for the manifestation of the abnormally puffy X chromosomes (Figure 1a; Kar and Pal, communicated). Introduction of this region into a marker X chromosome results in the manifestation of puffy X chromosomes, which although abnormally decondensed, do not show increased transcriptive activity (Kar and Pal, communicated).

At 18°C, about 50% of the X chromosomes from a single pair of salivary glands of third instar BM2 male larvae

In the second experiment, primary density did not affect survival rate (df = 6, F = 2.03, from an ANOVA analysis). The result is consistent with the first experiment (Figure 1). The mean proportions of flies emerging from eggs transferred after 48 hr into both groups of vials with high and low densities were $79.0 \pm 2.8\%$ and $68.3 \pm 5.9\%$, respectively. Despite significant environmental effects, small but significant effects of initial treatment were found (Table 2). It seems, therefore, that the accumulation of toxic materials, which is density dependent, may be an important factor for survival rate of eggs in this inbred line.

Acknowledgment: This work was supported by NIH grant POIAG08761. We thank L. Xiu for his technical assistance.

References: Fukui, H.H. 1990, Ph.D. Thesis, Bowling Green State University, Bowling Green, Ohio; SAS Institute, Inc., 1985, SAS Users Guide: Statistics, Version 5.0, SAS Institute Inc.; Weber, K.E. and L.T. Diggins 1990, Genetics 125:585-597; Zar, J.H. 1984, Biostatistical Analysis (2nd ed.), Prentice-Hall, Inc., NJ.

The polytene X chromosome of third instar male larvae of *D. melanogaster* appears more diffuse and puffy than the autosomes and the female X chromosomes (Dobzhansky, 1957). This distinctive property of the male X chromosome is the morphological manifestation of dosage compensation, the phenomenon by which the

Figure 1. (a) "Puffy" polytene X chromosome (X) of third instar male larvae from BM2. In this and subsequent panels, bar represents 10 μ m. (b) Puffy X chromosome (X) after 20 minutes of heat shock of BM2 male larvae. Arrow indicates the heat shock puffs 87A and 87C. (c) Narrow chromosome (X) appearing after heat shock of BM2 male larvae.

Table 1. Effect of heat shock on the X chromosome morphotypes of male third instar BM2 larvae

| Duration of heat shock (min) | X chromosome morphotypes ^a \pm S.D. | | | |
|---------------------------------|--|-----------------|-----------------|-----------------|
| | Normal | Puffy | Intermittent | Condensed |
| Control | 0 | 43.3 \pm 10.1 | 24.1 \pm 9.39 | 32.6 \pm 8.05 |
| Experimental | 20 | 51.3 \pm 6.4 | 12.7 \pm 4.03 | 16.2 \pm 3.55 |
| Experimental | 35 | 74.4 \pm 8.5 | 3.4 \pm 1.43 | 13.7 \pm 5.30 |

^aNormal, puffy, intermittent represent the X chromosome morphotypes observed in third instar male larvae. Condensed chromosomes appear, in addition to these morphotypes, in polytene spreads after the larvae are heat shocked.

show complete or random decondensation of the X chromosome (termed "puffy" and "intermittently puffy", respectively), while the remaining X chromosomes appear identical to the morphology observed in polytene spreads from wild type male larvae.

Since the puffy X chromosome resembles a giant puff, we were interested in testing the effect of heat shock on these chromosomes. Third instar BM2 male larvae, reared at 18°C and heat shocked at 37°C for 20 and 35 minutes, show an abnormal response to heat shock. (a) Although a reduction in the number of puffy and intermittently puffy X chromosomes is observed, puffy X chromosomes could be scored after both 20 and 35 minutes of heat shock (Table 1, Figure 1b). (b) In addition to the normal, puffy and intermittently puffy types of X chromosomes, a fourth type of X chromosome becomes evident after heat shock (Figure 1c). These X chromosomes appear reduced in width and are most often characteristically twisted and folded onto themselves as shown in Figure 1c. Polytene chromosome spreads from third instar female larvae do not show such narrow X chromosomes after heat shock. In order to determine whether the narrow X chromosomes result as an effect of heat shock on the mutation in BM2, recombinants bearing a marker X chromosome, in which the *Bar* region was replaced with the 16A region of BM2, were heat shocked. Polytene chromosome spreads from such larvae showed puffy, intermittently puffy, normal and narrow X chromosomes indicating that the narrow X chromosomes appear due to the effect of heat shock on the same mutation that is responsible for the manifestation of the puffy X chromosomes.

From these results it appears that a mutation mapping at the 16A region of the X chromosome of BM2 controls the sex-specific decondensation of the haploid male X chromosome, without affecting its transcriptional activity. We are now using this mutation to determine if the process of transcriptional hyperactivation and X chromosome decondensation are independently regulated processes leading to dosage compensation.

Acknowledgments: A major portion of the work reported here was done in the laboratory of Prof. A.S. Mukherjee. We thank him for his constant guidance and encouragement. CSIR Associateship (grant no. 9/137(192)/91-EMR-I) to AK is duly acknowledged.

References: Dobzhansky, Th. 1957, *Chromosoma* 8: 691;



Mukherjee, A.S. and W. Beerman 1965, *Nature* 207:785; Ashburner, M. 1972, In: *Developmental Studies on Giant Chromosomes* (W. Beermann, ed.), no. 4, pp. 101, Berlin, Springer-Verlag; Zhimulev, I.F. et al. 1987, *Chromosoma* 55:121; Lakhota, S.C. and A. Mishra 1982, *Ind. J. Exp. Biol.* 20:643; Mazumdar, D. et al. 1978, *The Nucleus*; Mukherjee, A.S. and Ghosh, M. 1986, *Genet. Res.* 48:65-75; Bose, D. and A. Duttaroy 1986, *Chromosoma*, 94:87.

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87A and 87C (Figure 1) and 63C, 67F and 67B of 3L (not shown) in polytene chromosome squashes (processed according to Ashburner, 1967) of zero hour prepupae. The SE strain has been reared at 25°C (60% r. humidity) for more than 18 years (191 generations) in culture medium composed of 15 g of agar, 50 g of integral rye flour, 100 g of sugar, 100 g of fresh yeast, 11 ml of propionic acid and 1000 ml of distilled water to which was added 31 mg/kg of sodium selenite. As a control we analysed puffing patterns of polytene chromosomes from prepupae of the Petropolis strain (PE) reared in the same medium without selenium. Both strains (SE and PE) are derived from the offspring of four inseminated females collected in a fruit market at Porto Alegre city, Southern Brazil (29° 10' and 30° 10' S; 51° 16' and 51° 05' W).

Although the phenomenon seems to be transient, since only 1 of the 25 prepupae analysed showed heat shock puffs, it is noticeable the potential of selenium to promote this response. Biological effects of selenium are yet well known. In *Drosophila*, Ting and Walker (1969), Walker and Bradley (1969), and Ahmed and Walker (1975) described alterations in the crossing over distribution along the X chromosome after selenocystein or selenomethionin treatment, and Chapco *et al.* (1978) observed damage effects on fecundity of the flies after the same treatment.

One of us (F.L.) was able to detect incipient reproductive isolation between the SE and PE strains (Lewgoy and Stark, 1992). This fact was probably due to genetic divergence induced by the selection imposed by selenium.

We think that this finding is important because the production of heat shock proteins could be suggested as a means to protect the larvae against the selenium toxic effects. In fact, as the mortality induced by the selenium treatment is high, individual abilities to face toxic agents could be selected. This hypothesis, however, needs to be tested.

Acknowledgments: Thanks are due to CNPq, FAPERGS, FINEP and PROPESP-

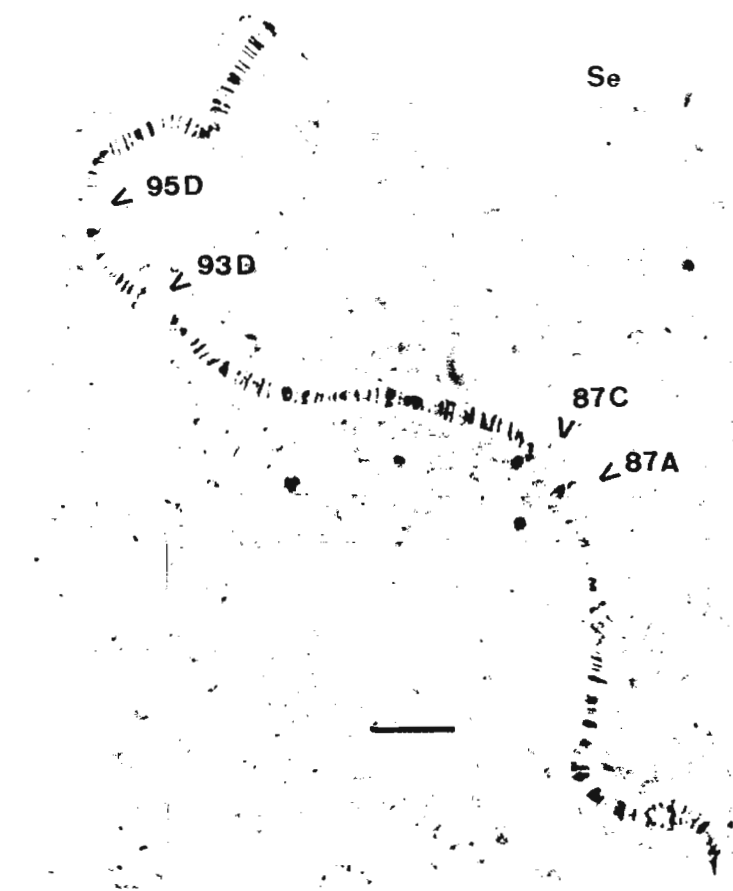


Figure 1. Heat shock puffs induced by selenium in chromosome 3R of *Drosophila melanogaster*, strain SE. Bar = 10 um.

UFRGS for grants.

References: Ahmed, Z.W. and W.R. Walker 1975, Can. J. Gen. Cytol. 17:55-56; Ashburner, M. 1967, Chromosoma 27:47-63; Chapco, W., S.G. Jones, and W.B. McConnel 1978, Can. J. Genet. Cytol. 20:555-565; Lewgoy, F. and A. Stark 1992, Austral. J. Statist. 34:11-17; Lindquist, S. 1986, Ann. Rev. Biochem. 55:1151-1191; Lindquist, S. and E. Craig 1988, Ann. Rev. Genet. 22:631-677; Nover, L. 1991, Heat Shock Response. CRC Press; Ritossa, F. 1962, Experientia, 18:571-573; Ting, K.P. and G.W.R. Walker 1969, Genetics, 61:141-155; Walker, G.W.R. and A.M. Bradley 1969, Can J. Genet. Cytol. 11:677-688.

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Drosophila kikkawai, a subcosmopolitan or a
cosmopolitan species?

There is some disagreement among the authors, concerning the status of *Drosophila kikkawai* around the world. In an attempt to contribute to the elucidation of this problem, we checked the literature on the distribution of this species. We found that Carson (1965) referred to *Drosophila kikkawai* as a species with extensive distribution but subcosmopolitan. Lachaise (1974b) mentioned its presence in Nearctic, Neotropical

and Afrotropical zones. It was cited also by Tsacas (1979) in the Afrotropical, Oriental, Australasian and Neotropical regions, but the author considered *Drosophila kikkawai* as a subcosmopolitan species. Parsons and Stanley (1981) once again considered *Drosophila kikkawai* a subcosmopolitan species, because it is present in five (Palearctic, Neotropical, Australasian, Afrotropical and Oriental) of the six biogeographical zones considered by the authors.

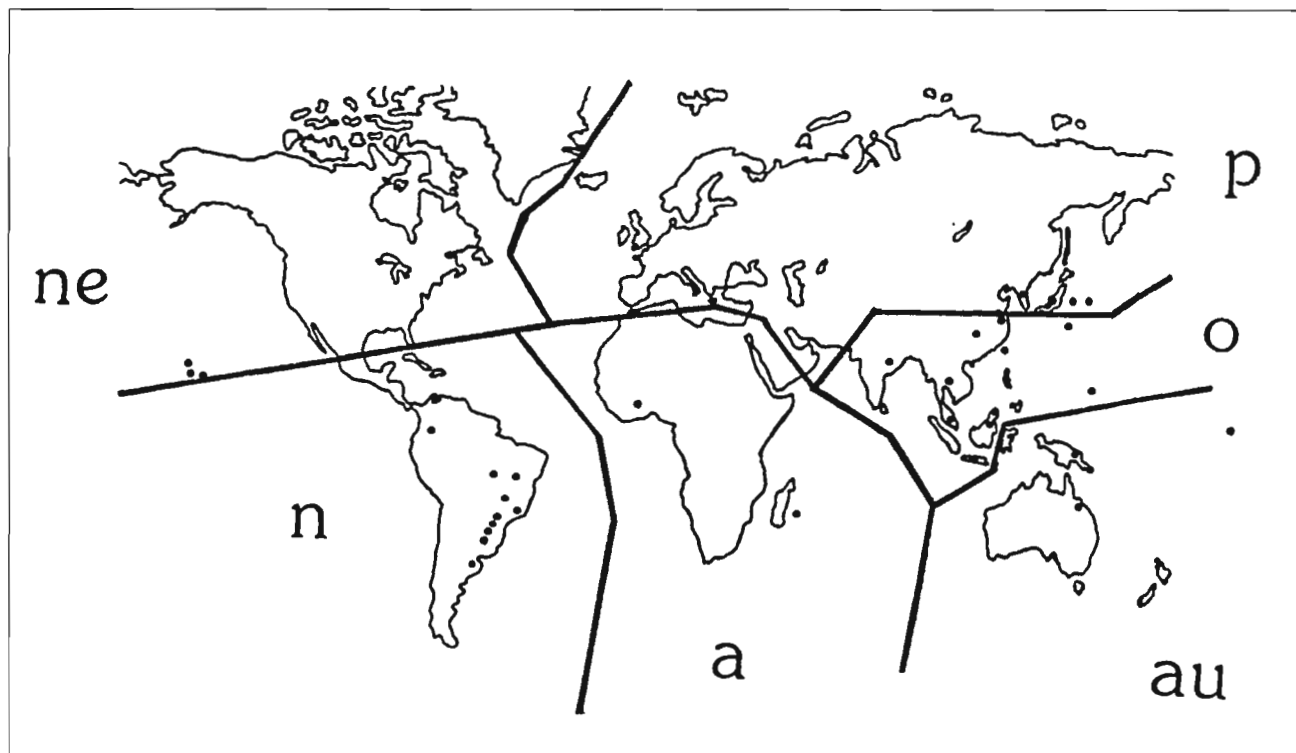


Figure 1 - The distribution of the *Drosophila kikkawai* in the six biogeographic zones.
Ne = Nearctic; N = Neotropical; P = Palearctic; O = Oriental; Au = Australasian; A = Afrotropical

Table 1 (following pages). The geographic situation of these places was obtained in the *Atlas Mirador Internacional* and in the *Guia do Terceiro Mundo*. N = Neotropical; Ne = Nearctic; P = Palearctic; A = Afrotropical; O = Oriental; Au = Australasian.

TABLE 1 - Research on the geographic origin of the samples of *Drosophila kikkawai*.

| AUTHOR, YEAR | GEOGRAPHIC ORIGIN OF THE SAMPLE City/ State/ Country | BIOGEOGRAPHIC REGION | | | | | |
|-----------------------------------|---|----------------------|----|---|---|---|----|
| | | N | Ne | P | A | O | Au |
| Meijere, 1916 | Java | | | | | X | |
| Duda, 1924 | Taiwan/China | | | | | X | |
| | Java | | | | | X | |
| Malloch, 1934 | Samoa | | | | | | X |
| Kikkawa, 1936 | Hukuoka/Japan | | | | X | | |
| | Isigakizima/Japan | | | | X | | |
| | Kumamoto/Japan | | | | X | | |
| | Matuyama/Sikoku/Japan | | | | X | | |
| | Shuri/Okinawa/Japan | | | | X | | |
| | Taihoku/Taiwan | | | | X | | |
| Kikkawa & Peng, 1938 | Amami/Oshima Is./Japan | | | | X | | |
| | Hukuoka/Japan | | | | X | | |
| | Isigakizima/Japan | | | | X | | |
| | Kumamoto/Japan | | | | X | | |
| | Matuyama/Sikoku/Japan | | | | X | | |
| | Miyanozyo/Japan | | | | X | | |
| | Naha/Japan | | | | X | | |
| | Oita/Japan | | | | X | | |
| | Saipan/South-Sea Is. | | | | | X | |
| | Simoda/Japan | | | X | | | |
| | Sintiku/Taiwan | | | X | | | |
| | Taihoku/Taiwan | | | X | | | |
| | Tokuyama/Japan | | | X | | | |
| | Tomioka/Japan | | | X | | | |
| | Yuasa/China | | | | | X | |
| Osima, 1940 | Amakusa/Japan | | | X | | | |
| | Isigakizima/Japan | | | X | | | |
| | Kanabo/Japan | | | X | | | |
| | Matuyama/Sikoku/Japan | | | X | | | |
| | Ningpo/China | | | | | X | |
| | Oita/Japan | | | X | | | |
| | Saipan/South-Sea Is. | | | | | X | |
| | Simoda/Japan | | | X | | | |
| | Yuasa/China | | | | | X | |
| Tan, 1942 | China | | | | | X | |
| Tan & Hsu, 1944 | China | | | | | X | |
| Freire-Maia, 1947; 1948 | Paraná/Brazil | X | | | | | |
| | Rio de Janeiro/Brazil | X | | | | | |
| | São Paulo/Brazil | X | | | | | |
| Pavan & Cunha, 1947 | Paraná/Brazil | X | | | | | |
| | Rio de Janeiro/Brazil | X | | | | | |
| | São Paulo/Brazil | X | | | | | |
| Freire-Maia, 1949a; c; 1952; 1953 | Goiás/Brazil | X | | | | | |
| and Freire-Maia et al, 1954 | Minas Gerais/Brazil | X | | | | | |
| | Paraná/Brazil | X | | | | | |
| | Rio Grande do Sul/Brazil | X | | | | | |
| | Santa Catarina/Brazil | X | | | | | |
| | São Paulo/Brazil | X | | | | | |

TABLE 1 - Research on the geographic origin of the samples of *Drosophila kikkawai*.

| AUTHOR, YEAR | GEOGRAPHIC ORIGIN OF THE SAMPLE City/ State/ Country | BIOGEOGRAPHIC REGION | | | | | |
|---------------------------------|---|----------------------|----|---|---|---|----|
| | | N | Ne | P | A | O | Au |
| Freire-Maia 1949b | Honolulu/Hawaii/USA | | X | | | | |
| | São Paulo/Brazil | X | | | | | |
| Tan <i>et al</i> , 1949 | China | | | | | X | |
| Ward, 1949 | China | | | | | X | |
| Patterson & Stone, 1952 | South America | X | | | | | |
| | Pacific Ocean | | X | | | | |
| | Oriental Region | | | | | X | |
| Burla, 1954 | Gaspar/Santa Catarina/Brazil | X | | | | | |
| | Japan | | | X | | | |
| Okada, 1956 | Japan | | | X | | | |
| Freire-Maia, 1964a | Hawaii/USA | | X | | | | |
| | Paraná/Brazil | X | | | | | |
| | Rio de Janeiro/Brazil | X | | | | | |
| | São Paulo/Brazil | X | | | | | |
| Freire-Maia & Freire-Maia, 1964 | Bahia/Brazil | X | | | | | |
| | Buenos Aires/Argentina | X | | | | | |
| | Hawaii/USA | | X | | | | |
| | Paraná/Brazil | X | | | | | |
| | Rio de Janeiro/Brazil | X | | | | | |
| | São Paulo/Brazil | X | | | | | |
| Mather, 1968a | Jesselton/Sabah/Malaysia | | | | | X | |
| | Tawau/Sabah/Malaysia | | | | | X | |
| Mather, 1968b | Jesselton/Sabah/Malaysia | | | | | X | |
| | Madang/New Guinea | | | | | | X |
| | Tawau/Sabah/Malaysia | | | | | X | |
| Baimai, 1969 | Jesselton/Sabah/Malaysia | | | | | X | |
| | Madang/New Guinea | | | | | | X |
| | Sandakan/Sabah/Malaysia | | | | | X | |
| | Tawau/Sabah/Malaysia | | | | | X | |
| Gupta, 1969 | Jesselton/Sabah/Malaysia | | | | | X | |
| | Madang/New Guinea | | | | | | X |
| | Tawau/Sabah/Malaysia | | | | | X | |
| Baimai, 1970 | Philippines | | | | | X | |
| Mather, 1970 | Philippines | | | | | X | |
| Bock & Wheeler, 1972 | China | | | | | X | |
| Lakhotia & Mukherjee, 1972 | Brazil | X | | | | | |
| Lachaise, 1974a; b; 1975; 1976 | Ivory Coast/Africa | | | | X | | |
| David & Tsacas, 1975 | Reunion Is./Africa | | | | X | | |
| Toda, 1976 | Chichi/Bonin Is. | | | | | X | |
| Belo & Gallo, 1977 | São Paulo/Brazil | X | | | | | |
| Bock, 1977 | Townsville/Australia | | | | | | X |
| Roy & Lakhotia, 1977; 1978 | Brazil | X | | | | | |
| Tsacas & David, 1977 | Brazil | X | | | | | |
| | Republic of Singapore | | | | | X | |
| | Reunion Is./Africa | | | | X | | |
| Belo & Oliveira Filho, 1978 | São Paulo/Brazil | X | | | | | |
| David <i>et al</i> , 1978 | Brazil | X | | | | | |
| | Republic of Singapore | | | | | X | |

TABLE 1 - Research on the geographic origin of the samples of *Drosophila kikkawai*.

| AUTHOR, YEAR | GEOGRAPHIC ORIGIN OF THE SAMPLE City/ State/ Country | BIOGEOGRAPHIC REGION | | | | | |
|-----------------------------|--|----------------------|----|---|---|---|----|
| | | N | Ne | P | A | O | Au |
| | Ivory Coast/Africa | | | | X | | |
| | Reunion Is./Africa | | | | X | | |
| Baimai, 1979a | Bankok/Thailand | | | | | X | |
| | Songkla/Thailand | | | | | X | |
| Kittawee & Baimai, 1979 | Thailand | | | | | X | |
| Singh & Gupta, 1979 | India | | | | | X | |
| Baimai & Chumchong, 1980 | Bankok/Thailand | | | | | X | |
| | Goroka/New Guinea | | | | | | X |
| | Chia-I/Taiwan | | | | | X | |
| | Heron Is./Australia | | | | | | X |
| | Nan Tou/Taiwan | | | | | X | |
| | Peng-Hu Is./Taiwan | | | | | X | |
| | Taipei Is./Taiwan | | | | | X | |
| | Leticia/Colombia | X | | | | | |
| | Luzon/Philippines | | | | | X | |
| | Madang/New Guinea | | | | | | X |
| | Mato Grosso/Brazil | X | | | | | |
| | Amami/Oshima Is./Japan | | | X | | | |
| | Pintung/Taiwan | | | | | X | |
| | Oahu/Hawaii/USA | | X | | | | |
| | Palau/Auluptagel Is. | | | | | X | |
| | Paraná/Brazil | X | | | | | |
| | Kolonia/Ponape | | | | | X | |
| | Samut Songkhrum/Thailand | | | | | X | |
| | São Paulo/Brazil | X | | | | | |
| | Seoul/Korea | | | X | | | |
| | Songkhla/Thailand | | | | | X | |
| | Townsville/Australia | | | | | | X |
| | Wangtakrai/Thailand | | | | | X | |
| | Wau/New Guinea | | | | | | X |
| Baimai <i>et al</i> , 1980 | Bankok/Thailand | | | | | X | |
| | Goroka/New Guinea | | | | | | X |
| | Leticia/Colombia | X | | | | | |
| | Luzon/Philippines | | | | | X | |
| | Mato Grosso/Brazil | X | | | | | |
| | Amami/Oshima Is./Japan | | | X | | | |
| | Pintung/Formosa | | | | | X | |
| | Oahu/Hawaii/USA | | X | | | | |
| | Palau/Auluptagel Is. | | | | | X | |
| | Kolonia/Ponape | | | | | X | |
| | Seoul/Korea | | | X | | | |
| | Townsville/Australia | | | | | | X |
| | Wau/New Guinea | | | | | | X |
| Lakhotia & Mishra, 1980 | Colombia | X | | | | | |
| Singh & Gupta, 1980 | India | | | | | X | |
| Valente <i>et al</i> , 1989 | Porto Alegre/Brazil | X | | | | | |

In the review of the Lemeunier *et al.* (1986), however, which considered only five biogeographical regions, the authors reported the occurrence of *Drosophila kikkawai* in every zone, Oriental, Australasian, Afrotropical, Palearctic and Neotropical.

In order to rule out doubts, we collected data from some of the papers that referred to the origin of the samples of *Drosophila kikkawai*. Table 1 shows all of them in chronological order, considering the existence of six biogeographic zones - Neotropical (N), Nearctic (Ne), Palearctic (P), Afrotropical (A), Oriental (O) and Australasian (Au). The map (Figure 1) summarizes Table 1. Although some of the points plotted on the map are close to the limits of the biogeographic regions, their location, however, is unmistakable. They correspond to the records of the following authors: Freire-Maia (1949b), Patterson and Stone (1952), Freire-Maia (1964a), Freire-Maia and Freire-Maia, (1964), Baimai and Chumchong (1980) and Baimai *et al.* (1980) in the Nearctic zone, and the ones of Kikkawa (1936), Kikkawa and Peng (1938), Osima (1940), Burla (1954), Okada (1956), Baimai and Chumchong (1980) and Baimai *et al.* (1980) in the Palearctic.

Since *Drosophila kikkawai* seems to be present in every zone, we support the hypothesis of the cosmopolitan status of this species.

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Parkash, R. and J.P. Yadav. M.D. University, Rohtak, India. Latitudinal clinal variation in *Zaprionus indianus* populations.

The population genetic studies on Afro-tropical and Indian colonizing populations of *Zaprionus* species are totally lacking. Since gel electrophoresis has helped in elucidating the genetic structure of colonizing populations of diverse taxa, the present studies were

made to analyze the extent of genic divergence at seven loci in colonizing populations of *Z. indianus* from the Indian

Table 1. Distribution patterns of allelic frequencies at seven polymorphic loci in twelve natural populations of *Zaprionus indianus*.

| Locus | Alleles | Ernakulam 10° | Bangalore 12° 58'N | Tirumala 13° 40'N | Hyderabad 17° 20'N | Nagpur 21° 09'N | Bhopal 23° 16'N | Jaipur 26° 55'N | Rohtak 28° 94'N | Roorkee 29° 52'N | Dehradun 30° 19'N | Chandigarh 30° 43'N | Jammu 32° 74'N |
|----------------|---------|------------------|-----------------------|----------------------|-----------------------|--------------------|--------------------|--------------------|--------------------|---------------------|----------------------|------------------------|-------------------|
| <i>Acph-1</i> | F | — | .013 | — | — | — | — | — | — | — | .02 | — | — |
| | F | .69 | .64 | .66 | .61 | .60 | .59 | .52 | .513 | .51 | .49 | .43 | .433 |
| | M | — | — | — | — | — | — | .04 | .02 | .05 | .01 | .03 | .02 |
| | S | .31 | .334 | .34 | .39 | .40 | .41 | .44 | .432 | .43 | .46 | .42 | .494 |
| | S' | — | .013 | — | — | — | — | — | .035 | .01 | .02 | .12 | .053 |
| <i>Acph-2</i> | N | 84 | 184 | 77 | 96 | 90 | 50 | 123 | 155 | 135 | 108 | 98 | 84 |
| | F | .92 | .87 | .89 | .96 | .92 | .89 | .80 | .79 | .81 | .78 | .76 | .75 |
| | S | .08 | .13 | .11 | .04 | .08 | .11 | .20 | .21 | .19 | .22 | .24 | .25 |
| | N | 84 | 120 | 90 | 65 | 90 | 50 | 92 | 80 | 108 | 102 | 64 | 102 |
| <i>Mdh-1</i> | F | .61 | .66 | .68 | .73 | .77 | .75 | .83 | .82 | .84 | .84 | .85 | .88 |
| | S | .39 | .34 | .32 | .27 | .23 | .25 | .17 | .18 | .16 | .16 | .15 | .12 |
| | N | 112 | 100 | 80 | 170 | 98 | 95 | 84 | 62 | 90 | 107 | 109 | 114 |
| <i>Ao</i> | F | .05 | .02 | .06 | .03 | .01 | .02 | .01 | .03 | .04 | .05 | — | .02 |
| | F | .73 | .69 | .70 | .66 | .62 | .60 | .60 | .58 | .56 | .55 | .52 | .52 |
| | S | .22 | .29 | .24 | .31 | .37 | .38 | .39 | .39 | .40 | .40 | .48 | .46 |
| | N | 53 | 105 | 85 | 80 | 72 | 82 | 76 | 74 | 110 | 80 | 50 | 78 |
| <i>Odh</i> | F | .65 | .71 | .73 | .69 | .65 | .70 | .75 | .76 | .75 | .78 | .73 | .63 |
| | S | .35 | .29 | .27 | .31 | .35 | .30 | .25 | .24 | .25 | .22 | .27 | .37 |
| | N | 88 | 84 | 77 | 110 | 120 | 80 | 80 | 90 | 140 | 100 | 88 | 95 |
| <i>Adh</i> | F | .25 | .34 | .36 | .40 | .43 | .45 | .44 | .49 | .48 | .48 | .52 | .55 |
| | S | .75 | .66 | .64 | .60 | .57 | .55 | .56 | .51 | .52 | .52 | .48 | .45 |
| | N | 52 | 132 | 64 | 88 | 43 | 42 | 72 | 95 | 103 | 92 | 54 | 94 |
| α -Gpdh | F | 0 | .03 | .04 | .06 | .04 | .03 | .09 | .12 | .14 | .15 | .20 | .18 |
| | S | 1.0 | .97 | .96 | .94 | .96 | .97 | .91 | .88 | .86 | .85 | .80 | .82 |
| | N | 60 | 85 | 70 | 80 | 98 | 72 | 64 | 68 | 68 | 102 | 47 | 108 |

Latitudes are given below the populations

Table 2. Latitudinal correlation(*r*) of changes in allelic frequencies at seven polymorphic loci in *Z. indianus* populations.

| | <i>Acph-1</i> ^S | <i>Acph-2</i> ^S | <i>Mdh-1</i> ^F | <i>Ao</i> ^S | <i>Odh</i> ^F | <i>Adh</i> ^F | α -Gpdh ^F |
|------------------|----------------------------|----------------------------|---------------------------|------------------------|-------------------------|-------------------------|-----------------------------|
| <i>r</i> -values | .95* | .83 | .98* | .95* | .33 | .95* | .89* |

* Significant at 5% level

Table 3. A comparison of data on mean number of alleles (A), proportion of polymorphic loci (P), observed and expected heterozygosity (*H_o*/*H_e*) in *Z. indianus* and three colonising* sympatric *Drosophila* species from the Indian sub-continent.

| Species | A | P | <i>H_o</i> / <i>H_e</i> |
|-----------------------------|------|------|---|
| <i>Z. indianus</i> (12) | 2.15 | 0.87 | 0.33/ 0.35 |
| <i>D. melanogaster</i> (11) | 1.52 | 0.45 | 0.25/ 0.29 |
| <i>D. busckii</i> (6) | 1.73 | 0.52 | 0.21/ 0.23 |
| <i>D. immigrans</i> (10) | 1.39 | 0.37 | 0.12/ 0.11 |

No. of populations given in parenthesis. *based on published and unpublished data of author's laboratory.

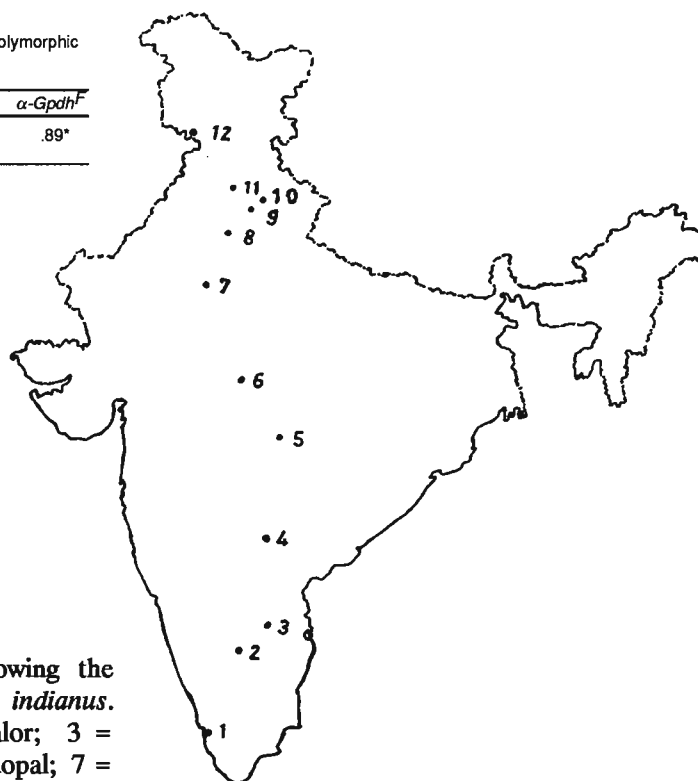


Figure 1. Map of Indian subcontinent showing the collection sites for wild caught individuals of *Z. indianus*. The sites include: 1 = Ernakulam; 2 = Bangalore; 3 = Tirumala; 4 = Hyderabad; 5 = Nagpur; 6 = Bhopal; 7 = Jaipur; 8 = Rohtak; 9 = Roorkee; 10 = Dehradun; 11 = Chandigarh; 12 = Jammu.

subcontinent. *Z. indianus* represents a genus related to *Drosophila* and occurs widely in the Afro-tropical regions as well as the Indian subcontinent. *Z. indianus* was described from India and could be collected almost throughout the Indian subcontinent (Gupta, 1970). The population samples of *Z. indianus* were bait-trapped from twelve latitudinally varying sites (Figure 1). Wild-caught males, as well as F_1 individuals from isofemale lines of *Z. indianus*, were analyzed

electrophoretically (Smith, 1976). The homogenates of single individuals were analyzed electrophoretically in 12% starch gels at 250 V, 30 mA at 4°C for four hours. The gel slices were stained for six different gene-enzyme systems (Harris and Hopkinson, 1976). The genetic basis of banding patterns was interpreted from the segregation ratios of electrophoretic phenotypes of the parents as well as F_1 and F_2 progeny of several genetic crosses.

The data on the distribution of allelic frequencies of the seven polymorphic loci have been given in Table 1. The *Acph* locus is represented by two common alleles in addition to a few rare alleles in different populations. The *Acph-2* and *alpha-Gpdh* loci revealed one common and one rare allele in the southern populations, while one common and one less frequent allele were found in the northern populations. *Mdh-1*, *Adh* and *Odh* loci revealed two common alleles in all the populations, while the *Ao* locus revealed two common and one rare allele.

