DROSOPHILA INFORMATION SERVICE

61

June 1985

Material contributed by
DROSOPHILA WORKERS

and arranged by
P. W. HEDRICK

Prepared at the

DIVISION OF BIOLOGICAL SCIENCES
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For information regarding submission of manuscripts or other contributions to Drosophila Information Service, contact P.W. Hedrick, Editor, Division of Biological Sciences, University of Kansas, Lawrence, Kansas 66045 - USA.

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TECHNICAL NOTES

ERRATA

DIS 60 (1984), **DIRECTORY**, page 282: Telephone number for La Jolla, Calif 92093: U of California-San Diego, Biology Dept -- should read: Tel 619-452-.

DIS 60 (1984), page 141: Mather, W.B. & A.K. Pope, "Inversions from Chiang Mai, Thailand. 2nd Report." For inversions P_6, Q_6 and R_6, read: Y_6, Z_6 and A_7.

DIS 60 (1984), page 165: Poole, J.H. & L.K. Dixon, "Drosophila peroxidases: II. Isozyme kinetics, and optimum conditions for assays utilizing p-phenylenediamine." [Bottom of page, should read:] For the H_2O_2 substrate, acid-PO had the highest apparent K_m=13.5±5.2 mM. Neutral-PO had the lowest apparent K_m=31±16.3 µM. Alkaline-PO's apparent K_m was 110±65 µM. For the PDA ....

Notice of Publication: The Genome of **Drosophila melanogaster** (progress report), Volume 62 DIS, 1985
by Dan L. Lindsley and Georgianna Zimm, Department of Biology,
University of California-San Diego, La Jolla, California 92093.

A preliminary revised edition of "Genetic Variations of **Drosophila melanogaster**," (Redbook), will be published by DIS in Volume 62 (September 1985), Part 1 (Genes A-K); at least one more volume is expected the following year (1986: Volume 64). Below is the Preface from the first volume:

> This issue of DIS contains the current manuscript version of the revised edition of "Genetic Variations of **Drosophila melanogaster**," which is to be entitled "The Genome of **Drosophila melanogaster**." It includes genes with initial letters A-K and the deficiencies.
>
> Our purposes in presenting it in this form at this time are two-fold: the first is to make available to Drosophila researchers the fruits of our labors to date, and the second is to elicit comments, additions, and corrections (including typographical errors) to what we have produced so far. We invite all workers to provide us with additions and corrections to any material contained within and also to provide us with fully-prepared entries describing loci or rearrangements that we may have missed or have not covered yet (e.g., those with initial letters L through Z and classes of rearrangements or special chromosomes not included here). Edited Xerox copies of the accompanying pages will suffice.
>
> It is our purpose to stop accepting new material at the end of this year (December 31, 1985) and to try to have the information as complete as possible through 1985. Anyone wishing to have new material included in this volume should have it in our hands before the end of this year. Our target date for completion of the volumes is April 1987.

NOTE: Standing orders will receive Vol. 62 as usual; cost is the same as for regular issues--$5.00 if paid in advance; $6.00 if invoicing is required.

26th ANNUAL DROSOPHILA RESEARCH CONFERENCE

Held April 1-4, 1985, at the Sharaton Charleston Hotel in Charleston, South Carolina.
Below is a list of invited speakers, topics and workshops:

Plenary Session - Chairman: Jo Jack — Monday, April 1
Speakers: Bruce Baker, Tom Kaufman, Ken Tartof

Concurrent Sessions: — Tuesday, April 2
Pattern formation and homeotic genes (Rob Denell, Tom Kornberg)
Enzyme biochemistry and genetics (Janis O'Donnell, John Rawls)
Chromosome structure and gene organization (Pierre Spierer)
Neurobiology and behavior (Ron Konopka, Doug Kankel)
Population genetics and ecology (Wyatt Anderson, Andrew Clark, John Jaenike)
Chromosome transmission and gene regulation (Scott Hawley, Leonard Robbins)

Poster Session (72 posters on display) — Tuesday evening, April 2

Concurrent Sessions: — Wednesday, April 3
Early development (Tony Mahowald, Kathryn Anderson)
Heat shock (John Lis, William Bendena)
Gene expression and structure I (Elliot Meyerowitz)
Gene expression and structure II (Spiros Artavanis-Tsakonas)
Transposable elements and hybrid dysgenesis (Michael Simmons, Linda Strausbaugh)

Plenary Session - Chairman: Lillie L. Searles — Thursday, April 4
Speakers: Jerry Coyne, John Lucchesi, Jim Posakony,
Jose Campos-Ortega, Beth Raff, Matt Scott

27th Annual Drosophila Research Conference will be held April 11-13, 1986 at the **Asilomar Conference Grounds in Pacific Grove, California.** Accommodations will be available for evening arrivals on Thursday, April 10. Program and registration materials will be sent out in October. If you do not receive the mailing, you should contact Susan Germeraad or Larry Sandler [addresses below]. Due to remodeling in progress at Asilomar, on-site accommodations may be somewhat limited. Off-site housing will be arranged for late registrants.

Contact: Susan Germeraad, Biology Dept, University of Santa Clara, Santa Clara, CA 95053
or Larry Sandler, Genetics Dept, University of Washington, Seattle, WA 98195.

Request for Materials: Request for light micrographs of salivary gland chromosomes of **D.subobscura.**

We are revising the LM chromosome map of **D.subobscura** by LM analyses of squash preparations (for constructing a LM photo map) and by EM analyses of surface spread polytene (SSP) chromosomes (see Research Note: Kalisch & Bohm, this issue). Since we mainly are interested in doing the EM analyses of SSP chromosomes, we are making a request for light micrographs of salivary gland chromosomes of squash preparations. Contributors are kindly invited as co-authors. We are anxious to receive the following material: (1) Light micrographs of well-extended (and if possible straight) salivary gland chromosomes from late third instar larvae with division (if possible subdivision) sectioning and the negative material. (2) Any information about additional bands or corrections (in comparison with the chromosome map of Kunze-Muhl & Muller 1958) on the basis of LM or EM analyses.

Please send material to: W.-E. Kalisch, Institut fur Genetik, Ruhr-Universitat, Postfach 10 21 48, D-4630 Bochum 1, FRG.

Stock Request: We are interested in analyzing various suppressor and enhancer loci and have been unable to locate a stock containing the mutation e(w^e) (enhancer of white-eosin). If anyone has such a stock or knows where it can be obtained, please contact Robert Coyne, Dept. of Biochemistry, Harvard University, 7 Divinity Ave., Cambridge, Massachusetts 02138.

Materials Available: Since 1981 I have maintained a strain of sepia-eyed **D.melanogaster** and a strain of wildtype **D.simulans** on coarse-grained and on fine-grained media. Graininess, in this case, refers to the size of individual food particles: Brewers yeast is blended into beaten egg whites and baked thoroughly. The hardened "meringue" is forced through a coarse sieve after which coarse and fine particles are separated by resifting with a finer sieve.

A test carried out in early 1984 did not reveal any obvious change in competitive ability between the different strains of flies when raised together on coarse, fine or mixed medium. One more test is planned for the coming year, after which the strains will be discarded.

Anyone interested in examining these flies for morphological or allozymic differences can obtain subcultures merely by writing: Bruce Wallace, Biology Dept., VPI & SU, Blacksburg, VA 24061.

Materials Available: For Sale: First eight volumes of series **Genetics and Biology of Drosophila**; i.e., Volumes 1a, 1b, 1c, 2a, 2b, 2c, 2d and 3a. All in perfect condition. Must go as a set. Contact: Dr. J.D. Agnew, Dept. of Genetics, University of the Witwatersrand, Box 1176, Johannesburg 2000, South Africa. Tel (011) 716-2161.

Materials Available: Extra copies of D.I.S. are available to Drosophila workers or laboratories needing to complete their files. Dr. Poulson (Yale) has the following copies available: volumes 37, 38, 40, 46, 47, 48, 52, 53, 55, 56, 57 and 58. Write to: D.F. Poulson, Department of Biology, P.O. Box 6666, Yale University, New Haven, Connecticut 06520.

SALARIES IN 1962

excerpt from DIS 37:148, 1963:

About 110 Drosophila geneticists attended the annual Drosophila conference, held this year at St. Louis University, November 2 to 4 [1962]. The next meeting will be held in East Lansing at Michigan State University at a date to be announced.

A poll was taken of the salaries of persons holding positions at North American colleges and universities. Contributors were unidentified. Despite a number of possible biases in this sample, the results are summarized here:

Twelve months salaries were arbitrarily reduced by 20% and scored as 9 months salaries.

		Mean	Range	
11	*Graduate Students and Research Assistants*	$ 2,300	$2,000-	2,800
3	*Research Associates*	6,530	5,200-	8,000
4	*Instructors*	6,160	5,200-	7,500
12	*Assistant Professors*	7,250	5,400-	10,400
7	*Associate Professors*	9,080	7,800-	10,400
12	*Professors*	10,410	7,200-	15,000
49				

NEW DIS TYPE STYLE: The DIS office has changed word processors this past year (the old one was finally retired after many years of service). With the new processor, we are able to offer right margin justification and proportional space type (a definite enhancement). Also, external diskette storage is the main feature of the new word processor; this allowed us to prepare proof copies for authors at a much earlier time before our press deadline and also afford authors to make more changes in their contributions. Boldface printing is now used for species names. The new machine is also easier on the Editorial Assistant (Gil) who prepares all the manuscripts.

CALL FOR MATERIAL FOR DIS 63

Contributions are now being accepted for DIS 63, June 1986. Please double-space all contributions, and otherwise follow the format of DIS 60 & 61. Make sure complete information is included with every section of submissions--which will be separated from one another.

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

Sections you may contribute to are:

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

Note: A bibliography section is not being offered at this time since Dr. Herskowitz is no longer performing this task. The DIS office will continue to collect any bibliographic data you wish to send, holding it in reserve in the meantime.

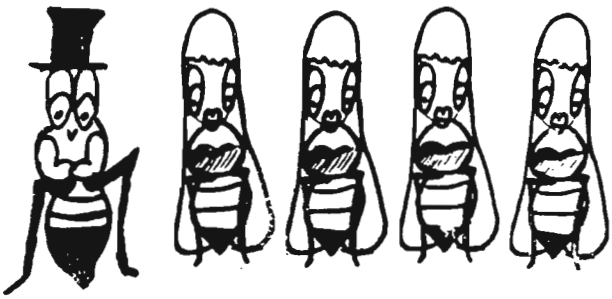
DEADLINE FOR DIS 63 IS APRIL 1, 1986.

ORDER COPIES OF DIS IN ADVANCE if possible. *Subscribers paying in advance with the enclosed form or by written request will pay $5.00 per copy.* If the DIS office has to send out an invoice in December, the cost will be $6.00 per copy. Those who need additional copies of invoices are asked to photocopy the form to conserve on postage and paper work; if this is not suitable, please contact us and we will furnish a standard invoice (invoiced copies of DIS are $6.00/copy). You may also request a standing order at this time. Orders may be air-mailed at your expense. NOTE: Checks for DIS should be drawn on a U.S. bank. The office still has on hand the following volumes:

38, 39, 40, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 (Stock List), 58, 59, 60.

CHANGE OF ADDRESS FOR SUBSCRIPTIONS: please notify us before June 1st of each year; after this date we send all orders to the printer to be mailed automatically from their office and cannot cancel any mailings at that time. If you will be on leave, let us know your temporary address; many offices will not forward books. NOTE: when sending a change of **temporary** address, please give your old address as well as the new one to avoid duplications.

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



Since this begins DIS's 51st year of publication, we would like to reprint the following:

¤ ¤ ¤ FOREWORD [to Volume 11, 1939] ¤ ¤ ¤

With this eleventh issue of the circular the Drosophila Information Service is beginning the sixth year of its existence. This seems an opportune occasion to review the work accomplished so far.

The primary purpose of this service was to establish a means of contact between various Drosophila workers scattered throughout the numerous research laboratories the world over. During the last decade genetics witnessed rapid development which can hardly be matched by any of the other sciences. In this wave of progress Drosohila played an important role. Because of its unequaled possibilities as material for study of a great number of problems, the use of Drosophila extended rapidly among geneticists. A number of newly established laboratories began to use it exclusively and a number of old laboratories started work on new problems with Drosophila. As a consequence of this increased activity the intimate contact which existed between the original Drosophila workers was lost with the result that much valuable material was not properly utilized and a great deal of effort was duplicated. Uncorrelated work in many widely scattered laboratories was bringing about a confusion in nomenclature of mutants and in the use of symbols. To remedy this situation Drosophila Information Service was started. By acting as a clearing house for new information it quickly straightened out many of the discrepancies, helped to keep up a unified system in Drosophila nomenclature and contributed towards its improvement. It also helped to disseminate information in regard to research techniques which otherwise would not be available except through personal visits or correspondence.

Another important aim of the DIS was to facilitate the exchange of stock cultures for research purposes. The original Drosophila workers established the policy of a free exchange of material among all actively interested in Drosophila research. This became an unwritten law which is contributing more than any other single factor toward the usefulness of Drosophila as research material. By publishing a list of stocks kept at various laboratories the DIS bulletin makes these stocks readily available to the whole group.

DIS is a cooperative undertaking. For each issue of the bulletin the editors send out a circular letter to every Drosophila worker known to them requesting for specific information to be printed in that issue. Only those answering the request are placed on the mailing list for the particular issue. The aim of the DIS is not so much to include every Drosophila laboratory but rather to circulate useful information among laboratories cooperating in the project. Bulletins are distributed free of charge. They are sent to cooperating Drosophila workers and to a selected list of laboratories doing resarch work in genetics. The size of the edition for every issue is determined by the number of replies received. Because of limited facilities, no extra copies are printed, as a rule, and bulletins are not available for general distribution. Frequent request for bulletins are received from geneticists but they cannot be satisfied.

So far two bulletins have been issued yearly, which makes 10 bulletins during the five years of its existence. Eight of them were general, containing the current material on nomenclature, description of symbols, reports on new mutants, linkage data, stock lists, notes on current research and laboratory technique, Drosophila bibliography, and directory of cooperating Drosophila workers. Two were special issues, one devoted to laboratory methods and techniques and the other containing a revised list of melanogaster mutants. From time to time "Work Sheets" are inserted into the bulletins. Of the twelve prepared so far, five were diagrammatic reference drawings of the whole fly or of parts of it and seven were reprints of various published salivary gland chromosome maps. Extra copies of these Work Sheets are available for research purposes.

The first issue of the DIS was printed in 120 copies. As the number of cooperating Drosophila workers increased the size of the edition was increased with each successive issue, so that this issue is being printed in 270 copies. This is close to the maximum which can be handled properly with the facilities now available. Special issues are printed in larger editions, of the Melanogaster Mutants issue, for example, 500 copies were prepared.

The growth of the DIS is best illustrated in the following summary of the geographical directory sections published in the various issues showing the number of laboratories (L) and the total number of workers (W) doing research with Drosophila for each country. The directory of this issue presents a fair picture of the distribution of Drosophila research among various countries.

The death of Calvin B. Bridges left a big gap among Drosophila geneticists and is an irreparable loss to DIS where he was particularly active in checking up stock lists, mutant lists and linkage data. Drosophila workers cooperating in the DIS project will have to put their best efforts into these departments of the bulletin to maintain the standard set by him.

¤ ¤ ¤ ¤ ¤ ¤ ¤ ¤

ODE TO A FLY

A fly is, it appears to me,
Of much use scientifically.
Drosophila, though not a pest,
Is prey to the geneticist;
This little dipteran it seems,
Has myriads of mutant genes,
Bestowed quite philanthropically,
By radio-activity.

These alleles of bizarre effect,
Are often useful to detect,
Locations of uncharted genes,
In company with other means,
Like squashing of salivary glands,
And counting chromosomal bands,
In polytenic genic strands,
That lurk within those larval glands.

It is of course most importune,
To have a fly die far too soon,
Although once information's found,
The fly in question will be drowned.
To combat the demise of flies,
A technique that the truth belies,
Is utilised - a minor miracle,
Described in brief as "mouth-to-spiracle".

When oft upon my tile they lie,
I spare a quick thought for each fly,
And wonder what it's thinking on,
In its restricted ganglion;
I'm sure they would prefer to be,
In company with you and me,
Free to take the chance to flee,
To some inviting winery.

---Malcom Stewart
Macquarie University
North Ryde, N.S.W., Australia

CREDITS: We would like to bring the attention of our Drosophila readers to the fine graphics on display in this issue of DIS. The fly drawings on page viii were contributed by C. Katz of Lawrence, Kansas (on the left) and by Marten Bos (on the right), University of Groningen, Netherlands.

The "kid" drawings are by Elfin Szychowski, Lawrence, Kansas. Elfin is a boy scout who has been collecting the stamps that we receive on our DIS mail in the office and he wanted to show his appreciation for all the good "collectable" stamps. Since he's no longer a kid himself, he went back to his "former" style of drawing to produce these fine graphics.

The "cloned" news on page 203 is reprinted from a local humor-paper called the "City Moon", by Roger Martin and David Ohle.

Engels, W.R. University of Wisconsin, Madison, USNA. Guidelines for P-element transposon tagging.

The use of P element insertion mutations for cloning was suggested by Bingham et al. (1982) and first used by Searles et al. (1983). Since then it has been used many times, usually with success. Briefly, the procedure is to (i) recover mutations in the target locus by screening progeny of P-M dysgenic hybrid flies, (ii) confirm that the mutations are P element insertions, (iii) select a set of clones with P homology from a cloning vector library of the mutant stock, and (iv) use in situ hybridization to identify the clone carrying genomic flanking DNA from the target locus.

The critical step is often the recovery of the P insertion. The following guidelines are based on experience in several laboratories:

1. Experiment size: The frequency of P insertion mutations is highly locus-specific. It ranges from greater than 10^{-3} for singed to essentially zero for Adh. (Heterochromatic sites seem to be less frequently hit by P elements.) For most loci, a screen of at least 10,000 chromosomes is needed for a high probability of success.

2. Selection of M strains: Since virtually all standard laboratory strains are M, the maternally-contributing strain can be chosen primarily on the basis of convenient markers and chromosome rearrangements. However, blotting or in situ hybridization is recommended to confirm the absence of P homology.

3. Selecting the P strains: Not all P elements are equal in their transposase productivity. Therefore, the choice of a P strain should be made on the basis of transposase activity rather than P sequence homology. A good measure of transposase activity is the degree of sn^W mutability in the progeny of crosses to sn^W(M) females (Engels 1984). Five P strains with high transposase activity by this criterion are available from this laboratory.

4. Selection of target chromosome: Studies of insertion rates into the singed locus (unpubl.) show that the P and M chromosomes in dysgenic flies are both equally likely to acquire P insertions. Therefore, to simplify eventual recovery of the right DNA clone, the crossing scheme should be such that the target chromosome is of M strain origin.

5. Dysgenic flies: P elements transpose in both sexes, but it is usually more convenient to use dysgenic males. To avoid GD sterility, the dysgenic flies should be grown at 21° or less.

6. Use of several P strains: Since P strains appear to vary in their target site specificity, it is best to use several independently derived P strains. For example, a screen of 10,000 chromosomes might involve five P strains with 2000 chromosomes each. The reason for this differential site specificity is not known.

7. Stabilizing mutations: Hybrid dysgenesis can be suppressed in the mutant stocks by appropriately controlling cytotype and the sources of P transposase (Engels 1983). If there is to be a long time lag between the mutagenesis and DNA extraction, it is better to avoid further dysgenesis by maintaining the mutation in a P-cytotype stock. Compound-X and balancer stocks of the P cytotype are available and described elsewhere in this volume. Another alternative is to cross away all the complete P factors and keep the mutation in an M-cytotype stock with no source of P transposase. Lethal mutations should be stabilized immediately to avoid dysgenesis-induced reversions that might cause confusion in scoring. For example, dysgenic males can be crossed to tester females of the P cytotype so that the mutations will be recovered in non-dysgenic flies.

8. Determining mutation rates: Since the primary goal is to obtain at least one mutation, estimating the mutation rate is normally considered to be of secondary importance. However, the necessary counting and record keeping needed to obtain such estimates require little additional effort, and can provide useful information. Since many P element transpositions occur premeiotically, it is desirable to identify clusters as opposed to independent events. Ideally, each dysgenic fly should be test-crossed separately. When this is not practical, small mass matings are preferable. When estimating mutation rates, all members of a cluster should be counted, but the family and cluster sizes need to be taken into account in determining the precision of the estimate (Engels 1979).

9. Confirmation of P insertion: In situ hybridization can be used to confirm that the mutation has a P element at the cytological site of the target locus. In addition, it is often helpful to confirm that the mutation reverts in response to dysgenesis. For example, if the mutation is in a stock known to have transposase-producing P elements, mutant males can be crossed to M strain females with reversions occurring in the germline of the progeny. Such reversions are usually excisions or partial deletions of the inserted P element (Voelker et al. 1984; Daniels et al. 1985), but they might also be secondary insertions or chromosome rearrangements (unpubl.). Any revertants recovered from this screen should be saved for later use in demonstrating that the cloned DNA actually corresponds to the target gene.

References: Bingham, P.M., M.G. Kidwell & G.M. Rubin 1982, Cell 29:955-1004; Daniels, S., M. McCarron, C. Love & A. Chovnick 1984, Genetics 109:95-117; Engels, W.R. 1979, Envir. Muta. 1:37-43; _____ 1983, Ann. rev. Genet. 17:315-344; _____ 1984, Science 226:1194-1198; Engels, W.R. & C.R. Preston 1979, Genetics 92:161-173; Searles, L.L. & R.S. Jokerst, P.M. Bingham, R.A. Voelker & A.L. Greenleaf 1983, Cell 31:585-592; Voelker, R.A., A. Greenleaf, H. Gyurkovics, G. Wisely, S. Huang, L. Searles 1984, Genetics 107:279-294.

SPECIAL REPORT: Molecular and population biology of hybrid dysgenesis.
A report of the meeting organized by Gabriel A. Dover and Margaret G. Kidwell, held in Cambridge, England, September 24-26, 1984.

Submitted by:
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The fascination that hybrid dysgenesis holds for molecular biologists, geneticists, evolutionary biologists, and even theoretical biologists, stems from its phenomenology, now firmly established to result from the behavior of specific classes of transposable elements. The transposition of these elements is strictly regulated in ways which can be experimentally manipulated, even though they are not yet fully understood. A recent meeting in Cambridge, England, brought together just such a collection of biologists to report new findings and to exchange their views on some controversial issues.

Hybrid dysgenesis is the term given to a collection of interrelated phenomena that are observed in the offspring of matings between certain strains of **Drosophila melanogaster**. The unifying observation is that the various manifestations are most readily seen when the males from one type of strain are mated to females of the other, and that the induction of dysgenic traits is limited almost exclusively to the germ line. These dysgenic traits include mutation, chromosome breakage, male recombination, and sterility.

There are two independent systems of hybrid dysgenesis that have been identified: the P-M system, mediated by the P family of transposable elements, and the I-R system, for which I elements are responsible. The elements can exist in the germ line in either of two states, quiescent or active. When P elements are quiescent, they are said to reside in a cellular environment called the P cytotype. This is the condition characteristic of flies from a P strain. The active state, characterized by high rates of transposition, is elicited by the M cytotype, a property of flies belonging to an M strain. All P strains have functional P elements in their genomes, but a fly carrying only a few functional P elements in its genome may still retain the M cytotype,i.e., the elements are still active. But this apparent exception is a special case, and like most exceptions to rules, it proved to be instructive.

In the I-R system, the I elements of inducer (or I) strains transpose at high frequency only when introduced into a reactive environment which is characteristic of R strains. (For the sake of discussion, we shall refer to this reactive environment as the R cytotype.) I element activity is further limited only to the female offspring, whereas P elements are active in both males and females.

In both systems there are distinct paternal and maternal contributions to the production of the dysgenic hybrid. P-M hybrid dysgenesis occurs when P strain males are crossed to M strain females, introducing functional P elements into the M cytotype. Similarly, I-R dysgenesis requires I strain males be mated to R strain females. Reciprocal crosses, or mating inter se within a strain, produce normal offspring. It is this nonreciprocal nature of the dysgenic cross that has led to the concept of cytotype being an extrachromosomal condition that regulates the transposition of the P or I elements. The inheritance of cytotype is somewhat complex. An individual's cytotype is determined both by its own genotype and by the cytotype of its mother.

The structure of P and I elements.

The workshop opened with a summary of P element structure presented by K. O'Hare, which he and G. Rubin had published in 1983 (Cell 34: 25-25). P elements fall into two broad classes, intact and defective. The intact elements share a conserved 2.9 kilobase (kb) sequence which is flanked by terminal inverted repeats of 31 base pairs (bp). One of the DNA strands specifies four open reading frames, covering about 90% of the sequence. Only the intact elements are capable of supplying the transacting function which is required for transposition. Defective elements are missing sequences internal to the 2.9 kb element, though they retain the inverted repeats. They can transpose, but only when supplied with transposase from an intact element. All P strains contain intact P elements, and usually also contain defective elements. Most common laboratory M strains have neither intact nor defective P elements. However, one can distinguish these "true" or "pure" M strains from M' strains (also called pseudo-M strains), which have defective P elements in their genomes.

The role of the four open reading frames encoded by the 2.9 kb element was described by R. Karess, who recently published experiments with G. Rubin (Cell 38: 135-146) showing that they are required for the production of P transposase. They found that frameshift mutations in any one of the four reading frames were each sufficient to eliminate transposase activity, as assayed in vivo. Efforts to complement the lesions by combining pairs of mutant elements within one genome failed to reconstitute the transposase, suggesting that all four reading frames contribute to a single polypeptide carrying the transposase act-

ivity. They also examined the transcripts of P elements in P strain embryos and in embryos of P male x M female dysgenic hybrids. A large number of transcripts were detectable, but most appeared to be derived from the defective elements that predominate in P strains. However they were able to show that two transcripts, a major 2.5 kb species and a less abundant 3 kb RNA, came from the nondefective P elements, and both RNAs included in their sequence the greater portion of the four reading frames. The clear suggestion is that one of these transcripts represented the transposase messenger, having spliced together sequences from each of the reading frames into a single large coding region.

The structural analysis of the I elements, though not yet complete, is rapidly catching up with that of P elements. This work was reported at the meeting by D. Finnegan, C. Vaury, and A. Bucheton. Some of it had been published recently (Cell 38:153-163). I elements also appear to come in intact and defective varieties. The sequence of one intact I element, which is being determined in Finnegan's lab, is 5.4 kb long. Two large open reading frames have been identified so far, both on one of the DNA strands. This element created a 12 bp duplication at its site of insertion. However, there does not appear to be a repeated structure, either direct or indirect, at its termini. The smaller I elements may be deleted for internal sequences, or may lack one or both of the elements' ends, something rarely if ever found among defective P elements.

Distribution of P and I elements in strains and species.

Both intact and defective I elements are found in the genomes of I strains, while R strains contain only defective elements. H. Sang has found that a 2.3 kb fragment internal to the 5.4 kb element can be used to distinguish DNA from I and R strain flies. This fragment is present about 15 times in the genome of an I strain fly, but is apparently absent from the genomes of R strains. This presumably means that all defective I elements present in R strains have sustained deletions or some other rearrangement of sequences within this 2.3 kb region. All strains of **D.melanogaster** so far examined have the smaller I elements. A comparison of DNA blots from R strains collected at different localities suggests that there is some considerable conservation of the structure and the genomic location of many of the deleted elements in these strains. This suggests that the defective I elements of R strains are essentially immobile, and have been so for quite a while. Moreover, the chromosomal distribution of I elements in these strains is highly nonrandom. In situ hybridization to polytene chromosomes reveals that nearly all the defective I elements reside in or near the centric heterochromatin. In contrast, the elements of I strains are found on the euchromatic arms as well as the heterochromatin. One possible explanation for this unexpected finding is that only intact 5.4 kb elements are able to move, and that defective elements have been trapped in heterochromatin where they are less likely to have harmful genetic effects. In P strains, where it is clear that both defective and intact elements are mobile, the distribution is nonrandom as well, but in the opposite way: there is an unexpected dearth of P elements residing in the centric heterochromatin regions. It has also been observed that P elements introduced by injection into M strain genomes prefer euchromatic sites of integration.

Many standard laboratory strains have no P-element-related sequences at all. Yet, most M strains derived from recently collected field isolates do contain defective P elements. The meeting organizer G. Dover reported on an extensive study carried out in collaboration with D. Black and M. Kidwell, in which samples from a number of different wild populations had been maintained in a series of parallel sublines for several generations and characterized phenotypically according to their ability to induce or suppress dysgenesis. While most of the sublines of each isolate behaved identically, there were occasional exceptions where the P factor activity or the cytotype appeared to have changed. The molecular nature of the P elements in the genome of each subline is currently being investigated. It is already clear from these experiments that all of the M strains, in their collection at least, contain large numbers of defective P elements, and that these may well affect the properties of the strains when tested in a dysgenic cross. This conclusion was echoed in the studies reported later by M. Kidwell and M. Simmons, and proved to be one of the more significant findings reported at the conference.

If one looks further afield, in the genomes of other members of the **melanogaster** species group, one finds corresponding differences in the distribution of I and P elements. A. Bucheton has used Southern transfer experiments to examine the genomes of many related **Drosophila** species, and has found that I element sequences are very widespread. There appear to be I elements in **D.mauritania, D.erecta, D.simulans, D.yacuba**, and **D.tessieri.** In **D.simulans**, he found the 2.3 kb fragment that correlates with the presence of functional I elements. Thus I elements, and possibly intact ones, are not confined to **D.melanogaster.**

P elements have not been found in the close relatives of **D.melanogster.** But at the Cambridge meeting it was revealed for the first time that P elements are found in the more distantly related South American **Drosophila** of the **willistoni** group. L. Strausbaugh, in collaboration with S. Daniels (see PNAS 81:6794-6797), reported that nine of the ten members of the **D.willistoni** species group had P sequences in their genomes. These include the sibling species **D.willistoni, D.tropicalis, D.equinoxialis, D.pavlovskiana,**

and the six semispecies of **D.paulistorum.** Whether any intact P elements are found in these species is yet to be determined. The provocative epidemiology of P and I element distributions was discussed more fully later on. But in general, it appears that I elements arose before the radiation of **Drosophila** into its dozens of related species, while P elements seem to be more recent arrivals.

While P elements have not been found naturally in **D.simulans**, they can be introduced artificially by injection into the embryonic germ line, whereupon they integrate into the chromosomes and are transmitted to subsequent generations. N. Scavarda and S. Daniels (in collaboration with L. Strausbaugh) have both succeeded in introducing intact P elements into **D.simulans** by this method. Scavarda has also put the **rosy** gene of **D.melanogaster** into **D.simulans** by using a P element as the transformation vector (PNAS 81:7515-7519). This tour de force opens the door to interspecific gene transfer among dipterans, and possibly beyond. Recently, W. Dickerson and colleagues have published (Cell 38: 147-151) the successful introduction of a functional P element into the genome of **D.hawaiiensis.** Thus the barriers to P transposition, at least within the genus **Drosophila**, appear to be at the level of mating, rather than of gene expression.

Are there other systems of dysgenesis besides I-R and P-M? J. Thompson tried to identify new dysgenic combinations by pairwise crossing of many different field isolates and examining the rates of visible and lethal mutation production on the X. There was some suggestion of elevated mutation rates in certain combinations of crosses.

C. Langley described what may be a dysgenic phenomenon that C. Hinton had found in the species **D.annassae**, (described in Genetics 106: 631-653). In certain lines, he observed that females produced numerous mutants in their offspring, most of which had altered eye morphology. These mutations, called **Om** mutations, appear to be caused by the insertion of a 5.4 kb mobile element, whose sequence was repeated 10-40 times in the genomes of wild fly populations, whereas lab stocks had few or none. While much of this phenomenology is reminiscent of the behavior of I elements (including the size of the insert), this tantalizing account stopped short of identifying the culprit.

Transposition and rearrangements.

What is the mechanism of transposition of P and I elements? Is it replicative or conservative? How are the chromosomal rearrangements generated? Several groups reported data that shed some light on these issues. The bulk of the evidence supports a replicative mechanism, involving the transfer of sequence information, rather than the physical transfer of an element from one site to another.

W. Engels and W. Benz have attempted to measure accurately the rate of acquisition and loss of P elements during dysgenesis. They followed a P element-containing X chromosome, maintained patroclinously with attached-X M strain females containing no P elements at all, for many generations. They found that new insertions on the X outnumbered excisions from the X by a factor of 1.5-3.0. If one considers that the elements can transpose to any site in the genome, but were only being counted if they integrated on the X, then the preponderance of transposition over excision becomes greater still. This experiment provides good circumstantial evidence that transposition is replicative, that is, a P element can send a copy of itself to a new site while still remaining at its old site. But the issue is far from settled.

Transposition of I elements also appears to be replicative. A. Pelisson described experiments in which chromosomes derived from an R strain were found to have acquired inducer activity, after being associated with a single I element on the X chromosome for a single generation. Apparently, this single I element was able to "contaminate" the R chromosomes very efficiently, but without any obvious changes in its own structure or position. Although a nonreplicative process of "chromosome contamination" cannot be ruled out, these experiments favor a replicative mechanism.

Besides transposing, both P and I elements mediate chromosome rearrangements. When rearranged chromosomes are examined by in situ hybridization with P or I element probes most, but not all, of the breakpoints are labeled. (Small defective elements would not be detected by this approach.) While there is not yet a demonstration of the total absence of P element sequences at the breakpoints, it is clear from experiments reported by K. O'Hare, that there can be a net loss of P sequences during the rearrangement. W. Engels and C. Preston examined cytologically nearly 800 P-mediated rearrangements of the X chromosome (recently published in Genetics 107: 657-678). Most were simple inversions, involving two chromosomal breaks, and usually at resident P element sites. But with decreasing fequency, they found others, involving 3, 4, or even 5 breakages and rejoinings. Some classes of these more complex rearrangements could not have been generated from sequential 2-break events. The implication from this observation was that there must have been a "simultaneous" breakage at several sites, followed by random joining of the broken ends. This analysis makes a cointegrate model of chromosome breakage implausible; however it does not reveal whether chromosome breakage arises from abortive P element transposition.

F. Sobels and J. Eeken presented evidence concerning the effects of mutagens on P element activity. Three chemical mutagens had no measurable effects, but x-rays seemed to enhance the frequency of P-

mediated chromosome breakage. In addition, the repair-deficient mutations mei-9 and mei-41 had significant effects on P-mediated mutation processes. These facts show that both genetically and environmentally altered conditions can affect the frequency of P-mediated events. No comparable information on the behavior of I elements currently exists.

Regulation.

What determines whether an element will transpose? Could the R and M cytotypes be the presence of a positive effector of transposition, or do they correspond to the absence of inhibitors that are found in the restrictive environments of P and I strain flies? One of the thorniest problems in the study of hybrid dysgenesis is the nature of these maternally contributed components, and it featured prominently in the presentations at the Cambridge meeting.

G. Picard summarized many of the experiments dealing with reactivity in the I-R system. Ultimately, this property is determined by the chromosomes, but the maternal effect is very strong. Up to 15 generations may elapse before the chromosomal contribution predominates. R strains may differ in their levels of reactivity and crosses between them yield offspring whose reactivity is like that of the mother. Yet replacement of the chromosome of one strain by those of another will eventually override this maternal influence. Reactivity also can be weakened by environmental factors. Aging of females from a strong R strain before mating them reduces the reactivity of their daughters. Similarly, rearing the flies at a high temperature weakens their reactivity. The changes produced by these treatments are cumulative over generations, but can be reversed by restoring the flies to their original culture conditions. These facts have been interpreted to mean that reactivity is due to extrachromosomal particles which have a limited ability to replicate independently of the chromosomes. The crucial question is whether strong reactivity is associated with the presence or absence of these particles (i.e., are the particles positive or negative regulators of I activity), and here no information is available to discriminate between these two alternatives. In either case, the presence of the hypothetical extrachromosomal particles must be functionally dependent on the chromosomes, since the genome ultimately controls the reactivity level.

Environmental factors also influence the switching between P and M cytotype that occurs to a greater or lesser extent in the offspring and descendants of the dysgenic cross between a P strain male and an M strain female. Early work by Engels and others had suggested that cytotype was affected primarily by the number of P elements in the genome and by the cytotype of the mother. To this must now be added the effects of age and high temperature. D. Anxolabehere and G. Periquet reported that both these factors tend to attenuate the maternal contribution, and thus make the cytoplasmic component relatively less important than the genome in ultimately determining the cytotype of the fly.

There are also genomic modifiers of the M cytotype. The M' strains, described by M. Kidwell, show a consistently lower level of dysgenesis than true M strains in the standard P male X M female cross. The modifiers in these strains could be identical to the defective P elements that reside in their genomes. Kidwell synthesized lines that contained chromosomes from both an M' strain and a true M strain, and found that each M' chromosome individually contributed to a reduction in the level of dysgenesis. However, in contrast to the inheritance of the strong and weak R strains, and in contrast to the inheritance of true P and M cytotype, the distant maternal ancestry of these synthetic lines had no effect on the ultimate level of the M cytotype. The absence of a maternal effect suggests that the apparent "intermediate" level of cytotype of the M' flies is a consequence exclusively of the genotype, with no extrachromosomal component at all.

W. Engels presented a study which for the first time provides evidence for the long-suspected notion that the cytotype determining factor is contributed by the P element itself. In what for many people studying the population biology of mobile genetic elements must be a dream experiment, he followed the fate of a single nondefective P element that had been introduced into an otherwise pure M strain genome. The classical expectation for a transposable element, if it is considered as a parasitic piece of DNA, is that the number of elements in the genome will increase until the point where its numbers confer too high a mutational burden. One way to stop the suicidal transposition of P elements is by establishing the restrictive condition, the P cytotype. Engels and C. Preston followed several replicate fly lines containing at first a single active P element, counting the increasing number of elements by in situ hybridization to the polytene chromosomes in subsequent generations. In one line, the number of elements gradually grew to approximately 20 in about 15 generations, and then stabilized. This line later proved to have the P cytotype and possessed strong P factor activity. In another line, however, the number of elements grew only slowly at first, and then explosively, until the line died out, apparently without ever establishing the P cytotype. The other lines retained the M cytotype and lost their initial P activity.

These experiments offer the first demonstration that an intact P element is sufficient to evoke the P cytotype, and as a corollary, that P and M cytotypes correspond to the presence and absence, respectively, of an inhibitor; the nature of the inhibitory substance, however, is as mysterious as ever. They also

point out that we do not yet know the whole story. What controls whether the elements will build in number slowly or rapidly? Why are 20 elements only sometimes enough to bring about the P cytotype?

M. Simmons presented a provocative model for cytotype that provides a way for understanding many of the preceding observations on the behavior and inheritance of cytotype and reactivity. The model independently predicts that the restrictive condition will correspond to the presence of an inhibitory substance.

The model of cytotype that Simmons offered for the P-M system was based on the results of "transposase titration" experiments he had performed. The main finding was that the transposition frequency of a specific defective P element in the presence of an intact element could be reduced if the genome also contained chromosomes bearing defective P elements from an M' strain. Simmons interpreted this to mean that some of the transposase generated by the intact element was bound by the defective P's, presumably at their terminal repeats; thus, it could no longer act on a specific target element.

He then generalized this idea by proposing that the P cytotype is the result of transposase titration. In Simmons' model, the P cytotype is mediated by extrachromosomal defective P elements, thereby accounting for the limited maternal inheritance of cytotype. Simmons also proposed that the generation of these extrachromosomal elements be dependent on the P transposase. This would explain why strains with at least one intact P element are able to evolve the P cytotype, although many generations may actually be required. The titration model is a modification of the positive feedback regulator model proposed by O'Hare and Rubin (Cell 34: 25-35). According to that model, the P element encodes a regulator which represses transposition, but which enhances its own transcription. This positive feedback loop leads to further regulator synthesis, eventually bringing about the P cytotype. In Simmons' model, the extrachromosomal elements perform the role of the regulator, so there is no need to postulate another P-encoded protein. This is one way of dealing with the discovery by Karess and Rubin that all four of the P element's reading frames are involved in coding for the transposase.

The titration model of cytotype also explains some of the other data presented at the conference. The reduced level of dysgenesis seen with Kidwell's M' stocks, and the absence of a reciprocal cross effect in her experiments, may be caused by the titration of transposase by the defective P elements residing on the M' chromosomes, and since there are no functional P elements, the M' strains produce no extrachromosal P elements. The effect of age and temperature in accelerating the switch from M to P might also be explained by the Simmons model, by saying that these factors encourage the amplification of the extrachromosomal P element population. The titration model provides a new way of looking at cytotype. It may also contribute to an understanding of reactivity in the I-R system. At the very least, it demands a search for extrachromosomal P elements.

There are other levels of regulation of P and I element activity besides cytotype. The participants of the workshop discussed the physiological and developmental regulation that is responsible for some of the phenomena pertaining to dysgenesis. P. Eggleston drew attention to the similar developmental profiles of P and I element action. Both types of elements can induce sterility in their hosts; both are mobilized in the germ line but are not activated in somatic cells. Somehow the elements are able todistinguish the germ line from the soma. Moreover, the I element is able to distinguish males from females. J. Brookfield reported on a possible case of somatic P element activity, perhaps resulting from some modification in the P element structure; however the data he had were still incomplete.

Evolutionary biology.

There was considerable discussion of evolutionary issues at the conference. The underlying questions were whether or not P and I elements can confer any sort of advantage on their carriers, and just when did P and I elements invade the species. T. MacKay and J. Thoday each presented evidence that dysgenesis-induced mutatioins can contribute significantly to variation in a quantitative character. MacKay's studies dealt with abdominal bristle number (recently published in Genetical Research 44: 231-237). Using the time-honored scheme of selecting for high and low values, she showed that populations derived from dysgenic hybrids had a much greater response to selection than those derived from nondysgenic controls. The total variance for bristle number was also consistently greater in the dysgeneic populations. This suggested that the mutational input from dysgenesis was great enough to provide new variation on which selection could act.

Does this mean that dysgenesis is an evolutionary windfall for the genus **Drosophila**? Some have argued that any mechanism that provides variation is, in evolutionary terms, a good thing. In the long run there may be some advantage to an elevated mutation rate, but the cost of all the deleterious mutations must also be kept in mind. If this is high enough, some mechanism for regulating the activity of transposable elements could be expected to evolve. B. Charlesworth presented a theoretical analysis of the prospects for such an event. The equations argue that unless there is restricted recombination between elements on a chromosome, or unless a significant fraction of all transpositions produce dominant lethal effects, there will be little chance for a regulatory mechanism to evolve. However, if this mechanism con-

trols the movement of many different transposon families, then these restrictions do not apply. G. Dover suggested that, in a wild population, differences in the number of P elements per genome at each generation during a peiod of spread might be insufficient to cause fitness differences between individuals, in which case the population would accumulate P (or I) elements cohesively (see the model discussed in Genetics 208: 501-521). C. Langley considered a related problem: the extinction of a transposable element family. In the P-M and I-R systems, for instance, there are intact and defective elements. If all the intact elements mutate into defectives, the transposition function will be lost. Langley's theoretical analysis shows that if a genome has just a few intact elements, this functional extinction can take a very long time.

Perhaps the simplest view of P and I elements is that they are genomic parasites. This is compatible with the idea that both types of elements invaded **D.melanogaster** from an outside source. In the P-M syshe P cytotype is common in North and South America, and central Africa. There are also some P populations in Australia and the Far East, but in the Mideast and in central Asia, the M cytotype is comnogastermon. D. Anxolabhere and G. Periquet have studied the molecular composition of strains isolated from different locations in Europe and Asia; the strains from western Europe have more P element homology than those from Asia. Thus the geographical distribution of cytotype coincides with a molecular cline. The problem is how to interpret the data. Does the pattern suggest that the P element invasion began in Asia and spread westward, ultimately reaching North and South America, and leaving behind M' populations whose elements had become largely defective? Or did the invasion occur the other way around, with the elements originating in the west, perhaps even in the Americas, and spreading to eastern localities where the M' strains represent the advancing wavefront? The finding of P elements in many South American species favors the latter hypothesis, but the issue is far from settled. In addition, there is the question of timing. Since true M strains lack P elements altogether, they must have escaped the invasion; it seems quite unlikely that they were invaded at one time and then subsequently lost all traces of their elements. A more plausible scenario is that these strains were taken into captivity before the invasion reached them, leaving us with laboratory specimens of the pre-invasion condition.

Then what of the I element? Did it follow a similar pattern? Here the analysis is complicated by the fact that all **D.melanogaster** strains seem to possess I element sequences. It would appear, therefore, that the I element is a very old invader, if it is one at all. This is consistent with the presence of I elements in related species. On this view, the reactive strains of **D.melanogaster** would represent cases of functional extinction which occurred during their propagation in the laboratory. Alternatively, they might represent the primitive condition, and I strains could have arisen by a second invasion, or perhaps by some form of recombination or gene conversion that reconstituted the functional element from portions of different defective ones. These are all viable possibilities, since the existing data cannot discriminate among them. Clearly, the search for the right version of the I element's natural history, and for that of the P element also, will be an interesting venture.

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Thompson, V. Roosevelt University, Chicago, Illinois USNA. Documentation of the electrophoresis revolution in Drosophila population genetics.

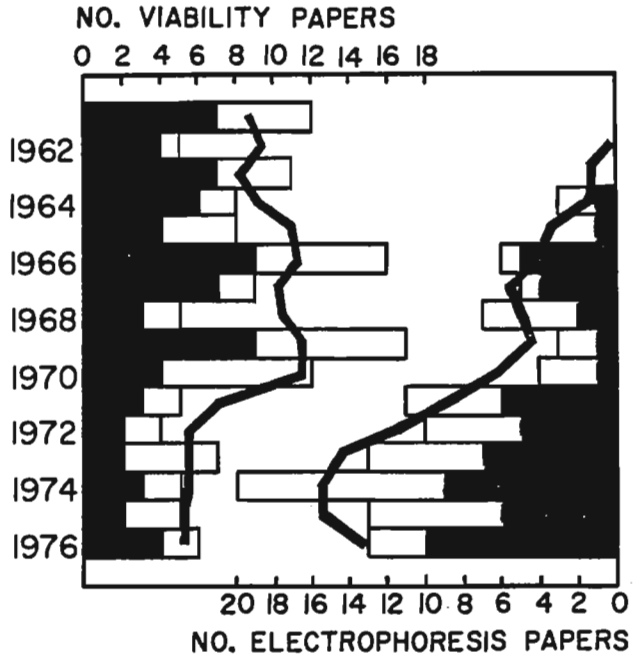


Figure 1. Publication of papers based on Drosophila whole chromosome viability measurements and Drosophila protein electrophoresis during the period 1961-1976. Bars from left represent viability papers while those from right represent electrophoresis papers. The solid portion of each bar represents papers in the journal Genetics while the open portion of each bar represents papers in the other nine journals examined (Amer. Natur.; Can. J. Genet. Cytol.; Evolution; Genet. Res.; Genetica; Hereditas; Heredity; Jap. J. Genet.; PNAS USA). The heavy lines connect three generation moving averages to emphasize longterm trends.

In 1966 Hubby & Lewontin demonstrated the existence of substantial electrophoretically detectable enzyme variation in **D.pseudoobscura.** Their work set off a major trend in experimental Drosophila population genetics that dominated the next decade. It is common wisdom among drosophilists that the Hubby-Lewontin papers reoriented the field in a sudden and not necessarily wholly productive way.

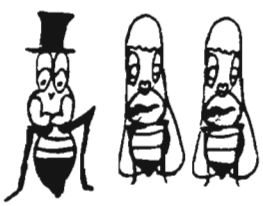
To provide one basis for assessing the changes in Drosophila population genetics during the "electrophoresis revolution" I have tabulated the publication of relevant papers in ten journals during the years 1961-1976. I provide data on two sorts of papers, those involving electrophoresis applied to assessing genetic variation and those involving the measurement and manipulation of whole chromosome viabilities by traditional balancer techniques. Such balancer techniques were the core experimental method of American population geneticists prior to the extensive application of protein electrophoresis.

The results appear in summary form in Figure 1. I have included the data for the journal Genetics as a distinct category because about half of the papers appeared there. In addition, during the period in question it was the preferred place of publication for American population geneticists and it may be taken as an important reflection of major trends in the field.

The data indicate that the period 1970-1971 witnessed a fairly dramatic change in publication patterns. The rate of publication of papers based on chromosome viability measurements dropped from about nine to ten per year during the nineteen sixties to only half that rate in the nineteen seventies. At the same time, the rate of publication of electrophoretic papers increased from about five per year in the late nineteen sixties to about three times that rate in the nineteen seventies. It appears that electrophoretic work did displace work using the traditional balancer techniques.

I suggest that in response to the Hubby-Lewontin observations a sizable portion of Drosophila population geneticists completed traditional projects they had underway, published the results (perhaps accounting for the burst of viability studies published in 1969-1970), and shifted their efforts to electrophoretic studies which began to reach print in numbers beginning in 1971. Since electrophoretic techniques permitted many Drosophila population geneticists to move to work on other organisms, the present data must understate the magnitude of the shift in population genetics research.

References: Hubby, J.L. & R.C. Lewontin 1966, Genetics 54:577-594; Lewontin, R.C. & J.L. Hubby 1966, Genetics 54:595-609.



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Cloned DNA by chromosome location, May 4, 1985, Revised. (See earlier lists: DIS 59:1-9; DIS 60:1-9)
Previous editions in: Genetic Maps, S.J. O'Brien (ed.) Cold Spring Harbor Press 1984

LOCATION	CLONE NUMBER	STOCK OF ORIGIN & OTHER INFORMATION	REF.
X chromosome			
1B1-2		Walk in Maniatis Library, Canton S, yellow achete loci	8
1B1,2-4,5		yellow, achaete, scute loci	57
1B	y+p13-2,yp3A2	yellow locus	74
1B	λy1	yellow locus	77
1B5,8 and 2E	cos 4P	Oregon R, 70kb	57
1B11-13		su(s) locus	15
1B		adm134E8	34
2B1,2-5,6		230Kb Maniatis library, occ locus	24
2E2-F3		fs(1)K10, crooked neck, pcx, kurz loci, 200Kb	98
2EF		fs(1)pecanex, pcx1	80
3B1,2		per locus, 40 Kb	9
3B2 to 3C2		Oregon R, microdissection, 200Ks, white locus	62
3B	mDm112 C 10	Oregon R	1
3BC	M187	0-6 hrs	84
3C		25Kb distal to white locus	95
3C,12	λm1.2	White locus from Maniatis library	3
3C6,8		Canton S, Notch locus	9
3C7		Notch locus	46
3C11,12		sgs 4 locus	45
3C7,3D1	pKdm 6B3	Intermolt I RNA	34
3, also 3R91	S24	Canton S	16
3,4	adm 136G5		34
4BC	mDm 109A7	Oregon R	1
4BC		40Kb walk, no receptor potential locus	90
4C	M97	8-24 hrs	84
4F5A	pkdm 35D12	late IV RNA	34
45 and 62	adm 106A10		34
4F5A	adm 139C12		34
5AB	adm 126D6		34
5C			12
5D		ribosomal protein 7/8 locus, Maniatis library	85
5EF, and 63F64A	adm 140C11		34
5F (prox.)	B70	maternal restricted transcript	69
6F5		Sex lethal locus	66
7A		99Kb, Maniatis library	67
7B3,4		Oregon R, cut locus 100kb	29
7D5,6 and short walk distal		90Kb, Canton S, Oregon R, fs(1) homeotic locus, 1(1)myospheroid locus	21
7D	m58	0-6 hrs	84
7E6,7F1,2		150 kb overlapping	44
78	adm 132H10		34
8A		100 kb overlapping	44
8D	PLZ-p	lozenge locus	2
8F9A	PYp1	Canton S, yolk protein 1 locus	2
8F9A	PYp2	Canton S, yolk protein 2 locus	2
8	56	Canton S	16
10A		vermilion: tryptophan oxygenase locus	79
10B			91
10C1,2	λDmRpII-1	RNA polymerase II largest subunit	51
10EF	adm 134A3	late V RNA	34
10EF, and 32AC	adm 130E12		34
10F	adm 10F.1	minor heat shock cDNA from Kc cells	39
11A		gastrulation defective locus, Maniatis library	38
12B,C	PYP3	Canton S, yolk protein 3 locus	2

LOCATION	CLONE NUMBER	STOCK OF ORIGIN & OTHER INFORMATION	REF.
12DE	pDt17R*	Ser 7 tRNA locus	27
12DE	pDt27*	Ser 4 tRNA locus	27
12DE	pDt73*	Ser 4-7 tRNA loci	27
12E	pDt16*	Ser 4-7 tRNA loci	27
12F1,2, Y	Dm2L1	tandem repeated 2L1 sequence	75
12F	λ32-10	tRNA locus	58
12,13	adm 136F10		34
12	S21b	Canton S	16
14BC	adm 132B8		34
14D	M75	0-1 hrs	84
15A1		rudimentary locus, 90Kb Maniatis library	30
15A,B	548	Oregon R+ Head Specific RNA	31
15B		ribosomal protein S 18 locus	85
15F	λf1	forked locus	77
16B3-5	PTE-1		2
16EF		Maniatis library: 50kb, Shaker locus	40
16F17	adm 135H4		34
17AB	λdmpt 61		58
18CD	A57	maternal restricted transcript	69
18D	λDmG21	G6PD locus, Oregon R+	28
19EF20AB	DCg2	collagen-like gene, from Maniatis library	25
19F	pDt67R	Arg tRNA locus	27
2L			
21B	adm 142G5		34
21C		double sex cognate	88
21D	pD957		3
21F22A	adm 123D12, 123H3, 128B8		34
22B/C	adm 129E7		34
22F1,2		130 Kb, decapentaplegic complex	55
23A3-7		70 Kb, Maniatis library	59
23BC	B13	maternal restricted transcript	69
23E	pDt5*	Ser 7 tRNA locus	27
24C	mDm101A10	Oregon R	1
25BC	mDm109D3	Oregon R	1
25C	DCg-1	collagen-like gene, from Maniatis library	25
25D	150-3(λ)	blastoderm-specific poly(A) RNA	47
25D1-4	MH5	from Gelbart library	6
26A7-9		beta galactosidase locus	6
26A	DmcMM115	vitelline locus, Oregon R ovaries, cDNA	72
26A	LS1	vitelline locus, Oregon R embryos genomic	72
26AB	A20	minor site at 88D	69
27C		GAR transformylase, GAR synthetase, AIR synthetase	7,86
27D	λ39-1	(Repetitive, also hybridizes to 91C and 43A, tRNA locus)	58
27F	adm 125G11		34
28A	551	Oregon R+, Head Specific RNA	31
28C	538	Oregon R+, Head Specific RNA	31
28C	λdmpt 49		58
28D9-12		CDNA, Kc cells	8
29A	pDt59R*	Lys 5 tRNA locus	27
29B1-4		CDNA, Kc cells	8
29C		SRC homologous	61
30B	λdmpt 75		58
30DE	adm 136D3		34
30EF	λdmpt 104		58
31A	mDm 106A10	Oregon R	1
31A	M35	0-6 hrs	84
31C	adm 134G6		34
31C33 B	adm 142H3		34
31F, and 39F	adm 142F4		34
32AB	503	Oregon R+, Head Specific RNA	31

LOCATION	CLONE NUMBER	STOCK OF ORIGIN & OTHER INFORMATION	REF.
32CD	231	Maniatis library, myogenic cell RNA	63
33AB		extra sex combs locus; 250 kb	70
33B	adm 124D9		34
33B	M146	0-1,2-5,3-5	84
34AB	A34		69
34C	DmcMM99	vitelline locus, Oregon R ovaries, cDNA	72
34F	527	Oregon R+, Head Specific RNA	31
35B3-5	AC	alcohol dehydrogenase locus, Maniatis library	52
35B	mdm 103D5	Oregon R	1
35C36	adm 125E7		34
36B		myosin heavy chain locus, Maniatis library	13
36B		walked from myosin heavy chain locus	20
37B9C1,2		Df(2L)hk18	35
37B13-37C5	λDdc-1 thru-20	dopa decarboxylase locus, 100 Kb	35
37BC	DmcMM109	Oregon R ovaries, cDNA	72
38A6	2E2		35
39CD		cDNA, definitive ribosomal protein from Spradling and Mahowald library	17
39DE		histone locus	54
39E, and 2L Base	adm 136D9		34
2L base (chromocenter)	adm 106H5, 123C3		34
2 R			
2R base, and 3L base (Chromocenter)		adm 130B2	34
42A	mDm 106F8	Oregon-R	1
42BC	λst11-205.16		83
42E	pDt 61	tRNA-Lys-2 locus	27
42EF	adm 126F7, 127A10		34
43AB	555	Oregon R+, Head Specific RNA	31
43BC	B17	maternal restricted transcript	69
43DE	B45	maternal restricted transcript	69
44C	λe8 e9	Oregon R, EMBL 4 library	71
44CD	536	Oregon R+, Head Specific RNA	31
44D	λDmLCP1-13	larval cuticle protein loci: 50kb	36,37
44EF,64B	M51	0-24 hrs	84
44F	129E7		3
44	L10	Canton S	16
45A	mDm103H10	Oregon R	1
45A	mDm108C7	Oregon R	1
45B	M199	0-3.5 hrs	84
45D, and chromocenter	mDm108A8		1
46B	B41	maternal restricted transcript	69
46DF, and chromocenter	236	Maniatis library, myogenic cell RNA	63
46E	549	Oregon R+, Head Specific RNA	31
47E	528	Oregon R+, Head Specific RNA	31
47F		50 Kb	14
47F48D	217	Maniatis library, myogenic cell RNA	63
48A		engrailed locus, Canton S 208 Kb	14
48B	pDt74	Met 2 tRNA locus	27
48C	adm 132A7		34
48E	adm 135E10		34
48F	543	Oregon R+, Head Specific RNA	31
49A	p500, p2.2	calmodulin locus	12
49A12B3		possible site aristapedioid locus	73
49C	mDm101D3	Oregon R	1
49CD	mDm101D12	Oregon R	1

LOCATION	CLONE NUMBER	STOCK OF ORIGIN & OTHER INFORMATION	REF.
49DE	adm 140D1		34
49E5F1		possible site aristapedioid locus	73
49F	Dm1606	muscle specific Troponin C locus (ca++ binding site), Maniatis library 35Kb	22
50B	adm 142E9		34
50C	mDm3021	Oregon R	1
50CD	adm 133H7,136F9, 138G8, 130H8		34
50F		double sex cognate	88
50	L6	Canton S	16
51A	S34	Canton S	16
51B	S14	Oregon R+, Head Specific RNA	31
51CD	A19	maternal restricted transcript	69
51D	adm 134E2		34
51DE	mDm102F11	Oregon R	1
51DE	mDm102B6	Oregon R	1
52A1,2	M144	0-1 hrs	84
52A3,6	M137	0-1 hrs	84
52B	mDm107A2	Oregon R	1
52D6,15	M222	07, 0-6	84
52D		(not double sex cognate)	88
52DF	adm 139H3		34
53CD	λDm32 (Class A)	Amy pseudogene, from Maniatis library	33
53F	λdmpt 116		58
53	L23	Canton S	16
54A1B1(54A)	λDm 65 (Class B)	Amy duplication locus, Canton S	33
54E	adm 54E.1	Minor heat shock cDNA	39
54F55A	adm 110A4, 132C9, 132E11, 132E12, 132G5, 134A4, 135D12		34
55BCD	adm110G1, 110H1, 132D6		34
55F	B32	maternal restricted transcript	69
56C	DTB2	β tubulin locus, from Maniatis library	25
56D412	KV 2-70a	β tubulin locus, from Maniatis library	22
56D	pTu56	β1 tubulin locus, Oregon R, EMBL 4 library	71
56EF	adm 135H8		34
56F	λDmt 56-6	$tRNA^{Gly}$ locus	58
57B			12
57C	525	Oregon R+, Head Specific RNA	31
57C		Punch locus, 60Kb	92
57C		Tudor locus, 200Kb	93
57F		v-erbB, EGF receptor protein locus	60
58F	adm 132A3, 135D10, 135E6		34
60A (distal)	A8		69
60A (prox.)	B6	maternal restricted transcript	69
60A	adm 125C2		34
60A, and nucleolus	adm 106H6		34
60B	pTu60	β3 tubulin locus, Oregon R, EMBL 4 library	71
60BC	B50	maternal restricted transcript	69
60C6-8	KV 1-11	β tubulin locus, from Maniatis library	22
60C	DTB3	β tubulin locus, from Maniatis library	25
60F3		Kruppel locus microdissected from Oregon R, 50Kb	97
3 L			
61A1-3	mDm105F3	Oregon R	1
61F		double sex cognate, Maniatis library	88
62A	adm 112C10		34
62A	λ48-9	tRNA locus	58
62AB, 97C	adm 140F12		34
62CD	203	Maniatis library, myogenic cell RNA	63
62D	adm 142F6		34
62E		ribosomal protein L12 locus	85
63AC	227	Maniatis library, myogenic cell RNA	63

LOCATION	CLONE NUMBER	STOCK OF ORIGIN & OTHER INFORMATION	REF.
63B	bDm 4L	Oregon R, hsp 83 locus	39
63BC	λ6	Canton S, hsp 83 locus	10
63BC	pPW244, 301, 330	Oregon R, hsp 83 locus	10
63F	adm 63 F.1	minor hsp locus	39
63-66	S7	Canton S	16
64B	Drsrc	SRC homologous	60,61
64B	pDMRS64B	ras oncogene locus Maniatis library	76
64BC, and chromocenter	mDm104C1	Oregon R	1
64C	DHSV4	RAS homologous	60
64C	λdmpt 85		
64F	mDm106E3	Oregon R	1
64F	λdmpt 120		58
64F, 66C	adm 126B4		34
64F/65A	adm 135G4		34
65C	adm 111F10		34
66CD	adm 106E3		34
66D9-10	.8247, 30152, 3019		32
66D10-15		Oregon R, 85kb	57
66D11-15		100 kb overlapping	44
66D	507	Oregon R+, Head Specific RNA	31
66D	547	Oregon R+, Head Specific RNA	31
66F	λdmpt 121		58
67A5-7 to 67B1,2		Walk from Maniatis library	22
67B	λ88	Canton S, loci of hsp 22, 23, 26 and 28	10
67B	λDmp 67	hsp loci & flanking transcripts, from Canton S	43
67B	J1	includes hsp 28, 23, 26 loci, Oregon R	42
67C	DTA2	α tubulin locus, from Maniatis library	25
67C4,5 + 24A,B?	M98	0-1 hrs	84
67DE	gt11.205.23		83
68C1-5	λcDm2021	In(3L)HR15	1
68C3-7; 68E	λbDm2054	Df(3L)vin	1
68C4-6	aDm 1501-10	sgs 3,7,8	1
68C7-15	mDm148F7	Oregon R	1
68C	pkdm 2G6	intermolt II RNA	34
68C	pkdm 2C1	intermolt III RNA	34
68C	pkdm 1H2	intermolt IV RNA	34
68C	adm 134C10		34
68EF	adm 133H1		34
69D	M37	0-24 hrs	84
69F	270	Maniatis library, myogenic cell RNA	63
69	L3g	Canton S	16
70A	adm 107A4		34
70AB	adm 128C11, 132B3		34
70BC	B20	maternal restricted transcript; minor homology to 2F-3A and chromocenter	69
70BC	pDt 55*	Val 4 tRNA locus	27
70C	adm 29D11		34
70D4,5	Q111,Q131,Q112	60Kb walk, Maniatis library frizzled locus	81
71A	2-5 (λ)	gastrula-differential poly(A) RNA	47
71AB	adm 123C4		34
71C3.4D1.2		EIP 28/29 locus	26
71 CE	λcDm 20,21,22,23,24	ecdysone induced late puff from Maniatis library	24
71DE	adm 134A9, 134A11, 134C11		34
71DE	pkdm 46B7	late I RNA	34
71DE	pkdm 38C9	late II, III RNA	34
71DE	pkdm 38C4	late II, III RNA	34
72BC	557	Oregon R+, Head Specific RNA	31
72DE	λdmpt 115		58
73A		transformer locus	87

LOCATION	CLONE NUMBER	STOCK OF ORIGIN & OTHER INFORMATION	REF.
73A		double sex cognate, not transformer locus	88
73B	Dash	Abelson SRC homologous	60,61
73D	adm 73D.1	minor heat shock locus	39
73DEF	521	Oregon R+, Head Specific RNA	31
74EF		early ecdysone responding puff, 300 Kb from Maniatis library	56
75C	adm 135F3		34
75	S39	Canton S	16
76A	adm 132D11		34
76DE	B48	maternal restricted transcript	69
76F	mDm 104G3	Oregon R	1
79B			12
79E1,2	13E5	Or,R PBR322	16
80C		Kc cells	8
3 L base (chromocenter)	adm 139A10		34
3 R			
3 R base (chromocenter)	adm 128F12		34
82A	S6-7	from Maniatis library	22
82F	506	Oregon R+, Head Specific RNA	31
83A	adm 136E4		34
83AB	adm 140E12		34
83A,B	pDt 66R2	Lys 5 tRNA locus	27
83B	adm 123G4		34
83C	mDm 105 B9	Oregon R	1
83CD	B21,B31	maternal restricted transcript	69
83F	adm 140C1		34
84A,B	pDt 12	Lys 5 tRNA locus	27
84A,B	pDt 39*	Lys 5 tRNA locus	27
84A4,5 to 84C1,2		Antennapedia complex, 440 Kb	49
84B1-3		Maniatis library: 240 Kb	50
84B3-6	λDm 2.55a	α tubulin locus, from Maniatis library	22
84B3-C1,2		75 Kb, Maniatis library, double sex locus	48
84B	DTA 1	α tubulin locus, from Maniatis library	25
84BC	adm 123D11		34
84C8	DmA 3a, 4a, 4b, 5a, 5b	Glucose dehydrogenase locus, eclosion genes A-D, 152Kb, Maniatis library	4
84D3,4	1,3,6,10	30 kb from Maniatis library, overlaps Val 3b tRNA locus	19
84D4-8	λDm 5-1	α tubulin locus, from Maniatis library	22
84D	mDm 104H7	Oregon R	1
84D	pDt 78 RC*	Val3btRNA locus	27
84D	DTA 4	α tubulin locus, from Maniatis library	25
84E1,2		105 Kb, double sex locus and flanking, Maniatis library	48
84E11-12 to F4-5		Maniatis library: 240 Kb	50
84F2,3		In(3R)Hu	50
84F	B34	maternal restricted transcript	69
85A	λ50-8	tRNA locus	58
85A		100Kb walk	78
85C	p85C	Arg tRNA locus	27
85C	λm 1:2	from Gelbart library	6
85D6-12	DTB 4	β tubulin locus, from Maniatis library	25
85D	KV 1-22	β tubulin locus, from Maniatis library	22
85D	542		16
85D	DHSV 7	RAS homologous	60
85D		β2 tubulin locus, Oregon R, EMBL 4 library	71
85D,E		double sex cognate	88
85E	M253	0-1 hr	84
85E6-10	λDm 5-22	α tubulin locus, from Maniatis library	22
85E	DTA 3	α tubulin locus, from Maniatis library	25

LOCATION	CLONE NUMBER	STOCK OF ORIGIN & OTHER INFORMATION	REF.
85E	mDm 3008	Oregon R	1
85	adm 123B10		34
86B4C1		In(3R)Hu	50
86	adm 35E6		34
86	S35g	Canton S	16
87A7	pPW 223	Oregon R, hsp 70 locus	10
87A7		hsp 70 locus subclone	39
87A	56H8	hsp 70 locus and flanking	41
87A	GB	Hsp70, Sn cell DNA	42
87AB	540	Canton S	16
87C1	pPW232, pPW229	Oregon R, hsp 70 locus	10
87C1	132E3	hsp 70 locus and flanking	41
87C	G3	hsp 70, Sn cell DNA	42
87CF, 94D	adm 125G5		34
87D	mG31	Hsc 70; Oregon R	42
87D5-87E5		315 kb overlapping, rosy and Ace loci	5
87E			12
88B	adm 88B.1	minor heat shock cDNA	39
88C	mDm 104D12	Oregon R	1
88D		double sex cognate	88
88E	mG34	Oregon R; hsc 70 locus	42
88F2-5	λDM 85	3 tropomyosin loci	22
88F		250 kb walk, actin locus	11
88F	λdmpt 73	tropomyosin locus	58
88F			12
88	S32	Canton S	16
89B	pDt 14*	Val 4, Phe 2 tRNA loci	27
89BC	EV27		23
89E1-4		400 kb walk, bithorax complex	18
90BC	pkdm 7E5	Intermolt V RNA, sgs locus	34,24
90BC	pDt 92RC*	Val 4 tRNA locus	27
90BC	pDt 120 RC*	Val 4 tRNA locus	27
90BC	pDt 41 RC4*	Val 3b, Pro tRNA loci	27
90BC	λbDm 1508	Oregon R, Hogness library	24
90BC	pDt 48*	Val 3b, Pro tRNA loci	27
90C	λ49-4	repetitive, also 85C and 84D, tRNA locus	58
91D	mDm 103G4	Oregon R	1
91	S24	Canton S, also X3	16
92A	mDm 101F8	Oregon R	1
92CD	512	Oregon R+, Head Specific RNA	28
92E	adm 124B10		34
92	S12g	Canton S	16
92B8-11		rhodopsin (nina E) locus	99,100
93B		90 Kb walk	78
93D		60 Kb walk	78
93D	adm 129F5		34
94A	adm 134C5, 135D2		34
94E	λdmpt 123		58
94F	0018	oocyte RNA locus 16Kb insert	41
94F95A	156-1 (λ)	blastoderm-differential poly(A) RNA	47
95B	mDm 108E11	Oregon R	1
95D	pPW227	Oregon R, hsp 68 locus	10
95D	λ15	Canton S, hsp 68 locus	10
96A	adm 137A2		34
96D	mDm 107D4	Oregon R	1
96F97A	adm 126D12		34
96F97C	adm 132C4, 132E7, 132H4		34
97A	λdmpt 50		58
97EF	DTB1	β tubulin locus, from Maniatis library	25
97F	KV 3-12	β tubulin locus, from Maniatis library	22
98,99	L2	Canton S	16

LOCATION	CLONE NUMBER	STOCK OF ORIGIN & OTHER INFORMATION	REF.
98E	B8	maternal restricted transcript	69
98F	M55	0-2.5 hrs	84
99B		Homeo box	50
99C5-6	559	transient receptor potential locus, 45 kb	68
99C	559	Oregon R+, Head Specific RNA	31
99CF	adm 129B8		34
99D	153-1 (λ)	blastoderm-specific poly(A) RNA	47
99D	rpro 49	ribosomal protein locus	53
99E1-3	36-1 (λ)	blastoderm-differential poly(A) RNA	47
99E	λDm 11-9	myosin light chain locus Maniatis library	22
99E	adm 132G9		34
99F	adm 142D9		34
100AB	5D7	OR.R PBR322	16
100B	mDm 103 F1	Oregon R	1
100B	λdmpt 31		58
100B	516	Oregon R+, Head Specific RNA	31
100C1-7	mDm 102A3	Oregon R	1
100C		double sex cognate, Maniatis library	88
100CD	11B2	cell death-5 locus	94
100D	mDm 105 H1	Oregon R	1
100F	λ5c,λR15,λgt11.205.16	microtubule-associated protein locus	83
100	S2	Canton S	16
4th Chromosome			
102C, also chromocenter	mDm 108 D1	Oregon R	1
102CD	116H2		3
102EF	λdmpt 101		58
Multiple			
3C, 5A, 5C, 8B-9D, 11A, 30F		1.688 satellite tandem repeats	96
5C, 42A, 57A, 79, 87F/88A, 88F	adm 105C6, 105G9, 108D11	Actin repeated locus	34
5CD, 24F, 30EF, 63F/64A	adm 136H5		34
21E, 82E, 95AC	pDm U1.4d U1 RNA	coding seq.	22
telomeres + β heterochromatin	T-A		22
	T-F		22
mitochondrial	710	Hind III C/Ch21A	22
	13	EcoRI C+B/Ch 4A	22
	23	EcoRI B /Ch 4A	22
	41	EcoRI C /Ch 4A	22
X base, 30F, 48 D/E, 96	adm 135D5		34
25A/C, 44D, 64F65A, 66CD, 67B, 99C, 99F	adm 8G8, 26H2, 135A8, 135A10	"Jonah"	34
44D and chromocenter	pDm 2/3-1	"Beagle"	37
48CD, 60A, 100C	adm 128A7		34
50BC, 50F, 58/59	adm 135D11		34
87C1, 42B, chromocenter	cDm 703	alpha beta repeated locus	39
Telomere	8-19T	Oregon R, EMBL 1 library	71

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Corrections to:

Chromosome rearrangements available for "jumping" with one breakpoint known within a cloned sequence.

May 31, 1984. (see DIS 60:10-13, 1984)

Target Breakpoints	Cloned Locus	Stock Description	Ref.
	[Breakpoint should read as follows:]		
X chromosome			
3A6-8	3B1-2 (from per)	Df(1)64j4	7
3C1-2	3C11-12 (Sgs-4)	$Df(1)wN^{fm20}$	9
3C6	3C11-12 (Sgs-4)	$Df(1)N^{5419}$	9
3C4	3C11-12 (Sgs-4)	$Df(1)dm^{75e19}$	9

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Transformed lines available for cloning or deleting DNA at specific chromosome sites.
May 4, 1985. Revised.
(see DIS 60:13-16, 1984)

Cytological location of inserts	Transformant strain	Markers/genes present in construction	Ref.
X Chromosome			
1B	g71:1	$Sgs3^+$, ry^+14	
1CD	BS2.71-2	$chorion^+$, ry^+	5
1F	R702.1	ry^+	5
1F	R704.2	ry^+	5
1F	DA24-14	Adh^+, Ddc^+	4
2B	Tf(1)Gr304-1	ry^+	1
2B13-18	g711:2	$Sgs3^+$, ry^+	14
3B	Tf(1)GR420-3	ry^+	1
3F	g71dx:2	$Sgs3^+$, ry^+	14
4B	cHBΔ-59	Hsp70-lacZ, ry^+	8
4D	R405.1	ry^+	5
6F	S6.9-2	$chorion^+$, lac^+, ry^+	5
7D	R403.1	ry^+	5
8BC	tAP-25,3.2	Adh^+	2
8E	cHBΔ-59	Hsp70-lacZ, ry^+	8
9A-D	R404.2	ry^+	5
9B		ry^+	7
9B	tAP-24B,3.2	Adh^+	2
9B	SB2.1-5	$chorion^+$. ry^+	5
9C	S6.9-9	$chorion^+$, $lacZ^+$, ry^+	5
9E	R701.1	ry^+	5
9E	cHBΔ-23	Hsp70-lacZ, ry^+	8
10BC	tAP-20,3.2	Adh^+	2
12A	tAP-17, 4.8	Adh^+	2
12BC	SRS3.9-1	$chorion^+$, ry^+	5
12D	R301.2	ry^+	5
12E	cHBΔ-73	Hsp70-lacZ, ry^+	8
13A	g71dx:1	$Sgs3^+$, ry^+	14
13A-C,14F,69A,98C	BS2.7-5	$chorion^+$, ry^+	5
13CD	SB2.1-6	$chorion^+$, ry^+	5
15DE	BS2.7-10	$chorion^+$, ry^+	5
16BC	S6.9-11	$chorion^+$, $lacZ^+$, ry^+	5
16D		ry^+	7
17DE	B1-2	w^+	3
18A	R704.3	ry^+	5
18A	g7:1	$Sgs3^+$, ry^+	14
18D	BS2.7-3	$chorion^+$, ry^+	5
19A	cHBΔ-89	Hsp70-lacZ, ry^+	8
19E	+65	Hsp70, ry^+	8
20A	Adh^{hs20A}	Adh^+, hsp 70	6
20A, 54A	2	$Act88F^+$	13
Chromosome II			
21D	R602.1	ry^+	5
21DE	tAP-10,4.8	Adh^+	2
22A	R604.1	ry^+	5
22B	w20.2	w^+	10
23A	+65	Hsp70, ry^+	8
23BC	g5:2	$Sgs3^+$, ry^+	14
25C	R401.3	ry^+	5
26B	D4	$rp49^+$, ry^+	9
28A	BS2.7-11	$chorion^+$, lac^+, ry^+	5
29B	R308.1	ry^+	5

Cytological location of inserts	Transformant strain	Markers/genes present in construction	Ref.
30A	28C-B	ry^+ hsp28	11
30A, 78D	cHBΔ-89	Hsp70-lacZ, ry^+	8
30C	S6.9-3	$chorion^+$, lac^+, ry^+	5
31B	cHBΔ-89	Hsp70-lacZ, ry^+	8
32BC	BS2.7-13	$chorion^+$, ry^+	5
32CD	cp70ΔB	Hsp70-lacZ, ry^+	8
32F	g6:5	$Sgs3^+$, ry^+	14
34D	g7:4	$Sgs3^+$, ry^+	14
34EF	g711:1	$Sgs3^+$, ry^+	14
35DE,45A,59E	S11.4-1	$chorion^+$	5
36A		Hsp26-lacZ, ry^+	8
36C	tAP-8C,4.8	Adh^+	2
37A		Hsp26-lacZ, ry^+	8
37BC		Adh^+ Hsp82	12
38B	cHBΔ-59	Hsp70-lacZ, ry^+	8
38BC	tAP-19,4.8	Adh^+	2
38D, 84F	1	$Act88F^+$	13
38E, 57F		ry^+	7
39B	28P-C	ry^+ hsp28	11
39BC	S6.9-8	$chorion^+$, lac^+, ry^+	5
39EF	AR4-2	w^+, ry^+	3
39E	B4	$rp49^+$, ry^+	9
39E,40F (2L)	AR4-3	w^+, ry^+	3
42A	R301.1	ry^+	5
42A	tAP-13,4.8	Adh^+	2
42A	+411	Hsp70, ry^+	8
42AB	R303.1	ry^+	5
42DE		ry^+	7
42E	R305.1	ry^+	5
42F	S6.9-4	$chorion^+$, lac^+, ry^+	5
42F	8X-C	ry^+ hsp28	11
43	R704.1	ry^+	5
43C	R304.1	ry^+	5
43CD	+411	Hsp70, ry^+	8
43E,44CD	S6.9-7	$chorion^+$, lac^+, ry^+	5
43E	g7:7	$Sgs3^+$, ry^+	14
44E	R3.9-4	$chorion^+$, ry^+	5
44F	cp70ZT	Hsp70-lacZ, ry^+	8
45A, 22B	w20.10	w^+	10
45AB	F4	$rp49^+$, ry^+	9
45E	DR-18	ry^+, Ddc^+	4
45E, 77A	cHBΔ-73	Hsp70-lacZ, ry^+	8
47A	tAP-18, 4.8	Adh^+	2
47A	A1-1	w^+, ry^+	3
47C	cHBΔ-89	Hsp70-lacZ, ry^+	8
48AB	g7:3	$Sgs3^+$, ry^+	14
48B		Hsp26-lacZ, ry^+	8
48C	R703.1	ry^+	5
48D	DR-9	ry^+, Ddc^+	4
49B		Hsp26-lacZ, ry^+	8
49D	-51	Hsp70, ry^+	8
49D	A1	$rp49^+$, ry^+	9
49D	S38M-5	$s38^+$,$M13^+$,ry^+	5
49EF	S3.8-6	$chorion^+$, ry^+	5
49F	tAP-9,4.8	Adh^+	2
50B	R306.1	ry^+	5
50B	S6.9-6	$chorion^+$, lac^+, ry^+	5
52A	DR-12	ry^+, Ddc^+	4

Cytological location of inserts	Transformant strain	Markers/genes present in construction	Ref.
52B	cHBΔ-89	Hsp70-lacZ, ry^+	8
52B	tAP-21,3.2	Adh^+	2
52D	SRS3.9-1	$chorion^+$, ry^+	5
52F	R402.1	ry^+	5
52F	S6.9-10	$chorion^+$,$lacZ^+$,ry^+	5
53A	S38M-6	$s38^+$,$M13^+$,ry^+	5
53E	R3,9-1	$chorion^+$, ry^+	5
53EF	SB2.1-1	$chorion^+$, ry^+	5
53F	g4:1	$Sgs3^+$, ry^+	14
K10;p(cos9)	fs(1)K10+, pcx^+, crn^+, krz^+		15
54C	Adh^{hs54c}	Adh^+, hsp 70	6
56D	R3.9-6	$chorion^+$, ry^+	5
56F	DR-15	ry^+, Ddc^+	4
56F	DR-5	ry^+, Ddc^+	4
58EF	R3.9-5	$chorion^+$, ry^+	5
58F	cp70ΔB	Hsp70-lacZ, ry^+	8
58F,59C	tAP-7A,4.8	Adh^+	2
59B	A3-1	w^+, ry^+	5
60A	R302.1	ry^+	5
60AB	g1	$Sgs3^+$, ry^+	14
60B	BS2.7-4	$chorion^+$, ry^+	5
60C		ry^+	7
60C	C2	$rp49^+$, ry^+	9
60E	S38Z-1	s38,lacZ,ry	5
60E	tAP-15A, 4.8	Adh^+	2
60F	S3.8-4	$chorion^+$, ry^+	5
Chromosome III			
61A	Bg61	ry^+, $hsp70^+$, $lacZ^+$	8
61A	S38Z-5	$s38^+$,$lacZ^+$,ry^+	5
61C	Adh^{hs61C}	Adh^+, hsp70	6
61D	cp70ΔB	Hsp70-lacZ, ry^+	8
61D	28P-A	ry^+ hsp28	11
61E	SRS3.9-4	$chorion^+$, ry^+	5
62A			
62AB	tAP-27	Adh^+	2
63BC	28C-A	ry^+ hsp28	11
63C	S38Z-6	$s38^+$,$lacZ^+$,ry^+	5
64F-65A	SB2.1-3	$chorion^+$, ry^+	5
64C	R405.1	ry^+	5
64C	tAP-7B, 4.8	Adh^+	2
64D	Bg64 (lethal)	ry^+, $hsp70^+$, $lacZ^+$	8
64F	cHBΔ-73	Hsp70-lacZ, ry^+	8
65AB	w47.1 N8	w^+	10
65D	28A-B	ry^+ hsp28	10
65F	g711:3	$Sgs3^+$, ry^+	14
66A	28X-D	ry^+ hsp28	11
66D-67A	DR-17	ry^+, Ddc^+	4
66E1,2	Tf(3L)GA6.0-1	Adh^+	1
68A	R7.7-1	$chorion^+$, ry^+	5
68C	28X-A	ry^+ hsp28	11
68C	g7:5	$Sgs3^+$, ry^+	14
68C	g71:2	$Sgs3^+$, ry^+	14
68D	S38Z-3	$s38^+$,$lacZ^+$,ry^+	5
68E	g6:4	$Sgs3^+$, ry^+	14
69CD	tAP-12,4.8	Adh^+	2
70AB	S3.8-3	$chorion^+$, ry^+	5
70C	S11.4-2	$chorion^+$	5
71AB	-51	Hsp70, ry^+	8

Cytological location of inserts	Transformant strain	Markers/genes present in construction	Ref.
71C	S38M-1	$s38^+$,$M13^+$,ry^+	5
71F	g7:2	$Sgs3^+$, ry^+	14
71F	tAP-11, 4.8	Adh^+	2
75CD	R502.1	ry^+	5
75D	R706.1	ry^+	5
76A	S38M-4	$s38^+$,$M13^+$,ry^+	5
78BC	R603.1	ry^+	5
79E	+411	Hsp70, ry^+	8
80A	B1,F2	$rp49^+$,ry^+	9
82A	w47.4L	w^+	10
82B	Adh^{hs82B}	Adh^+, hsp 70	6
82BC	tAP-8B, 4.8	Adh^+	2
82BC	g4:3	$Sgs3^+$, ry^+	14
82C	28X-E	ry^+ hsp28	11
83A		ry^+	7
83BC	SB2.1-2	$chorion^+$, ry^+	5
83F	R3.9-3	ry^+	5
83F	S38M-2	$s38^+$,$M13^+$,ry^+	5
84BC, 96B	S3.8-1	$chorion^+$, ry^+	6
84C		ry^+	7
84D		Hsp26-lacZ, ry^+	8
84D	28X-B	ry^+ hsp28	11
84E	cHBΔ-194	Hsp70-lacZ, ry^+	8
84E	g6:2	$Sgs3^+$, ry^+	14
84F	g71:3	$Sgs3^+$, ry^+	14
84F	A3	$rp49^+$, ry^+	9
85A	R309.1	ry^+	5
85AB	814,CH8	sry^+, ry^+	9
85D	g6:1	$Sgs3^+$, ry^+	14
85D	BS2.7-7	$chorion^+$, ry^+	5
85F	tAP-15B, 4.8	Adh^+	2
86D	R311.1	ry^+	5
86D	g7:6	$Sgs3^+$, ry^+	14
86E	S38Z-2	$s38^+$,$lacZ^+$,ry^+	5
87A	R307.1	ry^+	5
87AB	28A-C	ry^+ hsp28	11
87CD	C1-1	w^+	3
87E		ry^+	7
87F	R308.2	ry^+	5
87F	R404.1	ry^+	5
87F	DRI-15	ry^+, Ddc^+	4
88A	cHBΔ-23	Hsp70-lacZ, ry^+	8
88C	S6.9-5	$chorion^+$, lac^+, ry^+	5
88E	BS2.7-9	$chorion^+$, ry^+	5
88E	R401.2	ry^+	5
88F	cp70ΔB	Hsp70-lacZ, ry^+	8
88F	S6.9-1	$chorion^+$, lac^+, ry^+	5
89A	+204	Hsp70, ry^+	8
89A	B1-1	w^+	3
89B	BS2.7-6	$chorion^+$, ry^+	5
89B	g7:8	$Sgs3^+$, ry^+	14
90CD	S3.8-2	$chorion^+$, ry^+	5
90EF	DR-1	ry^+, Ddc^+	4
91B	cHBΔ-194	Hsp70-lacZ, ry^+	8
91C	A2-1	$w^=$, ry^+	3
91D	g4:2	$Sgs3^+$, ry^+	14
91F-92A	S3.8-5	$chorion^+$, ry^+	5
92A	R3.9-2	$chorion^+$, ry^+	5

Cytological location of inserts	Transformant strain	Markers/genes present in construction	Ref.
92BC	B2-1	w^+	3
92F	BS2.7-2	chorion$^+$, ry^+	5
93AB	R310.1	ry^+	5
93D	SB2.1-4	chorion$^+$, ry^+	5
93D6,7	g5:1	Sgs3$^+$, ry^+	14
94B	28P-D	ry^+ hsp28	11
95A	R601.1	ry^+	5
95C, 26B	D1	rp49$^+$, ry^+	9
95D	BS2.7-8	chorion$^+$, ry^+	5
95D		ry^+	7
95F	28A-A	ry^+ hsp28	11
96B	S38M-3	s38$^+$,M13$^+$,ry^+	5
96	DR-2	ry^+, Ddc$^+$	4
97A	tAP-16, 4.8	Adh$^+$	2
97AB	+65	Hsp70, ry^+	8
97AB	28-term	ry^+ hsp28	11
97B	28P-B	ry^+ hsp28	11
97F		ry^+	7
98A	BS2.7-1	chorion$^+$, ry^+	5
98B	cHBΔ-89	Hsp70-lacZ, ry^+	8
98C	R602.1	ry^+	5
98C	R705.1	ry^+	5
99A	g6:3	Sgs3$^+$, ry^+	14
99AB	DA24-44	Adh$^+$, Ddc$^+$	4
99D	SRS3.9-3	chorion$^+$, ry^+	5
99D,1 other	SRS3.9-5	chorion$^+$, ry^+	5
100D		ry^+	7
100F	A4-4	w^+, ry^+	3
Chromosome IV			
chromocenter	R401.1	ry^+	5

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Alatortsev, V.E. and E.V. Tolchkov.* Institute of Molecular Genetics, USSR Academy of Sciences, Moscow 123182 USSR; *Institute of Microbiology, Riga, USSR. Complementation analysis of rearrangements affecting the 2D-F region of the **D.melanogaster** X-chromosome.

Twelve complementation groups were detected in the 2D3-2F5 region of the X-chromosome by the saturation method. The complementation map of this region, based on the earlier (Gvozdev et al. 1973, 1975a, 1975b; Gerasimova 1976; Tolchkov et al. 1984; Tolchkov & Gvozdev 1984) and present data is shown in Figure 1.

The extent of deficiencies was determined for the following rearrangements: Df(1)pn7a, Df(1)pn38, T(1;3)pn12, In(1)pn45 (for a description of the rearrangements, see Ilyina et al. 1980) and Df(1)2T, Dp(1;Y)3T, Dp(1;Y)43T (described by Gorelova, see Belyaeva et al. 1982).

It should be noted that Dp(1;Y)3T and Df(1)pn7a rearrangements reveal only partial complementation with lethals of the first and third groups, respectively.

The Df(1)TEM-304 chromosome carries the pn mutation (Gerasimova 1976).

As Df(1)TEM-501 complements the kz group, it either carries two deletions (one to the right, the other to the left of kz) or the deletion is accompanied by a transposition of the kz^+ gene into the same X-chromosome.

T(1;3)pn12 and In(1)pn45 fail to cover complementation groups 1 to 7 and 4 to 8, respectively. These findings may be explained by a microdeletion or by the position effect.

The Df(1)2T deletion complements all mutations of the given region, although its right breakpoint has been localized cytologically in 2E1,2 (Belyaeva et al. 1982).

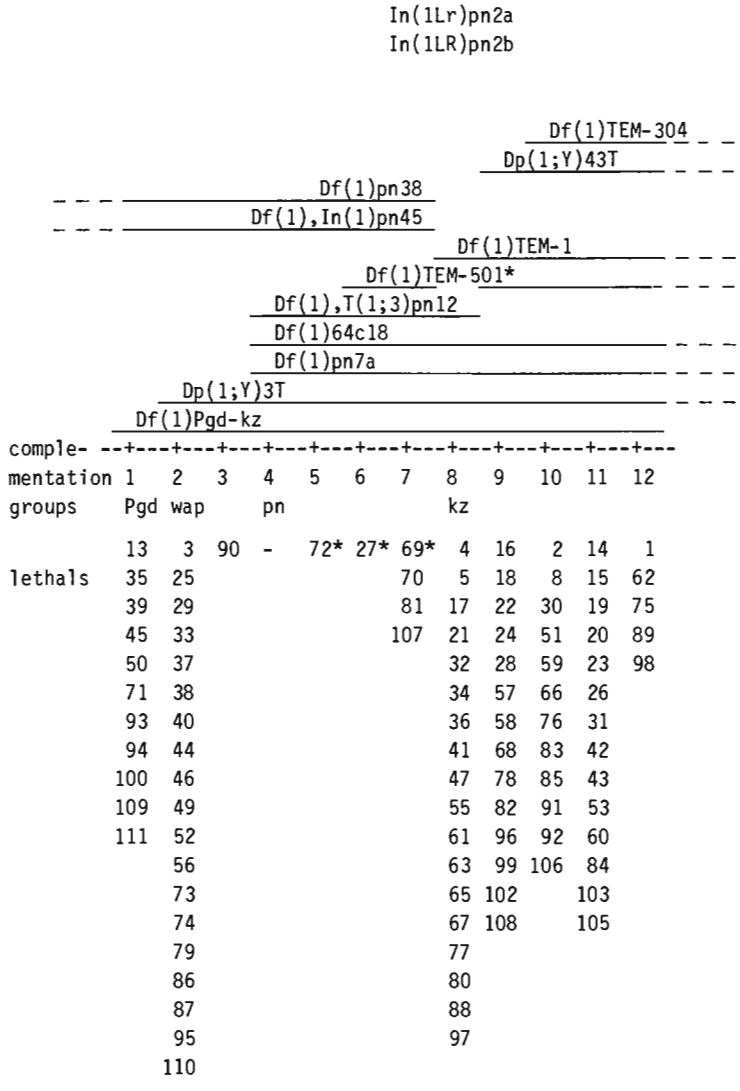

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Figure 1. Fine genetic map of the 2D3-2F5 region. The complementation group arrangement is oriented from the distal to the proximal end of the X-chromosome. Straight lines above the complementation group numbers indicate the absence of complementation, for both deletions and duplications. Asterisks (*) mark lost lethals and rearrangement. Arcs connect the complementation groups not localized in relation to each other.

Alexandrov, I.D. Research Institute of Medical Radiology, Obninsk, USSR. Modification of radiation-induced rates of intra- and intergenic changes at the yellow locus of **Drosophila melanogaster** by the genotype, caffeine, actinomycin-D and radiation quality.

As a part of the larger project on radiation genetics of the specific loci, 98 radiation-induced visible yellow mutations have been discovered in different experiments designed for estimating the effects of various biological, chemical and/or physical variables on the relative proportion of intragenic versus intergenic changes induced by the Low- or High-LET radiation at the locus in question. All the yellow mutations have at first been classified by dint of genetical analysis into 3 main and regularly occurring mutant types (Alexandrov 1984): (1) sterile F_1 visibles (SV), (2) transmissible visibles with recessive lethality inseparable from the yellow phenotype (LV), and (3) transmissible and viable in homo- or hemizygote visibles (VV). According to the data obtained, 17 out of 98 mutations scored were fully SV and 18 out of 81 were LV. Polytene chromosome analysis of 58 VV preserved was carried out and the number of VV associated (VV^{ch}) as well as unassociated (VV^{g}) with chromosome alterations was detected (see results in this issue elsewhere, Alexandrov et al.).

Consummation of this research has made it possible now to estimate the relative proportion of intra-locus changes (VV^{g}) versus all intergenic (that is chromosome SV, LV and VV^{ch}) alterations scored as yellow mutations after action of the variables studied (Table 1). As seen, VV^{g} arise nearly as frequent as chromosome changes in the wildtype (D-32, D-18) male germ cells (the post-meiotic stages as a whole)

TABLE 1.

Conditions of experiment (radiation, dose, modifier used, genotype of male treated, No. of F_1 progeny scored	Number of mutations							
		Chromosome changes:						
	VV^{g}	SV	LV	VV^{ch} Deletions	VV^{ch} Inversions	VV^{ch} Translocations	Lost before analysis	Total a.m.f.
1. gamma-rays, 40 Gy, D-32 and D-18, No.= 177716								
	20	8	4	5	4	2		
[a.m.f.] =	[2.8]			[3.2]				[6.0]
2. Caffeine (0.2%) + gamma-rays, 40 Gy, D-32, No.= 46647								
	5	1	2	2	1		1	
[a.m.f.] =	[2.7]			[3.2]				[6.4]
3. Actinomycin-D [100µg/ml) + gamma-rays, 40 Gy, D-32, No.= 24508								
	0	2	5	4	1		1	
[a.m.f.] =	[0.0]			[12.2]				[13.2]
4. gamma-rays, 40 Gy c(3)G, No.= 56109								
	1	3	5	3		1	3	
[a.m.f.] =	[0.42]			[5.3]				[7.1]
5. 0.35-0.85 MeV fission neutrons, 10 Gy D-32, No.= 51078								
	1.9	3	1	3		1		
[a.m.f.] =	[1.9]			[15.7]				[17.6]
6. ^{252}Cf, 14 Gy D-32, No.= 14682								
	3		1					
[a.m.f.] =	[14.6]			[4.8]				[19.4]
7. 0.85 MeV fission neutron, 10 Gy + gamma-rays, 10 Gy, D-32, No.= 6814								
	1							
[a.m.f.] =	[7.3]							

a.m.f. = Average mutation frequency, locus/r x 10^{-8}.

after gamma-irradiation. Pre-treatment of the D-32 males with caffeine does not modify this picture. However, pre-treatment with actinomycin-D (other things being equal) significantly enhances the occurring of the chromosome yellow mutations and reduces the yield of the gene ones. It would appear that the transformation of the pre-mutational lesions into chromosomal damages and less effective repair of the latter have both simultaneously taken place. These taken together (our own data and the well-known fact that actinomycin-D is bound with GC-repeats of DNA) argue that the chromosome changes, except for VV^g, are initiated by the damages of the repetitive DNA sequences surrounding and/or interspersing the functional genetic unit. Therefore, as it follows from our own experimental data with neutrons (see Table), the quality (mode and perhaps density) of initial lesions predetermines the occurrence of rearrangements also. Thus both factors (i.e., the feature of the initial lesions determined by the radiation quality and the nature of the primarily damaged DNA sequences) appear to be decisive in the processing of the radiation-induced chromosome changes of all kinds.

However, the modifying effect of some other variables studied prove to be new and unpredictable. In particular, the combined irradiation by neutrons and gamma-rays (consecutive or simultaneous in the case of ^{252}Cf) increases the yield of VV^g, but not a chromosome alteration. On the other hand, in the gamma-irradiated c(3)G post-meiotic male germ cells (repair-deficient mutant as proposed by Watson 1972), there was a very marked decrease in the frequency of the VV^g in comparison with that in the gamma-irradiated wild-type germ cells.

One obvious question that needs to be answered is whether these variatioins in the specific-locus radiomutability are conditioned by the variables as such or are due to the feature of the locus studied. To answer this question, the spectrum and frequency of visibles for another loci will need to be studied under the same experimental conditions. Such genetical and cytogenetical analysis of the white, black and cinnabar mutations scored simultaneously with yellow ones is in progress now.

Acknowledgement: I am grateful to Dr. G.M. Obaturov, Head of the Department of Medical Physics, for his help and encouragement. My thanks also to my colleagues: Mrs. M.V. Alexandrova, Research Assistant (Cytogenetics); Mrs. N.I. Kosigina, Curator of Stocks; Obninsk 249020: Research Institute of Medical Radiology, Laboratory of Neutron Radiology, Komarova st., 4, USSR.

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Andrade, C.A.C. and A.P. Gupta. Instituto de Biologia da UFRJ, Rio de Janeiro, Brazil.

Studies on bristle number in hybrids between strains of **D.capricorni** from Brazil.

Drosophila capricorni is related to the "willistoni" species group but distinguishable from each other in externally visible characters (Dobzhansky 1951). It usually prefers cooler and humid regions. In Brasil, it extends from northern part to the southern most state. It has rarely been found in the summer while it occurs in abundance during the winter season (pers. comm. from Prof. A.R. Cordeiro), and was confirmed partly by our data.

The object of the present research work was to examine the variance of bristle number in parental, F_1's and F_2 classes. For this purpose, several strains of **D.capricorni** were collected in July 1978 and February 1979 from Itatiaia (Resende, RJ), and were maintained in the laboratory as isofemale lines. A total of 13 strains (six from July 1978 and seven from the year 1979; these lines were maintained at 18°C for 540 and 980 days, respectively, before the commencement of the investigation) were utilized for the research work. Crosses in various combinations between strains (within and between the year of collection) were made to yield F_1 and F_2 classes. A total of 60 parental and 60 F_1's; 60 parental and 60 F_2 classes were analyzed at each of the two temperatures: 18° and 25°C. The parental and F_1's were placed simultaneously at each temperature, using 50 eggs for each of the five or more replicates. Similar procedure was followed for the parental and F_2 classes. The bristle number on 4th and 5th sternites, and the left and right esternopleurals were counted on the same individual in both sexes for each of the parental, F_1 and F_2 classes. In general, 8-10 males and 8-10 females from each of the three to six replicates for each class were examined. (However, there were cases where the sample size was very small which did not alter the final results.) The variance of the difference in bristle number between 4th and 5th sternites, and between left-right esternopleurals was computed. It was observed that the F_1's or F_2's had either equal, greater or smaller value of the variance when compared with their parental classes. However, in general, no significant difference in variance between parental and F_1's, and between parental and F_2's was observed for each sex at each temperature. Thus, these results indicated that the F_1's and F_2's were developmentally as buffered (stable/homeostatic) as their parental classes for the number of bristles examined even though the strains utilized in this experiment were maintained for the two different periods.

In addition, we looked for the pattern for frequency for the phenotypic variance. For this purpose, the variance values for the difference in esternopleural bristle number were grouped into three class intervals: 0-1, 1-2, and >2, for parental, F_1 and F_2 classes for each sex at each temperature. At each temperature the major part of the variance values in both the sexes occurred in the central class (1-2). The frequency in the case of the females grouped in the class greater than two was larger when compared with those of the males at either temperature. On comparing the class interval >2 between temperatures, a slight increase at 18° was observed than at 25°C for each sex. However, no specific pattern on this aspect could be noticed in the case of the crosses made between the two periods of collection. Comparison of the parental with F_1's yielded almost homogeneity for the frequencies in each of the three classes of variance. Overall, F_2's grouped in the central class, 1-2, were in larger number when compared with those of F_1's for each sex at 18°C. No consistent pattern for such a comparison was observed at 25°C.

Likewise, the values of the variance for the difference in bristle number between the two sternites were also classified, into three classes: 2-6, 6-10, and >10, in order to search for pattern. At 25°, the majority of the phenotypic variance for the parental, F_1's and F_2's occurred in the central class (6-10) for each sex. While at 18°, such a frequency was spread into two classes (2-6 and 6-10), indicating a reduced number of phenotypic variances in the class greater than 10 at this temperature. No concludable pattern was observed for the comparison of F_1's or F_2's with their parental classes. The other aspects are under investigation.

This work was supported by Research Grants from CNPq (Proc. 40.0312/79 and 40.2525/81) and FUJB (UFRJ) awarded to Prof. Anand Prakash Gupta. We thank Stavna Uchoa for typing the manuscript.

Reference: Dobzhansky, Th. 1951, in:Genetics and the Origin of Species, 3d ed rev, Columbia Univ, New York.

Andrews, K. and C. Chihara. University of San Francisco, California, USNA. An overproducer of a third instar urea-soluble cuticle protein in **Drosophila melanogaster.**

Third instar larvae of **Drosophila melanogaster** show a standard electrophoretic pattern for the urea-soluble cuticular proteins (Fristrom et al. 1978; Chihara et al. 1982) (Figure 1, lanes 1 and 4). The bands are designated from slowest (top) to fastest (bottom) as L_3CP-1, 2, 2a, 3, 4, 5, 6, 7, 8, respectively.

The overproducer variant of L_3CP-5 (named L_3CP5^{op}) was isolated from an Austin Species Center stock acquired from Kuala Lumpur, Malaysia. Several rounds of pair matings produced a stock which gave consistently dark bands at the position of protein 5, (125 samples). The intensity of the variant L_3CP-5 was checked against wild type using a gel scanner with integrator. The density of the L_3CP-5 band relative to the L_3CP-4 band of both stocks was compared and the following ratios were obtained (Figure 1):

5^{op}: Mean density ratio of Bands 5:4 = 1.95(±0.65):1 ;n = 67
+/+: " " " " " = 0.97(±0.13):1 ;n = 7

The L_3CP5^{op} variant is at least partially dominant. When it was crossed to wild type the 5 band remained visibly darker than the wild type. This can also be seen in the crosses described below.

To make sure the darkened 5 band was due to overproduction of L_3CP-5 and not to the introduction of a new protein with the same electrophoretic mobility, the L_3CP5^{op} variant was crossed with, and then backcrossed to, a recessive mutant known as omega. Omega is a putative processing gene whose product, when inactive, results in the non-processing of L_3CP-5; as a result, L_3CP-5 migrates much more slowly than wild type L_3CP-5. This slow band is designated as the omega band (Figure 1, lanes 2 and 3).

Density comparisons between the L_3CP-omega band and L_3CP-4 were made as above (Fig. 2).

Omega/omega: Mean density ratio of omega:4 = 1.04:1, with a high value of 1.40 and a low of 0.84. This is comparable to the values for +/+, bands 5:4 shown above.

5^{op} omega/5^{+} omega: Mean density ratio of Omega:4 = 2.4:1, with a high of 3. and a low of 1.83 (Fig. 1, lane 5).

Wild type omega recombinant: Mean density ratio of Omega:4= 1.25:1, with a high of 1.67 and a low of 0.83.

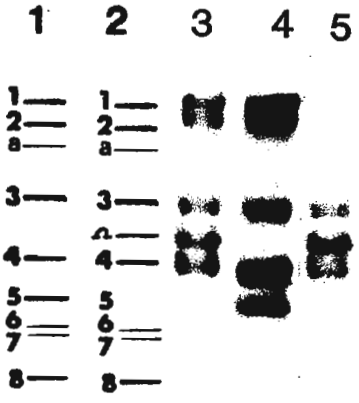

Figure 1. **Lane 1:** Schematic wild type banding pattern with numbers. **Lane 2:** Schematic omega homozygote pattern. **Lane 3:** Homozygous omega with wild type for gene 5. **Lane 4:** Typical wild pattern. **Lane 5:** L_3CP5^{op} heterozygote with homozygous omega, note dark omega band.

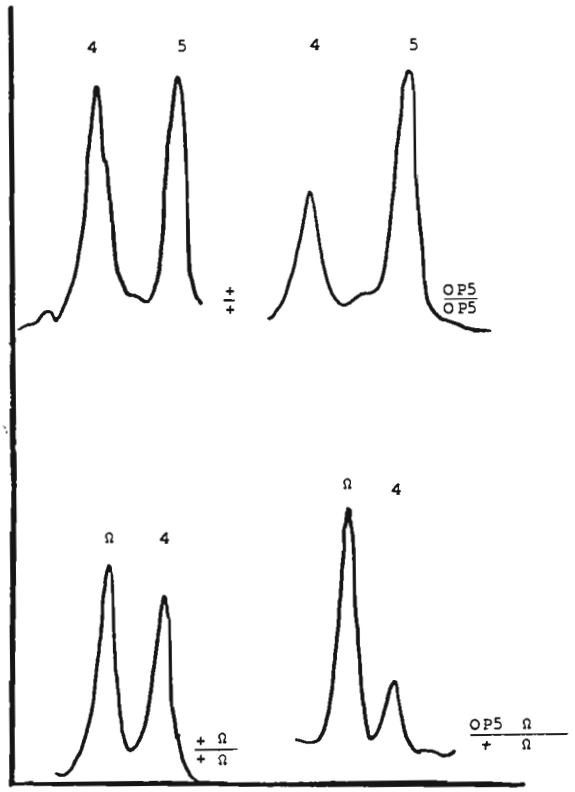

TABLE 1. Schema for the production of L_3CP5^{op} - Omega recombinants with results of the cross.

$$\frac{5^{op}}{5^{op}} \times \frac{omega}{omega} \text{ ------> } \frac{5^{op} \quad +}{+ \quad omega} \times \frac{+ \quad omega}{+ \quad omega}$$

		phenotype	#
Parentals -	$\frac{5^{op} \; +}{+ \; omega}$ ------>	5^{op}	20
	$\frac{+ \; omega}{+ \; omega}$ ------>	omega	19
Crossovers-	$\frac{5^{op} \; omega}{+ \quad omega}$ ------>	5^{op} omega	6
	$\frac{+ \quad +}{+ \; omega}$ ------>	+	1

Figure 2. Densitometer tracings as described in text.

While the numbers of recombinants are small they are consistent with an estimate of at least 15 map units between the omega and 5^{op} genes (Table 1). The gene for L_3CP-5 maps also maps at least 15 map units away from the Omega gene (Chihara & Kimbrell, in manuscript). The data are consistent with the L_3CP5^{op} locus being very close to the L_3CP5 gene.

This work was supported by NSF grant #PCM 8119181 to CJC.

References: Fristrom, Hall & Watt 1978, Biochem 19:3917; Chihara, Silvert & Fristrom 1982, Dev.Biol. 89:379.

Barker, J.S.F. and D.K. Fredline. University of New England, Armidale, Australia.

Reproductive biology of **Drosophila buzzatii.**

Drosophila buzzatii and other cactophilic Drosophila provide a valuable model system for studies in population and evolutionary genetics (Barker & Starmer 1982), because they are specific to the cactus niche, feeding and breeding in rotting arms, fruits and cladodes of various cactus species. Studies of **D.buzzatii** in Australia have concentrated on allozyme polymorphism and factors affecting genetic variation at the allozyme loci. As a basis for study of the fitness of genotypes at allozyme loci, three preliminary experiments were done to provide data on the reproductive biology of the species.

In the first experiment, time to copulation, copulation duration, time from copulation to first egg and female fecundity in the 6.5 days after copulation were recorded. The flies used derived from 100 females collected at Breeza, N.S.W., and were generation 3 of mass culture in the laboratory. The medium used for culture and in all experiments reported here was autoclaved cactus-yeast-sucrose-agar. Using previously collected and stored (at 25°C) virgin flies, 82 pairs were set up (without etherization) over a 2.5 hr period (1400-1630 hr) at 20°C in empty 7.5 x 2.5 cm vials. All females were 30-48 hr old at initiation of these pair meetings, while males in the first 48 were 9 days old and in the last 34, 5 days old. Thirty-four of the 82 pairs had copulated by 1705 hr, when observation ceased. Because of the longer average observation period for 9-day old males, male ages were compared for proportion copulating in 30 min or in 60 min. Neither difference was significant, and the overall proportions were 0.207 and 0.244, respectively. Copulation duration ranged from 44 to 236 seconds. Again age of males had no significant effect and the overall average was 138.2±47.2 seconds.

Table 1. Egg production data for **D.buzzatii** females after an observed first copula.

Time from mean copula (days)	Proportions of females: Laying in this period	Laying for first time	Cumulative first laying	Mean no. eggs/ female	
0.08	0.03	0.03	0.03	0.62	6.88
0.58	0.41	0.38	0.41	6.26	
1.08	0.76	0.38	0.79	12.74	22.95
1.58	0.47	0.00	0.79	10.21	
2.08	0.76	0.12	0.91	37.15	47.15
2.58	0.50	0.03	0.94	10.00	
3.58	0.94	0.03	0.97	51.38	
4.58	0.91	0.00	0.97	50.97	
5.58	0.94	0.03	1.00	62.59	
6.58	0.91	0.00	1.00	48.32	

At the end of the observation period, small plastic spoons with medium and live **Saccharomyces cerevisiae** were added to each vial of the pairs that had copulated, and the pairs kept subsequently at 25°C. At 2000 hr, the flies were transferred to clean vials with fresh spoons. This was repeated at 12 hr intervals for 2.5 days, and then at 24 hr intervals for a further 4 days. The numbers of eggs on each spoon were counted after removal from the vials, and the spoons kept in plastic boxes at 25°C for 48 hr, when the numbers of unhatched eggs were counted.

With 1600 hr as the mean time of copulation, and taking 1800 hr and 0600 hr as mean egg laying times, egg production results (Table 1) are given for each period from the mean time of copulation. The average time from copulation to first egg (again male ages not significantly different) was 1.23±1.05 days. Delayed initiation of egg laying (up to 5.58 days) may have been due to an unsuccessful first copula, but with a later re-mating. The average total number of eggs per female for the 6.5 day period was 289.3±128.7, with a range of 23-449. Hatchability did not differ among egg collection periods, and equalled 99.6% of all eggs laid.

Markow (1982) has described sexual dimorphism for age at reproductive maturity in **D.mojavensis** and other cactophilic Drosophila species, with females reaching maturity some days earlier than males. As no data were available for **D.buzzatii**, the second experiment was set up to estimate age at reproductive maturity for each sex, indirectly as age at first egg-laying, by pairing young females with older males and vice versa. The flies used were again from the Breeza population at generation 3 of laboratory culture. Older flies, 5 days old when pair matings were set up, had been collected as virgins (< 22 hr old) under ether anaesthesia and kept at 25°C. Young flies, emerged during a 12 hr period (2000 hr - 0800 hr), were transferred without etherization to fresh media vials. From 1100-1200 on the same day, single pair matings were set up without etherization, in vials with egg-laying spoons containing cactus-yeast-sucrose-agar

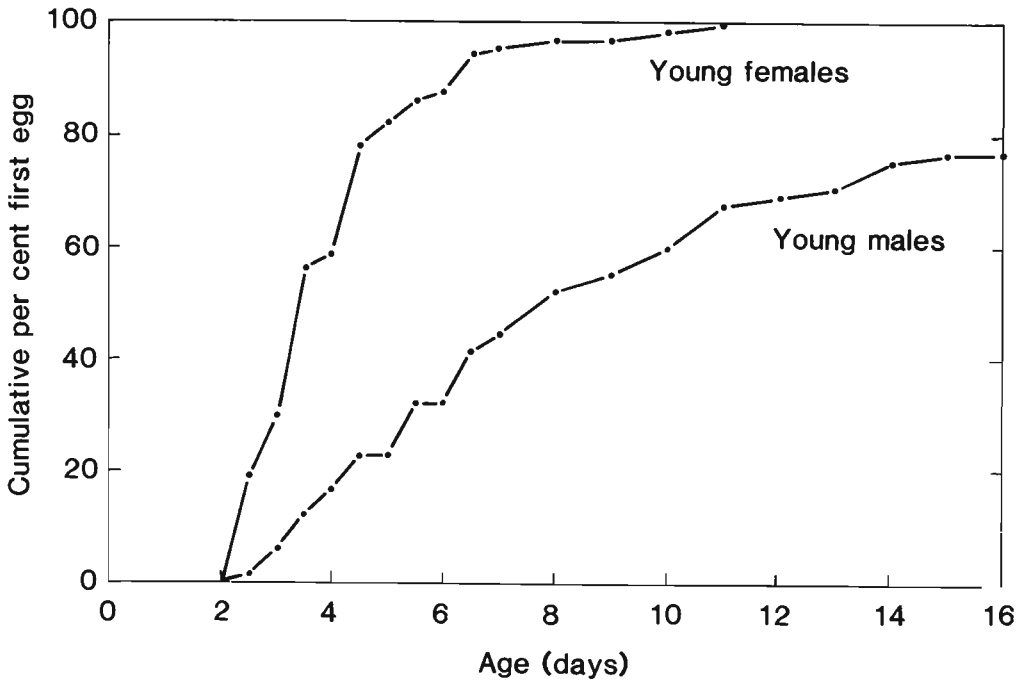

Figure 1. Cumulative percentage of first egg laying for young females paired with older males, and for young males paired with older females.

medium and live **S.cerevisiae:** 78 5-day old ♂ x young ♀, and 68 young ♂ x 5-day old ♀. At 2000-2100 hr that day, then every 12 hr for 7 days and subsequently every 24 hr, the pairs were transferred to clean vials with fresh spoons. After each transfer, spoons were checked for eggs. Where eggs had been laid, the numbers were counted and spoons kept as above to check hatchability. Pairs were discarded as they completed 3-4 days egg laying with normal hatchability. A number of vials were not included in the data set because of death of one of the pair, or loss of a fly at spoon transfer.

For six females, none of the first eggs laid hatched. These were assumed to be virgin eggs and were not counted as first egg-laying. Four of these females subsequently laid normally hatching eggs but no more eggs were laid by the other two.

Results were obtained for 73 young females paired with older males, and all had commenced egg laying by 11 days of age (Figure 1). The average age at first egg was 4.14±1.67 days.

For the young males with older females, only 77% of the pairs (50 of 65 possible) had produced eggs by the time the males were 16 days old (Figure 1). The average age of these 50 males at the first egg of their paired females was 7.33±3.41 days. The remaining 15 pairs that had produced no eggs were separated, paired with new 7-day old virgins, and kept until eggs were produced or until death (with virgin replacement where necessary). Eggs were produced by the females paired with nine of the 15 (originally) young males, but only three of the 15 (originally) older females produced eggs when paired with fresh males (and two of these laid only one egg).

These results show that male **D.buzzatii** reach reproductive maturity at a later age than females, but some of the apparent delay for males must be ascribed to continued rejection of younger males by older females. With this test system of continuous pairing and no choice, familiarity breeds contempt. Allowing for the estimated mean time from copulation to first egg (1.23 days), the average age of females at insemination was 3 days, while the average age at which males first successfully mated was at least 6 days).

The third experiment estimated lifetime fecundity and mortality schedules of flies maintained on two yeast species, viz. **S.cerevisiae** and **Pichia cactophila** (one of the two most common yeast species isolated from cactus rots in Australia: Barker, East, Phaff & Miranda 1984). The flies tested were first generation progeny of some 100 wild caught females from Trinkey, N.S.W. Pair matings were set up in

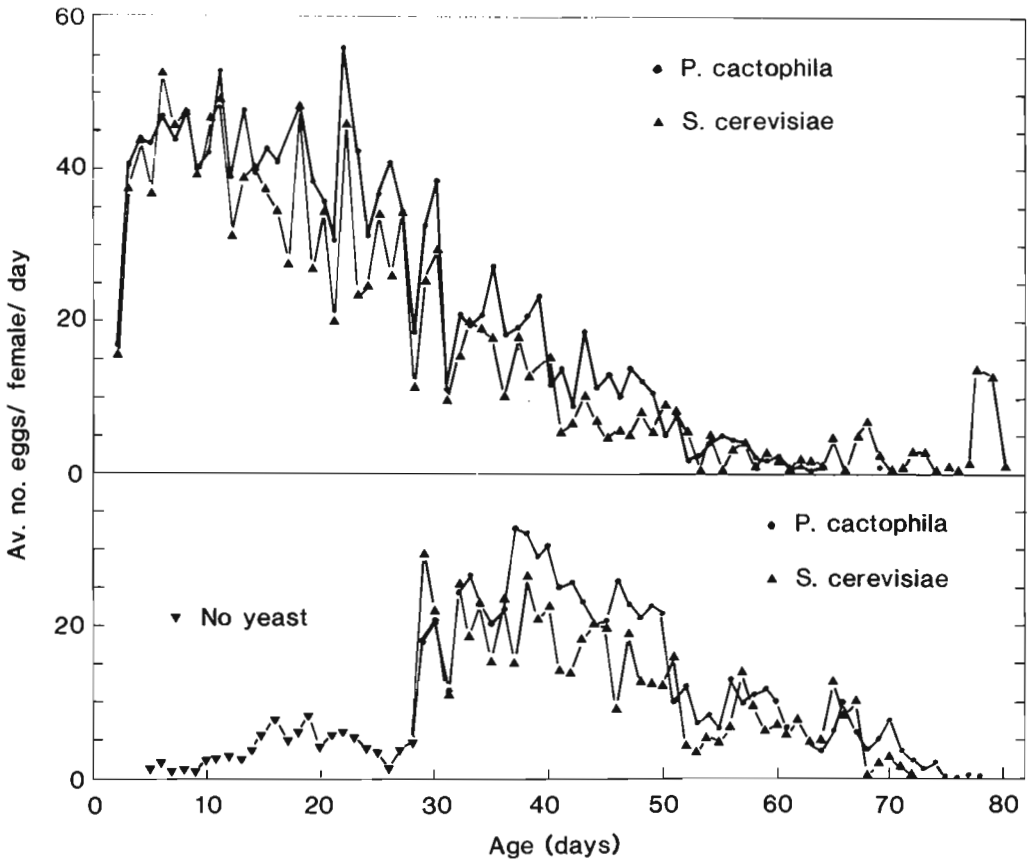

Figure 2. Fecundity schedules for females maintained on (1) **P.cactophila**, (2) **S.cerevisiae**, and (3) no yeast to 28 days of age, and then on **P.cactophila** or **S.cerevisiae.**

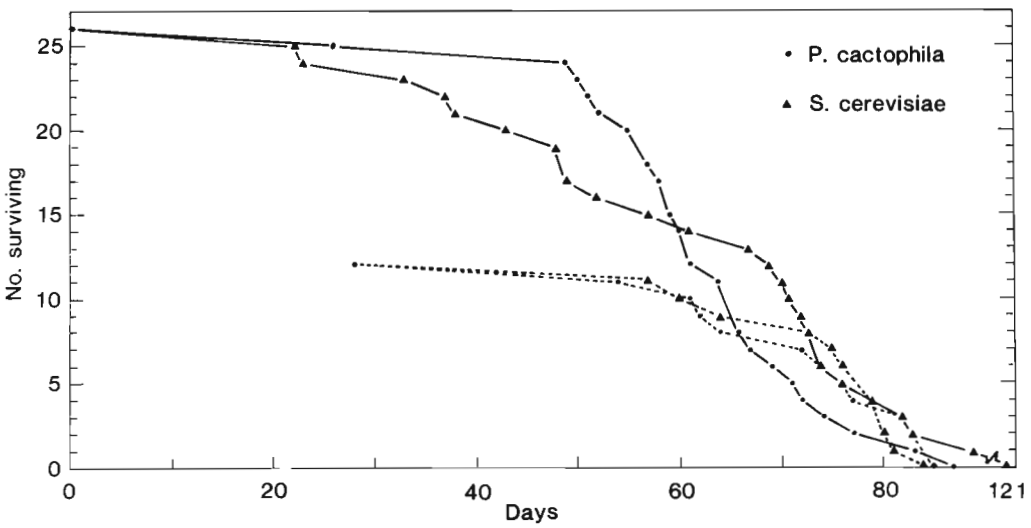

Figure 3. Numbers surviving for females maintained on (1) **P.cactophila**, (2) **S.cerevisiae**, and (3) no yeast to 28 days of age, and then on **P.cactophila** or **S.cerevisiae** (broken lines).

Table 2. Average number of eggs per initial female (standard deviation), cumulated to various ages, and to death of all females.

	Average eggs per female to ages:					
Treatment	9 days	16 days	23 days	30 days	37 days	TOTAL
P.cactophila	329 (133)	631 (254)	926 (368)	1157 (469)	1271 (530)	1455 (608)
S.cerevisiae	315 (164)	548 (306)	769 (449)	938 (531)	1035 (582)	1147 (649)

	28 days	35 days	42 days	
no yeast/ **P.cactophila**	100 (97)	252 (191)	434 (343)	712 (581)
no yeast/ **S.cerevisiae**		204 (176)	319 (286)	525 (538)

vials with egg-laying spoons as before, with the members of each pair deriving from different isofemale lines, but with approximately equal representation of the isofemale lines in each of three treatments: (1) **S.cerevisiae**; (2) **P.cactophila**; (3) no yeast. Twenty-six pairs were set up for each treatment, with males 3-7 days old and females 0-24 hr old. Spoons were changed daily and eggs counted. Average fecundity per day was calculated only for those pairs where both were still alive. Females on **P.cactophila** maintained early peak fecundity for longer and had higher total fecundity than those on **S.cerevisiae**, although the latter maintained egg production for longer (Figure 2 and Table 2).