The electrophoretic data revealed that the allelic frequencies of *Acph-1^S*, *Acph-2^S*, *Mdh-1^F*, *Ao^S*, *Adh^F*, and *alpha-Gpdh^F* were found to be significantly positively correlated with the latitude (Table 2). The observed clinal variation in the geographic populations of *Z. indianus* suggested adaptive changes in allelic frequencies along the climatic and ecogeographic north-south gradient of the Indian subcontinent. The data on the overall genetic indices (*A*, *P* and *Ho/He*) in all the geographic populations of *Z. indianus* were characterized by a higher amount of genic polymorphism, i.e. all the loci revealed polymorphism and displayed a high heterozygosity. The extent of genic polymorphism in *Z. indianus* populations was significantly higher, as compared to the levels observed in other colonizing *Drosophila* species (Table 3).

The present data on clinal variation at polymorphic loci in Indian populations of *Z. indianus* further supported and validated the hypothesis that the occurrence of latitudinal clines across different drosophilid populations provides evidence of natural selection maintaining clinal allozymic variation. Since various colonizing drosophilids differ significantly in their evolutionary history, the existence of parallel clinal allelic frequency changes at many polymorphic loci could be due to the action of latitudinally related environmental gradients.

The present observations on the patterns of extensive genic polymorphism in geographic populations of *Z. indianus* were in agreement with the niche-width variation hypothesis or the environmental amplitude hypothesis (Karin and Guttman, 1979; Steiner, 1980; Parsons, 1983). This hypothesis suggested that the amount of genetic variation could be regarded as an adaptive strategy in spatio-temporally varying heterogeneous environments. *Z. indianus* populations are found throughout the year in climatically diverse geographic regions of the Indian subcontinent. Thus, the observed high levels of genetic indices in *Z. indianus* populations could be causally related with the heterogeneous environmental conditions along north-south axis of Indian sub-continent.

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Yadav, J.P. and R. Parkash. M.D. University, Rohtak, India. Ethanol tolerance and ADH gene frequencies in Indian populations of *Zaprionus indianus*.

The natural food resources of most drosophilids consist of fermenting fruits. Since the larvae are often physically immersed in such media, they are required to cope with short chain alcohols at various concentrations (Hoffman, 1984; Chambers, 1988; David, 1988; Geer et

al., 1989). *Z. indianus* populations were found to exploit a variety of fermenting fruits in nature, and thus resembled *D. melanogaster*. Since ADH is known to be involved in the utilization and detoxification of exogenous alcohols (Chambers, 1988; David et al., 1986), the present studies were made to analyze the extent of ethanol tolerance in geographic populations of *Z. indianus*.

The data on ethanol utilization, ethanol tolerance, as well as *Adh^F* gene frequencies of five geographic populations of *Z. indianus* are given in Table 1. The effect of ethanol vapors in a closed system on the longevity has been expressed as LD_{50} hrs. The south Indian population of Bangalore revealed minimum increase in longevity (48 hrs) as compared with a higher increase (264 hrs) in a Dehradun population when adult individuals were exposed to 4% ethanol. The other three populations (Hyderabad, Bhopal and Chandigarh) revealed intermediate responses. A similar trend has been reflected by LT_{50} max./ LT_{50} control data, i.e. the *Z. indianus* populations along the north-south axis varied in the range of 0.8 to 5.28. The LC_{50} ethanol concentrations were calculated from the mortality curve at different ethanol concentrations (Table 1). The LC_{50} values revealed clinal variations in the range of 4% to 8.5% ethanol, i.e. southern populations depicted lower ethanol tolerance as compared with the northern populations. The data on larval ethanol preference behavior towards a range of ethanol concentrations (1% to 15%) are given in Table 1. The larval individuals

of species populations of *Z. indianus* revealed higher ethanol tolerance than those of adults, but the patterns of clinal variation were found to be similar both for adult as well as larval stages.

The ethanol tolerance and allelic frequencies at the *Adh* locus in the Indian geographic populations of *Z. indianus* revealed a clinal pattern, i.e. an increase in the frequency of *Adh^F* alleles as well as ethanol tolerance with increasing latitude (Figure 1). The ethanol tolerance levels were found to be parallel in both the larval and adult stages, thus, suggesting that in nature the larval stage could be an effective target of natural selection. The statistical correlations were found to be significantly high among larval as well as adult ethanol tolerance versus *Adh^F* allelic frequency (Table 2).

Table 1. Data on ethanol tolerance indices (adult and larval ethanol threshold concentrations), LC₅₀ ethanol concentration, increase in longevity (LD₅₀ hours and LT₅₀ maximum/LT₅₀ control) and *Adh^F* frequency in five natural populations of *Z. indianus*.

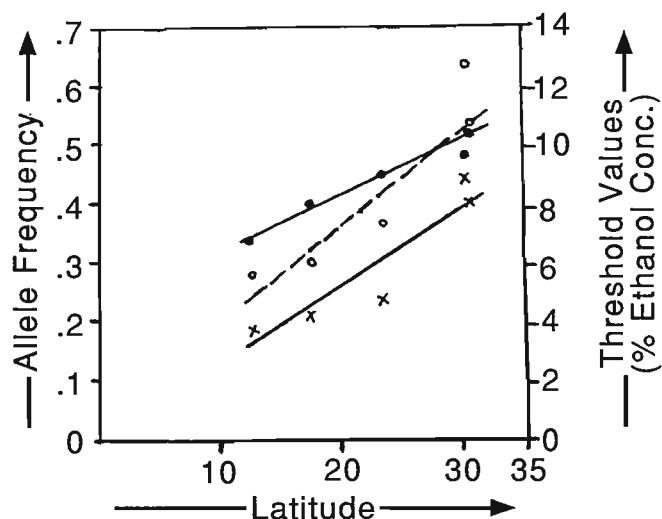
| Populations | Ethanol conc. threshold values (in %) | | LC ₅₀ ethanol conc. (in %) | Increase in longevity at 4 % ethanol conc. | | Allele Freq. <i>Adh^F</i> |
|-------------------------|---------------------------------------|--------|---------------------------------------|--|---|-------------------------------------|
| | Adult | Larvae | | LD ₅₀ hrs | LT ₅₀ max / LT ₅₀ control | |
| Chandigarh (30° . 43'N) | 8.0 | 10.6 | 7.6 | 192 | 3.0 | 0.52 |
| Dehradun (30° . 19'N) | 8.8 | 12.7 | 8.5 | 264 | 5.28 | 0.48 |
| Bhopal (23° . 16'N) | 4.7 | 7.4 | 6.6 | 101 | 1.40 | 0.45 |
| Hyderabad (17° . 20'N) | 4.2 | 6.0 | 6.0 | 72 | 1.06 | 0.40 |
| Bangalore (12° . 58'N) | 3.8 | 5.6 | 4.0 | 48 | 0.8 | 0.34 |

Table 2. Correlation coefficient (r) values between latitudes and biological variables (*Adh^F* frequency and ethanol tolerance) in populations of *Zapionus indianus*.

| Parameter | r-values |
|--|----------|
| Latitude versus <i>Adh^F</i> | .974* |
| Latitude versus ethanol tolerance (adult) | .928* |
| Latitude versus ethanol tolerance (larvae) | .929* |
| Ethanol tolerance (adult) versus <i>Adh^F</i> | .844* |
| Ethanol tolerance (larval) versus <i>Adh^F</i> | .827* |
| Ethanol tolerance, adult versus larval | .988* |

*Significant at 5% level.

Figure 1. Latitudinal relationship of *ADH^F* allelic frequency (solid line, solid circles), ethanol threshold concentrations for adult individuals (solid line, crosses), as well as larvae (dotted line, open circles) of different geographical populations of *Z. indianus*.



The present observations on clinal variation at the *Adh* locus in *Z. indianus* validate and support the hypothesis that occurrences of parallel or complementary latitudinal clines among different colonizing drosophilids, as well as across continents, provide strong evidence of natural selection maintaining such allozymic variation (Jones, 1980; Lemeunier *et al.*, 1986; Chambers, 1988). The geographic populations of *Z. indianus* revealed significant genetic divergence in their potential to utilize ethanol. The adult longevity periods were found to increase significantly at 1% to 4% for south Indian populations and 1% to 8% for north Indian populations of *Z. indianus*. The ethanol threshold values were found to vary clinally in the range of 3.8% to 8.8% in case of adults and 5.6% to 12.7% for larvae in the geographic populations of *Z. indianus* from south to north localities. The LC₅₀ values revealed clinal variation in the range of 4% to 8.5% ethanol, i.e. southern populations depicted lower ethanol tolerance as compared with the northern populations. The larval individuals of *Z. indianus* populations revealed higher ethanol tolerance than those of adults, but the pattern of clinal variation was found to be similar for both the adult and larval stages. The ethanol utilization indices, as well as ethanol tolerance threshold values, in larval and adult individuals were found to vary latitudinally in different Indian populations of *Z. indianus*.

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Yadav, J.P. and R. Parkash. M.D. University, Rohtak, India. Esterase variability in *Zapionus indianus* populations.

Electrophoretic analysis of genetic structure of some colonizing species had helped in elucidating the genetic potential for colonizing as well as in understanding biogeographical origin of such species (Endler, 1986). However, such studies have not been

attempted on the colonizing drosophilids of the Indian subcontinent. *Zapionus indianus* constitutes one of the most successful colonizing species of the Indian subcontinent. It had been argued that the genus *Zapionus* evolved from close to the *immigrans* species group radiation and that various *Zapionus* species might have originated in the Afro-tropical continent and later on colonized other tropical continents such as India (Throckmorton, 1975; Tsacas *et al.*, 1981). The present studies were made to analyze the extent of genic divergence at esterase loci in colonizing populations of *Z. indianus* collected from twelve latitudinally varying sites from the Indian subcontinent.

Figure 1. Starch gel electrophoretic patterns of polymorphic esterases in homogenates of single individuals of *Zapionus indianus*. Electrophoretic data on segregation behavior of esterase banding patterns in parents and progenies of genetic crosses for different populations are shown in A to C. The starch gel electropherograms (D to I) depict esterase polymorphisms in isofemale lines derived from natural population samples. *Est-1* and *Est-2* zones are characterized by single and triplet bands of varying mobilities while other zones depict single bands and two-banded patterns. Three esterase zones (*Est-1*, *Est-3* and *Est-7*) are often represented by low intensity bands as compared to other EST zones. The origin as well as direction of migration of esterases (from cathode to anode) have been shown in all the gels.



Gel slices stained for esterases have revealed seven polymorphic zones of activity. The electrophoretic phenotypes of different esterase zones have been depicted in Figure 1. The data on the distribution of allelic frequencies of esterase polymorphic loci in twelve natural populations of *Z. indianus* have been given in Table 1. All seven polymorphic esterase loci revealed significant variation in allelic frequency in the geographical populations of *Z. indianus*. The esterase-1 locus showed two common alleles in south Indian populations and one frequent and one rare allele in northern populations. The *Est-2* locus revealed two common alleles in all the geographical populations in addition to one or two rare alleles. Three esterase loci (*Est-3*, *Est-4*, and *Est-6*) depicted two common alleles in all of the populations. *Est-5* and *Est-7* loci revealed one common and one rare allele in the southern populations while the northern populations showed two common alleles. In order to find out the relationship between changes in gene frequencies and latitude, correlation coefficients (r) were calculated and are given in Table 2. The frequencies of *Est-1*^F, *Est-2*^S, *Est-3*^S, *Est-4*^S, *Est-5*^S, *Est-6*^S, *Est-7*^S showed positive correlations with the latitude. Thus, significant clinal variation patterns were observed at all of the *Est* loci except *Est-2*.

The twelve populations of *Z. indianus* sampled along the 22° latitudinal (north-south) axis of the Indian

subcontinent differ in their ecogeographic conditions, such as extent of precipitation, day length, temperature, and humidity. Various geographical populations of *Z. indianus* do experience gene flow and are adapted to local ecogeographic factors. The southern populations of *Z. indianus* have revealed significant allelic frequency differences at all seven *Est* loci when compared with the northern populations. Since the genetic background of the different Indian colonizing populations of *Z. indianus* could be assumed to be similar, the observed genic divergence at seven *Est* loci might be due to the geographic environmental gradient along the north-south axis of this country. Since gene flow is expected to influence allelic frequency changes in an identical manner at all the esterase polymorphic loci, the observed patterns of geographic differentiation in *Z. indianus* can not be explained on the basis of gene flow (Wills, 1980; Spiess, 1989).

Table 1. Data on the distribution of allelic frequencies at various polymorphic esterases loci in twelve natural populations of *Zaprionus indianus*.

| Locus | Alleles | Ernakulam 10° | Bangalore 12°58'N | Tirumala 13°40'N | Hyderabad 17°20'N | Nagpur 21°09'N | Bhopal 23°16'N | Jaipur 26°55'N | Rohtak 28°94'N | Roorkee 29°52'N | Dehradun 30°19'N | Chandigarh 30°43'N | Jammu 32°74'N |
|-------|---------|------------------|----------------------|---------------------|----------------------|-------------------|-------------------|-------------------|-------------------|--------------------|---------------------|-----------------------|------------------|
| EST-1 | F | .71 | .77 | .79 | .76 | .74 | .78 | .83 | .90 | .92 | .93 | .92 | .95 |
| | S | .29 | .23 | .21 | .24 | .26 | .22 | .17 | .10 | .08 | .07 | .08 | .05 |
| | N | 57 | 55 | 63 | 72 | 96 | 72 | 84 | 68 | 88 | 86 | 80 | 83 |
| EST-2 | F | — | — | — | — | — | — | — | .02 | .04 | .03 | .03 | — |
| | M | .61 | .57 | .63 | .67 | .64 | .66 | .58 | .48 | .47 | .49 | .44 | .39 |
| | S | — | .07 | .11 | .04 | .05 | — | .02 | .06 | .09 | .05 | .12 | .19 |
| | S' | .39 | .36 | .26 | .29 | .31 | .34 | .40 | .42 | .38 | .43 | .38 | .38 |
| | N | 57 | 86 | 64 | 76 | 90 | 57 | 72 | 113 | 88 | 86 | 80 | 83 |
| EST-3 | F | .82 | .78 | .79 | .72 | .80 | .74 | .70 | .65 | .67 | .68 | .61 | .60 |
| | S | .18 | .22 | .21 | .28 | .20 | .26 | .30 | .35 | .33 | .32 | .39 | .40 |
| | N | 44 | 56 | 78 | 60 | 70 | 57 | 50 | 54 | 88 | 86 | 80 | 83 |
| EST-4 | F | .67 | .68 | .70 | .65 | .66 | .68 | .71 | .73 | .75 | .78 | .76 | .77 |
| | S | .33 | .32 | .30 | .35 | .34 | .32 | .29 | .27 | .25 | .22 | .24 | .23 |
| | N | 57 | 66 | 60 | 76 | 72 | 57 | 50 | 54 | 88 | 86 | 80 | 83 |
| EST-5 | F | 1.0 | .92 | .93 | .87 | .77 | .75 | .65 | .59 | .57 | .60 | .56 | .54 |
| | S | — | .08 | .07 | .13 | .23 | .25 | .35 | .41 | .43 | .40 | .44 | .46 |
| | N | 57 | 64 | 42 | 69 | 65 | 57 | 60 | 68 | 88 | 86 | 80 | 83 |
| EST-6 | F | .65 | .68 | .66 | .60 | .55 | .53 | .50 | .48 | .46 | .44 | .42 | .40 |
| | S | .35 | .32 | .34 | .40 | .45 | .47 | .50 | .52 | .54 | .56 | .58 | .60 |
| | N | 57 | 64 | 60 | 69 | 65 | 57 | 60 | 68 | 88 | 88 | 80 | 83 |
| EST-7 | F | 1.0 | .97 | 1.0 | .96 | .92 | .91 | .88 | .86 | .85 | .83 | .84 | .81 |
| | S | — | .03 | — | .04 | .08 | .09 | .12 | .14 | .15 | .17 | .16 | .19 |
| | N | 57 | 60 | 60 | 70 | 65 | 57 | 60 | 68 | 88 | 86 | 80 | 83 |

F, F, M, S and S' represent faster, fast, medium, slow and slower electromorphs respectively. N = sample size.

Table 2. Latitudinal correlation(*r*) of esterase allelic frequencies in *Z. indianus* populations.

| | Est-1 ^F | Est-2 ^S | Est-3 ^S | Est-4 ^F | Est-5 ^S | Est-6 ^S | Est-7 ^S |
|------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| <i>r</i> -values | .89* | .55 | .89* | .81* | .99* | .98* | .98* |

* Significant at 5% level

The observed patterns of latitudinal clinal variation at the seven *Est* loci in Indian populations of *Z. indianus* could be explained on the basis of natural selection mechanisms. The occurrence of equilibrium clines results from the action of natural

selection that causes clinal variation in gene frequencies along a continuously varying environmental gradient (Endler, 1986). The steepness of such clinal variation depends upon the range of environmental gradient. The observed latitudinal clines and gene frequency changes at all the esterase loci in Indian populations of *Z. indianus* revealed statistically significant correlations with latitude. Thus, it may be argued that observed geographical variation at *Est* allozymic loci seem to represent equilibrium or adaptive gene clines under the dictates of spatially varying natural selective pressures (Singh and Rhomberg, 1987; Jiang *et al.*, 1989; Spiess, 1989).

References: Endler, J.A. 1986, *Natural Selection in the Wild*, N.J.: Princeton University Press; Throckmorton, L.H. 1975, The phylogeny, ecology and geography of *Drosophila*, In: *Handbook of Genetics*, pp. 421-469, R.C. King (ed.), New York: Plenum Press; Tsacas, L., D. Lachaise and J.R. David 1981, In: *The Genetics and Biology of Drosophila*, pp. 1-97, M. Ashburner, H.L. Carson and J.N. Thompson, jr. (eds.), New York: Academic Press; Wills, C. 1981, *Genetic Variability*, Oxford: Clarendon Press; Singh, R.S. and L.R. Rhomberg 1987, *Genetics* 117:255-271; Jiang, C., J.B. Gibson and H. Chen 1989, *Heredity* 62:193-198.

Parkash, R., Indu Sharma and Neena, Biosciences Department, M.D. University, Rohtak, India. Enzyme polymorphism in *Drosophila melanogaster*.

Several investigations have been made on the electrophoretic analysis of the cosmopolitan sibling species *D. melanogaster* and *D. simulans* populations from various temperate regions of the world (Girard *et al.*, 1977; Singh *et al.*, 1982). On the contrary, there are

no reports on the electrophoretic analysis of genic variations occurring in *D. melanogaster* populations of the Indian subcontinent. The present report describes the patterns of genic variations for seven gene-enzyme systems in wild populations of *D. melanogaster*.

Table 1. Data on distribution of observed and expected genotypes, sample size, allelic frequencies, heterozygosities (observed / expected), effective number of alleles (n_e), Wright's coefficients (f) and G-values for fit to Hardy-Weinberg expectations at seven polymorphic loci in a wild population of *Drosophila melanogaster*.

| | Observed / expected genotypes | | | Sample Size | Allelic frequency | | Heterozygosity obs. / exp. | n_e | f | G-values |
|----------------|-------------------------------|--------------|--------------|-------------|-------------------|------|-------------------------------|-------|-------|----------|
| | SS | FF | FS | | S | F | | | | |
| APH-3 | 8.0 / 11.36 | 38.0 / 41.31 | 50.0 / 43.33 | 96 | .34 | .66 | .52 / .45 | 1.82 | -0.15 | 2.36 |
| EST-6 | 98.0 / 95.4 | 5.0 / 2.13 | 23.0 / 28.5 | 126 | .87 | .13 | .18 / .23 | 1.30 | 0.19 | 4.00* |
| ADH | 8.0 / 7.30 | 60.0 / 59.14 | 40.0 / 41.56 | 108 | .26 | .74 | .37 / .38 | 1.62 | 0.03 | 0.14 |
| ODH | 14.0 / 6.48 | 93.0 / 85.44 | 32.0 / 47.08 | 139 | .216 | .784 | .23 / .34 | 1.51 | 0.32 | 12.62* |
| AO | 7.0 / 2.49 | 98.0 / 93.93 | 22.0 / 30.58 | 127 | .14 | .86 | .17 / .24 | 1.31 | 0.28 | 8.29* |
| α -GPDH | 10.0 / 8.12 | 75.0 / 73.13 | 45.0 / 48.75 | 130 | .25 | .75 | .34 / .37 | 1.57 | 0.08 | 0.83 |
| MDH-1 | 96.0 / 96.87 | 0.0 / 0.55 | 16.0 / 14.58 | 112 | .93 | .07 | .14 / .13 | 1.14 | -0.07 | 1.24 |

* Significant at 5% level

Table 2. Comparison of data on the allelic frequencies at seven polymorphic loci in *D. melanogaster* populations.

| Populations* | EST-6 | | APH-3 | | ADH | | α -GPDH | | ODH | | AO | | MDH | |
|-----------------------------------|-------|------|-------|------|------|------|----------------|------|------|------|------|------|------|------|
| | F | S | F | S | F | S | F | S | F | S | F | S | F | S |
| 1. Canada (Hamilton) (43.3° N) | 0.54 | 0.46 | 1.0 | — | 0.76 | 0.24 | 0.81 | 0.19 | 0.93 | 0.07 | 0.89 | 0.11 | — | 1.0 |
| 2. U.S.A. (Texas) (25.8° N) | 0.64 | 0.36 | 0.98 | 0.02 | 0.14 | 0.86 | 0.68 | 0.32 | 0.62 | 0.38 | 0.50 | ** | — | 1.0 |
| 3. West Africa (6.3° N) | 0.63 | 0.37 | 0.98 | 0.02 | 0.03 | 0.97 | 1.0 | — | 0.87 | 0.13 | ** | 0.57 | 0.14 | 0.86 |
| 4. Vietnam (11° N) | 0.77 | 0.23 | 0.94 | 0.06 | 1.0 | — | 0.77 | 0.23 | 1.0 | — | 0.77 | 0.23 | — | 1.0 |
| 5. India (28.94° N) | 0.73 | 0.27 | 0.66 | 0.34 | 0.74 | 0.26 | 0.75 | 0.25 | 0.78 | 0.22 | 0.86 | 0.14 | 0.07 | 0.93 |

* Data on population 1 to 4 belongs to Singh *et al.* (1982)⁶ while population 5 refers to the present study. F & S refer to fast and slow electromorphs / allozymes or allelic variants. ** refer to many other alleles at this locus.

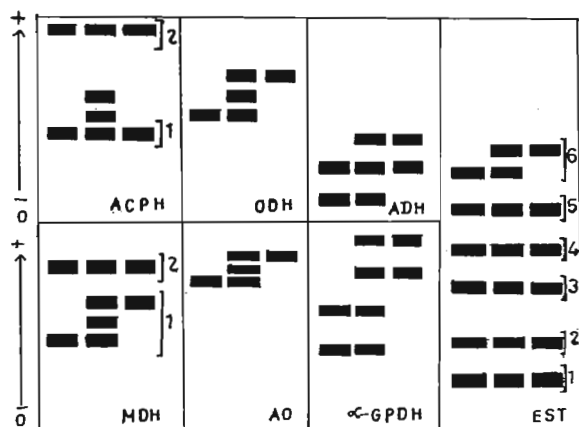


Figure 1. Schematic representation of electrophoretic phenotypes of seven gene-enzyme systems in single individuals of *Drosophila melanogaster*. Single-banded and three-banded phenotypes (APH, MDH, AO and ODH) represent homozygous and heterozygous genotypes, respectively. Two-banded ADH and α -GPDH phenotypes represent homozygous genotypes. Arrow indicates the direction of current flow.

Electrophoretic phenotypes of seven gene-enzyme systems in single individuals of *D. melanogaster* have been represented in Figure 1. The data on the distribution of enzyme genotypes, allelic frequencies, observed and expected heterozygosity, Wright's coefficient (F), and log-likelihood X^2 test for fit to Hardy-Weinberg expectations at the seven loci are given in Table 1. The range of heterozygosities observed at the various loci correlate well with the incidence of

number as well as allelic frequency distribution patterns. The positive and negative values of the Wright's index (F) indicate deficiency and excess of heterozygotes, respectively. Data in Table 1 reveal that the population deviates from Hardy-Weinberg equilibrium at *Est-6*, *Odh* and *AO* loci. Electrophoretic analysis of the *Mdh* locus in *D. melanogaster* has revealed the occurrence of one most common allele (0.93) and one rare allele (0.07), while each of the other six loci are represented by two frequent alleles.

The incidence of the frequent allele at most of the polymorphic loci is also in agreement with other studies, but the allelic frequency patterns are not in agreement with those of the populations of temperate regions (Table 2). Such differences in allelic frequency patterns at some loci (*Adh* and *alpha-Gpdh*) could be argued on the basis of earlier reported evidence of the action of temperature as an agent of natural selection (Oakeshott *et al.*, 1982; Alahiotis *et al.*, 1977). The observed low level of genic polymorphism at the *Mdh* locus concurs with the functional constraint hypothesis that the glucose metabolizing enzymes which are involved in crucial metabolizing pathways are least variant when compared to non-glucose-metabolizing enzymes (Harris and Hopkinson, 1976; Ferguson, 1980). Present studies need to be extended to several ecogeographical populations of *D. melanogaster*.

References: Girard, P., L. Palabost and C. Petit 1977, *Biochem. Genet.* 15:589; Singh, R.S., D.A. Hickey and J.R. David 1982, *Genetics* 101:235; Harris, H. and D.A. Hopkinson 1976, *Handbook of Enzyme Electrophoresis in Human Genetics*, Amsterdam: North Holland; Ferguson, A. 1980, *Biochemical Systematics and Evolution*, New York: Wiley; Oakeshott, J.G., J.B. Gibson, P.R. Anderson, W.R. Knibb, D.G. Anderson and G.K. Chambers 1982, *Evolution* 36:86; Alahiotis, S., S. Miller and E. Berger 1977, *Nature* 269:144.

Parkash, R., Neena and Indu Sharma. Biosciences Department, M.D. University, Rohtak, India. Esterase polymorphism in some *Drosophila* species.

The multifunctional esterases constitute an important gene-enzyme system and are known to regulate a variety of tissue functions although their indigenous substrates are largely unknown. The data on genetic polymorphism at esterase coding loci are avail-

able for several organisms, but there is little information on the degree and patterns of esterase polymorphism in *Drosophila* species of the Indian subcontinent. The aim of the present report is to examine the extent of genetic variation at esterase coding loci in six *Drosophila* species. Electrophoretic phenotypes of esterases (E.C. 3.1.3.1) in six *Drosophila* species are represented in Figure 1. Species specific esterase patterns comprise segregating and nonsegregating zones of activities. Esterase bands of a nonsegregating (nonvariant) zone do not involve any mobility difference and appear in all individuals of a species. Segregating esterase zones are characterized by alternating single bands (of faster and slower mobility) and two-banded patterns. The esterase banding patterns of parents and progeny of several species-specific genetic crosses reveal that the segregating zones are under the control of distinct loci. The patterns of esterases in *D. takahashii* and *D. nepalensis* seem to be homologous since both these species have depicted similarities in the number, intensity and electrophoretic mobility values of all the seven esterase zones (Figure 1). Likewise, the esterase profiles of three species of the *ananassae* species subgroup (*D. malerkotliana*, *D. bipectinata* and

Table 1. Observed and expected esterase (*Est*) genotypes, allelic frequencies, heterozygosity, Wright's inbreeding coefficient (f), effective number of alleles (n_e) and application of log-likelihood χ^2 test (G-values) for Hardy-Weinberg expectations at polymorphic esterase loci in six *Drosophila* species.

| Species | Esterase genotypes (obs. / exp.) | | | Sample Size | Allelic frequency | | Heterozygosity Obs. / exp. | f | n_e | G-value |
|-------------------------|----------------------------------|------------|------------|-------------|-------------------|------|-------------------------------|--------|-------|---------|
| | FF | SS | FS | | F | S | | | | |
| <i>D. melanogaster</i> | | | | | | | | | | |
| <i>Est-6</i> | 6 / 13.39 | 48 / 55.21 | 69 / 54.39 | 123 | 0.33 | 0.67 | 0.56 / 0.44 | 0.44 | 1.79 | 9.19* |
| <i>D. takahashii</i> | | | | | | | | | | |
| <i>Est-7</i> | 12 / 10.9 | 66 / 65.5 | 52 / 53.5 | 130 | 0.29 | 0.71 | 0.40 / 0.41 | 0.02 | 1.70 | 0.17 |
| <i>D. nepalensis</i> | | | | | | | | | | |
| <i>Est-7</i> | 22 / 25.9 | 42 / 46.0 | 76 / 68.6 | 140 | 0.43 | 0.57 | 0.54 / 0.49 | -0.10 | 1.96 | 0.74 |
| <i>D. malerkotliana</i> | | | | | | | | | | |
| <i>Est-1</i> | 34 / 34.13 | 12 / 11.8 | 40 / 40.1 | 86 | 0.63 | 0.37 | 0.46 / 0.47 | 0.002 | 1.87 | 0.03 |
| <i>Est-4</i> | 24 / 22.17 | 20 / 18.9 | 38 / 41.0 | 82 | 0.52 | 0.48 | 0.46 / 0.50 | 0.07 | 2.0 | 1.2 |
| <i>Est-5</i> | 34 / 33.1 | 14 / 12.4 | 38 / 40.5 | 86 | 0.62 | 0.38 | 0.44 / 0.47 | 0.06 | 1.9 | 0.38 |
| <i>D. bipectinata</i> | | | | | | | | | | |
| <i>Est-1</i> | 51 / 47.3 | 15 / 11.4 | 39 / 47.0 | 105 | 0.67 | 0.33 | 0.37 / 0.41 | 0.09 | 1.7 | 1.77 |
| <i>Est-4</i> | 51 / 41.5 | 26 / 16.0 | 31 / 50.9 | 108 | 0.62 | 0.38 | 0.29 / 0.47 | 0.38 | 1.89 | 15.49* |
| <i>Est-5</i> | 24 / 23.5 | 15 / 14.5 | 36 / 37.0 | 75 | 0.56 | 0.44 | 0.48 / 0.50 | 0.03 | 1.97 | 0.05 |
| <i>D. ananassae</i> | | | | | | | | | | |
| <i>Est-1</i> | 35 / 37.7 | 10 / 13.0 | 50 / 44.3 | 95 | 0.63 | 0.37 | 0.53 / 0.47 | -0.128 | 1.87 | 1.66 |
| <i>Est-2</i> | 16 / 12.9 | 52 / 48.8 | 44 / 50.3 | 112 | 0.34 | 0.66 | 0.39 / 0.45 | 0.124 | 1.81 | 1.69 |
| <i>Est-3</i> | 33 / 25.9 | 36 / 28.1 | 39 / 54.0 | 108 | 0.49 | 0.51 | 0.36 / 0.50 | 0.28 | 2.0 | 8.42* |

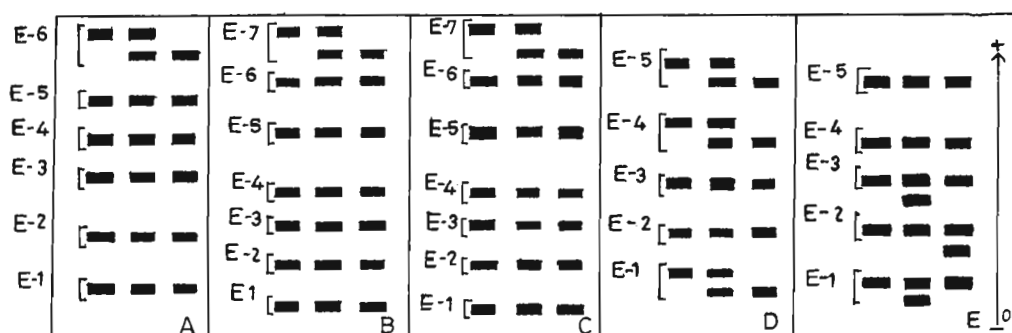
* Significant at 5 percent level.

D. ananassae) have revealed striking similarities on the basis of electrophoretic migration, substrate specificity and staining intensities of all the five esterase zones (Figure 1). Thus, the esterase electrophoretic phenotypes of closely related species of a *Drosophila* species subgroup have revealed homology relationships.

The data on distribution of esterase genotypes, allelic frequencies, observed and expected heterozygosity, Wright's inbreeding coefficient (F) and log-likelihood X^2 test for fit to Hardy-Weinberg expectations at polymorphic esterase zones in six *Drosophila* species are given in Table 1. The range of heterozygosities observed at most of the esterase loci correlate with the allelic frequency distribution patterns (Table 1). The species-specific population samples did not reveal the occurrence of null or rare alleles. The lower F values at most of the species-specific polymorphic esterase loci (except *Est-3* in *D. ananassae*) indicate that the populations are randomly mating. Data in Table 1 reveal that there are deviations from Hardy-Weinberg equilibrium, as well as excess homozygotes at the *Est-6* locus in *D. melanogaster* and the *Est-3* locus in *D. ananassae*. All the polymorphic esterase loci depict diallelic variation patterns. Except *D. melanogaster*, the polymorphic *Est* loci are represented by two common alleles. Polymorphic *Est* loci in four *Drosophila* species (*D. takahashii*, *D. nepalensis*, *D. malerkotliana*, and *D. bipectinata*) have revealed fit to Hardy-Weinberg expectations.

Electrophoretic analysis of esterases in six *Drosophila* species has revealed that species-specific esterases are coded by polymorphic, as well as, monomorphic *Est* loci. Since esterases are known to regulate a variety of tissue functions, such as reproduction, juvenile hormone metabolism and insecticide degradation, the observed species-specific esterase heterogeneity seems to confer species adaptability to possible changes in the environment. A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95, and accordingly all the variable *Est* loci are truly polymorphic in the species analyzed. The observations of the genetic basis, subunit structure and the occurrence of the most common allozymic variants at the *Est-6* locus in *D. melanogaster* concur with those reported earlier, but the allelic frequencies are not in agreement. The observed high levels of genic polymorphism at polymorphic *Est* loci concur with the functional constraint hypothesis that the non-glucose-metabolizing enzymes are highly polymorphic when compared with glucose-metabolizing enzymes. The observed diallelic frequency distribution patterns, as well as species-specific population structures at the polymorphic *Est* loci, may be interpreted in terms of balancing natural selection. The present studies need to be extended to several species-specific ecogeographical populations so as to assess the role played by various evolutionary forces in the maintenance of genic diversity at the polymorphic esterase loci.

Figure 1. Schematic representation of horizontal starch gel electrophoretic phenotypes of esterases in species-specific single individual homogenates. Homozygous and heterozygous *Est* genotypes are represented by single-band and two-band patterns, respectively.



The *Est* banding patterns of *D. melanogaster* served as marker stock. The species include: a. *Drosophila melanogaster*; b. *D. takahashii*; c. *D. nepalensis*; d. *D. malerkotliana* and *D. bipectinata*; e. *D. ananassae*. Arrow indicates the direction of current flow. The electrophoretic mobility of the species-specific esterase zones have been represented as R_f values, i.e. distance travelled by an esterase band/zone divided by the distance travelled by the bromophenol blue dye front.

Naseerulla, M.K. and S.N. Hegde. Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore 570-006, India. A new inversion in *Drosophila malerkotliana* from Varanasi, India.

Drosophila malerkotliana is a member of the *bipectinata* complex of the *ananassae* subgroup of the *melanogaster* species group. The polytene chromosome complement of this species contains four long autosomal arms and two short X-chromosome arms (Bock, 1971).

Chromosomal polymorphism in *D. malerkotliana* has been studied by Bock (1971) and Jha and Rahman (1972). Bock (1971) has reported four autosomal inversions, while Jha and Rahman (1972) have reported five. No X-

chromosome inversions were detected.