The females maintained without yeast had very low fecundity (Figure 2), indicating that yeast feeding is almost essential for egg production. Egg numbers for the first 4 days are not shown in Figure 2, as the spoons for this treatment were inadvertently not changed until day 4. In this period, about half the females had laid, and the average number of eggs for all females was 25.2. Apparently some females had fed on yeast before being set in the pair matings. In the next 7 days, samples of spoons where eggs were laid were retained to check for yeast growth and hatchability of eggs. No yeast was detected and hatchability was normal. On day 28, the pairs of this treatment were separated in two groups,one subsequently maintained on **P.cactophila** and the other on **S.cerevisiae**. The numbers of eggs laid increased immediately, with average numbers/female/day higher than for females of the same age maintained throughout on live yeast. One or two females in each of the four groups laid no eggs, and the highest total egg numbers recorded were 2608 for **P.cactophila**, 2077 for **S.cerevisiae**, 1478 for no yeast/**P.cactophila** and 1716 for no yeast/**S.cerevisiae.**

The mortality schedules were determined for all females in each treatment. When a male died, he was not replaced, but the female was maintained until death. Survival curves for each treatment (Figure 3) show that survival on **P.cactophila** was very high to 50 days of age and then decreased rapidly, while on **S.cerevisiae**, the numbers surviving decreased more evenly from 20 days of age. For the no yeast treatment, one female died at seven days and one at 25 days, and the survival curves for the 12 females put onto each yeast at 28 days of age are very similar. The mean ages at death were 62.2±12.2 for **P.cactophila**, 61.9±22.0 for **S.cerevisiae**, 64.8±23.1 for no yeast/**P.cactophila** and 65.7±22.9 for no yeast/**S.cerevisiae.**

Although there are no differences in mean lifespan between females maintained on the two yeast species, the fitness of **D.buzzatii** is higher on the cactophilic yeast, at least in terms of higher initial average fecundity and survival. Comparison of these fitness components for flies maintained on other naturally occurring cactophilic yeasts would be of interest.

Work supported by a grant from the Australian Research Grants Scheme. We are grateful to Tom Starmer for assistance with copulation observation, Darren Schafer for egg counting and Jenny Druitt, Annette Royal and Chris Leger for patient preparation of thousands of egg-laying spoons.

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Bel, Y. and J. Ferré. University of Valencia, Spain. Regulation of eye-pigment metabolism in **Drosophila melanogaster:** effect of high doses of aromatic amino acids in the diet.

It has been reported that the "in vivo" hydroxylation of aromatic amino acids (phenylalanine, tyrosine and tryptophan) requires 5,6,7,8-tetrahydrobiopterin, a pteridine derivative. In **Drosophila melanogaster**, the synthesis of this cofactor shares some metabolic steps with the synthesis of the red eye-pigments (also pteridine derivates). For this reason we considered it interesting to see how the pteridine pathway responded to high doses of aromatic amino acids in the diet. Since tryptophan is a precursor of xanthommatin (brown pigment of **Drosophila** eyes), metabolites of this pathway were also analyzed.

Increasing doses of L-tyrosine, L-tryptophan and L-phenylalanine were given to the larvae till the moment of pupation. Pupae were transferred to new non-supplemented media and 9-day old adults analyzed for eye-pigment metabolites.

Viability was found diminished when phenylalanine was used (21 adults per 100 eggs with the highest load), whereas no change in viability was found in flies fed with similar doses of tyrosine and tryptophan. Tyrosine was also found not to affect developmental time. Tryptophan and phenylalanine delayed pupation and eclosion times. Phenylalanine caused longer delays and lower synchronization of the eclosion times.

Pteridines were measured in extracts of wild type flies raised in media supplemented with the different amino acids. Table 1 summarizes the results obtained with the highest loads (266.7 mg/ml of food). Regarding xanthommatin metabolites, xanthurenic acid increased when flies were fed with tryptophan and decreased in flies fed with phenylalanine. Xanthurenic acid 8-O-glucoside and kynurenic acid also appeared in chromatograms of flies fed with tryptophan (these two metabolites are not detected in chromatograms of the wild type raised in standard media). No change was found using tyrosine. Xanthommatin biosynthesis was already known to be enhanced when tryptophan was added to the medium, and inhibited when phenylalanine and tyrosine were used instead (Puckett & Petty 1980).

Table 1. Levels of pteridines in flies raised in media supplemented with different amino acids (266.7 mg/ml of food). "Drosopterins" estimation was carried out after selective extraction in acidified ethyl alcohol (Real et al. 1985). The other pteridines were estimated after thin-layer chromatography on cellulose. + = like in the non-supplemented control; 1- = diminished; 1+ = increased; values in parenthesis need further confirmation.

	Supplemented amino acid		
Pteridines	L-Tyrosine	L-Tryptophan	L-Phenylalanine
"Drosopterins"	(+)	1-	1-
Isoxanthopterin	+	1-	1-
H_2-Biopterin	1+	(1+)	1-
Biopterin	1+	+	1-
Pterin	1+	+	?
Sepiapterin	1+	+	1-
Acetyldihydrohomopterin	+	+	1-

The decrease of pteridine and xanthommatin biosynthesis in flies raised on phenylalanine media seems to be a consequence of a general toxic effect of this amino acid on the development of the insect. However it is worth noting that a blue fluorescent spot (probably a pteridine) appears overlapping with pterin in chromatograms of flies raised in phenylalanine media. The accumulation of this metabolite could be the result of a specific response of the pteridine pathway to phenylalanine loads. This possibility is currently being investigated in our laboratory.

References: Puckett, L. & K. Petty 1980, Biochem. Genet. 18:1221-1228; Real, M.D., J. Ferre & J.L. Mensua 1985, DIS 61 (this issue).

Belote, J. and M.McKeown. University of California at San Diego, LaJolla, USNA.

Post-replicative repair of an X-ray damaged chromosome following fertilization in **Drosophila melanogaster.**

During the course of an X-ray mutagenesis screen designed to find mutations in the scarlet (st: 3-44.0) region, we recovered a chromosome rearrangement whose properties suggested the occurrence of an unusual mutational event. In that screen, males of the genotype Ki roe p^p were given an X-ray dose of 4000r and mass mated to st^{82c3} e virgin females. After three days the bottles were cleared of parents, and the resulting progeny were subsequently scored for scarlet-eyed flies. Among the mutations obtained was an allele, st^{g18}, recovered in a scarlet-eyed F_1 male. This male, when mated to tester females carrying a deficiency for the scarlet region, $Df(3L)st^{81K17(2)}$ (73A2-73D1·2), balanced over TM6b, Hu Tb e ca, yielded no st Ki progeny. The lack of st Ki flies among the F_2 progeny could mean that the irradiated Ki roe p^p chromosome, in addition to being mutant for st, is also mutant for an essential gene (or genes) that is not complemented by the $Df(3L)st^{81K17(2)}$ chromosome (for example, st^{g18} could be a deficiency that deletes not only st^+ but also other essential loci in the 73A2-73D1·2 region). The appearance of st^+ Ki progeny in the F_2 suggested that the F_1 male was a $st^-//st^+$ mosaic in which the eyes were st^- and at least part of the germ line was st^+.

While the production of mosaic flies from X-irradiated sperm is not that uncommon an occurrence, our cytogenetic observations on the st^{+g18} chromosome indicated that the mutational event giving rise to the $st^{g18}//st^{+g18}$ F_1 male was, indeed, unusual. Salivary gland chromosome squashes were prepared from $st^{+g18}/+$ larvae. Figure 1a shows the bizarre pairing configuration that was observed in the 72E-75A region. Chromosome spreads in which the homologues were well-stretched and asynapsed revealed that this rearrangement is an inversion of region 72E1·2 to 74F4-75A1 that is tandemly duplicated. Since mature sperm (i.e., haploid) had been irradiated, the insertion of this duplicated material must have occurred after zygotic chromosome replication and, thus, could be related to the same event that generated the st^- tissue in the F_1 mosaic male. This explanation is consistent with evidence demonstrating that chromosome breaks induced in spermatozoa do not rejoin before fertilization (Muller 1940; Helfer 1940; Kaufmann 1941). Under our hypothesis the material duplicated in one chromatid was donated at the expense of the other chromatid, implying that the genotype of the recovered mosaic male was:

$$Dp(3L)st^{+g18}/st^{82c3}\ e//Df(3L)st^{g18}/st^{82c3}\ e\ .$$

The existence of this deficiency chromosome was proven by the subsequent recovery of st^- chromosomes from some of the Ki Hu Tb flies that were saved from the F_2 generation. The F_1 mosaic male must have therefore had a $st^-//st^+$ mosaic germline. Cytological analysis showed that this st^{g18} chromosome is deleted for the same region (72E1·2 to 74F4-7A1) that is tandemly duplicated in $Dp(3L)st^{+g18}$ (Figure 1b).

The induction in mature sperm and recovery of both a deficiency and the complementary duplication chromosomes from the same individual can be explained by the occurrence of post-replicative repair of a broken chromosome in the zygote (Figure 2). In this case, the four broken ends were ligated back together in the

Figure 1. Photomicrographs of orcein-stained salivary gland chromosomes from: (a) $Dp(3;3)\ st^{+g18}/+$, and (b) $Df(3L)\ st^{g18}/+$ larvae. Arrows point to the 72E-75A region of chromosome arm 3L.

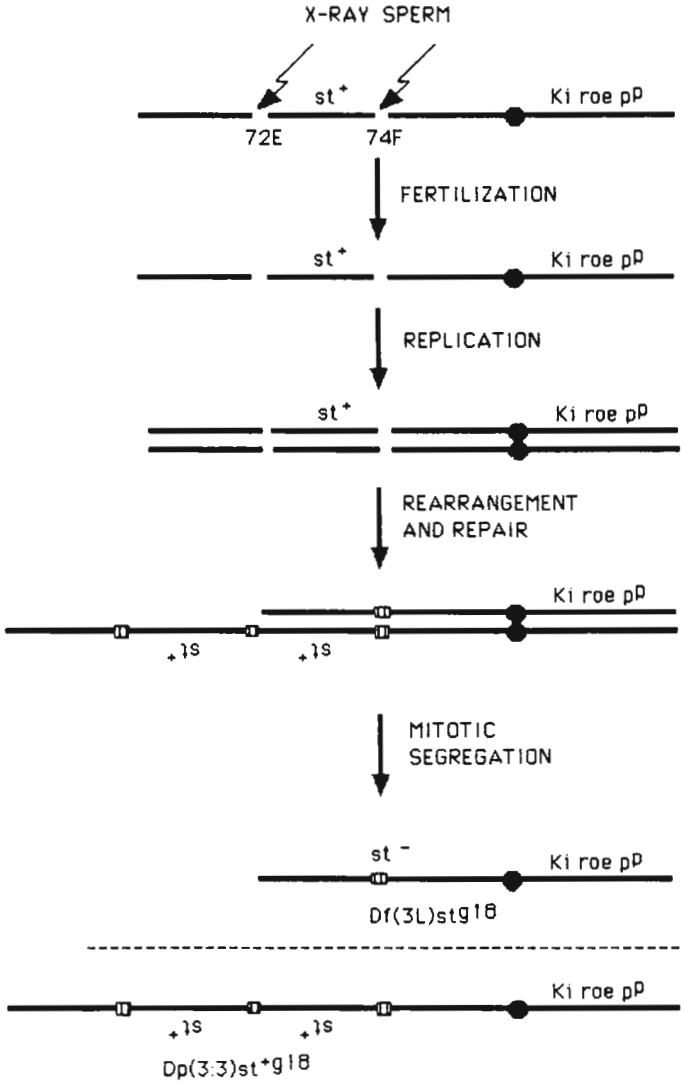


Figure 2. Model for the generation of the duplication and deficiency chromosomes shown in Figure 1.

wrong order, resulting in one chromatid with an inverted tandem duplication, and another chromatid with a deletion. Segregation of these sister chromatids at mitotic anaphase resulted in an individual that was mosaic for these chromosome rearrangements. The occurrence of post-replicative repair is also evident from the observation that the Df(3L)st^{g18} chromosome carries an inversion between regions 65A1·2 and 99A1·2, whereas the Dp(3L)st^{+g18} chromosome carries no such inversion (data not shown).

A similar interpretation invoking chromatid exchange occurring after replication in the zygote was proposed in 1969 by Leigh & Sobels (cited in Sankaranarayanan & Sobels 1976) to explain their recovery of homo-isochromosomes following irradiation of post-mitotic male germ cells.

Flies homozygous for the Dp(3L)st^{+g18} chromosome can survive to the adult stage, although their viability is low. The observation that these flies exhibit normal sexual phenotypes and are fertile is noteworthy, since they should carry four wild-type doses of the transformer (tra, 3-45) locus, a sex determination regulatory gene whose function is required in females, but not in males, for normal sexual development (Sturtevant 1945; Baker & Ridge 1980).

Acknowledgements: The Df(3L)st$^{81K17(2)}$ and st^{82c3} mutations were provided by Dr. M.M. Green. This work was supported by a Helen Hay Whitney Fellowship to M.M. and by research grants from NSF (PCM-8202812) and NIH (GM07199).

References: Helfer, R.G. 1940, PNAS 26:3; Kaufmann, B.P. 1941, PNAS 27:18; Muller, H.J. 1940, J. Genet. 40:1; Sankaranarayanan, K. & F. Sobels 1976, in:The Genetics and Biology of Drosophila, v1c:1089-1250; Sturtevant, A.H. 1945, Genetics 30:297; Baker, B.S. & K. Ridge 1980, Genetics 94:383.

Bierniaux, C., J. Lechien, E. Depiereux and A. Elens. F.N.D.P., Namur, Belgium.

Temperature and efficiency of a disruptive selection for phototactism.

In a previous paper (Dubucq et al. 1984) it was assumed, as a tentative hypothesis, that a disruptive selection for phototactism, using the Benzer method (1967) or the Kekic method (1981), could be more efficient at 30°C than at 25°C. Indeed, the range of the final distribution of the flies in the various chambers (Kekic test) or test tubes (Benzer test) was greater at 30°C than at 25°C, and consequently it was hoped that it should be easier to separate the most phototactic flies from the less phototactic ones.

From the same initial population "Namur", four strains have been obtained by disruptive selection. We have used the Benzer method modified by Tompkins et al.(1978), and the tests were carried on at 30°C and at 25°C. All the flies were raised and maintained at 25°C, but for the "population I" the tests were done at 30°C from the 5th till the 15th generation, whereas they were always done at 25°C for the "population II". The selection intensity was always the same: 10% (the most phototactic ones or the less phototactic ones, respectively) of at least 100 flies of same sex tested together were taken as parents for the next generation. At the beginning (September 1983) and at the end (May 1984) of the selection experiments, 5 tests have been done using the non-selected population "Namur". In the same way, 5 tests were done with the four strains resulting from the selection procedure. The comparison between the experimental results

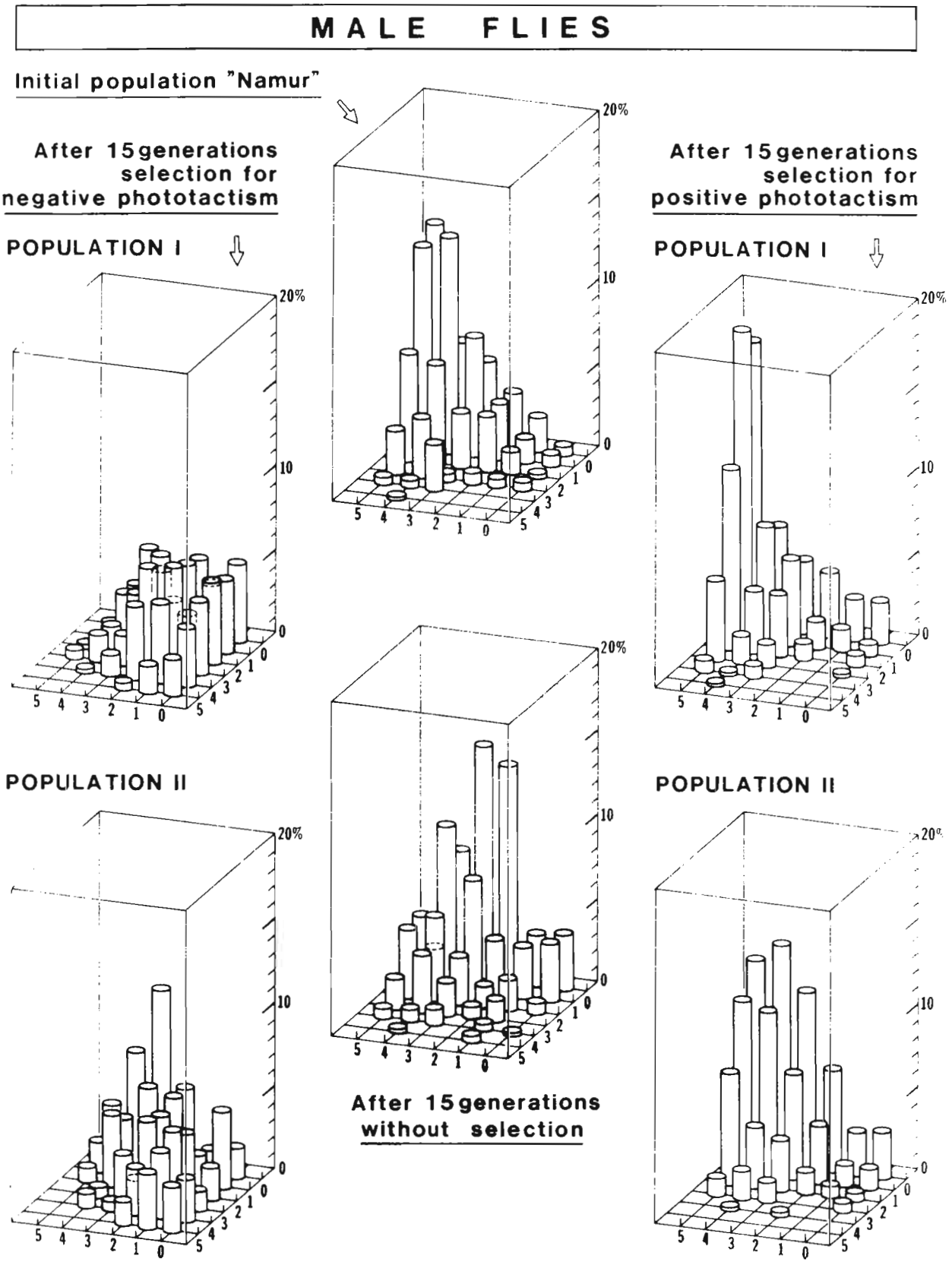


Figure 1. Countercurrent Benzer test for phototactism: final distribution of the flies. Ordinate: mean number of flies in the test tubes, in percent of the total. Front view abscissa: number of positive responses (toward light). Side view abscissa: number of negative responses (from light).

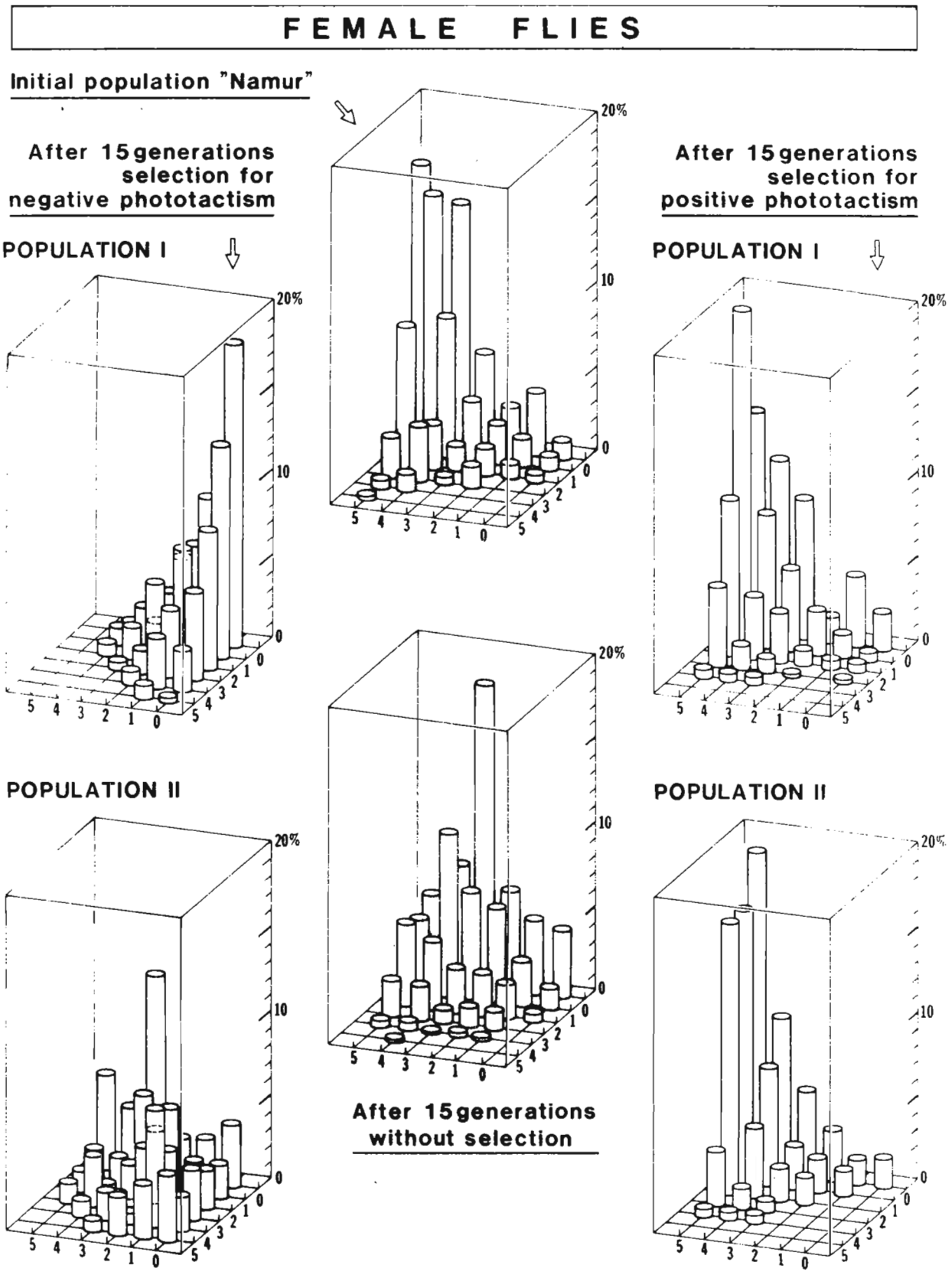


Figure 1 (continued).

were done by chi-square tests. The results are shown in Fig. 1. The unselected stock population "Namur" appears significantly less phototactic in May 1984 than in September 1983. In both populations I and II, the selection for positive phototactism has succeeded: after 15 generations, the resulting strains are significantly more phototactic than the population "Namur" as it appears from the September 1983 initial tests and as well in the May 1984 final experiments. The selection for negative phototactism seems to have succeeded in the population II only: some flies seem to be really attracted by darkness. For the population I, the difference with the population "Namur" consists essentially in a greater proportion of flies which are locomotrically less active and remain in the starting test tube. A 30°C temperature certainly increases the excitement and the agitation of the flies, and consequently influences the segregation between the "runner" and the "sluggish"flies. It doesn't seem to influence the phototactism in itself.

References: Benzer, S. 1967, Proc. Nat. Acad. Sci. 58:1112; Dubucq,D., E. Depiereux & A. Elens 1984, DIS 60:87; Kekic, V. 1981, DIS 56:178; Tompkins, L., J.A. Fleischman & G. Sanders 1978, DIS 53:211.

Bihari, B. and J.P. Gupta. Banaras Hindu University, Varanasi, India. Records of Drosophila species from three different areas of Madhya Pradesh, India.

As a matter of fact, there has been a conspicuous gap in our knowledge of world distribution of Drosophila where India is concerend. It is only very recently that interest has grown considerably in this field in India. However, a vast area of the Indian subcontinent still awaits exploration. Madhya Pradesh is one of the 22 states of India which has been largely neglected for such studies. Although it was Bachli (1973) who made for the first time a cursory survey of Drosophilid fauna of Kanha national park (M.P.) and collected 17 species of Drosophilidae. Since then nothing could be known about the Drosophilid species inhabiting this state of India.

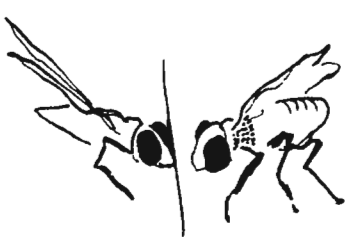
During the present study, collections were undertaken at three different localities, namely Betul, Bilaspur and Shahdole, using different fermenting fruits as baits and also by net sweeping over wild vegetation. Altogether eleven species of Drosophila were collected from these areas as shown in Table 1. Based on the collection data, it was found that there lies a remarkable similarity in the species spectrum of these three areas. Although similar distribution of species suggests that similar, but obviously not that similar, ecological niches are also utilized by these species in these areas.

Table 1. Drosophila species collected from Madhya Pradesh (India) during August 1984.

Species	Sub-genus	Locations			
		Betul	'Bilaspur'	'Shahdole'	Total
D.kikkawai	Sophophora	32	16	24	72
D.malerkotliana	"	91	80	65	236
D.jambulina	"	71	52	64	187
D.punjabiensis	"	56	43	48	147
D.bipectinata	"	42	13	29	84
D.biarmipes	"	75	91	35	201
D.takahashii	"	56	39	47	142
D.latifshahi	Scaptodrosophila	49	64	26	139
D.bryani	"	42	17	59	118
D.brunea	"	4	3	--	7
D.nasuta	Drosophila	47	19	62	128

This work has been supported by a research grant from the D.S.T. Government of India to J.P.G.

Reference: Bächli, G. 1973, Vjschr. Naturf. Ges. Zürich 118:29-30.



Boerema, A.C. and R. Bijlsma. University of Groningen, Haren, Netherlands. The effect of substituting artificial laboratory food by orange food on allozyme polymorphisms.

Little is known about the natural breeding sites of **Drosophila melanogaster**, but it is generally assumed to breed mainly on rotting and fermenting fruit. Boerema & Bijlsma (1984) measured egg-to-adult survival of **D.melanogaster** on different kinds of fruit. Reasonably good viabilities were observed on most fruits, even when mixed with agar. In the present study the influence--if any--of substituting the standard laboratory food by orange food on a number of allozyme polymorphisms was examined.

For the experiments two cage populations were used: the Death Valley population (DV) and the Riverside population (RV). These populations were both established in March 1981 by mixing the offspring of a number of isofemale lines (10 pairs per line) which had been caught in Death Valley and Riverside (for a description of the sites, see Coyne et al. 1983) in March and June 1980, respectively. The DV population was started with 83 and the RV population with 94 isofemale lines. After establishment the cages were kept undisturbed on standard laboratory food for more than one year at 25°C in order to ensure random mixing of the lines. Thereafter both cages were quadruplicated: of each population two cages were still provided with standard laboratory food, the other two with orange food. The standard food contained 18 g agar, 54 g sucrose, 32 g dried dead yeast and 13 ml of a nipagin solution (10 g nipagin in 100 ml ethanol 96%) in 1 liter water. The orange food consisted of 720 g ground orange (without peel) and 450 ml of agar solution (1% w/v) in which 6.5 ml nipagin solution.

The allele frequencies of the following allozyme loci were monitored at intervals by electrophoresis: glucose-6-phosphate dehydrogenase (G6pd), 6-phosphogluconate dehydrogenase (Pgd), alcohol dehydrogenase (Adh) and α-glycerophosphate dehydrogenase (α-Gpdh). All four loci were found to be polymorphic in both of the original populations and each exhibited a fast (F) and a slow (S) allele (for electrophoretic procedures, see Eisses et al. 1979). Significance of allele frequency changes in the cages in relation to time was tested by Spearman rank coefficient.

The results are shown in Table 1. The data for Pgd are omitted from this table as all cages turned out to be nearly fixed for the F allele with frequencies fluctuating between 0.95 and 1.00 F. The other three loci showed less extreme allele frequencies. The results differed between enzymes and/or populations.

Table 1. F frequency determined at intervals for the G6pd, Adh and α-Gpdh loci for the two populations both on standard food and orange food. Gene frequencies were determined on 84 females (G6pd) or 84 males (Adh and α-Gpdh).

DEATH VALLEY POPULATION:

	G6pd-F				Adh-F				α-Gpdh-F			
Time in	Standard food		Orange food		Standard food		Orange food		Standard food		Orange food	
months	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2
0	0.67	0.67	0.67	0.67	0.78	0.78	0.78	0.78	0.79	0.79	0.79	0.79
5	0.64	0.77	0.61	0.67	0.80	0.79	0.81	0.80	0.87	0.83	0.75	0.73
9	0.68	0.78	0.59	0.51	0.73	0.82	0.65	0.68	0.87	0.84	0.82	0.64
12	0.73	0.72	0.57	0.75	0.74	0.73	0.69	0.67	0.83	0.79	0.83	0.64
15	0.63	0.74	0.64	0.55	0.74	0.73	0.68	0.58	0.86	0.77	0.86	0.67
18	0.55	0.66	0.57	0.60	0.75	0.74	0.64	0.65	0.85	0.86	0.86	0.60
21	0.66	0.69	0.65	0.40	0.76	0.68	0.55	0.55	0.89	0.82	0.87	0.70
24	0.60	0.71	0.63	0.58	0.69	0.76	0.67[a]	0.65[a]	0.92	0.82	0.83[b]	0.51[a]

RIVERSIDE POPULATION:

	G6pd-F				Adh-F				α-Gpdh-F			
Time in	Standard food		Orange food		Standard food		Orange food		Standard food		Orange food	
months	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2	cage 1	cage 2
0	0.57	0.57	0.57	0.57	0.84	0.84	0.84	0.84	0.95	0.95	0.95	0.95
2	0.74	0.63	0.61	0.80	0.71	0.83	0.80	0.81	0.87	0.96	0.96	0.94
6	0.75	0.54	0.70	0.67	0.81	0.81	0.85	0.71	0.88	0.95	0.96	0.82
9	0.63	0.54	0.55	0.55	0.70	0.82	0.73	0.74	0.88	0.95	1.00	0.82
12	0.61	0.61	0.56	0.68	0.71	0.78	0.80	0.72	0.88	0.96	1.00	0.89
15	0.64	0.61	0.63	0.61	0.63	0.77	0.70	0.69	0.83	0.88	0.98	0.92
18	0.52	0.61	0.65	0.61	0.63	0.68	0.71	0.69	0.82	0.92	0.99	0.83
21	0.65	0.61	0.71	0.62	0.63[a]	0.73[a]	0.72[a]	0.74	0.84[a]	0.94	0.91	0.99

a = significant decrease, $P < 0.05$. b = a significant increase, $P < 0.05$.

(1) For G6pd the allele frequencies were found to be fairly constant over the two year period for both populations; there were some fluctuations but none of the cages showed a significantly consistent change. At the end of the experiment no divergence between the two food situations was observed.

(2) For Adh the RV populations showed a decrease in F frequency in the course of the experiments; this decrease was significant for both cages with standard food and for cage 1, with orange food. The change was in the same order of magnitude in all cages, so again no divergence between standard food and orange food was observed. The DV populations also showed a decrease in F frequency, but the decrease was found to be only significant for the cages provided with orange food. This indicated some divergence between the two types of food, but the difference in allele frequency at the end of the experimental period was, however, small.

(3) For α-Gpdh the situation was ambiguous. The RV population showed a significant decrease in F frequency in one standard food cage only (cage 1) while the other three cages did not change significantly. The DV population showed significant changes for both orange food cages. The change, however, was in opposite directions: cage 1 showed an increase while cage 2 showed a decrease in F frequency. These results also did not indicate a consistent divergence between the two food situations.

In conclusion, it can be said that, compared to standard food, orange food did not significantly alter the behavior of these allozyme polymorphisms and that it did not change selective differences--if any--with respect to these loci and/or linked fitness genes. Only in one of the cases (DV: Adh) a possible divergence was observed.

References: Boerema, A.C. & R. Bijlsma 1984, DIS 60:62-63; Coyne, J.A., J. Bundgaard & T. Prout 1983, Am. Nat. 122:474-488; Eisses, K.T., H. van Dijk & W. van Delden 1979, Evol. 33:1063-1068.

Botella, L.M. and J.L. Ménsua. University of Valencia, Spain. Can crowding promote larval diapause in Drosophilids?

Chymomyza costata is a Drosophilid closely related to **Drosophila** genus which shows light-dependent larval diapause (Hackman et al. 1970). This species is located in Northern European regions. Larval diapause appears in 3rd instar larvae when the period of light is under a certain minimum according to the strain. This diapause can only be interrupted by cold treatment for two months. The present work has analyzed the relationship between diapause and other phenomenon of larval arrest promoted by crowding, the larval stop in 3rd instar larvae of **Drosophila melanogaster** (Mensua & Moya 1983). For this purpose **Chymomyza costata** was bred under crowded conditions which give rise to a larval stop in Drosophila. Two different strains of **Chymomyza costata** (VKL & TODA), kindly supplied by Dr. Rihimaa, were employed. TODA comes from a mass capture carried out in Tomazaki (Japan) in August 1983, while VKL was captured in Kuopio (Finland) in June 1981. Crowded cultures were set up by seeding 30 larvae in 5 x 0.8 cm vials with 0.5 ml of Lakovaara medium (Lakovaara 1969). Uncrowded controls were also taken by seeding 30 larvae in 10 x 2.5 cm vials with 5 ml of the same medium. Temperature was kept at 19°C and light was constant to avoid diapause. Crowding was interrupted by overfeeding (Moya & Mensua 1983) on days 17th, 21st, 25th and 29th in VKL strain, and on days 17th, 21st, 25th, 29th and 33rd in TODA strain. The total population was divided in this way in inner and outer subpopulations. A total of five replicates were made for each strain and overfeeding.

Table 1 shows survival obtained in inner populations (adults emerged in the small vials), in outer subpopulations (adults emerged in the overfeeding vials) and total survival, as well as developmental times corresponding to inner and outer populations. As can be seen from this table, hardly were flies recovered in inner populations. Moreover, most of total survival is due to outer populations which nevertheless is below 50% in all the overfeedings. Developmental times are progressively delayed as overfeedings are later. In the VKL strain, the highest elongation of development obtained by difference between the longest time in crowded experiments and the uncrowded culture is about 21 days, while in TODA where overfeedings were prolonged until the 33rd day, the maximum lengthening is about 29 days. These elongations, though noticeable, would seem too short to be considered as a true diapause. In order to enhance the developmental arrest, the following experiment was designed. Ten crowded cultures for each strain were seeded as usual on the 3rd November 1984. On the 40th day of culture, the remaining larvae were extracted and seeded again in fresh food (5 x 0.8 cm vials with 0.5 ml food) by groups of 30 larvae keeping in this way the same degree of initial crowding. This operation has been repeated every 40 days. The results obtained have been striking in both strains, but especially in VKL. A large number of larvae does not pupate, remaining as 3rd instar larvae for a period of time in principle not determined. In fact, as of this date (15th March 1985),120 larvae from VKL strain (40% of the number initially seeded), and 30 larvae from TODA strain (10%) remain alive in these cultures.

Table 1. Mean survival in inner, outer and total populations, and mean developmental time in inner and outer subpopulations of the Drosophilid **Chymomyza costata.**

Strain	Overfeeding (days)	Survival			Developmental Time	
		Inner	Outer	Total	Inner	Outer
VKL	Uncrowded control	--	17.0±1.3	17.0±1.3	--	32.18±0.44
	13	0.0±0.0	8.2±2.1	8.2±2.1	--	40.84±0.98
	17	0.7±0.5	11.5±2.9	12.5±2.8	34.50±0.50	41.91±2.10
	21	1.5±0.9	5.5±3.0	7.0±2.9	36.83±0.17	47.93±1.65
	25	2.0±1.0	10.7±0.7	12.7±0.7	42.10±1.45	53.42±0.64
TODA	Uncrowded control	--	17.7±0.9	17.7±0.9	--	32.11±0.17
	17	0.0±0.0	11.5±3.5	11.5±3.5	--	46.39±1.87
	21	0.0±0.0	13.5±1.3	13.5±1.3	--	44.35±1.94
	25	0.5±0.5	13.7±2.8	14.2±2.6	38.50± *	47.57±1.07
	29	2.0±2.0	10.7±3.9	12.8±2.3	38.75± *	52.01±1.94
	33	3.0±2.4	14.5±2.5	17.5±4.6	40.50±2.51	61.29±1.20

* values obtained from a single vial.

The conclusion is that larvae are in diapause, but the mechanism which makes them enter diapause is different from that previously described in literature so far for this species (Basden 1954; Hackman et al. 1970). In relation to this view, it seems that larval stop might be regarded as a kind of diapause in non-diapausing species, such as **D.melanogaster**, but that is also present in diapausing species such as **Chymomyza costata**, promoted by crowding.

References: Basden, E.B. 1954, Proc. Royal Ent. Soc. London 29:7-9; Hackman, W., S. Lakovaara, A. Saura, M. Sorsa & K. Vepsalainen 1970, Ann. Ent. Fenn. 36:1-9; Lakovaara, S. 1969, DIS 44:128; Mensua, J.L. & A. Moya 1983, Heredity 51:347-352; Moya, A. & J.L. Mensua 1983, DIS 59:90-91.

Botella, L.M. and J.L. Ménsua. University of Valencia, Spain. A comparison of the urea and uric acid content between crowded and uncrowded cultures of **D.melanogaster** throughout development.

Uric acid, main biotic residue coming from the nitrogen catabolism in **Drosophila** (Botella et al. 1984a), plays an important role in competition phenomena for food, in such a way that it may account at least partially for the low survival and delayed development, as well as larval stop obtained in these conditions (Botella et al. 1984). On the other hand, urea, also present in small quantities in Drosophila cultures (Botella et al. 1984, 1985) is able to mimic the kinds of responses obtained in crowded cultures, for it decreases larva-adult survival and increases mean developmental time (Botella et al. 1983). An analysis of urea and uric acid has been carried out in the present work in order to compare uric acid and urea contents in crowded and uncrowded cultures throughout development. Crowded cultures consisted of 5 x 0.8 cm vials with 0.5 ml of Lewis medium seeded with 70 larvae. Uncrowded cultures consisted of 10 x 2.5 cm vials with 5 ml of Lewis medium seeded with 70 larvae. Both kinds of cultures were incubated at 19°C at 85% relative humidity and constant light. Analysis of urea and uric acid were carried out following the methods described elsewhere (Botella et al. 1984). The expression giving larval, pupal and adult content of both products is:

C(mg/100ml) = (Sample absorbance/Standard Absorbance) X (C Standard) X (1/dilution factor), where dilution factor (d.f.) is: d.f. = (Sample Volume)/(Sample Volume + Sodium Acetate Volume).

Sample volumes were estimated from larval, pupal and adult densities, as well as their respective body weights. These data appear in Table 1. The results of uric acid and urea determinations throughout the different stages of development are also shown in Table 1. As can be seen, when comparing the results between crowded and uncrowded cultures the following observations deserve to be pointed out: (i) uric acid concentrations in larvae from uncrowded cultures are higher than those of larvae bred in crowded conditions; (ii) uric acid concentrations in pupae rise as their development progress (as a consequence of a lack of external excretion), while urea concentration remains more or less the same, this being true for both crowded and uncrowded cultures. It is worth mentioning here that the level of both products is higher in crowded than in uncrowded conditions in pupal stage, but for the urea content in mature pupae where both kinds of cultures show approximately the same level; (iii) the main component of the newly born adults excretion is uric acid present in high levels, and close to that obtained in 33-day old pupae from crowded cultures. Recently emerged adults, when incubated at 25°C for 20 hr show a high uric acid content, similar in both kinds of cultures, and close to the uric acid concentration at the end of the pupal stage. Urea concentrations are slightly above this level.

Table 1. Urea and uric acid concentrations in larvae, pupae and adults from crowded and uncrowded cultures of **Drosophila melanogaster** throughout development.

Stage	Development (days) crowded	uncrowded	Body Density	Mean weight (mg) (20 individuals) crowded	uncrowded	Uric acid level (mg/100ml) crowded	uncrowded	Urea level (ml/100ml) crowded	uncrowded
Larvae	13	12	1.05±0.01	22.8±1.0	34.7±1.8	31.7±3.4	142.6±5.0	18.5±3.5	13.5±1.8
	25	--		22.1±3.0		22.1±4.1		27.0±2.7	
Pupae	24	14	0.85±0.04	17.0±0.8	28.6±1.0	121.4±8.8	80.4±5.5	25.9±2.9	3.5±1.5
	33	20		13.0±0.3	32.3±0.9	140.7±7.9	99.7±4.3	14.9±2.6	18.6±6.0
Adults	Excretion of newly emerged adults		--	--	--	169.7±30.4	173.3±21.0	20.8±4.7	11.9±7.0
	20 hr old adults		0.97±0.1	9.2±0.6	20.4±0.6	173.9±31.3	161.2±12.8	29.2±4.0	33.3±7.1

The differences found between larvae, pupae and adults from crowded and uncrowded cultures are explained as follows: (i) larvae from crowded cultures must be metabolically less active than those from uncrowded ones; (ii) when uncrowded larvae approach pupation they must eliminate a large amount of their uric acid content, while crowded larvae should activate their metabolism in order to pupate producing as a result a large amount of uric acid, similar to the level found in larvae from uncrowded cultures; (iii) all pupae that are able to attain adult stage must be included within a physiological optimal range which allows them to emerge as adults. These physiological limits would in turn explain the similarity in the uric acid content found in adults coming from crowded and uncrowded cultures.

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Carracedo, M.C. and P.Casares. Universidad de Oviedo, Spain. Hybridization between **Drosophila melanogaster** and **D.simulans** in competition experiments.

Most of the papers dealing with interspecific competition between the pair of sibling species **D.melanogaster** and **D.simulans**, assumed that the possibility of interspecific hybridization is negligible. This assumption is based on the supposition that in these competition cultures, in which males and females are present, the frequency of hybridization must be very low or null, since each individual has a chance to elect (free-choice) a partner of its own species. This situation is different from the well-known methods of "no-choice" (one sex of each species), where the females are forced to accept a foreign male, and the frequency of hybridization reaches, in some instances, high values (Manning 1959; Watanabe et al. 1977; Carracedo & Casares 1985). We have carried out a study on interspecific competition between a "sparkling-poliert" laboratory mutant of **D.melanogaster** and a "white" strain of D.simulans derived from a natural population. All competition tests were made at an adult density of 10 pairs and with relative species frequencies of 0.1-0.2....0.9. A factorial 2 x 2 design was carried out by using two temperature regimes for the first factor, room variable temperature or fixed 21°C. As the second factor, two types of adults were utilized, based on that their larval development were in mono-specific or bi-specific cultures. For each combination of temperature-development-frequency, five replications were made. Ten pairs of virgin adult flies coming from mono- or bi-specific cultures were placed in culture bottles for 10 days. At the end, the females were placed into individual vials and their progenies examined. The appearance of wild phenotypes was taken as evidence of hybridization. We have only examined the females of **D.melanogaster** because in a simultaneous study with the same strains, the hybridization between **simulans** females and **melanogaster** males (no-choice method) was null (Carracedo & Casares 1984).

Table 1 shows the values of hybridization (i.e., hybridized females divided by total females), which represents the sum of all the relative frequencies for each of the four factorial combinations. These values, as percentages,were submitted to a weighted analysis of variance in the logit scale with the transformation for small size suggested by Snedecor & Cochran (1967), and the results are also shown in Table 1. The factorial effect of the conditioned adult development was not significant, which differs from the results of Eoff (1973), but the temperature showed a significant effect, being hybridization higher at room temperature than at 21°C. This result appears to be related with better general fitness showed by **D.simulans** in the former temperature regime (Carracedo 1984). It is of interest to ascertain whether the values

Table 1. Values of hybridized **melanogaster** females in each of the 2x2 factorial combinations, previous larval development and temperature, and results of a weighted analysis of variance of the percentages of hybridization in the logit scale.

previous development	room temperature	21°C
mono-specific cultures	9/225	4/225
bi-specific cultures	13/225	5/225

factorial effect of temperature: 0.862±0.385 (t=2.23, $p < 0.05$)

factorial effect of development: 0.318±0.359 (t=0.88 n.s.)

Table 2. Number and percentage of hybridized **melanogaster** females for each species frequency. Data of the 2x2 factorial combinations were pooled.

number of **D.melanogaster** pairs								
9	8	7	6	5	4	3	2	1
number of **D.simulans** pairs								
1	2	3	4	5	6	7	8	9
hybridized/ total **melanogaster** females								
0/180	1/160	2/140	2/120	7/100	3/80	6/60	4/40	6/20
percentage of hybridization								
0%	0.62%	1.43%	1.67%	7%	3.75%	10%	10%	30%

of hybridization are related with the relative frequencies of both species. Owing to the low values of hybridization found in some frequencies, we have grouped the data of the four factorial combinations under the supposition that in each combination, the possible effect of species frequency was the same.

Table 2 shows the percentages of hybridized females in each relative frequency and the total number of examined females. Apparently, when the relative frequency of **D.melanogaster** diminished, the number of **melanogaster** females hybridizing with **simulans** males increased. To confirm this, we have obtained a weighted linear regression of the percentages of hybridization in the logit scale on the frequencies of **D.melanogaster**, using an iterative routine that yields maximum likelihood estimates (Finney 1971). The 0.1-0.2 and 0.3 frequencies were grouped to increase the expectatives. Regression was highly significant (b=5.6±0.9 in logits) and the data fit well with the model (chi-square = 7.02 with 5 degrees of freedom, no-heterogeneity). Therefore, the hybridization of **melanogaster** females increased when its frequency in the mixture decreased. If we suppose that any female may be simultaneously courted by all present males, then the probability of mating between **melanogaster** females and **simulans** males increases with the decrease of the relative frequency of **D.melanogaster**. This was confirmed by the notable fact that when the relative **melanogaster** frequency was 0.1 the value of hybridization (30%) was not different from that one found in a "no-choice" test carried out with these same strains of **D.melanogaster** and **D.simulans** (Carracedo & Casares 1984).

Summarizing, our results showed that the possibility of hybridization between **D.melanogaster** and **D.simulans** in competition cultures can not be rejected, especially when the relative frequency of **D.melanogaster** is low. Thus, if hybridization occurs, the **melanogaster** progeny may be lower than expected. Furthermore, hybrids could not be detected because they have reduced viability, particularly at the commonly used temperature of 25°C (Sturtevant 1920). In this way, results of competition could be erroneously imputed to factors other than hybridization.

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Carracedo, M.C. and P. Casares. Universidad de Oviedo, Oviedo, Spain. A study on the dynamics of crossing between **Drosophila melanogaster** females and **Drosophila simulans** males.

Pontecorvo (1942), Manning (1959) and Barker (1967), among others, have shown that hybridization between **D.melanogaster** females and **D.simulans** males is more frequent when flies are aged a few hours than 3 or more days. As a possible explanation it has been suggested that young females have not well developed their sexual discriminative sense and may mate with almost any courting male. Nevertheless, it is also probable that when very young male and female flies of both species are kept together and they mature in proximity, they may become accustomed to each other and facilitate interspecific mating, once sexual

Table 1. Average percentage of hybridization between **D.melanogaster** females and **D.simulans** males, in different periods of time. ML and MH = lines of **D.melanogaster**. SL and SH = lines of **D.simulans**.

	Days									
Cross	1	2	3	4	5	6	7	8	9	10
ML x SL	0	0	0	4	0	4	0	4	0	4
ML x SH	0	0	0	8	4	0	4	0	0	0
MH x SL	0	64	60	48	40	48	52	44	48	48
MH x SH	0	16	56	84	68	48	64	76	80	64

maturity has been reached. In previous works, we used adult flies aged 6 hr, which remained together for a period of 5 (Carracedo & Casares 1985b) or 10 days (Carracedo & Casares 1984; 1985a); at the end of these periods of time, we obtained the frequency of hybridized females. But by this procedure, we do not know if the observed hybridization mainly occurred in the first 24-28 hr, or if, on the contrary, the number of hybridized females of **D.melanogaster** increased day after day due to persistent courtship by **D.simulans** males. These two different phenomena deserve examination because we have found that isofemale lines extracted from single populations of **D.melanogaster** and **D.simulans** show large differences in frequency of hybridization (Carracedo & Casares 1985b).

The present study has been carried out with the aim of determining the dynamics of heterospecific matings throughout a 10-day period. We used two isofemales lines of each **D.melanogaster** and **D.simulans** species, chosen on the basis of their showing a low (L) or high (H) value of hybridization (Carracedo & Casares 1985b) and named ML and MH, respectively, for **D.melanogaster** and SL and SH for **D.simulans.** The schedule utilized was as follows: five **D.melanogaster** females and five **D.simulans** males, aged 2 hr, were placed in vials with food in which they remained together for ten different periods of time, ranging from 1 to 10 days. At the end of each period, females were individually placed in vials. The presence of hybrid progeny in the vials was taken as evidence of heterospecific mating. This scheme was initiated for each of the four possible directions of crossing between the 2 x 2 lines of males and females. All the experiments were replicated 5 times in a single block, and carried out at room temperature.

To avoid confusion with the use of the word "age", we must state that there is a clear difference between, for example, saying that maximum hybridization occurs when we use females "aged 3 days", or saying "on the third day of life", since, obviously, in the first case the observation is on day-4 of life; whereas in the second, it is on day-3. In future, we will utilize this last meaning. The table shows the average percentages of hybridization for crossings and periods of time. When the ML-line was utilized as female parent, the frequency of hybridization was, independently of the two male lines, extremely low: from 500 females tested at different periods, only 7 left hybrid progeny. The other **D.melanogaster** line, MH, gave a very different result. In the cross MH x SL, a multiple comparison of percentages revealed that values from 2 to 10 days were not significantly different (X^2 = 4.52; df=9). That is, the maximum hybridization was attained on day-2 of coexistence, and more time did not increase this value. In the other cross, MH x SH, the percentages from 3 to 10 days were not significantly different (X^2 = 12.08; df=9) and so, the maximum of hybridization was attained on day-3.

In summary the results show: (1) No interspecific mating occurs on day-1; unpublished data have shown that males of **D.simulans** are sexually active within this period of 24 hr, but females of **D.melanogaster** are practically unreceptive; the above result suggests that young **D.melanogaster** females are not raped by **D.simulans** males. (2) Females play a more important role in hybridization than males, as previously demonstrated (Parsons 1975; Carracedo & Casares 1985b; Casares & Carracedo 1985). (3) Hybridization does not increase with time. This suggests that, in these species, interspecific mating does not depend on male persistence. (4) As previously suggested (Carracedo & Casares 1984), there seems to be a temporal disagreement between the age at which females attain sexual receptivity, and their sexual specific discriminative ability.

Finally, we have obtained for the MH-line the maximum frequency of hybridization with each **D.simulans** line. This has been performed by calculating the average of the non-different percentages. In the cross MH x SL, the maximum hybridization, attained on day-2, is 50.22% (113/225), whereas in the cross MH x SH it is 67.50% (135/200) and it is attained on day-3. These two percentages are significantly different (X^2 = 4.48; df=1; $p < 0.05$) which point out that "speed" in hybridization is not in agreement with the maximum value attained. This result might be imputed to different behaviour of the male lines, which reveals the complexity of the behavioural mechanisms involved in the precopulatory isolation between these two sibling species.

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Casares, P. and M.C. Carracedo. Universidad de Oviedo, Spain. Hybridization between sympatric and allopatric populations of **Drosophila melanogaster** and **D.simulans.**

The speciation process is in essence the formation of different sets of gene combinations reproductively isolated. Reproductive isolation comprises pre-mating and post-mating mechanisms. The former, sexual isolation, encloses all the mate recognition signals and stimuli that prevent the wastage of gametes between two coadapted gene pools. Two models are generally accepted for the origin of sexual isolation in allopatric speciation: in the first, isolation arises as a by-product of genetic divergence owing to local adaptation of allopatric populations. In the second, isolation appears as a direct product of natural selection acting in sympatry, selection that prevents the appearance of sterile, inviable, or poorly adapted hybrids. The sexual isolation mechanisms strengthen the initial post-mating barriers of reproductive isolation, a process generally referred to as "reinforcement" or more exactly, "reproductive character displacement" (Brown & Wilson 1956).

If there were natural selection for sexual isolation between related species, this isolation should be greater among sympatric than allopatric species populations. The pair of sibling species **D.simulans** and **D.melanogaster** are a good material to test this possibility. They show courtship displays that differ more quantitatively than qualitatively (Manning 1959; Bennet-Clark & Ewing 1969). Although the two species are cosmopolitan, **D.simulans** was practically unknown in Japan before 1972. Since then, this species has extended its geographical distribution and today it is found in coexistence with **D.melanogaster** in wide areas of Japan.

In 1982 Dr. T.K.Watanabe kindly sent us some isofemale lines of **D.melanogaster** (MKc) and **D.simulans** (SKc) sympatric populations caught in Kochi, and isofemale lines of one population of **D.melanogaster** (MKf) from Kofu which presumably had never been in contact with **D.simulans.** A first approach to examine sexual isolation between these populations is to assess the rate of hybridization under laboratory conditions. Because the break of sexual isolation is rare when pairs of the two species are in the same vial, we have used the no-choice method, only one sex of each species being present in the tests, as follows: five males and five females aged 24 hrs were left together for 3 days in a bottle with standard baker's yeast food. At the end, females were individually placed in vials, the frequency of hybridization being measured by the number of females out of five that left fertile eggs. Eight isofemale lines of each population were employed.

In general, the hybrid crossing between **melanogaster** males and **simulans** females occurs at an extremely low frequency (Sturtevant 1919). To confirm this, 4 lines, at random, of SKc were tested in the mentioned cross-direction with 4 lines of MKc, the remainder of 4 SKc lines do it with 4 MKf lines. Two replications were achieved for each test. None of the 80 **simulans** females was hybridized.

In the other cross-direction, **simulans** males and **melanogaster** females, a factorial 8x8 design was carried out with isofemale lines of both allopatric SKc x MKf and sympatric SKc x MKc species populations. A fully randomized system was followed to achieve two replications of all tests in a single experimental block. The arc-sine transformed data with the suggested corrections for small size (Snedecor & Cochran 1967) were subjected to factorial analysis of variance. Results appear in Table 1. The average of hybridization between sympatric populations was 42.3% while only an 8.3% occurred between allopatric ones. In both cases the male and female components of variation were significant. Notably, the sexual isolation was higher between allopatric than sympatric populations of **D.melanogaster** and **D.simulans.** This fact did not support the hypothesis that reproductive character displacement had been operating in these populations. This might lead us to think that sexual isolation between these species could have been originated through the general process of genetic differentiation and divergence of allopatric populations. Watanabe (pers. comm.) has found that different levels of sexual isolation (hybridization) between populations of **D.simulans** and **D.melanogaster** were unrelated with the number of years they have been in sympatry.

Table 1. Analysis of variance of the arc-sine transformed percentages of hybridization between sympatric and allopatric populations of **D.melanogaster** and **D.simulans.** Bottom, maximum-minimum value of hybridization for each sex and type of cross.

	TYPE OF CROSS			
source of variation	sympatric x sympatric M.S.	F	allopatric x allopatric M.S.	F
males	1523.68	4.65***	133.73	2.62*
females	1001.62	3.06**	194.11	3.81**
males x females	414.69	1.27	58.36	1.14
error	327.49		50.95	
range of the means (in angles)				
for males	30.73-58.48		12.18-22.18	
for females	29.88-48.82		13.77-22.84	

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Our results show that there was intrapopulational genetic variation for hybridization in both species. This has been already noticed in some reports (Eoff 1975, 1977; Carracedo & Casares 1985). However, since these species appear to be almost completely isolated in nature, natural selection for sexual isolation seems to be improbable. Further work is necessary to understand the origin of sexual isolation between these sibling species.

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Castro, J. and J.L. Ménsua. University of Valencia, Spain. Effect of the seeding site on viability and developmental time of three genotypes of **Drosophila melanogaster.**

Viability and larva-to-adult developmental time are two important components of fitness in Drosophila and they can be related to phenomena such as larval facilitation (Lewontin 1955; Beardmore 1963; Bos et al. 1977; Bos 1979), as well as to concepts of microniches (Tosić & Ayala 1981) and microenvironments (Barker 1971).

Viability and developmental time of three strains of **Drosophila melanogaster** in an uncrowded situation, but with different seeding sites in vials, were studied.

The strains employed here were: a wild strain and two mutant strains for eye colour; cardinal (III, 75.7) and sepia (III, 26.0). A total of 72 newly hatched larvae ±2 hr old were seeded into 10 x 2.5 cm vials with 10 ml of boiled yeast medium according to the following ways: (1) All larvae seeded at the same time

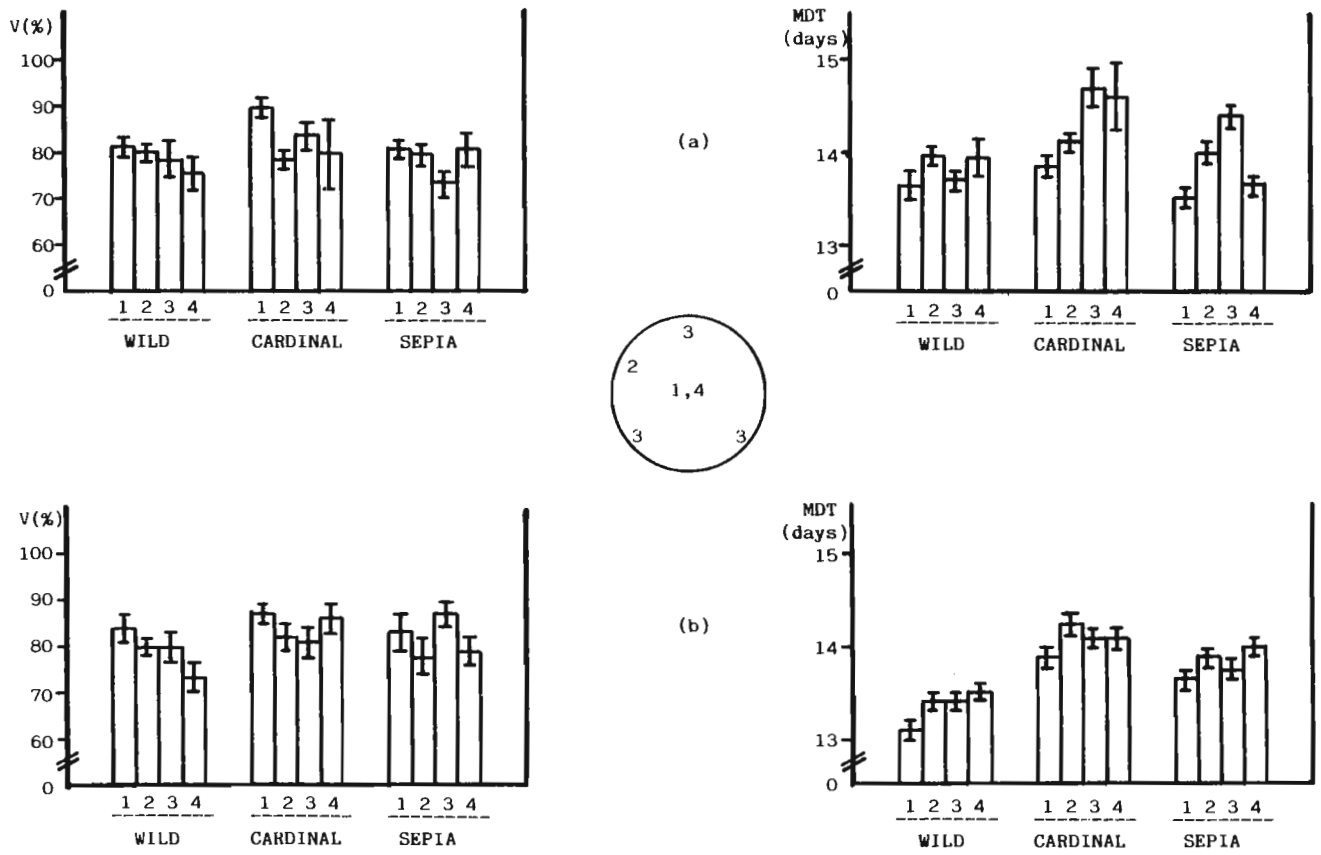

Figure 1. (a) Viabilities (V) and Mean Developmental Times (MDT) according to the four different seeding sites of the strains wild, cardinal and sepia in monocultures. (b) The same as before, but in tricultures. The circle represents a vial with the different seeding sites.

in the centre of food by means of an incision practised with a lancet. (2) All larvae seeded at the same time in a side of the medium just beside the vial wall with incision on food. (3) Larvae seeded in 3 groups of 24 larvae each, separated as far as possible with incision on food. (4) All larvae seeded on the centre of food, without incision. In this case larvae were placed on a piece of paper (0.5 x 0.5 cm) which was put on the surface of food.

Monocultures and tricultures (24 larvae for each strain) were carried out. A total of 8 replicates for monocultures and 10 replicates for tricultures were made. Cultures were incubated at 25±1°C and at 60±5% relative humidity. Data were analyzed by ANOVA and Student-Newman-Keuls test.

Figure 1a shows viabilities (V) and mean developmental times (MDT) for the three strains in monocultures. Viability shows only significant differences among the different seeding sites in the cardinal strain which has a higher viability when seeding in site 1. As regards MDT, cardinal and sepia strains show differences, cardinal being faster in 1 and 2 situations and sepia in 1 and 4.

Figure 1b shows viabilities (V) and mean developmental times (MDT) in tricultures. Though wild strain shows a slight decrease in viability when seeded in the 4th way, no significant differences among viabilities appear. As regards MDT, statistical tests show that wild strain is faster in situation1; the MDT of the cardinal strain remain unchanged in all situations, and sepia is faster in situation 1 and slower in the 4th.

In monocultures, wild strain seems to be unaffected by the seeding sites. Cardinal strain, on the other hand, is slower in situations 3 and 4, it showing that, perhaps it is more sensitive than wild strain to gregarism and to help which may represent the incision of the medium. These ideas are supported by the highest viability exhibited by cardinal in situation1. It seems that sepia has higher sensitivity to gregarism than wild strain though not face to cardinal strain. The incision does not change its response.

In tricultures, viabilities do not show differences among the different seeding sites, while mean developmental times show a phenomenon of facilitation among the strains, mean developmental times being lower in tricultures than in monocultures. The first seeding method gives rise to the fastest developmental rate in the three strains. This result supports some kind of mutual facilitation. This facilitation is present in spite of the existence of the different competitive abilities of genotypes being reflected as differences among the MDT. In this way, the concept of larval facilitation is extended. Moreover, this effect seems to be important for the understanding of genetic polymorphisms. Since in our uncrowded cultures facilitation is put into evidence, it may be thought that under more restrictive conditions its role may be determinant. However, when food and space are limited, facilitation might be hidden under other factors more relevant.

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Chanteux, B., J. Lechien, C. Dernoncourt-Sterpin, M. Libion-Mannaert, S. Wattiaux-De Coninck and A. Elens. F.N.D.P., Namur, Belgium. Ethanol metabolizing enzymes subcellular distribution, in **D.melanogaster** flies homogenates.

A method of homogenization and subcellular fractionation originally described for Rat liver (de Duve et al. 1955) has been slightly modified and used for Drosophila flies homogenates (Liétaert et al. 1984). First, a nuclear fraction (N) is separated from a total cytoplasmic extract (E). From the cytoplasmic extract, four fractions are isolated: a heavy mitochondrial fraction (M), a light mitochondrial fraction (L), a microsomal fraction (P), and a final supernatant (S). The same reference enzymes have been used as for Rat liver: cytochrome c oxidase and malate dehydrogenase for mitochondria, acid phosphatase and beta-galactosidase for lysosomes, NADPH cytochrome c reductase for endoplasmic reticulum, and catalase (which plays a part in ethanol metabolism) for peroxisomes.

Five **D.melanogaster** genotypes have been considered: the strain y v f mal^{bz} lacks aldehyde oxidase (AO) but has a normal alcohol dehydrogenase (ADH) activity; the strain $bAdh^{n4}$ lacks both AO and ADH; the HA and LA lines result from a long term selection for "male sexual activity" combined with brother-sister mating which has given, after 330 generations, a "highly active" line HA and a "lowly active" line LA; the wild e^{+} strain is used as a control. These three last genotypes are endowed with normally high ADH and AO activities. All these genotypes differ in tolerance to ethanol, in oviposition preference for ethanol supplemented mediums, and in larval preference for ethanol supplemented or acetaldehyde supplemented mediums, in relation with their ADH activity level (Deltombe-Liétaert et al. 1979; Hougouto et al. 1982; Depiereux et al. 1985). Adult flies (from 5 to 10 days of age) were used.

The specific activities of the reference enzyme and of the main ethanol metabolism enzymes are shown in Table 1, for the five genotypes. The distribution pattern of the same enzymes for each

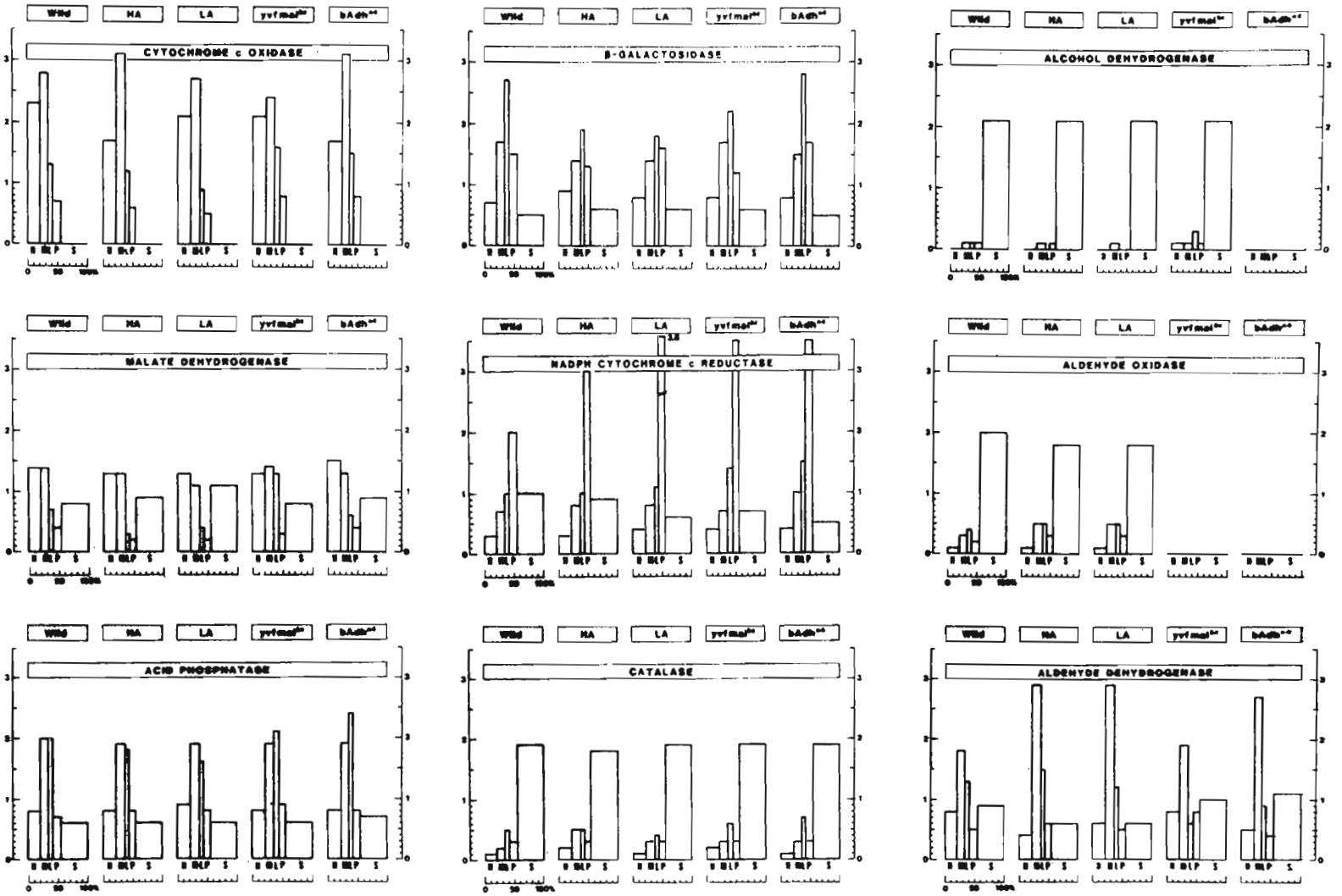

Figure 1. Distribution pattern of enzymes. Ordinate: relative specific activty of fractions (percentage of total recovered activity/percentage of total recovered proteins). Abscissa: relative protein content of fractions (cumulatively from left to right). **N**: nuclear fraction, **M**: heavy mitochondrial fraction, **L**: light mitochondrial fraction, **P**: microsomal fraction, **S**: supernatant.

Table 1. Enzymes specific activities (percentage of total recovered activity/percentage of total recovered proteins), for the five genotypes. The specific activities are given in units per gram proteins. For acid phosphatase, beta-galactosidase, and aldehyde dehydrogenase, the values have been multiplied by 10^2.

STRAINS:	wild e^+		HA		LA		yvf mal^{bz}		bAdh^{n4}	
ENZYMES	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
Cytochrome c oxidase	0.1636	0.0160	0.1395	0.0368	0.1695	0.0535	0.1454	0.0254	0.1496	0.0391
Malate dehydrogenase	2.1352	0.5756	1.6434	0.3473	1.4681	0.3460	2.0636	0.4652	2.4134	0.3949
Acid phosphatase	2.7093	0.4037	2.4348	0.8748	2.9246	1.7960	3.3772	0.8349	3.0263	0.7651
Beta galactosidase	0.7077	0.1623	0.9545	0.0940	0.8137	0.1656	0.6525	0.864	0.9235	0.1410
NADPH cytochrome c reductase	0.1790	0.0344	0.1314	0.0099	0.1219	0.0173	0.1563	0.0324	0.1206	0.0384
Catalase	0.0529	0.0130	0.0474	0.0241	0.0579	0.0089	0.0674	0.0087	0.0638	0.0240
Alcohol dehydrogenase	0.3032	0.0280	0.1350	0.0345	0.1535	0.0322	0.3262	0.118	-	-
Aldehyde oxidase	5.5915	1.0535	2.5416	1.0281	2.4723	0.5790	-	-	-	-
Aldehyde dehydrogenase	2.8450	2.2997	3.8454	1.6485	4.3905	0.6647	2.1533	0.0115	5.1252	1.1137

of the subcellular fractions obtained by differential centrifugation are shown in Fig. 1. A high proportion of cytochrome c oxidase is present in M and N fractions. Malate dehydrogenase is found in M and N fractions and also in the soluble fractions. The presence of large amounts of the two mitochondrial enzymes in N fractions denotes a high sedimentation coefficient and the presence of large mitochondria. About 40% of the lysosomal enzymes are recovered in M and L fractions. However, the relative specific activities are about the same in L and M fractions for acid phosphatase. Beta galactosidase is most purified in L fraction. A high proportion of the two enzymes is present in the soluble fraction. Catalase is mainly recovered in S fraction and seems not to be associated with a particulate fraction. By electron microscopy, peroxisomes have not been detected in the isolated fractions. NADPH cytochrome c reductase is most purified in the microsomal fraction P. In **D.melanogaster** flies endowed with ADH activity, this enzyme is mainly recovered in the soluble fractions. In wild e^+ strain and in the HA and LA lines, most of AO is unsedimentable, but a low proportion of the enzyme is associated with heavy and light mitochondrial fractions, specially in HA and LA flies. Aldehyde dehydrogenase (ALDH) seems to be associated with mitochondria, but it is also present in the S fraction; perhaps two different ALDH exist in **D.melanogaster** (Garcin et al. 1983) and are localized as in Mammals (Dawson 1983): an essentially mitochondrial ALDH and another soluble one.

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Evidence that the locus of a novel type of suppressor mutation regulates transcription of the white locus.

Members of the w^{sp} class of mutant alleles at the white locus (w^{sp1}, w^{sp2}, w^{2p3}, and w^{sp4}) apparently map outside of and 5' to the white transcription unit and exert tissue-specific effects on white expression (Zachar & Bingham 1982; O'Hare et al. 1983; Davison et al. 1985; Figure 1). The mutations causing these alleles, thus, appear to affect regulatory sequences. The suppressor-of-white-spotted mutation [su(w^{sp})] was isolated as a partial revertant of w^{sp1} and proved to be a suppressor of w^{sp1} mapping distal to white on the X chromosome (W. Gelbart, pers. comm.).

We have extended the genetic analysis of su(w^{sp}) and find it to map approximately at position 0.16 (assuming a map position of 0 for yellow and 1.5 for white) based on the recovery of 2 crossovers on the y-su(w^{sp}) interval and 17 on the su(w^{sp})-white interval.

We further find that su(w^{sp}) strongly suppresses (restores to nearly wild type) the mutant eye color phenotype produced by all four of the w^{sp} mutations, but exerts no detectable effect on the eye color phenotypes produced by any other tested white alleles. The non-w^{sp} alleles tested were w^{bf}, w^{b1}, w^{a1}, w^{a2}, w^{a3}, w^{ch}, w^{sp55} and w^+. su(w^{sp}) effects on expression of any of the non-w^{sp} mutant alleles tested comparable in magnitude to its effects on the w^{sp} alleles would have been readily detected as demonstrated by gene dosage experiments. The non-w^{sp} alleles tested are distinguishable in structure from

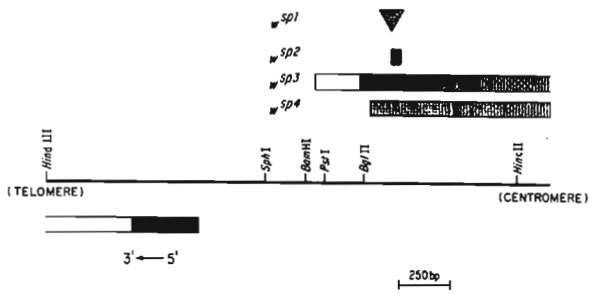

Figure 1. Physical map of relationship of w^{sp} mutations to the 5' end of the major white transcription unit. The central line with appended restriction sites represents white locus DNA sequences extending from coordinates +3.1 (HindIII) to +5.5 (HincII) on the conventional white locus map (see Zachar & Bingham 1982). The lower bar indicates the position of the 5'-most known exon (solid portion) and intron (open portion) of the major white transcript (Pirrotta & Brockl 1984; Davison et al. 1985). The stippled triangle represents the position of the B104 insertion responsible for the w^{sp1} mutation and the three stippled bars represent the extents of deletion lesions presumably responsible for the w^{sp2}, w^{sp3}, and w^{sp4} alleles (Zachar & Bingham 1982; Davison et al. 1985).

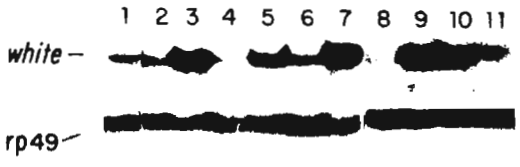

Figure 2. Effects of the su(w^{sp}) mutation on accumulation of white locus transcripts in adult head tissues. Shown are the results of hybridization to polyadenylated RNAs (10 micrograms per channel) isolated from adult heads with white locus or rp49 DNA sequence probes. The genotypes analyzed were as follows by lane: [1] w^{+}; [2] w^{sp1}; [3] su(w^{sp})w^{sp1}; [4] w^{sp2}; [5] su(w^{sp})w^{sp2}; [6 and 8] w^{sp3}; [7 and 9] su(w^{sp})w^{sp3}; [10] su(w^{sp})w^{a2}; [11] w^{a2}. The RNA blot was cut in half; the top portion was hybridized with the white probe and the bottom portion (as a control for the amount of polyadeylated RNA) was probed with the rp49 probe. The white probe has approximately 900 bases of homology to the major white transcript and is a combination of fragments from -1.2 to -0.4 kb and from +3.1 to +6.6 kb on the conventional white locus map (see Figure 1). The rp49 probe is the 0.6 kb EcoRI to HindII fragment containing most of the transcription unit (O'Connell & Rosbash 1984). The su(w^{sp}) mutations produce elevation in white transcript levels in adult body tissues in these genotypes quite similar to those shown here for head tissues (results not shown). The procedures for RNA blot analysis and tissue and RNA isolations are as described in Bingham & Zachar (1985) except that culture temperature was carefully controlled between 21° and 22°C here.

the w^{sp} alleles by having an intact w^{sp} region (Zachar & Bingham 1982; Figure 1). Numerous allele-specific suppressors are known in Drosophila (Lindsley & Grell 1968). In contrast to su(w^{sp}), the specificity of previously characterized suppressors is based on interaction with specific transposons (Bender et al. 1983; Modollel et al. 1983; L. Searles & R.A. Volker, pers. comm.). Thus, suppressor-of-white-spotted represents the first identified member of a novel class of suppressor mutation whose allele specificity is detemined by the portion of the target locus mutationally altered.

We have investigated the effects of su(w^{sp}) on accumulation of white transcripts in mature adult tissues. We find that w^{sp1}, w^{sp2}, w^{sp3}, w^{a2} and w^{+} alleles produce indistinguishable levels of a 2.6 kb transcript in adult head tissues and adult body tissues (Figure 2 and results not shown). This transcript is indistinguishable in size and transcriptional polarity from the major white transcript previously observed in unfractionated w^{+} tissues (O'Hare et al. 1983; Pirrotta & Brockl 1984). The su(w^{sp}) mutation produces a substantial elevation in white transcript levels in mature adult head and body tissues (Figure 2 and results not shown) in the cases of all tested genotypes (Figure 2).

In summary, the suppressor-of-white-spotted mutation is partially recessive and results in elevation of adult white transcript levels. The w^{sp} mutations cause apparently tissue-specific effects on white expression in immature adult eye tissues and the suppressor-of-white-spotted mutation largely relieves this effect without having a measurable effect on eye pigment deposition engendered by white alleles having an intact w^{sp} region.

On the basis of these results, we propose the following model for the function of the su(w^{sp}) locus product and the w^{sp} genetic element in regulating white expression in adult tissues: the su(w^{sp}) locus causes the production of a repressor active in several (and possibly all) adult tissues, mature and immature. The repressor is partially responsible for defining the levels of white transcripts in most of these tissues. However, in immature eye pigment cells (where eye pigment deposition occurs) the effect of this repressor on white transcription is completely antagonized by the action of a positive effector requiring or acting at the regulatory genetic element inactivated by w^{sp} mutations. This positive effector is present in immature adult eye pigment cells but exerts little or no effect on white transcript levels in mature adult tissues (much later).

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Changes of DNA replication pattern of the polytene chromosomes of **Drosophila melanogaster** resulting from chromosomal rearrangements.

Bender et al. (1971) showed that replication of a duplicated region of polytene chromosome is changed as a result of a rearrangement. A similar situation has also been noted by Kalisch & Haegele (1973) who also observed that, at comparable replication phase, the labelling intensity of duplicated subdivision is different from those of homologous subdivisions in +/+ chromosomes even when the different DNA amount of both genotype was taken into consideration. Ananiev & Gvozdev (1974), on the basis of their work on DNA replication in a Eu-heterochromatin rearrangement of Drosophila, also indicated that the replicative behaviour of a replicating unit of the transposed region is altered from the parental type. The following sets of experiments were therefore carried out to characterize the factor or factors presumably responsible for determining the replication pattern in greater detail by genetically dissecting the units. For this pur-

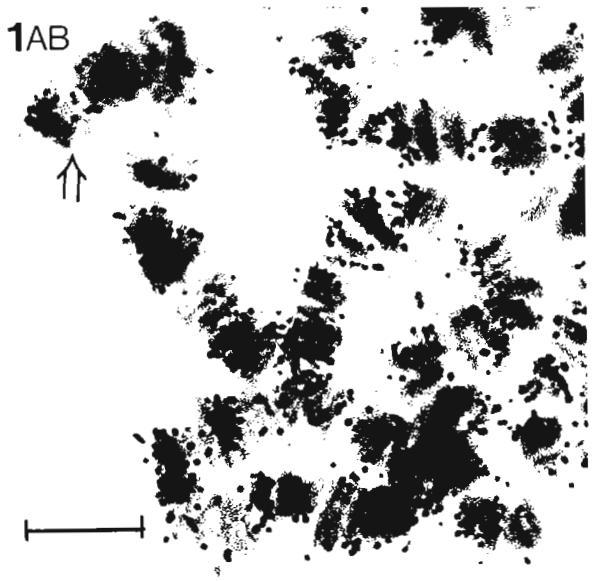

Figure 1. ^{3}H-TdR labelled autoradiogram of inversion heterozygote In(1)d149, 1(1)J1/+, showing the 3D pattern in 1(1)J1/+. Arrow indicates asymmetric distribution of label at 1AB site. Scale indicates 10 µm.

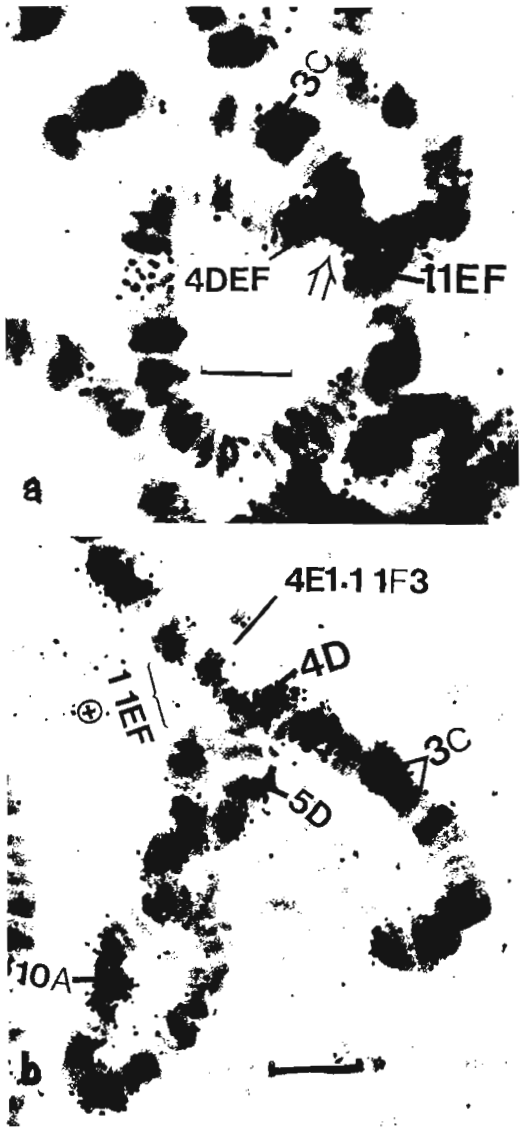

Figure 2. ^{3}H-TdR labelled autoradiograms of the X chromosomes of the inversion heterozygote In(1)d149, 1(1)J1/+. (a) An early pattern in In(1)d149/+. Arrow indicates absence of label at the point of break. (b) A terminal pattern in In(1)d149/+. Arrow points to the asynapsed homologues segments, one of which is unlabelled and the other labelled. Scale indicates 10 µm.

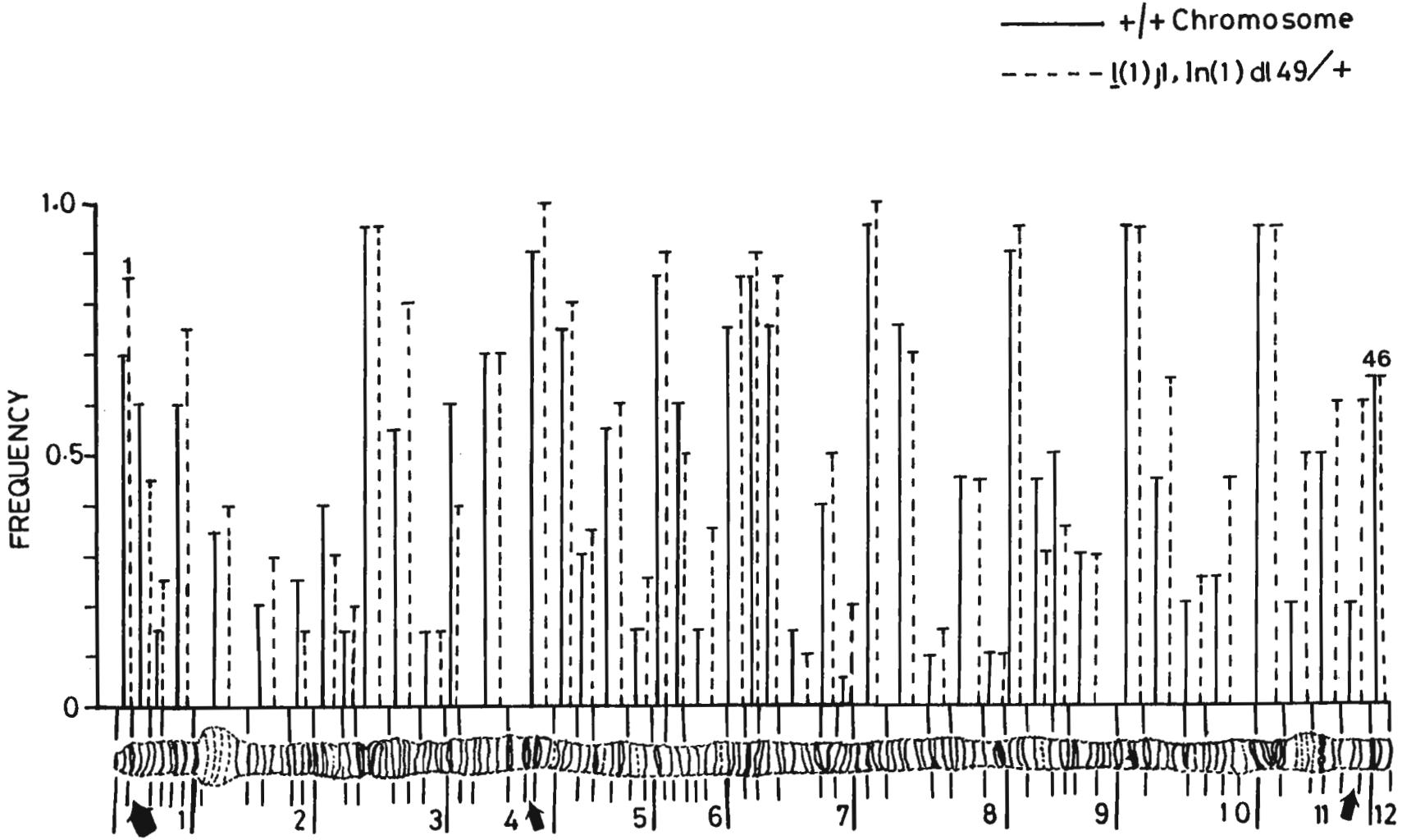

Figure 3. Histograms showing the frequencies of labelling of different replicating units in the normal and 1(1)J1, In(1)d149 bearing X chromosomes. Arrows at the bottom indicate the break points. Number 1 and 46 represent first and 46th replicating units.

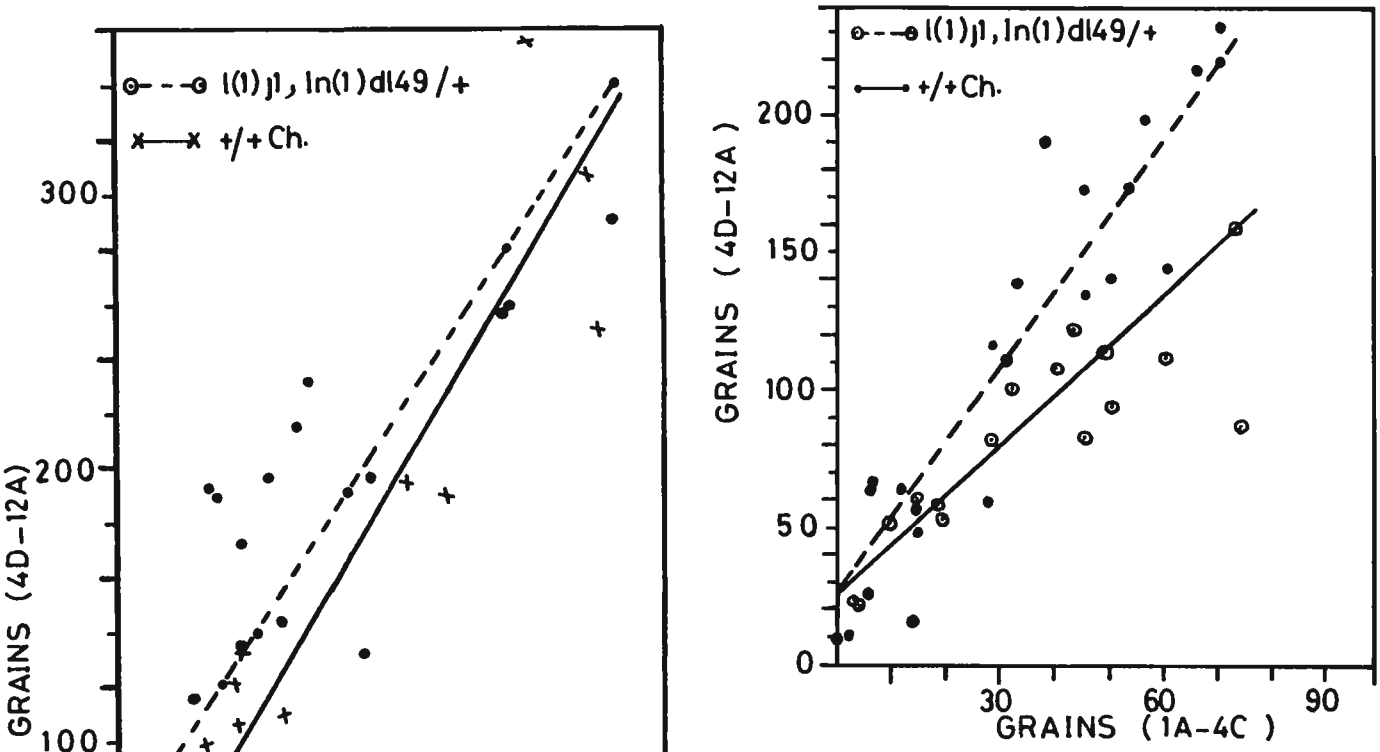

Figure 4. Relationship between the ^{3}H-TdR induced grain numbers on the segment 1A-4C and those on the segment 4D-12A of the X chromosome in +/+ and 1(1)J1, In(1)d149/+ genotypes of **D.melanogaster.** (a) For all late patterns. (b) For 2D-1D pattern only. Lines represent the calculated slopes.

pose, DNA replication in genetically altered sequences such as those involving breaks within a replicating unit, has been examined.

For this series of investigations, the wild type strain (Oregon R^{+}) and the inversion heterozygote In(1)J1, y w $1z^{s}$/+ stock of **D.melanogaster** were used. Excised salivary glands from late third instar larvae were dissected out in buffered Drosophila Ringer (pH 7.2) and incubated in 10 µl of Ringer's containing 5 µCi of ^{3}H-thymidine (Conc. 500 µCi/ml, Sp. activity 6,600 mCi/ml obtained from BARC, Bombay, India). Cytological preparations of chromosomes were then made and processed for autoradiography.

In this investigation, segment 1A-12A of the X chromosome have been analyzed for their order of replication in two altered sequences from the stock 1(1)J1, In(1)d149. In inversions, 1(1)J1, In(1)d149, the regions 1A5-1B5 and 4E1-11F2 have been inverted as two independent inversions, the resulting sequences in the two regions being 1A1-4.1B5-1A5. 1B6-4D7-11F2-4E1.11F3.....20. Thus, here an altered sequence has formed within the two replicating units (Lakhotia & Mukherjee 1970) 1A and 1B and this alteration involves about 8 bands. On the other hand, the unit 4DEF has been split at 4D7-4E1, the new order of replicating units being 4D7-11F2 and 4E1.11F3. These inversions have been examined only in heterozygous condition due to the presence of the lethal mutant.

The autoradiogram in Figure 1 reveal the 3D pattern at the terminal phase of replication. The distribution of the grain on the tip of the X chromosome reveals that there is clear asymmetry in the distribution of lebel on the two homologues, one of which appears to finish the replication a little earlier than the other. Since it is known that the telomeric end of the X is always late replicating, it is reasonable to assume that half of the tip part of the X chromosome which shows relatively lower labelling (Fig. 1) or absence of labelling, is the altered sequence of the chromosome, bearing 1(1)J1. One reservation to be

made, however, is that the analysis does not rule out the possibility of existence of a deletion as the cause of its slight early finishing.

Figures 2a and b present an initial and a terminal labelling pattern, respectively, of the paired X chromosomes in In(1)d149/+ heterozygote. No study on the homozygous female bearing the inversion has been possible as the inversion bearing X also carries a lethal on the tip of it. In figure 2a, which shows an early pattern, the unit 4DEF, being late replicating is unlabelled in both homologues, whereas 11EF, being early replicating is labelled completely across the bands. In contrast, in Figure 2b (in late phase of replication) the 4D is completely labelled on both homologues, while 4F-4E1.11F3 segment is labelled on the inverted segment but in the normal homologue the 11EF part is unlabelled. The 4EF section is also labelled in the normal homologue (hidden under 4D in the band of the loop in Fig. 2b).

The histogram of the frequencies of labelling [obtained from 22 labelled nuclei of middle to late pattern of replication, each in control and 1(1)J1, In(1)d149/+ genome] presented in Figure 3, corroborates the observations on the autoradiogram. Here in the inversion bearing chromosome, the frequency of 4D1-7 (to the left of the arrow on the segment 4) remains unaltered, whereas that of 4EF.11F3 drastically increases by a factor of about 3. Thus, it appears that in this case the late replicating property is inherent in the 4EF section. It may be noted that some other sites (namely 6DEF, 7F, 9C, 10B, 10DEF and 11B) also show some difference in frequency from normal.

No data on intensity of site-wise labelling has been presented for this inversion heterozygote, as such intensity data may be incorrect due to introduction of change in geometrical configuration of the segments involved which might arise out of pairing in the inversion heterozygote. However, a total grain count data over 1A-4BC, which is outside the inversion, and that over 4D-12A which includes the inversion, has been presented in Figures 4a and b. It is interesting to note there that when analysis is made considering all middle to terminal pattern labelled chromosomes (3D-1D type), the regression slopes do not show significant deviation. However, when the analysis is delimited by considering only the grain range of 0-90, a limit up to which no super-imposition or "coincidence" is detectable, the regression slope of grain number in +/+ and the inversion heterozygotes significantly deviated from each other (Fig. 4b). This finding is a strong evidence to support that the alteration in the sequence of the constituent subunits of the replicating unit affects the terminal patterns more significantly than the initial patterns. The results of the present investigation have therefore revealed that the rearrangement of polytene chromosomes might affect the kinetics of DNA synthesis by establishing new patterns of control.

This work is financially supported by University Grants Commission and Indian National Science Academy research grant to R.N.C.

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Effect of α-amanitin on the DNA synthesis in the polytene chromosome of **Drosophila melanogaster.**

In the giant cells of dipteran larvae, α-amanitin, a potent inhibitor of eukaryotic RNA polymerase II, inhibits chromosomal RNA synthesis but not the nucleolar RNA synthesis at a concentration range of 20 to 30 µg/ml (Beermann 1971; Chatterjee & Mukherjee 1982, 1984). α-amanitin is also known to inhibit DNA synthesis in eukaryotes (Montecuccoli et al. 1973; Chatterjee & Mukherjee 1977). It has been suggested that the toxin, α-amanitin inhibits the initial and middle part of S phase probably through the inhibition of the primer RNA. An investigation was therefore undertaken to find out whether the inhibition of DNA synthesis by α-amanitin is causally related to the inhibition of RNA polymerase II by the inhibitor. For this reason, parallel pulse labelling with ^{3}H-UR and ^{3}H-TdR of the two contralateral glands of a pair was performed.

For this series of investigations, salivary glands from late third instar larvae of **Drosophila melanogaster** were dissected out in buffered Drosophila Ringer (pH 7.2) and incubated for 20 minutes in α-amanitin (Conc. 20 µg/ml). One gland from the pair was transferred to ^{3}H-UR (300 µCi/ml, Sp. activity 7500 mCi/mM obtained from BARC, Bombay, India) containing Ringer and the remaining one was transferred to ^{3}H-Tdr Ringer (Conc. 500 µCi/ml, Sp. activity 6500 mCi/m mole, obtained from BARC, Bombay, India). Cytological preparations of chromosomes were then made and processed for autoradiography.

Results of these experiments are presented in Table 1. Since it was noted that the amatoxin fails to produce a selective inhibition of DNA synthesis only in male or only in female nuclei, the data from both sexes have been pooled together. In Table 2, the glands receiving ^{3}H-TdR were designated as 'A' (contralateral gland) and those receiving ^{3}H-UR, in the contralateral counterparts were coded as 'B'. Data presented in Table 1, show that as it was observed earlier (Chatterjee & Mukherjee 1977), α-amanitin

Table 1. Analysis of the frequency of ^{3}H-TdR labelled chromosomes and nucleoli in the contralateral glands of **D.melanogaster** after in vitro treatment with alpha-amanitin.

	Number of ^{3}H-Tdr labelled nuclei						Number of ^{3}H-UR labelled nuclei		
Gland number	Total number of nuclei observed	Only nucleoli labelled pattern (NL)	Early pattern (DD-2C)	Mid pattern (3C-3D)	Terminal pattern (2D-1D and chromocentre labelled)	Gland number	Total number of nuclei observed	Only nucleoli labelled pattern	Both chromosomes/nucleoli and nucleolus labelled
1A	123	--	--	9 (7.31%)	3 (2.45%)	1B	125	27 (21.60%)	23 (18.40%)
2A	90	1 (1.11%)	19 (21.11%)	2 (2.22%)	6 (6.67%)	2B	72	12 (16.44%)	14 (19.15%)
3A	53	1 (1.88%)	--	3 (5.66%)	3 (5.66%)	3B	74	24 (32.43%)	18 (24.32%)
4A	100	--	--	1 (1.00%)	2 (2.00%)	4B	26	--	--
5A	66	--	--	41 (62.12%)	17 (25.75%)	5B	21	14 (66.62%)	7 (33.33%)
6A	43	--	--	2 (4.65%)	--	6B	19	--	3 (15.79%)
7A	98	--	--	--	--	7B	86	--	1 (1.16%)
8A	104	--	2 (1.92%)	24 (23.08%)	37 (35.58%)	8B	50	2 (4.00%)	3 (6.00%)
9A	90	1 (1.11%)	2 (2.22%)	5 (5.55%)	11 (12.22%)	9B	134	44 (32.84%)	42 31.34%)
10A	58	9 (15.51%)	3 (5.17%)	2 (3.45%)	1 (1.72%)	10B	181	44 (24.31%)	84 (46.41%)
11A	71	1 (1.11%)	4 (5.63%)	8 (11.27%)	24 (33.80%)	11B	104	50 (48.08%)	52 (50.00%)
12A	56	--	--	--	--	12B	55	--	1 (1.82%)
13A	55	--	--	2 (3.64%)	1 (1.82%)	13B	45	2 (4.44%)	14 (31.11%)
14A	71	--	--	--	--	14B	121	17 (14.05%)	5 (4.13%)
15A	90	--	--	9 (10.00%)	7 (7.79%)	15B	35	6 (17.14%)	4 (11.13%)
16A	133	--	--	12 (9.02%)	8 (6.02%)	16B	79	6 (7.59%)	4 (5.06%)

drastically inhibits the initial phase of replication in the majority of the glands. Only in a few cases (see Table 1, Gland Nos. 2, 3, and 9-11), a small amount of early phase of DNA replication was observed. It was observed that the percentage of ^{3}H-UR labelled nuclei (both chromosome and nucleolus) ranged from approximately 1 to 48 (Table 1). Detailed analysis of the data presented in Table 1 reveals that there is no correlation between the ^{3}H-UR labelling on the nucleolus and the ^{3}H-TdR nucleolus labelled (NL) pattern. This is expected, since α-amanitin does not inhibit the activity of RNA polymerase I, which is responsible for rRNA (nucleolar RNA) synthesis. On the other hand, in the majority of cases, there is a reasonable correlation between the frequency of ^{3}H-TdR labelled nuclei and the frequency of ^{3}H-UR labelled cells (both chromosome and nucleolus labelled) in α-amanitin treated salivary glands (see Gland Nos. A and B, 1, 6, 7, 9, 12-16). In two pairs (Nos. 5 and 8), lack of correlation between ^{3}H-UR labelled chromosomes and ^{3}H-TdR labelled cells, have been noted. In the two glands there was a higher frequency of 3C-1D pattern of labelling in ^{3}H-thymidine labelled glands. This result is interesting because it appears from the data that α-amanitin, a RNA polymerase II inhibitor, is unable to interfere with the late phase of replication.

Table 2 presents statistical evaluation of the data in Table 1. The statistical evaluation of the data based on the correlation coefficient, clearly corroborates the above interpretation. The analysis of correlation was from the percent of frequencies of ^{3}H-TdR labelled cells and ^{3}H-UR labelled chromosome, as well as from the percent of frequencies of 3D-1D patterns. Results reveal that the measured values of correlation coefficients (r_i) of the three successive combinations (see Table 2) are 0.47, 0.35 and 0.93, respectively. All these values are positive and transcribe the fact that the correlation between the percent of ^{3}H-TdR labelled cells and that of ^{3}H-UR labelled chromosomes, as well as between the

Table 2. Analysis of the degree of correlation between ^{3}H-TdR and ^{3}H-UR labelling frequency in contralateral experiments of salivary gland chromosome of **Drosophila melanogaster** after in vitro treatment with alpha-amanitin.

Gland number	Frequency of ^{3}H-TdR labelled nuclei	Frequency of ^{3}H-UR labelled chromosomes	Frequency of mid to terminal pattern of ^{3}H-TdR labelled nuclei	Slope (a)	Intercept (b)	Correlation coefficent (r)	Multiple correlation coefficient ($r_{1.23}$)
1A/1B	9.75	18.40	9.75				
2A/2B	31.11	19.14	8.89				
3A/3B	13.21	24.32	12.22				
4A/4B	3.00	0.00	3.00				
5A/5B	87.88	33.33	87.88				
6A/6B	4.65	15.79	4.65	$b_{12.3}$=0.25	0.85	r_{12}=0.47	0.94
7A/7B	0.00	1.16	0.00	$b_{13.2}$=0.89		r_{23}=0.35	
8A/8B	60.50	6.00	58.66			r_{13}=0.93	
9A/9B	21.11	31.34	17.70				
10A/10B	25.86	46.41	5.17				
11A/11B	52.11	50.00	45.07				
12A/12B	0.00	1.00	0.00				
13A/13B	5.45	31.11	5.45				
14A/14B	0.00	4.13	0.00				
15A/15B	17.78	11.43	17.78				
16A/16B	15.04	5.06	15.04				

percent of ^{3}H-TdR labelled cells and that of 3C-1D patterns are significant at 5% level (Fisher's 'r' significant test Table). On the other hand, the correlation coefficient for the relation between the percent of ^{3}H-UR labelled chromosomes and that of 3D-1D patterns of ^{3}H-TdR labelling is not significant (r = 0.35). It appears therefore that there is a reasonably good correspondence between the proportion of ^{3}H-TdR labelled cells and that of ^{3}H-uridine labelled chromosomes. Conversely, therefore, the inhibition of ^{3}H-UR incorporation parallels the inhibition of ^{3}H-TdR incorporation by α-amanitin. A multiple correlation test on the percent of ^{3}H-uridine labelled chromosomes, the percent of all ^{3}H-thymidine labelled cells and the percent of 3D-1D patterns, yields a high positive correlation coefficient ($r_{12.3}$ = 0.94). Thus, the result shows clearly that the in vitro treatment of salivary glands with α-amanitin fails to intercept the labelling of late patterns (3D-1D), but drastically interferes with the initiation of replication. Therefore, the result of contralateral experiments suggest the inhibition of the initial pattern by α-amanitin is causally related to the inhibition of RNA polymerase II. So, on the basis of these observations, it may be suggested that the synthesis of RNA and initiation of DNA synthesis are closely coupled, and that disorder in the first process affects the process of initiation of DNA synthesis.

This work is financially supported by University Grants Commission and Council of Scientific and Industrial Research grants to R.N.C.

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Collins, M.F. and J.K. Hewitt. University of Birmingham, Birmingham, England. Correlations between the elements of male courtship behavior in a series of inbred lines of **Drosophila melanogaster** derived from the same population.

The male mating behavior of a series of 16 inbred lines of **D.melanogaster** derived the Texas (Barnes & Kearsey 1970) population was recorded using the time-sampling technique described in Collins, Hewitt & Gogarty (1984). The male courtship behavior in pairs of three day old male and female flies was scored, using Texas 15 females as a common tester genotype. The experiment was carried out in a fully randomised block design, consisting of ten blocks of five replicates. All courtships were recorded within the first four hours of a 12 hour day/night light cycle at 25°C.

Table 1. Means (±S.E.) of the male courtship behavior of a series of inbred lines derived from the Texas population.

Inbred line	CI	OI	WVI	LACI	COPI	NoCop
Texas 1	0.752 (0.0145)	0.339 (0.0114)	0.229 (0.0122)	0.065 (0.0022)	0.119 (0.0095)	12
Texas 3	0.653 (0.0126)	0.393 (0.0138)	0.129 (0.0105)	0.040 (0.0044)	0.091 (0.0071)	7
Texas 5	0.728 (0.0130)	0.330 (0.0100)	0.240 (0.0118)	0.049 (0.0051)	0.109 (0.0084)	11
Texas 6	0.756 (0.0141)	0.335 (0.0141)	0.210 (0.0109)	0.072 (0.0020)	0.139 (0.0118)	14
Texas 7	0.735 (0.0145)	0.351 (0.0114)	0.221 (0.0084)	0.056 (0.0041)	0.107 (0.0100)	12
Texas 8	0.805 (0.0110)	0.317 (0.0145)	0.261 (0.0141)	0.102 (0.0049)	0.125 (0.0118)	15
Texas 9	0.727 (0.0126)	0.325 (0.0182)	0.246 (0.0141)	0.058 (0.0022)	0.098 (0.0114)	10
Texas 10	0.682 (0.0114)	0.408 (0.0095)	0.122 (0.0118)	0.060 (0.0041)	0.092 (0.0095)	8
Texas 15	0.798 (0.0114)	0.320 (0.0158)	0.227 (0.0110)	0.116 (0.0037)	0.135 (0.0126)	15
Texas 17	0.700 (0.0122)	0.335 (0.0114)	0.205 (0.0114)	0.064 (0.0036)	0.096 (0.0095)	10
Texas 18	0.720 (0.0155)	0.369 (0.0105)	0.186 (0.0122)	0.044 (0.0032)	0.121 (0.0105)	12
Texas 19	0.751 (0.0118)	0.358 (0.0122)	0.195 (0.0109)	0.070 (0.0030)	0.128 (0.0077)	12
Texas 20	0.770 (0.0122)	0.329 (0.0100)	0.248 (0.0134)	0.074 (0.0035)	0.119 (0.0095)	12
Texas 25	0.721 (0.0184	0.348 (0.0100)	0.192 (0.0118)	0.074 (0.0035)	0.107 (0.0084)	10
Texas 27	0.771 (0.0179)	0.319 (0.0122)	0.244 (0.0100)	0.078 (0.0057)	0.130 (0.0084)	14
Texas 28	0.821 (0.0179)	0.311 (0.0141)	0.272 (0.0141)	0.100 (0.0022)	0.138 (0.0109)	15

Each mean is based on the mean of ten blocks. Indices are: proportion of observation periods male engaged in orientation of female (OI), wing vibration (WI), licking and attempted copulation (LACI) and copulation (COPI). CI = OI + WI + LACI + COPI. NoCop is number of pairs (out of 50) achieving copulation within 10 minutes.

Table 2. Correlation matrix between courtship elements.

	CI	OI	WVI	LACI	COPI	NoCop
CI		-0.65^{**}	0.68^{**}	0.85^{***}	0.78^{***}	0.92^{***}
OI			-0.87^{***}	-0.64^{***}	-0.25^{NS}	-0.50^{*}
WVI				0.46^{NS}	0.21^{NS}	0.50^{*}
LACI					0.60^{*}	0.75^{***}
COPI						0.88^{***}
NoCop						

Pearson product-moment correlations (r_{14}):
*significant at 5%; ** significant at 1%;
***significant at 0.1%; and NS = non-significant.

Table 1 shows the means and standard errors for the courtships of each of the 16 inbred lines. Significant differences between inbred lines were found for all measures of courtship beahvior. These differences reflect additive genetic variation (and possibly i-type epistatic variation) within the Texas population for these courtship measures. Differences in mating speed and courtship success are therefore evident within a set of inbred lines derived from the same population.

The data on the courtship elements were subjected to correlational analysis. Correlations were computed across inbred lines. The correlation matrix between the courtship elements is shown in Table 2. Positive correlations were found between all the courtship elements except for OI which was negatively correlated with CI, WVI, LACI, COPI and the number achieving copulation. This negative correlation suggests that a high score for orientation is predictive of a less successful courtship pairing and is further supported by results from a diallel cross on courtship behavior where dominance was for high overall courtship, WVI, LACI and COPI but for decreased orientation (Collins & Hewitt 1984). This idea is furthered mirrored in that the unsuccessful courtship by males of fertilised females is composed mostly of orientation (see Collins, Hewitt & Gogarty 1984). Bastock (1956) demonstrated that yellow males were less successful courters than wildtype males and that the yellow courtship was characterised by a high level of orientation behavior. Obviously males must pass through the orientation phase for successful courtship but must move quickly to the more important later courtship elements and should not remain at the orientation level which would reflect poor male courtship or female unreceptivity. These results indicate that it is important to distinguish between orientation and the other courtship elements in any investigations of male courtship behavior.

This work was supported by a SERC research studentship to MFC; MFC also acknowledges continued support through a SERC postdoctoral fellowship.

References: Barnes, B.W. & M.J. Kearsey 1970, Heredity 25: 1-10; Bastock, M.A. 1956, Evolution 10: 421-439; Collins, M.F., J.K. Hewitt & J.G. Gogarty 1985, D.I.S. 61; Collins, M.F. & J.K. Hewitt 1984, Heredity 53:321-337.

Collins, M.F., J.K. Hewitt and J.F. Gogarty.
University of Birmingham, Birmingham, England. Time-sampling courtship behavior in **Drosophila melanogaster.**

Biometrical genetic analysis requires scores on many individuals to provide adequate data for statistical analysis and parameter estimation. To measure the courtship behavior of individual files continuously over long periods is therefore impractical, and so procedures are needed which allow behavior to be time-sampled while yielding scores that reflect reliably the behavior under observation.

A plastic mating wheel of diameter 10cm with ten separate observation chambers (Hotta & Benzer 1976) each of area 0.64^2 and volume $0.38cm^3$ was used to observe courtship behavior. The flies were first lightly anaesthetised with CO_2 to facilitate transfer to the wheel using a soft paint brush. The wheel itself is such that it consists of a plastic disc for the females with ten holes which are initially out of register with those for the males. Two further outer discs with one hole each allow flies to be placed in either male or female chambers. The flies are allowed to accommodate to the wheel for five minutes. The two inner discs are then rotated to bring the male and female chambers into register and then the courtship of flies is noted. After each test the wheels are dismantled and washed thoroughly.

For all courtship tests the male behavior was categorised into four easily identifiable components: orientation, wing vibration, licking and attempted copulation, and copulation itself. Licking and attempted copulation were scored together as one category as licking is a behavior of short duration which would not be detected reliably by a time-sampling procedure and moreover it is known that licking is usually followed by attempted copulation. A score was given to a pair of flies every 30 seconds. The male courtship behavior was recorded over a ten minute period giving each male 20 courtship scores.

A courtship index (CI) is defined for a given male as the proportion of observation periods that showed any courtship behavior. Four further courtship indexes are defined for each behavioral element as the proportion of observation periods that showed any orientation (OI), wing vibration (WVI), licking and attempted copulation (LACI), and copulation itself (COPI). These four indices sum to the total courtship index. The copulation index should of course be a good indicator of mating speed.

An experiment was performed to test the validity of the time-sampling procedure described above. The courtship behavior of single pairs of flies in one chamber of a wheel was video-recorded for 10 minutes. The behavior was decoded using a computer as an event recorder. The continuous observation (CO) and

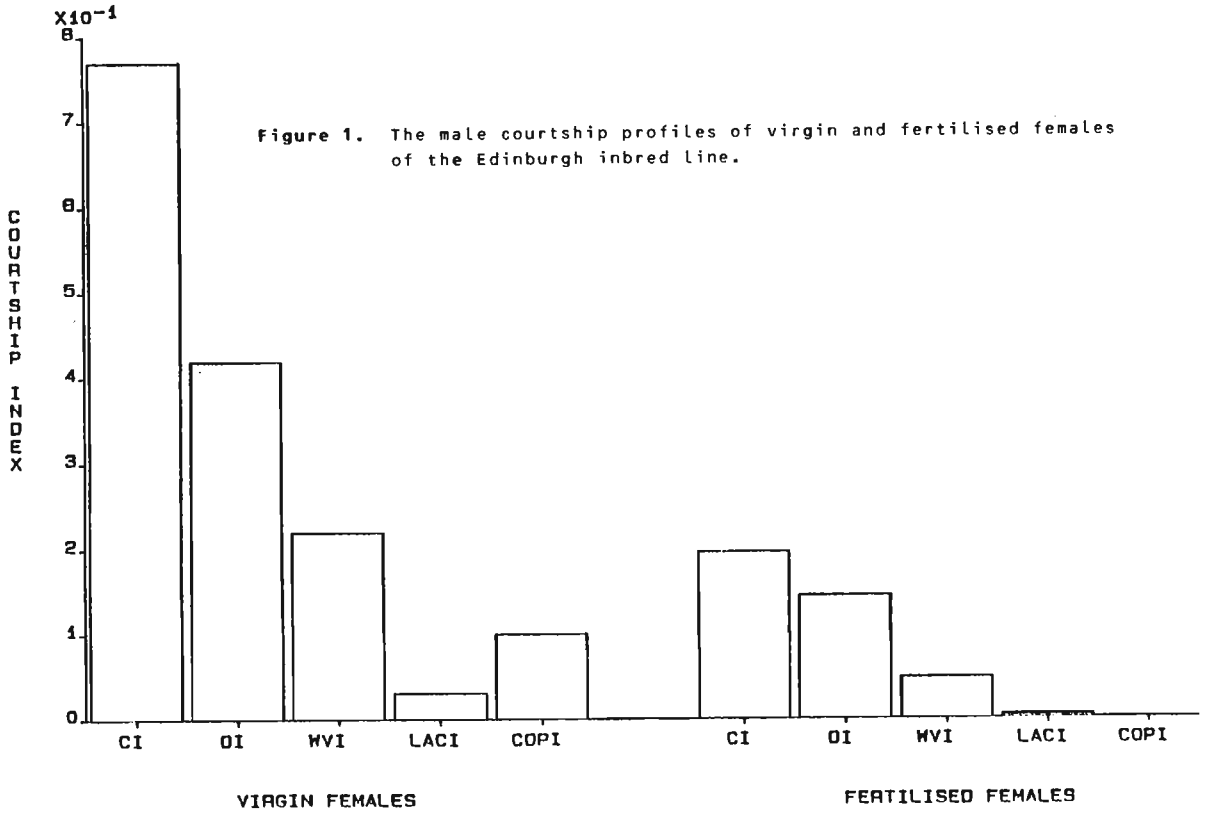

Figure 1. The male courtship profiles of virgin and fertilised females of the Edinburgh inbred line.

Table 1. Group means and between individual correlations for continously observed and time-sampled courtship behavior.

Behavior	Mean proportions of observations in each category of behavior (N=80)				Correlations across individuals*
	Continuous		Time-sampled		
	Mean	S.E.	Mean	S.E.	
Orientation (OI)	0.392	0.027	0.381	0.024	0.94
Wing Vibration (WVI)	0.113	0.013	0.114	0.015	0.90
Licking & attempted copulation (LACI)	0.019	0.002	0.028	0.004	0.51
Copulation (COPI)	0.248	0.023	0.250	0.022	0.99
Overall courtship (CI)	0.772	0.028	0.773	0.028	0.96

*Correlations were computed within each inbred line (N=20 per genotype) and pooled across inbred lines. All are significant at the 1% level.

the derived time-sampling (TS) scores were thus obtained from viewing the videotape once. The courtship behavior of twenty pairs of three day old flies of each of four inbred lines of **D.melanogaster** was recorded. Analysis of this data is presented in Table 1.

Highly significant correlations were found between time-sampled and continuously observed courtship. As would be expected, the behavior which is the least reliably time-sampled is licking and attempted copulation, the behavior which has the least total duration and occurs in the shortest bouts. It should also be noted that the very close agreement between the mean time-sampled proportion of observations spent in a particular courtship element and the mean proportion of time spent in a particular courtship phase for the continuously observed data would, of course, be expected for a reliable measure averaged over 80 pairs of flies. Nevertheless it is strong evidence for the reliability and validity of the time-sampling technique. We can conclude that time-sampling gives good agreement with continuous observation for proportional time spent in particular categories of behavior, except where they are infrequent or of short duration. The courtship index scores have been subjected to tests for skewness and kurtosis and no significant departures from a normal distribution were found and hence the data require no transformation.

A further experiment was designed to test this time-sampling procedure. Could the time-sampling technique reliably detect the often cited effect that virgin males court fertilised females less vigourously than virgin females (Connolly & Cook 1973)? The male courtship behavior of fifty virgin and fifty fertilised females of one inbred strain was time-sampled for ten minutes. Figure 1 shows the male courtship profiles of these virgin and fertilised females. The results clearly show a marked reduction in the male courtship of fertilised females for each courtship element. Not surprisingly no males copulated with a fertilised female within the ten minute observation period and furthermore well over 75% of the overall courtship of the fertilised females was spent in orientation with little progression to the more important courtship elements. The method is therefore sensitive to the differences in courtship intensity caused by exposure to virgin or mated females.

The economy of the method makes courtship amenable to the detailed analyses of biometrical genetics (see Collins & Hewitt 1984). Further application of the method should permit analysis of the role of genotype-environment interaction in mating behavior. It is also hoped that the method should be useful in the screening for further mutants of courtship behavior and possibly in the study of the effects of drugs on courtship behavior. A more detailed presentation of this work has been submitted to Behavior Genetics.

This work was supported by SERC research studentships to MFC & JFC, MFC also acknowledges continued support through a SERC postdoctoral fellowship.

References: Collins, M.F. & J.K. Hewitt 1984, Heredity 53:321-337; Connolly, K. & R. Cook 1973, Behav. 64: 142-166; Hotta, Y. & S. Benzer 1976, P.N.A.S. 73: 4154-4158.

Cook, J.L. and D.T. Kuhn. University of Central Florida, Orlando, Florida USNA. Spatial distribution of tuh abnormalities.

Transformations of the eye-antenna to posterior abdominal tergites and genitalia appear in the tumorous-head strain (tuh-lh; tuh-3). A casual analysis would lead one to assume that the distribution patterns are random. The objective of this communication is to show that the defects seem to respect at least some of the developmental restrictions shown for the eye-antenna (Baker 1978; Campos-Ortega & Waitz 1978).

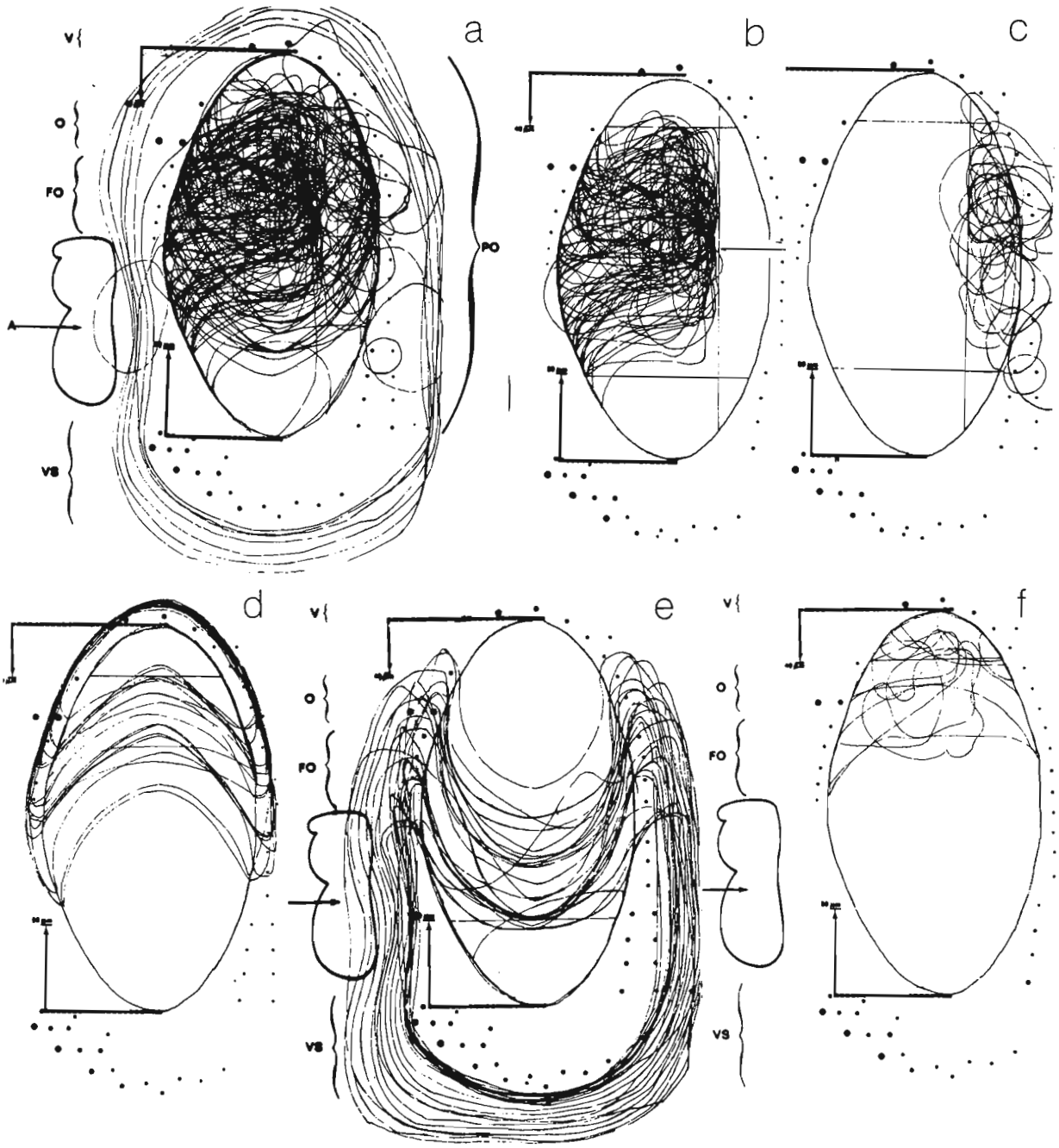

Figure 1. Distribution of selected tuh defects in the eye region. **V**, vertical bristles; **O**, orbital bristles; **FO**, frontorbital bristles; **A**, antenna; **VS**, vibrissae; **PO**, postorbital bristles.

Two hundred flies were hydrolyzed and the heads placed in lactophenol on microscope slides. Camera lucida drawings depicting the head were made using a wild-M8 stereoscopic microscope X80. For each group of head bristles emcompassing the eye, average number of bristles was determined from the drawings. An optical comparator (reticle graduated to 0.1 mm) was used to determine distances between diverse groups of bristles and those within a group. Data from 200 heads were averaged to produce a "typical" eye. Conversion factors were applied to each tuh eye to adjust its dimension to that of the model eye and the locations of the defects determined. The 200 tuh abnormalities selected for analysis were prescreened to insure that appropriate bristle markers were represented for accurate spatial mapping.