In this report we describe a new paracentric inversion heterozygote in *D. malerkotliana* population from Varanasi, Uttar Pradesh, India. Comparison with the reference map of salivary gland chromosomes of *D. malerkotliana* constructed by Jha and Rahman (1972) shows that the new inversion lies between the regions 38 to 43 of the left arm of the second chromosome and involves about 16% of the euchromatic length of the arm. This new inversion has been named IIL_C, the microphotograph of which is shown in Figure 1 and the location depicted in Figure 2.

Figure 1. Microphotographs of the IIL_C inversion heterozygote.

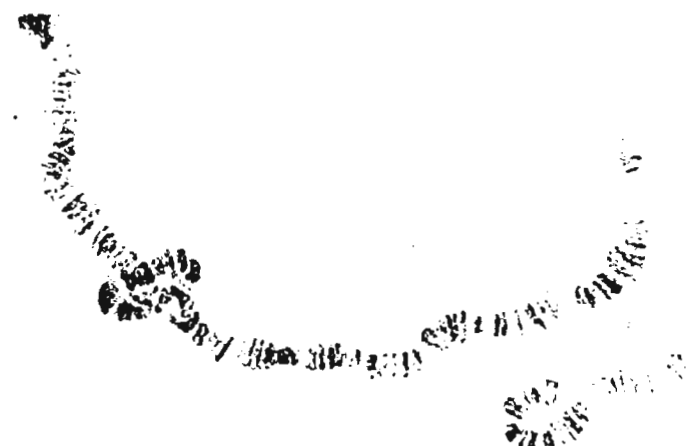
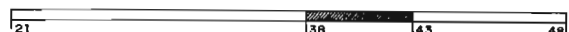


Figure 2. The location of the IIL_C inversion.



On chromosome IIL, Bock (1971) has reported two inversions, one median in position and another in the regions between 26 to 46. Jha and Rahman (1972) have detected two inversions, namely IIL_A, which is subterminal in position extending from region 22 to 29, and IIL_B, extending from region 39 to 46. Therefore, the new inversion IIL_C, which lies between the regions 38 to 43, is a smaller one compared to the inversions on IIL reported by Bock (1971) and Jha and Rahman (1972).

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References: Bock, I.R. 1971, *Chromosoma* 34:206-229; Jha, A.P. and S.M.Z. Rahman 1972, *Chromosoma* 37:445-454.

Naseerulla, M.K. and S.N. Hegde. Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore-570 006, India. Microclimatic variations at two enzyme loci in *Drosophila malerkotliana* and *Drosophila bipectinata*.

In recent years extensive studies have been made on enzyme polymorphism. The analysis of such polymorphisms either in *Drosophila* or any other species of organisms has a major relevance to our understanding the pattern of genetic diversity. Since information on molecular differentiation of isozymes as related to variable microclimatic conditions is wanting, an attempt

is made to compare populations from microclimatic regions of each of *D. malerkotliana* and *D. bipectinata* with regard to electrophoretic variation in two enzymes, alkaline phosphatase (Aph) and glucose 6-phosphate dehydrogenase (G6-PD).

A total of four populations of *D. malerkotliana* and four of *D. bipectinata* have been used in the present study. The collection localities of both the species are Mysore Airport (8 km south of Mysore), Bogadi (8 km west of Mysore),

Krishnarajasagar (19 km northwest of Mysore) and Srirangapatna (16 km north of Mysore). The wild caught males were immediately utilised for isozyme analysis while wild caught females were kept individually in food vials to build up isofemale lines. Equal number of males and females from each of the isofemale line were used for the present study, and all individuals of a single isofemale line were considered as a single genome from nature.

Polyacrylamide gel slabs were prepared using solutions made as described by Davis (1964). Electrophoresis was carried out at 4°C for 2 h with 80 volts and 3 h with 100 volts for Aph and G6-PD, respectively. Staining procedures adapted were of Ayala *et al.* (1972) and Gillespie and Kojima (1968) for Aph and G6-PD, respectively.

Number of loci present in each population and the number of alleles present in each locus were estimated following the procedure of Hegde and Krishnamurthy (1982-83). Allelic frequencies at different loci were calculated following Ayala *et al.* (1972), and heterozygosity per individual (H) was calculated using the formula of Singh and Coulthart (1982). Heterogeneity in allelic frequencies was calculated using G-statistics (Sokal and Rohlf, 1981).

Table 1. Allelic frequencies at Aph-locus in various populations of *D. malerkotliana* and *D. bipectinata*.

| Species | Population | Total number of individuals sampled | Allelic frequencies | | | | | Heterozygosity per individual |
|-------------------------|------------------|-------------------------------------|---------------------|---------------|---------------|---------------|---------------|-------------------------------|
| | | | Null | 0.85 | 0.90 | 0.95 | 1.00 | |
| <i>D. malerkotliana</i> | Mysore Airport | 80 | — | 0.186 | 0.053 | 0.300 | 0.460 | 0.662 |
| | Bogadi | 100 | 0.015 | 0.240 | 0.090 | 0.130 | 0.525 | 0.641 |
| | Krishnarajasagar | 75 | — | 0.162 | 0.122 | 0.304 | 0.412 | 0.697 |
| | Srirangapatna | 30 | — | 0.260 | 0.100 | 0.140 | 0.500 | 0.580 |
| | Average | 71.25 ± 12.790 | 0.003 ± 0.003 | 0.212 ± 0.019 | 0.091 ± 0.125 | 0.218 ± 0.042 | 0.474 ± 0.021 | 0.645 ± 0.021 |
| | G-value | | 2.06 | 0.17 | 12.90* | 31.74* | 88.05* | 122.17* |
| <i>D. bipectinata</i> | Mysore Airport | 59 | — | 0.292 | 0.215 | 0.185 | 0.308 | 0.740 |
| | Bogadi | 55 | 0.064 | 0.282 | 0.073 | 0.173 | 0.408 | 0.864 |
| | Krishnarajasagar | 55 | 0.027 | 0.309 | 0.127 | 0.027 | 0.508 | 0.629 |
| | Srirangapatna | 30 | 0.043 | 0.400 | 0.043 | 0.086 | 0.428 | 0.646 |
| | Average | 49.75 ± 5.760 | 0.003 ± 0.012 | 0.321 ± 0.023 | 0.114 ± 0.033 | 0.118 ± 0.032 | 0.413 ± 0.036 | 0.719 ± 0.047 |
| | G-value | | 5.42 | 1.00 | 4.98 | 10.28* | 2.46 | 0.36 |

* $p < 0.05$

Table 2. Allelic frequencies at G6-PD locus in various populations of *D. malerkotliana* and *D. bipectinata*.

| Species | Population | Total number of individuals sampled | Allelic frequencies | | | Heterozygosity per individual |
|-------------------------|------------------|-------------------------------------|---------------------|---------------|---------------|-------------------------------|
| | | | 1.00 | 1.08 | Null | |
| <i>D. malerkotliana</i> | Mysore Airport | 50 | 0.500 | 0.480 | 0.020 | 0.519 |
| | Bogadi | 105 | 0.505 | 0.438 | 0.057 | 0.550 |
| | Krishnarajasagar | 70 | 0.500 | 0.500 | — | 0.500 |
| | Srirangapatna | 50 | 0.500 | 0.500 | — | 0.500 |
| | Average | 68.75 ± 11.230 | 0.501 ± 0.001 | 0.479 ± 0.013 | 0.019 ± 0.014 | 0.517 ± 0.010 |
| | G-value* | | 1.06 | 1.95 | 6.41 | 1.67 |
| <i>D. bipectinata</i> | Mysore Airport | 45 | 0.500 | 0.490 | 0.010 | 0.510 |
| | Bogadi | 55 | 0.500 | 0.482 | 0.018 | 0.518 |
| | Krishnarajasagar | 45 | 0.500 | 0.500 | — | 0.500 |
| | Srirangapatna | 50 | 0.500 | 0.470 | 0.030 | 0.528 |
| | Average | 48.75 ± 2.073 | 0.500 ± 0.000 | 0.486 ± 0.005 | 0.015 ± 0.005 | 0.514 ± 0.005 |
| | G-value* | | 3.86 | 5.74 | 2.10 | 5.89 |

* G-value not significant at 5 % level.

The number of individuals sampled from each population, different alleles present at each locus, their frequencies in the population, heterozygosity per individual, and G-values are given in Tables 1 and 2.

In *D. malerkotliana* the allelic frequencies at the Aph locus showed significant variation (at 5% level) between different populations analysed for the alleles Aph^{0.90} (G = 12.90), Aph^{0.95} (G = 31.74) and Aph^{1.00} (G = 88.05) and also for heterozygosity per individual (G = 122.17) while in *D. bipectinata* only the Aph^{0.95} allele showed significant interpopulation variation (G = 10.28; Table 1).

In both *D. malerkotliana* and *D. bipectinata*, though there were differences in allelic frequencies and heterozygosity per individual for G6-PD, they were all insignificant (Table 2).

There were four alleles for Aph (the hydrolytic enzyme) and only two for G6-PD (the glycolytic enzyme) in both *D. malerkotliana* and *D. bipectinata*. Kojima *et al.* (1970) have suggested that since the hydrolytic enzymes act on substrates which come directly from the external environment, the substrate environment of these enzymes is highly heterogeneous, and this heterogeneity of the substrates maintains a high level of allelic variation. On the other hand the enzymes of the glycolytic pathway are specific and hence less variable. Selection is weak against variation in the hydrolytic enzymes while it is more stringent against variation in the glycolytic enzymes (Prakash, 1977). According to Yang *et al.* (1972) there is a positive correlation between the number of alleles which occur in a species and the probability of there being interpopulation differences. Hence our results agree with Kojima *et al.* (1970), Prakash

(1977), Yang *et al.* (1972) and several other workers.

The populations analysed were from areas surrounding Mysore city, all situated within a radius of 25 km. There is no physical barrier as such between these populations. In addition, the macroclimatic factors like temperature, rainfall, humidity and altitude are the same for these localities. In spite of these similarities, there was a statistically significant difference in the frequency of the Aph enzyme in both the species under study. According to Reddy (1973), populations of a species are kept adapted to their environments and always preserve a high plasticity which enables them to respond to changes in the microenvironmental factors. Similar observations, but with regard to chromosomal variations, have been made by Dobzhansky (1947), Nirmala (1973), Singh and Das (1990) and other workers. Hence, the differences in the frequency of the Aph enzyme reflect the genetic differentiation in order to adapt to ambient microhabitats.

Acknowledgments: Authors are thankful to the Chairman, Department of Studies in Zoology, Manasagangotri, Mysore for facilities and MKN is grateful to UGC for financial assistance.

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Alahiotis, Stamatis N. Department of Biology, University of Patras, Patras, Greece. On the interspecific hybridization between *Drosophila melanogaster* sibling species.

In a recent paper published by Naveira (1991) the successful construction of F₁ fertile hybrids between *D. mauritiana* females and *D. simulans* (and *D. melanogaster*) males, even in its extremely rare fashion (Goulielmos and Alahiotis, 1988) is disputed. According to Naveira this situation is questionable because "the

homogenates analyzed (for electrophoretic studies) were groups of flies rather than single flies".

Based on this criticism we have to underline that:

We use three species-diagnostic genes (located on the three major chromosomes, X, 2nd, 3rd), for 6-Phosphogluconate dehydrogenase (*6-Pgd*), alcohol dehydrogenase (*Adh*) and aldehyde oxidase (*Aldox*). A strong criterion to verify the hybrid status is to reveal in the same hybrid, within the same generation, electromorphs from different (parental) species. For the investigation of this situation, it is valid to use coelectrophoresis of one fly from each species and hybrid; and this was done in our study. In each coelectrophoretic experiment referred to in our paper (Goulielmos and Alahiotis, 1988) we used one fly. For example, when we denote "coelectrophoresis between *D. mauritiana* and *Masi*" we mean electrophoresis of a sample (channel) constituted with one *D. mauritiana* fly and one *Masi* fly. This has to become clear. Thus, the coexistence in each *Masi* fly of the *D. mauritiana* *6-Pgd*, the *D. simulans* (or *D. mauritiana*) *Adh* and the *D. simulans* *Aldox* electromorphs is a very strong verification of its interspecific hybrid status. An analogous situation also stands for *Masi-2*. It is obvious that such data cannot be attributed to a contamination scenario.

On the other hand the various unexpected abnormalities and irregularities in several electromorphs of ADH, the shift of the ALDOX and ADH isozymes (something which could be due to modification factor(s)), the three (instead of two) banded 6-PGD electromorphs revealed in only five females originating from the backcross of *D. mauritiana* males with females from the cross female *Masi* X *Mame* male (those three bands corresponded to three species) strongly support situations which have been observed in interspecific hybrids (e.g., Phillipp *et al.*, 1983; Dickinson, 1980).

Further data from the study of *Masi*, *Masi-2* and *Mame* hybrids have been published by Kiliass, Goulielmos and Alahiotis (1989); Karvountzi, Goulielmos, Kalpaxis and Alahiotis (1989) and Alahiotis (1987) give additional evidences supporting their hybrid status. These data have not been into consideration by Naveira (1991). Thus, it was found that for various fitness components (fecundity, fertility, viability, developmental time, sex ratio, oviposition rhythm), each parental species (*D. melanogaster*, *D. simulans*, *D. mauritiana*) and hybrid (*Masi*, *Masi-2*, *Mame*) exhibit characteristic values (for each component). In many cases the hybrids did not exhibit intermediate values, compared with their parental species. For example, in terms of the eggs produced per fly (fecundity) the corresponding values for *D. mauritiana*, *D. simulans* and *Masi* are: 18.68 ± 2.34 , 16.91 ± 3.68 and 10.92 ± 2.75 , while these values for fertility (hatched eggs per fly) are 15.6 ± 1.65 , 16.19 ± 3.86 and 9.27 ± 2.62 , respectively (Kiliass *et al.*, 1989). In relation to the sex ratio the normal analogy 1:1 for the parental species departs significantly (female > male; $p > 0.01$) for all hybrids while in crosses involving *Masi* and *Mame* extreme deviations (2:1, 4:1) have been observed, even 30 generations after

their origin (Goulielmos and Alahiotis, 1988; Kiliyas et al., 1989).

Additional data based on kinetic and acclimation studies (Karvountzi et al., 1989) revealed characteristic behavior of the hybrids and their parental species in terms of Lactate Dehydrogenase (LDH) and cytoplasmic and mitochondrial Malate Dehydrogenase (cMDH, mMDH) specific activity and K_m values. Thus, the LDH specific activity ($\Delta OD \text{ min}^{-1} \text{ mg pr}^{-1}$) values for *D. mauritiana*, *D. simulans* and *Masi* are 32 ± 1.5 , 12 ± 0.5 and 20 ± 1.1 , respectively, while the K_m malate values (mM) of mMDH are around 1 mM for the parental species and about 5 times higher in the *Masi* (4.80 mM) at 14°C and ten times higher (9.75 mM) at 28°C.

Another criticism done by Naveira (1991) refers to no availability of the hybrids to other workers for further tests. This is not true again, since they have been sent for other studies. For example, there are data showing the existence of an extremely high number of virus-like particles in the nuclei of hybrid cells (unpublished data), something which is not true for the parental species. For the time being an effort is made to identify these particles. On the other hand these hybrids have been differentiated already and tend to turn to one parental species. For example, *Masi-2* resembles now *D. simulans* and *Mame* resembles *D. melanogaster*. Specific experiments dealing with enrichment of the hybrids' genome with genome coming from their parents, 35 generations after the hybrid construction, showed: i) a linear accumulation of the parental genome and ii) significant difference regarding this interaction with the two parents. For example, the sample regression coefficient of *Masi-2* is $b = 3.85$ for *D. mauritiana* and $b = 6.62$ for *D. simulans*. This is the reason for which *Masi-2* resembles to *D. simulans* and not to *D. mauritiana*. Gradual differentiation (with the generations) of *Masi-2* has also been revealed by scoring the percentage of successful matings of this hybrid with its parental species. This behavior is also typical of interspecific hybrids.

All these data strongly support the hybrid status of *Masi*, *masi-2* and *Mame*. As we had underlined (Goulielmos and Alahiotis, 1988), the phenomenon is very rare and could be attributed possibly to the specific *D. mauritiana* strain (stock number gif 163.1) we used. We do not declare that F_1 hybrids of *D. mauritiana* females and *D. simulans* males are always fertile. We had described an exception which is very rare. We succeeded in producing such hybrids just two times (or three when *D. melanogaster* is involved) in spite of extremely many efforts. Deviations from an expected situation can happen. For example, the rate of hybridization between *D. melanogaster* females and *D. simulans* males was found to be very high (2.90%) in one natural population (Gavros-Achaia-Greece, 1973) and very low (0.75%) in another (Cephalonia-Greece, 1973). The differences found in the field were verified in the laboratory by crossing *D. melanogaster* and *D. simulans* originating from the respective natural populations; the hybridization percentage was found to be 42% (using 231 cultures) for the Gavros-Achaia collection and 6% (using 12 cultures) for the Cephalonia collection (Alahiotis, 1978).

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Chatterjee, S. and B.N. Singh. Banaras Hindu University, Department of Zoology, Varanasi, India. Variation in the male sex comb teeth number in four species of the *Drosophila bipectinata* complex.

The *bipectinata* species complex of the *ananassae* subgroup of the *melanogaster* species group consists of four closely related and morphologically similar species: *Drosophila bipectinata*, *D. malerkotliana*, *D. parabipectinata* and *D. pseudoananassae* which are distributed in the Australian or Oriental biogeographic

zones. *D. bipectinata* is most widespread ranging from India to Fiji and Samoa in the Pacific and the other three are less widespread (Bock and Wheeler 1972). Females of these four species are indistinguishable but males are distinguishable with the help of the structure of sex combs and the abdominal colouration. However, abdominal colouration can not distinguish all the species from each other. Interspecies hybridization among the members of the *bipectinata* complex has been reported (Gupta 1973) and the degree of sexual isolation varies among the species (Bock 1978; Singh et al. 1981, 1982). Singh and Chatterjee (1992) found no evidence for character displacement for reproductive isolation between *D. bipectinata* and *D. malerkotliana*.

The structure of sex combs in the males of all four species is depicted in Figure 1. Teeth are arranged singly or in rows to form sex combs on the first and second tarsal segments of prothoracic legs. During the present study, a large number of strains of these four species were used to know the variation in the number of sex comb teeth. In each strain,

Table 1. Mean number of sex comb teeth in four species of *Drosophila bipectinata* species complex.

| Species / strain | Mean number of teeth \pm SE | Range |
|--------------------------------|-------------------------------|---------|
| <i>D. malerkotliana</i> | | |
| 1. Varanasi (BHU) | 6.48 \pm 0.11 | 4 - 9 |
| 2. Varanasi (UL) | 7.28 \pm 0.09 | 5 - 9 |
| 3. Mirzapur | 7.11 \pm 0.10 | 5 - 10 |
| 4. Nagaland | 6.37 \pm 0.11 | 4 - 11 |
| 5. Mysore | 7.97 \pm 0.12 | 6 - 11 |
| 6. Brazil | 7.06 \pm 0.10 | 5 - 10 |
| 7. Baripada | 6.22 \pm 0.10 | 4 - 9 |
| <i>D. bipectinata</i> | | |
| 1. Varanasi (BHU) | 14.97 \pm 0.14 | 11 - 19 |
| 2. Varanasi (UL) | 13.86 \pm 0.15 | 11 - 19 |
| 3. Nepal | 14.07 \pm 0.12 | 11 - 17 |
| 4. Townsville, Australia | 12.83 \pm 0.15 | 10 - 16 |
| 5. Baripada | 12.77 \pm 0.13 | 9 - 17 |
| 6. Mysore | 13.82 \pm 0.17 | 10 - 19 |
| <i>D. parabipectinata</i> | | |
| 1. Mysore | 11.33 \pm 0.15 | 8 - 14 |
| 2. Cambodia | 11.48 \pm 0.10 | 9 - 14 |
| 3. Indonesia | 13.15 \pm 0.13 | 10 - 17 |
| 4. Mauritius | 13.59 \pm 0.15 | 10 - 19 |
| <i>D. pseudoananassae</i> | | |
| 1. Chiang Mai, Thailand | 9.08 \pm 0.15 | 5 - 12 |
| 2. North Queensland, Australia | 9.04 \pm 0.14 | 6 - 13 |

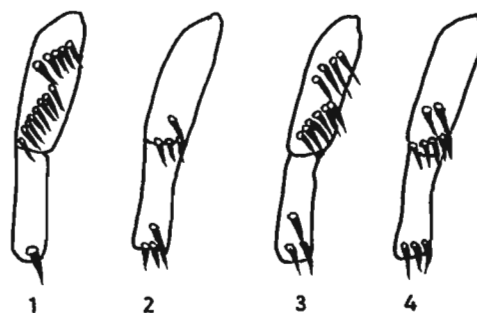


Figure 1. Male sex combs in four species of the *bipectinata* complex. 1, *D. bipectinata*; 2, *D. malerkotliana*; 3, *D. parabipectinata*; 4, *D. pseudoananassae*.

the number of sex comb teeth on right prothoracic leg was counted in 100 males and the mean number of teeth was calculated. Table 1 shows the mean number of sex comb teeth and range of variation in different species. It is evident that all the four species differ with respect to the number of sex comb teeth and there is intraspecies variation also. Further, there is considerable individual variation in the number of teeth in all strains of the four species. In *D. malerkotliana*, the number of teeth ranges from 4 to 11, *D. bipectinata* from 9 to 19, *D. parabipectinata* 8 to 19 and *D. pseudoananassae* 5 to 13. This is consistent with polygenic inheritance of sex comb teeth in *Drosophila* as it has been suggested by Crossley and Taylor (1985) based on mid parent value of sex comb teeth number in hybrids produced by interspecific hybridization in the *bipectinata* complex.

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Milanović, M., G. Bojić-Stamenković and M. Andjelković. University of Belgrade, Institute for Biological Research, 11060 Beograd, 29. Novembra 142, Yugoslavia. Adaptive significance of amylase polymorphism in *Drosophila*. IX. Alpha-amylase structural gene polymorphism in *Drosophila busckii*.

Electrophoretic techniques have revolutionized the status of empirical studies in population genetics, systematics and evolutionary biology. Electrophoretic banding patterns can be transformed into allelic and/or non-allelic genetic variations used to describe the genetic structure of natural populations (MacIntyre, 1986).

Natural populations of different *Drosophila* species are usually polymorphic for the *Amy* structural gene coding for alpha-amylase (EC 3.2.1.1. alpha-1,4-glucan-4-glucanohydrolase). Different degree of *Amy* polymorphism detected and a number of alleles or isozymes varied between the species as well as between the populations within a species (Doane *et al.*, 1975; Hickey, 1979; Doane *et al.*, 1987; Andjelković *et al.*, 1987).

Drosophila busckii is the only known species of the subgenus *Dorsilopa*. The species is widely distributed in the world and is found in associations with domestic habitats of man (i.e., on decaying materials especially those rich in starch and glycogen, the two major alpha-amylase substrates). We have found it of interest to perform the studies on gene-enzyme system of amylase in this species, since it has been poorly investigated so far. Using agar gel

electrophoresis, Prakash (1973) found that amylase in *D. busckii* was monomorphic. In this paper we present an electrophoretic survey of *Amy* structural gene polymorphism in two *D. busckii* natural populations.

The two natural populations of *D. busckii* have been sampled from Ub (30 km south from Belgrade) and from Slanci (Belgrade suburb), respectively. The flies were grown on potato medium (Bojić-Stamenković *et al.*, 1992) for one generation before electrophoresis. F_1 offspring of freshly sampled flies were studied. Adult flies were homogenized in 10 μ l 5 mM Tris-acetate buffer pH 7.8. Continuous rod electrophoresis was performed using 7.5% acrylamide gel (Hames, 1981), for 160 min at 4°C and 5.5 mA/gel. Under these conditions, the studied enzyme moves toward the cathode. The identification of alpha-amylase on the gels was done by the amyloclastic method for qualitative determination (Doane, 1967).

Analysis of the samples of *D. busckii* natural populations revealed four amylase electromorphs designated according to their electrophoretic mobility as VS (very slow), S (slow), F (fast) and VF (very fast). The frequencies of different combinations of amylase electromorphs found in the two populations are listed in Table 1. It can be seen that VS electromorph always appeared together with S, F and VF in the following combinations: VS/S/F, VS/S/VF and VS/F/VF. The frequency of individuals with the given combinations was very low (0-5%). Some minor accompanying bands were observed by Doane *et al.* (1975) for *Amy* electromorphs in *D. hydei*. The same authors speculated that more anodal electromorphs might arise by deamidation of GLN and ASN similar to human and chicken amylase (Karn and Malacinski, 1978). Thus, VS electromorphs observed in our experiment could also be formed by deamidation. So, we presumed that only 3 amylase electromorphs in *D. busckii* can be considered as a product of different alleles termed S, F and VF, according to their electromorph analogues (Andjelković *et al.*, 1987).

Analyses of different combinations of amylase electromorphs (alleles) in the two populations studied revealed a high frequency of the S/F/VF genotype (26.4% and 40.7% in Slanci and Ub populations, respectively). Such a high frequency of individuals with 3 alleles is very rare in *Drosophila* populations (Hickey, 1979; Andjelković *et al.*, 1987). Genotypes with more than two alleles could have evolved by *Amy* gene duplication, as demonstrated for some *Drosophila* species (Dainou *et al.*, 1987; Doane *et al.*, 1987). Since the number of amylase electromorphs or alleles is a poor indicator of *Amy* gene duplication, this conclusion calls for more precise evidence.

On the basis of our results it could be concluded that in *D. busckii* species *Amy* structural gene is polymorphic. In contrast to our results Prakash (1973) found a monomorphic *Amy* structural gene in this *Drosophila* species. This discrepancy could be explained by different electrophoretic conditions applied. The present paper provides only preliminary data for further examinations of *Amy* locus in *D. busckii* at biochemical and genetical level, which are currently in progress.

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Stark, W.S.¹, G. Brown¹, D. Rodriguez¹ and J.P. Carulli².

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²Harvard University, Boston, MA U.S.A. Visual structure in a strain of *Drosophila virilis* which lacks R7.

of long standing interest in development of R7 in mutants of *Drosophila melanogaster* (e.g., Cagen and Zipurski, 1992).

Table 1. Frequencies of *Amy* electromorph combinations in two *D. busckii* natural populations

| Electromorphs | Locality | | | |
|---------------|----------|-------|-----|-------|
| | Slanci | | Ub | |
| | n | p | n | p |
| S/F | 7 | .049 | 1 | .007 |
| S/VF | 3 | .020 | 2 | .015 |
| F/F | 4 | .028 | 3 | .022 |
| F/VF | 51 | .354 | 44 | .326 |
| VF/VF | 33 | .229 | 22 | .163 |
| S/F/VF | 38 | .264 | 55 | .407 |
| VS/S/F | 7 | .049 | 4 | .030 |
| VS/S/VF | 1 | .007 | 2 | .015 |
| VS/F/VF | — | — | 2 | .015 |
| N | 144 | 1.000 | 135 | 1.000 |

n = electromorph samples, p = electromorph frequency, N = flies sampled

We are pursuing interest in molecular evolution in *Drosophila* species (Carulli *et al.*, 1993; Carulli and Hartl, 1992; Neufeld *et al.*, 1991) with electron microscopy (EM) of the *D. virilis* compound eye. Interestingly, a white eyed *D. virilis* stock lacks the R7 photoreceptor in every ommatidium, significant because

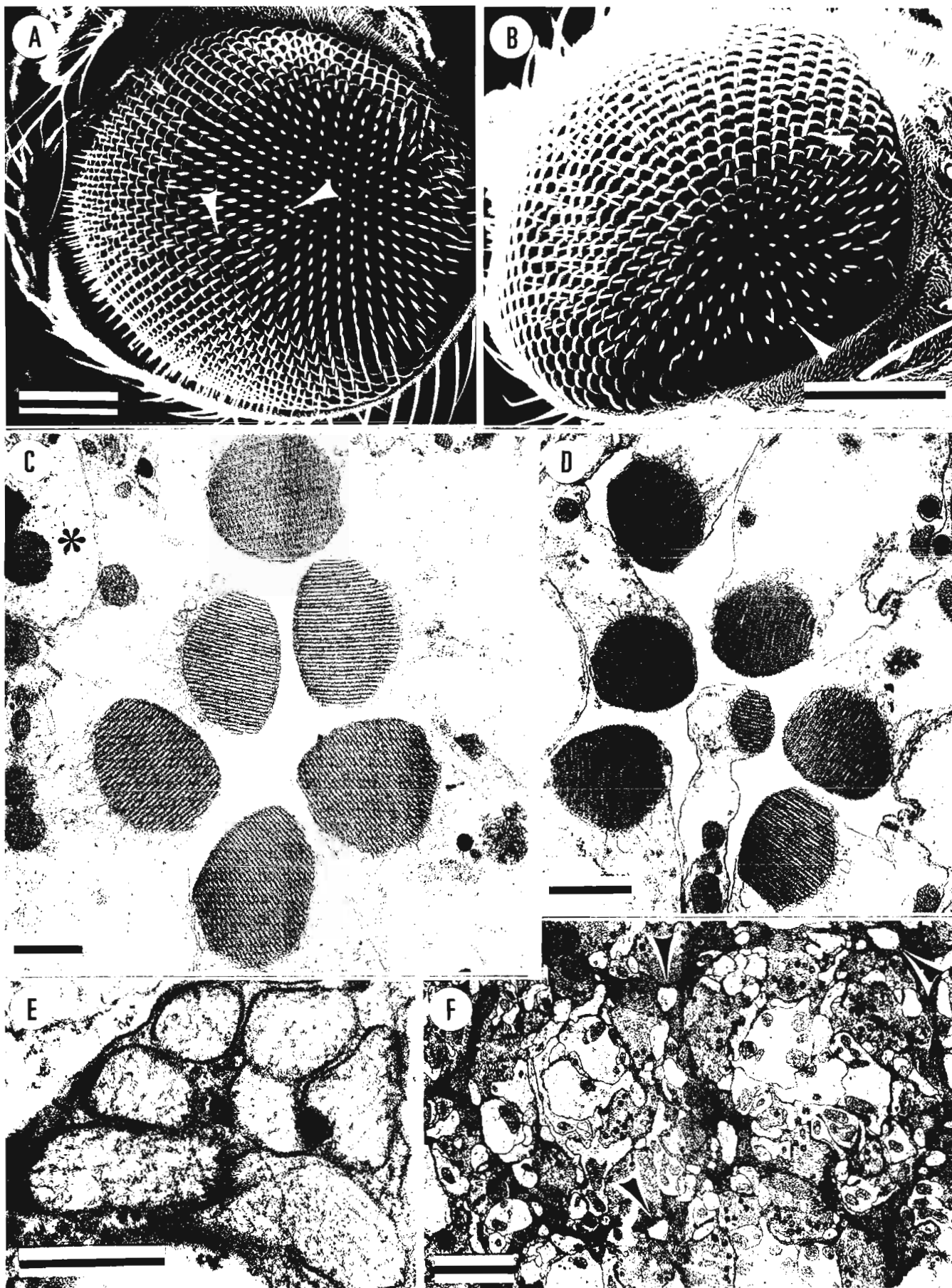
We obtained this unusual white eyed *D. virilis* and a red eyed control from Bowling Green Stock Center. A white eyed control strain which does have R7 (called Ds11w9) was obtained from Elena Lozovskaya in the laboratory of Daniel L. Hartl, then at Washington University. For comparison, the well-studied (since Harris *et al.*, 1976) *sev* (= *sevenless*) mutant of *D. melanogaster* was also used. Transmission EM was done using our standard protocols (e.g., Stark and Sapp, 1987; Stark *et al.*, 1988) with aldehyde prefix followed by osmium postfix. Dehydration, embedding, sectioning and lead and uranium staining were as published. A JEOL model 1200 transmission EM (operated at 80 kV) was used to view the specimens. Scanning EM (SEM) was performed on several specimens. Since the surfaces to be viewed were cuticular, fixation was not necessary. Heads, dissected at the neck, were gradually dehydrated in a series of 30, 50, 70, 95, and 100% ethanols. After critical point drying (Ladd), heads were fixed to stubs with copper tape and gold coated (Fullman EMS-76 Minicoater) and viewed on a JEOL model JSM-35 scanning EM.

The ultrastructure of the control *D. virilis* (having R7) is much like that of normal *D. melanogaster* (not shown). The *D. virilis* strain which lack R7 is shown in the accompanying plate (Figure 1. A and C-F). Figure 1 A (calibration bar = 100 μ m) is an SEM of a typical eye of *D. virilis*. Eye reductions down to bar shapes were occasionally observed, but, in most eyes, there are irregularities (arrowheads), especially places where there are two hairs instead of one. Because we thought this mutant might be homologous to *sev D. melanogaster*, where such surface abnormalities had not been noted yet, we looked at *sev*. Figure 1 B (bar = 100 μ m) is an SEM of the eye of *sev D. melanogaster* showing the same sort of irregularities (arrowheads) as in *D. virilis*. Figure 1 C (bar = 1 μ m) is a distal cross section of the *D. virilis* ommatidium at the level of R1-6 nuclei (*) showing just six rhabdomeres. Multivesicular bodies (MVBs), involved in membrane turnover in *D. melanogaster* (Stark *et al.*, 1988), abound (in cells at the 5, 7 and 10 o'clock positions). Figure 1 D (bar = 1 μ m) is a proximally sectioned ommatidium of the *D. virilis* strain which lacks R7. R8 inserts from the 6 o'clock position. Conspicuously lacking is the axon of R7 which would be observed in this plane of section if R7 were present. We note (one o'clock position) a curious "eye color pigment granule" such as those which invaginate from secondary pigment cells into retinula cells in *D. melanogaster* (Stark and Sapp, 1988) and *D. mauritiana* (Stark *et al.*, 1989), despite the lack of eye color pigments in white-eyed flies. Figure 1 E (bar = 1 μ m) is a cross section of a pseudocartridge just beneath the basement membrane in the lamina ganglionaris. This structure consists of photoreceptor axons from one ommatidium before they branch to their individual destinations in the lamina and medulla. There are 7 axons as had been reported for *sev D. melanogaster* (Stark and Carlson, 1985). In flies with all 8 retinula cells per ommatidium, the pseudocartridge has 8 axons (e.g., Stark and Carlson, 1983, 1985). Figure 1 F (bar = 1 μ m) is a cross section of optic cartridges in the lamina ganglionaris showing two full cartridges (top, left and right) and portions of several others (mainly at the bottom). Centered in each cartridge are the L cells, and their lucent processes abound. Of intermediate electron density are the R cell terminals with the capitate projections serving as advertising landmarks. Around and separating cartridges are the so-called (Trujillo-Cenoz and Melamed, 1966) "epithelial" glia of high electron density. In the lamina, a fly with all 8 retinula cells would show R7/8 axons passing as a pair through the glia on their way to their synaptic destinations in the medulla (e.g., Stark and Carlson, 1983, 1985). By contrast only one axon, that of R8, is seen at this site (Figure 1 F, arrowheads), as had been noted earlier for *sev D. melanogaster* (Stark and Carlson, 1985).