Camera lucida drawings are shown in Fig. 1a-f for the abnormalities. A compositie of defects seen in Fig. 1b,c,f and a group of abnormalities surrounding the entire eye are presented in Fig. 1a. Three restrictive margins were found. Figure 1a,b and c indicate that a large number of irregularities are confined to either the anterior or posterior side of a dorso-ventrally oriented restriction line that lies slightly posterior to the medio-lateral axis of the eye. Those abnormalities exhibited in half-heads demonstrating extensive reductions of the ommatidial number fail to observe this line. At about 130 µm down the longitudinal axis from the dorsal-most eye a region of high activity (HA) was observed (Fig. 1b). It lies on the anterior-posterior restriction line slightly above the horizontal bisector of the eye and expresses numerous abnormalities. Approximately 40 µm ventral to the top of the eye, there is a rather nebulous horizontal restriction line inasmuch as abnormalities more frequently exceed it than the previously described line (Fig. 1b,c,d and f). In the lower quadrant, roughly 50 µm from the bottom of the eye, there is another weak restrictive region (Fig. 1b,c and e). Small, isolated abnormalities circumscribed by ommatidia were occasionally documented in this area. The abnormalities in Fig. 1d and 1e exceed the dorsal and ventral restriction lines. Deviations in this neighborhood generally border a reduced eye.

Some previously described restriction lines correspond to regions frequently observed by the tuh defects. However, no specific abdominal tergite was confined to any of these regions although 8th tergite was expressed only in the anterior eye.

Supported by NIH Grant AGO-1846, NSF Grant PCM-8403124, ACS-Fla. Div. summer research fellowship to JLC.

References: Baker, W.K. 1978, Dev. Biol. 62:447-463; Campos-Ortega, J.A. & M. Waitz 1978, Wilhelm Roux's Arch. 184:155-170.

De Frutos, R. and L. Pascual. University of Valencia, Spain. Weak points and ectopic pairing in polytene chromosomes of **Drosophila subobscura.**

Intercalary heterochromatin in polytene chromosomes has been related to different biochemical and cytological features, such as ectopic pairing, tendency to break, late replication, etc. In a first attempt to detect intercalary heterochromatin sites in polytene chromosomes of **Drosophila subobscura**, the tendency to break (weak points) and ectopic pairing, were analyzed.

A total of 1152 slightly squashed nuclei of larval salivary glands were observed by optic microscopy analysis. 714 breaks and 374 ectopic contacts were detected. Centromeric contacts were not included because most nuclei showed centromeric pairing. Only ectopic contacts between not-centromeric or between non-centromeric and centromeric regions were taken into account. Weak points and ectopic contact sites are indicated in Fig. 1. Their location is based on the standard salivary gland chromosome map of Kunze-Mühl & Müller (1958). The map includes the situation of weak points or ectopic pairing sites only, but not their frequencies. A total of 63 weak points and 151 contact sites were identified. The number of weak points is clearly lower than ectopic contacts. In general, break points coincide with sites of ectopic pairing. The distribution of both features does not seem to be erratic. Clusters of them are found in some regions, for instance, the proximal half of the J and U chromosomes. On the other hand, weak points do not coincide with the boundaries of inversions. These chromosome arrangements are very frequent in **Drosophila subobscura.** The various types of weak chromosome points described by Zhimulev et al. (1982): breaks, semibreaks, constrictions and shifts, were found in polytene chromosomes of this species. Depending on the regions, they tend to show one or another type of break. For instance, 23E site generally shows constrictions, 27C shifts, etc. With respect to the regions involved in each of the ectopic contacts, they tend to take place between neighbouring zones of the same chromosome. Most of them occur between strong bands. Chromatin threads arise from either intact or broken large bands. However, in a few cases a tangle of threads arise from a whole interband, for instance, the whole of section 47. Also, threads arise from active puffs or the Balbiani ring with a very low frequency. Furthermore, in many cases

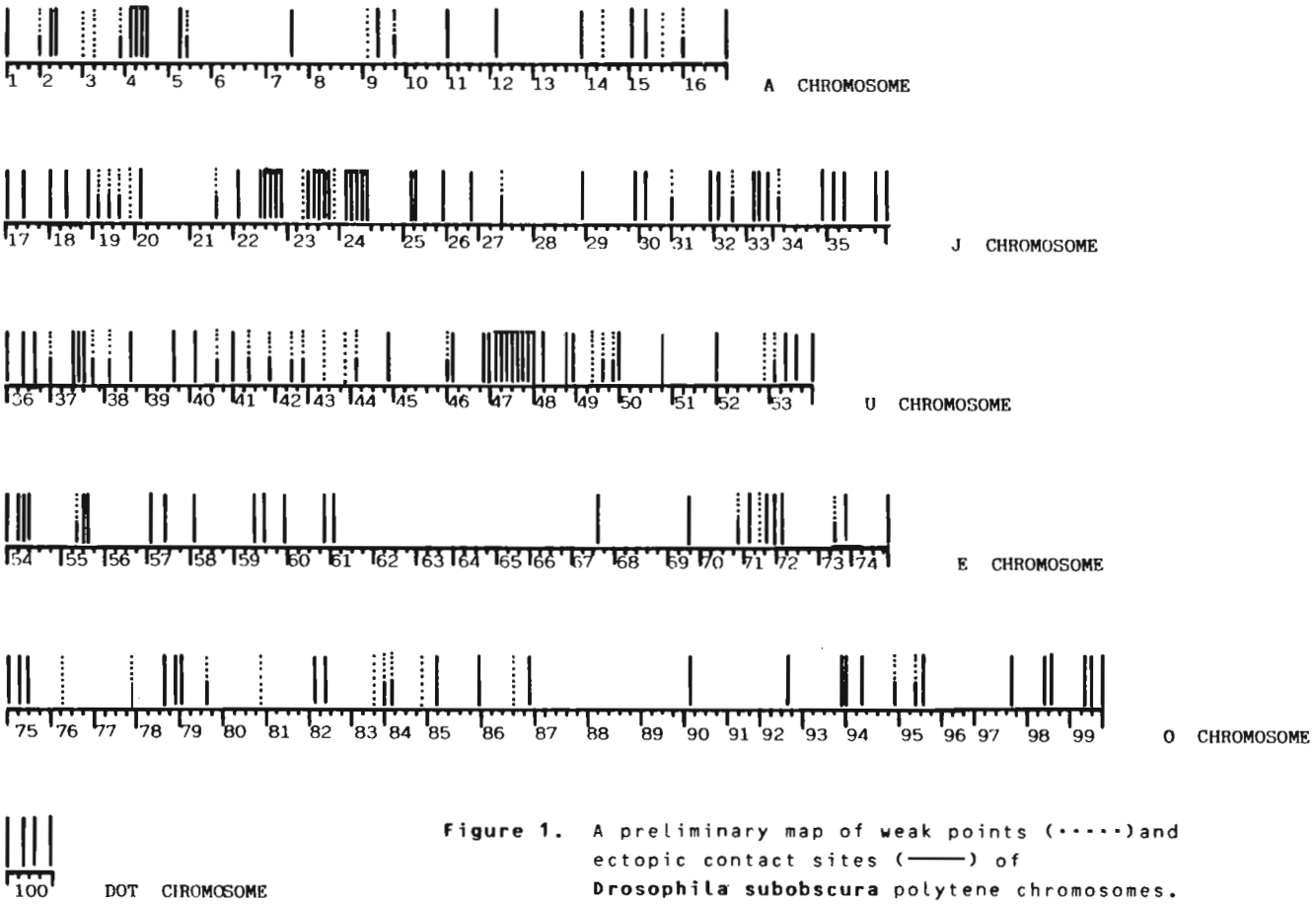

Figure 1. A preliminary map of weak points (·····)and ectopic contact sites (——) of **Drosophila subobscura** polytene chromosomes.

breaks, constrictions and ectopic contacts, are located close to active puffs. If both features could be taken as an indication of intercalary heterochromatin, it can be suggested that intercalary heterochromatin does not affect the gene expression of neighbouring regions.

References: Kunze-MÜhl, E. & E. MÜller 1958, Chromosoma 9:559-570; Zhimulev, I.F. et al. 1982, Chromosoma 87:197-228.

Del Puerto, G.A., J.J. Culich and C.J. Chihara.
University of San Francisco, California USNA.

Last larval instar cuticle protein patterns and their use for the identification of twenty-one species of Drosophila.

Ten different third instar larval cuticle proteins have been described for **D.melanogaster** and have been labelled as L3CP 1-9 (Figure 1). Genetic studies have shown that the production of each of these proteins is under the control of a different locus. L3CP 1-4 have been located on the second chromosome, whereas L3CP 5, 6, and 8 have been placed on the third chromosome (Fristrom et al. 1978; Snyder et al 1981, Chihara et al. 1982; Chihara & Kimbrell, unpubl.). Studies have shown that in related species some of the cuticle proteins seem to be shared among species in so far as the amino acid constitution of the proteins is concerned (Hackman 1971, 1976; Willis et al. 1981). Recently, electrophoresis has become an invaluable tool for insect systematics. Most of these studies have focused on genetic variation of enzymatic loci and have helped in the description and identification of different species (Avise 1974; Berlocher 1984).

Twenty-one different species of Drosophila belonging to the groups **melanogaster, obscura, virilis,** and **repleta** were studied for their urea soluble, last instar larval cuticle proteins using P.A.G.E. Each species was found to have a unique cuticle protein pattern that can be used to establish larval species identity. In addition, we have also developed consensus on last larval instar cuticle protein patterns for four species: **D.simulans, D.persimilis, D.pseudoobscura,** and **D.virilis.** Our results suggest that it is possible to identify a particular species of Drosophila based on its last instar larval cuticle protein pattern.

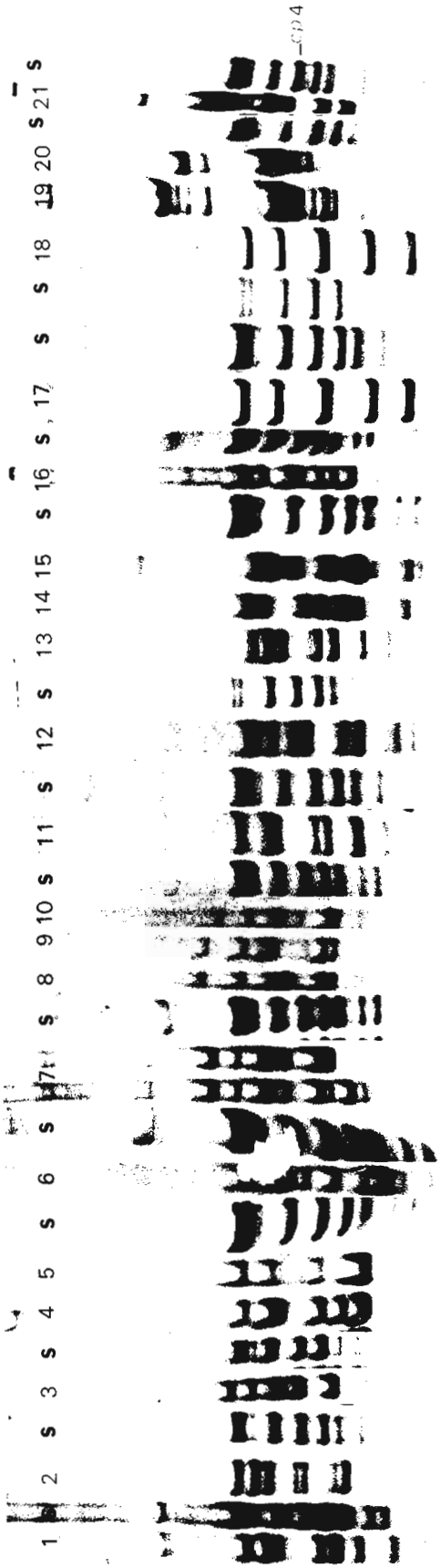

Figure 1. Cuticle protein patterns of Drosophila species studied. Cuticle protein bands are read from top to bottom with the first dark band representing the first protein band; white dots indicate variant bands within a species. (S) **D.melanogaster** standard, (1) **D.simulans** consensus, (2) **D.mauritiana**, (3) **D.yakuba**, (4) **D.lutescens**, (5) **D.prostipennis**, (6) **D.takahashii**, (7) **D.ananassae**, (8) **D.bipectinata**, (9) **D.pseudoananassae nigrens**, (10) **D.pseudoananassae pseudoananassae**, (11) **D.jambulina**, (12) **D.kikkawai**, (13) **D.auraria**, (14) **D.rajasekari**, (15) **D.ficusphila**, (16) **D.eugracilis**, (17) **D.persimilis** consensus, (18) **D.pseudoobscura** consensus, (19) **D.americana**, (20) **D.virilis** consensus, (21) **D.hydei**, (cp 4) Third instar larval cuticle protein 4 of **D.melanogaster**.

Fifty-six stocks, representing thirty-five different locations in nineteen different countries world-wide were used in this study. All stocks were purchased from the National Drosophila Species Resource Center, Bowling Green State University, Ohio, with the following exceptions: (1) four **D.simulans** stocks (Guatemala, Morro Bay, South Africa, and Kushla-F) were obtained from the California Institute of Technology, (2) **D.persimilis, D.pseudoobscura**, and **D.mauritiana** were obtained from Dr. T. Prout at U.C. Davis, and (3) **D.virilis** (wild type) was purchased from the Carolina Biological Supply Company. Our control stock, an Oregon R strain of **D.melanogaster**, has been maintained at the University of San Francisco, CA., since 1976. We call this strain the "standard" since it is invariant for all the cuticle proteins, and is the stock against which all other Drosophila species are compared.

Late last instar larvae of all species were recovered for dissection. Cuticles were recovered and extracted according to Chihara et al. (1982). All protein extractions were assayed by vertical gel electrophoresis using nondenaturing gels as described by Chihara et al. (1982). All samples were run in cold Borate buffer pH 8.6 (18.2 g/lt Boric Acid) or cold Tris-Glycine buffer pH 8.6 (3.03 g/lt Tris, 14.4 g/lt Glycine). Denaturing SDS gels were made according to Laemmli (1970). Gels were 17.5% Acrylamide, 0.46% Bis-acrylamide. Running buffer was Tris-Glycine (as described above) with 0.1% SDS. All gels were stained with 0.01% Coomasie Brilliant Blue G-250 or R-250 and were subsequently dried for future reference. Immunodiffusion was performed using the Ouchterlony procedure in 1% Agarose-Tris gels. Antibody against the third instar larval cuticle proteins of **D.melanogaster** was used and is described by Chihara et al. (1982).

The mobility of the cuticle proteins of all species was determined in relation to the mobility of L3CP 4 of **D.melanogaster.** Although thousands of wild genomes have been screened for cuticle protein variants of **D.melanogaster** none has ever been recovered for L3CP 4. It was for this reason that mobility values (R4) were determined relative to the mobility of L3CP 4 of **D.melanogaster.** Migration distances were measured on dried gels, using a centimeter ruler calibrated in millimeters, from the top of the separating gel to the mid-line of each protein band. The R4 was calculated as the ratio of the distance of protein migration to the distance of L3CP 4 migration. Accordingly, L3CP 4 of **D.melanogaster** had an R4 of 1.00, slower migrating proteins an R4 of less than 1.00, and faster migrating pro-

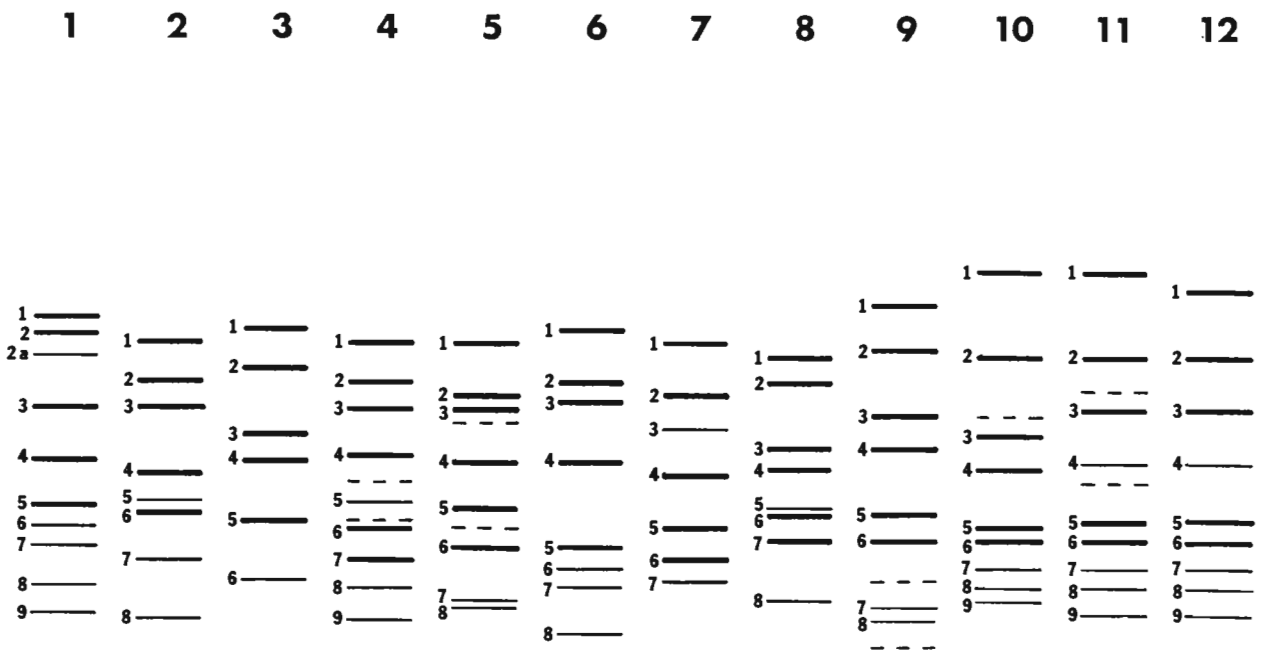

Figure 2. Comparison of last larval instar cuticle protein patterns. Subgroup **melanogaster:** (1) **D.melanogaster** standard, (2) **D.simulans** consensus, (3) **D.yakuba,** (4) **D.mauritiana.** Subgroup **takahashii:** (5) **D.lutescens,** (6) **D.prostipennis,** (7) **D.takahashii.** Subgroup **suzukii:** (8) **D.rajasekari.** Subgroup **ananassae:** (9) **D.ananassae,** (10) **D.bipectinata,** (11) **D.pseudoananassae nigrens,** (12) **D.pseudoananassae pseudoananassae.** Dashed lines represent variant protein bands. See Table 1 for individual protein band R4 values.

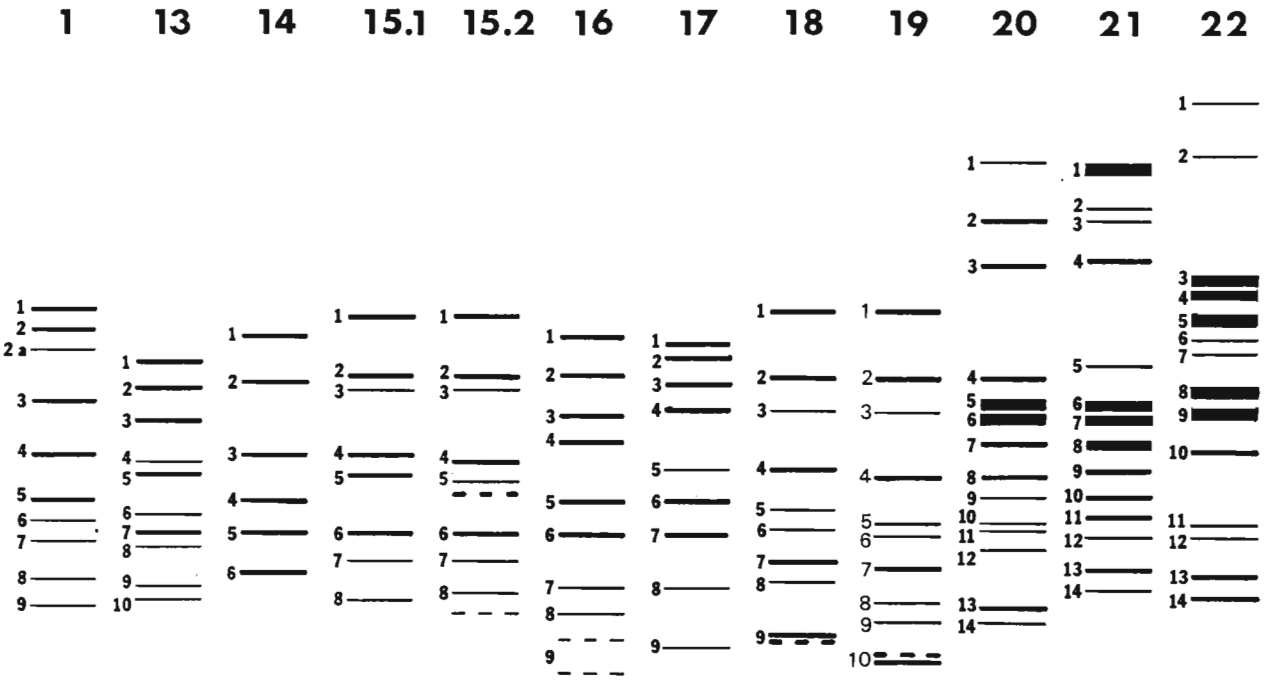

Figure 3. Comparison of last larval instar cuticle protein patterns. (1) **D.melanogaster** standard. Subgroup **ficusphila:** (13) **D.ficusphila.** Subgroup **eugracilis:** (14) **D.eugracilis.** Subgroup **montium:** (15.1) **D.jambulina** 3116.66, (15.2) **D.jambulina** 3117.6, (16) **D.kikkawai,** (17) **D.auraria.** Subgroup **obscura:** (18) **D.persimilis** consensus, (19) **D.pseudoobscura** consensus. Group **virilis:** (20) **D.virilis** consensus, (21) **D.americana.** Subgroup **hydei:** (22) **D.hydei.** Dashed lines represent variant protein bands. See Table 2 for individual protein band R4 values.

Table 1. Relative mobility values (R4) for Drosophila species in the Group **melanogaster.**

Protein	LANE: 1	2	3	4	5	6	7	8	9	10	11	12
1	0.78	0.82	0.80	0.82	0.82	0.80	0.82	0.84	0.76	0.71	0.74	0.74
2	0.81 0.84[a]	0.88	0.86	0.88	0.90	0.88	0.90	0.88	0.83	0.84 0.93[a]	0.84 0.89[a]	0.84
3	0.92	0.92	0.96	0.92	0.92 0.94[a]	0.91	0.95	0.98	0.93	0.96	0.92	0.92
4	1.00	1.02	1.00	0.99 1.03[a]	1.00	1.00	1.02	1.01	0.98	1.01	1.00 1.03[b]	1.00
5	1.07	1.06	1.09	1.06 1.09[b]	1.07 1.10[b]	1.13	1.10	1.07	1.08	1.11	1.09	1.09
6	1.11	1.08	1.18	1.11	1.13	1.16	1.15	1.08	1.12 1.18[a]	1.12	1.12	1.12
7	1.13	1.15		1.15	1.21	1.19	1.18	1.12	1.22	1.16	1.16	1.16
8	1.19	1.24		1.19	1.22	1.26		1.21	1.24 1.28[b]	1.19	1.19	1.19
9	1.23			1.24						1.21	1.23	1.23

Legend: Lane numbers on top as designated in Figure 2.

Footnotes: Lane 1: **a** = R4 value for L3CP 2e. Lane 4: **a** = R4 value for putative fast variant of L3CP 4; **b** = R4 value for putative slow variant of L3CP 6. Lane 5: **a** = R4 value for L3CP 3 as seen in stock 3388.1; **b** = R4 value for putative fast variant of L3CP 5. Lane 9: **a** = R4 value for putative fast variant of L3CP 6 as seen in stock SB 18.8D; **b** = R4 value for faint band observed below L3CP 8 in stock SB 18.8C. Lane 10: **a** = R4 value for protein band observed below L3CP 2 in stock 3054.3E. Lane 11: **a** = R4 value for faint protein band observed above L3CP 3; **b** = R4 value for putative fast variant of L3CP 4.

Table 2. Relative mobility values (R4) for Drosophila species in the Groups **melanogaster, obscura, virilis,** and **repleta.**

Protein	LANE: 13	14	15.1	15.2	16	17	18	19	20	21	22
1	0.86	0.82	0.79	0.79	0.82	0.83	0.78	0.80	0.55	0.56	0.46
2	0.90	0.89	0.88	0.88	0.88	0.85	0.88	0.90	0.64	0.62	0.54
3	0.95	1.00	0.90	0.90	0.94	0.89	0.93	0.94	0.71	0.64	0.73
4	1.01	1.07	1.00	1.01	0.98	0.93	1.02	1.04	0.88	0.70	0.75
5	1.03	1.12	1.03	1.04 1.06[a]	1.07	1.02	1.08	1.11	0.92	0.86	0.79
6	1.09	1.18	1.12	1.12	1.12	1.07	1.11	1.14	0.94	0.92	0.82
7	1.12		1.16	1.16	1.20	1.12	1.16	1.19	0.98	0.94	0.84
8	1.14		1.22	1.21 1.24[b]	1.24	1.20	1.19	1.23	1.03	0.98	0.90
9	1.20				1.28[a] 1.33[b]	1.29	1.27 1.28[a]	1.26 1.30[a]	1.06	1.02	0.93
10	1.22							1.32	1.10	1.06	0.99
11									1.11	1.09	1.10
12									1.14	1.12	1.12
13									1.23	1.17	1.18
14									1.25	1.20	1.21

Legend: Lane numbers on top as designated in Figure 3.

Footnotes: Lane 15.2: **a** = R4 value for putative fast variant of L3CP 5; **b** = R4 value for faint band observed below L3CP 8. Lane 16: **a** = R4 value for L3CP 9 as seen in stock 3046.6; **b** = R4 value for L3CP 9 as seen in stock 3014.4. Lane 18: **a** = R4 value for putative fast variant of L3CP 9 as seen in stocks KE K3 and KE K22. Lane 19: **a** = R4 value for putative slow variant of L3CP 10 as seen in stocks AR 148 and HH 21.

teins an R4 greater than 1.00. Consensus patterns were assigned to the species listed above by calculating mean R4 values for all invariant protein bands from their respective stocks. The stock best matching the mean R4 values was designated as the consensus for that species.

Our results indicate that the last instar larval cuticle protein patterns for each Drosophila species is unique, and that species within the same subgroup share proteins with similar mobilities. All species appeared to have between six to ten protein bands with the exception of **D.virilis, D.americana,** and **D.hydei** where 14 bands were visualized. Putative fast or slow variants for some of the bands were observed in nine different species. The cuticle protein patterns and number of protein bands per species are diagrammed in Figures 2 and 3. The R4 values for each protein band and the character of the variant bands are given in Tables 1 and 2. The protein bands which migrated toward the anode were numbered consecutively for each species with the least electronegative band numbered 1; therefore, band numbers should not be taken to indicate any homology between species, but only as relative mobilities. The protein bands in Figure 1 are labelled with white dots to indicate variant bands.

Although **D.melanogaster, D.simulans, D.mauritiana,** and **D.yakuba** (Subgroup **melanogaster**) are considered sibling species, **D.melano-**

Table 3. Molecular weight ranges for Drosophila species.

Species	Molecular weight ranges
D.melanogaster	8.5K - 22K
D.simulans	less than 6K - 22K
D.mauritiana	6K - 19K
D.yakuba	7K - 18K
D.lutescens	5.9K - 21.7K
D.takahashii	5.9K - 22K
D.ananassae	9.9K - 22K
D.bipectinata	9.9K - 21.8K
D.pseudoananassae	9.9K - 21.5K
D.jambulina	9K - 18K
D.kikkawai	less than 6K - 18K
D.auraria	less than 6K - 18K
D.rajasekari	less than 6K - 22K
D.ficusphila	less than 6K - 22.5K
D.eugracilis	9.9K - 22K
D.persimilis	10K - 22K
D.pseudoobscura	10K - 22K
D.americana	less than 6K - 24K
D.virilis	8K - 24K
D.hydei	12K - 21.5K

gaster and **D.simulans** share only two proteins with identical mobilities, whereas **D.mauritiana** and **D.simulans** share six (Figure 2 and Table 1, lanes 1-4). The results obtained suggest that related structural genes may be shared by all four species. If this is the case, our results agree with studies on enzymes which conclude that **D.simulans** and **D.mauritiana** are more closely related to each other than to **D.yakuba** and **D.melanogaster** (Eisses et al. 1979; Gonzalez et al. 1982).

D.lutescens, D.prostipennis, and **D.takahashii** (Subgroup **takahashii**) have similar electrophoretic patterns; however, their mean R4 values differ slightly. These patterns are most clearly distinguished from one another by the position of L3CP 3 in **D.takahashii** and of L3CP 5 in **D.lutescens** (Figure 2 and Table 1, lanes 5-7).

D.ananassae, D.bipectinata and **D.pseudoananassae** (Subgroup **ananassae**) also have similar cuticle protein patterns as visualized on polyacrylamide gels. The main difference that permits visual discrimination of the subspecies **pseudoananassae** and **nigrens** is the variation in L3CP 1 and the two additional faint bands in **D.pseudoananassae nigrens** (Figure 2 and Table 1, lanes 9-12).

D.jambulina, D.kikkawai, and **D.auraria** (Subgroup **montium**) do not have similar overall electrophoretic patterns on the gels; however, some of the protein bands between these three species have the same mobilities (Figure 3 and Table 2, lanes 15.1-17).

The overall pattern for **D.persimilis** and **D.pseudoobscura** (Subgroup **obscura**) seems at first glance to be identical; however, the main difference between the two sibling species is the mobility of L3CP 8 (**D.persimilis** R4=1.19 and **D.pseudoobscur**a R4=1.23) which allowed for quick visual discrimination of the two species (Figure 3 and Table 2, lanes 18 and 19).

D.americana and **D.virilis** (Group **virilis**) have very similar electrophoretic patterns on polyacrylamide gels. Proteins thirteen and fourteen of **D.virilis** have faster mobilities than the same protein bands of **D.americana**; this last difference, together with the position of protein band 2, of **D.americana** allows for visual discrimination of the two species (Figure 3 and Table 2, lanes 20 and 21).

D.rajasekari, D.ficusphila, D.eugracilis, and **D.hydei** (Subgroups **suzukii, ficusphila, eugracilis,** and **hydei,** respectively) were not compared to any other species within their subgroup; however, last instar larval cuticle protein patterns and their respective R4 values for these species are given in Figures 2 and 3; Tables 1 and 2.

Our results show that the third instar larval cuticle proteins of all species in the Group **melanogaster** are antigenically related to the third instar larval cuticle proteins of **D.melanogaster.** It is possible that all species in this group share common structural genes for at least some of the third instar larval cuticle proteins. In contrast, Groups **obscura, virilis,** and **repleta** did not react with **D.melanogaster** third instar antibody.

For the majority of species, most proteins fall within the range of **D.melanogaster** (8K-22K); however, many species showed either very small proteins or heavier proteins (below 6K or above 22K).

In conclusion, our results indicate that the last instar larval cuticle protein patterns for each Drosophila species is unique, and that species within the same subgroup share proteins with similar mobilities. At the same time, our work is consistent with that of others who have shown that protein components of Drosophila species are similar, and that protein patterns of closely related species are more similar and probably share more protein components than distant species of Drosophila (Hackman 1971). Our results suggest that with the use of PAGE, a species (and even a subspecies) of Drosophila can be easily identified by visual comparison of the overall pattern of its last larval instar cuticle proteins with that of **D.melanogaster,** or with closely related species, and secondarily by calculating R4 values for each protein.

References: Avise, J.C. 1974, Syst. Zool. 23:465-481; Berlocher, S.H. 1984, Ann. Rev. Entom. 29:403-433; Chihara, C.J., D.J. Silvert & J.W. Fristrom 1982, Devel. Biol. 89:379-388; Eisses, K.T., H. van Dijk & W. van Delden 1979, Evolution 33:1063-1068; Fristrom, J.W., R.J. Hill & F. Watt 1978, Biochem 17:3917-3924; Gonzalez, A.M., V.M. Cabrera, J.M. Larruga & A. Gullon 1982, Evolution 36:517-522; Hackman, R.H. 1971, in:Chemical Zoology (Florkin & Scheer, eds.), Academic Press, New York, p1-62; Hackman, R.H. 1976, in:The Insect Integument (Hepburn, ed.), Elsevier, p107-119; Laemmli, U.K. 1970, Nature 277:680-685; Snyder, M., J. Hirsch & N. Davidson 1981, Cell 25:165-177; Willis, J.H., J.C. Regier & B.A. Debrunner 1981, in:Current Topics in Insect Endocrinoloogy and Nutrition (Bhaskaran et al., eds.), Plenum, New York, p27-46.

Di Pasquale Paladino, A., P. Cavolina and A. Vella.
Universitá di Palermo, Italy. Lack of response to selection for high and low penetrance in the tu-pb stock of **Drosophila melanogaster.**

Remarkable changes with regard to the penetrance of the melanotic tumor character have been shown to occur periodically in the tumorous stock of tu-pb of **Drosophila melanogaster**, Figure 1.

In the search of verifying if this variability was to attribute to the choice of different modifying genes, two selection experiments--one for high penetrance (Line H) and another for low penetrance (Line L)--in standard conditions of rearing were made. From the results obtained (Figure 2) no modification can be shown in divergent sense with the respect to the tumoral incidence in the two lines H and L.

Nevertheless considerable swingings of the percentage of tumorous individuals, equally affecting the two lines, are recordable during the 14 generations. The lack of response to the directional selection could be explained with a very high genetic homogeneity of the modifying genes or with their absence; one could also think that the genome structure is such that doesn't allow reassortment of modifier genes. In any case, percentage swingings should constitute a purely phenotypic variability, the manifestation of tumors in the individuals of the tu-pb stock being susceptible of changes because of factors extraneous to the genome.

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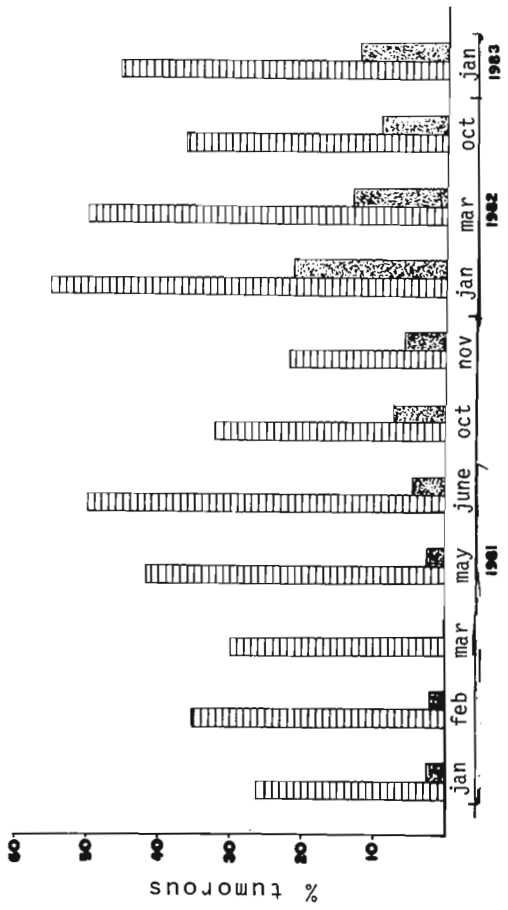

Figure 1. Histogram showing tumor penetrance variations during two years; females (dashed bar), males (hatched bar).

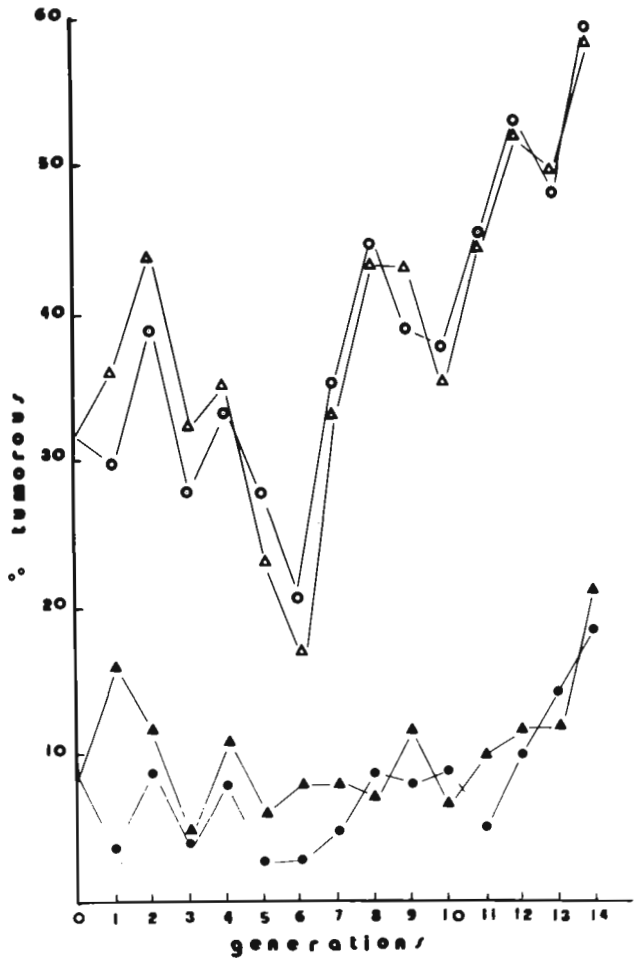

Figure 2. Percent response to selection for high Δ and low **O** penetrance in females and for high ▲ and low ● penetrance in males.

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Location of a factor with effect on sternopleural bristle number in **Drosophila melanogaster.**

An analysis of genes responsible for the difference in sternopleural bristle number between two inbred lines of **D.melanogaster** is presented. The fact that the lines are inbred and have not been selected for the trait as were those lines used in previoius studies (Breese & Mather 1957; Thoday et al. 1964; Spickett & Thoday 1966; Robertson 1967; Davies 1971) makes unlikely that the located polygenes be linked complexes in coupling phase.

Inbred lines were derived from a wild strain of **D.melanogaster** by 60 generations of brother x sister matings. The two analyzed lines, numbered 1 and 4, show a mean bristle number per sternopleura in females of 9.45 ± 0.18 and 7.30 ± 0.25, respectively (limiting the study to females simplifies the process by eliminating complications due to sex dimorphism or to environmental or genetic interactions with sex).

All possible homozygous combinations of major chromosomes from lines 1 and 4 were synthesized according to the method employed by Kearsey & Kojima (1967). The balancer chromosomes used were Binscy for the X chromosome, SM5 Cy for chromosome II and TM3 Sb Ser for chromosome III (for description of all special chromosomes used in this study, see Lindsley & Grell 1968). The effects of the three major chromosomes on bristle number per sternopleura and their contributionis to the total variance are given in Table 1. Chromosome III shows the greatest effect, the effect of chromosome II and the interaction of chromosome II-chromosome III are also significant.

Chromosome III heterozygous effect was determined by counting sternoleural bristle number in 20 females of each of the constitutions 1/1, 1/4 and 4/4 for chromosome III in a line 1 background. Results of bristle number counts (9.45 ± 0.20 for 1/1; 8.30 ± 0.18 for1/4 and 7.65 ± 0.15 for4/4) show that chromosome III from line 4 has a different effect than its homologue from line 1 even in heterozygosis.

In order to investigate the distribution of genetic differences between third chromosomes of the two lines (1 and 4) in respect to sternopleural bristles, synthetic chromosomes which consisted of two known portions of the two studied third chromosomes were constructed according to the method of Breese &

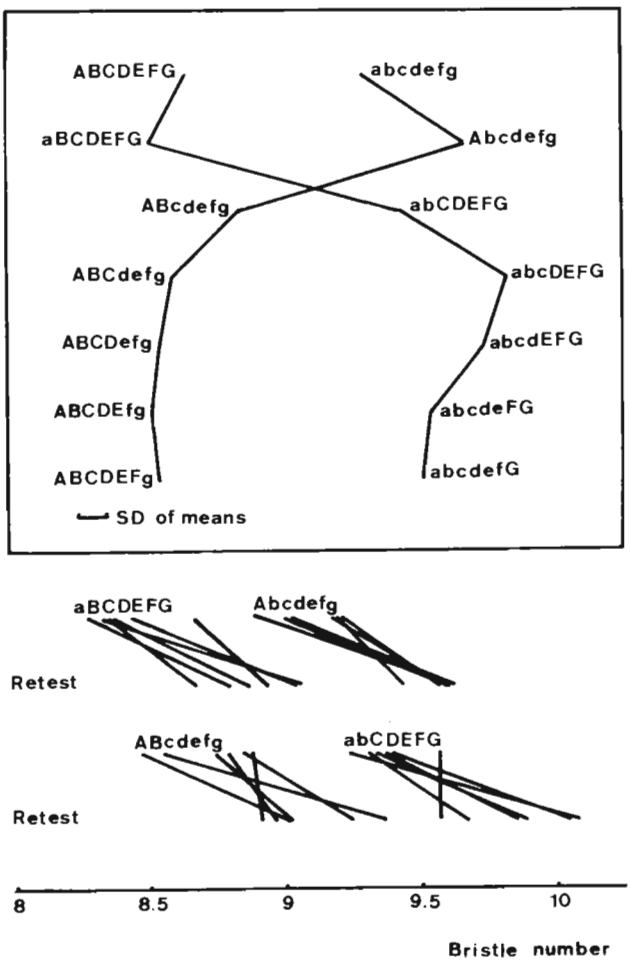

Table 1. Chromosome substitution analysis.

Source of Variation	d.f.	M.S.	Mean Effect	
I	1	2.80	+0.26	
II	1	4.00	-0.31*	
III	1	128.80	-2.14***	S.D.=
I-II	1	0.60	-0.11	±0.14
I-III	1	0.40	-0.09	
II-III	1	8.60	-0.46***	
I-II-III	1	1.40	+1.79	
Error	152	0.78		

*P < 0.05; **P < 0.01; ***P < 0.001

Figure 1. Mean sternopleural bristle number of a chromosome from each of the indicated classes of synthetic chromosomes III (above) and of six chromosomes from each one of the recombinant chromosomic classes between h and a contiguous marker in two successive blocks of measures (below).

Table 2. Analysis of segregation between chromosomes from Abcdefg, ABcdefg, aBCDEFG and abCDEFG classes.

Source of Variation	d.f.	M.S.
Between chromosomes within class:		
Abcdefg	5	0.42
ABcdefg	5	1.54
aBCDEFG	5	1.51
abCDEFG	5	0.76
Block	1	160.46***
Chromosome-Block	23	1.66***
Within chromosome and block	2832	0.73

***P < 0.001

Mather (1957). The marker chromosomes used were the chromosome rucuca which bears the recessive genes ru, III-0.0; h, III-26.5; th, III-43.2; st, III-44.0; cu, III-50.0; sr, III-62.0; e^s, III-70.7; and ca, III-100.7 (the markers th and st were treated as a composite locus) and the chromosome ruPrica. These two chromosomes were put into a line 1 background and chromosomes III 1 and 4 were taken from the chromosome substitution lines that have chromosomes I and II from line 1, so all synthetic chromosomes were obtained in a line 1 background. Six chromosomes of each single recombinant class were made so that chromosome III was being treated as consisting of seven segments to be identified by letters (A,a), (B,b) ... (G,g) from left to right. Capital letters and small letters indicate segments from the 4 and 1 lines, respectively (e.g., chromosomes of class ABCdefg have the left segment from line 4 and the right from line 1, the junction lying between st and cu loci, with possibly a short piece from rucuca interposed between 4 and 1 segments). Different chromosomes of the same class could differ in the points at which recombination has taken place.

Results of chromosome III analysis are summarized in Fig. 1. Mean bristle number given by a chromosome of the indicated class in partial heterozygosis with a chromosome III from line 1 (abcdefg) is presented in the Figure (above) (means are based on counts of sternopleura bristle number of 60 females divided in two blocks; standard deviation of the means is based on the MS interaction chromosome-block). The segment with the greatest effect is the (B,b) which includes the locus of the h marker, the other segments having a small or null effect over the trait. To determine at which side of h the genes with effect are located, the means of the 24 synthetic chromosomes recombinant at one or the other side of the h locus (chromosomes of the aBCDEFG, abCDEFG, Abcdefg and ABcdefg classes) were estimated. Counts were made on 30 females in each of two consecutive blocks (lower part of the Figure). No segregation was found between chromosomes within each class (Table 2) indicating that the effect is closely linked to the h locus.

The data reported show that there is a segment in chromosome III which, as a mean, includes the map locations from 13.25 cM to 35.25 cM that has a major effect on sternopleural bristle number. The effect of such a segment must be due to a factor which maps near the h locus (26.5 cM) since no recombination was observed between this marker and the factor under study.

We can not establish that the located factor is a functional unit contrary to some of the genes located in other studies which show qualitatively different effects (Spickett & Thoday 1966), but, within the limits defined by the experimental method used, it behaves as a segregational unit. If this unit is a complex it must be composed of linked genes in a coupling phase that could not be brought together by selection. So this factor with a major effect on sternopleural bristle number must be segregating as such in the original population.

The (B,b) segment is one of greatest effect on sternopleural bristle number in Breese & Mather's analysis (Breese & Mather 1957). Furthermore, in the locations corresponding to the (B,b) segment, 17 factors out of 38 factors affecting sternopleural bristle number on chromosome III were located by Davies (1971) and the two genes found by Thoday et al. (1964) in this chromosome map in this region too. All these results point to the idea that those genes which influence this trait are found grouped in specific chromosomal regions as was suggested by Davies (1971).

References: Breese, E.L. & K. Mather 1957, Heredity 11: 373-395; Davies, R.W. 1971, Genetics 69: 363-375; Kearsey, M.J. & K. Kojima 1967, Genetics 56:23-37; Lindsley, D.L. & E.H. Grell 1968, Genetic Variation in D.melanogaster, Carnegie Inst. of Wash., Publ. No. 627; Robertson, A. 1967, in: Heritage from Mendel, p. 265-280 (ed. R.A. Brinck), Univ. of Wisconsin Press; Spickett, S.G. & J.M. Thoday 1966, Genet. Res. Camb. 7: 96-121; Thoday, J.M., J.B. Gibson & S.G. Spickett 1964, Genet. Res. Camb. 5: 1-19.

Duttagupta, A.K., D. Mutsuddi and M. Mutsuddi (Das). University of Calcutta, India. Replication in X chromosomal segmental aneuploids in Drosophila.

In all the Drosophila species, while the male-X chromosome in polytene nuclei shows puffy appearance and early completion of replication, the female-X chromosome exhibits a similar state of chromatin condensation and synchronous pattern of replication like that of their autosomal counterparts. From our earlier works (Duttagupta et al. 1984) the idea was deduced that in **D.melanogaster** the male-X chromosome can recognize with up to 62% segment as its "duplication". As in these experiments duplicated segments were added to 1X individuals from the proximal side towards the tip linearly, study of distal and intercalary duplications of comparable length became essential to know whether the "tolerance of male duplication" would always remain within the same range. We started experiments with intercalary duplications of variable length to verify the above notion.

In the present investigation, with the help of T(1;3)$w^{vco}v$ f/C1B stock, three aneuploid conditions, viz. (i) individuals with 1.05 X-chromosomal segments, (ii) individuals with 1.95 X-chromosomal segments,

Figure 1. Morphology of the salivary gland chromosomes of **D.melanogaster** showing the female X chromosomes (a) deficient for the region $2C_1$ to $3C_4$ and (b) duplicated for the same segment which remains as translocated segment to the 3rd chromosome. Note in each case, each part of X chromosomes represents similar diameter and staining intensity in comparison to that of autosomal segments. **x** = X chromosome. **A** = autosome. Arrow indicates the aneuploid region.

Figure 2. Autoradiograms showing synchronous pattern of ^{3}H-TdR labelling on the X chromosomes and autosomes in individuals with (a) 1.95 X chromosomal segment and (b) 2.05 X chromosomal segment. **x** = X chromosome. **A** = autosome. Arrow indicates the aneuploid region.

and (iii) individuals with 2.05 X-chromosomal segments were constructed. In the first and third case, the segment $2C_1$ to $3C_4$ was added (as translocated segment to 3rd chromosome) to 1X and 2X individuals, respectively, and in the second case 2X individuals were deficient for the similar segments. Replication pattern of the salivary gland chromosomes were studied after pulse labelling with ^{3}H-thymidine (sp. activity: 17,400 uCi/mM, BARC, Trombay, India; Cons. 500 uCi/ml, exposure time - 20 days).

Our results reveal that in individuals with 1.05 X-chromosomal segments, both the entire X and the X chromosomal fragment involved in duplication, displays both puffy appearance and asynchronous replication pattern in comparison to that of autosomal segments. On the other hand, in individuals with 1.95 and 2.05 X-chromosomal segments, each part of X chromosomes, so far morphology and replicationi is concerned, represents a typical female X chromosome (Figures 1a-b; 2a-b). Such results indicate that 1X individuals could recognize small fragments as its duplication, regardless of the position of the duplicated segments. However, further works with large intercalary duplications would provide a more clear picture and such works are in progress.

This work is supported by a UGC minor research project to Debasish Mutsuddi.

Reference: Duttagupta, A.K., M. Mutsuddi and D. Mutsuddi1984, DIS 60:97-98.

Engels, W.R. University of Wisconsin, Madison, USNA. A set of P cytotype balancer stocks.

Multiply rearranged balancer chromosomes in P-cytotype stocks are often useful for maintaining dysgenesis-induced mutations while avoiding further changes in P element numbers and positions. I constructed two such stocks for the X chromosome and one for each major autosome. The resulting balancer stocks are:

M-5(P), w^a B; π_2 , C(1)DX,y f /FM7(P) y sn^{x2} B; π_2 ,
CyO(P), S^2 cn^P bw/π; π_2 , TM6B(P), e Tb ca/π-lethal; π_2 .

The strain π_2 is a strong P strain described previously (Engels & Preston 1979) and the symbol π refers to individual chromosomes from this stock. Tb is described by Craymer (1980),and cn^P is a strong cinnabar allele that was EMS-induced by C.R. Preston (pers. comm.) in 1977. The TM6B chromosome, constructed by L. Craymer (pers. comm.), is thought to be the most effective crossover suppressor available for the third chromosome. The other balancers and the markers they carry are all described in DIS or Lindsley & Grell.

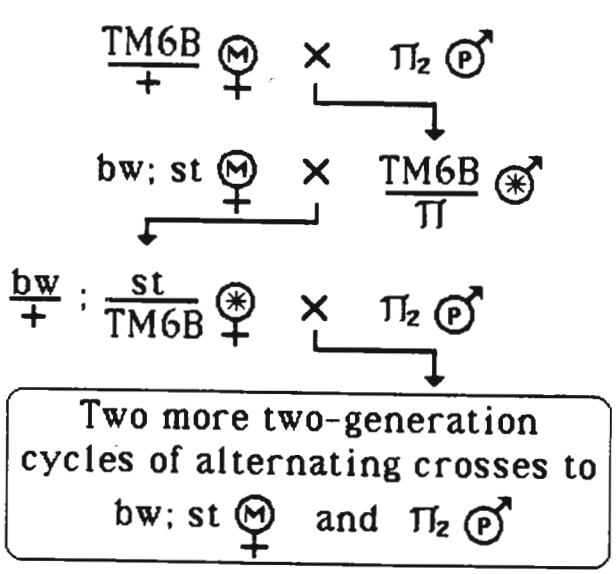

Figure 1. Crossing scheme to generate an M-5 chromosome with P elements. Each fly is designated as **P** or **M** according to its classification in the **P-M** system, or else as * to indicate it is dysgenic. All crosses were performed at 21°.

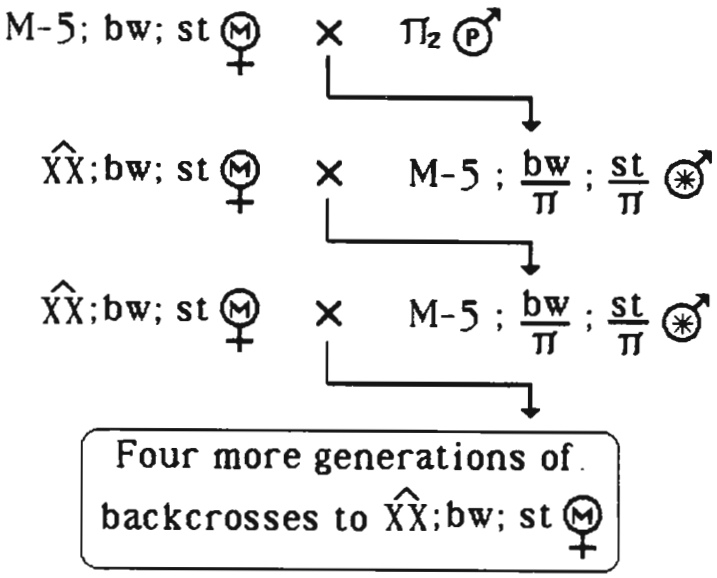

Figure 2. Crossing scheme to generate a TM6B chromosome with P elements. The designations **P**, **M** and * are the same as in Figure 1.

Transpositions of P elements from chromosomes of the P strain, π_2, onto the balancer chromosomes was achieved through a series of "chromosome contamination" crosses in which the balancer was passed at least six times through dysgenic flies in the presence of π_2 chromosomes. Figures 1 and 2 show the procedure for the M-5(P) and TM6B(P) stocks; the others are similar.

Following the "contamination" crosses, each balancer was tested by in situ hybridization to a P element probe to ensure that it had acquired numerous P elements. At least four sites were observed on each of the balancers. In most cases the "contamination" steps were carried out in multiple replicates so that the chromosome with the greatest number of acquired P hybridization sites could be selected.

The remainder of the genome was then replaced by chromosomes from the π_2 stock through a series of backcrosses to π_2 ♀♀. In the case of M-5(P), the balancer was also made homozygous.

Finally, the cytotype of each stock was determined using the sn^W test (Engels 1984). Females from the stock to be tested were crossed to sn^W(P) males, and the resulting sn^W-bearing daughters (or sons in the case of C(1)DX/FM7(P)) were progeny-tested to measure the rate of mutations to sn^e and sn^+. All stocks were confirmed to have the P cytotype, as indicated by the lack of sn^W mutability.

References: Craymer, L. 1980, DIS 55:197-200; Engels, W.R. & C.R. Preston 1979, Genetics 92:161-175; Engels, W.R. 1984, Science 226:1194-1196.

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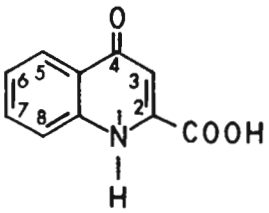
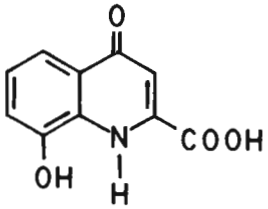
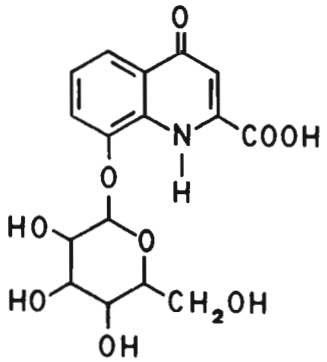
Characterization of a novel quinoline in **Drosophila melanogaster**: xanthurenic acid 8-O-β-D-glucoside.



kynurenic acid



xanthurenic acid



cardinalic acid
(xanthurenic acid 8-O-ß-D-glucoside)

The biosynthesis of xanthommatin, the brown eye pigment of **D.melanogaster**, can be represented by a series of reactions: L-tryptophan → kynurenine → 3-hydroxykynurenine → xanthommatin: Two branch points give rise to other metabolites: xanthurenic acid is derived from 3-hydroxykynurenine and kynurenic acid from kynurenine. The cardinal mutant is deficient in xanthommatin and extracts of the head contain an abnormal fluorescent component that is shown by two dimensional thin layer chromatography. The cardinal mutant shows a fluorescent pattern like that of the wild type, except that the former has an additional blue fluorescent spot with R_f values of 0.65 in isopropanol/2% ammonium acetate (1:1) and 0.53 in 3% ammonium chloride. This unknown compound has been called "cardinalic acid".

To obtain pure "cardinalic acid" for structural studies, the following purification procedure was set up: A Drosophila head extract (from the mutant pr cd which accumulates more cardinalic acid than the cd mutant) at pH 7 was loaded onto a column of Dowex AG 50W (H^+ form) equilibrated with water.

Table 1. pK_a values of the quinolines found in Drosophila, as detected by changes in the UV absorbance.

	Cardinalic acid	Kynurenic acid	Xanthurenic acid
$pK_a(N)$	1.8	2.2	1.8
$pK_a(O_4)$	11.0	11.2	7.3
$pK_a(O_8)$	-	-	12.3

Figure 1. Structure of the quinolines from Drosophila.

Most of the fluorescent components of the extract were retained and cardinalic acid was eluted with water in the first fractions. Fractions containing cardinalic acid were loaded onto ECTEOLA-cellulose column (Cl^- form) equilibrated with water. Cardinalic acid was retained as a thin blue fluorescent band on the top, and eluted with 0.01M HCl. Cardinalic acid obtained by this procedure was shown to be free of any contaminant that could interfere with the subsequent assays.

The ultraviolet spectra from cardinalic acid were very similar to those of xanthurenic and kynurenic acid. Cardinalic acid and xanthurenic acid had similar fluorescent color (emission maximum wavelength around 480 nm). Studies of their acid-base properties gave similar pK_a values for cardinalic acid and kynurenic acid (Table 1), suggesting the same free substituents for both molecules (Figure 1). These results pointed at the possibility that cardinalic acid was an 8-derivative of xanthurenic acid. The infrared spectrum gave strong peaks in the region around 100 cm^{-1}, suggesting the presence of a sugar in the molecule. The chemical hydrolysis gave xanthurenic acid and glucose. This was further confirmed by hydrolysis of cardinalic acid with β-glucoside; since the products were xanthurenic acid and glucose, the linkage to the glucose was established. This is the first time that a glucoside of xanthurenic acid has been characterized from natural sources.

Acknowledgements: This research was supported in part by the Office of Health and Environmental Research, U.S. Dept. of Energy, under contract of DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

Ford, S. and L. Tompkins. Temple University, Philadelphia, Pennsylvania USNA. An assay to measure the consumption of attractants in solution.

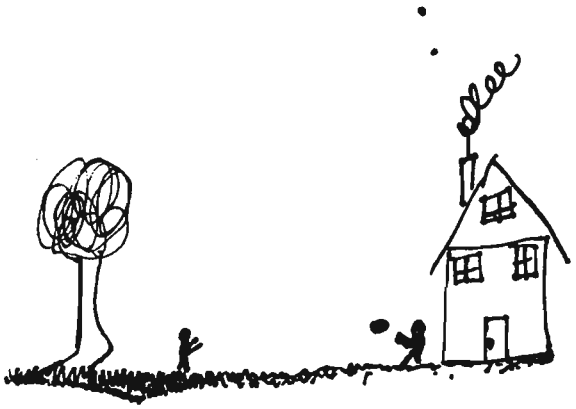
Falk and Atidia (1975) described an assay that measured the responses of **Drosophila melanogaster** males to repellents in solution. We have developed a similar assay in which the consumption of different attractants can be measured. 25-35 adult male flies, 3-5 days old, are starved for 18 hours in a 95 x 25 mm shell vial that contains water-saturated filter paper. After starvation, the vial is inverted onto a 35 x 10 mm plastic culture dish that contains 2 ml of a medium consisting of 1.5% agar, 1 M sucrose, glucose or fructose, water and red food coloring (3 drops/10ml). The flies are allowed to feed on the medium for 1 hour, after which they are anesthetized with carbon dioxide and the number of flies that have red-colored abdomens is determined.

We have observed that 99±0, 97±1, and 98±1 % of the Oregon R males had red abdomens after being tested with sucrose, glucose and fructose, respectively (15 groups of flies were tested with each attractant). However, Oregon R females are somewhat less attracted to the stimuli; 62±3, 58±2, and 64±4 % have responded to sucrose, glucose and fructose, respectively (15 groups were tested with each attractant).

We have also used this assay to screen populations mutagenized with EMS and have recovered two X-linked mutations that affect the responses of males to 1 M sucrose; specifically, 64±2 and 57±3 % of the males have red-colored abdomens (15 groups of males from each mutant stock were tested).

This research was supported by NIH grant GM 28998.

Reference: Falk, R. & J. Atidia 1975, Nature 254:325-326.



Garnett, D.J. Lovesgrove House, Aberystwyth, Wales, U.K. Detection of colour mutations in Drosophila by biochemical methods.

A sample of male $^{+}/+$ **D.melanogaster** were treated with EMS in the way described by Lewis & Bacher (DIS 43:1 93) for 15 hr and then mated with XXY strain (as Jenkins 1967). The 24hr old X_1 were scored for mutations and homogenised in batches of 25, in 1cc of 0.04M HCl. The solutions were filtered and analysed with a Unicam 1800 spectrophotometer at 200nm (5µl per 1.2cc curvette). No attempt was made to fractionate the solution and mutations throughout the body can be picked up; however, due to the fluorescent nature of the pterin eye pigments, mutations occurring elsewhere were less apparent at these higher wavelengths. It was hoped that this kind of analysis could be routinely used in this laboratory, for detecting internal changes, where any organ with distinct pigmentation is involved, e.g., eyes, malpigian tubes, etc.