We want to determine the gene mutation causing the lack of R7. Three well studied genes in *D. melanogaster* have mutations leading to absence of R7 cells: *sev* (see above), *bride of sevenless* (*boss*) and *seven in absentia* (*sina*) (e.g., Basler and Hafen, 1991; Rubin, 1992). A simple Mendelian cross showed that it is X-linked (as it is in *D. melanogaster*, Harris *et al.*, 1976); *sev* is the only one of the three known to be X-linked in *D. virilis* (Lozovskaya *et al.*, 1993; Ashburner, 1989). Thus, our *D. virilis* mutant could be *sev*. Preliminary Northern analysis suggests that *sev* is expressed at a higher level in our mutant than in controls as probed with *D. virilis sev*. This may or may not contradict the suggestion that the mutant could be *sev*. Genes other than *sev*, *boss* and *sina* may be mutant, and further work is needed.

Acknowledgments: Supported by NIH grant EY07192 and NSF grant BNS 88 11062 to WSS and NIH pre-doctoral Training Grant EY07108 to JPC. Part of the cost of maintaining the EM facility was defrayed by the University of Missouri - Columbia Ophthalmology Department's Research to Prevent Blindness grant, and we thank Michael Norberg, the EM supervisor. We thank Dr. Jerry White for assistance with the SEM. We thank Darren Hombs for help in the dark room, Stephen Harrison of Prof. G. Rubin's lab for the *sev* probe and John Hiebert in Prof. J. Birchler's lab for help with the genetics and molecular experiments.

References: Ashburner, M. 1989, *Drosophila: A Laboratory Handbook*, Cold Spring Harbor Laboratory Press; Basler, K. and E. Hafen 1991, *Bioessays*, 13: 621-631; Carulli, J., D.-M. Chen, W.S. Stark and D.L. Hartl 1993, *J. Molec. Evol.*, submitted; Carulli, J.P. and D.L. Hartl 1992, *Genetics* 132: 193-204; Harris, W.A., W.S. Stark and J.A. Walker 1976, *J. Physiol. (Lond.)* 256: 415-439; Lozovskaya, E.R., D.A. Petrov and D.L. Hartl 1993, *Chromosoma*, in press; Neufeld, T.P., R.W. Carthew and G.M. Rubin 1991, *Proc. Nat. Acad. Sci. USA* 88: 10203-10207; Rubin, G.M. 1992,



Mech. Dev. 37: 37-42; Stark, W.S. and S.D. Carlson 1983, Cell Tiss. Res. 233: 305-317; Stark, W.S. and S.D. Carlson 1985, Int. J. Insect Morphol. Embryol. 14: 243-254; Stark, W.S. and S.D. Carlson 1985, Dros. Inf. Serv. 61: 164-166; Stark, W.S. and R.J. Sapp 1988, Can. J. Zool. 66: 1301-1308; Stark, W.S. and R.J. Sapp 1987, J. Neurogenet. 4: 227-240; Stark, W.S., R.J. Sapp and D.S. Haymer 1989, Pig. Cell Res. 2: 86-92; Stark, W.S., R.J. Sapp and D. Schilly 1988, J. Neurocytol. 17: 499-509.

Stoltenberg, S.F. and J. Hirsch. University of Illinois, Urbana-Champaign, Illinois USNA. F_2 hybrid analysis and the phenotypic correlation between geotaxis and mate preference in evolved laboratory populations.

occurred were positively assortative, a significant departure from the 50% expectation when mate preference is not related to geotaxis phenotype. This phenomenon has remained stable for at least 80 generations and was demonstrated in two independent laboratories.

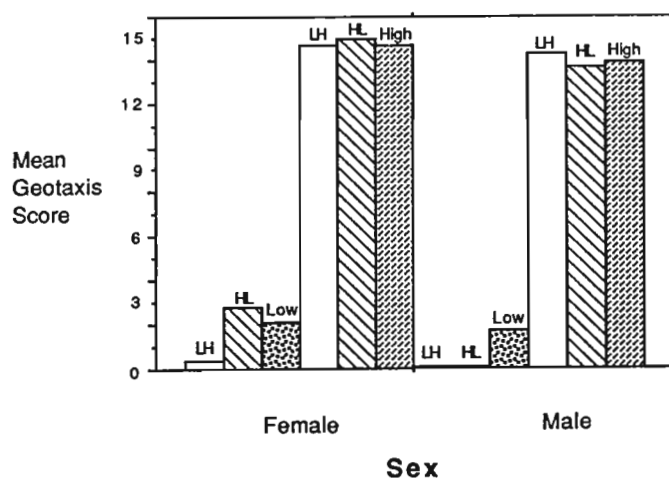
Thus, there is a correlation between geotactic performance and mate preference in the High and Low lines; as homogamic matings are more likely than heterogamic. In order to assess relations among the genetic correlates of the correlated phenotypes, we performed an F_2 hybrid analysis (Tully et al., 1982). F_2 generation hybrids between the High and Low lines permit the independent assortment of unlinked genes and the recombination of gene linkages. If the correlated phenotypes are related to the same genetic system(s) their correlation should persist through the hybrid F_2 generation. Whereas the attenuation or disappearance of the correlation in the F_2 disconfirms such a relationship.

Lofdahl et al. (1992) demonstrated that the negatively (High) and positively (Low) selected geotaxis lines studied by Hirsch and his colleagues since 1957 showed significant (partial) premating reproductive isolation. In Elens and Wattiaux (1964) mating chambers approximately 60% of the matings that

Table 1. F_2 hybrid matings: geotaxis extreme category versus sex (Chi-square test for association = 0.007, 1 df, $p > 0.90$).

| | Male | | |
|--------|-------|-------|-----|
| | High | Low | |
| Female | H 145 | L 137 | 282 |
| | L 142 | H 136 | 278 |
| | 287 | 273 | 560 |

Figure 1. Comparison of mean geotaxis scores from geotaxis lines (Low, High Generation 703) and F_2 generation assayed for mate preference (LH, HL).



First we made reciprocal crosses of the High and Low lines to produce F_1 generation individuals to be used as parents in the next round of crosses (Low female x High male = F_{1LH} , High female x Low male = F_{1HL}). Then, we crossed those F_1 individuals to produce F_2 offspring as follows (note: reciprocal crosses were not made): F_{1LH} female x F_{1LH} male = F_{2LH} ; F_{1HL} female x F_{1HL} male = F_{2HL} . Samples of approximately 230 2-3 day old F_2 individuals (collected within 4-6 h of eclosion and stored separately according to sex) were assayed for geotactic performance (general culturing and geotaxis testing procedures have been described previously Ricker and Hirsch, 1985). We assayed a total of 6189 flies for geotaxis.

To test the mating preferences of F_2 generation individuals that were phenotypically similar to the High and Low geotaxis lines, in each sample the 26 most extreme scorers (of each sex and geotaxis extreme) were kept. In all but one case (extreme low HL females), mean geotaxis scores of the F_2 generation individuals tested for mate preference were at least as extreme as the High and Low lines (see Figure 1). These flies were then marked by wing clipping (counterbalanced) and aged until 5-7 days old. Mate preference testing was similar to that described previously (Lofdahl et al., 1992).

Results indicate that F_2 generation hybrids, with geotaxis phenotypes comparable to those of the High and Low lines, mate without respect to geotaxis phenotype (i.e. mate preference was statistically independent of geotaxis phenotype, see Table 1). Of 560 matings, 50.2% (281) were homotypic (i.e., HxH, LxL). This sample size has a power

of 96% to detect a difference between 50% and 60% homotypic matings at $\alpha = 0.05$ (Cohen, 1988).

We interpret these data as evidence that, although a phenotypic correlation between geotactic performance and mate preference exists in the High and Low lines, it appears that their genetic correlates are independent. Thus, it appears that geotaxis and mate preference are influenced by separate genetic systems. Ongoing work in our lab is geared toward a better understanding of the mating behavior and the genetic correlates of geotaxis in the High and Low lines. Samples of the High and Low lines are available to interested investigators who contact Jerry Hirsch.

References: Cohen, J. 1988, Statistical Power Analysis For The Behavioral Sciences, Lawrence Erlbaum Associates, Inc.; Elens, A. and J.M. Wattiaux 1964, Dros. Inf. Serv. 39:118-119; Lofdahl, K.L., D. Hu, L. Ehrman, J. Hirsch and L. Skoog 1992, Anim. Behav. 44:783-786; Ricker, J.P. and J. Hirsch 1985, J. Comp. Psych. 99:380-390; Tully, T., S. Zawistowski, and J. Hirsch 1982, Behav. Genet. 12:181-191.

Regional *Drosophila* Research Conference Programs

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

34th Annual *Drosophila* Research Conference, 31 March - 4 April 1993, San Diego, California

The 34th Annual *Drosophila* Research Conference was held at the Town and Country Hotel, San Diego, California. The Program Chair was Gerry Rubin, University of California at Berkeley. The registrant list (as of 8 March 1993) was 989; additional individuals registered on site. The 357 page Program and Abstracts volume lists 11 plenary session talks, 72 slide presentations, and 610 poster presentations.

Larry Sandler Memorial Lecture:

Schneider, David S. (Department of Molecular and Cell Biology, University of California at Berkeley). Purification of a morphogen involved in dorsal-ventral patterning.

Plenary Lectures:

Mahowald, Tony (Department of Molecular Genetics and Cell Biology, University of Chicago). Cryobiological preservation of *Drosophila* embryos.

Jan, Lily (Department of Physiology and Biochemistry, University of California at San Francisco). Studies of potassium channels.

O'Farrell, Pat (Department of Biochemistry, University of California at San Francisco). Developmental control of the cell cycle.

Tjian, Robert (Department of Molecular and Cell Biology, University of California at Berkeley). Assembly of transcriptional regulators, coactivators and basal factors into an initiation complex: lessons from *Drosophila*.

Levine, Mike (Biology Department, University of California, San Diego). Transcription stripes in the embryo.

Montell, Denise (Department of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland). Border cells: a model system for the study of cell differentiation and migration.

Karpen, Gary (Department of MBVL, The Salk Institute, La Jolla, California). Molecular genetic analysis of chromosome inheritance.

Evans, Ron (The Salk Institute, La Jolla, California). Functional studies on nuclear receptors in *Drosophila*.

Simon, Mike (Stanford University, Stanford, California). Signaling through the Sevenless protein tyrosine kinase receptor.

Anderson, Kathryn (Department of Molecular and Cell Biology, University of California at Berkeley). Dorsal-ventral patterning in embryogenesis.

Lehmann, Ruth (Howard Hughes Medical Institute, Whitehead Institute, Cambridge, Massachusetts). Germline determination and pattern formation.

***Drosophila* Population Biology, 21 - 24 September 1992, Bodington Hall,
The University, Leeds, LS2 9JT UK.**

This 3-day meeting, organized by A.J. Davis of the *Drosophila* Population Biology Unit (headed by Professor Bryan Shorrocks), Pure and Applied Biology, University of Leeds, covered wild *Drosophila* and their interactions with the parasitoids and predators, behaviour, taxonomy, molecular biology and genetics, but excluded studies that were purely molecular biological or genetic. Biologists working with drosophilids are poorly served by the European *Drosophila* Congresses (which are dominated by molecular biology) and this meeting provided them with a forum.

Dr. Andrew J. Davis, *Drosophila* Population Biology, Pure and Applied Biology, The University, Leeds, West Yorkshire, LS2 9JT UK.

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email: PAB6WD@UK.AC.Leeds.CMS1 (Janet network)

Aspi, Jouni (Department of Genetics, University of Oulu, Oulu, 90570 Finland). Reproductive ecology of the boreal *Drosophila virilis*-group species.

Beuk, Paul L. Th.¹ and Brian R. Pitkin² (¹Natuur Historische Museum, Raamsteeg, Leiden, Netherlands. ²Natural History Museum, South Kensington, London SW7 5BD UK). A new identification key for British Drosophilidae.

Bouletreau-Merle, J. (Laboratoire de Genetique des Populations (associé au CNRS), Université Claude Bernard, Lyon I, 69622 - Villeurbanne, France). Clinal and seasonal modifications in the reproductive potential of *D. melanogaster*.

Burla, Hans and Gerhard Bachli (Zoological Museum, University of Zurich-Irchel, Winterthurerstrasse 190, 8057 Zurich, Switzerland). Aggregated reproduction.

Cariou, M.L., J.L. Da Lage, A. Tadlaoui and E. Chanut (Laboratoire de Biologie et Genetique Evolutive, CNRS, 91198 Gif sur Yvette, France). The amylase gene family in *Drosophila*: Evolution and regulation.

David, J.R., B. Moreteau, A. Alonso-Moraga and P. Capy (Laboratoire de Biologie et Genetique Evolutive, CNRS, 91198 Gif sur Yvette, France and Department of Genetics, University of Cordoba, 14071 Cordoba, Spain). Phenotypic plasticity helps to demonstrate the microspatial patchiness of natural populations.

Davis, A.J., M.E. Varley, R.H.A. Baker and I.C.W. Hardy (*Drosophila* Population Biology, Pure and Applied Biology, The University, Leeds LS2 9JT UK, 18 Apsley Road, Oxford OX2 7QY UK, CSL-MAFF, Hatching Green, Harpenden AL5 2BD UK, and Population Biology, Leiden University, 2300 RA Leiden, Netherlands). Distribution of parasitoids of *Drosophila* in the British Isles.

Davis, A.J. and B. Shorrocks (*Drosophila* Population Biology, Pure and Applied Biology, The University, Leeds, West Yorkshire, LS2 9JT UK). Species coexistence in *Drosophila*: The role of oviposition behaviour.

Davis, Andrew J. and Tong-Xu Peng (*Drosophila* Population Biology, Pure and Applied Biology, The University, Leeds, LS2 9JT UK, and Institute of Entomology, Guangzhou, Guangdong, P.R. China). Guild size, aggregation and coexistence of *Drosophila* exploiting fruit in subtropical forests of Guangdong, Southern China.

van Dijken, M.J. and J.J.M. van Alphen (Department of Population Biology, University of Leiden, P.O. Box 9516, 2300 RA Leiden, The Netherlands). The ecological significance of differences in the searching behaviour of two coexisting species of *Drosophila* parasitoids.

Dubreuil, Gladys Ruiz¹, Barrie Burnet² and Kevin Connolly² (¹Austral University, Valdivia, Chile. ²University of Sheffield, England). Aggregated oviposition by *Drosophila melanogaster*: Adult and larval behaviour in populations under divergent selection.

Dytham, C. and B. Shorrocks (*Drosophila* Population Biology Unit, The University, Leeds, LS2 9JT UK). Maintaining polymorphisms in *Drosophila* populations: A cellular automaton model.

Eijs, Irene (University of Leiden, Department of Population Biology, Kaiserstraat 63, P.O. Box 9516, 2300 RA Leiden, The Netherlands). Niche differentiation in *Drosophila* parasitoids: The place of *Leptopilina australis*.

Kraaijeveld, Alex R. and Jacques J.M. van Alphen (University of Leiden, Department of Population Biology, Kaiserstraat 63, P.O. Box 9516, 2300 RA Leiden, The Netherlands). *Asobara tabida* vs. *Drosophila melanogaster*: A coevolutionary arms race?

- Lachaise, Daniel (Laboratoire de Biologie et Genetique Evolutive, CNRS, 91198 Gif sur Yvette cedex, France). Sperm diversity and evolution in *Drosophila*.
- Law, Graham (*Drosophila* Population Biology Unit, Pure and Applied Biology, The University, Leeds, LS2 9JT UK). *Drosophilidae* exploiting rotting plants in a Scottish wetland habitat: Occurrence and distribution over patches.
- Lemeunier, F., J.L. Da Lage, S. Aulard, M. Arienti and L. Tsacas (Laboratoire de Biologie et Genetique Evolutive, CNRS, 91198 Gif sur Yvette cedex, France). Insular speciation: The new *Drosophila ercepeae* complex.
- McNamee, S. and C. Dytham (*Drosophila* Population Biology Unit, Department of Pure and Applied Biology, Baines Wing, University of Leeds, Leeds, LS2 9JT UK). Morphometric discrimination of the sibling species *Drosophila melanogaster* (Meigen) and *Drosophila simulans* (Sturtevant) (Diptera: *Drosophilidae*).
- Mitchell, Paul (School of Sciences, Staffordshire University, Stoke on Trent, ST4 2DE UK). *Drosophila* generation time and resource turnover time as factors affecting the stability of competitive coexistence.
- O'Brien, Lynne and David Coates (Pure and Applied Biology, The University, Leeds, LS2 9JT UK). First catch your fly ... then squash it.
- Pitkin, Brian R. (Department of Entomology, Natural History Museum, South Kensington, London SW7 5BD UK). Host preferences of *Scaptomyza* flies in Britain.
- Sevenster, Jan G. and Jacques J.M. van Alphen (University of Leiden, Department of Population Biology, Kaiserstraat 63, P.O. Box 9516, 2300 RA Leiden, The Netherlands). A life-history trade-off in *Drosophila* species and community structure in a variable environment.
- Shorrocks, Bryan (*Drosophila* Population Biology Unit, Department of Pure and Applied Biology, University of Leeds). Coexistence in *Drosophilids*: The role of time and space.
- Shorrocks, Bryan (*Drosophila* Population Biology Unit, Department of Pure and Applied Biology, University of Leeds). Priority experiments with *Drosophila*: Some laboratory and field experiments.
- Speirs, Douglas (University of Dundee, Dundee, Scotland). Parasitism and competition in population cages (poster).

The 1992 Midwest *Drosophila* Conference, 9 - 10 October 1992, Allerton Park, Illinois

The 1992 Midwest *Drosophila* Conference was organized by Hugh Robertson, Department of Entomology, University of Illinois, Urbana, IL 61801.

- Eissenberg, Joel, Thomas Hartnett and James Powers (St. Louis University). Molecular genetics of HP1, an essential heterochromatin-associated protein with dosage-dependent effects on position-effect variegation.
- Roseman, Robin, and Pamela Geyer (University of Iowa). Effects of mutations in the *suppressor of Hairy-wing* locus on formation of heterochromatin.
- Lampe, Dave, and Judy Willis (University of Illinois). A cuticular protein gene from the *Cecropia* moth.
- Robertson, Hugh (University of Illinois). The *mariner* transposon in other insects.
- Dellavalle, Robert, and Susan Lindquist (University of Chicago). *Hsp70* mRNA processing and turnover.
- Nogueron, Isabel, Jeffrey Otto, and Gail Waring (Marquette University). Processing of *dec-1* eggshell gene products in *D. melanogaster* and related species.
- Stark, William, Gary Brown, De-Mao Chen, J. Christianson, and Ronny Lee (St. Louis University). Does glycosylation deficiency interfere with rhodopsin synthesis or routing?
- Granok, Howard, and Sarah Elgin (Washington University). The role of GAGA factor in chromatin structure and gene expression of *hsp26*.
- Rogulski, Ken, and Iain Cartwright (University of Cincinnati). Multiple interacting elements confer ecdysone responsiveness within the small heat shock gene locus.
- Blackman, Ron, Dave Bergstrom, Chris Garwacki, and Rich Shelby (University of Illinois). *dpp*, *tnd*, enhancers, promoters and other good stuff.
- Zahner, Joe, Sarah Tanenbaum, and Chris Cheney (Washington University). *quartet*, small GTPases, and localization of *nanos* mRNA.
- McCormick, Jenny, and Ruthann Nichols (University of Michigan). Developmental expression of the neural peptide TDVDHVFLRFamide.
- Wong, Lei, and Doug Coulter (St. Louis University). An *odd-skipped* homolog which is expressed in embryonic termini.
- Lu, Quin, Lori Wollrath, and Sarah Elgin (Washington University). Misalignment of a positioned nucleosome disrupts chromatin structure and expression of the *hsp26* gene.

- Shaffer, Chris, Carolyn Craig, Robert Clark, and Sarah Elgin (Washington University). Heterochromatin protein 1, a highly conserved protein, is a critical component for position effect variegation.
- Roseman, Robin, Eric Johanson, Chris Rodesch, Mike Bjerke, Rod Nagoshi, and Pamela Geyer (University of Iowa). Increased mutagenic rates by mobilization of a P element containing the *suppressor of Hairy-wing* binding region of the *Gypsy* retrotransposon.
- Eunkyung, Bae, Scott Patton, Pamela Geyer, and Rod Nagoshi (University of Iowa). Interactions between genes that regulate somatic and germ line sexual differentiation.
- Flickinger, Tom, Beth Mittendorf, and Helen Salz (Case Western University). Molecular analysis of *deadhead*, a maternal-effect gene required for head development.
- Romanelli, Anthony, Jennifer Soriano, Ian Boussy, and Ron Woodruff (Loyola University). P elements in natural populations: the eastern Australian P-Q-M cline revisited.
- Jupe, Eldon, and Iain Cartwright (University of Cincinnati). Microdomain of unrestrained supercoiling at a heat shock gene locus.
- Noel, Patricia, and Iain Cartwright (University of Cincinnati). Characterizing a novel gene from chromosomal locus 31A.
- Hart, Marilyn, Lei Wong, and Doug Coulter (St. Louis University). Similarities between *odd-skipped* and a closely linked gene: will the real pair-rule gene please stand up.
- Albrecht, Elizabeth, and Helen Salz (Case Western University). The *snF* gene is required to establish the female-specific splicing pattern of *Sex-lethal*.
- Kapoun, Ann, Filippo Randazzo, and Thomas Kaufman (Indiana University). Identification of positive, negative, and pairing sensitive regions of the homeotic gene *proboscipedia*.
- Kelsey, Cynthia, and Robert Holmgren (Northwestern University). Localization of the *cubitus interruptus* Dominant product and its role in development.
- Parks, Annette, P.J. Kooh, Rudi Truner, and Marc Muskavitch (Indiana University). *Delta* dynamics during developmental decisions.
- Chu-LaGraff, Quynh, and Chris Doe (University of Illinois). *wingless* controls neuroblast formation in the CNS.
- Nichols, Ruthann, Indy Lim, and Liz Norris (University of Michigan). Expression and activity of the *drosulfakinin* (*dsk*) gene and related genes.
- Incardona, John, and Terry Rosenberry (Case Western University). Cell biology of acetylcholinesterase.

Announcements

National *Drosophila* Species Resource Center Stock List

Kathleen Matthews, University of Indiana.

The stock list of the National *Drosophila* Species Resource Center at Bowling Green is now available on FlyBase. See the document [flybase/stocks/stock-centers/species-center.doc](#) for further information. Instructions for using FlyBase are given in the *Drosophila* Information Newsletter (DIN) reprints that follow.

Guide to Contributors

Drosophila Information Service prints short research and technique articles, descriptions of new mutations, stock lists, directory information, and other material of general interest to *Drosophila* researchers. The current publication schedule for regular issues is annually in July. Special issues will also be prepared on an irregular schedule.

Manuscripts, orders, and inquiries concerning the regular annual DIS issue should be sent to James Thompson at the address at the front of this issue.

Submission: Submissions are accepted at any time, but the deadline for the annual July issue will be about 1 April or until the issue is full. To help minimize editorial costs, proofs will not be sent to authors, unless there is some question that needs to be clarified or they are specifically requested by the author at the time of submission.

Manuscripts should be submitted in duplicate. If possible, a 5.25" or 3.5" diskette with the manuscript in ASCII, in a major IBM-compatible word processing format such as WordStar 2000 or WordPerfect, or in Macintosh Word would

be very much appreciated.

Format: Manuscripts are now being entered into computer files so that a diskette version can eventually be distributed with the printed copy. In order to make the text as simple as possible for different printer formats and search commands, we have tried to minimize the use of special symbols (e.g., μ is used for microliter, "female" and "male" are written out in the text in place of the short-hand symbols, Greek letters are written out, and so forth). The meaning should be clear from the context in all cases.

Citation of References: Citation should be by name and date in the text of an article (Smith, 1989; Smith et al., 1990). At the end of the article, references should be listed alphabetically by author; titles will not be included except for books, unpublished theses, and articles in press.

Stock Lists, Specialized Bibliographies, and Long Technical Articles: Long or complex material can generally not be accepted unless it is submitted on diskette, with a printed copy for editorial guidance. Special justification is needed for material, such as bibliographic lists, that are often readily available by other means. A page charge of \$30.00 per page will be requested to help defray publication costs of accepted material. Inquiries about format and content are welcomed.

Figures: Both line drawings and half-tone illustrations will be accepted, but we request a special charge of \$10.00 per half-tone illustration to help cover the cost of their preparation for printing.

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Special Issues of Drosophila Information Service

DIS 69 (1991) is a special issue. As long as copies are available, it can be obtained for \$13.00 plus shipping by sending payment to: William Gelbart, Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138-2097 U.S.A.

DROSOPHILA INFORMATION NEWSLETTER

The following material is reprinted from DIN volumes 6-10, appearing between April 1992 and April 1993. The complete tables of contents of all five issues are included here, but out of date material, or material expected to be covered elsewhere in this issue, has not been reprinted.

The Drosophila Information Newsletter has been established with the hope of providing a timely forum for informal communication among Drosophila workers. The Newsletter will be published quarterly and distributed electronically, free of charge. We will try to strike a balance between maximizing the useful information included and keeping the format short; priority will be given to genetic and technical information. Brevity is essential. If a more lengthy communication is felt to be of value, the material should be summarized and an address made available for interested individuals to request more information. Submitted material will be edited for brevity and arranged into each issue. Research reports, lengthy items that cannot be effectively summarized, and material that requires illustration for clarity should be sent directly to Jim Thompson for publication in DIS. Materials appearing in the Newsletter will be reprinted, in unedited form to the extent space permits, in the next issue of DIS. Back issues of DIN are posted on the Indiana fileservers in the directory fly/news. Material appearing in the Newsletter may be cited unless specifically noted otherwise.

Material for publication may be submitted in any of the following formats - Macintosh Microsoft Word or MacWrite, MS-DOS WordPerfect, or text/ASCII file. Figures and photographs cannot be accepted at present. Send material, in order of preference, as E-mail (addresses below), on floppy disk, or as laserwriter or typed hard-copy (not bit-mapped). Technical notes should be sent to Carl Thummel, all other material should be sent to Kathy Matthews. The e-mail format does not allow special characters to be included in the text. Both superscripts and subscripts have been enclosed in square brackets; the difference should be obvious by context. Bold face, italics, underlining, etc. cannot be retained. Please keep this in mind when preparing submissions. To maintain the original format when printing DIN, use Courier 10cpi font on a standard 8.5" x 11" page with 1" margins.

Drosophila Information Newsletter is a trial effort that will only succeed if a broad segment of the community participates. If you have information that would be useful to your colleagues, please take the time to pass it along.

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ANNOUNCEMENTS VOL. 6-10

13th EUROPEAN DROSOPHILA RESEARCH CONFERENCE

Dear Colleague, It may come as a surprise to you to find the first circular of the 13th EDRC in your mailbox some 20 months before it takes place, but the organizing committee felt that it would be necessary to contact you as soon as possible. As you may already have heard, our Swiss colleagues had to give up their plans to organize the next EDRC, due to unforeseen and unsolvable organizational problems at the University of Zurich. The Crete laboratory was asked whether it could take over, and we agreed. The 13th EDRC is, thus, scheduled for September 12-17, 1993 in Crete.

Since the month of September is still high season on the island, it is extremely difficult for us, at this time, to let you know the exact location of the Conference. We are trying our best to find accommodation for the constantly rising number of European drosophilists and also to guarantee low prices for rooms in a place that will be conveniently close to an airport. This requires long searching and a lot of bargaining on our side. We understand that scientists would like

to keep their freedom of scheduling their attendance until the very last moment but these circumstances make it mandatory for us to plan a long way ahead. Therefore, in our next circular, which will be mailed fall 1992, we will ask for a preregistration, i.e. a more or less final decision as to whether you would like to attend, and whether you would want us to book hotel rooms for you. After the final registration deadline in February or March 1993 we will no longer be in a position to offer any help with lodging and you will be on your own. Bear in mind, though, that during last year most of the hotels in Crete were overbooked until the beginning of November.

Finally, you will also have noticed that the Conference will last one day longer than usual. This means that there will be more opportunities for oral presentations. Although, again, no final commitments will be required we would welcome your preferences in this matter (poster or oral presentation) soon after the second circular has been received by you. For more information please contact:

13th European Drosophila Research Conference
c/o Dr. K. Louis
Insect Molecular Genetics Group
Institute of Molecular Biology and Biotechnology
P.O.Box 1527, GR-711 10 Heraklion, Crete, Greece
fax: 0030-81-231308
E-mail: FLIES@GRIMBB or FLIES@MYIA.IMBB.FORTH.GR

AN OPEN LETTER FROM JAMES CROW

There is a growing concern among population geneticists regarding their communications with non-population geneticists, a problem which is perceived to affect the process of reviewing research grant applications. An ad hoc committee has been formed to prepare a series of mini-reviews that set forth the strengths and accomplishments of the various subjects and methods in population genetics. This is seen as a useful exercise for those in the field, as well as providing information for outsiders who must form judgements about research projects.

The effort is being coordinated by Bruce Weir; other members of the committee are Francisco Ayala, Allan Campbell, Brian Charlesworth, Michael Clegg, Marcus Feldman, Daniel Hartl, Mary Claire King, Martin Krietman, Charles Langley, Richard Lewontin, Glenys Thomson, and Bruce Walsh. For information on submitting material for these reviews before the July 31, 1992 deadline, please contact: Dr. Bruce Weir, Dept. of Statistics, North Carolina State U., Raleigh, NC 27695-8203, (919)515-3574, FAX/7591.

ENHANCER-TRAP STOCKS AVAILABLE

Dan Lindsley has made a set of 232 enhancer-trap stocks carrying autosomal mini-w[+] inserts (Bier et al., Genes & Dev. 3:1273) available through the Bloomington stock center. The stocks were generated by undergraduate students and are a random set (i.e., nothing has been removed based on phenotype). 84 lines (42 on 2, 42 on 3) express lacZ in the testis. Of the remaining 148 lines, 80 have inserts on 2 and 68 have inserts on 3. These stocks will be maintained by the stock center for a limited time. If you are interested in screening these lines you should plan to obtain them from the stock center within the next 18 months.

DROSOPHILA NEWSGROUP ON BIOSCI

A BIOSCI/Bionet newsgroup for discussion of Drosophila biology has been proposed. At this writing, the vote is not yet complete. The following assumes that sufficient YES votes will be received by BIOSCI to establish the group. The DROSOPHILA group will not replace the DIN mailing list at present, but a copy of each DIN issue will be posted to this group, so that you can read it via Usenet should you prefer. It is hoped that this newsgroup will foster more active discussion of Drosophila research methods and materials. A newsgroup with similar orientation for Arabidopsis researchers is quite successful in meeting needs of rapid information exchange between labs on methods, materials and questions of scientific interest in this area. Michael Ashburner, Dept. of Genetics, U. of Cambridge, Cambridge, England (MA11@GEN.CAM.AC.UK), will serve as the Discussion Leader and be "responsible for ensuring a reasonable level of activity". The name of the group will be bionet.genome.drosophila (on Usenet) or bionet.drosophila or DROSOPHILA (on mailing lists). Discussions of non-genetic as well as genetic aspects of Drosophila are encouraged.

If you are not a current reader of these BIOSCI/Bionet newsgroups, here are some instructions for starting out. Please ***DO NOT*** send requests for newsgroup subscriptions or information about BIOSCI/Bionet to the DIN editors

- we cannot help you. You MUST direct your requests to BIOSCI at the addresses provided below.

New users of BIOSCI/bionet may want to read the "Frequently Asked Questions" or "FAQ" sheet for BIOSCI. The FAQ provides details on how to participate in these forums and is available for anonymous FTP from net.bio.net [134.172.2.69] in pub/BIOSCI/biosci.FAQ. It may also be requested by sending e-mail to biosci@net.bio.net (use plain English for your request). The FAQ is also posted on the first of each month to the newsgroup BIONEWS/bionet.announce immediately following the posting of the BIOSCI information sheet.

Most of the following information is from the BIOSCI ELECTRONIC NEWSGROUP NETWORK INFORMATION SHEET for the Americas and Pacific Rim countries. If you are located in Europe, Africa, or Central Asia, please request that version of the BIOSCI information sheet by sending e-mail to the Internet address: biosci@net.bio.net.

Introduction: The BIOSCI newsgroup network was developed to allow easy worldwide communications between biological scientists who work on a variety of computer networks. By having distribution sites or "nodes" on each major network, BIOSCI allows its users to contact people around the world without having to learn a variety of computer addressing tricks. Any user can simply post a message to his/her regional BIOSCI node and copies of that message will be distributed automatically to all other subscribers on all of the participating networks, including the Internet, USENET, BITNET, EARN, NETNORTH, HEANET, and JANET.

E-mail Subscription Requests and other Information: If you need to receive BIOSCI messages by e-mail, please send all subscription requests, subscription cancellations, or any other questions about using BIOSCI to the Internet address: biosci@net.bio.net (Americas & Pacific) or biosci@daresbury.ac.uk (Europe). As your request will be read by a human, there is no need for special syntax in your message. Simply state the name of the newsgroup to which you would like to subscribe.

Please be sure to use the biosci address above and ****DO NOT**** request subscriptions by posting messages to the newsgroup mailing addresses. Posting to the newsgroup addresses sends copies of your request to hundreds of people around the world and wastes network resources. **PLEASE NOTE THAT IF YOU HAVE ACCESS TO USENET NEWS YOU DO NOT NEED AN E-MAIL SUBSCRIPTION!!** Simply read and post to the newsgroups in the "bionet" newsgroup hierarchy using your USENET news software (e.g., readnews, rn, vnews, ANU-NEWS, postnews).

All interested users are strongly encourage to explore getting usenet news software at your site. The software is in the public domain, and you will find it much more convenient than subscribing to newsgroups by e-mail. Please consult your systems manager or contact biosci@net.bio.net for assistance if needed.

Canceling E-mail Subscriptions: If you have subscribed to a newsgroup and are now leaving an institution or changing your e-mail address, it is **IMPERATIVE** that you send a note to biosci@net.bio.net and cancel your subscription! Non-existent addresses or overflowing mailboxes cause computer mail programs to send back "daemon" messages which might bother everybody on the newsgroup. We will immediately remove any address causing such a problem, but would prefer it if you would notify us in advance as a courtesy to the rest of the user community. (The DIN editors would greatly appreciate the same courtesy. Please see instructions at the beginning of this issue for unsubscribing to DIN).

Posting Messages to Newsgroups: Messages can either be posted into the USENET newsgroups using "postnews" or similar software or they can be submitted by electronic mail. To post a message to the Drosophila group by e-mail send your message to dros@net.bio.net (Americas, Asia, Pacific) or dros@daresbury.ac.uk (Europe and Africa). Messages to the Drosophila group will be posted directly to the newsgroup without editorial intervention.

USENET users who use the "postnews" or similar software on their local computer should be sure to set the message distribution to "world" or "bionet" or else your message may not be distributed beyond your local computer. USENET newsgroups are read using, e.g., the "readnews," "rn," or "vnews" software on UNIX systems. USENET news software is in the public domain and is available for most UNIX systems. A public domain USENET news software package named ANU-NEWS is also available for VAX/VMS systems. Your local BIOSCI node can point you towards acquiring the software for use on your computer system.

EUROPEAN DROSOPHILA RESEARCH CONFERENCE

We would like to remind all interested drosophila workers that the deadline for registration for the 13 European Drosophila Research Conference was Monday, March 15. Those of you who forgot to register have still the opportunity to do so at the regular fee, if we get the complete registration by April 5. Participants of the us fly meeting can register there by giving the registration form and a cheque for the conference fees to Dr. Michael Ashburner. The complete registration package can be obtained by requesting it from: FLIES@GRIMBB.BITNET or FLIES@MYIA.IMBB.FORTH.GR or from Michael at San Diego.

Registration Fees (in US\$)

| | BEFORE APRIL 5 | | AFTER APRIL 5 | |
|---------------------|----------------|-----------|---------------|------------|
| | DR | SR | DR | SR |
| Regular | 725 (480) | 850 (665) | 900 (665) | 1150 (790) |
| Student | 600 (400) | NA | 790 (480) | NA |
| Accompanying Person | 545 (360) | NA | 665 (400) | NA |

Figures in brackets: Minimum Down Payment, DR: Double Room, SR: DR Single Occupancy
WE HOPE TO SEE YOU IN CRETE -- KITSOS LOUIS.

COMMENTARY VOL. 6-10

ARE THE ANNUAL DROSOPHILA RESEARCH MEETINGS GETTING TOO BIG?

Frank M. Butterworth, Oakland U., Rochester, MI. BUTTERWO@VELA.ACS.OAKLAND.EDU.

Yes. They are not only too big, but also they are too expensive. My recommendation is to limit the size either by having a registration cut-off number or limiting the number of attendees from a given lab, OR having several simultaneous meetings with maybe only 4-5 specific topics instead of 10-15. Maybe you'll have to do all three. If you have to have concurrent sessions, the meeting is still too big. They should be given in cheaper places such as university campuses which rent out their facilities in the summer. There is absolutely no reason why we have to put so much financial stress on underfunded scientists and students.

For the past decade the meetings have slowly but inexorably become more populous and are slowly losing their ability to do what they're supposed to: namely to provide an EFFECTIVE vehicle for the EFFICIENT exchange of information. At the risk of sounding condescending, meetings to be EFFECTIVE AND EFFICIENT must be limited in size - limited numbers of plenary talks with NO concurrent sessions, limited costs, limited numbers of attendees, and limited space. These limitations would keep people in close proximity during meals and coffee breaks and assure plenty of time for people to meet as many others as they wish and plenty of time for leisurely discussion.

It is a lot of work to host and plan a meeting, and I think the makers and shakers of the "fly society" are doing a great job (thanks folks!), but at the same time are losing track of things. We all know what makes a good meeting, but I feel our leaders need some feedback from us followers. After all aren't we the ones who make you leaders?

The fly meetings are popular because they are good. However they WERE great! Now I feel they have reached the point where they no longer perform their intended function. If small meetings are good, why are big meetings bad? Because they are incredible time wasters. At the last ASCB meeting I attended, I spent at least fifty percent of my time going somewhere, trying to get to a concurrent session, trying to find someone, trying to make reservations for dinner, traveling around in cabs and shuttle buses. Next time you're at a large meeting stop for a moment and watch. What do you see? Weary people by the hundreds walking back and forth, almost aimlessly. Not talking science, but walking! Walking is good, but this is ridiculous. Big meetings also create information overload. In one day you can get enough to saturate your scientific curiosity receptors. In two days you're reduced to the ranks of the aimless walkers. I will bet that if you can set up a science exchange efficiency quotient (SEEQ) you'd find that big meetings will get a low score, small meetings will get a high score. Its time to limit the annual meeting size. Thanks for reading this. I hope in the next DIN we get more dialogue on this topic.

[Editors' note: More discussion of this issue is welcome. However, the people who make decisions about meeting formats are the members of the Drosophila board. Your opinion about the annual meeting should be conveyed to your regional representative to the board. See DIN Vol. 2 or DIS for the name and address of your representative.]

REQUESTS FOR MATERIALS VOL. 6-10

NATURAL ORCEIN

Michael Ashburner, Dept. of Genetics, U. of Cambridge, Downing St., Cambridge CB2 3EJ, England. 44-223-333969, FAX/333992, MA11@PHX.CAM.AC.UK.

Does anyone know a source for Natural Orcein? Gurr's (that is BDH) now only stock synthetic. If you let me know directly then I will broadcast the information in the next issue of the Newsletter. Thank you.

SURVIVAL DATA

Matthew Witten, UT System Center For High Performance Computing, Balcones Research Center, 1.154 CMS, 10100 Burnet Rd., Austin, TX 78758-4497, USA. 512-471-2472, FAX/2445 or 2449, M.WITTEN@HERMES.CHPC.UTEXAS.EDU or M.WITTEN@UTCHPC.BITNET.

I am actively involved in a long term project on the analysis of survival data. Many people use *Drosophila* and keep survival distributions under various experimental conditions. I am collecting multispecies datasets on survival of all species and would welcome receipt of reprints/preprints/actual data. We have an electronic submission system SURVIVAL@CHP.UTEXAS.EDU or SURVIVAL@HERMES.CHPC.UTEXAS.EDU. If you have any questions, please contact me.

REDBOOK WANTED

Roger J. Harris, Dept. Biology, U. of Oregon, Eugene, OR 97403, USA. 503-346-5090, FAX/2364, RJHARRIS@OREGON.UOREGON.EDU.

I would like to purchase a used copy of "Genetic variations of *Drosophila melanogaster*" by Lindsley & Grell (1968), Carnegie Institute Publications No. 627. I will pay any reasonable price. If your lab has a spare copy please contact me.

UPDATES AND CORRECTIONS TO THE REDBOOK

Kathy Matthews, Dept. of Biology, Indiana U., Bloomington, IN 47405. 812-855-5782, FAX/2577, MATTHEWK@UCS.INDIANA.EDU.

As you probably know, the new Redbook, "The Genome of *Drosophila melanogaster*" by Dan Lindsley and Georgianna Zimm, Academic Press, San Diego, is now available (call 1-800-321-5068 to order directly from the publisher). Dan and Georgianna have done an admirable job. The book is a pleasure to use, and all of our scientific lives are made easier by having access to the vast amount of information it contains. Producing this volume was a huge task and future compilations will be increasingly difficult. Dan and Georgianna have passed the curatorial torch on to Michael Ashburner who, for reasons unknowable, is willing to perform this vital service to our community. Nevertheless, Michael cannot do it alone. We as individuals need to take an active role in updating information in our fields of expertise. With the hope of making that a bit easier, I am asking that you submit new/updated/corrected Redbook information to me by e-mail (only!). I will post your information on the stock center file server where it will be available to Michael and the rest of us as well. Corrections, new mutations, and other appropriate information that you provide will also be published in DIN.

WILD STRAINS OF DROSOPHILA MELANOGASTER

John Ringo, Dept. Zoology, U. of Maine, Orono, ME 04469, USA. 207-581-2556, FAX/2537, RINGO@MAINE.

I am seeking wild strains of *Drosophila melanogaster* captured above the 45th parallel. The higher the latitude the better. I hope to obtain strains from a wide range of geographic locations. Both flies and information about whom to contact directly for flies will be deeply appreciated.

UNSTABLE RING X

Kathy Matthews, Dept. of Biology, Indiana U., Bloomington, IN 47405, USA. 812-855-5782, FAX/2577, MATTHEWK@INDIANA.EDU.

The Bloomington Stock Center would like to have a line of R(1)2, w[vC] that is sufficiently unstable to provide a useful number of mosaics, but not so unstable that it is extremely difficult to maintain. If you have such a line, please call me.

LABORATORY STOCK LISTS WANTED

Kathy Matthews, Dept. of Biology, Indiana U., Bloomington, IN 47405-6801, USA. 812-855-5782; FAX/2577; MATTHEWK@INDIANA.EDU.

One goal of the FlyBase project (see below) is to simplify the process of identifying potentially useful mutations and then locating stocks that carry those mutations. To this end, we would like to incorporate the stock collections of as

many individual laboratories into FlyBase as possible. If you are willing to make your laboratory stock list available, with the understanding that only stocks not available from any of the funded stock centers should be requested from your lab, please contact me. I would like to have computerized lists now for immediate incorporation into FlyBase. Hardcopy lists, if typed, are also useful; I will convert these to machine-readable format as time permits.

MATERIALS AVAILABLE VOL. 6-10

MOLECULAR CLONING OF GENOMIC DNA FROM THE 60CD REGION

Philip J. Gotwals and James W. Fristrom, Dept. of Molecular and Cell Biology, U. of California, Berkeley, CA 94720.

In two separate chromosomal walks, we have recovered 230 kilobases of genomic DNA in the chromosomal region uncovered by Df(2R)Px2 (60C1/2-60D9/10). One walk was initiated by jumping from the centromere-distal to the centromere-proximal breakpoint of Df(2R)Px2 using a beta3-tubulin probe (Kimble et al, (1991) Genetics 126:991). We have recovered nearly 100 kilobases of overlapping genomic DNA, primarily carried in cosmids, around the proximal breakpoint.

The other walk was initiated from within Df(2R)Px2 using a fragment from the muscarinic acetylcholine receptor (MAR) gene (Shapiro et al., (1989) PNAS 86:9030). We have recovered nearly 130 kilobases of overlapping DNA, housed in both phage and cosmids, surrounding the MAR gene.

Anyone interested in obtaining clones from these walks or information regarding the region should contact: Philip J. Gotwals, HHMI, Bld. E17-225, 40 Ames St., MIT, Cambridge, MA 02139, USA. (617) 253-6452; eMAIL: PJGOTWALS@wccf.mit.edu

COMPILATION OF DROSOPHILA CDNA AND GENOMIC LIBRARIES

Carl Thummel, HHMI, 5200 Eccles Institute of Human Genetics, Bldg. 533, U. of Utah, Salt Lake City, UT 84112, USA. 801-581-2937, FAX/5374, CTHUMMEL@HMBGMAIL.MED.UTAH.EDU

The following is a listing of Drosophila cDNA and genomic libraries that are currently available and in common use. Please do not request shipment of a library unless you have an immediate use for it - many contributors are concerned about the time and money involved in mailing their libraries. Also, please inquire with local colleagues before requesting a library since many of these libraries are already widely distributed.

cDNA LIBRARIES

--Nick Brown, Wellcome/CRC Institute, Tennis Court Rd, Cambridge CB2 1QR UK. Phone: 44-223-334128; FAX: 44-223-334089, Email: NB117@MB1.BIO.CAM.AC.UK

Vector/Insertion/Complexity/mRNA source

pNB40/see ref./3x10⁵/0-4 hr embryo
pNB40/see ref./3x10⁶/4-8 hr embryo
pNB40/see ref./3x10⁵/8-12 hr embryo
pNB40/see ref./1x10⁶/12-24 hr embryo
pNB40/see ref./3x10⁶/imaginal discs

The Drosophila strain used is an isogenic second chromosome stock: dp cn bw, from the Gelbart lab. Ron Blackman has made a genomic library from this same strain (see below). The vector is a pUC based plasmid with a SP6 promoter at the 5' end of the cDNA and a T7 promoter at the 3' end of the cDNA. The cloning strategy was directional and designed to maximize the number of full-length cDNAs. A useful diagnostic of full-length cDNAs is a non-coding G nucleotide at the 5' end, after the polyC tract; the origin of this nucleotide is, however, unknown.

Reference: Brown, N.H., and F.C. Kafatos (1988) Functional cDNA libraries from Drosophila embryos. J. Mol. Biol. 203: 425-437.

--Steve Russell, Dept. of Genetics, Univ. of Cambridge, Downing St., Cambridge, CB2 3EH UK. Phone: 44-223-337733, FAX: 44-223-333992, Email: SR120@MOLECULAR-BIOLOGY-1.BIOLOGY.CAMBRIDGE.AC.UK.BITNET

All libraries were made with RNA isolated from Oregon R strain
Vector/Insertion/Complexity/mRNA source

NM1149/RI/2x10⁶/Male 3rd instar larvae
NM1149/RI/6x10⁵/Female 3rd instar larvae
NM1149/Directional: RI-HIII/3x10⁶/Adult male heads
NM1149/Directional: RI-HIII/1x10⁶/Adult female heads
lambda gt11/RI/3x10⁵/Testes

--Charles P. Emerson, Jr. or Mary Beth Davis, Biology Dept., Univ. of Virginia, Charlottesville, Virginia, 22901 USA. Phone: 215-728-5283 (Emerson); 215-728-5284 (Davis); FAX: 215-728-2412, Email: emerson@castor.rm.fccc.edu or davis@castor.rm.fccc.edu

Vector/Insertion/Complexity/mRNA source/Titer

lambda gt10/RI/1x10⁶/late pupae/1x10¹⁰

Blunt-ended cDNA was ligated to EcoRI adaptors, then ligated to EcoRI digested gt10 lambda arms. We have isolated cDNA clones corresponding to MHC isoforms that were lengths of 5940 and 5500 bases.

Reference: George, E.L., M.B. Ober, and C.P. Emerson, Jr. (1989) Functional domains of the *Drosophila melanogaster* muscle myosin heavy-chain isoform are encoded by alternatively spliced exons. *Mol. Cell Biol.* 9: 2957-2974.

--Bruce Hamilton, Division of Biology 216-76, California Institute of Technology, Pasadena, CA, 91125, USA. Phone: 818-356-8353; FAX: 818-449-0756, Email: BAH@citromeo.bitnet or BRUCE@seqvax.caltech.edu

Library name/Vector/Insertion/Complexity/mRNA source

Head M/lambda EXLX/ApaI-SacI/1.1x10⁷/Oregon R adult heads
Head P/lambda EXLX/ApaI-SacI/9x10⁶/Oregon R adult heads
Head 1.2/lambda EXLX/ApaI-SacI/2.7x10⁶/Oregon R adult heads
Head 2.0/lambda EXLX/ApaI-SacI/1.2x10⁶/Oregon R adult heads
Adult/lambda EXLX/ApaI-SacI/>1x10⁶/Oregon R adults
0-24 mojo/lambda EXLX/ApaI-SacI/3.4x10⁶/Can S, 0-24 hr embryos

All libraries were cloned directionally into the ApaI-SacI sites of lambda EXLX, as described in ref. 1, with internal restriction sites protected. Lambda EXLX allows in vivo excision of plasmid DNA using a CRE/loxP site-specific recombination system. This vector also allows regulated expression of the insert DNA as a phage T7 gene 10 N-terminal/cDNA fusion protein, under the control of a T7 RNA polymerase promoter (1). The Head 1.2 library was prepared from cDNAs that were size-selected for molecules 1.2 kb or larger by fractionation through an agarose gel. Head 2.0 contains cDNAs that are 2 kb or larger. The cDNA for the Adult library was not size-fractionated. The Adult and mojo libraries were published in ref. 1. The Head M and Head P libraries are unpublished, but I have asked people who use them to refer to ref. 1, since they were constructed in the same way and in the same vector. The two size-selected libraries, Head 1.2 and Head 2.0 were published in ref. 2, which also describes a rapid screening procedure that is very straightforward.

References:

1. Palazzolo et al (1990) *Gene* 88, 25-36.
2. Hamilton et al (1991) *Nucl. Acids Res.* 19, 1951-1952.

--Tom Kornberg, Department of Biochemistry, University of California, San Francisco, CA 94143 USA. Phone: 415-476-8821, FAX: 415-476-3892, Email: tomk@ucsf.cgl.edu

Our cDNA libraries were prepared from RNA isolated from Oregon R animals, with the cDNA sequences inserted into the EcoRI site of lambda gt10. Libraries will be shipped by Federal Express. Requests should be accompanied by an appropriate Federal Express Authorization Number.

Stage/Library designation/Complexity

0-3 hr embryo/D/300,000
3-12 hr embryo/E/500,000
12-24 hr embryo/F/300,000
1st and 2nd instar/G/200,000
early 3rd instar/H/300,000
late 3rd instar/I/300,000
early pupal/P/300,000
late pupal /Q/300,000
adult male/R/300,000
adult female/S/300,000

Reference: Poole, S., Kauvar, L.M., Drees, B., and Kornberg, T. (1985) The engrailed locus of Drosophila: Structural analysis of an embryonic transcript. Cell 40: 37-43.

--Carl S. Thummel, Dept. of Human Genetics, 5200 Eccles Institute, Bldg. 533, University of Utah, Salt Lake City, Utah, 84112 USA. Phone: 801-581-2937, FAX: 801-581-5374, Email: cthummel@hmbgmail.med.utah.edu

Vector/Insertion/Complexity/mRNA source

lambda gt10/RI/1x10⁶/larval tissues cultured in vitro with cycloheximide + ecdysone
lambda ZAP/RI/3x10⁵/late 3rd instar larvae
lambda ZAP/RI/4x10⁵/0-1 day prepupae

The late third instar cDNA library is available in two size-fractionations that are enriched for either 1-3 kb or 3-6.5 kb cDNAs. Both libraries, however, do contain some smaller inserts.

--Peter Tolias, Public Health Research Institute, 455 First Ave., New York, New York, 10016 USA. Phone: 212-578-0815, FAX: 212-578-0804, Email: tolías@wombat.phri.NYU.EDU

Vector/Insertion/Complexity/mRNA source

lambda gt22A/SalI-NotI/5x10⁵/Canton S ovaries, stages 1-14
This is a cDNA expression library in which the inserts are directionally cloned. A SalI site is present at the 5' end and a NotI site is at the 3' end.

--Kai Zinn, Division of Biology, 216-76, Caltech, Pasadena, CA 91125, USA. Phone: 818-356-8352, FAX: 818-449-0679, Email: kai@seqvax.caltech.edu

Vector/Insertion/Complexity/mRNA source

lambda gt11/EcoRI/1.2x10⁶/Oregon R, 9-12 hr embryos

The complexity is an underestimate for larger cDNAs, since it was >5X size-selected for cDNAs larger than 1.8 kb. The complexity could thus be as high as 6×10^6 for these larger inserts.

GENOMIC LIBRARIES

--Winifred W. Doane, Dept. of Zoology, Arizona State University, Tempe, Arizona 85287-1501 USA. Phone: 602-965-3571, FAX: 602-965-2012, Email: icwwd@asuacad

Vector/Insertion/Complexity/DNA source

pWE15/BamHI/4x10⁴-1x10⁶/Amy[1,6] mapP[12] strain of *D. melanogaster*

This cosmid vector contains a T3 and T7 promoter on either side of the insertion site, to facilitate the preparation of end-specific probes for chromosomal walking.

Reference: Thompson, D.B., and Doane, W.W. (1989) A composite restriction map of the region surrounding the Amylase locus in *Drosophila melanogaster*. *Isozyme Bull.* 22: 61-62.

--Ron Blackman, Dept. of Cell and Structural Biology, 505 S. Goodwin Ave., Univ. of Illinois, Urbana, Illinois 61801 USA. Phone: 217-333-4459, FAX: 217-244-1648, Email: Ron_Blackman@qms1.life.uiuc.edu

Vector/Insertion/Complexity/DNA source

lambda EMBL3/BamHI/1x10⁶/Adult *Drosophila virilis*

lambda EMBL3/BamHI/1x10⁶/Embryonic *D. melanogaster*, see below

Both libraries were prepared by MboI partial digestion of the DNA and insertion into the BamHI site of lambda EMBL3. The inserts can be excised by digestion with SalI. Titer is approximately 5×10^9 pfu/ml. The *D. melanogaster* genomic library is made from animals that are isochromosomal for chromosome 2, dp cn bw. The same strain was used by Nick Brown for his cDNA libraries.

--Howard Lipshitz, Division of Biology, 156-29, California Institute of Technology, Pasadena, CA 91125, USA. Phone: 818-356-6446, FAX: 818-564-8709, Email: HDL@ROMEO.CALTECH.EDU

Vector/Insertion/Complexity/DNA source

Charon 4/EcoRI/6x10⁵/Canton S embryos

This is the original *Drosophila* genomic library from the Maniatis lab. It has been amplified several times but is still useful for most purposes.

Reference: Maniatis et al., The isolation of structural genes from libraries of eucaryotic DNA. *Cell* 15: 687-701.

DATABASES/COMPUTING VOL. 6-10

MORPHOLOGICAL MUTANTS OF DROSOPHILA MELANOGASTER: A PRELIMINARY DATABASE FROM THE 'RED BOOK'

Roger J. Harris, Dept. of Biology, U. of Oregon, Eugene OR 97403-1210, USA. 503-346-5090, FAX/2364, RJHARRIS@OREGON.UOREGON.EDU.

Lindsley and Grell's (1972) 'Genetic variations of *Drosophila melanogaster*', also known as the 'Red Book', is a unique tool for geneticists. Besides 3000 or so mutants there are lists for chromosome aberrations, special chromosomes

and other genetic variants from a 'wild-type' genotype. The mutation data is listed by code, name, location, origin, discoverer, references, phenotype and cytology. The salivary chromosome drawings and accompanying gene map enable the worker to quickly locate a mutant phenotype to a particular chromosomal region.

Recently, the 'Red Book' has been updated (by Lindsley and Zimm) so the information now includes data since 1972 (Brutlag, pers. comm.). This was recently published as 'The Genome of *Drosophila melanogaster*' and is also available as a draft in recent special DIS issues. The new book is available from the publishers or as text files from GenBank, but as it is free text it is inaccessible for computer surveys. A detailed appendix does not exist (Ashburner, pers. comm.). To classify mutations by effects on particular traits, it is necessary to go through each description that lists phenotypic effects. To do this with the free text would be extremely tedious, so I coded the data to make computer searches more efficient.

Towards this aim, I put all the data on 1016 mutations that affect morphology into a database much smaller than that of the free text. This article is to announce the completion of preliminary work on the database. Work remains but the database is available for use by others (details below).

Compilation of data into the database: First, I got the updated 'Red Book' by file transfer from GenBank via a VAX mainframe computer. Mutations that affect morphology were entered into the database. I used the MSEXcel spreadsheet on a Macintosh microcomputer. The information in the database format is much easier to access and manipulate than the free text.

The data for each mutation is entered in the same order as in the 'Red Book': gene code, gene name, chromosome, position, origin, phenotype and cytology. I included only mutations that affect adult external morphology, so many details in the GenBank data are omitted. Mutations that affect internal adult structure are excluded, as are behavioral, biochemical and embryonic mutants and mutations which influence the expression of other mutations, such as those at suppressor loci. For brevity, alleles beside the first listed in the 'Red Book' are also omitted from the database. This will be a problem for investigations of pleiotropic effects or if mutations for specific experimental purposes are needed. I should emphasize the database will be expanded. The effects for alleles other than the first listed, as well as other omitted data are in separate databases that are incomplete at the time of writing.

Some mutations in the original 'Red Book' are not listed in the new version because they were found to be part of a gene complex and so were subsumed under the name of the complex (e.g., scute is now listed under ASC, achaete-scute complex, rather than sc). In my database, I included the mutations from the first book and noted to which complex they belonged. The corresponding variant listed under the complex is cross-referenced. For example, the entry for the 'allele' scute at the ASC complex is under scute and not ASC, but the scute entry notes it is a part of the ASC complex.

The important difference between my database and the free text is that the phenotypic effects of the mutations are coded. This was to lessen the amount of memory occupied by the data. The total amount of memory occupied is 181K, whereas the original listing needs 4.2 MBytes (S. Maulik, pers. comm.). The coding speeds up data entry and forces the reduction of descriptions to a minimum. Thus there are no synonymous or redundant terms in the code. There are a total of 149 different effects listed. Single effect descriptors are reduced to three letters by use of the first three consonants (e.g., white is 'wht'). This was to have a code which was shorter than the word itself, yet still intuitively meaningful. I did not use a numerical code because of the latter condition.

The body parts affected by the mutations were coded with four letters and numbers. Table 1 lists the major body part groups. The list of all coded morphological structures is too large to include here but all those pictured in the fly drawings in Lindsley and Grell (1972: 472) are used in the database.

The aim for coding the body parts was to have a hierarchical structure to the code. The body part affected is the first letter of the code which is always upper case. The second letter is the trait group which the mutation affects. The third and fourth letters (the fourth position may be a number) are unique identifiers for a particular body part. For example, "Wv15" is the fifth (5) longitudinal (1) wing (W) vein (v). Mutational effects on different body segments are separated by a solidus (/) and trait groups within a segment, by a comma. Multiple effects on the same trait are separated by a comma.

Here is a typical example of an entry. The entry for the effects of the mutation bowed: "W bwd dwn, sml/B sml/E blg". Note that the part affected is listed first, the result of the mutation second. The wings (W) are bowed (bwd) down (dwn) and smaller (sml) than normal. The body (B) size is reduced (sml) and the eyes are bulging (blg).

There is also a 'Notes' column which is for data on viability, fertility and development time. This column may include information about other phenomena which may influence expression such as gene interaction and temperature effects. For bowed these are: "OL/V 75%", which means the mutant phenotype overlaps (OL) the wild-type and that viability (V) is 75% of the wild-type.

Use of the database: While the code was established I compiled a dictionary. This allows mutations to be studied for particular phenotypic effects. For example, if I wanted to know which mutations caused pink eyes, I would look up

'pink' in the dictionary and find the code "pnk", then do a computer search for the string, "pnk". With this, it is possible to quickly find mutants at different loci with similar phenotypic effects. The converse dictionary can be used to look up any code for the right description.

With the dictionary the data can be quickly searched for information on specific effects, body parts affected or important effects on fitness. My immediate aim is to update Braver's (1956) list of mutations by body parts affected. With the database I can construct a list with a more detailed hierarchy of parts affected and greatly expand the original list.

Limitations of the data set: There are some limitations that arise from the way the data is structured. The effects are not listed separately if there are multiple effects on the same trait. In the above example, if I wanted to search for mutations that cause smaller wings, the search string "W sml" would not have found bowed. Instead one would have to use the string ",sml" (comma included) and then omit entries which did not have W preceding sml. This flaw in the database can be amended by adding the trait code before the effect code. In the example, this would give "W bwd dwn, W sml/B sml/E blg". This would increase the amount of memory taken but simplify searches for entries with a particular code. I will incorporate this modification into a second version of the database.

A more basic limitation is the loss of information necessary to streamline the database. As noted above, for brevity I omitted descriptions of alleles beside the one first listed in the 'Red Book'. This is usually the first allele found at a locus. Certain non-morphological effects of listed alleles are not in the list. Variation in description is also lost. For example, quantitative differences of allelic effects are excluded, so a description of small wings will be "W sml" whether the effect is strong or weak.

Improvements could be made to the biological basis of the database structure. For example, the hierarchical groups of traits are arbitrary and for convenience. Thus, mutations that affect eyes are in a separate group from those that affect other head structures, even though eyes are part of the head. So the hierarchical groups do not correspond to imaginal discs or homeotic segments, although it should be easy in principle to modify the database accordingly.

These shortcomings may be important in some applications but I think the database will be useful for studies which need a general survey of morphological mutational effects.

Availability: To obtain the database send a MacIntosh formatted 3 1/2-inch floppy disk (DD) to the author with return postage. The database will soon be available as a text file by anonymous FTP. The data will include the dictionaries as well as the listing of mutations.

Acknowledgements: I thank Kathy Matthews and I. L. Heisler for helpful comments on the manuscript.

Table 1. List of major body parts listed in the 'Red Book' database. The list below does not include the specific structures within body parts, (e.g., orbital bristles on head) which are in the database.

Body part: head, antenna, eyes, thorax, legs, wings, abdomen, cuticle, body, bristles, hairs.

Non-morphological traits: lethality, viability, fertility, development, sterility.

EUROPEAN STOCK CENTER AT UMEA STOCK LIST

An electronic version of the European Drosophila Stock Center's 1991-1992 stock list is now posted on the Indiana file server in the file Umea.txt.

CYTOLOGICAL FEATURES DATABASE, VERSION 9207

Sally Amero, Dept. of Molecular and Cellular Biochemistry, Loyola U. Medical Center, 2160 South First Ave., Maywood, IL 60153. 708-216-3365, FAX/8523, YM60SAA@luccpua.bitnet.

This is a database of polytene chromosome sites that have been found to bind antibodies to particular Drosophila proteins. The database is maintained by Sally Amero and has been formatted by Michael Ashburner. It is available by anonymous ftp from the Indiana fileservers (ftp.bio.indiana.edu) in the directory fly. The database is kept in two files: Amero.txt is the database itself and Amero.Refs are the references for the database. The file Amero.doc contains information about the organization of Amero.txt.

FLYBASE - A DROSOPHILA GENETIC DATABASE, RELEASE 9301

The FlyBase Consortium (see below for names and addresses)

{Editors' note: The following document has been edited for DIN; section 6 has been omitted, section 8 has been truncated to include only a list of subdirectories and not the files within them, and section 9 has been omitted. The

complete document is available from FlyBase as described below.}

CONTENTS OF THIS DOCUMENT

1. What is FlyBase
2. The FlyBase Consortium
3. How to contact FlyBase
4. How to obtain FlyBase
5. How to reference FlyBase
6. Differences between printed and computer versions of FlyBase {omitted}
7. Allied databases
8. The structure of FlyBase {truncated}
9. Detailed description of FlyBase {omitted}
10. Future plans for FlyBase
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12. Full addresses of members of the FlyBase Consortium
13. The copyright of FlyBase
13. Acknowledgements

1. WHAT IS FLYBASE

FlyBase is a comprehensive database for information on the genetics and biology of *Drosophila*. It is, or will be (see below), available in several different formats. That released now is a series of flat files in which different data are displayed. FlyBase includes (by permission of Academic Press) all of the material of the Redbook, i.e. The Genome of *Drosophila melanogaster* by D.L. Lindsley and G. G. Zimm (Academic Press, 1992). A short introduction to FlyBase is to be found in the file flybase/about-flybase.txt.

2. THE FLYBASE CONSORTIUM

FlyBase is being built by a Consortium of researchers funded by the National Institutes of Health. This Consortium includes both *Drosophila* biologists and computer scientists. The Consortium is split between four sites, at Harvard, Cambridge (England), Bloomington and Los Angeles. In addition the Consortium has very close links with the National Center for Biotechnology Information in Washington and with several other workers who provide us with data, either for FlyBase itself or for one of its allied databases. The members of the Consortium are:

- o Biological Laboratories, Harvard University:
William Gelbart (PI)
Wayne Rindone
Joe Chillemi
- o Dept. of Genetics, University of Cambridge:
Michael Ashburner
Rachel Drysdale
Aubrey de Grey
- o Dept. of Biology, Indiana University, Bloomington:
Thomas Kaufman
Kathy Matthews
Don Gilbert
- o Dept. of Biology, University of California, Los Angeles:
John Merriam
Beverley Matthews
Soon-Young Huh
- o National Center for Biotechnology Information, NIH, Washington:
Carolyn Tolstoshev

3. HOW TO CONTACT FLYBASE

FlyBase has established a central e-mail address to which all communications and questions can be sent. This is:

- o FLYBASE@NUCLEUS.HARVARD.EDU

Communications or questions about the Indiana files server may be addressed to:

- o FLYBASE@BIO.INDIANA.EDU

We very much welcome corrections and additions to the data in FlyBase, comments about the types of data the we now (or should) make available or about the structure of FlyBase. FlyBase is meant to serve the Drosophila community. Only if we receive some feedback from the community will we know how best to do this. Many of the working papers between members of the FlyBase Consortium are publicly available (see below). The full mail addresses, with telephone and fax numbers and e-mail addresses, of the members of the Consortium are given in the penultimate section of this document.

4. HOW TO OBTAIN FLYBASE

FlyBase will be made available in several different ways and formats. These will include direct access to FlyBase servers, versions for stand alone access on different computer platforms, flat files and as printed text (as special issues of Drosophila Information Service, of which DIS 69 was a prototype) (see "Future plans for FlyBase"). In its present form FlyBase is only available as a series of flat files although these can be browsed and queried interactively using publicly available software (see below). The prime archive of FlyBase is maintained on a publicly accessible computer at the Department of Biology, Indiana University (IUBio). The files can be obtained in two ways, either interactively using a Gopher Client (see below) or by anonymous FTP (File Transfer Protocol). A subset of FlyBase files are also kept on several other computers, from where they are available either interactively or by anonymous FTP. If all of this is mysterious to you, contact Don Gilbert, Wayne Rindone or Aubrey de Grey, by mail or phone, for help (see below for contact numbers).

WAIS/Gopher: By far the easiest way to access FlyBase is with a Gopher Client. Gopher is a program that runs on a variety of computer platforms (including Macs). To use Gopher you need three things - a suitable computer, access to Internet and a Gopher Client. We cannot help you for the first of these but in view of the plans to make FlyBase available using X-windows software we recommend that, if purchasing, you buy a computer that can support X-windows. For Internet access you must consult your local computer advisors. For those without direct network access there are commercial companies that provide Internet access across telephone lines using modems. The Gopher Client software is available by anonymous ftp from boombox.micro.umn.edu, in the directory /pub/gopher/, or from ftp.bio.indiana.edu, in the directory /util/gopher/.

Services on the IUBio Gopher host are also available using WAIS client software. WAIS is the Wide Area Information System. Client software is available for a variety of computer platforms by FTP from IUBio (in the directory /util/wais) and ftp.think.com. It may be convenient, if your main use of Gopher is to search FlyBase, to set up your Gopher client so that access to Indiana is the default. Two of the great advantages of using Gopher are (a) that it allows you to search files interactively and (b) that you need not understand the structure of the FlyBase files. What Gopher provides is an interactive search of the flat files of FlyBase and the ability to transfer all or part of any file back to your home computer. FlyBase is accessible from the Gopher hole at Indiana (IUBio) and most of the files (but not those from the Redbook) are also accessible from the Gopher hole at the Biozentrum in Basel. The link to add to your Gopher server to tunnel to Indiana is:

```
Name=IUBio Biology Archive, Indiana University (experimental)
Type=1
Port=70
Path=1/
Host=ftp.bio.indiana.edu
```

The link for the Basel Biozentrum host is:

```
Name=bioftp EMBnet Switzerland (experimental)
Type=1
Port=70
Path=
Host=bioftp.unibas.ch
```

The WAIS source for the Indiana archive (IUBio) is:

```
(:source
:version 3
:ip-address "129.79.224.25"
:ip-name "ftp.bio.indiana.edu"
:tcp-prot 210
:database-name "INFO"
:cost 0.00
:cost-unit none
```

:maintainer "archive@bio.indiana.edu"

:description "

This WAIS service includes several indexed Biology Information sources, including Genbank nucleic acid sequence databank, Drosophila genetics, Biosci/Bionet network news, and others.

File Transfer Protocol (FTP): FlyBase is available by File Transfer Protocol (FTP) from several sources. However only Indiana has the complete set of files. The other sites have most files except those that include the Redbook data. Note that since most of these machines run Unix, the commands and names of directories and files are case sensitive. The FTP servers from which FlyBase is now available are:

FTP.BIO.INDIANA.EDU (129.79.224.25). Login with the username anonymous and use your e-mail address as password. FlyBase is in the directory flybase/.

NCBI.NLM.NIH.GOV (130.14.20.1). Login with the username anonymous and use your e-mail address as password. FlyBase is in the directory repository/FlyBase.

FTP.EMBL-HEIDELBERG.DE (192.54.41.33). Login with the username anonymous and use your e-mail address as the password. FlyBase is in the directory /pub/databases/flybase.

SUNBCD.WEIZMANN.AC.IL (132.76.64.79). Login with the username anonymous and your e-mail address as the password. FlyBase is in the directory /pub/databases/flybase.