Wild type - 0.323 ± 0.004

0.125M EMS treated culture - 0.356; 0.025M EMS treated culture - 0.395.

It was found in the course of these experiments that substantial discrepancies occurred when old, killed flies were used as controls for freshly prepared extracts; the solutions, made up as above, keep well at -18°C, less well at room temperature.

I thank the Wellcome Trust and Sigma Chemicals Co.

References: Stein, S.P. & E.A. Carlson 1980, DIS 55:139; Jenkins, J.B. 1967, Mut. Res. 4:90-92.

Garnett, D.J. Lovesgrove House, Aberystwyth, Wales, U.K. A new method of mutagen application to Drosophila eggs and larvae.

During the course of my work with mosaic eye mutants, I found that chemically induced mutations occurring through development can be shown by treating the embryos and larvae with the compound. Larvae are isolated and the chorion is wetted with the mutagen using an innoculating loop. The larvae are left for 5-10 minutes and are then dried with filter paper (the larvae can be preimmersed in dilute NaOCl to remove the membrane). Embryos were deposited onto microslides coated with a yeast paste hydrated with 0.25M EMS plus 0.1% sucrose and Nipagin. Eggs are counted by transilluminating the slides, so that the percentage survival can be calculated, giving some idea of the toxicity and the mutagenicity of the chemical. The embryos and the larvae are left at 25°C to develop and are scored for mutations 24 hrs after emergence. If the eggs are all deposited in a short time (Gupta 1980, DIS 55:152), the dose (concentration x time) to each will be constant.

It was also shown that insoluble powders could be tested by working into a yeast paste, 1% sucrose, on which the adult males feed for 24 hrs.

Gazaryan, K.G., S.D. Nabirochkin and E.N. Shibanova. Institute of Molecular Genetics, USSR Academy of Sciences, Moscow State University, USSR. Induction with high frequency of site-specific visible mutations in the MR-strain of **D.melanogaster** by DNA injected into the polar plasm of early embryos.

In an earlier study we obtained the eye-deformed **(edf)** mutation, an abnormal development of the eye-antennal disk, by introducing the Rous sarcoma virus (RSV) into **D.melanogaster** eggs (Gazaryan et al. 1981, 1982). RSV DNA cloned in pBR322 (pPrC11, see Ambartzumian et al. 1982) rendered similar mutagenic effect (Gazaryan et al. 1984). Neither homologous DNA, nor pBR322 caused visible mutations with such a frequency (1-2 mutants among ∿ 200 flies of F_2 progeny of each of 1 to 5 injected embryos (F_0). At that time we were dealing with the wild-type Oregon R stock of **D.melanogaster**. In the present study we used **D.melanogaster** (T-007) (MR-strain) for similar experiments. The following DNAs were introduced into the polar zone of eggs at stages 7 to 8 (70-80 min. p.o. at 25°C): pPrC11 plasmid (see above), pBR322, **D.melanogaster** DNA, rat liver DNA. To activate the MR-factor, the males of T-007 strain were crossed with wild-type Oregon R females. The results are listed in the Tables.

The most important thing is that the introduction of pPrC11 containing RSV DNA insert into the polar plasm of the MR-strain embryos causes the mutation in one locus with an extraordinarily high frequency: in up to 50% of the injected embryos. The mutation alters the development of eye facets (about 1/3 of the eye facets are fused) and has been denoted fe^{m} (fused eye, moderate). Apart from this,

Table 1. Mutant lines isolated in experiments on injection of cloned RSV DNA (plasmid pPrC11) into polar plasm of MR-strain (T-007) embryos with repressed and activated MR-factor.

Exp No	recipient line	material injected	Number of embryos injected	F_0 flies survived	mutant lines isolated	mutations
1	T-007	pPrC11	3050	77	5	Or (Orange eyes); dominant, x-chromosome
					2 (9.1%)	fe^m (fused eyes, moderate) recessive, 2nd chromosome
-"-	-"-	saline	2230	83	0	--
2	T-007/ Oregon R	pPrC11	560	82	39	fe^m
					2	Or
					1 (50%)	white
3	-"-	-"-	460	37	16	fe^m
					1	fe^w (fused eyes, weak)
					(45%)	recessive, 2nd chromosome
4	-"-	Oregon R DNA	800	78	11	fe^w
					2	white
					1 (20%)	Or
5	-"-	Rat liver DNA	840	75	10	fe^w
					5	fe^m
					2 (20%)	w
6	-"-	pBR322	830	80	11	fe^w
					2	Or
					(∿ 15%)	
-"-	-"-	saline	440	75	0	--

the Orange eyes (**Or**) and white mutations occur with a considerably lower frequency (see Table 1). A test for allelism has shown independently obtained fe^m mutations to belong to one complementation group. The injection of rat DNA, pBR322 and homologous DNA into the eggs caused the same three mutations, but the **fe** mutation occurred far less frequently and showed a lower expressivity (about 5-10% of facets are fused), so this phenotype was denoted fe^w (weak expression) to distinguish them from fe^m (moderate) phenotype caused by pPbC11. It is interesting that the same mutations (and **white** among them) occur in homozygous T-007 stock embryos where the MR-factor is repressed (see e.g., Bregliano & Kidwell 1983) but the frequency of the **fe** mutation is no higher than that of the other two. It is very important that in the T-007/Oregon R stock, where the MR-factor is active, no mutations are observed at the said loci in our samples if no DNA is introduced (saline is injected instead), but the mutations are there (the white among them) if any of the above-mentioned DNAs is injected. This means that not only the **fe** locus, which is here described for the first time as a potentially unstable one in the MR-strain, but also white, already known to be unstable in this system (Colins & Rubin 1982) show a higher mutation frequency in the presence of foreign DNA than under the action of the MR-factor alone (in the control series). Unlike the **fe** locus, white do not distinguish between DNAs of different origin, although one cannot be sure that such distinctions might not transpire if a greater number of strains are analyzed. The **fe** locus behaves in a different manner: (1) its mutability varies depending on whether oncogenic or other DNA is used; (2) activation of the MR-factor leads to a dramatic increase of its mutability, although the mechanism of this increase is still obscure.

Some of the mutant lines obtained in our previous works appeared unstable (Gazaryan et al. 1981, 1984). So, we checked the stability of phenotypic expression of 16 arbitrarily selected (from over 100 obtained) fe^m mutant lines. Up to 5th generation no changes were observed. Then, between 5th and 7th generations the phenotype of the flies in 7 lines was altered, reversed to the normal, or acquired new ab-

Table 2. Secondary mutations arose as a "burst of instability" in 5th-7th generation flies of a fe^m line.* (r = recessive; d = dominant)

Mutation	Reduced area of the facets (%)	other changes	inheritans, chromosoma
fe^w (fused eye, weak)	5-10	-	r, 2nd
edf (eye deformed)**	15-20	cuticular outgrowths on altered part of eyes	r, 2nd
Fe^s (fused eye, strong)	50-60	-	d, 2nd
Pd (Palp duplication)	70-80	Palp duplication	d, 2nd
Ce (Changed eye)†	70-80	Facts on cuticular outgrowths	?
Ad (Antennal dupl.)†	90-100	Antennal duplication	?
Od (Ocular dupl.)	90-100	Mini eye in altered region	d, 2nd
Er (Eye reduced)**	100	-	d, 2nd
Cw (changed wings)	-	Local thickening of wing veins	d, 3rd
Curly			

* In ∿ 90% of the same generation flies fe^m mutation reversed to normal.
**For more detailed characteristics see Gazaryan et al. 1981, 1982.
† the line is lost.

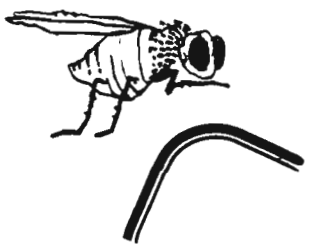
normalities (secondary mutations). In 3 lines we observed a kind of general instability, the "burst" of phenotypic changes: about 90% of the flies reversed to normal phenotype, about 5% maintained **fe^m** appearance and about 5% gave rise to the set of new mutations (some of them maintaining the **fe^m** together with new ones). Six out of nine secondary mutations were the same type as **fe**, e.g., led to abnormal development of the eye-antennal disk derivatives. They are characterized by more extensive fusion of facets (up to 100%) and in addition in some of them we found duplications of some of eye-antennal structures (Table 2). So, we have at present in total nine mutations in presumably one gigant locus which alter this disk development. They represent a row with gradual increase of the effect (facet fusing, mainly). Only three of them, those with weaker effects (**fe^m**, **fe^w**, edf) are recessive and arose as a consequence of the primary effect of the injected virus (Gazaryan et al. 1981, 1984) or DNA (see Table 1). The secondary mutations more strongly affect the eye-antennal disc development and most of them represent dominant mutations (Table 2).

We attempted to find the RSV-specific sequence in the DNA of the mutants. The result of in situ hybridization of ^{32}P DNA of RSV with polytene chromosomes and of Southern blot-hybridization with DNA of **fe^m** mutants were negative. However, dot-hybridization revealed in some of mutant lines (mainly in those which exhibited instability) some amount (0.2-0.4 copies per haploid drosophila genome) of RSV-specific sequence.

How can foreign DNAs specifically induce mutations (see also Gershenson et al. 1971) and what is the reason of the higher effectivity of oncogenic virus DNA?

The instability of mutations strongly suggests that they are insertional mutations. Two possibilities can be considered: (1) exogenous DNA is inserted into the loci concerned; (2) it binds proteins (e.g., repressors) mobilizing genomic IS-elements (transposons). In situ hybridization of labeled Dm5002 plasmid containing **copia** element (Dunsmuir et al. 1980) with polytene chromosomes shows that in the mutants described here the sites of **copia** localization are changed compared with the control larvae. This observation suggests that the mobilization of endogeneous elements is actually induced by the DNA injected in embryo.

References: Gazaryan, K.G., A.K. Shahbazyan, N.S. Neznanov, S.G. Smirnova, F.L. Kisselev & A.G. Tatosyan 1981, Dokl. Acad. Nauk SSSR (USSR) 258: 1224-1227; Gazaryan, K.G., A.K. Shahbazyan, N.Yu. Sakharova & S.G. Smirnova 1982, DIS 58:64-65; Ambartzumian, N.S., A.G. Tatosyan & G.N. Yenikolopov 1982, Molec.Biol. (USSR) 16:1183-1188; Gazaryan, N.G., S.D. Nabirochkin, A.K. Shahbazyan, E.N. Shibanova, T.I. Tichonenko, L.V. Gening & V.A. Goltzov 1984, Genetica (USSR), 20:1237-1244; Bregliano, G.C. & M.G. Kidwell 1983, in: Mobile Genetic Elements (ed. J.A. Shapiro) Acad. Press, N.Y., pp. 329-361; Colins, M. & G.M. Rubin 1982, Cell 30:71-79; Gershenson, S.M., Y.N. Alexandrov & S.S. Maluta 1971, Mutation Res. 11:163-173; Dunsmuir, B., W.Y. Brorein, M.A. Simon & G.M. Rubin 1980, Cell 21: 575-579.



Genova, G.K.[1] and E.P. Semionov.[2§] [1]Sofian University, Bulgaria. [2]Leningrad University, USSR. [§]Present address: Institute of Molecular Biology, Sofia, Bulgaria. Unstably localized nucleoli in **Drosophila melanogaster** salivary gland cells in various stocks.

Lately the phenomenon of "additional nucleoli" formation in the salivary gland cells of **D.melanogaster** was investigated in a series of works. In these cells a few nucleoli can be generated with varying sites of attachment to chromosomes, which do not coincide with the number and localization of the nucleolus organizers presented in one copy in each X and Y chromosomes. By means of in situ hybridization and incorporation of labelled precursors, it was shown that additional nucleoli contained ribosomal RNA genes actively replicated and transcribed (Ananiev et al. 1981). In our research we had for an object to compare the distribution maps of the "unlawful" nucleoli in flies with different genotypes.

The comparative analysis of nucleoli attachment sites in the salivary gland cells (stained with methylgreen-pyronin) was carried out in the third instar larvae from the stocks LA and LA$^+$ differing in number of physiological characteristics (Khuguto et al. 1980; Gvozdev et al. 1981). 219 and 202 nucleoli attachment sites (about 4000 cells are analysed) along the polytene chromosomes were localized for larvae from LA and LA$^+$ stocks, respectively. The highest frequency of nucleoli occurs in the telomeric regions of all chromosomes, corresponding to 2-3 divisions of Bridges' map. Therefore we recorded on Figure 1 the picture of the nucleoli localization only for the five distal divisions in telomeric regions of the chromosomes X, 2 and 3. It is evident, that within these regions we consider the attachment of nucleoli occurs to discrete sites, including in sum the greater part of the chromosome telomeric regions; and euchromatic as well as sites assigned to the intercalary heterochromatin in each big chromosome (X,2,3) can serve as nucleoli attachment sites. At the same time there are particular chromosome sites to which the nucleoli were connected most frequently: the distal points of each telomere, the divisions 3C,56EF (all these sites according to a number of criteria belong to intercalary heterochromatin), chromocenter (the nucleoli connected with the nucleolus organizer - 20CD - were not registered). Single small chromosome regions (59F, 61B), on the contrary, have never served as nucleoli attachment sites, although in the adjoining regions this event was relatively often registered. We emphasize that the type of the connection site distribution of unstably localized nucleoli along the chromosomes of the LA and LA$^+$ larvae is similar. Along with this, in particular sites (60E-F, 100F) the frequency of nucleoli connection for these stocks is different. The average number of nucleoli per nucleus was the same in males and females from both stocks.

Acknowledgements: The authors express their deep gratitude to Dr. A.F. Smirnov and Dr. L.Z. Kaidanov for their help in carrying out the present work.

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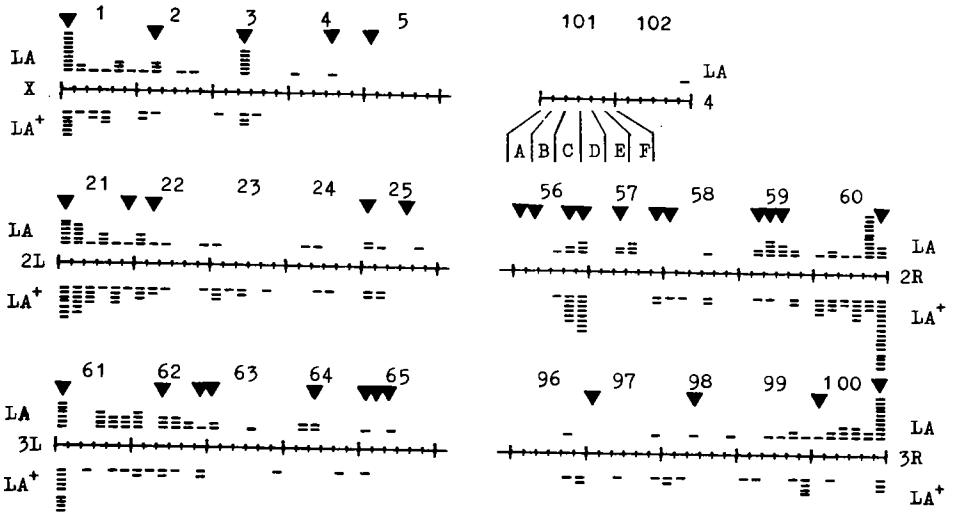


Figure 1. Distribution of nucleoli attachment sites along the telomeric regions of the salivary gland chromosomes. With numbers 1,2,3... are marked the number of the divisions of the Bridges' chromosome map. X,2L,...4 - chromosome symbols. Arrowheads point at the intercalary heterochromatin regions according to Hannah (1951) and Kaufmann & Iddles (1963). One horizontal dash corresponds to one recorded event of nucleolus attachment to the given polytene chromosome site. Fairly frequently nucleoli were connected with chromocenter (about 15% of the registered additional nucleoli; they are not included in the data mentioned above).

Gerasimova, T.I. and L.V. Matyunina. Inst. of General Genetics, USSR Academy of Sciences, 117908 Moscow, USSR. Simultaneous reversion of three unstable alleles at loci yellow, white, singed.

Among derivatives of unstable ct^{MR2} stockof great interest is the line $y^{MR19}w^{MR19}ct^{MRpN19}$ which contains three unstable mutations at loci yellow (0.0; 1AB), white (1.5; 3C2) and cut (20.0; 7B3-4). This line has a specific feature: the vast majority of revertants are triple and carry mutations at the locus singed (21.0; 7D1-2) $-y^+w^+ct^+sn$. Among 19,200 flies of the homozygous line $y^{MR19}w^{MR19}ct^{MRpN19}$, we observed seven independent revertants of the $y^+w^+ct^+sn$ type and only one $y^+w^+ct^{MRpN19}$ revertant; i.e., coordinated mutational events involving four loci occurred in the chromosome $y^{MR19}w^{MR19}ct^{MRpN19}$: mutations at the loci yellow, white, cut reverted to the wild type and simultaneously there was a mutation at the locus singed.

In the progeny from $y^{MR19}w^{MR19}ct^{MRpN19}$ females mated to males of the P-containing line (MRh12/Cy); the reversion frequency increased up to 2×10^{-3}. The effect of triple reversion remained but concomitant sn mutations disappeared. This indicates that the effect of triple reversion discovered in this work as well as the effect of double reversion discovered earlier (Gerasimova et al. 1984a) are associated with the nature of insertion mutations and concomitant specific mutagenesis depends strongly on the genotype and may change as the autosomal background is changed.

The allele ct^{MRpN19} and other ct-alleles arising from the parental line ct^{MR2} are induced by MDG4 (Gerasimova et al. 1984b). The nature of the yellow mutation is also found out (Gerasimova, DIS: this issue). The molecular nature of the transposon at the locus white is unknown, unfortunately.

The $y^{MR19}w^{MR19}ct^{MRpN19} \rightarrow y^+w^+ct^+sn$ reversion results in MDG4 excision from 7B region of the cut locus and MDG2 excision from the 1AB region of the locus yellow. X-chromosomes of the line $y^{MR19}w^{MR19}ct^{MRpN19}$ and of five independent revertants $y^+w^+ct^+sn$ were analyzed by in situ hybridization with MDG1, MDG2, MDG3, MDG4, copia, 5 to 20 individuals being analyzed in each case. The results are presented in Table 1. As seen from Table 1, the effect of triple reversion is a result of "transpositional bursts" (Gerasimova 1984c) which are accompanied by transposition of four mobile elements (MDG1, MDG2, MDG3, MDG4) and involve changes at four loci (y, w, ct, sn). The number of such transpositional events in the X-chromosome amounted to 18 including MDG excisions and incisions. Analysis of the data presented in Table 1 suggests a conclusion about high specificity of transpositions in the given family of related lines. Coordinated appearance or disappearance of several MDG hybridization sites was observed: for instance, simultaneous disappearance of three sites of MDG2-1AB, 2B, 3A hybridization in all analyzed revertants or simultaneous appearance of four new hybridization sites in the X-chromosome for MDG3 in the three independent revertants.

The nature of the effects of double and triple reversion as well as of coordinated disappearance or appearance of sites of hybridization of different mobile elements remains completely obscure, but it seems to be related to the structure of MDG themselves and to the nucleotide sequence of target genes (sites).

References: Gerasimova, T.I. 1981, Mol. Gen. Genet. 184:544-547; Gerasimova, T.I., L.V. Matyunina, Y.V. Ilyin, & G.P. Georgiev 1984a, Mol. Gen. Genet. 194:517-522; Gerasimova, T.I., Y.V. Ilyin, L.J. Mizrokhi, L.V. Semjonova & G.P. Georgiev 1984b, Mol. Gen. Genet. 193:488-492; Gerasimova, T.I., L.Y. Mizrokhi & G.P. Georgiev 1984c, Nature 309:714-716.

Table 1. Distribution of in situ hybridization sites on X-chromosomes of the $y^{MR19}w^{MR19}ct^{MRpN19}$ line and its derivatives.

Line	MDG1	MDG2	MDG3	MDG4	Number of transpositions
$y^{MR19}w^{MR19}ct^{MRpN19}$	3C,8A,15D, 19E,20	1AB,2B,3A, 5A,19E,20	2B,3E	7B	
$y^+w^+ct^+sn^{L1}$	3C,12A*, 19E,20	3C,5A,19E	8E,9E*	-	18
$y^+w^+ct^+sn^{P4}$	3C,9D*,20	5A,20	9A*,9E*, 13A*,19E*	-	15
$y^+w^+ct^+sn^{P5}$	3C,18A,20	5A,20	9A*,9E*, 13A*,19E*	-	15
$y^+w^+ct^+sn^{P6}$	3C,9D*,20	5A,20		-	9
$y^+w^+ct^+sn^{L2}$	3C,12A*, 19E,20	5A,20	9A*,9E* 13A*,19E*	-	14

"*" = similar sites of hybridization of different MDG in X-chromosomes of independent sn mutants.

Gerasimova, T.I. and L.V. Matyunina. Inst. of General Genetics, USSR Academy of Sciences, 117908 Moscow, USSR. Unstable mutations at the locus yellow induced by the mobile element MDG2.

The unstable line ct^{MR2} (Gerasimova 1981) is characterized by "transpositional bursts": mass simultaneous transpositions of different mobile elements in the same germ cell resulting in insertion mutagenesis (Gerasimova et al. 1984a). Insertion mutagenesis spontaneously occurring in the ct^{MR2} line and its derivatives is characterized by high locus specificity (Gerasimova 1984b). The locus yellow (0.0; 1AB) is one of the most frequently mutating loci in the "ct^{MR2} system".

15 independent yellow mutations have been obtained from the ct^{MR2} line and its derivatives. X-chromosomes in 6 y mutants and 2 y^+ revertants were analysed by the method of in situ hybridization with ^{3}H-DNA of mobile elements MDG1, MDG2, MDG3, MDG4, and copia. The results of hybridization with MDG2 are given in Table 1. In all 6 independent yellow mutations, the site of hybridization for MDG2 was discovered in the 1AB region of the locus yellow (Figure 1). In the two y^+ revertants, no such hybridization site was found. The results obtained show with high probability that mutations at the locus yellow are induced by MDG2.

Among the yellow mutants and their revertants three lines successively obtained from each other were studied in detail: $y^{MR19}{}_w{}^{MR19}{}_{ct}{}^{MRpN19}$ → $y^+w^+ct^+sn^{L2}$ → $y^{MR19a}{}_w{}^{MR19a}{}_{sn}L2$. The initial line $y^{MR19}{}_w{}^{MR19}{}_{ct}{}^{MRpN19}$ contains MDG2 in the 1AB region (two sites, Figure 1). The reversion to $y^+w^+ct^+sn^{L2}$ is accompanied by MDG2 excision from the 1AB region of the locus yellow. In course of repeated mutagenesis in $y^{MR19a}{}_w{}^{MR19a}{}_{ct^+sn^{L2}}$, MDG2 returns to this region: 1AB (both sites, Figure 1). Thus, repeated mutagenesis at the locus yellow is also associated with

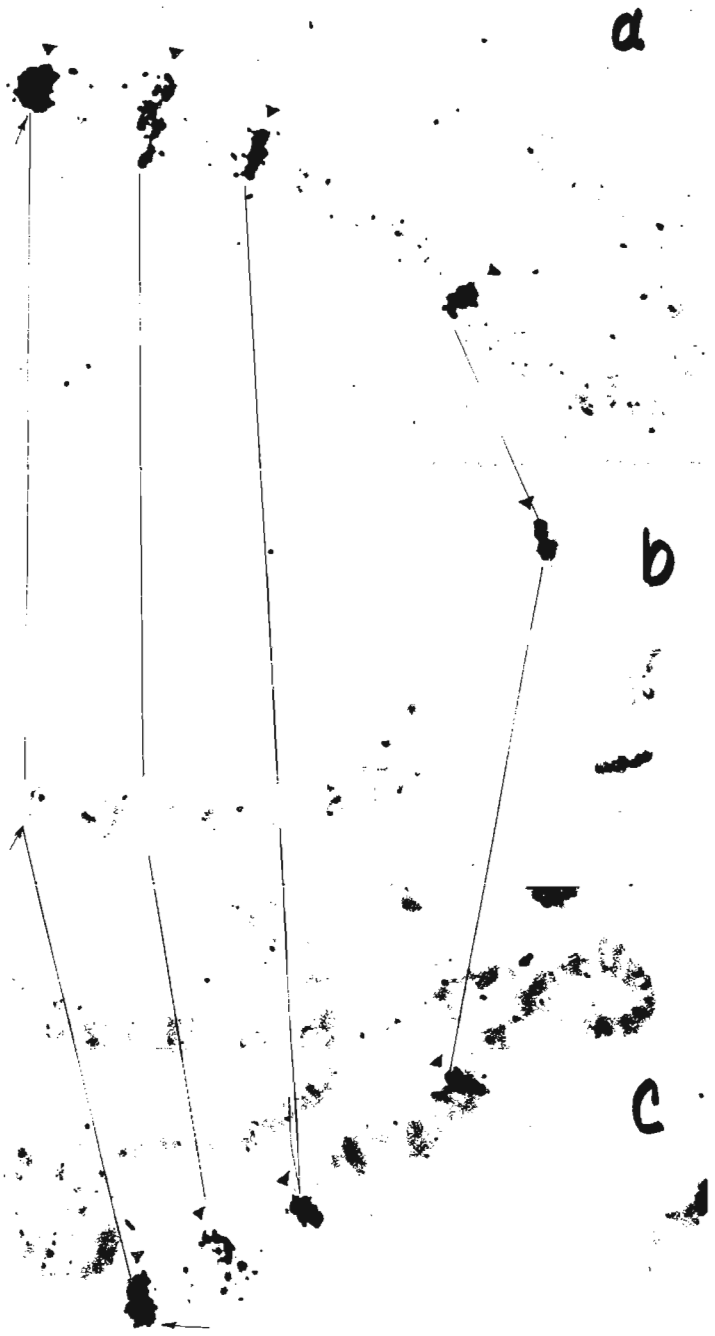


Table 1. Distribution of MDG2 hybridization sites on X-chromosomes of yellow mutations and their revertants.

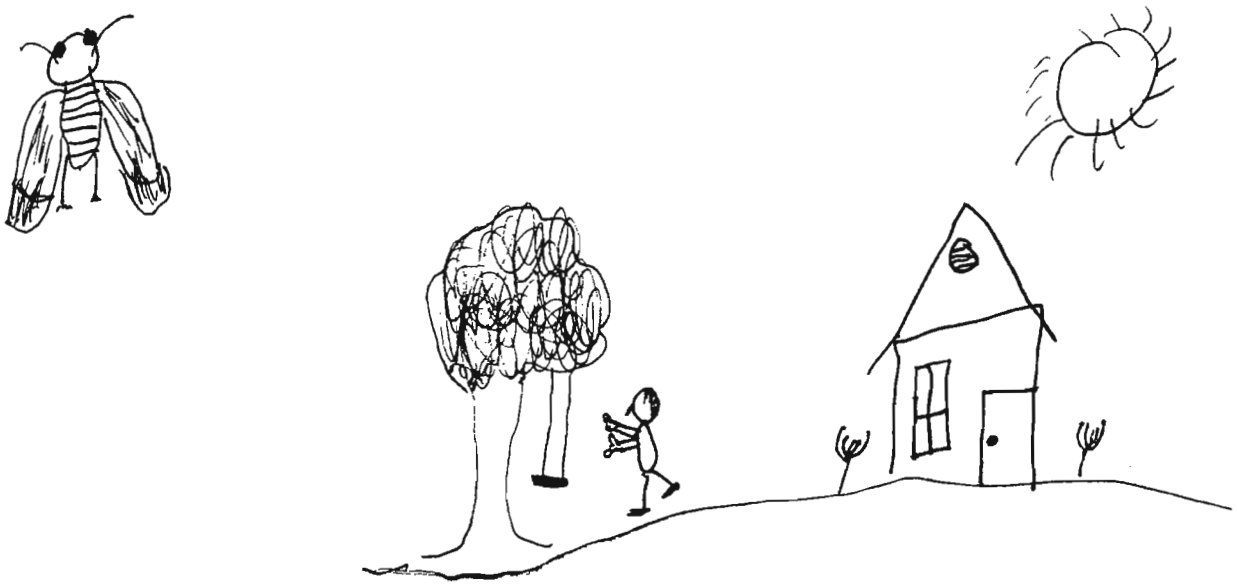
Line	MDG2
ct^{MR2}	2B, 3E, 4D, 20
$y^{MR2}ct^+$	1AB, 4D, 10C, 11A, 13F, 20
y^+ct^{MR2}	2B, 4A, 4D, 19E, 20
$y^{MR7}{}_w{}^{MR7}ct^+$	1AB, 2B, 3A, 5A, 19E, 20
$y^{MR14}{}_w{}^{MR10}ct^+$	1AB, 2B, 4A, 6F, 19E
$y^{MR19}{}_w{}^{MR19}{}_{ct}{}^{MRpN19}$	1AB, 2B, 3A, 5A, 19E, 20
$y^+w^+ct^+sn^{L2}$	5A, 20
$y^{MR19a}{}_w{}^{MR19a}{}_{ct^+sn^{L2}}$	1AB, 2B, 3A, 5A, 20
$y^{MR19b}{}_w{}^{MR19b}{}_{ct}{}^{MRpN19b}$	1AB, 2B, 3A, 5A, 20

Figure 1. The results of in situ hybridization of MDG2 with X-chromosomes of the mutant $y^{MR19}{}_w{}^{MR19}{}_{ct}{}^{MRpN19}$ (**a**); its revertant $y^+w^+ct^+sn^{L2}$ (**b**); and back mutant $y^{MR19a}{}_w{}^{MR19a}{}_{ct^+sn^{L2}}$ (**c**). The arrow points out the 1AB region of the locus yellow; the triangles designate the sites of hybridization 1AB, 2B, 3A, 5A.

MDG2. It is of interest that the two other sites of MDG2 hybridization (2B, 3A) located in the X-chromosome of $y^{MR19}{}_w{}^{MR19}{}_{ct}{}^{MRpN19}$ line disappear in sn mutants in the course of reversion and appear again in the course of repeated mutagenesis in $y^{MR19a}{}_w{}^{MR19a}{}_{ct^+}{}_{sn}{}^{L2}$ (Figure 1). A possible cause of such shuttle of transposons may be the existence of transpositional molecular memory, i.e., preservation of short transposon fragments (MDG2 in the given case) in the target site (locus).

Modolell with coworkers (1983) showed earlier that the collection mutation y^2 was induced by MDG4. In our collection of y mutants no MDG4 transpositions to the locus yellow were found. This indicates that the high specificity of insertion mutations and transpositions of mobile elements strongly depends on the genotype.

References: Gerasimova, T.I. 1981, Mol. Gen. Genet. 184:544-547; Gerasimova, T.I., L.J. Mizrokhi & G.P. Georgiev 1984a, Nature 309:714-716; Gerasimova, T.I., L.V. Matyunina, Y.V. Ilyin & G.P. Georgiev 1984b, Mol. Gen. Genet. 194:517-522; Modolell, J., W. Bender & M. Meselson 1983, 80:1673-1682.



Ghosh, A.K. University of Calcutta, India.

Transcriptional activity of an autosomal arm (2L) in trisomic condition in **Drosophila melanogaster.**

The principal object of this present investigation is to measure the transcriptional activity of thirty different chromosomal segments of trisomy for the entire left arm of the second chromosome by using ^{3}H-Uridine autoradiography technique. The dissected glands were incubated in 500 µCi/ml of ^{3}H-Uridine (Specific activity 12,700 µCi/mM, BARC, Trombay) and processed for autoradiography as described previously (Lakhotia & Mukherjee 1969). Trisomy for 2L were generated by crosses between stocks C(2L)dp; F(2R)bw and Oregon R^+.

Results revealed that thirteen out of thirty diferent chromosomal segments (21A to 35F of 2L) synthesize equal amount of transcripts in both diploid and trisomy for 2L stock, whereas some segments behave in a dose-dependent manner (hyperploid/euploid - 1.5). The remaining segments of trisomy-2L(T2L) show increased or decreased transcriptional activity (see Figure 1).

Thus Devlin's enzymetic study of trisomy for 2L stock and the results of our transcriptional study suggest that at least some segments of the autosomal arm in the trisomic have the ability to maintain the transcriptional activity at the same level as in the disomic. This is in agreement with Devlin's (1982) proposition of autosomal dosage compensation. However, as this is true regardless of the sex, this phenomenon of compensation is different from the haplo-X compensation in **Drosophila** male.

This phenomenon is true also for other major autosomal arms, e.g., 3L.

References: Devlin, R.H. et al. 1982, PNAS 79:1200-1204; Lakhotia, S.C. & A.S. Mukherjee 1969, Genet. Res. Camb. 14:137.

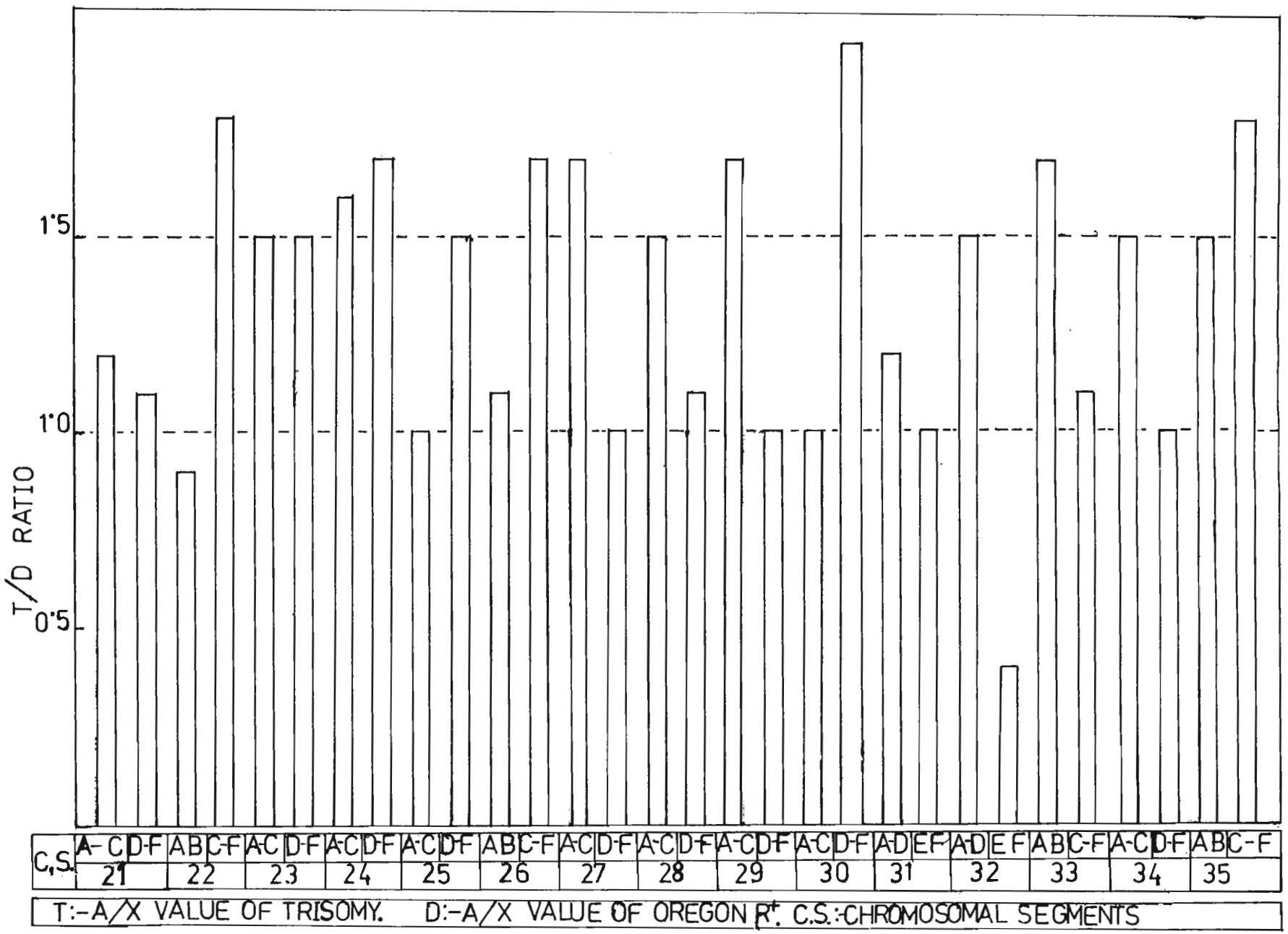


Figure 1. Histogram showing the level of compensation and level of dose-dependence of different sites of the left arm of second chromosome in trisomic condition. The segment 1A to 4F of X chromosome of the same nucleus has been used as reference.

Ghosh, M., S. Banerjee and A.S. Mukherjee.
University of Calcutta, India. Activity of the X chromosome of the reinverted mosaic mutant larvae of **D.melanogaster** in in vitro culture.

Ashburner (1972, 1973) and Ashburner et al. (1974) have shown puffing activity in polytene chromosomes of Drosophila larval salivary gland grown in vitro synthetic culture medium with ecdysone. Majumdar & Mukherjee (1980) have reported induced puffing activity and replication of polytene chromosome in gland grown in modified Schneider's medium.

We have examined the puffing activity of the X chromosomes in male and female larval salivary gland of the mutant strain, $In(1)BM^2fB^{15}$, reinverted mosaic of **Drosophila melanogaster**, grown for 24-48 hr in Schneider's medium without yeast hydrolysate. In this mutant strain the X chromosome in in vivo, is extraordinarily hyperactive in male, and in extreme condition highly flabby and stumpy (Ghosh et al. 1982). In squash preparations of salivary glands of this strain, the X chromosome of the male appears in three morphological expressions, viz. (a) extremely wide, stumpy and puffy (flabby); (b) intermittently puffy

Figure 1. (a) Flabby X chromosome, (b) Intermediate X chromosome and (c) Near normal X chromosome of the mutant male $In(1)BM^2$ fB^{15} (rv, mosaic) grown in vivo.

Figure 2. (a) Flabby X chromosome, (b) Intermediate X chromosome and (c) Near normal X chromosome of the male mutant larval glands grown in synthetic culture medium for 24 hr.

Table 1. Frequencies of different classes of X chromosomal activity in In(1)BM^{2}fB^{15} reinverted mosaic male larval salivary glands grown in Schneider's medium and in vivo.

	Total no. of cells examined	(%) freq. of X chromosomal types		
		Flabby	Intermediate	Near normal
A] In vivo				
10°C	1430	62.2	31.5	6.3
18°C	2534	50.0	24.9	25.1
23°C	1770	24.8	50.1	25.1
B] In vitro (23°C)				
24 hr	1700	25.0	37.7	37.3
48 hr	1242	18.7	31.4	49.9

in sections of the chromosome; and (c) nearly normal puffy structure as in wild type male. Thus, the different nuclei manifest as mosaic expression for the X chromosome. All other autosomes appear normal. In squash preparations from female larval glands, all chromosomes are normal. Such expression has been explained as an expression of some modulator for dosage compensation (Ghosh & Mukherjee 1983, and in prep.).

The intention of the present investigation was to find out whether in the simplified culture medium such extraordinary hyperactivity of the male X chromosome of the mutant strain is maintained and expressed as mosaic.

Salivary glands were dissected out from male and female late third instar larvae, and transferred to a T-flask containing Schneider's medium (minus yeast hydrolysate) and kept at 23°±1°C in an incubator, for 24 hr and 48 hr. Thereafter, the glands were transferred to Drosophila Ringer (pH 7.2) after giving an interval of 5 min for acclimatization. Squash preparations of chromosomes were made as described (Lakhotia & Mukherjee 1969).

The mosaic expression of the X chromosome of the mutant male larva in vivo is presented in Figure 1a-c and that of the X chromosome of male larval gland grown in vitro culture is shown in Figure 2a-c. Evidently, all three classes of nuclei, containing flabby, intermediate and near-normal condition of the X chromosome in 1X2A nuclei, are manifested as observed in vivo.

However, as evident from Table 1, the frequencies of these classes of nuclei are slightly different in in vitro culture, as compared to in vivo. The results in Table 1 reveal that the extreme flabby condition of the X chromosome of the mutant is predominant in in vivo specially when grown at 10°C. In in vitro culture, more near-normal class appear, especially after 48 hr of culture.

Apart from the difference in the frequency of the three classes, it is quite evident that the mosaic condition of the extra hyperactive X chromosome is maintained in in vitro culture, i.e., even outside the larval body. It may be suggested that continuous presence and supply of larval hormone such as ecdysone may not be needed to maintain the extraordinary hyperactivty of the X chromosome in the male. The mosaic expression appears also to be cell autonomous.

References: Ashburner, M. 1972, Chromosoma 38:255-282; _____ 1973, Devel. Biol. 35:47-61; Ashburner, M., C. Chihara, P. Metzer & G. Richards 1974, Cold Spr. Harb. Symp. Quant. Biol. 38:655-662; Ghosh, M. & A.S. Mukherjee 1983, XVth Int. Congr. Genet. Abstr. 178:104; Ghosh, M., D. Bose & A.S. Mukherjee 1982, Vth All Ind. Cell Biol. Conf. Abstr. 28:17; Majumdar, D. & A.S. Mukherjee 1980, DIS 55:159-160.

Ghosh, M. and A.S. Mukherjee. University of Calcutta, India. DNA replication in the X chromosome of In(1)BM^2 (rv,mosaic) of **Drosophila melanogaster.**

It has been well documented that as in all eukaryotes, in Drosophila DNA replication is also initiated at multiple initiation sites (Blumenthal et al. 1974; Lakhotia & Mukherjee 1970). Cytologically, such multiple initiation is manifested as disperse labelling of ^{3}H-thymidine on puffs and interbands (Lakhotia & Mukherjee 1970; Chatterjee & Mukherjee 1975; Haegele & Kalisch 1974) and such labelling pattern is called DD pattern (which is identified by disperse label on puffs and interbands and lack of label on chromocentric heterochromatin). Earlier works from this laboratory have revealed that the X chromosome in Drosophila male is early replicating (Lakhotia & Mukherjee 1970) and also faster in rate of chain growth (Chatterjee & Mukherjee 1978). The X chromosome of the male in all Drosophila species examined is hyperactive, puffy and faster replicating (see Mukherjee 1982). While searching for the mechanism of the hyperactivity of the X chromosome, we came across with a mutant strain, In(1)BM^2--reinverted mosaic. The X chromosome in the male larval salivary gland of this strain is extremely puffy in 30 to 50% of the cells. It has been shown that the X is indeed superhyperactive as compared to that of the wild type, and synthesizes 3 to 4 times as much RNA as the individual X chromosomes of its female counterpart.

In this report we are presenting the replicative behaviour of the X chromosome in this strain. DNA replication has been monitored by autoradiography using ^{3}H-thymidine. Results shown in Table 1 reveal that while in the reinverted mosaic female the labelling frequencies of all sites except two (viz., 6DEF and

Table 1. Labelling frequencies of moderately late and very late replicating X chromosomal sites of Oregon R^+ male, female and reinverted mosaic male and female (data taken from 2D-1D labelling patterns).

Chromosomal sites	Oregon R^+ male	Oregon R^+ female	Reinverted mosaic male	Reinverted mosaic fem.
A] Late replicating sites				
1A	85	100	100	100
3C	90	100	100	100
11A	100	100	100	100
12EF	100	100	100	100
14A	70	95	55	95
15DEF	40	80	40	90
19EF	35	90	90	100
20AB	45	95	75	100
B] Moderate replicating sites				
3DE	15	50	35	45
4DEF	30	100	60	100
6DEF	50	85	75	65
7ABC	60	95	10	50
8ABC	85	75	80	100
9A	30	95	15	85
9C	10	90	30	85
12A	85	100	45	85
13A	40	95	90	95
13DEF	60	70	35	70
18A	45	60	35	60

Table 2. Labelling frequencies of relatively early replicating X chromosomal sites of Oregon R^+ male, female, reinverted mosaic male and female (data taken from 2D-1D type labelled patterns.

Chromosomal sites	Oregon R^+ male	Oregon R^+ female	Reinverted mosaic male	Reinverted mosaic fem.
1DEF	30	60	0	30
2AB	35	35	0	10
3A	15	50	0	45
4A	50	70	0	60
4BC	45	55	0	20
5CD	40	50	0	45
6A	35	70	5	70
7E	0	65	0	50
8E	20	30	10	30
8F	0	20	0	20
9EF	60	80	5	80
12D	20	40	5	40
14DE	25	35	0	45
16A	35	55	0	55
16DE	20	40	0	40
17A	10	20	10	40
17DEF	15	40	10	40
18DE	20	80	0	80
18F	5	60	0	60
19A	35	95	5	95
19D	10	75	0	75

7ABC) are similar to those in the wildtype (Oregon R^+) female, in the reinverted mosaic male, frequencies of all but the late replicating sites (1A, 3C, 11A and 12EF) are lower than those in the reinverted mosaic female. However, the frequencies for most of the sites are greater than those of the corresponding sites in Oregon R^+ male.

A critical evaluation of the percent frequencies of the labelling presented in Tables 1 and 2, reveal that the different replicating sites or clusters can be classified into three groups. In Table 1 are shown the two classes: (A) those which are late replicating sites and (B) those which include early to moderately late replicating. The sites included in (A) do not show considerable change in labelling frequency in the mutant male and female. The sites included in (B) contain regions which do not show a change in the mutant or show an increased labelling frequency in the male. In contrast, the sites presented in Table 2, which include mostly early replicating regions in normal male, are highly early replicating in the mutant male such that no labelling is virtually observed on the X chromosome of the mutant male, while they do not reveal a considerable change in the X of the mutant female.

Thus, nearly 50% of the replicating sites of the X chromosome of the mutant male shows a relatively more early replicating character as compared to the early replicating property of the Oregon R^+ male.

References: Blumenthal, A.B., H.J. Kriegstein & D.S. Hogness 1974, Cold Spr. Harb. Symp. Quant. Biol. 38:205-223; Chatterjee, S.N. & A.S. Mukherjee 1975, Ind. J. Exp. Biol. 13:452-459; ____ 1978, Ind. J. Exp. Biol. 16:1027-1031; Haegele, K. & W.E. Kalisch 1974, Chromosoma 47:403-413; Lakhotia, S.C. & A.S. Mukherjee 1970, J.Cell Biol. 47:18-33; Mukherjee, A.S. 1982, Curr. Sci. 51(5):205-212.

Glätzer, K.H. and P.-M. Kloetzel.* University of Düsseldorf and *University of Heidelberg, FR Germany. Antigens of cytoplasmic RNP particles of **D.melanogaster** can be localized on distinct Y chromosomal structures in spermatocytes of **D.hydei.**

RNP particles can be isolated from the cytoplasm of Drosophila cells. The particles have been characterized in detail both biochemically and structurally (Schuldt & Kloetzel 1985): the particles consist of 16 polypeptides within the MW range 23000-35000 dalton and contain three small RNAs with as yet unknown function. In the electron microscope the RNP particles have a ring like appearance with an outer diameter of 12 nm and an inner core of 3 nm. With the help of antibodies raised against the different particle proteins it was shown that at least one of the small heat shock proteins of Drosophila must be a genuine part of the 19s particles.

Despite the cytoplasmic location of the RNP particles their constituent antigens are found specifically concentrated on Y chromosomal structures in male germ cells, i.e., in nuclei of primary spermatocytes of Drosophila. In particular, antibody Dm 28K2 reacts very strongly with the Y chromosomal "pseudonucleolus" and "clubs" in spermatocyte nuclei of **D.hydei** (Fig. 1a-c). This specificity can now be used to look at different translocation stocks in order to test for example whether or not the translocated Y fragment still carries the segment responsible for the formation of the "clubs". As known from light microscopical observations, the morphology of a single Y chromosomal structure changes considerably if the whole complement of the Y chromosome is not present in the spermatocyte nucleus. This can also be seen in the electron microscope. The ultrastructure of RNP in spermatocyte nuclei in certain translocation stocks indicates that the presence of a Y section per se is not sufficient to guarantee the normal spatial relation of RNP structures to the Y chromatin (Glaetzer et al., in prep.). This is also evident by indirect immunofluorescence with the antibody Dm 28K2. Despite the presence of the "clubs" the morphology and distribution of this nuclear compartment is greatly altered in these strains (Fig. 1d,e). Even in translocation stocks which by cytogenetical data should carry the same Y chromosomal segment, the "clubs" differ characteristically (data not shown). Another example is stock 701/17 supposed to contain mutated "nooses", i.e., "granular nooses" (Hess 1970). As seen in Fig. 1f the spermatocyte nucleus clearly shows "club"-specific material. Thus, this stock should be reinvestigated with respect to the translocated Y chromosomal segment.

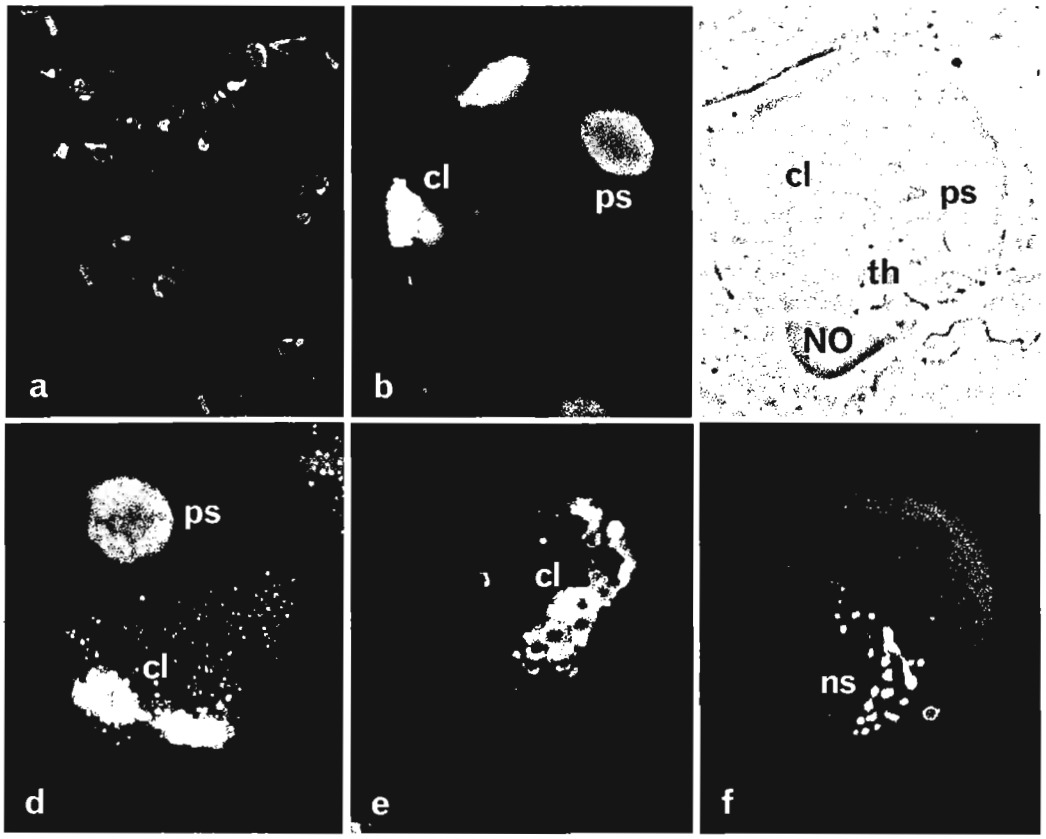


Figure 1. Localization of antigen Dm 28K2 in nuclei of primary spermatocytes by indirect immunofluorescence. (a) squash of wild type spermatocytes, survey photograph to show the identical immunoreaction in several nuclei; (b) a wild type nucleus enlarged to show the specific reaction of antibody Dm 28K2 with distinct Y chromosomal structures; (c) the same nucleus as depicted on b in phase contrast; (d) nucleus of a genotype lacking "tubular ribbons"; (e) nucleus with a Y fragment carrying only "nooses" and "clubs"; (f) nucleus with a Y fragment supposed to carry "granular nooses" **cl**: clubs, **ns**: nooses, **ps**: pseudonucleolus, **th**: threads, **NO**: nucleolus organizer. Magnifications: a) x250, b-f) x1125.

The application of specific antibodies against gene products that play a role in developmental processes, here spermiogenesis in Drosophila, offers a good opportunity for elucidating the role of particular components involved in that process (cf. Glaetzer 1984; Melzer & Glaetzer, this issue). For example, there are indications that the cytoplasmic RNP particles have a regulatory function (Kloetzel et al., in prep.). This is paralleled by the localization of similar polypeptides on specific Y chromosomal formations known to exert regulatory functions in spermiogenesis.

Supported by DFG grant GI 104/2-3 and DFG grant (Forschergruppe Gen-Expression) Ba 384/18-4.1. to P.M.K.

References: Glaetzer, K.H. 1984, Mol. Gen. Genet. 196:236-243; Hess, O. 1970, Mol. Gen. Genet. 106:328-346; Schuldt, C. & P.-M. Kloetzel 1985, Devel. Biol., in press.

González, A. and J.L. Ménsua. University of Valencia, Spain. Allelic rates and population sizes of two populations of **D.melanogaster** from cellar and vineyard.

Captures of **D.melanogaster** were carried out simultaneously in two sites, one cellar and one vineyard, both located in Requena (Valencia) in the east of Spain. In a previous study of these two populations, which are approximately four kilometres away, the relative viabilities of heterozygotes and homozygotes, frequency of lethals and D:L relation of third chromosomes were compared (Gonzalez & Mensua 1983). In this study the allelism rates of lethal third chromosomes from vineyard and cellar are presented, both intra- and interpopulations.

The effective size (N_e) of both populations was estimated according to Nei (1968). This formula assumes that the degrees of dominance of lethal genes and the mutation rates to lethals (u) per locus are the same for all loci:

$$\hat{N}_e = (1-\hat{I}_g) / 4(\hat{I}_g U-u) \ ,$$

where: I_g stands for the allelism rate of lethal genes. Can be estimated by:

$$I_g = \ln(1-I_C Q^2) / (\ln (1-Q)^2) \ .$$

I_C stands for the allelism rate of lethal chromosomes. Q is the frequency of the lethal chromosomes. U is the total lethal mutation rate; Wallace (1968) estimated this for third chromosome 0.005. u is the lethal mutation rate per locus; values of 10^{-5} and 0.20×10^{-5} were used in our calculation; the former estimate comes from the number of lethal producing loci per second chromosome (n=500); this value is assumed also for the third chromosome. The latter estimate is based on n=2.400 (Judd et al. 1972).

The results of allelism test are shown in Table 1. Low frequencies of allelism are observed in the two populations and between populations. There are no significant differences among the three estimates (5% level). Clusters of allelic lethals did not exist in any of the two populations.

The part of the allelism observed which is caused by chance mutations and the part due to consanguinity were estimated in the vineyard and cellar populations according to Wallace (1966), Table 2.

The allelism due to chance mutations was similar in both populations. The greater frequency of allelism observed in the cellar compared to the vineyard, although the difference between these frequencies is not significant, can be attributed essentially to the greater consanguinity inside the cellar habitat.

Table 1. Allelism tests of lethal third chromosomes from cellar and vineyard populations.

Populations crossed	No. of lethal chromosomes	No. of crosses completed	No. of allelic crosses	Frequency of allelic crosses
cellar x cellar	38	703	6	0.00853±0.00347
vineyard x vineyard	40	780	5	0.00641±0.00286
cellar x vineyard	79	1520	5	0.00329±0.00147

Table 2. Estimated values of allelic frequencies of lethals due to chance mutations and consanguinity.

	I_C	I_N	I_F
Vineyard			
n = 500	0.0064	0.0027	0.0037
n = 2400		0.00057	0.0058
Cellar			
n = 500	0.0085	0.0026	0.0059
n = 2400		0.00055	0.0079

I_C = Allelism frequency of lethal chromosomes; I_N = a.f. due to chance mutations; I_F = due to consanguinity.

Table 3. Estimates of the effective populations sizes.

Population	Cellar	Vineyard
Q	0.2452	0.2759
I_c	0.0085	0.0064
I_g	0.0065	0.0047
$N_e(u=10^{-5})$	11000	18500
$N_e(u=0.2x10^{-5})$	8000	11500

The estimates of effective sizes are presented in Table 3. Greater values were obtained for the vineyard population than for the cellar population.

Differences in the allelic rates of cellar and vineyard populations do not exist although the distance between these populations is relatively great. This fact raises the possibility that these are two sub-populations of the same population, and that owing to the greater consanguinity the cellar population has a smaller effective size than the vineyard population.

References: Gonzalez, A. & J.L. Mensua 1983, DIS 59:43-44; Judd, B.H., H.W. Shen & T.C. Kaufman 1972, Genet. 71:139-156; Nei, M. 1968, P.N.A.S. 60:517-524; Wallace, B., The American Naturalist 100:565-578; Wallace, B. 1968, Genet. 60:389-393.

Gupta, J.P. Banaras Hindu University, Varanasi, India. Further additions to the list of drosophilid species from India.

The present communication is in continuation of the previous report appearing in 1981 (DIS 56:50) concerning the drosophilid species described and recorded from India. In the present list an attempt has been made to include all those species recorded thereafter and also the changes which have been made recently regarding the taxonomic status of certain species.

Genus **Acletoxenus** Von Frauenfeld

Genus **Amiota** Loew

1. **indicus** Malloch, 1929
2. **apodemata** Gupta and Panigrahy (submitted)
3. **creberii** Singh, 1976
4. **pictus** (Coquillett, 1904)

Genus **Cacoxenus** Loew

5. **perspicax** (Knab, 1914)
   Cacoxenus punctatus Duda, 1924
   Syn. Ref. McAlpine, 1968, Canad. Entomol. 100(5):514.

Genus **Chymomyza** Czerny

6. **pararufithorax** Vaidya and Godbole, 1973
   vaidyai Okada 1976, Junior Syn. Ref. Okada, 1981, Kontyu 49:171.

Genus **Hypselothyrea** de Meijere

7. **aptera** Papp, 1979

8. **fascipennis** de Meijere, 1906

Genus **Leucophenga** Mik

9. **pentapunctata** Panigrahy and Gupta, 1982
10. **abbreviata** (de Meijere, 1911)
11. **albofasciata** (Macquart, 1851)
    albicincta (de Meijere, 1908)
    Syn. Ref. Bock, 1979, Aust. J. Zool. Suppl. Ser. 71:4.
12. **angusta** Okada, 1956
13. **bellula** (Bergroth, 1894)
    guttiventris (de Meijere, 1908), Syn. Ref. Bock, 1979, Aust. J. Zool. Suppl. Ser. 71:25.
14. **insulana** (Schiner, 1868)
    New Comb. for **D.insulana** Schiner, Ref. Okada, 1977, Cat. Dipt. Orient. Reg. III:347.
15. **pectinata** Okada, 1968
16. **regina** Malloch, 1935
17. **rimbickana** Singh and Gupta, 1981
18. **globosa** Okada, 1965

Genus **Nesiodrosophila** Wheeler & Takada

19. **lindae** Wheeler and Takada, 1964
    pleurostriata Singh and Gupta, 1981
    Syn. Ref. Okada, 1984, Kontyu, 51(1):32.

Genus **Pararhinoleucophenga** Duda

20. **maura** (de Meijere, 1911)

Genus **Phorticella** Duda

21. **flavipennis** (Duda, 1929)
    D.bicolovittata Singh, 1974, Syn. Ref. Wheeler, 1981-83, Add. Cat. World's Drosophilidae:15.

    Zaprionus striata Nirmala Sajjan and Krishnamurthy, 1975. Syn. Ref. Wheeler, 1981-83, Add. Cat. World's Drosophilidae:15.

Genus **Scaptomyza** Hardy

22. **elmoi** Takada, 1970
23. **himalayana** Takada, 1970

Genus **Stegana** Meigen

24. **crescentica** Gupta and Panigrahy (submitted)
25. **shirozui** Okada, 1971
26. **penihexata** Gupta and Panigrahy (submitted)

Genus **Zaprionus** Coquillett

27. **argentostriatus** (Bock, 1966)
    multistriatus Sturtevant, 1927, Syn. Ref. Wheeler, 1981-83, Add. Cat. World's Drosophilidae:16.
28. **grandis** (Kikkawa, 1938)
29. **indianus** Gupta, 1970 (for **indiana**)
    paravittiger Godbole and Vaidya, 1972. Syn. Ref. Okada, 1977, Cat. Dipt. Orient Reg. III:387.
30. **obscuricornis** (de Meijere, 1916)
    New comb. for **D.obscuricornis** de Meijere. Ref. Wheeler, 1981-83, Add. Cat. World's Drosophilidae:16.
31. **orissaensis** (Gupta, 1972)
    New comb. for **D.orissaensis** Gupta 1972. Ref. Wheeler, 1981-83, Add. Cat. World's Drosophilidae:16.

Genus **Drosophila** Fallen

Subgenus **Drosophila** Fallen, Str.