FTP.NIG.AC.JP (133.39.16.66). Login with the username anonymous and your e-mail address as password. FlyBase is in the directory /pub/db/flybase. Once logged in to an FTP server the following commands can be used to obtain one or more FlyBase files onto your own computer:

```
ftp> cd {directory name} (i.e. cd flybase if using IUBio)
```

```
ftp> get /documents/full.doc
```

```
ftp> get /genes/loci.txt
```

```
  . et cetera
```

```
  . or
```

```
ftp> mget *.txt (to retrieve all text files)
```

```
ftp> quit
```

For those without access to FTP there is a gateway between BITNET/EARN and the FTP part of IP at Princeton. This allows you to make an FTP request by BITNET/EARN mail, the file(s) requested from the remote site being forwarded to you as mail from Princeton. This gateway is known as BITFTP. For information on how you use it send the one-line message HELP to BITFTP@PUCC.BITNET. In brief, this service is used by sending a MAIL message (using BITNET) to BITFTP@PUCC as follows:

```
FTP ftp.bio.indiana.edu NETDATA
```

```
USER anonymous guest
```

```
<now the FTP commands as if you were doing this directly; see above>
```

```
QUIT
```

The files will then be returned to you by e-mail.

Netserver: These files are available from the Netserver at EMBL, and if you do not have the facility for FTP this is a way to get them. For general help and a listing of files on the EMBL Netserver send an e-mail message to NETSERV@EMBL-HEIDELBERG.DE with the text HELP FLYBASE. To obtain a particular file send an e-mail message with the text GET FLYBASE:FILENAME to NETSERV@EMBL-HEIDELBERG.DE, where FILENAME is one of the filenames listed above.

Direct logon access in the UK: In the UK FlyBase is available on both the SEQNET and HGMP facilities. The SERC SEQNET computing facility at the Daresbury Laboratory (UK.AC.DL.SEQNET) can be directly accessed via JANET. For an account write to Dr. Alan Bleasby, SERC Daresbury Laboratory, Warrington WA4 4AD, Cheshire or send e-mail to AJB@UK.AC.DARESBURY. FlyBase is kept in a directory called /data/flybase. The MRC Human Genome Mapping Project is also accessed via JANET (MENU.CRC.AC.UK). Applications for an account should be sent to The HGMP Resource Centre, Clinical Research Center, Watford Road, Harrow, Middx HA1 3UJ. Access to FlyBase is via the menu.

CD ROM: Most of the files of FlyBase (except those of the Redbook) are included in the NCBI Data Repository and EMBL CD-ROMs. These are released periodically and are available from the NCBI Data Repository, National Library of Medicine, Bldg. 38A, Rm 8N-803, NIH, Bethesda, MD 20894 ((1)-301-496-2475) or from the EMBL Data Library, Postfach 10.2209, 6900 Heidelberg, Germany (phone (49)-6221-387258; fax (49)-6221-387519). Dr. Amos Bairoch has

made this database available as ascii files on CD ROM. Contact Dr. A. Bairoch, Department of Medical Biochemistry, University of Geneva, Geneva, Switzerland. e-mail: BAIROCH@CMU.UNIGE.CH.

5. HOW TO REFERENCE FLYBASE

We suggest FlyBase be referenced in publications in the following manner: FlyBase (1993). A Drosophila Genetic Database. Available from the FTP.BIO.INDIANA.EDU network server.

6. DIFFERENCES BETWEEN PRINTED AND COMPUTER VERSIONS OF FLYBASE [omitted]

7. ALLIED DATABASES

It is both undesirable and impossible for literally all data on Drosophila to be kept within FlyBase. However, FlyBase wishes to encourage collaboration between other workers who are building different or more specialized Drosophila databases. For this reason FlyBase has established the concept of allied databases. These databases are explicitly attributed to their authors, who are responsible for the data they include. FlyBase encourages other members of the community to make their databases available associated to FlyBase. In particular, FlyBase encourages other database curators to cross-reference FlyBase to ensure consistency in, for example, gene names and symbols. FlyBase offers help to other curators in both ensuring nomenclatural consistency and in making their databases publicly available through FlyBase. The allied databases now available are listed in the detailed description of FlyBase, below.

8. THE STRUCTURE OF FLYBASE

The presently available version of FlyBase is a series of files arranged in a hierarchical structure of directories and subdirectories. An inconvenience of this is that file names can become very long. However, on Unix operating systems, access to a particular directory can be limited by using the cd (change directory) command. The command cd .. (i.e. cd followed by a space and then two periods) will take you up one directory level. Against the disadvantage of cumbersome file names this structure is very logical and easy to maintain. Files are of different types, indicated by the suffixes to their names:

| | |
|--------|--|
| .doc | an explanatory document, in plain text. |
| .txt | a file in plain text, may be data, documentation or other information. |
| .rpt | a formatted data file, suitable for viewing by people, in plain text. |
| .rtf | a rich-text file, best read with common word processors, but also readable by people, in plain text. |
| .gif | an image file (graphic interchange format). Use a gif viewer to see. |
| .ps | a postscript image file. Use a postscript viewer or printer. |
| .tar.Z | a Unix compressed archive file. Use uncompress and tar to extract. |
| .hqx | a Macintosh binhex archive file. Use stuffit to decompress. |
| .zip | an MSDos compressed archive file. Use unzip to extract. |

There now follows the complete structure of the FlyBase files as kept on the IUBio server. The structure may differ when FlyBase is mounted on other computers, but should reflect this structure in a logical way. A detailed description of the contents of each file is given in the next section of this document. Some files have yet to be implemented, but they have been listed here as it is expected that they will be available very soon. If you are using FlyBase through a Gopher client the details of this organization are irrelevant, as you will be presented with the available files by the interactive Gopher menu.

flybase/

- flybase/redbook
- flybase/redbook/genes
- flybase/redbook/lethals
- flybase/redbook/aberrations
- flybase/redbook/miscellany
- flybase/genes
- flybase/aberrations
- flybase/maps
- flybase/function
- flybase/clones
- flybase/stocks

- flybase/stocks/stock-centers
 - flybase/stocks/stock-centers/bloomington
- flybase/stocks/labs
- flybase/references
- flybase/miscellany
- flybase/sequences
- flybase/people
- flybase/news
 - flybase/news/news
 - flybase/news/oldnews
 - flybase/news/din
- flybase/documents
 - flybase/documents/full.doc {i.e. this document}
- flybase/working-papers
- flybase/allied-data

9. DETAILED DESCRIPTION OF FLYBASE {omitted}

10. FUTURE PLANS FOR FLYBASE

In this section of the documentation we indicate some of the future directions we are taking with the building of FlyBase. This text is supplemented by the papers in the files of flybase/working-papers. We encourage the fly community to respond to what we are doing - let us know (by e-mail to FLYBASE@NUCLEUS.HARVARD.EDU or by regular mail to any of us) if you think we are not doing something that should be done, or are doing something that should not be done. Only by feedback from the community will we produce a product of the greatest utility to all. As we have explained elsewhere in this document the present release of FlyBase is seen very much as a temporary measure, until the full relational schema has been implemented.

The relational schema: FlyBase is being built and will be maintained in a commercial relational database management system called Sybase. The design of the relational schema can be found in a series of files in flybase/working-papers/sybase-*. This schema was designed by Carolyn Tolstoshev. It is not yet stable - that is to say changes to the schema are still being made as a consequence of experience and discussion. The Harvard group are now implementing and testing this schema prior to the importation of data.

The data: Any database is only as good as its data and the way these data are interrelated. At present, FlyBase data are available as a series of independent tables with few relationships between them. Not only does this mean that there are major inconsistencies between tables (e.g. a gene may have one symbol in one table but another in a second) but also it means that the user cannot automatically go from e.g. the loci table to a stock table. One of the major tasks that is now being done is to force consistency between tables.

1. The bulk of the genetic data is now in two sets of directories, flybase/redbook - the text material of Lindsley and Zimm, and flybase/genes and flybase/aberrations (with flybase/maps, flybase/function, flybase/references). The Cambridge group is now integrating these two sets of tables into a single structure. This will, in effect, be the replacement of the Redbook. Since science does not stop simply because we are building this database the Cambridge group is also continuously updating the data, by scanning the literature.

2. There are now several different tables of clone data. These are being integrated and continuously updated by the Los Angeles group. This group is also developing software for the graphical display of molecular data.

3. References can now be found in three different sets of tables, those in flybase/redbook, flybase/genes and flybase/clones/clonelist.txt. Not only is there redundancy between these but each set differs in its reference format. The Cambridge group is dealing with this problem by building a single Drosophila reference file. The objective is to have as complete a bibliography on Drosophila biology as possible with all entries in uniform format. The sources of this bibliography are several: the published bibliographies (Morgan et al. 1925, Muller, Herskowitz and some smaller more specialized ones) are being read by an optical character reader; we have concluded a license agreement with MEDLINE giving us a retrospective download from 1966 (the year MEDLINE introduced computer files) with monthly updates from January 1993 (these entries will include abstracts) and Dr. G. Bachli's computer bibliography, which is especially strong on taxonomic and faunistic papers. When these have all been entered, duplicate entries removed and reformatted they will be checked against the large Drosophila offprint collection in Cambridge for errors and omissions. This reference table will serve all of the other tables of FlyBase. Users will be able to recover references from it in a variety of formats (e.g. that used by ENDNOTE).

4. The Bloomington group is working on the problem of stock lists, not only collecting stock lists from other laboratories (see flybase/stocks.doc) but also ensuring a consistency in format, so that all can be seen in a similar way. We hope to publish a recommendation for stock list format, with the hope that others will use it and reduce the problems we have in displaying stock list data. The second major problem with the stock lists is to ensure that they are consistent in the symbols used for genes, alleles, aberrations and insertions.

5. There are now several different files of addresses and/or e-mail addresses. These have been gathered from various sources. The aim is to have a single address file, in a consistent format. This work is being done in Bloomington.

6. The formal description of chromosome aberrations, in a manner suitable for manipulation by computer programs, is a difficult problem. One approach is that discussed in flybase/working-papers/aberration-syntax.txt. We are writing software that will allow efficient searching of the aberration tables for, e.g., breakpoints within a specific chromosome region, and allow the graphical display of aberrant chromosomes.

Output: Although FlyBase will be built and maintained in Sybase we expect few to have the skills to use it as such or to have the very considerable cash required for a Sybase operating license. For this reason we are building a number of output products that will make FlyBase available to as wide a community as possible. (The Sybase implementation will be publicly available should any users need it.)

1. The simplest output will be printed text. We have, in pre-Consortium days, experimented with this with a special volume of DIS (DIS 69) compiled by Michael Ashburner and edited (and distributed) by William Gelbart. It is our intention to publish such special issues of DIS whenever the amount of new data warrants. By doing this we will ensure that even those workers who have no access to computers or networks will not be disenfranchised. These issues of DIS will be produced as output from the Sybase tables.

2. The presentation of FlyBase as a series of ascii flat files on computer servers will be maintained. The prime server will be that at IUBio and the Bloomington group will continue to make improvements in access and display of these tables. By far the easiest way to access these files is by using a Gopher client (see "How to obtain FlyBase"). In addition these tables will be distributed, as now, to a number of major servers used by biologists and will be included on the CD-ROMs being distributed by NCBI, EMBL and others.

3. There is now strong interest in the development of software to display databases such as FlyBase interactively using X-windows systems. We are developing such tools for use with FlyBase. We are concentrating our efforts in two ways. The first is to exploit the software tools written at the NCBI for use with their Entrez system. The second is to develop the programs written by Richard Durbin and Jean Thierry-Mieg for the *C. elegans* database - acedb. acedb is now being modified for *Drosophila* data in Berkeley and we are collaborating with Suzanna Lewis to make these programs suitable for the display of FlyBase. These implementations of FlyBase will be available from the IUBio server (and probably from other servers), on CD-ROM and perhaps on floppy discs. To make use of them you will need a computer that can implement X-window software (or its equivalent). A color monitor would be a great advantage.

11. RELEASE NOTES

Since FlyBase is still kept as a series of independent tables the concept of a "release" or "version" of the database is difficult to apply. However, while this format continues we will signal new releases (as yearmonth) whenever we consider that there has been a sufficient change in data or organization to warrant it. New versions of particular tables may well be released without obvious notice. One way of finding out is to look at the date that a particular file was last modified on IUBio. This can be done by FTP using the dir command in the flybase directory. The last update of a file is automatically displayed by Gopher. The individual document files will be kept up to date and they will indicate any changes in organization or major changes in content. The last update of the .doc files is displayed on the top line of each.

Release 9301 of FlyBase is the first by the Consortium. It is released as a temporary measure to make the data that is in FlyBase already available to the community. In large part 9301 is simply a restructuring of data that had been previously available from IUBio and other sources. It includes the tables from the 9209 release of Michael Ashburner (see flybase/news/oldnews/1992.txt). These tables include 5321 loci, 4218 entries in the genetic map, 11940 aberrations and 3120 references. Note that of the 5321 loci, about 800 are not in Lindsley and Zimm (1992). The great majority of the references are also subsequent to Lindsley and Zimm. This release includes tables from the 3/06/1992 release of John Merriam's clone lists.

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13. THE COPYRIGHT OF FLYBASE

The files containing the text of Lindsley and Zimm (1992) The genome of *Drosophila melanogaster* are the copyright of Academic Press and are redistributed in FlyBase by their agreement. These files cannot be redistributed by users without the explicit permission of Academic Press. The copyright of FlyBase itself is held by the Genetics Society of America.

14. ACKNOWLEDGMENTS

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GOPHER ACCESS TO FLYBASE

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The easiest way to access FlyBase is with Gopher. For those of you who haven't heard of Gopher yet, the following excerpts from "GOPHER DIGS THROUGH THE INTERNET FOR YOU" by Eric Schlene, which appeared in UCS

Monitor, Volume 4, Number 2 (March 15, 1993), published by University Computing Services, Indiana University, should be useful.

"Developed at the University of Minnesota, Gopher is a network navigation tool. The name "Gopher" conveys much about the technology. If you need something off the network, let Gopher "go for" it. Gopher will burrow through the network to retrieve what you asked for.

Like its mammalian namesake, Gopher is hardy and aggressive. It can adapt to a variety of computing environments and has a world-wide range. Gopher servers, computers where Gopher goes to find files, are popping up all over the globe. Known collectively as "Gopherspace," these servers offer information and services you can access over the Internet.

How Gopher works: Like many new applications, Gopher is a client/server product. The client part runs on your computer (e.g., PC, Mac, UNIX workstation). And the server part runs on computers elsewhere on the Internet. At your command, your client 'opens electronic tunnels,' finding the computers where the goodies you want are stored.

If you like to explore electronically, you're going to love Gopher. There are countless links to libraries, computing centers, universities, corporations, and organizations all over the world. The only hard part about using Gopher is quitting!"

You may also find the README file provided by the developers of Gopher to be helpful:

In this directory are subdirectories containing the current versions of internet Gopher clients and servers. Unfortunately, there is minimal documentation for the software; but have faith, we are working on it.

The internet Gopher uses a simple client/server protocol that can be used to publish and search for information held on a distributed network of hosts. Gopher clients have a seamless view of the information in the gopher world even though the information is distributed over many different hosts. Clients can either navigate through a hierarchy of directories and documents -or- ask an index server to return a list of all documents that contain one or more words. Since the index server does full-text searches every word in every document is a keyword.

If you want to test a gopher client without setting up your own gopher server you should configure the client to talk to 'gopher.micro.umn.edu' at port 70. This will allow you to explore the distributed network of gopher servers at the University of Minnesota. You can try the Unix client by telneting to consultant.micro.umn.edu and logging in as 'gopher'.

If you decide to run a gopher server and would like it to appear in the list of 'other gophers' at gopher.micro.umn.edu send an e-mail to gopher@boombox.micro.umn.edu and we will add your gopher to the list of gophers.

Bug reports, comments, suggestions, etc. should be e-mailed to the Gopher development team at: gopher@boombox.micro.umn.edu

If you don't have access to a Gopher client, ask your local computing support center to help you get set up. The Gopher client software is available by anonymous ftp from boombox.micro.umn.edu, in the directory /pub/gopher/, or from ftp.bio.indiana.edu, in the directory /util/gopher/. FlyBase is accessible from the Gopher hole at Indiana (IUBio) and most of the files (but not those from the Redbook) are also accessible from the Gopher hole at the Biozentrum in Basel. It may be convenient, if your main use of Gopher is to search FlyBase, to set up your Gopher client so that access to Indiana or Basel is the default. The link to add to your Gopher server to tunnel to Indiana is:

Name=IUBio Biology Archive, Indiana University (experimental)

Type=1

Port=70

Path=1/

Host=ftp.bio.indiana.edu

The link for the Basel Biozentrum host is:

Name=bioftp EMBnet Switzerland (experimental)

Type=1

Port=70

Path=

Host=bioftp.unibas.ch

Services on the IUBio Gopher host are also available using WAIS client software. WAIS is the Wide Area Information System. Client software is available for a variety of computer platforms by FTP from IUBio (in the directory /util/wais) and ftp.think.com. The WAIS source for the Indiana archive (IUBio) is:

```
(:source
:version 3
:ip-address "129.79.224.25"
:ip-name "ftp.bio.indiana.edu"
:tcp-prot 210
:database-name "INFO"
:cost 0.00
:cost-unit none
:maintainer "archive@bio.indiana.edu"
:description "
```

Once you reach FlyBase with Gopher you can copy files to your local machine or you can search files interactively. The top FlyBase menu will look like this on your screen:

Internet Gopher Information Client v1.00

Flybase

```
--> 1. About Flybase [14Jan93, 5kb].
    2. -----/
    3. Genes/
    4. Functions/
    5. Aberrations/
    6. Clones/
    7. Maps/
    8. Sequences/
    9. -----/
   10. People/
   11. Stocks/
   12. Miscellany/
   13. -----/
   14. Documents/
   15. News/
   16. References/
   17. Working papers/
   18. Allied fly data/
   19. Redbook (Genome of Drosophila)/
```

When you choose a topic a new menu of subtopics and/or available files will be displayed. Choosing a file from a Gopher menu returns the file to your local machine. Menu items ending with <?> allow you to search that part of the database for specific information. Gopher searches are supported for the topics Genes, Clones, People, Stocks, News (back issues of DIN), Allied fly data, and Redbook.

Gopher is able to retrieve information very rapidly because it searches indexes of the data files. Any file can be retrieved with Gopher, but only files that have been indexed for Gopher's use can be searched with Gopher. Indexes based on standard rules of English did not work well for Drosophila nomenclature, as those of you who tried to find stocks with the original IUBio Gopher server discovered. Don Gilbert has modified the indexing rules of the IUBio Gopher so that Drosophila genotypes can be more effectively searched. There are still a few bugs in the system, but you will find it significantly improved. Plain language queries work well for retrieving information from most of FlyBase, but some tricks are helpful when searching the stock lists. The following information should help you construct an effective query.

* An asterisk (*) serves as a wild card when placed at the end of a character string. Wild cards cannot precede or be imbedded in a character string.

- * Word delimiters are: -/{ }.:~*";|. These symbols will not be recognized as literal characters in a search string.
- * Stock numbers are preceded by a pound sign (#) in all of the lists used by Gopher.
- * All of the stock lists used by Gopher mark superscripts with brackets ([]).
- * All cytological map positions are entered in the database with three digit division numbers and two digit band numbers so that these alphanumeric items will sort numerically. For example, 67C4-6 is recorded as 067C04-06, and 3C2 as 003C02. Cytological information is available for Bloomington stocks only at present. Gopher search software is still under development and is not always accurate when searching on cytological locations.
- * Examples:

To find a stock by its stock number precede the number with the pound sign (e.g., #617) for Bowling Green stocks, Umea stocks, and stocks from the main collection at Bloomington. Precede the stock number with #P (e.g., #P43) for transposon stocks from Bloomington.

To find all available alleles of a gene, type the gene symbol followed by * (e.g., lt*). To find a specific allele, type the gene symbol followed by the superscript in brackets (e.g., lt[14]).

Find all stocks with breakpoints in a given division by asking for the three digit division number followed by * (e.g., 084*). Add the subdivision letter (e.g., 084B*) to find all breakpoints within a given subdivision.

Most published P stocks available from Bloomington can now be found by searching for the laboratory acquisition name (e.g., ms(3)neo6, A146.3M3, l(3)A344.1M3, B12-3-5, l(3)B9-3-53). Stocks carrying specific constructs can be found by searching for the construct abbreviations, such as lacW, lArB, pW8, A92, or Car20, although this search may also turn up stocks that contain modified versions of the desired construct.

As with rearrangements in the main collection, cytological map positions for insertions are entered as three digit division numbers and two digit band numbers. To find all insert in 32D, ask for 032D* [see gopher-README.doc in Stocks/ for updated information].

32 by searching for 032*, plus each subdivision (032A*, 032B*, etc.) as described above.

TECHNICAL NOTES VOL. 6-10

AN IMPROVED PROBOSCIS EXTENSION ASSAY

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Flies extend their proboscis in response to a sugar solution applied to the tarsal or labellar taste hairs. This proboscis extension reflex has been extensively used as an assay for gustatory response. In conventional methods, the fly is bound by wax or myristic acid (Tompkins, L. & Barnhart, K. J., 1982, DIS 58: 171-172), the test solution is then applied to the tarsi or labella by cotton-tipped applicator. An alternative method is to mount the fly into a plastic micropipet tip with the head and first pair of legs protruding out of the tip bore (Vargo, M. & Hirsch, J., 1982, DIS 58: 174). Both methods are tedious and slow, unsuitable for large-scale mutant screens. In addition, the flies are being tested under strain, which may affect their response. We have developed a method for testing the proboscis extension reflex in free-walking flies.

A 1.5% agarose solution containing test sugar is boiled and cooled to 50-60°C. A 1 ml plastic pipet is cut to 10 cm length. The sugar-agarose solution is sucked up from one end to 4.5 cm high and then expelled. From the other end of the pipet, a 1.5% agarose solution is sucked up to 4.5 cm high and also expelled. A thin agarose coating is thus left along the inner surface of the pipet, with the blank agarose and sugar-agarose on opposite ends, separated by 1 cm to prevent mixing. At the blank agarose end, a plastic 1000 µl pipet tip (blue tip) is attached, with the tip cut to an opening of 2-3 mm in diameter to allow the fly to pass through. A starved and water-satiated fly is introduced into the test pipet through the blue tip. The pipet is then raised to a vertical position and an optic fiber illuminator is positioned above the pipet. The fly is induced to walk upward by negative geotaxis and positive phototaxis. The first section of blank agarose probably accustoms the fly to the agarose coating. When a wild type fly steps onto the sugar-agarose section, its proboscis extends repeatedly. The response can be easily observed without a microscope. This assay is simple, rapid, and the flies are recovered intact. About 60-100 flies can be tested per hour, making large scale mutant screening feasible. Using this assay, we have isolated several mutants with a defective response to sucrose. It is also possible to sequentially suck up agarose solutions with increasing concentrations of sugar, creating partially overlapping layers. This allows the response threshold to be determined in a single run. This research was supported by grant NSC-78-0203-B001-11 from the National Science Council, ROC.

IMMUNOSTAINING OF POLYTENE CHROMOSOMES

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Many people have contacted us regarding our protocol for immunostaining of polytene chromosomes. Our current protocol is listed below, in its entirety. We hope that this will be of use to others who are interested in identifying protein binding sites in the polytene chromosomes.

1. Preparation of 3rd instar larvae: Use bottles with rich medium (i.e. 8 g agar, 18 g dried yeast, 10 g soybean meal, 7 g molasses, 80 g malt extract, 80 g cornmeal and 6.3 ml propionic acid in 1 lt water). Add a large drop of live baker's yeast on top of the dried medium. Let the flies lay eggs to the point where larvae will hatch under uncrowded conditions (< 100 larvae/bottle) and grow larvae at 18[o]C. For salivary gland preparations, use crawling 3rd instar larvae.

2. Chromosome squashes: Dissect two pairs of salivary glands in solution 1. Try to get rid of most of the fat body without separating the two glands. Using a tungsten needle with a hook, transfer the glands to a drop of solution 2 on a siliconized coverslip. Fix the glands homogeneously by moving them with the tungsten needle for 10-30 sec in solution 2 (time needs to be adjusted for each individual antigen). Move glands into a droplet (40 ul) of solution 3 on a coverslip (Corning or equivalent quality, 22x22 mm, not siliconized) and leave them for 2 - 3 min. During this incubation, break up the glands and get rid of remaining chitinous structures of the pharynx using tungsten needles. Lower a poly-L-lysine treated slide onto the coverslip. Under the stereo-microscope, tap the coverslip with a pencil until cells are broken up. Hold the coverslip and spread the chromosomes using the eraser-end of the pencil. Remove excess fixative by pressing slides (coverslip down) onto blotting paper. Examine the preparation under phase contrast. Mark the position of the coverslip. After freezing slides in liquid nitrogen, flick off coverslip with a razor blade. Wash slides two times for 15 min. in PBS, slowly shaking the rack. Proceed with the immunostaining or keep the slides (up to one week) in 100% methanol or in 50% (w/v) ammonium sulfate at 4[o]C.

3. Immunostaining: Stored slides are washed 2 x 15 min. in PBS. Block for 1 hour in blocking solution at room temperature (rt). Add 40 ul affinity-purified primary antibodies (i.e. rabbit polyclonal antibodies; try dilutions in the range from 1:50 to 1:500 in blocking solution) to each slide. Cover with coverslip and incubate for 1 h at rt in a humid chamber. Rinse in PBS. Wash: 15 min in PBS, 300mM NaCl, 0.2% NP40, 0.2% Tween20-80; then 15 min in PBS, 400mM NaCl, 0.2% NP40, 0.2% Tween20-80. If background problems persist, NaCl conc. can be raised to 500mM. Shake rack thoroughly during washing. Rinse in PBS. Add 40 ul diluted secondary antibody (i.e. anti-rabbit IgG (Fc) HRP-conjugate, Promega cat.# W4011, 1:100 dilution) with 2% normal (goat) serum in blocking solution. Cover with coverslip and incubate for 40 min. at rt in humid chamber. Rinse in PBS. Wash exactly as described above for the primary antibody. Rinse in PBS. Add 100 ul 0.5 mg/ml DAB-solution (diaminobenzidine tetrachloride; Sigma # D5637) + 0.01% H[2]O[2] (Merck # 7210). Watch the color develop under phase contrast. Stop reaction by dipping slides in PBS. Wash 10 min. in PBS.

4. Cytology: Stain chromosomes for 10-20 sec. in Giemsa solution (Merck # 9204; 1:130 dilution in 10 mM sodium phosphate buffer pH 6.8). Rinse in distilled water. Mount in 99.5% glycerol and immediately examine the slides under the microscope - the giemsa stain fades within a few hours. Chromosomes can be washed in PBS and restained. For storage, slides can be frozen at -20[o]C. Entelan (EM Science) can be used as a permanent mounting solution. In order to increase the contrast between the signals and the chromomeres (important for black and white photography) DAB precipitates can be enhanced by applying a silver amplification system (Amersham). The enhancement is performed according to the manufacturer's protocol, except that the silver amplification step is shortened to about 1 min.

Solutions and reagents:

Solution 1: 0.1% Triton X-100 in PBS pH 7.5.

Solution 2: 3.7% formaldehyde, 1% Triton X-100, in PBS pH 7.5. The 37% formaldehyde stock solution used to make this solution is prepared as follows: 1.85g paraformaldehyde dissolved in 5 ml water, add 70 ul 1 N KOH, dissolve by boiling).

Solution 3: 3.7% formaldehyde, 50% acetic acid.

Important: Solutions 2 and 3 should be made fresh every 2 - 3 hours!

Blocking solution: 3% BSA, 10% non-fat dry milk, 0.2% NP40, 0.2% Tween20-80 in PBS. Do not worry about the turbidness of the solution. It still works!

Preparation of Poly-L-lysine coated slides for chromosome squashes: Start with 100 - 200 slides in racks. Soak slides in a corrosive detergent solution for 2 hrs. Wash under running tap water for 2 hrs. Wash in distilled water, two changes. Dip in 95% ethanol, two changes. Air dry. Dip slides into poly-L-lysine solution (slide adhesive solution, 0.1% w/v in water, Sigma Cat# P 8920). Withdraw rack, solution should wet slides uniformly and stay on slides. Air dry slides.

PLASMID VECTORS FOR EXPRESSING PROTEINS IN DROSOPHILA TISSUE CULTURE CELLS OR STUDYING ENHANCER FUNCTION

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Two plasmid vectors for expression of proteins in transfected *Drosophila* tissue culture cells have been constructed.

1) pMK26 is a vector for protein expression from the actin 5C promoter in transient transfections. It contains unique Sall, HindIII, EcoRV, and PstI sites downstream from the *Drosophila* actin 5C promoter and upstream from a fragment containing the SV40 splice site and poly(A) addition signals. It was derived from Bluescript+KS and pPac (Krasnow et al., Cell 57: 1031-1043, 1989).

2) pMK33 is a vector for inducible protein expression from the metallothionein promoter. This plasmid contains a bacterial hygromycin-resistance gene driven by the *Drosophila* copia promoter, allowing the selection of stable transformed cell lines. Unique XhoI, EcoRV, BamHI, and SpeI sites are located downstream from a *Drosophila* metallothionein promoter and upstream from the *Drosophila* actin 5C poly(A) addition signal. Open reading frames inserted into this multiple cloning site will be efficiently expressed upon addition of copper to the culture medium. This plasmid contains fragments from pPac, pHSX-MT (Kaufman et al., Cell 59: 359-371, 1989), and pcophy (Rio et al., Cell 44: 21-32, 1986).

We have also constructed a general purpose lacZ reporter plasmid for use in *Drosophila* and *Drosophila* tissue culture cells. This plasmid, pMK42, contains a minimal *Drosophila* Adh distal promoter (from -34 to +53 with respect to the transcription start site) driving a hybrid lacZ gene, consisting of the Ubx untranslated leader and initiation codon, the *E. coli* lacZ ORF, and SV40 splice and poly(A) addition signals. A unique Sall site is located upstream of the Adh TATA box for the insertion of foreign enhancer elements. pMK42 is a derivative of pD(delta)5'-34 (Heberlein et al., Cell 41: 965-977, 1985) and cP(beta)bx6.2 (Irvine et al., Development 111: 407-424, 1991).

All of these plasmids have been extensively tested (e.g. Koelle et al., Cell 67: 59-77, 1991). Further information and DNA is available upon request. Please direct inquiries to Betty Swyryd at the address and phone number given above.

MODIFIED pPac PLASMID FOR TRANSIENT EXPRESSION OF PROTEINS IN TRANSFECTED TISSUE CULTURE CELLS

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The pPac plasmid was constructed by Krasnow et al. (Cell 57: 1031-1043, 1989) to allow efficient expression of proteins in transfected *Drosophila* tissue culture cells. pPac contains a single unique BamHI cloning site located between the actin 5C promoter and poly(A) addition signal. To extend the usefulness of this plasmid, we have inserted a polylinker into this BamHI site. The modified vector, which we call pPacPL contains unique BamHI, EcoRV, SpeI, XbaI, KpnI, SacI, NotI, and HpaI restriction sites for the insertion of open reading frames. We will be happy to provide DNA and maps to anyone interested in using this modified vector.

GENETIC NOTES VOL. 6-10

NEW LAC-Z MARKED BALANCER

Scott Panzer, Alison Fong, and Steve Beckendorf, MCB: Genetics, U. of California, Berkeley, CA 94720, USA. 510-642-6973, FAX/7000; SPANZER@ENZYME.BERKELEY.EDU.

We constructed a new lac-Z marked balancer by jumping a P{eve-lacZ} onto SM6B, Cy Roi. Because eve-directed beta-gal synthesis starts in blastoderm embryos, embryos carrying the balancer may be distinguished histochemically from those that don't at many stages of development. (In contrast, the other lacZ marked second chromosome balancer we've come across, CyO-beta P{elav-lacZ}, is most useful only in embryos older than seven hours old). Flies and more information available upon request directly from us (send request by email if possible) or from the Bloomington stock center.

UPDATES AND CORRECTIONS TO THE REDBOOK

(Editor's note: if you have similar corrections/updates to the Redbook, please send them to KM. A cumulative file of these corrections will be posted on the Indiana fileserver.)

(1) *mhc*, myosin heavy chain -- The draft entry on *mhc*, published in DIS, volume 68, is accurate, but the entry in the final version of the "Genome of *Drosophila melanogaster*" includes an appended exon diagram of *mhc*, page 459, that incorrectly labels the five alternative exons 11's. In our primary publication describing the complete structure of *mhc*, George, et al. *Mol. Cell Biol.* 9, 2957-2974 (1989), we designated the order of these exons (5' to 3') to be 11e, 11a, 11b, 11c, 11d. (The diagram on page 459 incorrectly orders these exons 11a, 11b, 11c, 11d, 11e). -- Charles Emerson, emerson@castor.rm.fccc.edu

(2) *Khc*, kinesin heavy chain -- The symbol for the kinesin heavy chain gene, shown on p. 296 as *Kin*, is *Khc*. -- Bill Saxton

(3) p. 638: "shaven: see *svb*" should read "shaven: see *sv*"

(4) p. 109: add entry "*cel*, cell lethal: see *l(3)84Ab*" -- Ken Howard

(5) p. 259: add entry "*grh*, grainy-head: see *Ntf*" -- Larry Marsh

A NEW MUTANT OF *D. SEHELLIA*

Isaya Higa and Yoshiaki Fuyama, Dept. of Biology, Tokyo Metropolitan U., Hachioji-Shi, Tokyo 192-03, Japan. 81-426-77-2575, FAX/2559; A910741@JPNTMU00.BITNET.

A new white (*w*) mutant of *D. sechellia* spontaneously occurred in an iso-female strain originally collected in Plaslin Island, Seychelles in 1986. General features are the same as those of white of *D. simulans* and white[1] of *D. melanogaster*. Homozygote fertile and viability normal. Sex-linked and recessive. Does not complement white of *D. simulans*.

NEW RADIUS INCOMPLETUS ALLELE AND ITS LETHAL INTERACTION WITH HAIRLESS

Petter Portin and Mirja Rantanen, Laboratory of Genetics, Dept. of Biology, U. of Turku, SF-20500 Turku, Finland. SEPNE@SARA.CC.UTU.FI

In June 1992 we began to suspect that a spontaneous radius incompletus (*ri*) mutation had occurred in our *Ax[ts1]* stock. We mapped this mutation with the aid of *cu* and *es* mutations, and found that the new mutation really mapped to the position of *ri* (3-47.0). Our new mutation failed to complement *ri*, and consequently it was named *ri[92f]*.

Even earlier we had found that in the cross *ri[92f] cu es* x *H es cd/In(3R)P*, spr only non-ebony progenies appeared. Therefore we concluded that the interaction of *ri* and *H* is lethal even though both are in a heterozygous condition.

CORRECTIONS FOR REDBOOK

Dan Lindsley and Georgianna Zimm, Dept. of Biology, U. of California, La Jolla, CA 92093. 619-534-3109, FAX/0053.

p = page, L = left, R = right

p2L, line 12: choromosomes > chromosomes

p8R, ABO table footnote: Sander > Sandler

p25L, Ama-1: alpha-amanatin > alpha-Amanatin

p41R, zen: Location > location

p71R, bottom of page: Add entry "*bcd*:: see *ANTC*"

p100: Add entry "*Ubx[16K]* X ray Ramey *In(3R)79D;89B Ubx*"

p100: Add entry "*Ubx[42T]* X ray *In(3R)70D;89E Ubx*"

p100: Add entry "homozygous lethal" to last column of *Ubx[130]*.

p101: Add entry "; T(2;3) " to cytology column and entry "*Ubx*" to type column of *Ubx[A]*

p109: Add entry "extreme *Ubx*" to type column of *Ubx[U]*

p109R: Add entry "*cel*: see *l(3)84Ab*"

p128R: Add entry "*cry*: see *Su(Ste)*"

p128R: Add entry "*crystal*: see *Su(Ste)*"

p142L: Add entry "*da[12]* 7 recessive lethal"

p142L: Add entry "*da[13]* 7 recessive lethal"

p142L: Add entry "*da[14]* 7 recessive lethal"

p142L: Add entry "*da[15]* 7 recessive lethal"

p142L: Add entry "*da[16]* 7 recessive lethal"

p142L: Add entry "*da[17]* 7 recessive lethal"

p142L: Add entry "da[18] 7 recessive lethal"
 p142L: Add entry "da[19] 7 recessive lethal"
 p142L: Add entry "da[20] 7 recessive lethal"
 p142L, da table footnote: Add entry "7 = Grigliatti."
 p142L, da cytology: Change to "Placed in 32A by fine-structure deficiency analysis of region 31A-32A by Grigliatti et al."
 p183R: E(Sd) > E(SD)
 p201R, err alleles: Add entry "err[2] - err[4] also isolated."
 p201R, err cytology: Change to "Placed in 31E by fine-structure deficiency analysis of region 31A-32A."
 p249R, Gbeta13F location: 2-{54} > 2-{51}
 p255L: Add entry "Glucose-tasting-defective: see Gtd."
 p255L: Add entry "Glutamic acid decarboxylase: see Gad."
 p259R: Add entry "grh, grainy head: see Ntf."
 p259R: Add entry "groggy: see ggy."
 p268L, H references: Add entry "Plunkett, 1926, J. Exp. Zool. 46: 181-244."
 p268L, H references: Add (after "Development") "111: 89-104."
 p268R, H[17] (in table): Add entry "gamma ray Posakony/Groger"
 p268R, H[18] (in table): Add entry "gamma ray Posakony/Groger"
 p268R, H[19] (in table): Add entry "gamma ray Posakony/Groger"
 p268R, H[20] (in table): Add entry "gamma ray Posakony/Groger"
 p268R, H[21] (in table): Add entry "gamma ray Posakony/Groger"
 p268R, H[21] (in table): H[C] > H[C23]
 p268R, H[22] (in table): Add entry "gamma ray"
 p268R, H[22] (in table): Bang > Posakony
 p268R, H[22] (in table): H[C] > H[RP1]
 p268R, H[26] (in table): Add entry "X ray."
 p288L, inC alleles: InC[1] - InC[3] > inC[1] - inC[3]
 p309L (in l(1)2A table): l(1)2Af (bold face) > l(1)2Af (regular)
 p309L (in l(1)2A table): sta > sta (bold face)
 p509R, nod references: Genetics (submitted) > Genetics 125:115-27.
 p555L, pn: awk[K] > awd[K] (appears twice)
 p555R, pn: awk[K] > awd[K] (appears four times)
 p570R, qua: Nsslein- > Nusslein (diaeresis over the u)
 p570R, qua: f2qua[2] - qua[7] > qua[2] - qua[7] (in italics)
 p621L: Add entry "scabrous > sca"
 p621L: Add entry "shaven baby > sv"
 p740R: For the entry unk, see the CYTOGENETIC MAP, p1132.
 p1067: change figure explanation to "the third row shows the N- banding pattern (provided by Pimpinelli, Bonaccorsi, Dimitri, and Gatti)."
 p1068L: Change reference for figure explanation to "(Pimpinelli, Bonaccorsi, Dimitri, and Gatti)."
 p1069L: Change reference for figure explanation to "(Pimpinelli, Bonaccorsi, Dimitri, and Gatti)."
 p1069R (upper): Change reference for figure explanation to "(Pimpinelli, Bonaccorsi, Dimitri, and Gatti)."
 p1069R (lower): Change reference for figure explanation to "(Pimpinelli, Bonaccorsi, Dimitri, and Gatti)."

Notes appended to Redbook by attendees at the Philadelphia fly meeting:

exo: exocephalon is allelic to phm: phantom (Eberl)
 mat(2)N mutations are hypomorphic alleles of l(2)31Ei
 Sryc likely to correspond to wdn (Lepesant)
 fs(1)A107 renamed brn: braniac (can't read signature)
 fs(1)1621 renamed snf: simply not fertile (Saltz)
 Kin: Kinesin should be Khc: Kinesin heavy chain (Saxton)
 l(1)3Ac renamed trol: troll by Datta and Kandel {not l(1)trol as they suggest}
 l(3)73Ab will be named soon (Andrew)
 l(3)85Ee renamed hyd: hyperplastic discs (Shearn)
 l(3)SG29 renamed md: minidisks (Shearn)
 l(3)SG56 renamed qrt: quartet (Shearn)

New genes inserted into list by participants:

Chc: Clathryn heavy chain

dco: discs overgrown (see Developmental Biology 140: 413-429)

Gprk1

Gprk2

Pra: Paramyosin

rdgC: retinal degeneration C

tsh: teashirt

A change that we suggest:

ms(3)sa should be sa: spermatocyte arrest

Symbols used by Ashburner that I prefer over ours:

l(1)17Aa through l(1)17Ad instead of l(1)16Fa through l(1)16Fd

LanA, LanB, and LanC instead of Lam-A, Lam-B, and Lam-C

Pk17C instead of Pk?4

Pk45C instead of Pk?3

Pk53C instead of Pk?7

Pk64F instead of Pk?6

Pk91C instead of Pk?2

Other new synonymy:

Pkc2 is the same as inaC

sbl mutations are allelic to para

CORRECTIONS FOR THE REDBOOK

Dan Lindsley and Georgianna Zimm, Dept. of Biology, U. of California, La Jolla, CA 92093. 619-534-3109, FAX/0053, REDBOOK@JEEVES.UCSD.EDU, ZIMM@JEEVES.UCSD.EDU.

p = page, L = left, R = right

p 296L: Kin : Kinesin to Khc: Kinesin heavy chain

p 296L, Khc : Add entry " cytology: Located in 52F10-11."

p 296L, Khc : Remove " discoverer: Christensen."

p 296L, Khc phenotype: Kin to Khc

p 621L, Add entry "scabrous: : see sca"

p 626L, Sdh1 (table): de Jong to Lawrence

p 638R, shaven : svb to sv

p 659L, alphaSpec : Add entry "synonym: l(3)62Bd"

p 660R, spire : spi to spir

p 660R, Add entry "spitz : see spi"

p 779R, wg[l-14] table comments: Add entry "P-element insert"

p 779R, wg molecular biology: Omit wg[l-18]

p 779R, wg molecular biology table: Add entry below wg[1] "wg[l-14] located at origin of molecular map"

p 935R, In(2L)wg[P]: Omit "synonym: In(2L)wg[P] "

p 1051L, Tp(2;Y)L12 table cytology: 41A;43E to 41A;43A

p 1051L, Tp(2;Y)R70 table ref : 1,3 to 1

p 2618: T(Y2)A111 = T(Y;2)h14;028D

p 2634: T(Y;2)B66 = T(Y;2)Xhy[+];028C

p 2637: T(Y;2)B104 = T(Y;2)B[S]Xh;028D

p 2763: T(Y;2)R50 = T(Y;2)h1-2;028B

In addition, in The Genome of *Drosophila melanogaster*, we have ceased superscripting YL and YS to conform to usage for the autosomes; thus, YSX.YL, etc.

p 34 (table), Antp[*Ns-rv2*]: In(3R)81F;90BC to T(3;4)84B1-3;102F

p 34 (table), Antp[*Ns-rv72*]: Df(3R)84B3;84D to In(3R)84B3;84D

-- Paul Talbert, Basic Sciences M684, Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA 98104.
206-667-4509, FAX/5889.

Request for Updated Directory Information

We shall maintain an updated Directory file to be printed periodically in future issues. If there are changes, additions, or corrections to the Directory as printed in DIS 71, please provide the following information as soon as possible.

(I recommend that you submit your information on a photocopy of this page so that the original remains available to you for updating your directory listing.)

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Please mail to:

Drosophila Information Service
James N. Thompson, jr., Editor
Department of Zoology
University of Oklahoma

Drosophila Information Service volume 71 (July 1992) included a tentative version of a Directory of *Drosophila* Researchers drawn from a variety of sources. We took names from earlier directories, from the current subscriber list for DIS, and from information provided to us on directory information request forms sent world-wide and published in DIS volume 70 (July 1991). We are grateful to those who responded to our request for corrections and additions. Even with this assistance, however, it is clear that this directory is far from complete. It is weighted in favor of individuals and laboratories that read and support DIS. Hopefully those of you who read and support DIS will find it a useful source of information.

Although some important geopolitical changes were reflected in last year's directory, additional changes have occurred since then. We apologize for any errors we make in recording them here. The material submitted by researchers themselves is sometimes ambiguous, but in these changing times, that is understandable.

We will continue to maintain a Directory of *Drosophila* Researchers and will contribute information to the central *Drosophila* database being developed for international communication. Future versions of the Directory will be available from the Editor at cost, and updated versions will be printed periodically in future issues of *Drosophila* Information Service. We look forward to receiving information from you and your laboratory at any time.

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 Chatterjee, S.: India Calcutta
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 Chung, K.W.: Republic of Korea Seoul
 Chung, Y.D.: Republic of Korea Seoul
 Chung, Y.J.: Republic of Korea Seoul
 Chung, Y.L.: Republic of Korea Seoul
 Cicchetti, R.: Italy Rome
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 Clark, S.H.: U.S. Newington, Connecticut
 Clark, W.: U.S. Piscataway, New Jersey
 Claudianos, C.: Australia Canberra, A.C.T.
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 Cochrane, B.J.: U.S. Tampa, Florida
 Cohen, B.: U.S. New York, New York

COHEN — DE MOED

Cohen, J.: U.S. New York, New York
 Cohen, J.: U.S. Philadelphia, Pennsylvania
 Cohen, L.: U.S. Philadelphia, Pennsylvania
 Cohen, S.: U.S. Houston, Texas
 Collett, J.I.: U.K. Brighton
 Colley, N.: U.S. La Jolla, California
 Collier, C.: U.K. Cambridge
 Comerón, J.M.: Spain Barcelona
 Compos, A.: U.S. Cambridge, Massachusetts
 Connolly, K.J.: U.K. Sheffield
 Conrad, A.: Germany Bochum
 Conway, J.: U.S. Philadelphia, Pennsylvania
 Cook, K.R.: U.S. Iowa City, Iowa
 Cooley, L.: U.S. New Haven, Connecticut
 Corces, V.G.: U.S. Baltimore, Maryland
 Cornelius, G.: Germany Saarbrücken
 Coronella, J.A.: U.S. Tampa, Florida
 Correa, M.C.: Columbia Medellín
 Costello, W.J.: U.S. Athens, Georgia
 Cotsell, J.: Australia Canberra, A.C.T.
 Couderc, J.L.: France Clermont-Ferrand
 Counce, S.J.: U.S. Durham, North Carolina
 Courreges, V.C.: U.S. New London, Connecticut
 Coyle-Thompson, C.: U.S. Sylmar, California
 Coyne, J.: U.S. Chicago, Illinois
 Coyne, J.A.: U.S. Chicago, Illinois
 Craddock, E.: U.S. Purchase, New York
 Craig, C.A.: U.S. St. Louis, Missouri
 Craven, D.: Australia Sydney, N.S.W.
 Crews, S.: U.S. Los Angeles, California
 Cribbs, D.: France Toulouse
 Cripps, R.: U.S. San Diego, California
 Cronmiller, C.R.: U.S. Charlottesville, Virginia
 Crosby, M.A.: U.S. St. Paul, Minnesota
 Crossley, S.A.: Australia Clayton, Victoria
 Crowley, T.: U.S. New York, New York
 Cuenca, J.: Spain Burjassot (Valencia)
 Cummings, M.R.: U.S. Chicago, Illinois
 Curtis, D.: U.S. Cambridge, Massachusetts
 Dalby, B.: U.K. London
 Danilevskaya, O.N.: C.I.S. Moscow, Russia
 Dapkus, D.: U.S. Winona, Minnesota
 Das, P.C.: India Calcutta
 DasGupta, S.: India Calcutta
 Dastugue, B.: France Clermont-Ferrand
 David, R.: U.S. Cold Spring Harbor, New York
 Davies, P.L.: Canada Kingston, Ontario
 Davis, A.: U.K. Leeds
 Davis, P.S.: U.S. Research Triangle Park, N. C.
 Davis, R.L.: U.S. East Lansing, Michigan
 Davis, T.: U.K. Brighton
 De, A.: India Varanasi
 De Couet, G.: U.S. Honolulu, Hawaii
 De Toma, C.: Italy Bologna
 de Frutos, R.: Spain Burjassot (Valencia)
 de Jong, G.: Netherlands Utrecht
 de Moed, G.: Netherlands Utrecht

DEAROLF — ENGSTROM

ERIESSON — GALINDO

Dearolf, C.R.: U.S. Boston, Massachusetts
 Degelmann, A.: Germany Düsseldorf
 Demopoulos, N.: Greece Patras
 Deng, Y.: U.S. Summit, New Jersey
 Deorgieva, T.G.: Bulgaria Sofia
 Desplan, C.: U.S. New York, New York
 Dewees, A.A.: U.S. Huntsville, Texas
 Di Lemma, G.: Italy Palermo
 Di Pasquale Paladino, A.: Italy Palermo
 Dickinson, M.: U.S. Chicago, Illinois
 Dickinson, W.J.: U.S. Salt Lake City, Utah
 Diederich, R.J.: U.S. New Haven, Connecticut
 Dimitrova, M.L.: Bulgaria Sofia
 Dinardo, S.: U.S. New York, New York
 Dixon, L. K.: U.S. University Park, Pennsylvania
 Dixon, L.: U.S. Denver, Colorado
 Doane, W.W.: U.S. Tempe, Arizona
 Doctor, J.: U.S. Pittsburgh, Pennsylvania
 Dodd, D.M.B.: U.S. Wilmington, North Carolina
 Doe, C.: U.S. Urbana, Illinois
 Dooher, K.: U.K. Leeds
 Dorsett, D.: U.S. New York, New York
 Druger, M.: U.S. Syracuse, New York
 Drysdale, R.: U.K. Cambridge
 Dübendorfer, A.: Switzerland Zürich
 Dudas, S. P.: U.S. Detroit, Michigan
 Duffy, J.: U.S. Stony Brook, New York
 Duncan, G.: U.S. Lincoln, Nebraska
 Duncan, I.: U.S. St. Louis, Missouri
 Duncker, B.: Canada Kingston, Ontario
 Durica, D.S.: U.S. Norman, Oklahoma
 Dusenbery, R.: U.S. Detroit, Michigan
 Dushay, M.S.: U.S. Waltham, Massachusetts
 DuttaGupta, A.K.: India Calcutta
 Dutton, F.L.: U.S. Storrs, Connecticut
 Duyf, B.: Canada Guelph, Ontario
 Dyby, S.: U.S. Austin, Texas
 Dytham, C.: U.K. Leeds
 Eanes, W.: U.S. Stony Brook, New York
 Eberl, D.: U.S. Buffalo, New York
 Ebert, P.: U.S. New Haven, Connecticut
 Edery, I.: U.S. Waltham, Massachusetts
 Edgecomb, R.S.: U.S. Ithaca, New York
 Egelhaaf, M.: Germany Tübingen
 Ehrman, L.: U.S. Purchase, New York
 Eisen, A.: U.S. Atlanta, Georgia
 Eissenberg, J.C.: U.S. St. Louis, Missouri
 Eisses, K.Th.: Netherlands Utrecht
 Ekström, K.: Sweden Umea
 Elgin, S.C.R.: U.S. St. Louis, Missouri
 Ellis, H.M.: U.S. Atlanta, Georgia
 Emecen, G.: Turkey Ankara
 Enander, P.: Sweden Umea
 Endow, S.A.: U.S. Durham, North Carolina
 Engels, W.: U.S. Madison, Wisconsin
 Englert, D.C.: U.S. Carbondale, Illinois
 Engstrom, L.E.: U.S. Muncie, Indiana

Ericsson, C.: Sweden Stockholm
 Ertürk, N.H.: Turkey Ankara
 Etges, W. J.: U.S. Fayetteville, Arkansas
 Evert, H.T.: Australia Clayton, Victoria
 Factor, J.R.: U.S. Purchase, New York
 Falk, R.: Israel Jerusalem
 Falk, R.: U.S. Amherst, Massachusetts
 Fang, H.: China Republic of Nankang, Taipei
 Farkas, R.: U.S. Piscataway, New Jersey
 Fasano, L.: U.S. New York, New York
 Feany, M.B.: U.S. Boston, Massachusetts
 Feder, J.: U.S. Chicago, Illinois
 Fehon, R.: U.S. Durham, North Carolina
 Fehr, K.: U.S. Atlanta, Georgia
 Felsenfeld, A.: U.S. Bethesda, Maryland
 Ferrandon, D.: Germany Tübingen
 Ferré, J.: Spain Burjassot (Valencia)
 Ferrús, A.: Spain Madrid
 Fessler, J.: U.S. Los Angeles, California
 Filosa, M.F.: Canada West Hill, Ontario
 Finelli, A.: U.S. Piscataway, New Jersey
 Finkelstein, R.: U.S. Boston, Massachusetts
 Finnegan, D.J.: U.K. Edinburgh, Scotland
 Finnerty, V.M.: U.S. Atlanta, Georgia
 Fischbach, K.-F.: Germany Freiburg i.
 Fitch, C.: U.S. Seattle, Washington
 Flavell, A.: U.K. Dundee, Scotland
 Fleming, R.: U.S. Rochester, New York
 Flint, K. K.: U.S. Waltham, Massachusetts
 Flister, S.: Switzerland Basel
 Fogleman, J.: U.S. Denver, Colorado
 Follner, K.: Germany München
 Forbes, P.D.: U.S. Horsham, Pennsylvania
 Forbes, S.: U.K. Edinburgh, Scotland
 Force, A.: U.S. Detroit, Michigan
 Forsyth, T.R.: U.S. Morehead, Kentucky
 Foster, J.L.: U.S. Memphis, Tennessee
 Fowler, K.: U.K. Edinburgh, Scotland
 Frankham, R.: Australia Sydney, N.S.W.
 Frasch, M.: U.S. New York, New York
 French, V.K.: U.K. Edinburgh, Scotland
 Freriksen, A.: Netherlands Utrecht
 Friedman, T.B.: U.S. East Lansing, Michigan
 Frisch-Brandes, B.: U.S. Waltham, Massachusetts
 Fryxell, K.: U.S. Riverside, California
 Füger, M.: Germany Tübingen
 Fujimoto, K.: Japan Okayama
 Fujisawa, R.: Japan Tokyo
 Fukatami, A.: Japan Sakado, Saitama
 Fukui, H.H.: U.S. St. Paul, Minnesota
 Fukumitsu, T.: Japan Okayama
 Furman, D.P.: C.I.S. Novosibirsk, Kazakhstan
 Furukubo-Tokunaga, K.: Switzerland Basel
 Fuse, N.: Japan Misima
 Fuyama, Y.: Japan Tokyo
 Galiana, A.: Spain Burjassot (Valencia)
 Galindo, M.I.: Spain Burjassot (Valencia)

GALL — GREENLEAF

Gall, J. G.: U.S. Baltimore, Maryland
 Galloni, M.: Switzerland Geneva
 Galoyan, A.A.: C.I.S. Yerevan, Armenia
 Ganetzky, B.S.: U.S. Madison, Wisconsin
 Garcia-Bellido, A.: Spain Madrid
 Garcia-H., M.E.: Mexico Saltillo, Coahuila
 Garden, A.: Canada Victoria, B.C.
 Garen, A.: U.S. New Haven, Connecticut
 Gateff, E.: Germany Mainz
 Gatti, M.: Italy Rome
 Geer, B.W.: U.S. Galesburg, Illinois
 Gehring, W.J.: Switzerland Basel
 Geisler, R.: Germany Tübingen
 Gelbart, W.M.: U.S. Cambridge, Massachusetts
 Gendre, N.: Switzerland Fribourg
 Genovés, J.F.: Spain Burjassot (Valencia)
 Georgieva, T.G.: C.I.S. Moscow, Russia
 Gepner, J.: U.S. St. Paul, Minnesota
 Gerasimova, T.I.: U.S. Baltimore, Maryland
 Gergen, P.: U.S. Stony Brook, New York
 Geyer, P.: U.S. Iowa City, Iowa
 Ghosh-Mukherjee, S.: India Calcutta
 Giangrande, A.: France Strasbourg
 Gibson, W.: U.S. Research Triangle Park, N. C.
 Gibson, J.B.: Australia Canberra City, ACT
 Gillam, I.: Canada Vancouver, B.C.
 Giorgi, G.: Italy Bologna
 Girton, J.R.: U.S. Ames, Iowa
 Glätzer, K.H.: Germany Düsseldorf
 Gloor, H.: Switzerland Geneva
 Glover, C.V.: U.S. Athens, Georgia
 Godt, D.: U.S. Los Angeles, California
 Goerick, S.: Germany Bochum
 Goldstein, E.: U.S. Tempe, Arizona
 Goldstein, L.: U.S. Cambridge, Massachusetts
 Golic, K.G.: U.S. Salt Lake City, Utah
 Golic, M.: U.S. Salt Lake City, Utah
 Gomez, S. P.: U.S. Gainesville, Florida
 Gómez-Gómez, F.: Colombia Bogotá
 Gong, Y.: Netherlands Nijmegen
 González, A.: Spain Burjassot (Valencia)
 González, F.: Spain Burjassot (Valencia)
 González, R.: Spain Barcelona
 Goode, E.A.: U.S. Research Triangle Park, N. C.
 Gordesky-Gold, B.: U.S. Philadelphia, Pennsylvania
 Gordon, K.: U.S. Salt Lake City, Utah
 Gore, N.D.: U.S. Research Triangle Park, N. C.
 Gorodetsky, V.P.: C.I.S. Kishinev, Ukraine
 Gottlieb, J.F.: U.S. Purchase, New York
 Götz, K.: Germany Tübingen
 Graf, U.: Switzerland Zürich
 Graham, L.: Canada Kingston, Ontario
 Granok, H.: U.S. St. Louis, Missouri
 Graphodatsky, A.S.: C.I.S. Novosibirsk, Kazakhstan
 Graves, J.: U.S. Research Triangle Park, N. C.
 Green, C.: U.K. Cambridge
 Greenleaf, A.L.: U.S. Durham, North Carolina

GREENSPAN — HEIKKINEN

Greenspan, R. J.: U.S. Nutley, New Jersey
 Grenke, L.: U.S. Chicago, Illinois
 Griffith, L.: U.S. Waltham, Massachusetts
 Grigliatti, T.: Canada Vancouver, B.C.
 Grinbaum, Y.: Israel Jerusalem
 Gromko, M.: U.S. Bowling Green, Ohio
 Grossniklaus, U.: Switzerland Basel
 Grozdev, V.A.: C.I.S. Moscow, Russia
 Gryllis, C.: Canada Montreal, Quebec
 Gubb, D.: U.K. Cambridge
 Guerra, D.: Italy Bologna
 Guild, G.: U.S. Philadelphia, Pennsylvania
 Gundelfinger, E.: Germany Hamburg
 Gupta, J.P.: India Varanasi
 Gupta, K.K.: India Varanasi
 Gustafson, T.: U.S. Rochester, Michigan
 Gutierrez, R.: Spain Madrid
 Gutknecht, J.: Germany Tübingen
 Haapala, O.: Finland Turku
 Hadziselimovic, R.: (frm) Yugoslavia Sarajevo
 Haenlin, M.: France Strasbourg
 Haerry, T.: Switzerland Basel
 Hageman, J.: Netherlands Utrecht
 Halder, G.: Switzerland Basel
 Hales, K.: U.S. Chicago, Illinois
 Hall, D.: Australia Canberra, A.C.T.
 Hall, J.C.: U.S. Waltham, Massachusetts
 Hall, L.M.: U.S. Buffalo, New York
 Hamblen-Coyle, M.J.: U.S. Waltham, Massachusetts
 Handler, A.M.: U.S. Gainesville, Florida
 Hannah-Alava, A.: Finland Turku
 Hanske, M.: Netherlands Nijmegen
 Hao, H.: Canada Kingston, Ontario
 Hardy, R.W.: U.S. La Jolla, California
 Harhanghi, H.: Netherlands Nijmegen
 Hariyama, T.: Japan Sendai
 Harkins, B.: U.S. New Haven, Connecticut
 Harrelson, A.: U.S. Columbia, Missouri
 Harrington, M.: Canada Vancouver, B.C.
 Harris, R.J.: U.S. Eugene, Oregon
 Harshman, L.: U.S. Davis, California
 Hartl, D.: U.S. Cambridge, Massachusetts
 Hashimoto, C.: U.S. New Haven, Connecticut
 Hauschteck-Jungen, E.: Switzerland Zürich
 Hawley, R.S.: U.S. Bronx, New York
 Hayashi, I.: U.S. Duarte, California
 Hayashi, L.: U.S. Honolulu, Hawaii
 Hayashi, S.: Canada Vancouver, B.C.
 Hayashi, S.: Japan Misima
 Haymer, D.S.: U.S. Honolulu, Hawaii
 Haynes, S.R.: U.S. Bethesda, Maryland
 Hays, T.S.: U.S. St. Paul, Minnesota
 Heck, M.: U.S. Baltimore, Maryland
 Hedrick, P.W.: U.S. Tempe, Arizona
 Heemskerk, J.: U.S. Berkeley, California
 Hegde, S.N.: India Mysore
 Heikkinen, E.: Finland Oulu

HEIKKINEN — HU, D.

HU, K. — JUPE

Heikkinen, E.: Germany Tübingen
 Heilig, J.S.: U.S. Boulder, Colorado
 Heino, T.I.: Finland Helsinki
 Heimbeck, G.: U.K. Cambridge
 Heisenberg, M.: Germany Würzburg
 Heisler, I.L.: U.S. Eugene, Oregon
 Heitzler, P.: France Strasbourg
 Held, L. I., jr.: U.S. Lubbock, Texas
 Hellack, J.J.: U.S. Norman, Oklahoma
 Hengstenberg, R.: Germany Tübingen
 Hennig, W.: Netherlands Nijmegen
 Henrich, V.C.: U.S. Greensboro, North Carolina
 Hernandez, A.: Spain Burjassot (Valencia)
 Hernandez, M.E.G.: U.S. Hidalgo, Texas
 Hess, O.: Germany Düsseldorf
 Hickey, D.: Canada Ottawa, Ontario
 Hilfiker, A.: U.S. Atlanta, Georgia
 Hilfiker, D.: U.S. Atlanta, Georgia
 Hill, R.J.: Australia Sydney, N.S.W.
 Hilliker, A.: Canada Guelph, Ontario
 Hillman, R.: U.S. Philadelphia, Pennsylvania
 Hinz, U.: Germany Köln
 Hirose, S.: Japan Misima
 Hirsch, J.: U.S. Champaign, Illinois
 Hirsh, J.: U.S. Charlottesville, Virginia
 Ho, K.-F.: U.S. New York, New York
 Hochmuth, E.: Germany München
 Hochstenbach, R.: Netherlands Nijmegen
 Hoekstra-du Pui, M.L.L.: Netherlands Haren, Groningen
 Hoelzinger, D.: U.S. Tempe, Arizona
 Hoenigsberg, H.F.: Columbia Bogota
 Hofbauer, A.: Germany Würzburg
 Hofstetter, A.: Germany München
 Hoikkala, A.: Finland Oulu
 Hollenhurst, B.: U.S. Notre Dame, Indiana
 Hollocher, H.: U.S. Chicago, Illinois
 Holm, D.: Canada Vancouver, B.C.
 Holmgren, R.: U.S. Evanston, Illinois
 Homyk Jr., T.: U.S. Charlottesville, Virginia
 Honegger, M.: U.S., Salt Lake City, Utah
 Hong, K.J.: Republic of Korea Seoul
 Hooper, J.: U.S. Denver, Colorado
 Hopmann, R.: U.S. St. Louis, Missouri
 Hori, S.H.: Japan Sapporo
 Horris, P.M.V.: U.S. Davis, California
 Hosford, J.: U.S. Chestnut Hill, Massachusetts
 Hoshizaki, D.: U.S. Chicago, Illinois
 Hosoya, T.: Japan Tokyo
 Hossain, M.A.: Bangladesh Rajshahi
 Hotta, Y.: Japan Tokyo
 Houle, D.: U.S. Chicago, Illinois
 Houle, D.: Canada Toronto, Ontario
 Houtchens, K.: U.S. Honolulu, Hawaii
 Hovemann, B.T.: Germany Heidelberg
 Hoy, M.: U.S. Berkeley, California
 Hsieh, T.: U.S. Durham, North Carolina
 Hu, D.: China Beijing

Hu, K.: China People's Repb. of Hainan
 Huang, N.: U.S. Norman, Oklahoma
 Huang, S.-M.: U.S. Research Triangle Park, N. C.
 Huey, R. B.: U.S. Seattle, Washington
 Hultmark, D.: Sweden Stockholm
 Hung, M.-H.: China Republic of Nankang, Taipei
 Hunt, J.A.: U.S. Honolulu, Hawaii
 Hurley, J. B.: U.S. Seattle, Washington
 Hussain, A.F.: Iraq Baghdad
 Hyde, D.: U.S. Notre Dame, Indiana
 Iannini, A.: Colombia Bogotá
 Idili, C.: Italy Rome
 Imasheva, A.G.: C.I.S. Moscow, Russia
 Imberski, R.B.: U.S. College Park, Maryland
 Inaga, A.: Japan Sakado, Saitama
 Inocencio-Green, B.: U.S. Purchase, New York
 Inoue, Y.: Japan Osaka, Minoo
 Irick, H.: U.S. Davis, California
 Irizarry, B.: U.S. Rio Piedras, Puerto Rico
 Ish-Horowicz, D.: U.K. Oxford
 Ishii, S.: Japan Ibaraki Tsukuba
 Islam, M.S.: Bangladesh Rajshahi
 Isoda, K.: Germany Tübingen
 Isono, K.: Japan Sendai
 Ito, K.: Germany Mainz
 Ivannikov, A.V.: C.I.S. Novosibirsk, Kazakhstan
 Jack, J.W.: U.S. New York, New York
 Jackson, F.R.: U.S. Shrewsbury, Massachusetts
 Jacobs, J.R.: Canada Hamilton, Ontario
 Jacobs-Lorena, : U.S. Cleveland, Ohio
 Jacobson, J.W.: U.S. Houston, Texas
 Jaenike, J.: U.S. Rochester, New York
 Jaime, B.: Mexico Saltillo, Coahuila
 James, T.C.: U.S. Middletown, Connecticut
 Jan, L.: U.S. San Francisco, California
 Janning, W.: Germany Münster
 Jarman, M.: U.K. Brighton
 Jeffery, D.E.: U.S. Provo, Utah
 Jelisavcic, B.: (frm) Yugoslavia Belgrade
 Jenkinson, L.: U.K. Leeds
 Jeong, D.Y.: U.S. Eugene, Oregon
 Jeung, M.: U.S. Norman, Oklahoma
 Johansson, B.: Sweden Umea
 Jonathan, B.C.: U.S. Tucson, Arizona
 Johnsen, R.C.: U.S. Garden City, New York
 Johnson, D.: U.S. Washington, D. C.
 Johnson, G.: U.K. Cambridge
 Johnson, K.: U.K. Cambridge
 Johnson, W.A.: U.S. Iowa City, Iowa
 Jones, G.: U.S. Lexington, Kentucky
 Jones, C.J.: U.S. Coldspring Harbor, New York
 Jovanovska-Rizova, M.: (frm) Yugoslavia Skopje
 Jowett, T.: U.K. Newcastle Upon Tyne
 Juan, E.: Spain Barcelona
 Judd, B.H.: U.S. Research Triangle Park, N. C.
 Judd, D.: U.S., Salt Lake City, Utah
 Jupe, E.R.: U.S. Cincinnati, Ohio

KADLECOVA — KLARENBERG

Kadlecova, J.: Czechoslovakia BRNO
 Kaguni, L.: U.S. East Lansing, Michigan
 Kaidanov, L.Z.: C.I.S. St. Petersburg, Russia
 Kaiser, K.: U.K. Glasgow, Scotland
 Kalisch, W.-E.: Germany Bochum
 Kalthoff, K.: U.S. Austin, Texas
 Kambysellis, M.P.: U.S. New York, New York
 Kamdar, K.P.: U.S. Atlanta, Georgia
 Kamping, A.: Netherlands Haren, Groningen
 Kaneko, A.: Japan Tokyo
 Kaneko, M.: Japan Tokyo
 Kaneshiro, K.Y.: U.S. Honolulu, Hawaii
 Kang(Song), Soon JA.: Republic of Korea Seoul
 Kania, M.: U.S. Stony Brook, New York
 Kankel, D.R.: U.S. New Haven, Connecticut
 Kankel, K.: U.S. New Haven, Connecticut
 Kar, A.: India Pune
 Karch, F.: Switzerland Geneva
 Karkowski-Shuman, L.: U.S. University Park, PA
 Karpen, G.: U.S. San Diego, California
 Karpovsky, A.L.: Russian Fed. Dubna Moscow
 Kasprzak, A.: Australia Canberra, A.C.T.
 Katokhin, A.V.: C.I.S. Novosibirsk, Kazakhstan
 Katz, F.: U.S. Dallas, Texas
 Katzen, A.: U.S. San Francisco, California
 Kazuko, S.: U.S., Salt Lake City, Utah
 Keegan, L.: Switzerland Basel
 Kekic, V.: (frm) Yugoslavia Belgrade
 Kellerman, K.: U.S. St. Louis, Missouri
 Kennison, J.: U.S. Bethesda, Maryland
 Kernan, M.: U.S. La Jolla, California
 Kerridge, S.: France Marseille
 Khadem, N.: Greece Athens
 Khazaeli, A.A.: U.S. St. Paul, Minnesota
 Khechumian, R.K.: C.I.S. Yerevan, Armenia
 Kibart, M.: U.K. Sheffield
 Kidwell, M.G.: U.S. Tucson, Arizona
 Kiefel, P.: U.S. St. Louis, Missouri
 Kiehart, D.: U.S. Durham, North Carolina
 Kim, A.J.: Republic of Korea Seoul
 Kim, D.I.: Republic of Korea Taejeon
 Kim, N.W.: Republic of Korea Seoul
 Kim, S.Y.: Republic of Korea Seoul
 Kim, U.K.: Republic of Korea Seoul
 Kim, W.: Republic of Korea Choong Nam
 Kim, Y.-K.: U.S. Purchase, New York
 Kimura, M.T.: Japan Sapporo
 King, L.: U.S. Cambridge, Massachusetts
 King, R.C.: U.S. Evanston, Illinois
 Kirby, K.: Canada Guelph, Ontario
 Kirschfeld, K.: Germany Tübingen
 Kitagawa, O.: Japan Tokyo
 Kittel, M.: Germany Tübingen
 Kitthawee, S.: Thailand Bangkok
 Klämbt, C.: Germany Köln
 Klarenberg, A.: U.K. Leeds
 Klarenberg, A.J.: Germany München

KLINGLER — LATTER

Klingler, M.: U.S. Stony Brook, New York
 Kloter, U.: Switzerland Basel
 Klug, W.S.: U.S. Trenton, New Jersey
 Knössel, M.: Germany Tübingen
 Knust, E.: Germany Köln
 Koehler, W.: Germany Giessen
 Koepfer, H.R.: U.S. New York, New York
 Koizumi, K.: Japan Tokyo
 Kojima, S.: Japan Tokyo
 Kokoza, V.A.: C.I.S. Novosibirsk, Kazakhstan
 Kolia-Yakoumi, G.: Greece Athens
 Koliantz, G.: U.S. West Lafayette, Indiana
 Komatsu, A.: Japan Tokyo
 Komma, D.: U.S. Durham, North Carolina
 Kondo, S.: Switzerland Basel
 Kopyl, S.A.: C.I.S. Novosibirsk, Kazakhstan
 Korablinova, S.V.: Russian Fed. Dubna Moscow
 Korochkin, L.I.: C.I.S. Moscow, Russia
 Korol, A.B.: C.I.S. Kishinev, Ukraine
 Kosuda, K.: Japan Sakado, Saitama
 Kotarski, M.A.: U.S. Morgantown, West Virginia
 Kouriti, A.: Greece Athens
 Kovtyukh, L.P.: C.I.S. Kishinev, Ukraine
 Kozhemyakina, T.A.: C.I.S. Novosibirsk, Kazakhstan
 Kozlova, T.Yu.: C.I.S. Novosibirsk, Kazakhstan
 Kramers, P.G.N.: Netherlands Bilthoven
 Krasnow, M.: U.S. Stanford, California
 Kraus, K.: U.S. Decorah, Iowa
 Kreber, R.A.: U.S. Madison, Wisconsin
 Krebs, R.A.: Australia Armidale, NSW
 Krefer, O.: Germany München
 Kreitman, M.: U.S. Chicago, Illinois
 Krider, H.: U.S. Storrs, Connecticut
 Krimbas, C. B.: Greece Athens
 Kroeger, H.: Germany Saarbrücken
 Kuhn, D.T.: U.S. Orlando, Florida
 Kundu, J.K.: India Calcutta
 Kurenova, E.V.: C.I.S. Moscow, Russia
 Kuroda, M.: U.S. Houston, Texas
 Kurzik-Dumke, U.: Germany Mainz
 Kuzin, B.: U.S. Philadelphia, Pennsylvania
 Kwon, H.C.: Republic of Korea Seoul
 Kyba, M.: Canada Vancouver, B.C.
 Labonne, S.: U.S. Schenectady, New York
 Lachaise, D.: France Gif-sur-Yvette
 Laguna, M.: Colombia Bogotá
 Laird, C.: U.S. Seattle, Washington
 Lakhotia, S.C.: India Varanasi
 Lakovaara, S.: Finland Oulu
 Lang, A.: U.K. Leeds
 Lankenau, Dr.: U.S. Baltimore, Maryland
 Lankinen, P.: Finland Oulu
 Larruga, J.M.: Spain Tenerife
 Larsen, E.: Canada Toronto, Ontario
 Larsson, J.: Sweden Umea
 Latorre, A.: Spain Burjassot (Valencia)
 Latter, B.D.H.: Australia Lismore, N.S.W.