32. **acutisima** Okada,1956
33. **bimorpha** Singh and Gupta, 1980
34. **dominici** Dwivedi, 1982
35. **neoimmigrans** Gai and Krishnamurthy, 1982
36. **neomakinoi** Gupta and Singh, 1981
37. **notostriata** Okada, 1966
38. **novaspinofera** Gupta and Singh, 1979
39. **novazonata** Gupta and Dwivedi, 1980
40. **paralongifera** Gupta and Singh, 1981
41. **parazonata** Gupta and Dwivedi, 1980
42. **penidentata** Singh and Gupta, 1981
43. **penispina** Gupta and Singh, 1979
44. **peniclubata** Singh and Gupta, 1980
45. **pentastriata** Okada, 1966
46. **ramamensis** Dwivedi, 1979
47. **setitarsa** Gupta and Dwivedi, 1980
48. **tetradentata** Singh and Gupta, 1980
49. **fascipennis** Okada, 1967
50. **neokurokawai** Singh and Gupta, 1981

Subgenus **Scaptodrosophila** Duda

51. **bansadharae** Panigraphy and Gupta, 1983
52. **cristata** (Singh, 1976)
    New comb. for **Scaptomyza cristata** Singh, (probably Syn. of **D.silvalineata** Gupta and Ray-Chaudhuri, 1970) Ref. Wheeler, 1981, Genet. Bio. Droso. 3a:56.
53. **koraputae** Gupta and Panigrahy, 1982
54. **neomedleri** Gupta and Panigraphy, 1982
55. **paratriangulata** Gupta and Ray-Chaudhuri, 1970
56. **plumata** (Singh, 1976)
    New comb. for **Scaptomyza plumata** Singh, Ref. Wheeler, 1981, Genet. Bio. Droso. 3a:58.
57. **puriensis** Gupta and Panigraphy, 1982

Subgenus **Sophophora** Sturtevant

58. **agumbensis** Prakash and Reddy, 1978
59. **barbarae** Bock and Wheeler, 1972
60. **bhagamandalensis** Muniyappa, Reddy and Krishnamurthy, 1981
61. **biarmipes** Malloch, 1924
    rajasekari Reddy and Krishnamurthy, 1968, Syn. Ref. Bock, 1980, Syst. Entomol. 5:345.
    raychaudhurii Gupta, 1969. Syn. of **rajasekari** Reddy and Krishnamurthy, Ref. Bock & Wheeler, 1972, Univ. Texas Publ. 7213:23.
62. **brahmagiriensis** Muniyappa, Reddy and Krishnamurthy, 1981
63. **brevina** (Wheeler, 1981)
    New name for **brevis** Parshad and Singh, 1971. Ref. Wheeler, 1981, Genet. Bio. Droso. 3a:60.

Subgenus **Lordiphosa** Basden

64. **cauverii** Muniyappa, Reddy and Prakash, 1982
65. **elegans** Bock and Wheeler, 1972
66. **fruhstorferi** Duda, 1924
67. **gangotrii** Muniyappa and Reddy, 1981
68. **jagri** Prakash and Reddy, 1979
69. **madikerii** Muniyappa and Reddy, 1980
70. **microdenticulata** Panigrahy and Gupta, 1983
71. **nagarholensis** Prakash and Reddy,1980
72. **neotrapezifrons** Ranganath, Krishnamurthy and Hegde, 1983
73. **parabipectinata** Bock, 1971 (authorship error in earlier report)
74. **rhopaloa** Bock and Wheeler, 1972
    coonorensis Reddy and Krishnamurthy, 1973. Syn. Ref. Wheeler, 1981-83, Add. Cat. World's Drosophilidae:12.
75. **sahyadrii** Prakash and Reddy, 1979
76. **sampangensis** Muniyappa and Reddy, 1980
77. **trapezifrons** Okada, 1966
78. **unipectinata** Duda, 1924
79. **coei** Okada, 1966
    angusi Okada, 1977. Syn. Ref. Wheeler, 1981, Genet. Bio. Droso. 3a:54.

Harisanova, N.T. and K.H. Ralchev. University of Sofia, Bulgaria. The effect of 10°C temperature on the embryonic development of **Drosophila hydei.**

(Author's note: This note is related to and follows in sequence the note by Ralchev & Harisanova 1985.) The usual temperature for raising Drosophila is around 24°C though this organism can also live at temperatures ranging from 15 to 34°C (Hedman & Krogstad 1963; Powsner 1935).

We have investigated the influence of 10°C temperature upon the duration of **Drosophila hydei** embryonic development.

A number of embryos were collected within one hour employing the method already described in the preceding paper (Ralchev & Harisanova 1985, this issue). Each egg was put onto a drop of black-coloured agar as shown on Fig. 1. The embryos were kept at 10°C for different periods of time (4, 8, 12, 16, 20 and 24 hr) and transferred to 25°C after that. The control embryos were left to develop at 25°C. Sixty eggs were used for each experimental group.

Table 1. The effect of preserving **Drosophila hydei** eggs at 10°C on their embryonic development.

Time of preserving eggs at 10°C (hr)	Duration of the embryonic development at 25°C (hr)	Percentage of survival*
0(control)	27	89 ± 2.04
4	25	95 ± 0.86
8	24	89 ± 2.12
12	24	89 ± 2.63
16	24	93 ± 1.11
20	24	93 ± 3.47
24	24	82 ± 1.84

* The results are mean values from 6 to 8 experiments.

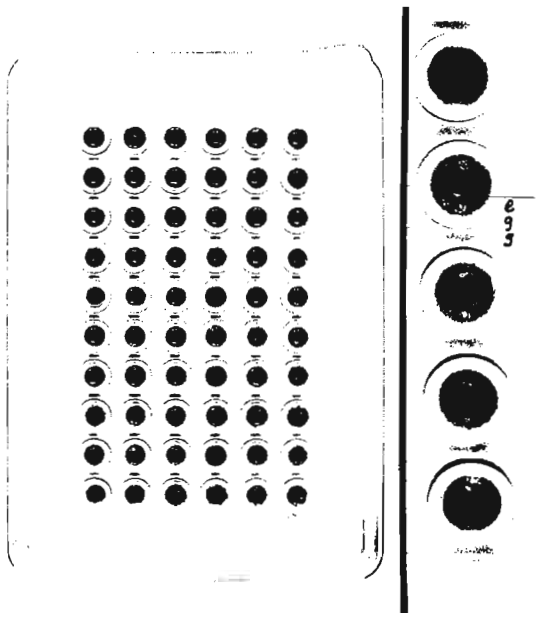


Figure 1. Chamber with black-coloured agar drops. Magnification: (a) 1.3x (b) 4x

We determined the moment of larva hatching after the incubation of embryos at 25°C and calculated the percentage of the hatched eggs. The results obtained are shown in Table 1.

It was obvious that preserving **Drosophila hydei** eggs at 10°C suppressed almost completely their embryonic development but did not influence the viability of the embryos.

We have used this finding in order to facilitate our experimental work when studying histone modifications in embryos at various stages of development.

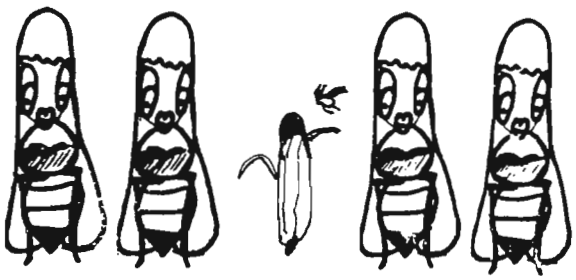
References: Hedman, S. & B. Krogstad 1963, Proc. Minn. Acad. Sci. 31:78-81; Powsner, L. 1935, Physiol. Zool. 474-530; Ralchev, K.H. & N.T. Harisonava 1985, DIS: this issue.

Itoh, N., P. Salvaterra and K. Itakura. Beckman Research Institute (City of Hope), Duarte, California USNA. Construction of an adult Drosophila head cDNA expression library with lambda gt 11.

To isolate neural protein genes, we constructed an adult Drosophila head cDNA library using lambda gt 11 which was shown to be a useful expression vector (Young & Davis 1983). RNA was extracted from frozen adult Drosophila (Canton S) heads by the Urea-LiCl method (Auffray & Rougeon 1980). Poly(A) containing RNA was prepared by oligo (dT) cellulose chromatography (Aviv & Leder 1972), and examined after electrophoresis on agarose gels containing methylmercuric hydroxide (Bailey & Davidson 1976). We did not detect any degradation in the poly(A) containing RNA sample. Double-stranded cDNA was synthesized from 16 µg of the poly(A) containing RNA as a template using AMV reverse transcriptase, E.coli DNA polymerase I (Klenow fragment) and S 1 nuclease (Wickens, Buell & Schimke 1978). The double-stranded cDNA was treated with EcoR1 methylase to protect EcoRI cleavage sites, and treated with E.coli DNA polymerase (Klenow fragment) to increase the number of flush-ended double-stranded cDNA molecules (Huynh, Young & Davis 1985). After ligation to EcoRI linkers, the double-stranded cDNA was digested with EcoRI and fractionated by Sephacryl-S1000 chromatography. Fractions containing double-stranded cDNA 800-5,000 base pairs long were pooled and precipitated by ethanol. Lambda gt 11 DNA was disgested with EcoRI and dephosphorylated by digestion with calf intestinal alkaline phosphatase. The double-stranded cDNA was ligated to dephosphorylated, EcoRI-cut lambda gt 11 DNA with T4 DNA ligase. The ligated lambda gt 11 was packaged, in vitro, using a lambda packaging mixture (from J. Forrest, Beckman Research Institute of the City of Hope). The package library was amplified as plate lysates on agar plates. The library contained 1.3×10^7 independent phage. Ninety-eight per cent of the phage produced white plaques on agar plates containing X-Gal and IPTG, indicating 98% of the phage were recombinants.

Recently, we have isolated 14 positive recombinant phage clones from the library by immunological screening with a mixture of three different monoclonal antibodies to choline acetyltransferase of Drosophila. The cDNA inserts ranged in size from 1,000 base pairs to 2,400 base pairs. We are currently trying further characterization of the cloned DNA.

References: Young, R.A. & R.W. Davis 1980, Proc.Natl.Acad.Sci. 80:1194-1198; Auffray, C. & F. Rougeon 1980, Eur.J.Biochem. 107:303-314; Aviv, H. & P. Leder 1972, Proc.Natl.Acad.Sci. 69:1408-1412; Wickens, M.P., G.N. Buell & R.T. Schimke 1978, J.Biol.Chem. 253:2483-2495; Bailey, J.M. & N. Davidson 1976, Anal.Biochem. 70:75-85; Huynh, T.V., R.A. Young & R.W. Davis 1985, in DNA Cloning: A Practical Approach (D.Glover, ed., IRL Press, Oxford) in press.



Ivanchenko, M., T. Braude-Zolotaryova and V. Kakpakov. Inst. of General Genetics of USSR, Moscow. Histone extracts from nuclei of established **Drosophila** cell lines contain two additional minor components.

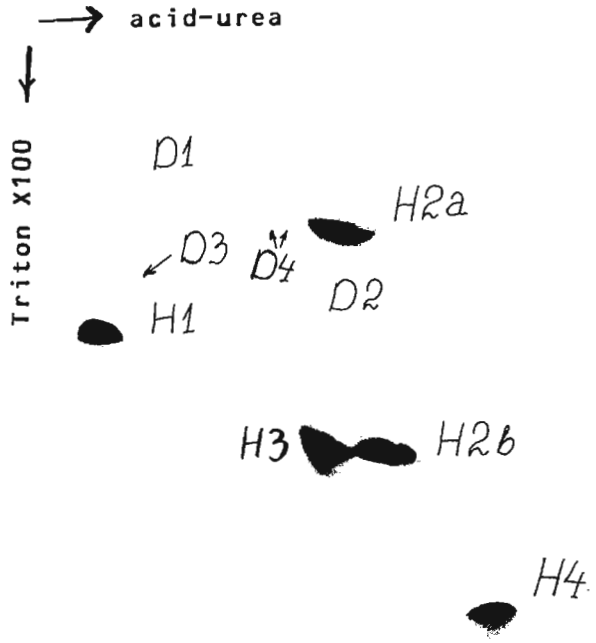


Figure 1. Two-dimensional gel electrophoresis of **D.virilis** cell culture histone extract. **D.melanogaster** culture cells contains the small amounts of D3 and D4 which are clearly visible only on silver-stained gels.

It is well known that minor histone-like proteins occur in nuclei from different tissues and species. These proteins have electrophoretic properties chracteristic for histones but their amino acid compositions are not closely related to any of the five major histone classes. Histone extracts of vertebrates may comprise three or four minor components (M1, M2, M3, M4) (Franklin & Zweidler 1977; Urban et al. 1979). In Drosophila two minor proteins have been discovered and designated D1 and D2 (Alfageme et al. 1975). In nuclei from Drosophila established cell lines 79f4DV3g (**D.virilis**) and 67j25D (**D.melanogaster**), we observed two additional minor proteins designated as D3 and D4 in Fig. 1.

Purified nuclei were prepared by a modification of a method previously described (Oliver & Chalkley 1972) but in the presence of mercaptoethanole and phenylmethylsulfonyl fluoride in all buffers. Histones were extracted from nuclei with 0.4N H_2SO_4. Two-dimensional gel electrophoresis was performed as described by Russanov et al. (1980), but in presence of 5.5M urea in the first dimension and 7.5M urea in the second one.

Similar to the inner histones, D1 and D2, D3 and D4 have high affinity to Triton X 100. They are present in diploid embryonic established cell lines of **D.virilis** and **D.melanogaster** but not in the whole embryos of these Drosophila species. Because, D3 and D4 may be specific proteins of Drosophila cell cultures.

The nature of D3 and D4 is not known.

References: Alfageme, C.R., A. Zweidler, A. Mahowald & L.H. Cohen 1975, J. Biol. Chem. 249: 3729-3736; Franklin, S.G. & A. Zweidler 1977, Nature 266: 273-275; Oliver, D. & R. Chalkley 1972, Exp. Cell Res. 73: 295-302; Russanova, V., Ch. Venkov & R. Tsanev 1980, Cell Differ. 9: 339-350; Urban, M.K., S.G. Franklin & A. Zweidler 1979, Biochem. 18: 3952-3960.

Ivanchenko, M. and L. Muhovatova. Institute of General Genetics, Academy of Sciences of USSR, Moscow. Modifications of **Drosophila** proteins D1 and D2.

Specific for Drosophila proteins D1 and D2 with unknown nature were discovered in the histone extracts from nuclei (Alfageme et al. 1975). D2 is nucleosomal and histone-like (Palmer et al. 1980). D1 resembles HMG proteins of vertebrates (Alfageme et al. 1980).

We have investigated electrophoretically the histone extract from nuclei of **D.melanogaster** cell culture line 67j25D. Cells were incubated 5h in medium containing ^{32}P-ortophosphate or ^{3}H-acetate. The nuclei were prepared as previously had been described (Oliver & Chalkley 1972) but with presence of mercaptoethanole and phenylmethylsulfonyl fluoride in all the buffers. Histones were extracted from nuclei with 0.4N H_2SO_4. Two dimensional electrophoresis was performed as previously described (Russanova et al. 1980) but in presence of 5.5M urea in 1-dimension and 7.5M urea in 2-dimension. For autoradiographic analysis, gels were dried in C_2H_5OH.

The protein D1, as the histones H2a, H1 and H4, incorporate ^{32}P-ortophosphate (Fig. 1) but the protein D2 incorporates ^{3}H-acetate (Fig. 2).

These qualities of D1 and D2 can help to resolve the problem of their nature and biological functions.

References: Alfageme, C.R., G.T. Rudkin & L.H. Cohen 1980, Chromosoma (Berl.) 78: 1-31; Alfageme, C.R., A. Zweidler, A. Mahowald & L.H. Cohen 1975, J. Biol. Chem. 249: 3729-3736; Oliver, D. & R. Chalkey 1972, Exp. Cell Res. 73: 295-301; Palmer, D., L.A. Snyder & M. Blumenfeld 1980, Proc. Natl. Acad. Sci. USA 77: 2671-2675; Russanova, V., Ch. Venkov & R. Tsanev 1980, Cell Differ. 9: 339-350.

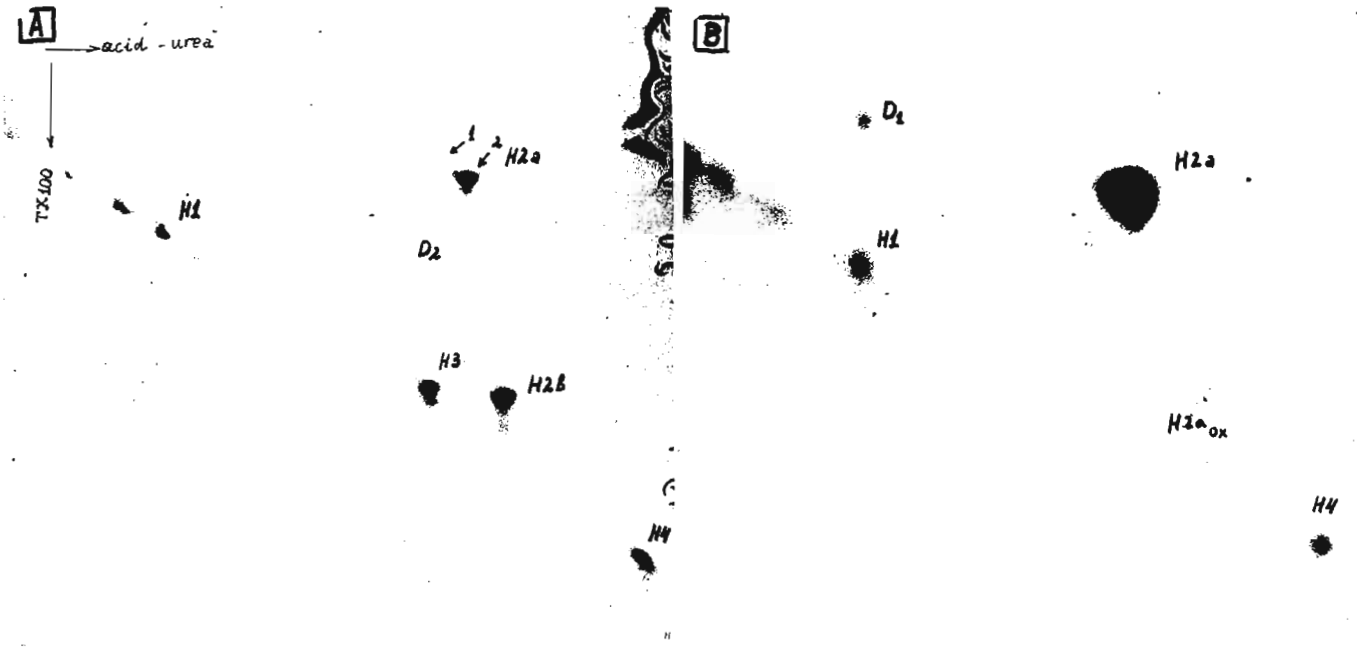


Figure 1. Incorporation of ^{32}P-ortophosphate into **D.melanogster** cell culture histone extract proteins. (A) Stained gel after two-dimensional electrophoresis. (B) Autoradiograph of the same gel.

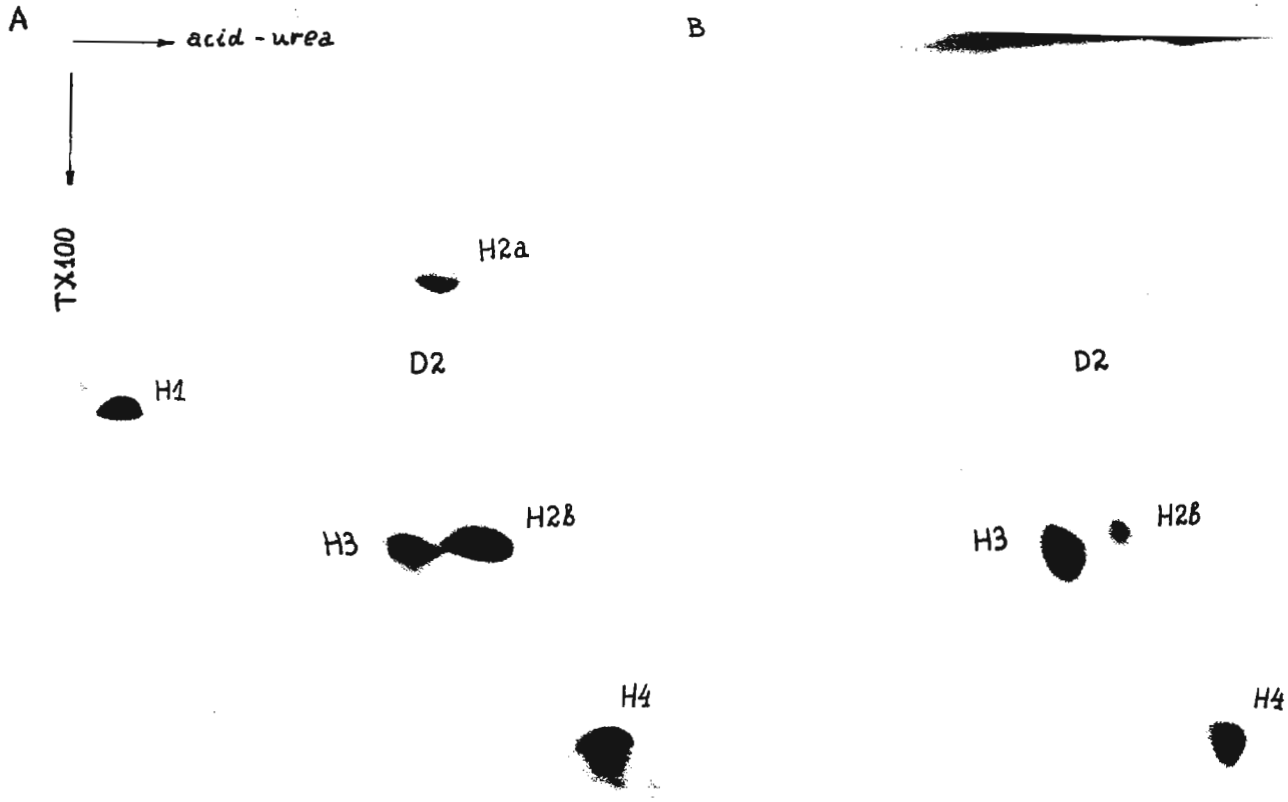


Figure 2. Incorporation of ^{3}H-acetate into **D.melanogaster** cell culture histone extract proteins. (A) Stained gel. (B) Autoradiograph of the same gel.

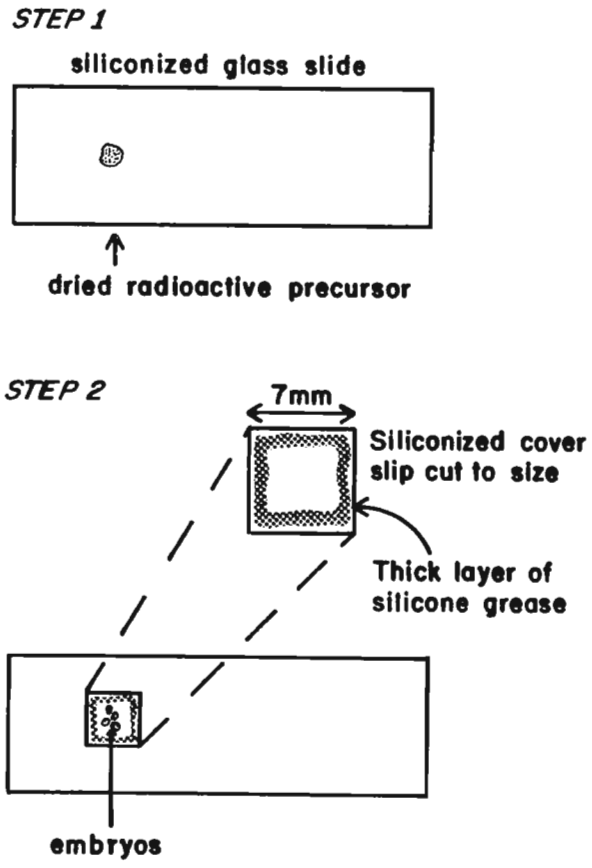
Jacobs-Lorena, M. Case Western Reserve University, Cleveland, Ohio USNA.

Radiolabeling of Drosophila embryonic RNA and protein to high specific activity.

With the recent emphasis on the molecular approach to the study of Drosophila embryogenesis, the availability of a method for the radiolabeling embryonic macromolecules to high specific activity is highly desirable. Although procedures for the labeling of embryonic proteins have been published (Zalokar 1973; Raff et al. 1982), the incorporation of radioactive precursors is relatively low or unknown.

We have devised a protocol for radiolabeling Drosophila embryonic RNAs and proteins to high specific activity. It is based on our previous observations with other tissues (e.g., ovaries) that the net incorporation of radioactive precursors into macromolecules relates directly to the concentration and not to the amount of labeled precursors in the surrounding medium. This paradigm was taken to its extreme in the procedure illustrated in Figure 1. Dechorionated embryos were placed over dried radioactive precursor. The embryos were then gently disrupted by crushing under light pressure. No buffer is used; the radioactive precursor is dissolved in the embryo's own fluid. This provides for the maximum possible concentration of labeled precursor. Incubation is at room temperature (about 22°C) and the contents are mixed occasionally by applying gentle pressure over the cover slip. This procedure has been used for labeling both RNA and protein.

The time course of incorporation of ^{3}H-uridine into RNA is illustrated in Figure 2. Incorporation proceeds for at least 1 hr. The mean incorporation of ^{3}H-uridine into RNA in 4 independent experiments was 5,100 (±1,300) CPM per embryo per 40 min for 20-hr old embryos. High incorporation was also obtained when ^{35}S-methionine was used as a precursor. The mean incorporation of ^{35}S-methionine into protein in 11 independent experiments was 68,900 (±26,400) CPM and 87,600 (±56,000) CPM per embryo per 40 min incubation at 22°C for 5-hr old and 19-hr old embryos, respectively.



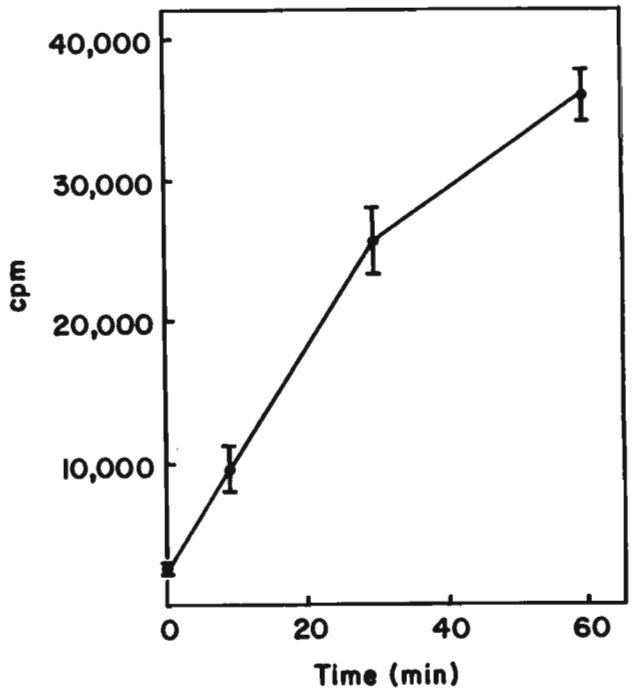


Figure 2. Time course of incorporation with ^{3}H-uridine. Groups of 5 embryos (20-hr old) were labeled with 1 uCi of ^{3}H-uridine for the specified length of time. At the end of the incubation the cover slip was flipped over and the contents were transferred with several 2 ul aliquots of 1% SDS to a filter paper disc. The discs were washed several times in the cold with 5% trichloroacetic acid and the insoluble radioactivity was quantitated by scintillation counting. The background was not substracted.

Figure 1. Set up for radiolabeling of Drosophila embryos. Step 1: a droplet of the radioactive precursor is placed over a siliconized glass microscope slide and dried under vacuum. Step 2: 5 to 15 hand-dechorionated embryos are placed over the dried labeled precursor; these are immediately covered with the cover slip and gentle pressure is applied over its center to disrupt the embryos and at the same time establish a hermetic seal by silicone grease.

For most purposes, analysis of the labeled macromolecules can be accomplished by dissolving the embryo in strongly denaturing detergents, such as sodium dodecyl sulfate (SDS). However, for certain applications this may not be desirable. For instance, we have recently used this labeling procedure to study the synthesis of ribosomal proteins at different stages of embryogenesis (Kay & Jacobs-Lorena, submitted). The two-dimensional gel system that was used for the analysis of ribosomal proteins is incompatible with the presence of SDS. Other denaturing agents such as urea resulted in incomplete extraction of the labeled proteins from the embryos. We therefore extracted the labeled embryos sequentially with a buffer containing Triton X-100 and then with 66% acetic acid, an excellent protein solvent. In this way the labeled proteins were completely extracted. The Triton was then removed by ether extraction. To introduce the labeled proteins into electrohoresis buffer, a Biogel P6 (BioRad) gel filtration column was prepared in a 1-ml disposable syringe and equilibrated in electrophoresis buffer. The column was placed in a test tube over a 1.5 ml conical tube. The radioactive sample (approx. 100 ul) was applied onto the column and centrifuged for 2 min at 1,800 RPM. All of the labeled ribosomal proteins were recovered in the first eluate, while non-incorporated label and undesired ions were retained in the column. The eluate could then be used directly for electrophoresis. Alternative procedures to change the ionic composition of the ribosomal proteins to that of electrophoresis buffer, such as precipitation with trichloroacetic acid or dialisys, were not satisfactory in that the recovery was low or unpredictable.

In summary, a simple procedure for very efficient radiolabeling and analysis of embryonic macromolecules is described. Because the embryos are disrupted, all tissues are equally exposed to the isotope allowing for uniform labeling of embryos at any developmental stage.

References: Limbourg, B. & M. Zalokar 1973, Dev. Biol. 35:382-387; Raff, E.C., M.T. Fuller, T.C. Kaufman, K.J. Kemphues, J.E. Rudolph & R.A. Raff 1982, Cell 28:33-40.

Jennings, N.J. and R.D. Seager. University of Northern Iowa, Cedar Falls, USNA.

Larval substrates of wild Drosophila.

For the past several years we have been sampling Drosophila populations from different communities near Cedar Falls, Iowa (Jennings et al., submitted to Proc. Iowa Acad. Sci.; Seager & Jennings 1984). In order to add to our knowledge of natural breeding sites of Drosophila, in conjunction with our 1983 collections we attempted to rear adult flies from probable larval substrates found in a lowland forest community. These data were gathered in order to determine the possible success of a more thorough future study of this type. In addition we observed adult flies on some of these substrates and collected (aspirated) them for later identification. The data we report here are preliminary since our fungal identifications have not been verified by taxonomists. For future collections we have enlisted the aid of a fungal expert.

The substrates from which adults were either reared or aspirated and the species involved are:

Agaricus sp.: **D.tripunctata** and **D.falleni** were both reared and aspirated and **D.testacea** was reared from this species.

Tremella sp.: **D.putrida** and **D.falleni** were both reared and aspirated from this species.

Juglans nigra (black walnut): **D.tripunctata** was aspirated from walnuts.

Morus sp. (mulberries): **D.affinis, D.falleni, D.putrida,** and **D.tripunctata** were all aspirated from fallen mulberries.

In addition, four fungal growths which we were unable to key were studied. **D.quinaria, D.putrida, D.tripunctata,** and **D.falleni** were all reared from at least one of these, and **D.putrida, D.tripunctata, D.falleni,** and **D.testacea** were all aspirated from at least one of these.

The lack of suitable larval substrates will limit the distribution of a species. **D.tripunctata** was very common in our collections from a lowland forest community but was not found in samples taken at the same time from a nearby sand prairie community (Seager & Jennings 1984). At least two of the substrates that this species apparently uses, walnuts and mulberries, are present in the lowland community but are absent from the sand prairie community. Thus the absence of a suitable larval substrate may restrict the distribution of **D.tripunctata.** This is a possibility that we will explore in more detail in the future.

Reference: Seager & Jennings 1984, DIS 60:182-184.

Kalisch, W.-E. and J. Böhm. Ruhr-Universität Bochum and Universität Tübingen, FR Germany.

The EM band-interband pattern of SSP chromosomes in **D.subobscura.**

We are revising the LM chromosome map of salivary glands from **D.subobscura** (Kunze-Muehl & Mueller 1958) on the basis of electron micrographs from surface spread polytene (SSP) chromosomes. The same preparation technique has already been used in **Chironomus** (Kalisch 1982; Kalisch & Whitmore 1983) and in **D.hydei** (Kalisch et al. 1985; Whitmore et al., this issue). However, due to the species specific chromosomal proteins we had to modify our SSP chromosome preparation technique for **D.subobscura.** The primary difference compared with the original technique (Kalisch et al. 1984) is in the shortening of the time needed for the acid pretreatment (4 min instead of 30 min in 3.18 M citric acid 1-hydrate and 8.82 M

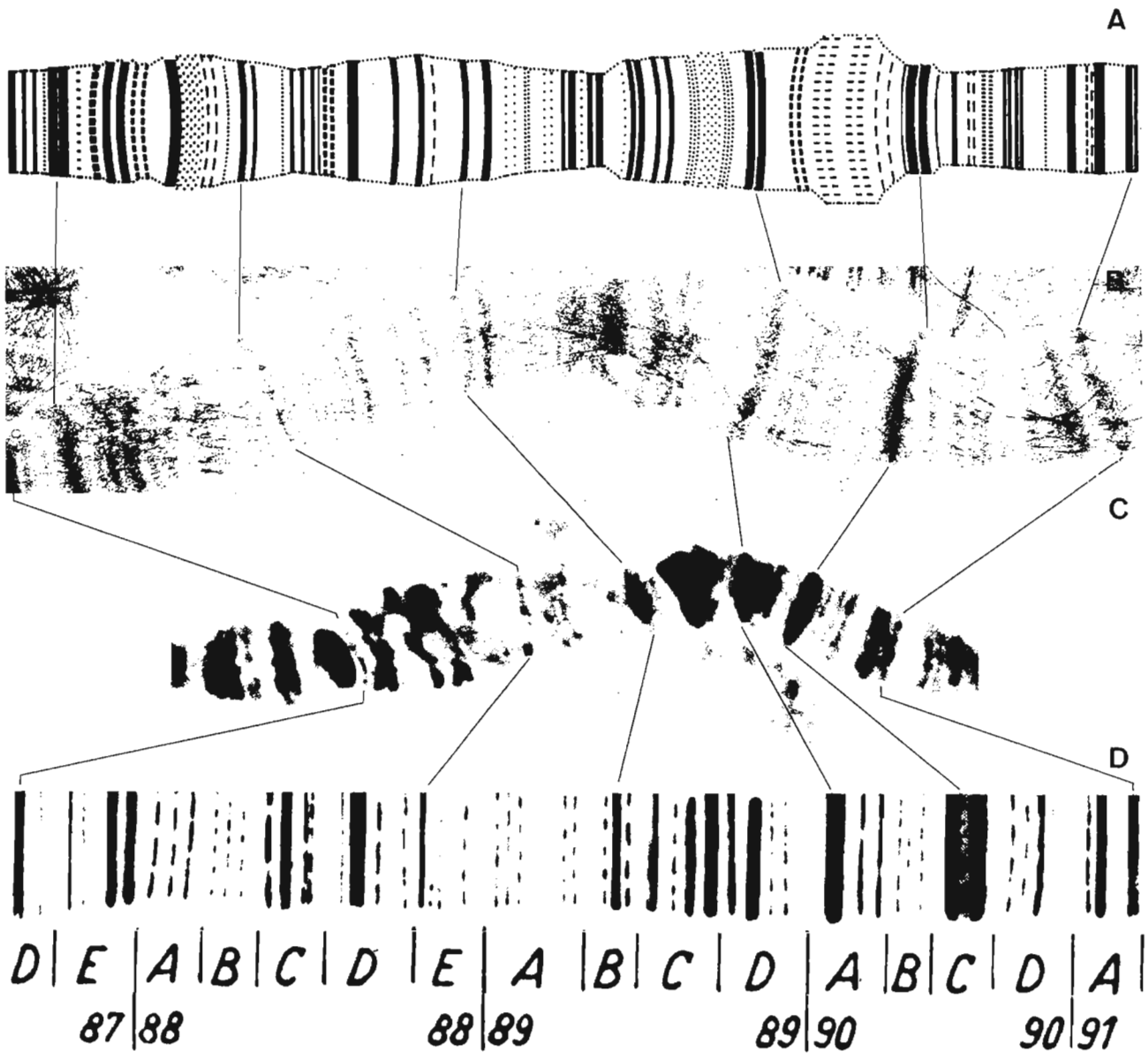


Figure 1. Salivary gland chromosome maps of divisions 88-90 in **D.subobscura.** (A) EM chromosome map based on five SSP chromosome preparations. (B) Electron micrograph of a SSP chromosome. x1600. (C) Light micrograph of a chromosome squash preparation (Pinsker & Sperlich 1981). (D) LM chromosome map based on a large number of chromosome squash preparations (Kunze-Muehl & Mueller 1958).

propionic acid) of the excised salivary glands. Further methodological modifications will be described in detail (in prep.).

To compare the patterns achieved by different chromosome preparation techniques, we have analyzed homologous polytene structures in light micrographs from squash preparations and in electron micrographs from SSP chromosome preparations. Fig. 1 shows our preliminary results for divisions 88-90 of chromosome O: (A) The computerized plot of the EM chromosome map from five SSP chromosome preparations, (B) an individual electron micrograph of a SSP chromosome, (C) an individual light micrograph of a squash preparation, and (D) the LM chromosome map based on many squash preparations (Kunze-Muehl & Mueller 1958).

The plot of the chromosome map shown in Fig. 1A is based on a simple BASIC program written for use with IBM PC XT (MS-DOS) and a Hewlett-Packard HP7475A plotter. The program is basically the same as the one we published for **D.hydei** (Reiling et al. 1984a,b). However, automatic plotting of chromosome outlines by connecting individual chromosome bands with dotted lines, has now been integrated into the program. Those who are interested in obtaining a copy of the program may do so by sending a 5.25" disk (double-sided, double-density, soft sector) to the first author.

Preliminary results: (1) The longitudinal spreading of the SSP chromosome preparation separates prominent bands and interbands from each other. By this, they are not 'fused' as usually depicted in squash preparations (e.g., compare subdivisions 89B-90A in Fig. 1 B and C). (2) The bands registered in the LM chromosome map (Kunze-Muehl & Mueller 1958) are depictable in individual SSP chromosome preparations. (3) Cytologic homology between the LM chromosome map of Kunze-Muehl & Mueller (1958) and the chromosomes in squash preparations as well as in SSP chromosome preparations is sometimes unusual for individual subdivisions as can be judged from the map example depicted in Fig. 1D. Due to this, we have decided to revise the entire map and to put more emphasis on the cytological peculiarities of the pattern (Fig. 1A). (4) The total number of bands in the EM chromosome map shows a ca. 40% increase (81:58) in comparison with the LM chromosome map in Fig. 1D. However, the number of additional bands of EM micrographs compared with LM micrographs differs strongly in individual chromosome subdivisions according to the number of submicroscopical bands and interbands (Kalisch et al. 1985).

For map construction of the entire genome, we still need light micrographs of squash preparations. Contributors are kindly invited as co-authors (for details, see REQUESTS in this issue).

References: Kalisch, W.-E. 1982, Genetica 60:21-24; Kalisch, W.-E. & T. Whitmore 1983, Cytobios 37:37-43; Kalisch, W.-E., T. Whitmore & H. Reiling 1984, Cytobios 41:47-62; Kalisch, W.-E., T. Whitmore & G. Schwitalla 1985, Chromosoma, in press; Kunze-Muehl, E. & E. Mueller 1958, Chromosoma 9:559-570; Pinsker, W. & D. Sperlich 1981, Genetica 57:51-64; Reiling, H., W.-E. Kalisch, T. Whitmore & K. Tegtmeier 1984a, Europ. J. Cell Biol. 34:336-338; Reiling, H., W.-E. Kalisch & T. Whitmore 1984b, DIS 60:172-174; Whitmore, T., G. Schwitalla & W.-E. Kalisch 1985, DIS 61 (this issue).

Kaplan, H. and T. Glover. Hobart College, Geneva, New York USNA. Frequency dependent selection utilizing Bar and sepia mutants of **Drosophila melanogaster.**

Frequency dependent selection has been observed in several species of Drosophila (see review by Ehrman & Probber 1978). When given a choice between two different types of males (different in genotype at a particular locus, reared at different temperatures, or collected from different geographic locations), females mate more frequently than expected with the type of male which is rare. Although this rare male mating advantage is well documented in **Drosophila pseudoobscura** (Ehrman 1966, 1967, 1968), reports of frequency dependent selection in **Drosophila melanogaster** are somewhat rarer. Markow et al. (1978) suggest that frequency dependent selection in **D.melanogaster** is not as common or as strong as reported for **D.pseudoobscura.**

A clear-cut example of strong frequency dependent selection in **D.melanogaster** has been observed using two eye mutant strains, Bar and sepia. Bar is a sex-linked dominant trait caused by a partial chromosome duplication and appears phenotypically as narrow or kidney-shaped red eyes. Sepia is a recessive point mutation on the third chromosome and appears phenotypically as reddish-brown to black eyes.

A total of 100 males in various Bar to sepia ratios were placed in half pint bottles containing culture media and 50 sepia virgin females. The flies were allowed to mate for 24 hr, after which each female was placed in a separate vial. The type of male which inseminated each female was determined by scoring the individual progenies produced by these females.

Table 1. Proportion of matings by Bar and sepia males at various ratios.

Male ratio Bar:sepia	No. females producing F_1	Bar male mating %	Sepia male mating %
9:1	45	75.6	24.4
7:3	47	66.0	34.0
6:4	45	71.1	28.9
1:1	44	68.2	31.8
4:6	42	69.0	31.0
3:7	40	45.0	55.0
1:9	44	25.0	75.0

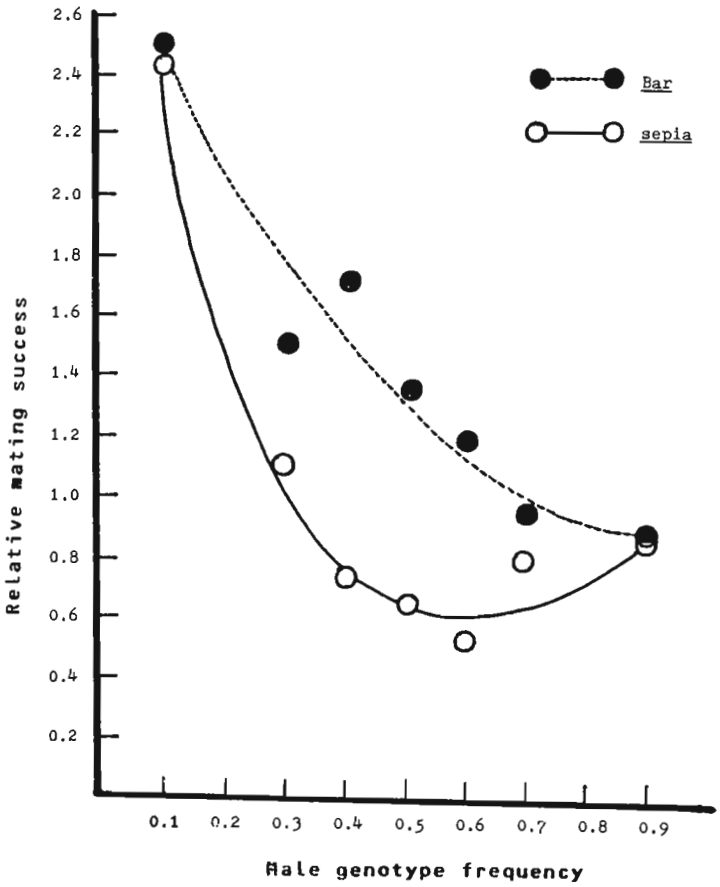


Figure 1. Relative mating success of Bar and sepia males at various genotype frequencies. Genotype frequency is the proportion of Bar or sepia males present during mating. Relative mating success (RMS) is the proportion of mating by one type of male divided by the proportion at which that type was present during mating. An RMS=1 indicates random mating. An RMS=2.5 indicates that the particular male strain mated 2½ times as often as would be expected if mating were random.

The results (Table 1 and Figure 1) indicate that both Bar and sepia males, when rare (comprising 10% of the population), mated nearly $2\frac{1}{2}$ times as often as would be expected if mating were random. As the frequency of Bar or sepia males increased, their relative mating success generally decreased (Figure 1). The data was analyzed by a statistical method suggested by Ayala (1972). Log transformations of the proportions of males that mated and the proportions of males present were made. Utilizing this transformed data, a linear regression analysis of the proportion of mated males on the proportion of males present was performed as both a test of frequency dependent mating and as an indicator of its strength. If mating is random, then the regression coefficient is expected to be 1 and if a rare male mating advantage exists, then the regression coefficient is expected to be less than 1. For this data the regression coefficient is 0.521±0.076 which is significantly less than 1 ($P<0.005$). Hence, it is concluded that this data constitutes an unequivocal example of frequency dependent selection in **D.melanogaster.**

References: Ehrman 1966, Anim.Behav. 14:332; _____ 1967, Amer.Nat. 101:415; _____ 1968, Genet.Res. 11:135; Ehrman & Probber 1978, Am.Sci. 66:345; Markow et al. 1980, Genet.Res. 35:59; Ayala 1972, Behav.Genet. 2:85.

Kidwell, M.G. and J.B. Novy. Brown University, Providence, Rhode Island USNA. The distribution of hybrid dysgenesis determinants in North American populations of **D.melanogaster.**

A collection of more than 200 isofemale lines from 21 locations in N. America was made in order to test their potential for various hybrid dysgenic traits. The majority of isofemale lines were established from natural population collections made during the 1977-79 period and tested shortly thereafter. Several additional lines collected before and after this period were included in some of the analyses. The number of isofemale lines tested per location varied from five to twenty. For the P-M system of hybrid dysgenesis, the traits tested were gonadal (GD) sterility, male recombination, and transmission ratio distortion. SF sterility was the only I-R system trait tested. Both hybrid dysgenesis components were tested with respect to the two sterility traits, using the standard Cross A and A* assays (Kidwell 1979). Lines were tested for male recombination and transmission ratio distortion in the second and third chromosomes by crossing with females from the multiple marked stock al b sp; ve st ca. F_1 males were then individually backcrossed to females of the multiple marked stock.

Table 1 presents means and standard errors for male recombination (Cross A) and GD sterility and SF sterility (Cross A and A*) for all locations sampled during the 1977-79 period. In Figure 1 the GD sterility values for Cross A and Cross A* are plotted for those locations from which at least ten isofemale lines were tested. It is seen that, with the exception of the Niagara, Ontario 1975 collection, all locations show rather similar distributions, indicating strong P cytotype and polymorphism for the P/Q types. The exceptional line from Niagara showed a pattern indicating a Q/M' polymorphism which is very common in many European populations (Anxolabéhère et al. 1984).

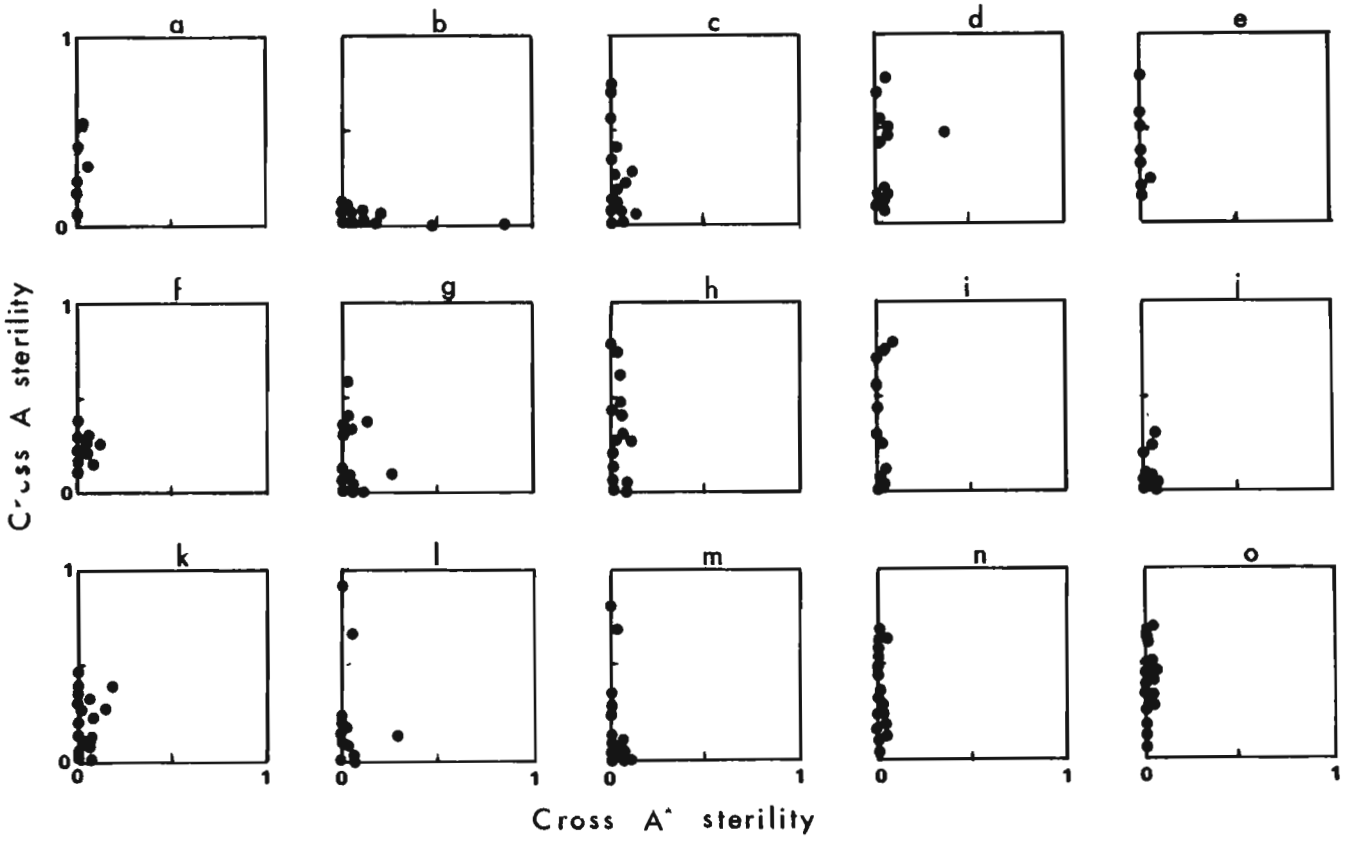


Figure 1. Distributions of GD sterility frequencies in isofemale lines collected at 15 N. American locations during the period 1971-82. **a.** Coolspring, Maryland 1971; **b.** Niagara, Ontario 1975; **c.** Amherst, Massachusetts 1977; **d.** Athens, Georgia 1977-78; **e.** Des Moines, Iowa 1977; **f.** Lubbock, Texas 1977; **g.** Madison, Wisconsin 1978; **h.** Raleigh, North Carolina 1977; **i.** Pequot Lakes, Minnesota 1977; **j.** Portland, Oregon 1979; **k.** Sonoma Valley, California 1979; **l.** St. Catherines, Ontario 1977; **m.** St. Paul, Minnesota 1979; **n.** El Rio, California 1982; **o.** Miami, Florida 1977-78.

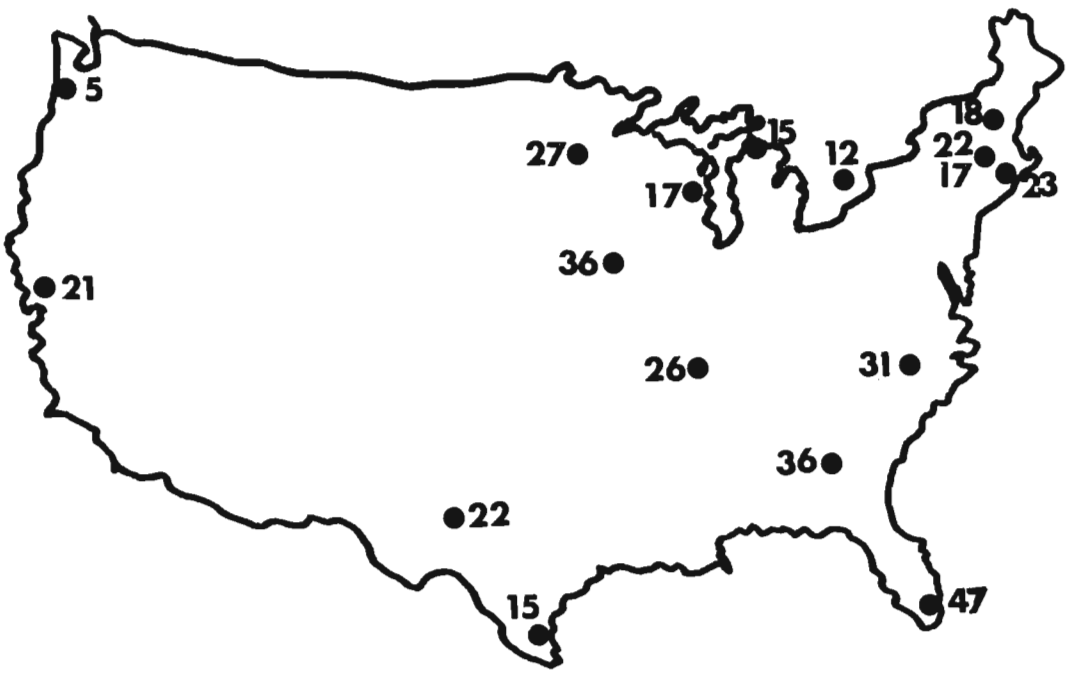


Figure 2. Geographical variation in P factor activity in N. America. The numbers are mean frequencies of GD sterility (Cross A) for isofemale lines collected at 17 locations during the 1977-79 period.

Table 1. Mean values and standard errors of male recombination, GD and SF sterilities in isofemale lines from those locations sampled during the period 1977-79.

Location	Year	MR %*	GD %	SF %
Amherst, MA	1978	2.2 ± 0.32	21.6 ± 5.60	61.8 ± 5.40
Athens, GA	1977	4.3 ± 0.73	36.4 ± 6.67	44.9 ± 6.22
Bowling Green, KY	1978	2.3 ± 0.60	26.4 ±10.46	49.1 ± 5.11
DesMoines, IA	1977	6.2 ± 1.49	35.6 ± 7.00	43.9 ± 8.43
Houston, TX	1979	2.6 ± 0.75	14.6 ± 7.57	60.3 ± 12.84
Lake Charlevoix, MI	1977	3.6 ± 0.55	15.4 ± 5.96	68.5 ± 13.22
Lubbock, TX	1977	3.0 ± 0.69	21.8 ± 2.53	46.9 ± 7.97
Madison, WI	1978	N.D.	16.9 ± 4.74	46.9 ± 4.80
Markert, MA	1977	2.1 ± 0.38	16.1 ± 3.79	38.6 ± 4.84
Miami, FL	1977	6.5 ± 3.20	46.8 ± 6.85	39.2 ± 13.09
Moultonborough, NH	1977	2.5 ± 0.56	18.1 ± 5.18	46.4 ± 6.93
Pequot Lake, MN	1977	2.3 ± 0.35	27.7 ± 7.56	46.8 ± 6.50
Portland, OR	1979	1.7 ± 0.32	5.0 ± 3.66	52.3 ± 10.68
Raleigh, NC	1977	4.4 ± 1.55	30.5 ± 5.93	43.3 ± 5.49
Sonoma, CA	1979	N.D.	21.5 ± 3.40	47.1 ± 5.55
St. Catherines, Ont.	1977	1.56± 0.33	11.9 ± 4.07	47.8 ± 6.72
Weymouth, RI	1977	1.99 ± 0.23	22.7 ± 6.76	61.7 ± 5.03

* % male recombination in chromosome 2 and 3 combined, uncorrected for clustering.

As shown in Table 1 and illustrated in Figure 2, there was some variability in mean values of GD sterility (Cross A) from location to location. The standard errors are fairly large and the sampling inadequate to enable any clear geographical pattern to be detected. However, it is noted that there is a tendency for high P factor activity to be found most frequently in southeast locations and low activity to be found in northwest locations. D. D. Home (pers. comm.) has independently collected data supporting the observation of low P activity in the northwest. A large majority of 66 lines collected in the Fraser Valley region of British Columbia proved to have Q strain characteristics.

The data for all four measured traits from the 1977-79 collections were used to compute correlation coefficients (Kendal's Tau). The results are summarized in Table 2. There was a highly significant positive correlation between GD sterility (Cross A) and male recombination (chromosomes 2 + 3) and a highly significant negative correlation between GD sterility and k_3 (a measure of transmission distortion in the third chromosome). All other correlations were not significant at the 95% level of probability.

Figures 3 and 4 present histograms for the overall distribution of GD sterility and male recombination, respectively, when all isofemale lines from different locations were pooled together. The difference in form of the two distributions might be explained by the greater sensitivity of male recombination, over that of GD sterility, to low levels of transposase enzyme. One striking implication of these distributions is that commonly used strong P strains, like Harwich and π_2, are very atypical of P strains in general. The distributions also provide further justification for treating Q strains as a weak subset of P strains.

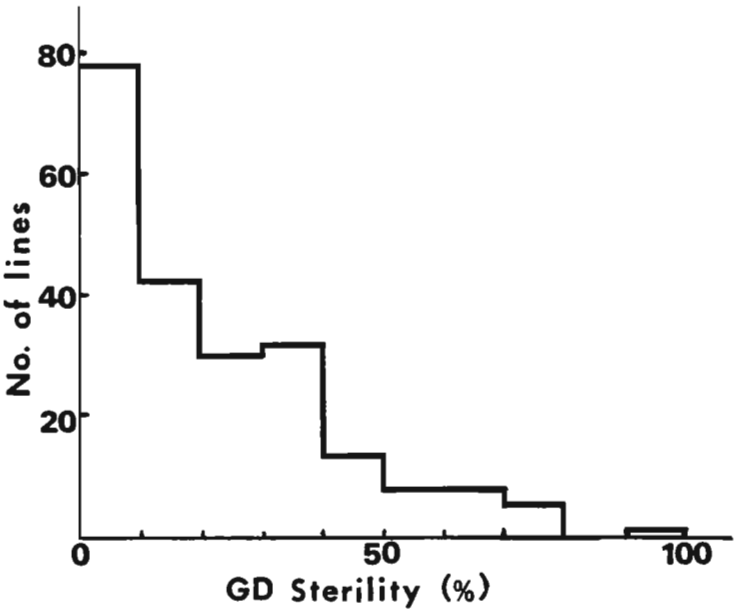


Figure 3. The pooled distribution of GD sterility frequencies (Cross A) for all those N. American isofemale lines tested during the period 1977-79.

Table 2. Rank sum correlation coefficients (Kendall's tau), and their respective probabilities (P) between location means for selected pair of dysgenic traits (1977-79 collections).

		Dysgenic traits				
		P-M				I-R
		GD	MR	k_2	k_3	SF
GD	τ	--	0.484	-0.126	-0.385	-0.238
	P	--	0.001**	0.218	0.009**	0.066
MR	τ	--	--	-0.053	-0.195	-0.123
	P	--	--	0.373	0.115	0.231

GD = gonadal sterility; MR = male recombination in chrom. 2 and 3; k_2 = transmission ratio distortion in chrom. 2; k_3 = transmission ratio distortion in chrom. 3; SF = SF sterility.

Figure 5 shows the GD sterility results of samples of isofemale lines collected every year at a single location, Weymouth Street, Providence, R.I., over almost a decade. The distributions of P factor activity and cytotype differ very little from year to year and are typical of those seen for other N. American locations (Figure 5). Although the mean GD sterility frequencies (Figure 6) vary from year to year, there is no clear temporal trend over the tested period.

SF sterility was the only I-R system trait tested. Not one of the more than 200 isofemale lines examined showed any clear indication of being other than inducer (I). However, the degree of inducer activity was variable. This is illustrated in Table 1 and Figure 7 which shows the overall distribution of Cross A SF sterility values for all isofemale lines tested.

The most important conclusion from this study is that there is very little qualitative variation from one location to another in N. American populations with respect to either P-M or I-R system properties. For the P-M system this represents a significant difference in distribution pattern from other continents, such as Europe, Asia and Australia, where extensive qualitative variability has been observed (Anxolabéhère et al. 1984; I. Boussy, pers. comm.).

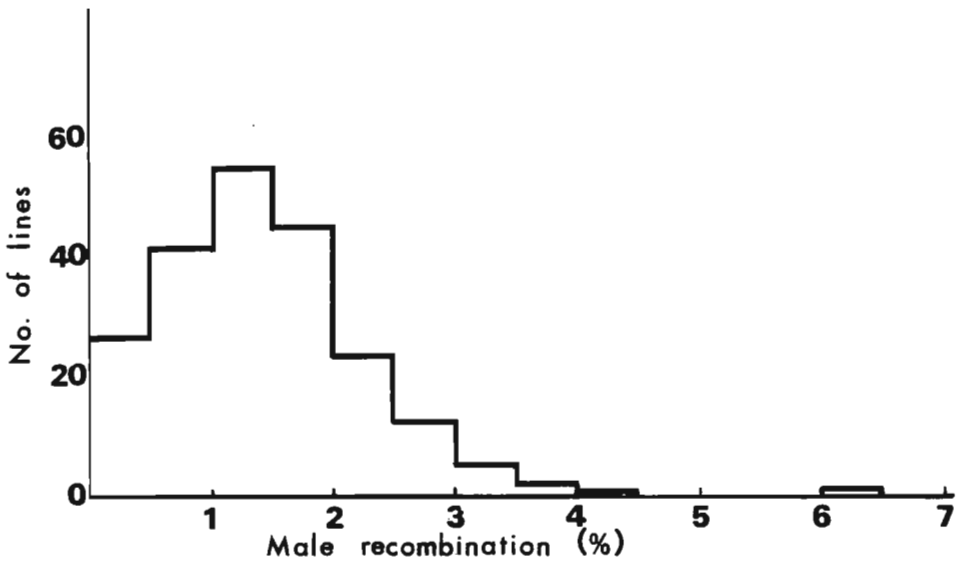


Figure 4. The pooled distribution of male recombination frequencies (Cross A) for all those N. American isofemale lines tested during the period 1977-79.