LAUGHON — MACDOWELL

Laughon, A.: U.S. Madison, Wisconsin
 Laurie, C.: U.S. Durham, North Carolina
 Lavery, K.J.: Australia Clayton, Victoria
 Lazebny, O.E.: C.I.S. Moscow, Russia
 Lee, B.: Canada Vancouver, B.C.
 Lee, C.C.: Republic of Korea Seoul
 Lee, T.J.: Republic of Korea Seoul
 Lee, W.: U.S. Baton Rouge, Louisiana
 Leemans, R.: Switzerland Basel
 Leibovitch, B.A.: C.I.S. Moscow, Russia
 Leigh-Brown, A.J.: U.K. Edinburgh, Scotland
 Leisner, J.: Denmark Lyngby
 Lemann, R.: U.S. Cambridge, Massachusetts
 Lengyel, J.: U.S. Los Angeles, California
 Lepesan, J.A.: France Paris
 Lessard, R.: U.S. Chicago, Illinois
 Letson, A.: U.S. Salt Lake City, Utah
 Lev, Z.: Israel Haifa
 Levan, G.: Sweden Goteborg
 Levine, L.: U.S. New York, New York
 Levis, R.: U.S. Seattle, Washington
 Levitan, M.: U.S. New York, New York
 Lewontin, R.: U.S. Cambridge, Massachusetts
 Li, M.-G.: U.S. St. Paul, Minnesota
 Lidholm, D.: U.S. St. Louis, Missouri
 Lim, C.-S.: U.S. Columbia, Missouri
 Lim, J.K.: U.S. Eau Claire, Wisconsin
 Lin, F.-J.: China Republic of Nankang, Taipei
 Lin, J.C.: U.S. Natchitoches, Louisiana
 Lin, S.-F.: China Republic of Nankang, Taipei
 Lindquist, S.: U.S. Chicago, Illinois
 Lindsley, D.L.: U.S. La Jolla, California
 Lipshitz, H.: U.S. Pasadena, California
 Lloyd, V.: Canada Vancouver, B.C.
 Locke, J.: Canada Edmonton, Alberta
 Lockett, T.J.: Australia Sydney, N.S.W.
 Loeschcke, V.: Denmark Aarhus
 Lofdahl, K.L.: U.S. Champaign, Illinois
 Löffler, T.: Germany Mainz
 Lohe, A.: U.S. Cambridge, Massachusetts
 Loosli, F.: Switzerland Basel
 López, A.J.: U.S. Pittsburgh, Pennsylvania
 López, A.: Spain Burjassot (Valencia)
 López, N.: Columbia Medellín
 Lord, P.: U.S. Chicago, Illinois
 Louis, C.: France St. Christol-les-Ales
 Loukas, M.: Greece Athens
 Lozovskaya, E.: U.S. St. Louis, Missouri
 Lu, Q.: U.S. St. Louis, Missouri
 Lucchesi, J.C.: U.S. Atlanta, Georgia
 Ludwig, M.: U.S. Tampa, Florida
 Lumme, J.: Finland Oulu
 Lyman, D.: U.S. New York, New York
 Lyman, R.F.: U.S. Raleigh, North Carolina
 Lytle, T.W.: U.S. Honolulu, Hawaii
 Lyu, M.S.: Korea Seoul
 MacDowell, K.: U.S. Atlanta, Georgia

MACHADO — MERRIAM

Machado, R. G.: Columbia Medellín
 Machova, H.: Czechoslovakia BRNO
 MacIntyre, R.J.: U.S. Ithaca, New York
 Magnusson, J.: Sweden Stockholm
 Maguire, M.: U.S. Austin, Texas
 Mahaffey, J.: U.S. Raleigh, North Carolina
 Mahowald, A.P.: U.S. Chicago, Illinois
 Maitra, S.: India Calcutta
 Mal'ceva, N.I.: C.I.S. Novosibirsk, Kazakhstan
 Malevanchuk, O.A.: C.I.S. Moscow, Russia
 Mann, R.S.: U.S. New York, New York
 Manna-Kundu, N.: India Calcutta
 Manso, F.: Argentina Buenos Aires
 Margulies, L.: U.S. Valhalla, New York
 Marinkovic, D.: (frm) Yugoslavia Belgrade
 Markova, B.A.: Bulgaria Sofia
 Markow, T.: U.S. Tempe, Arizona
 Maroni, G.P.: U.S. Chapel Hill, North Carolina
 Marsh, L.: U.S. Irvine, California
 Martin, P.F.: U.S. Philadelphia, Pennsylvania
 Martín-Campos, J.M.: Spain Barcelona
 Martin-Morris, L.: U.S. Seattle, Washington
 Martínez M.J.: Spain Burjassot (Valencia)
 Martinez, R.M.: U.S. Hamden, Connecticut
 Martínez, O.: Colombia Bogotá
 Maruo, F.: Japan Tsukuba, Ibaraki
 Masai, I.: Japan Tokyo
 Mason, J.M.: U.S. Research Triangle Park, N. C.
 Massie, H.R.: U.S. Utica, New York
 Masuda, K.-i.: Japan Okayama
 Matos, M.: Portugal Lisbon
 Matsuda, M.: Japan Tokyo
 Matsuura, E.T.: Japan Tokyo
 Matsuzaki, F.: Japan Tokyo
 Mathew, S.: India Varanasi
 Matthews, K.: U.S. Bloomington, Indiana
 Mattson, D.: U.S. St. Louis, Missouri
 Mazar-Barnett, B.: Argentina Buenos Aires
 McCarron, M.: U.S. Storrs, Connecticut
 McCarthy, P.C.: U.S. New Wilmington, PA
 McCrady, W.: U.S. Arlington, Texas
 McElwain, C.: U.S. Los Angeles, California
 McEvey, S.: Australia Sydney, N.S.W.
 McGrail, M.: U.S. St. Paul, Minnesota
 McGuffin, M.E.: U.S. Houston, Texas
 McKearin, D.: U.S. Dallas, Texas
 McKee, B.: U.S. Knoxville, Tennessee
 McKimmie, C.: U.K. Cambridge
 McNamee, S.: U.K. Leeds
 McRobert, S. P.: U.S. Philadelphia, Pennsylvania
 Medina, M.: Spain Madrid
 Meister, G.: Canada Vancouver, B.C.
 Ménsua, J.L.: Spain Burjassot (Valencia)
 Meredith, J.: U.S. Chicago, Illinois
 Mergny, J.-L.: Switzerland Basel
 Mermall, V.: U.S. St. Louis, Missouri
 Merriam, J.: U.S. Los Angeles, California

MERRIMAN — MURATA

Merriman, P.J.: U.S. St. Paul, Minnesota
 Mestres, F.: Spain Barcelona
 Miassod, R.: France Marseille
 Michael, C.P.: U.S. Atlanta, Georgia
 Michalopoulou, E.: Greece Patras
 Michelson, A.: U.S. Boston, Massachusetts
 Miedema, K.: Netherlands Nijmegen
 Miglani, G.S.: India Ludhiana
 Mikasa, K.: Japan Sakado, Saitama
 Miklos, G.L.G.: Australia Canberra, A.C.T.
 Miklos, M.: Australia Canberra, A.C.T.
 Milanovic, M.: (frm) Yugoslavia Belgrade
 Miller, K.: U.S. St. Louis, Missouri
 Milner, M.J.: U.K. St. Andrews, Scotland
 Milosevic, M.: (frm) Yugoslavia Belgrade
 Milosevic, N.: (frm) Yugoslavia Belgrade
 Minato, K.: Japan Misima
 Mindek, G.: Switzerland Zürich
 Miyamoto, T.: Japan Kagawa
 Mizrokhi, L.: U.S. Philadelphia, Pennsylvania
 Mndjoyan, E.O.: C.I.S. Yerevan, Armenia
 Mogami, K.: Japan Tokyo
 Mohler, J.P.: U.S. New York, New York
 Moltó, M.D.: Spain Burjassot (Valencia)
 Montagne, J.: Switzerland Basel
 Montaña, A.: Colombia Bogotá
 Montell, D.: U.S. Baltimore, Maryland
 Montgomery, M.: Australia Sydney, N.S.W.
 Moore, B.C.: U.S. Riverside, California
 Moore, J.A.: U.S. Riverside, California
 Moore, R.H.: New Zealand Wellington
 Morales, B.: U.S. Rio Piedras, Puerto Rico
 Moreno, A.: Colombia Bogotá
 Morin, K.: Canada Vancouver, B.C.
 Moriwaki, D.: Japan Tokyo
 Moriyama, E.N.: Japan Misima
 Morley, T.: U.K. Cambridge
 Morrison, W.J.: U.S. Shippensburg, Pennsylvania
 Morton, R.A.: Canada Hamilton, Ontario
 Moreteau, B.: France, Gif-Sur-yvette
 Moses, K.: U.S. Los Angeles, California
 Mottus, R.: Canada Vancouver, B.C.
 Mount, S.: U.S. New York, New York
 Moya, A.: Spain Burjassot (Valencia)
 Molina, J.L.M.: Spain Alicante
 Mrcalica, E.: (frm) Yugoslavia Nis
 Muckenthaler, F.A.: U.S. Bridgewater, Massachusetts
 Mueller, L.D.: U.S. Irvine, California
 Mukherjee, A.: India Varanasi
 Mukherjee, A.S.: India Calcutta
 Mukherjee, J.: India Calcutta
 Mukherjee, R.: India Calcutta
 Müller, E.: Germany Tübingen
 Muñoz, E.R.: Argentina Buenos Aires
 Munstermann, L.: U.S. Notre Dame, Indiana
 Murad, A.M.B.: Iraq Baghdad
 Murata, H.: Japan Misima

MURNIK — ORDÓÑEZ

Murnik, M.: U.S. Big Rapids, Michigan
 Muskavitch, M.A.T.: U.S. Bloomington, Indiana
 Mutsuddi, M.: India Varanasi
 Myszewski, M.E.: U.S. Des Moines, Iowa
 Nadarajah, L.: U.K. Brighton
 Nahmias, J.: U.S. Raleigh, North Carolina
 Narise, S.: Japan Sakado, Saitama
 Narise, T.: Japan Uravasu, Chiba
 Narveson, D.: U.S. St. Louis, Missouri
 Naseerulla, M.K.: India Mysore
 Nash, H.: U.S. Bethesda, Maryland
 Nash, W.G.: U.S. Frederick, Maryland
 Nason, J.: U.S. Rochester, Michigan
 Nauber, U.: Germany Goettingen
 Navia, M.: Colombia Bogotá
 Neckameyer, W.: U.S. St. Louis, Missouri
 Newfeld, S.J.: U.S. Cambridge, Massachusetts
 Nichols, R.: U.S. Ann Arbor, Michigan
 Nicholson, M.: U.S. Detroit, Michigan
 Nicoletti II, B.: Italy Rome
 Niño, I.: Colombia Bogotá
 Nirenberg, M.: U.S. Bethesda, Maryland
 Nishida, Y.: Japan Nagoya
 Nitasaka, E.: U.S. Cambridge, Massachusetts
 Nitta, K.: Japan Tokyo
 Noach, S.: Netherlands Utrecht
 Noël, P. J.: U.S. Cincinnati, Ohio
 Nokkala, C.: Finland Turku
 Nokkala, S.: Finland Turku
 Noll, M.: Switzerland Zürich
 Nomura, T.: Japan Osaka
 Noor, M.: U.S. Chicago, Illinois
 Noor, R.R.: Australia Armidale, NSW
 Norman, R.A.: U.S. Tempe, Arizona
 Nöthiger, R.: Switzerland Zürich
 Novitski, E.: U.S. Eugene, Oregon
 Nunnery, L.: U.S. Riverside, California
 Nusse, R.: U.S. Stanford, California
 Nüsslein-Volhard, C.: Germany Tübingen
 O'Brien, L.: U.K. Leeds
 O'Brien, S.J.: U.S. Frederick, Maryland
 O'Connor, M.: U.S. Irvine, California
 O'Donnell, J.M.: U.S. Tuscaloosa, Alabama
 O'Donnell, M.P.: U.S. St. Louis, Missouri
 O'Hare, K.: U.K. London
 O'Neill, S.: U.S. New Haven, Connecticut
 Ochoa-R., A.M.: Mexico Saltillo, Coahuila
 Odenwald, W.: U.S. Bethesda, Maryland
 Offenberger, M.: Germany München
 Oh, E.: U.S. Berkeley, California
 Ohnishi, E.: Japan Okayama
 Ohno, C.: Canada Kingston, Ontario
 Okada, T.: Japan Tokyo
 Okazaki, A.: Japan Tokyo
 Olivarez-S., G.: Mexico Saltillo, Coahuila
 Omelianchuk, L.V.: C.I.S. Novosibirsk, Kazakhstan
 Ordóñez, M.: Colombia Bogotá

ORR — PIERRE

Orr, A.A.: U.S. Davis, California
 Orr-Weaver, T.L.: U.S. Cambridge, Massachusetts
 Orvieto, L.: U.S. Chestnut Hill, Massachusetts
 Osgood, C.: U.S. Norfolk, Virginia
 Osheim, Y.: U.S. Charlottesville, Virginia
 Oster, P.: U.S. Bowling, Green, Ohio
 Otteson, D.C.: U.S. Ann Arbor, Michigan
 Packert, G.: U.S. Orlando, Florida
 Padgett, R.W.: U.S. Piscataway, New Jersey
 Paik, Y.K.: Republic of Korea Seoul
 Pak, W.: U.S. West Lafayette, Indiana
 Pal-Bhadra, M.: India Calcutta
 Palermo, A.M.: Argentina Buenos Aires
 Palmer, M.J.: U.S. Stillwater, Oklahoma
 Palter, K.: U.S. Philadelphia, Pennsylvania
 Pandey, M.B.: India Varanasi
 Pandey, P.: U.S. Rochester, Michigan
 Pannuti, A.: U.S. Atlanta, Georgia
 Papaceit, M.: Spain Barcelona
 Parádi, E.: Hungary Budapest
 Parício, N.: Spain Burjassot (Valencia)
 Parisi, M.: U.S. New York, New York
 Parkhurst, S.: U.S. Seattle, Washington
 Parks, S.: U.S. Baltimore, Maryland
 Paro, R.: Germany Heidelberg
 Partridge, L.: U.K. Edinburgh, Scotland
 Pascual, L.: Spain Burjassot (Valencia)
 Pascual, M.: Spain Barcelona
 Paterson, B.M.: U.S. Bethesda, Maryland
 Pathak, K.: India Calcutta
 Pattatucci, A.: U.S. Bethesda, Maryland
 Pauli, D.: Switzerland Geneva
 Pavkovic, S.: (frm) Yugoslavia Belgrade
 Perkins, L.A.: U.S. Boston, Massachusetts
 Pelliccia, J.: U.S. Lewiston, Maine
 Peng, T.: U.K. Leeds
 Pepling, M.: U.S. Stony Brook, New York
 Pérez, M.: Spain Burjassot (Valencia)
 Perez-Chiesa, Y.: U.S. Rio Piedras, Puerto Rico
 Periquet, G.: France Tours
 Perotti, M.: Italy Milano
 Perrimon, N.: U.S. Boston, Massachusetts
 Perrin-Schmitt, F.: France Strasbourg
 Peterson, J.: U.S. Chestnut Hill, Massachusetts
 Peterson, K.: U.S. Baltimore, Maryland
 Petkovic, D.: (frm) Yugoslavia Belgrade
 Petkovich, P.M.: Canada Kingston, Ontario
 Petri, W.H.: U.S. Chestnut Hill, Massachusetts
 Peunova, N.I.: C.I.S. Moscow, Russia
 Pezzoli, M.C.: Italy Bologna
 Pfeifer, T.: Canada Vancouver, B.C.
 Phillips, J.: Canada Guelph, Ontario
 Phillips, R.G.: U.K. Brighton
 Piano, F.: U.S. New York, New York
 Pick, L.: U.S. New York, New York
 Piedrafita, A.C.: Spain Burjassot (Valencia)
 Pierre, C.: U.S. St. Louis, Missouri

PINEDA — REGNER

Pineda, N.: Columbia Medellín
 Piñón, L. S.: Spain Lugo
 Piovant, M.: France Marseille
 Plehn, Chr.: Germany Bochum
 Podoplevova, M.L.: C.I.S. Novosibirsk, Kazakhstan
 Pokholkova, G.V.: C.I.S. Novosibirsk, Kazakhstan
 Polanco, M.M.E.: Colombia Bogotá
 Pongs, O.: Germany Bochum
 Ponimaskin, E.G.: C.I.S. Novosibirsk, Kazakhstan
 Poodry, C.: U.S. Santa Cruz, California
 Porter, A.: U.K. Leeds
 Portin, P.: Finland Turku
 Posakony, J.: U.S. La Jolla, California
 Possidente, B.: U.S. Saratoga Springs, New York
 Postlethwait, J. H.: U.S. Eugene, Oregon
 Powell, B.: U.S. Norfolk, Virginia
 Powell, J.R.: U.S. New Haven, Connecticut
 Pradel, J.: France Marseille
 Preat, T.: U.S. Waltham, Massachusetts
 Preiss, A.: Switzerland Basel
 Prevosti, A.: Spain Barcelona
 Price, J.: Canada Burnaby, B.C.
 Price, M.: Canada Kingston, Ontario
 Primus, J.P.: U.S. Atlanta, Georgia
 Protopopov, M.O.: C.I.S. Novosibirsk, Kazakhstan
 Prout, T.: U.S. Davis, California
 Pulido, D.: Spain Madrid
 Purcell, J.: U.S. Irvine, California
 Puro, J.: Finland Turku
 Purobit, S.: U.S. Davis, California
 Pursey, J.: U.S. Buffalo, New York
 Quan, F.: U.S. Portland, Oregon
 Quiring, R.: Switzerland Basel
 Quisada-Allue, L.A.: Argentina Buenos Aires
 Raab-Vetter, M.: Germany Tübingen
 Rabinow, L.: U.S. Piscataway, New Jersey
 Racobaldo, N.: U.S. Philadelphia, Pennsylvania
 Raff, E.C.: U.S. Bloomington, Indiana
 Raftery, L. A.: U.S. Charlestown, Massachusetts
 Ramel, C.: Sweden Stockholm
 Ramesh, S.R.: India Mysore
 Ramirez, E.: Spain Alicante
 Ramos, R.G.P.: Brasil Rio de Janeiro
 Ramos, S.: Spain Barcelona
 Rand, D.: U.S. Providence, Rhode Island
 Ranganath, H.A.: India Mysore
 Rasmuson, B.: Sweden Umea
 Rasmuson, M.: Sweden Umea
 Rasmuson-Lestander, Å.: Sweden Umea
 Rasooly, R.S.: U.S. Bronx, New York
 Rasumsson, K.: U.S. St. Paul, Minnesota
 Rawls, J.: U.S. Lexington, Kentucky
 Ray, P.: India Varanasi
 Raymnod, J.D.: U.S. St. Paul, Minnesota
 Real, L.: Spain Burjassot (Valencia)
 Regan, J.: U.K. Cambridge
 Regner, L.P.: Brazil Porto Alegrea

REICHERT — SALVATERRA

Reichert, J.: Germany Tübingen
 Reinikainen, J.: Finland Oulu
 Reiss, J.A.: U.S. Atlanta, Georgia
 Rendahl, K. G.: U.S. Waltham, Massachusetts
 Renkawitz-Pohl, R.: Germany Marburg
 Resendez-Perez, D.: Switzerland Basel
 Restifo, L.L.: U.S. Tucson, Arizona
 Resto, L.: U.S. Rio Piedras, Puerto Rico
 Reuter, G.: Canada Vancouver, B.C.
 Rey, M.: Argentina Buenos Aires
 Ribaudo, R.M.: Italy Palermo
 Richards, G.: France Strasbourg
 Richardson, E.: U.S. Chestnut Hill, Massachusetts
 Richmond, P.C.: U.S. Tampa, Florida
 Riihimaa, A.: Finland Oulu
 Ring, M.: Canada Vancouver, B.C.
 Robbins, L.G.: U.S. East Lansing, Michigan
 Robertson, H.: U.S. Urbana, Illinois
 Robinow, S.: U.S. Seattle, Washington
 Roca, A.: Spain Oviedo
 Rockwell, R.F.: U.S. New York, New York
 Rodin, S.N.: C.I.S. Novosibirsk, Kazakhstan
 Rodriguez, D.: U.S. Columbia, Missouri
 Rodriguez, H.: U.S. Rio Piedras, Puerto Rico
 Rodriguez, N.I.: U.S. Rio Piedras, Puerto Rico
 Rogulski, K.R.: U.S. Cincinnati, Ohio
 Rojas, N.: Colombia Bogotá
 Romans, P.: Canada Toronto, Ontario
 Romero, I.: Colombia Bogotá
 Roos, C.: Finland Helsinki
 Roote, J.: U.K. Cambridge
 Rosenstein, A. W.: U.S. Davis, California
 Rosset, R.: France Marseille
 Roth, H.: U.S. Los Angeles, California
 Rothenfluh, A.: Switzerland Basel
 Rothwell, K.: U.K. Edinburgh, Scotland
 Rouyer, F.: U.S. Waltham, MA
 Roy, J.K.: India Varanasi
 Rozas, J.: Spain Barcelona
 Rubin, G.M.: U.S. Berkeley, California
 Rudkin, G.T.: U.S. Philadelphia, Pennsylvania
 Ruiz, P.: Spain Burjassot (Valencia)
 Ruíz-García, M.: Colombia Bogotá
 Russell, C.: U.K. Brighton
 Russell, S.: U.K. Cambridge
 Rutherford, S.: U.S. La Jolla, California
 Ryerse, J.S.: U.S. St. Louis, Missouri
 Ryo, H.: Japan Osaka
 Ryskova, M.: Czechoslovakia BRNO
 Sagarra, E.: Spain Barcelona
 Sakonju, S.: U.S. Salt Lake City, Utah
 Sakul, B.U.: Turkey Ankara
 Sakul, H.: U.S. Davis, California
 Salam, A.M.: Bangladesh Rajshahi
 Salán, E.S.M.: Spain Lugo
 Salinas, C.: Canada Vancouver, B.C.
 Salvaterra, P.: U.S. Duarte, California

SAMSON — SINGH, B.K.

Samson, M.-L.: U.S. Piscataway, New Jersey
 Sanchez, L.: Spain Madrid
 Sander, M.: U.S. Research Triangle Park, N. C.
 Santamaría, J.: Colombia Bogotá
 Sato, T.: Japan Tokyo
 Saumweber, H.: Germany Köln
 Saura, A.: Sweden Umea
 Saura, A.O.: Finland Helsinki
 Savic, G.: (frm) Yugoslavia Pristina
 Savontaus, M.-L.: Finland Turku
 Sawamura, K.: Japan Tokyo
 Saxton, W.M.: U.S. Bloomington, Indiana
 Scanga, S.: Canada Toronto, Ontario
 Schaefer, M.: Germany Düsseldorf
 Schafer, U.: Germany Düsseldorf
 Scharloo, W.: Netherlands Utrecht
 Schejter, E.D.: U.S. Princeton, New Jersey
 Schier, A.: Switzerland Basel
 Schneiderman, A.M.: U.S. Ithaca, New York
 Schoin, A.G.: Japan Osaka
 Schubiger, G.: U.S. Seattle, Washington
 Schulz, H.F.: Germany Freiburg i.
 Schwarz, T.: U.S. Stanford, California
 Schweizer, P.: Switzerland Zürich
 Schwinck, I.: Germany Hamburg
 Schwinck, I.: U.S. Storrs, Connecticut
 Seager, R.D.: U.S. Cedar Falls, Iowa
 Sedrov, Y.: U.S. Philadelphia, Pennsylvania
 Seiger, M.B.: U.S. Dayton, Ohio
 Sekelsky, J.J.: U.S. Cambridge, Massachusetts
 Semeriva, M.: France Marseille
 Semionov, E.P.: Bulgaria Sofia
 Serban, N.: (frm) Yugoslavia Belgrade
 Sergeev, G.N.: C.I.S. Moscow, Russia
 Serr, M.: U.S. St. Paul, Minnesota
 Serra, L.I.: Spain Barcelona
 Shaffer, C.D.: U.S. St. Louis, Missouri
 Sharma, A.: India Varanasi
 Sharma, A.K.: India Ludhiana
 Shearn, A.: U.S. Baltimore, Maryland
 Sheehan, K.B.: U.S. Bloomington, Indiana
 Sheen, F.-m.: U.S. Seattle, Washington
 Sheinker, V.Sh.: C.I.S. Moscow, Russia
 Sherald, A.F.: U.S. Fairfax, Virginia
 Shieh, B.-H.: U.S. Nashville, Tennessee
 Shilo, B.: Israel Rehovot
 Shimada, K.: Japan Sapporo
 Shin, H.J.: Republic of Korea Seoul
 Shorrocks, B.: U.K. Leeds
 Shortridge, R.D.: U.S. Buffalo, New York
 Silva, F.: Spain Burjassot (Valencia)
 Simcox, A.A.: U.S. Columbus, Ohio
 Simmons, G.: U.S. New York, New York
 Simmons, M.J.: U.S. St. Paul, Minnesota
 Simpson, P.: France Strasbourg
 Sinclair, D.: Canada Vancouver, B.C.
 Singh, B.K.: India Naini Tal

SINGH, B.N. — STRECKER

Singh, B.N.: India Varanasi
 Singh, G.: U.S. Dallas, Texas
 Singh, J. N.: India Varanasi
 Singh, R.N.: India Bombay, Colaba
 Singh, R.S.: Canada Hamilton, Ontario
 Singh, S.: U.S. Buffalo, New York
 Skaer, H.: U.K. Cambridge
 Slee, R.: U.K. Edinburgh, Scotland
 Sliter, T.J.: U.S. Dallas, Texas
 Smith, L.: U.S. Waltham, Massachusetts
 Smith, P.D.: U.S. Detroit, Michigan
 Smouse, D.: U.S. Tempe, Arizona
 Sneddon, A.: U.K. Dundee, Scotland
 Sobrier, M.L.: France Clermont-Ferrand
 Sofer, W.: U.S. Piscataway, New Jersey
 Sokoloff, A.: U.S. San Bernardino, California
 Sokolowski, M.: Canada Toronto, Ontario
 Soll, D.: U.S. New Haven, Connecticut
 Song, E.S.: Republic of Korea Seoul
 Sorsa, V.: Finland Helsinki
 Soto, A.N.: U.S. Rio Piedras, Puerto Rico
 Soung, J.: U.S. Stony Brook, New York
 Sparrow, J.C.: U.K. York
 Sperlich, D.: Germany Tübingen
 Spiegelman, G.: Canada Vancouver, B.C.
 Spierer, A.: Switzerland Geneva
 Spierer, P.: Switzerland Geneva
 Spiess, E.B.: U.S. Chicago, Illinois
 Spofford, J.B.: U.S. Chicago, Illinois
 Spradling, A.C.: U.S. Baltimore, Maryland
 Srivastava, T.: India Varanasi
 Stamatis, N.: Greece Patras
 Stamenkovic-Radak, M.: (frm) Yugoslavia Belgrade
 Stanic, S.: (frm) Yugoslavia Kragujevac
 Stark, W.S.: U.S. St. Louis, Missouri
 Starmer, W.T.: U.S. Syracuse, New York
 Steele, F. R. U.S. Bethesda, Maryland
 Stein, D.: Germany Tübingen
 Steingrimsson, E.: U.S. Los Angeles, California
 Steinhauer, W.: U.S. Research Triangle Park, N. C.
 Steinmann-Zwicky, M.: Switzerland Zürich
 Stephan, W.: U.S. College Park, Maryland
 Stephanou, G.: Greece Patras
 Stephenson, E.: U.S. Tuscaloosa, Alabama
 Sterling, J.: U.S. Research Triangle Park, N. C.
 Stern, M.: U.S. Houston, Texas
 Stearns, S.: Switzerland, Basel
 Stevens, M.E.: U.S. Davis, California
 Stocker, R.F.: Switzerland Fribourg
 Stojiljkovic, V.: (frm) Yugoslavia Belgrade
 Stolina, M.R.: C.I.S. Kiev, Ukraine
 Stoltzenberg, S.F.: U.S. Champaign, Illinois
 Storti, R. V.: U.S. Chicago, Illinois
 Störtkuhl, K.: Switzerland Fribourg
 Strausbaugh, L.: U.S. Storrs, Connecticut
 Strauss, R.: Germany Tübingen
 Strecker, T.R.: U.S. Claremont, California

STRICKBERGER — TONZETICH

Strickberger, M.W.: U.S. Berkeley, California
 Strnadova, V.: Czechoslovakia BRNO
 Stromotich, G.: Canada Vancouver, B.C.
 Struhl, G.: U.S. New York, New York
 Struse, H.: U.S. Chicago, Illinois
 Sun, D.: U.S. Columbia, Missouri
 Sun, X.: Netherlands Nijmegen
 Sundaran, A.K.: India Varanasi
 Sunkel, C.E.: Portugal, Porto
 Sutakova, G.: Czechoslovakia Ivanka pri Dunaji
 Suter, B.: Canada Montreal, Quebec
 Suzuki, D.: Canada Vancouver, B.C.
 Sved, J.: Australia Sydney, N.S.W.
 Swift, H.: U.S. Chicago, Illinois
 Szauder, P.: U.S. Hershey, Pennsylvania
 Taira, T.: Japan Tokyo
 Takasu-Ishikawa, E.: Japan Tokyo
 Tamangi, M.: U.S. Philadelphia, Pennsylvania
 Tamarina, N.: U.S. Tampa, Florida
 Tamate, H.B.: Japan Ishinomaki
 Tamura, K.: Japan Hachioji, Tokyo
 Tan, J.: China People's Rep. of Shanghai
 Tanaka, Y.: Japan Osaka
 Tanda, S.: U.S. College Park, Maryland
 Tansey, T.: U.S. Washington, D.C.
 Tapadia, M.: India Varanasi
 Tartof, K.D.: U.S. Philadelphia, Pennsylvania
 Tautz, D.: Germany Münster
 Teare, J.M.: U.S. St. Louis, Missouri
 Tearle, R.: Australia Adelaide, S. Australia
 Technau, G.M.: Germany Mainz
 Temin, P.: U.S. Madison, Wisconsin
 Templeton, A.R.: U.S. St. Louis, Missouri
 Tener, G.: Canada Vancouver, B.C.
 Teramoto, L.: U.S. Honolulu, Hawaii
 Terol, J.: Spain Burjassot (Valencia)
 Terry, M. G.: U.S. Champaign, Illinois
 Terric, T.: (frm) Yugoslavia Belgrade
 Thomas, J.: U.S. San Diego, California
 Thompson, C.: U.S. Johnstown, Pennsylvania
 Thompson, D. B.: U.S. Tempe, Arizona
 Thompson, J.N., jr.: U.S. Norman, Oklahoma
 Thompson, V.: U.S. Chicago, Illinois
 Thörig, G.E.W.: Netherlands Utrecht
 Thummel, C.: U.S. Salt Lake City, Utah
 Ting, C.-T.: China Republic of Nankang, Taipei
 Tirronen, M.: Finland Helsinki
 Tobari, Y. N.: Japan Tokyo
 Tobin, S.: U.S. Oklahoma City, Oklahoma
 Tobler, H.: Switzerland Fribourg
 Toda, M.J.: Japan Sapporo
 Toddo, T.: Japan Osaka
 Tominago, H.: Japan Sakado, Saitama
 Tomkiel, J.E.: U.S. Baltimore, Maryland
 Tomlinson, A.: U.S. New York, New York
 Tompkins, L.: U.S. Philadelphia, Pennsylvania
 Tonzetich, J.: U.S. Lewisburg, Pennsylvania

TOPOL — WARRIOR, R.

Topol, J.: U.S. Pasadena, California
 Tracey, M.: U.S. Miami, Florida
 Traub, E.: Germany Tübingen
 Traut, H.: Germany Münster
 Trbusek, M.: Czechoslovakia BRNO
 Trehan, K.S.: India Ludhiana
 Tretyakova, I.V.: C.I.S. Novosibirsk, Kazakhstan
 Trevarrow, B.: U.S. Pasadena, California
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