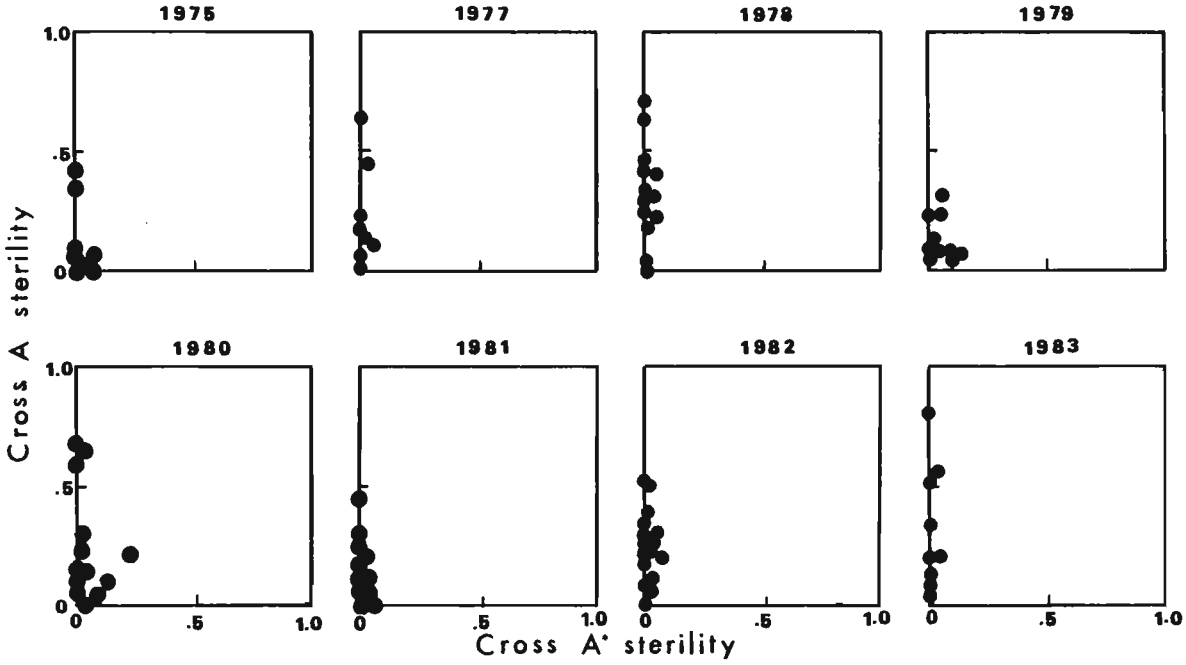


Figure 5. Distributions of GD sterility frequencies in isofemale lines collected from the same location, Weymouth St., Providence, Rhode Island, in different years during the 1975-1983 period.

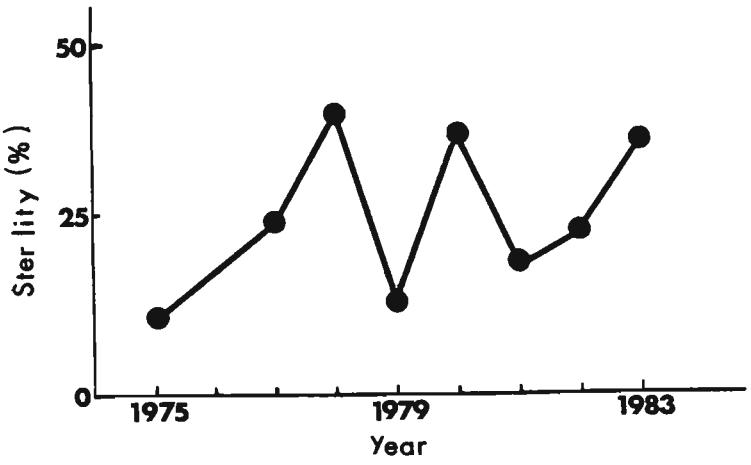


Figure 6. Mean frequencies of GD sterilities (Cross A) in isofemale lines collected from the Weymouth population in the period 1975-1983.

Acknowledgements: The authors sincerely thank the following for collecting and sending flies: P.T. Ives, M.T. Clegg, D. Cavener, W. Hollander, P. Fuerst, B. Rathcke, A. Allen, W.R. Engels, M.L. Tracey, Jr., R. Schaefer, H. Band, C. Laurie-Ahlberg, M. Green. This work was supported in part by NSF grant DEB 76-82630 and PHS grant GM-25399.

References: Anxolabéhère, D., K. Hu, D. Nouaud, G. Periquet & S. Ronsseray 1984, Génét. Sél. Evol. 16:15-26; Kidwell, M.G. 1979, Genet. Res. 33:205-217.

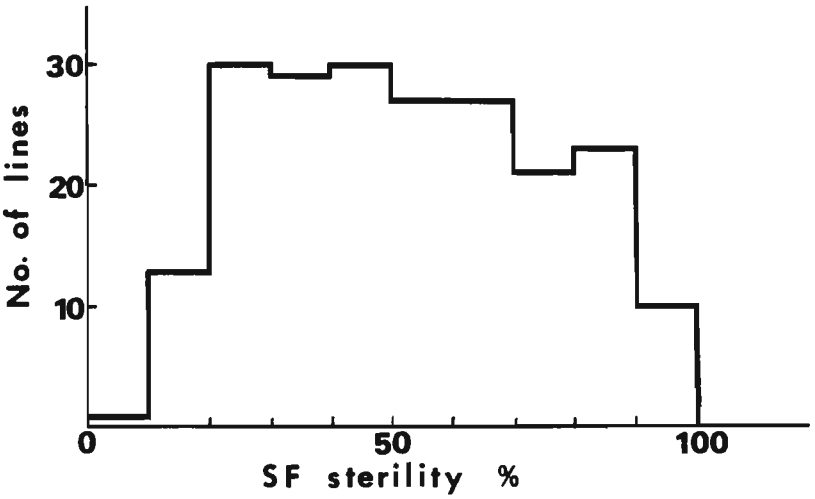
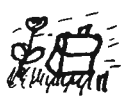


Figure 7. The pooled distribution of SF sterility frequencies (Cross A) for all those N. American isofemale lines tested during the period 1977-79.



Knoppien, P. University of Groningen, Haren, Netherlands. The number of males stored per vial, a possible source of bias in rare male experiments.

Rare male mating advantage has been defined as frequency-dependent male sexual fitness with the rare type of male favoured. The phenomenon has been described frequently, but it is still a subject of controversy (reviewed by Knoppien 1985). It has been suggested recently that in some cases the rare male effect may be merely an artifact of experimental methods, for instance, when males used for the mating tests are not sampled randomly with respect to place in the storage vial (Markow 1980). The possibility that alternately harming the rare and the common strain as a consequence of marking may produce a rare male effect is discussed by Knoppien (1985). The same applies to the many statistical pitfalls in this area. It is the purpose of this paper to discuss yet another possibility to create an artificial rare male effect.

The idea is based upon a finding of Van den Berg (1985, this issue). He found a profound effect of the number of males stored per vial on subsequent male courtship behaviour, directed to a decoy. This decoy consisted of a dead male body, deprived of its own odour, and supplied with female odour. Males stored singly preceding the test were shown to be much more persistent for a number of courtship elements (orientating and wing vibrating) than males stored together in numbers of 25 per vial. In a rare male experiment males may be stored preceding the mating test according to the number in which they are needed for each desired ratio, simply because this is a practical and convenient way of experimental setup. However, in this case the males needed for the low ratio of either type turn out to be the most persistent in courting. This may create an artificial rare male effect because the more actively courting males probably also will be superior in mating.

The following findings support this idea. It is predicted by the finding of Van den Berg that males previously stored singly are superior in mating to males stored together with other males. This prediction was tested in experiments in which two males, each with a different storage history, were competing for one female. For this purpose flies of **D.melanogaster** were derived from the Groningen population. Virgin males and females were etherized once to separate the sexes, and subsequently aged for 3-4 days at 25°C. Males were either stored as single males or in numbers of 10 per vial; all females were stored in numbers of 10 per vial. The wing of the former type of male was clipped throughout the experiment for identification. One female and two males were combined in vials, and mating was observed for maximally one hour. As soon as copulation took place, the mating couple was removed, and the type of the male was identified after the experiment. In the 90 runs of this experiment 89 matings took place: 53 by males stored singly and 36 by males stored in numbers of 10, which is significantly in favour of the singly stored males ($P<0.04$, binomial distribution, normal approximation, one-tailed test). Next a more direct test was made to decide whether the number of males stored per vial could cause bias in rare male experiments. Flies of **D.melanogaster** were derived from the Bogota population. Virgin males and females were etherized once to separate the sexes, and subsequently aged for 3-5 days at 25°C. Both sexes were stored in vials in numbers of either 1 or 9 to serve as "rare", respectively "common" flies. Throughout the experiment the wing of the rare flies was clipped for identification. Mating experiments were conducted by direct observation during one hour in slightly modified Elens-Wattiaux mating chambers (Van den Berg et al. 1984) with ten pairs of flies per run (1 male and 1 female rare, and 9 males and females common), while copulating pairs were not removed, thus allowing any individual male more than one copulation. In this experimental setup it was found that rare males are superior in mating to common males (Table 1). This can be shown to be significant with the test applied by Pruzan (1976) ($P<0.04$).

Table 1. Mating success of flies stored singly, used as rare (R) flies, compared with flies stored in numbers of 9, used as common (C) flies, determined by direct observation, with 10 pairs of flies per run.

Frequency of rare type	No. of runs	Matings ♀ x ♂ RR	RC	CR	CC	R♀	C♀	R♂	C♂
0.10	14	3	10	16	91	13	107	19	101

A preliminary for the interpretation of these findings is that wing clipping does not affect mating success. A mating disadvantage, if any, of clipped males seems most likely (Robertson 1982), which is the reason that only the males stored as a single male, from which superiority in mating was hypothesized, were clipped. Also a rare male effect for wing clipping seems unlikely (Ehrman 1966).

It is concluded that it is imperative to keep in mind that differential storage conditions can bias the outcome of a rare male experiment.

References: Ehrman, L. 1966, Anim. Behav. 14:332-339; Knoppien, P. 1985, Biol. Rev. 60:81-117; Markow, T.A. 1980, Behav. Genet. 10:553-556; Pruzan, A. 1976, Evol. 30:130-145; Robertson, H.M. 1982, Anim. Behav. 30:1105-1117; Van den Berg, M.J. et al. 1984, Behav. Genet. 14:45-61.

Kobayashi, K. and H. Watabe.* Toyoha Junior High School; *Hokkaido University of Education, Sapporo, Japan. Drosophilid fauna of Rishiri Island in northern Japan.

The study on the ecology of drosophilid flies in an island is advantageous to understand interspecific relationships (MacArthur & Wilson 1967). We made a periodical collection in the Island of Rishiri (183 km^2 in area) from August 15 to September 27, 1983. The Island (45°N, 141°E) is located in northern Japan. The collection was made using retainer-type traps baited with fermenting bananas. The traps were set up at four different environments, i.e., natural forest (NF), waterside (WS), grassland (GL) and human habitation (HH).

In total, 4763 specimens were obtained, involving 32 species belonging to 4 genera and 9 subgenera. The 14 common species are shown in Table 1.

Table 1. Drosophilid flies collected at four sites in Rishiri Island, 1983.

	Natural forest	Water-side	Grass-land	Human habitation	Total
Genus Leucophenga					
Subgenus Neoleucophenga					
L.quinquemaculipennis	49	18	1	--	68
Genus Drosophila					
Subgenus Hirtodrosophila					
D.confusa	25	36	8	--	69
Subgenus Dorsilopha					
D.busckii	1	--	2	45	48
Subgenus Sophophola					
D.melanogaster*	5	3	42	518	568
D.suzukii	50	15	73	19	157
D.auraria	--	7	15	162	184
D.bifasciata	369	290	237	99	995
Subgenus Drosophila					
D.funebris	--	1	1	224	226
D.lacertosa	12	66	21	9	108
D.immigrans	26	18	26	41	111
D.testacea	460	428	128	68	1084
D.nigromaculata	1	--	62	22	85
D.curvispina	12	37	51	47	147
D.histrio	206	348	149	165	868
Others	13	10	12	10	45
Total ind. No.	1229	1277	828	1429	4763
Total spp. No.	19	19	21	18	32

The abundant species were **D.testacea** (22.8% of total samples), **D.bifasciata** (20.9%) and **D.histrio** (18.2%), all of which have been found exclusively in the natural forests of northern Japan. In the Island, however, they were commonly collected at the sites GL and HH, which are not original habitats for these species. The other species are as follows: **Amiota stylopyga*** (NF), **A.albilabtris*** (GL), **A.furcata** (GL, HH), **A.trifurcata*** (NF, GL), **A.taurusata*** (NF), **A.kappa*** (GL), **A.conifera takadai*** (WS, GL, HH), **A.sp.*** (NF), **Scaptomyza pallida** (HH), **Drosophila coracina** (WS), **D.simulans*** (HH), **D.lutescens** (NF, WS), **D.moriwakii** (WS), **D.pengi*** (NF, WS, HH), **D.ezoana*** (NF), **D.kanekoi*** (GL), **D.bizonata*** (WS), **D.brachynephros** (NF, GL, HH). The asterisks in the above species and in Table 1 indicate the species new to the Island (Takada 1956; Kaneko et al. 1969). With regard to domestic species of Drosophila, the abundance of **D.funebris** is noticeable. This species has not been so much collected in any districts of Japan. **D.simulans**, which colonized in Japan about ten years ago, was discovered at Rishiri Island (cf. Watabe et al. 1980).

Considering the small number, this species might have recently invaded the Island. Up to the present, about 120 species have been recorded in northern Japan, one-fourth of which has been found in the Island during the survey. We will investigate the collection records about the drosophilid flies which have hardly visited the fruit-trap.

References: Kaneko, A., E. Momma & T. Tokumitsu 1969, J. Fac. Sci. (Hokkaido Univ.), Ser. VI (Zool.) 17:381-385; MacArthur, R.H. & E.O. Wilson 1967, in: The Theory of Island Biogeography, Princeton Univ. Press, New Jersey; Takada, H. 1956, DIS 30:154; Watabe, H., E. Momma & M.T. Kimura 1980, DIS 55:141-142.

Kosuda, K. Josai University, Sakado, Saitama, Japan. Mating activity of aged males in **Drosophila melanogaster.**

A body of evidence is accumulating that the male reproductive component of fitness, virility at young age, plays a more important role than fitness variables in preadult stages and female fertility (Petit et al. 1980; Brittnacher 1981; Sharp 1982, Kosuda 1983). Since mating activity of the aged individuals which have passed through the reproductive period is considered not to be subjected to natural selection, it is expected that the genetic variability in mating activity is much higher in aged individuals than in young ones. Mating activity of aged males (28 days old) in **Drosophila melanogaster** was measured under no competition among males for females in 29 lines homozygous for the second chromosome which were extracted from a natural population in Katsunuma, Japan. Males were individually placed into a mating vial together with 12 virgin females of a standard laboratory strain. After 24 hr, 10 out of 12 females were randomly chosen for sperm inspection. Twelve replicates each were made for 29 homozygous lines. Mating activity of heterozygotes was also measured utilizing progenies from the natural population.

Mating activity of aged males over the array of 29 lines was 1.85±0.25. Three lines exhibited sterility due to aging. All males from these lines are consistently sterile, although they are sexually active when they are young (Kosuda 1983). Analysis of variance disclosed the significant difference between lines, indicative of the genetic nature of the trait (Table 1). The frequency distribution of mating activity for homozygous aged males significantly differs from a Poisson distribution (Figure 1). The males exhibiting high mating activity were more frequent than that expected from a Poisson distribution. The fact implies that some aged males do not lose their mating activity by aging, although there is a general tendency that male mating activity decreases with increasing age (Stromnaes & Kvelland 1962).

Table 1. Analysis of variance for mating activity of the aged males among 29 homozygous lines.

Source	d.f.	S.S.	M.M.	F
Line	28	618.51	22.09	6.40*
Error	319	1099.02	3.45	
Total	347	1717.53		

* significant at 1% level.

The frequency distribution of heterozygous males was also given in Figure 2. Whereas the proportion of non-mating males in heterozygous ones was about 10% (20/201), about half of the homozygotes did not mate at all (153/348). None of the aged homozygous males mated more than 9 times, while 8 out of 201 heterozygotes did. The mean mating activity of aged heterozygotes was calculated to be 3.76±0.17. The difference in male mating activity between homozygotes and heterozygotes was statistically significant ($P<0.001$).

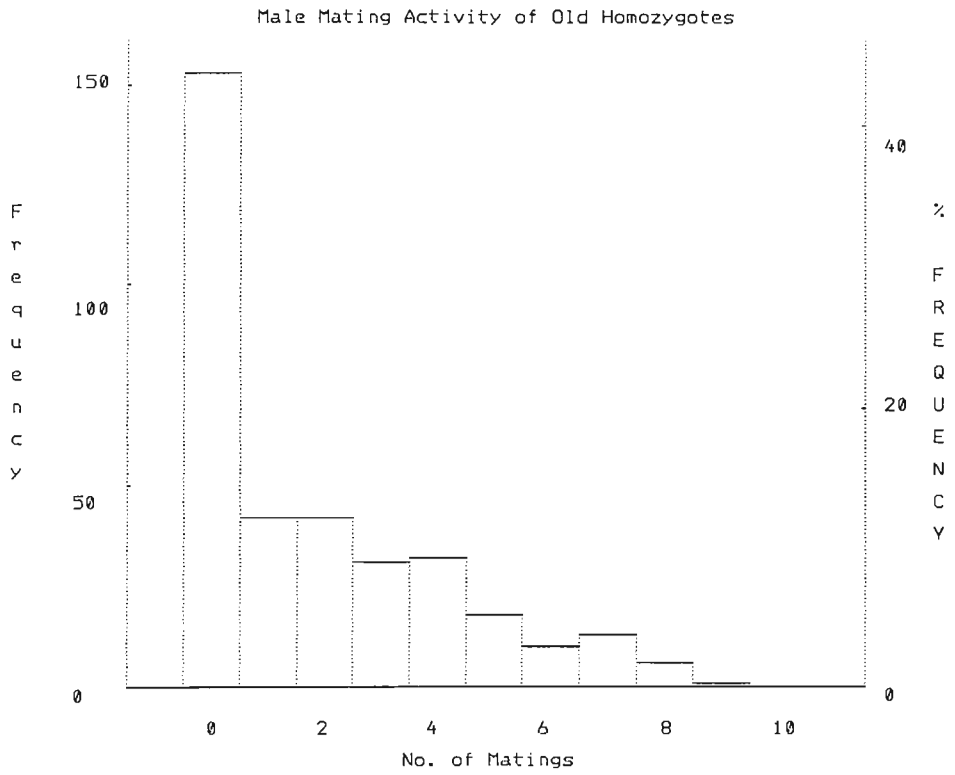


Figure 1. Frequency distribution of mating activity for homozygous males.

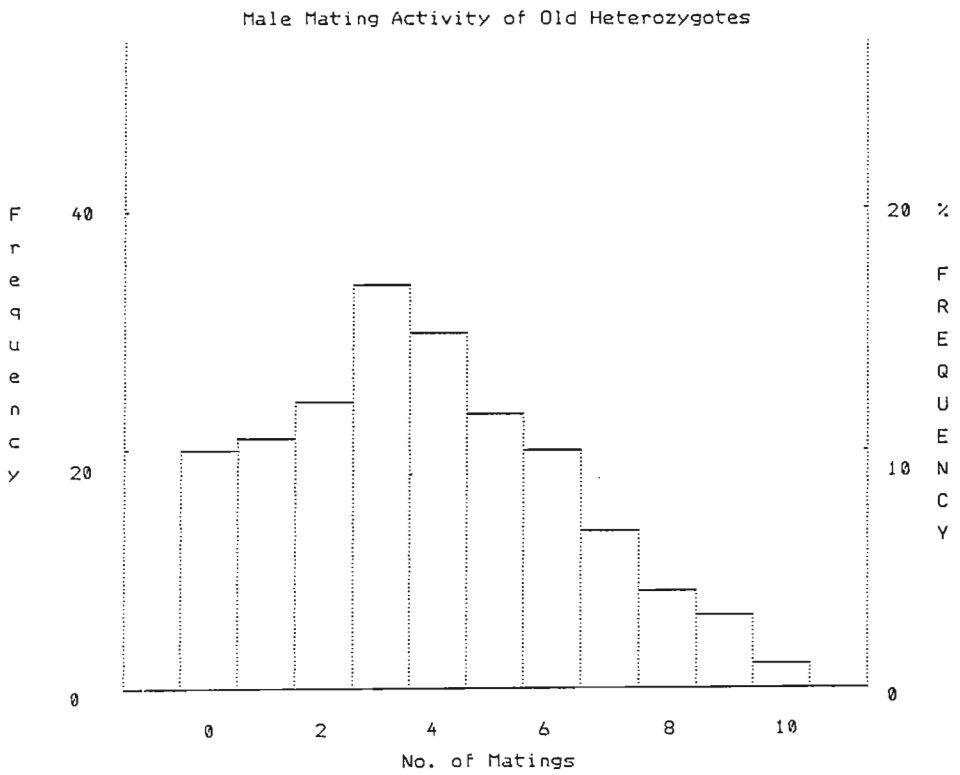


Figure 2. Frequency distribution of mating activity for heterozygous males.

References: Petit, C., P. Bourgeron & H. Mercot 1980, Heredity 45:281-292; Brittnacher, J.C. 1981, Genetics 97:719-730; Sharp, P.M. 1982, Genet. Res. 40:201-205; Kosuda, K. 1983, Experientia 39:100-101; Stromnaes, O. & I. Kvelland 1962, Hereditas 48:442-470.

Kumar, A. and J.P. Gupta. Banaras Hindu University, Varanasi, India. Further records of Drosophilid species from north-east India.

Table 1. Drosophilid species collected from different areas in Meghalaya and Arunachal Pradesh during September-October 1983.

Name of species	Subgenus	Locality	Number ♂♂/♀♀
Z.obscuricornis	Aprionus	Shillong & Tai	103
Z.multistriatus	"	Shillong	61
Liodrosophila penispinosa	"	Shillong & New Itanagar	9
D.rhopaloa	Sophophora	Tai	
D.sp.Nov.	"	"	9
D.ficusphila	"	"	6
D.bryani	Scaptodrosophila	"	88
D.sp.Nov.	Drosophila	Shillong	2
D.sp.Nov.	"	Tai	21
D.sp.Nov.	"	"	15
D.sp.Nov.	"	"	10
D.neoimmigrans	"	"	5
D.lacertosa	"	Shillong	31
D.tongpua	"	Tai	22
D.siamana	"	"	60
D.synpanishi	"	"	32
D.sternopleuralis	"	"	3
D.setitarsa	"	"	9
TOTAL			492

Recent surveying studies in different parts of north-east India (Singh & Gupta 1977; Gupta & Singh 1979; Dwivedi & Gupta 1979, 1980; Dwivedi et al. 1979; Singh & Gupta 1981) have indicated that this region possesses a fairly rich, and at present little known fauna of Drosophila and related genera of Drosophilidae. During the present study, intensive field collections were carried out in several previously unexplored areas in Meghalaya and Arunachal Pradesh. Altogether 18 species were collected as shown in Table 1. Among them, 5 species are detected as new to science, while 4 other species namely **D.tongpua, D.siamana, D.synpanishi, D.sternopleuralis** are recorded for the first time from India. An interesting feature of the collection data is that out of the total 18 species, 11 species are found to belong to the immigrans group of the subgenus Drosophila alone, indicating that resources are better utilized in this region by these species than the species of other genera of Drosophilidae.

This work has been supported by a research grant from the DST, Goverment of India to JPG.

References: Dwivedi, Y.N. & J.P. Gupta 1979, Entomon 4:183-187; ____ 1980, Proc. Ind. Acad. Sci. 89:85-89; Dwivedi, B.K. Singh & J.P. Gupta 1979, Oriental Insects 13(1-2):61-74; Gupta, J.P. & B.K. Singh 1979, Entomon 4(2):167-172; Singh, B.K. & J.P. Gupta 1977, Ent. Mont. Mag. 113:71-78; ____ 1981, Stud. Nat. Sci. 2(13):1-8.

Lechien, J., C. Bierniaux and A. Elens.
Facultes Univ. N.-D. de la Paix, Namur, Belgium.

Response to a disruptive selection for phototactism.

The present experiments use **D.melanogaster** populations as a model to measure the influence of the selection intensity on the response to selection. Flies of a wild strain, called "Waterloo" because it derives from adults captured on the historic battlefield of this name, have been submitted to a disruptive selection for positive and for negative phototactism using the Benzer method (1967), as modified by Tompkins et al. (1978). In both the negative and the positive directions, three selection intensity levels were used: 5%, 15%, or 25%, respectively, of the flies of each generation were selected. They become the parents of the following generation. The tests were done at 25°C and 60-80% R.H. The flies were 5 days old.

FEMALES toward light

from \ light	5	4	3	2	1	0
0	240	51	15	24	2	15
1	227	80	16	15	13	7
2	69	43	23	6	9	1
3	36	26	10		5	3
4	9	3	4	1	3	1
5	1	1	1	1		

MALES toward light

from \ light	5	4	3	2	1	0
0	369	44	14	15	6	5
1	154	53	11	3	2	3
2	88	46	25	4		
3	36	22	14	5	1	
4	15	6	5	1	1	
5	2	2	1	3	1	

Figure 1. Countercurrent distribution test: final distribution of the flies. Initial population "Waterloo".

Figure 1 shows the results of five tests on flies from the original wild population. Figure 2 and 3 show the results of similar tests done (five repetitions) on flies from the 5th generation after the beginning of selection. Even after so short a time, one observes a response in both the selection for negative phototactism and the selection for positive phototactism. In the strains selected for positive phototactism, the best response is obtained with a high selection intensity level (5% of the flies used as parents); for the male flies, the response is however the same at the 5% and the 15% intensity level. The greatest response is also obtained for the highest intensity level in the strains selected for negative phototactism. Strangely enough, no significant response was observed with the medium intensity level (15%) for the female flies. For the male ones, the response was the same at the highest and the medium selection intensity levels (5% and 15%). As in our previously published papers (e.g., Hougouto et al. 1983; Dubucq et al. 1984), chi-square tests were used for the comparison, after grouping data according to the schema given in Figure 4.

Intensity level: 5%

FEMALES toward light

from \ light	5	4	3	2	1	0
0	150	32	6			
1	35	16	1			
2	10	9	1	1		
3	6	1				
4	1					
5						

MALES towardlight

from \ light	5	4	3	2	1	0
0	113	35	10	6	2	1
1	82	24	4	2		
2	12	4			2	
3	10	1				
4		1				
5						

15%

from \ light	5	4	3	2	1	0
0	105	4	2	3		
1	97	29	5	1		
2	22	16	1	1		
3	10	6		2		
4	1	3				
5						

from \ light	5	4	3	2	1	0
0	139	25	9	1		
1	49	30	4	2		
2	20	13	3	2		
3	7	4	1			
4	1	4				
5		1				

25%

from \ light	5	4	3	2	1	0
0	207	78	27	8	1	
1	70	50	13	8	1	1
2	30	18	9	6	1	1
3	10	16	3	2	1	
4	4	3	1	1		
5	2	2	1			

from \ light	5	4	3	2	1	0
0	170	73	31	14	5	
1	78	39	15	12	5	
2	21	29	2	1	3	1
3	7	18	1	9		1
4	1	3	2	1		
5	2			2		

Figure 2. Countercurrent distribution test: final distribution of the flies. Selection for positive phototactism: 5th generation.

Intensity level

FEMALES

5%

from light \ toward light	5	4	3	2	1	0
0	71	27	8			
1	93	14	9	1	2	
2	34	24	1		1	1
3	20	7	2	3		
4	11	6	1	1		
5	8	2				

MALES

5%

from light \ toward light	5	4	3	2	1	0
0	46	34	11	1		
1	98	31	6			1
2	51	12	7	3	1	2
3	19	13	5	1	1	
4	16	4	1		1	
5	9	5		1		

FEMALES

15%

from light \ toward light	5	4	3	2	1	0
0	91	56	14	20	1	1
1	35	22	13	7	1	1
2	30	11	11	1	4	1
3	13	9	3	4	1	1
4	4	7	2	2		1
5	3	5			1	

MALES

15%

from light \ toward light	5	4	3	2	1	0
0	60	37	20	11	3	1
1	35	22	13	7	3	4
2	12	17	15	5	5	2
3	10	10	7	6	1	2
4	7	6		1	1	1
5	1	1	2			1

FEMALES

25%

from light \ toward light	5	4	3	2	1	0
0	71	27	8			
1	93	14	9	1	2	
2	34	24	1		1	1
3	20	7	2	3		
4	11	6	1			
5	8	2				

MALES

25%

from light \ toward light	5	4	3	2	1	0
0	46	34	11	1		
1	98	31	6			1
2	51	12	7	3	1	2
3	19	13	5	1	1	
4	16	4	1		1	
5	9	5		1		

Figure 3. Countercurrent distribution test: final distribution of the flies. Selection for negative phototactism: 5th generation.

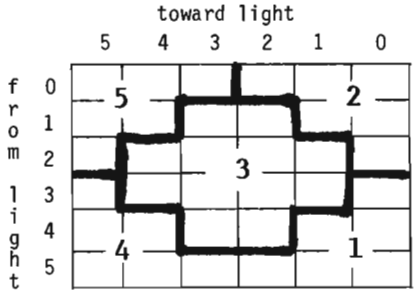


Figure 4. Data grouping before the chi-square test.

References: Benzer, S. 1967, Proc. Nat. Acad. Sci. 58:1112; Dubucq, D., E. Depiereux & A. Elens 1984, DIS 60:87; Hougouto, N. & A. Elens 1982, DIS 58:79; Tompkins, L., J.A. Fleischman & G. Sanders 1978, DIS 53:211.

Levitan, Max. Mount Sinai Medical Center, New York, New York USNA. Spontaneous chromosome aberrations in **D.robusta** since October 1960. II. Autosomal inversions among the first 559.

The chromosome breakage factor in the ST_y strain of **Drosophila robusta** has resulted in over 2500 new aberrations to date. Levitan (1964) began to describe the first 559, the ones on which the reports delineating the main features of the breakage factor (Levitan 1962, 1963; Levitan and Schiller 1963) were based. I listed there the X-chromosome inversions in this group, also the few simple deficiencies that had been observed. This report will describe the autosomal inversions in these 559. It will also make some corrections in the previous report because one XR inversion was inadvertently left out and some changes became necessary upon reexamination of the material for this report.

Some of the preliminary material of Levitan (1964) will be repeated here, with a few emendations, in order to understand the descriptions. Copies of the earlier report, which contains this material in greater detail, including the division of the chromosome arms into the unit sections (Figure 1) mentioned in the text, are available from the author on request.

Classification		Text Abbreviation
I.	Deficiencies	Df (arm)
II.	Paracentric Inversions	Ipa (arm)
III.	Pericentric Inversions	Ipe (chromosome)
IV.	Reciprocal Translocations (2 breaks)	T (arm:arm)
V.	Transpositions (insertions, shifts)	Tp (Df arm:Ins.arm)
VI.	Multiple breaks and miscellaneous	MB (arms involved)
VII.	Nucleolar aberrations*	Nuc [type*:arm(s)]

* As described in Levitan (1970).

"Newly Induced" or "Repeated". Aberrations seen only in one larva are referred to as "newly induced" to distinguish them from those seen more than once ("repeated") because the latter had been induced in an earlier generation and were being transmitted. In a few instances the single sighting occurred when less than six progeny of the cross were smeared, leaving a doubt of greater than 5% that it could be a "repeated" aberration, the other copies having missed by chance; such a single-appearing aberration is listed as "newly induced?" (rather than "repeated?") because most aberrations found to date have been "newly induced."

Cross or Experiment. The type of cross or experiment in which the aberration was encountered is noted according to the following outline:

A. Female(s) from stock ST_y or any of its subdivisions or substrains crossed to:
   1. A male from a natural population; thus A1(O.) means "ST_y female x Ohio male." Occasionally more than one female was used per cross.
   2. A male from a non-inducer laboratory stock.
   3. A male from an artificial population based on non-inducer stocks.
   4. A male from the same ST_y substrain.
   5. A male from a different ST_y substrain.
   6. A male descended from one of the above (A1-A5) crosses.
   7. A male from a category not mentioned above.

B. Female from an experimental population based on ST_y stocks.
   1. Female inseminated in the population.
   2. Despermed female crossed to:
      a. A male from nature, as in A1.
      b. A stock male, as in A2.
   3. Newly eclosed female crossed to:
      a. A male from nature, as in A1 or B2a.
      b. A stock male, as in A2 or B2b.

C. Female descended maternally from ST_y but karyotypically heterogeneous due to a previous outcross.
   1. Female inseminated in the stock.
   2. Newly eclosed female crossed to a male from the same stock.
   3. Newly eclosed female crossed to male from a non-inducer stock.

D. Female from an ostensibly non-inducer line, with no apparent contact with ST_y flies, their eggs, or debris, crossed to:
   1. A male from nature.
   2. A male from a non-inducer stock.
   3. A male from a cage developed from non-inducer lines.

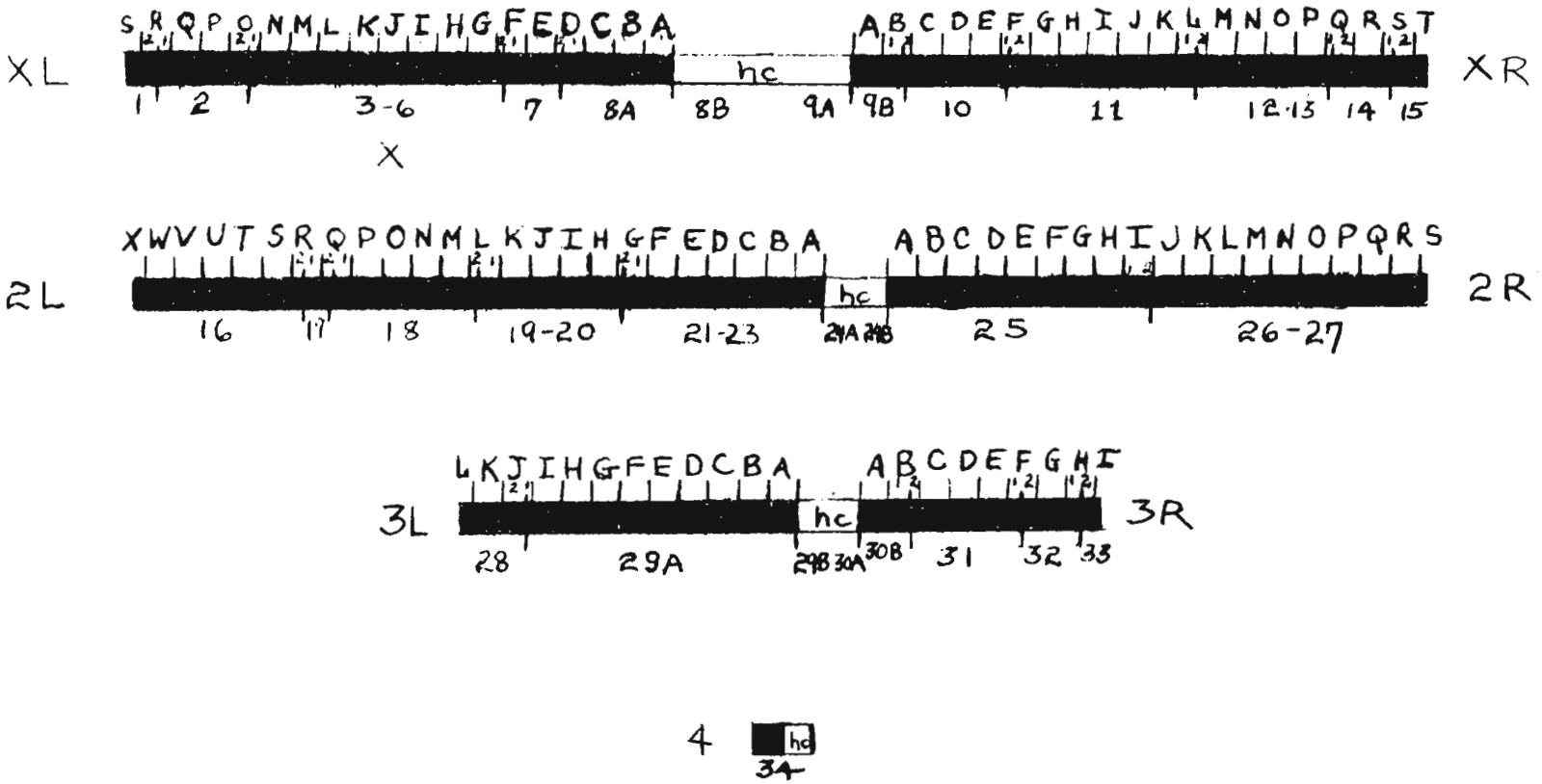


Figure 1. **D.robusta** Standard chromosome diagram showing the correspondence between the percentile (capital letter) divisions of each arm and the older numerical divisions based primarily on the natural inversions. The central heterochromatin of X should be perhaps a bit longer than shown.

E. Female from an ostensibly non-inducer stock, in an experiment attempting to transfer the inducing property, crossed to:
   1. A male from nature.
   2. A male from a non-inducer stock.
   3. A male from a non-inducer cage experiment.
F. ST_Y male, the "opposite number" in every respect to "A" above.
G. ST_Y cage male, "opposite number" in every respect to "B" above.
H. Male descended from ST_Y female, "opposite number" to "C" above.
I. Non-inducer stock male, "opposite number" to "D" above.
J. Non-inducer cage male, "opposite number" to "E" above.

Date. Year and month, in Bridges & Brehme (1944) notation, when slide was made. Chronological order may differ from the numerical order because the latter is based on when slide was read. For transmitted aberrations seen on more than one slide, date is of first sighting.

Acknowledgements. This work was supported by grants from N.S.F., by U.S.P.H.S. Research Career Program award GM-K3-16,689, and by Anatomy Department Research Fund G9-2780 of the Mt. Sinai Medical Center.

References: Bridges, C.B. & K.S. Brehme 1944, The mutants of **D.m.**, Carnegie Inst. Wash. Publ. 552; Levitan, M. 1962, Proc. Natl. Acad. Sci. (Wash.), 48: 930-37; _____ 1963, Nature 200: 437-38; _____ 1964, D.I.S. 39: 89-96; _____ 1970, Chromosoma 31: 452-61; Levitan, M. & R. Schiller 1963, Genetics 48: 1231-38.

Corrections to Entries in Levitan, Part I (1964)

Ipa(XL)1.	Also Ipa(XR)1, among others (Levitan, 1964), from the A1(O.) cross.
Ipa(XL)5.	A3 cross (not Ae).
Ipa(XL)18.	C2 cross (not C1). Ipe(2)49 (not 51).
Ipa(XR)3.	C2 Cross (not B2). Ipe(2)4 (not Ipa). Delete Ipa(2L)7, now known to be an MB, to be numbered later; add Ipa(3L)2.
Ipa(XR)4.	C2 cross (not B2).
Ipa(XR)6.	Ipa(2L)12 (not 13).
Ipa(XR)13.	Ipa(2L)36 (not 39).
Ipa(XR)14.	Ipe(2)44 (not 46).
Ipa(XR)17.	Ipa(2L)48 and Ipa(2L)49 (not 53 and 54).
Ipa(XR)18.	No. 325B. Newly induced. Breaks on XR-2 (inf.): prox. one in C; dist. one in I. Female larva. A3 cross, with a T(XR:2L) in same cells and a T(XL:3L) in a sib. 62h.
Ipe(X)6.	Ipe(2)15 (not 16).
Ipe(X)15.	Ipa(2L)27 (not 28).
Ipe(X)17.	Newly induced.
Ipe(X)18.	Ipa(3L)6 (not 5).

NEW ENTRIES: Autosomal Inversions: Paracentric Inversions: Chromosome 2: Left Arm

Abbreviations: N-I = newly induced. R = repeated. M = Male. F = Female. x = cross.

Ipa(2L) 1. #17. N-I. Breaks on 2L (obs): prox. one in K; dist. one in V. M larva. A1(Va.) x. 61h.
Ipa(2L) 2. #21. N-I. Breaks on 2L-1 (obs): prox. one in hc; dist. one in P. M larva. A1(Ga.) x. 61k.
Ipa(2L) 3. #22. N-I. Breaks on 2L (obs): prox. one in C; dist. one in N. M larva. A1(O.) x. 61k.
Ipa(2L) 4. #32. N-I. Breaks on 2L (obs): prox. one in H; dist. one in W. M larva. D1(Pa.) x. 61i.
Ipa(2L) 5. #47. R. Breaks on 2L (obs): prox. one in N; dist. one in S. First seen in A4 x. 62c. Has persisted in stocks over twenty years. Heterozygous with 2L in Figs. 1c, 1e and 2a of Levitan (1970).
Ipa(2L) 6. #56. N-I. Breaks on 2L-1 (obs): prox. one in A; dist. one in Q1. F larva. Same C2 x as Ipa(XR)3 (Levitan 1964), Ipe(2)4, Ipa(3L)1, Ipa(3L)2, and others. 62c.
Ipa(2L) 7. #73. N-I. Breaks on 2L-3 (obs): prox. one in hc; dist. one in S. F larva. A3 x. 62d.
Ipa2L) 8. #75. N-I. Breaks on 2L-1 (obs): prox. one in R1; dist. one in V. F larva. A3 x. 62d.
Ipa(2L) 9. #109. N-I. Breaks on 2L-1 (obs): prox. one in D; dist. one in O. M larva. A3 x. 62f.
Ipa(2L) 10. #118. N-I. Breaks on 2L-1 (obs): prox. one in hc; dist. one in N. F larva. A3 x. 62f.
Ipa(2L) 11. #122. N-I. Breaks on 2L-3 (obs): prox. one in the transposed hc of 2L-3; dist. one in R1. M larva. A3 x. 62f.
Ipa(2L) 12. #167. N-I. Breaks on 2L-1 (inf): prox. one in C; dist. one in R1. F larva. Same A3 x as Ipa(XR)6 and others (Levitan 1964). 62f.
Ipa(2L) 13. #169. N-I. Breaks on 2L-1 (obs): prox. one in hc; dist. one in J. M larva. A3 x. 62f.
Ipa(2L) 14. #179. R. Breaks on 2L (obs): prox. one in hc; dist. one in O. First seen in A3 x. 62f.
Ipa(2L) 15. #182. N-I. Breaks on 2L-1 (obs): prox. one in hc; dist. one in P. M larva. A3 x. 62f.
Ipa(2L) 16. #193. N-I. Breaks on 2L-1 (obs): prox. one in B; dist. one in H. M larva. A3 x. 62f.
Ipa(2L) 17. #202. N-I. Breaks on 2L-1 (obs): prox. one in R1; dist. one in W. M larva. A3 x. 62f.
Ipa(2L) 18. #226. N-I. Breaks on 2L-3 (inf): prox. one in P; dist. one in V. F larva. A3 x. 62f.
Ipa(2L) 19. #230. N-I. Breaks on 2L-1 (obs): prox. one in hc; dist. one in N. M larva. Same A3 x as Ipa(2L)20. 62f.
Ipa(2L) 20. #231. N-I. Breaks on 2L-1 (inf): prox. one in E; dist. one in K. F larva. Same A3 x as Ipa(2L)19. 62f.

Ipa(2L) 21. #233. N-I. Breaks on 2L-3 (inf): prox. one in J; dist. one in U. M larva. A3 x. 62f.
Ipa(2L) 22. #246. N-I. Breaks on 2L-1 (obs): prox. one in hc; dist. one in O. F larva. A3 x. 62g.
Ipa(2L) 23. #261. N-I. Breaks on 2L-1 (obs): prox. one in P; dist. one in M. F larva. Same A3 x as Ipe(2)24 and others. 62h.
Ipa(2L) 24. #266. N-I. Breaks on 2L-3 (inf): prox. one in K; dist. one in U. M larva. A3 x. 62g.
Ipa(2L) 25. #267. R. Breaks on 2L (obs): prox. one in hc; dist. one in I. F3 x. 62h.
Ipa(2L) 26. #271. N-I. Breaks on 2L-1 (obs): prox. one in hc; dist. one in Q1. M larva. A3 x. 62g.
Ipa(2L) 27. #294. N-I. Breaks on 2L-3 (inf): prox. one in P; dist. one in T. F larva. Same A3 x as Ipe(X)15 and others (Levitan 1964). 62g.
Ipa(2L) 28. #299. N-I. Breaks on 2L-1 (obs): prox. one in J; dist. one in I. F larva. A3 x. 62g.
Ipa(2L) 29. #350. N-I. Breaks on 2L-1 (inf): prox. one in F; dist. one in R2. F larva. A3 x. 62h.
Ipa(2L) 30. #361. N-I. Breaks on 2L (obs): prox. one in D; dist. one in W. F larva. A3 x. 62i.
Ipa(2L) 31. #363. N-I. Breaks on 2L-1 (obs): prox. one in hc; dist. one in H. F larva. A2 x. 62i.
Ipa(2L) 32. #371. N-I. Breaks on 2L (obs): prox. one in hc; dist. one in F. M larva. Same A1(O) x as Ipa(2R)20 and others and same cells as Ipa(2R)21. 62j.
Ipa(2L) 33. #386. N-I. Breaks on 2L (obs): prox. one in hc; dist. one in H. M larva. A1(N.C.) x. 62l.
Ipa(2L) 34. #394. R. Breaks on 2L containing Ipa(2L)5 (obs): prox.one in E; dist. one in U. In A1(N.C. and Va.) and F1(N.C.) x's. First seen 62j.
Ipa(2L) 35. #411. R. Breaks on 2L (obs): prox. one in I; dist. one in V. A1(Va.) x. 62k.
Ipa(2L) 36. #412. N-I. Breaks on 2L (obs): prox. one in hc; dist. one in N. M larva. Same A1(O.) x as Ipa(XR)13 (Levitan 1964). 62k.
Ipa(2L) 37. #420. R. Breaks on 2L (obs): prox. one in L2; dist. one in W. Seen in all 9 male larvae from F1(N.C.), H3, and B1 x's, but does not involve the Y. 62k.
Ipa(2L) 38. #447. R. Breaks on 2L (obs): prox. one in hc; dist. one in I. F1(N.C.) x. 62l.
Ipa(2L) 39. #452. N-I. Breaks on 2L (obs): prox. one in N; dist. one in W. M larva.B1 x. 62l.
Ipa(2L) 40. #475. R. Breaks on 2L (obs): prox. one in A; dist. one in K. F1(O.) x. 63d.
Ipa(2L) 41. #491. N-I. Breaks on 2L (obs): prox. one in hc; dist. one in F. F larva. B1 x. 63d.
Ipa(2L) 42. #494. R. Breaks on 2L (obs): prox. one in F; dist. one in J. B1 x's. 63c.
Ipa(2L) 43. #496. N-I. Breaks on 2L-1 (inf): prox. one in I; dist. one in W. M larva. A1(Va.) x. 62l.
Ipa(2L) 44. #508. R. Breaks on 2L (obs): prox. one in hc; dist. one in C. B1 x's. 63c.
Ipa(2L) 45. #509. N-I. Breaks on 2L (obs): prox. one in hc; dist. one in Q1. M larva. B1 x. 63c.
Ipa(2L) 46. #510. N-I. Breaks on 2L (obs): prox. one in A; dist. one in S. F larva. B1 x. 63c.
Ipa(2L) 47. #516. N-I. Breaks on 2L (obs): prox. one in D; dist. one in I. M larva. B1 x. 63c.
Ipa(2L) 48. #521. N-I. Breaks on 2L (obs): prox. one in C; dist. one in M. F larva. Same B1 x as Ipa(XR)17 (Levitan 1964) and Ipa(2L)49. 63c.
Ipa(2L) 49. #522. N-I. Breaks on 2L (obs): prox. one in G1; dist. one in J. M larva. Same B1 x as Ipa(2L)48. 63c.
Ipa(2L) 50. #527. N-I. Breaks on 2L-1 (obs): prox. one in J; dist. one in V. M larva. C2 x. 63c.
Ipa(2L) 51. #528. N-I. Breaks on 2L-1 (inf): prox. one in M; dist. one in I. F larva. C2 x. 63c.
Ipa(2L) 52. #532. N-I. Breaks on 2L (obs): prox. one in F; dist. one in T. M larva. C2 x. 63c.

Chromosome 2: Right Arm

Ipa(2R) 1. #36. R. Breaks on 2R-1 (obs): prox. one in A; dist. one in Q. C2 x. 62b.
Ipa(2R) 2. #38. R. Breaks on 2R (obs): prox. one in hc; dist. one in O. A4 x. 62a. Persisted in stock many years.
Ipa(2R) 3. #41. N-I. Breaks on 2R (obs): prox. one in E; dist. one in P. F larva. A4 x. 62b.
Ipa(2R) 4. #65. N-I. Breaks on 2R-1 (obs): prox. one in D; dist. one in I1. F larva. C2 x. 62c.
Ipa(2R) 5. #82. N-I. Breaks on 2R-1 (inf): prox. one in F; dist. one in R. F larva. A3 x. 62e.
Ipa(2R) 6. #83. R. Breaks on 2R (obs): prox. one in P; dist. one in R. F3 x. 62e. Linked to Ipa(2L)5.
Ipa(2R) 7. #120A. N-I. Breaks on 2R (obs): prox. one in N; dist. one in Q. M larva. A3 x. 62f. (Levitan 1970: Fig. 1c)
Ipa(2R) 8. #126. N-I. Breaks on 2R (obs): prox. one in L; dist. one in N. M larva. A3 x. 62f.
Ipa(2R) 9. #154. N-I. Breaks on 2R-1 (inf): prox. one in C; dist. one in R. F larva. Same A3 x as Ipa(2R)10 and others. 62f.
Ipa(2R) 10. #155. N-I. Breaks on 2R (obs): prox. one in J; dist. one in L. M larva. Same A3 x as Ipa(2R)9. 62f.
Ipa(2R) 11. #189. N-I. Breaks on 2R (obs): prox. one in F; dist. one in Q. M larva. A3 x. 62f.
Ipa(2R) 12. #210. N-I. Breaks on 2R (obs): prox. one in G; dist. one in J. F larva. Same A3 x as Ipa(XR)8 (Levitan 1964) and others. 62f.
Ipa(2R) 13. #229. N-I. Breaks on 2R (obs): prox. one in hc; dist. one in P. F larva. In same cells from an A3 x as Ipe(X)9 and Ipe(X)10 (Levitan 1964). 62f.

Ipa(2R) 14. #269. N-I. Breaks on 2R (obs): prox. one in M; dist. one in P. M larva. Same A3 x as Ipa(XL)10 (Levitan 1964) and others. 62g.
Ipa(2R) 15. #281. N-I. Breaks on 2R (obs): prox. one in hc; dist. one in O. M larva. Same A3 x as Ipe(2)28 and others. 62g.
Ipa(2R) 16. #291. N-I. Breaks on 2R (obs): prox. one in hc; dist. one in G. M larva. A3 x. 62g.
Ipa(2R) 17. #326. N-I. Breaks on 2R-1 (obs): prox. one in D; dist. one in H. F larva. A3 x. 62h.
Ipa(2R) 18. #344. N-I. Breaks on 2R (obs): prox. one in hc; dist. one in R. F larva. A3 x. 62h.
Ipa(2R) 19. #367. N-I. Breaks on 2R (obs): prox. one in O; dist. one in Q. M larva. A1(Va.) x. 62j.
Ipa(2R) 20. #370. N-I. Breaks on 2R (obs): prox. one in D; dist. one in G. M larva. Same A1(O.) x as Ipa(2R)21, Ipa(2L)32, and one other. 62j.
Ipa(2R) 21. #372. N-I. Breaks on 2R-1 (obs): prox. one in hc; dist. one in I1. M larva. Same A1(O.) x as Ipa(2R)20 and same cells as Ipa(2L)32. 62j.
Ipa(2R) 22. #378. N-I. Breaks on 2R (obs): prox. one in hc; dist. one in J. M larva. A1(O.) x. 62j.
Ipa(2R) 23. #408. N-I. Breaks on 2R-1 (obs): prox. one in hc: dist.one in H. M larva. Same C3 x as Nuc 5(1:2R). 62k.
Ipa(2R) 24. #414. N-I. Breaks on 2R (obs): prox. one in M; dist. one in P. M larva. A1(O.) x. 62k.
Ipa(2R) 25. #415. N-I? Breaks on 2R (obs): prox. one in F; dist. one in Q. F larva. A1(N.C.) x. 62k.
Ipa(2R) 26. #432. N-I. Breaks on 2R (obs): prox. one in E; dist. one in R. M larva. A1(N.C.) x. 62l.
Ipa(2R) 27. #446. R. Breaks on 2R (obs): prox. one in hc; dist. one in G. F1(N.C.) x. 62l. Probably linked to Ipa(2L)5, though the data do not rule out the possibility that it was carried by the N.C. female and linked to 2L-3.
Ipa(2R) 28. #479. N-I. Breaks on 2R (obs): both in Q. F larva. B1 x. 63c.
Ipa(2R) 29. #501. R. Breaks on 2R (obs): prox. one in P; dist. one in S. In a B3(Va.) and three B1 x's [in one Ipa(2R)30, in another Ipa(2R)31 also present]. 63c. 10±9.5% recombination in an Ipa(2L)5.S/S.Ipa(2R)29 homokaryous in X and 3.
Ipa(2R) 30. #502. N-I? Breaks on 2R (obs): prox. one in hc; dist. one in R. M larva. In one of the B1 x's with Ipa(2R)29. 63c.
Ipa(2R) 31. #515. N-I? Breaks on 2R (obs): prox. one in hc; dist. one in Q. F larva. In another B1 x with Ipa(2R)29. 63c.
Ipa(2R) 32. #535. N-I? Breaks on 2R-1 (obs): prox. one in A; dist. one in N. M larva. Same C2 x as Ipa(3L)10. 63c.

Chromosome 3: Left Arm

Ipa(3L) 1. #55. N-I. Breaks on 3L (obs): prox. one in E; dist. one in H. M larva. Same C2 x as Ipa(3L)2 and others [see Ipa(2L)6]. 62c.
Ipa(3L) 2. #58. N-I. Breaks on 3L (obs): prox. one in H; dist. one in J2. F larva. Same C2 x as above, q.v.
Ipa(3L) 3. #195. N-I. Breaks on 3L (obs): prox. one in hc; dist. one in F. F larva. A3 x. 62f.
Ipa(3L) 4. #239. N-I. Breaks on 3L (obs): prox. one in F; dist. one in J2. M larva. A3 x. 62g.
Ipa(3L) 5. #273. N-I. Breaks on 3L (obs): prox. one in A; dist. one in J2. M larva. A3 x. 62h.
Ipa(3L) 6. #402. N-I. Breaks on 3L (obs): prox. one in G; dist. one in I. M larva. Same A1(N.C.) x as Ipe(X)18 and others (Levitan 1964). 62k.
Ipa(3L) 7. #425. N-I. Breaks on 3L (obs): prox. one in A; dist. one in G. M larva. A1(N.C.) x. 62l.
Ipa(3L) 8. #465. N-I. Breaks on 3L (obs): prox.one in hc; dist. one in H. M larva. B1 cross. 63a.
Ipa(3L) 9. #468. R. Breaks on 3L (obs): prox. one in hc; dist. one in E. F1(Va.) and B1 x's. 63a.
Ipa(3L) 10. #534. N-I? Breaks on 3L (obs): prox. one in hc; dist. one in E. M larva. Same C2 x as Ipa(2R)32. 63c.

Chromosome 3: Right Arm

Ipa(3R) 1. #4. N-I. Breaks on 3R (obs): prox. one in D; dist. one in F2. M larva. A1(Ia.) x. 60l.
Ipa(3R) 2. #33. R. Breaks on 3R (obs): prox. one in D; dist. one in F2. D1, I1 and I3 x's. (I3 x proves it is not from nature.)
Ipa(3R) 3. #203. N-I. Breaks on 3R (obs): prox. one in hc; dist. one in G. M larva. A3 x. 62f.
Ipa(3R) 4. #486. N-I. Breaks on 3R (obs): prox. one in E; dist. one in G. F larva. B1 x. 63c.

Pericentric Inversionis: Chromosome 2

Ipe(2) 1. #11. N-I. Lt. break on 2L-1 (inf) in W; rt. break on 2R-1 (inf) in R. F larva. A3 x. 61g.
Ipe(2) 2 #16. R. Lt. break on 2L (obs) in U; rt. break on 2R (obs) in P. A3 and A1(Va.) x's. First seen 61h. Persisted in stock many years.
Ipe(2) 3. #44. R. Lt. break on 2L (obs) in A; rt. break on 2R (obs) in F. First seen in same A4 x as Df2 (Levitan 1964). 62b. Persisted in stock many years.
Ipe(2) 4. #57. N-I. Lt. break on 2L-3 (obs) in P; rt. break on 2R (inf) in C. M larva. Same x as Ipa(2L)6, etc. 62c.

Ipe(2) 5. #67. R. Lt. break on 2L (inf) in G1; rt. break on 2R (inf) in hc. A6 x (male from A1,Va.) 62d.
Ipe(2) 6. #72. N-I. Lt. break on 2L-1 (obs) in Q2; rt. break on 2R-1 (obs) in J. F larva. A3 x. 62d.
Ipe(2) 7. #74. N-I. Lt. break on 2L-3 (inf) in C; rt. break on 2R (inf) in M. F larva. A3 x. 62d.
Ipe(2) (No. 78) R. Probably natural. Lt. break on 2L-4 (obs) in hc; rt. break on 2R (obs) in I. D2 x. 62d. In stock derived from an inseminated female known to lack both 2L-4 and 78, collected by H.D. Stalker in Wagarville, Washington Co., Ala., 61g18/19.
Ipe(2) 8. #96. R. Lt. break on 2L-1 (obs) in E; rt. break on 2R (obs) in M. C2x. 62e.
Ipe(2) 9. #100. N-I. Lt. break on 2L-3 (obs) in F; rt. break on 2R-1 (obs) in G. F larva. C2 x. 62e.
Ipe(2) 10. #112. R. Lt. break on 2L (obs) in L2; rt. break on 2R (obs) in G. Two C2 x's; both 62d.
Ipe(2) 11. #138. N-I. Lt. break on 2L-1(inf) in Q2; rt. break on 2R(obs) in F. M larva. A3 x. 62f.
Ipe(2) 12. #140. N-I. Lt. break on 2L-3 (inf) in W; rt. break on 2R (obs) in D. M larva. C2 x. 62f.
Ipe(2) 13. #142. N-I. Lt. break on 2L-1 (obs) in A; rt. break on 2R-1 (inf) in C. F larva. C2 x 62f.
Ipe(2) 14. #143. N-I. Lt. break on 2L-3 (obs) in I; rt. break on 2R (obs) in G. M larva. C2 x. 62f.
Ipe(2) 15. #150. N-I. Lt. break on 2L-1 (inf) in U; rt. break on 2R-1 (inf) in C. F larva. Same A3 x as Ipe(X)6 (Levitan 1964). 62f.
Ipe(2) 16. #158. N-I. Lt. break on 2L-3 (inf) in A; rt. break on 2R (obs) in G. M larva. A3 x. 62f.
Ipe(2) 17. #178. N-I. Lt. break on 2L-3 (inf) in G2; rt. break on 2R (obs) in J. M larva. A3 x. 62f.
Ipe(2) 18. #188. R. Lt. break on 2L (obs) in L1; rt. break on 2R (obs) in F. Two A3 x's; both 62f.
Ipe(2) 19. #191. R. Lt. break on 2L (obs) in hc; rt. break on 2R (obs) in G. A3 x. 62f.
Ipe(2) 20. #206. N-I. Lt. break on 2L-1 (inf) in V; rt. break on 2R-1 (inf) in E. F larva. A3 x. 62f.
Ipe(2) 21. #234. N-I. Lt. break on 2L-1 (inf) in K; rt. break on 2R-1 (inf) in C. M larva. A3 x. 62f.
Ipe(2) 22. #235. N-I. Lt. break on 2L-3 (inf) in B; rt. break on 2R (obs) in O. F larva. A3 x. 62f.
Ipe(2) 23. #256. N-I. Lt. break on 2L-1 (obs) in H; rt. break on 2R-1 (inf) in E. M larva. A3 x. 62h.
Ipe(2) 24. #262. N-I. Lt. break on 2L-1 (inf) in W; rt. break on 2R-1 (inf) in P. F larva. Same x as Ipa(2L)23, etc. 62h.
Ipe(2) 25. #264. N-I. Lt. breakon 2L-1 (obs) in L1; rt. break on 2R (obs) in hc. M larva. A3 x. 62g.
Ipe(2) 26. #272. N-I. Lt. break on 2L-3 (obs) in J; rt. break on 2R (obs) in R. F larva. A3 x. 62g.
Ipe(2) 27. #276. N-I. Lt. break on 2L-1 (inf) in B; rt. break on 2R-1 (obs) in R. F larva. A3 x. 62h.
Ipe(2) 28. #280. N-I. Lt. break on 2L-1 (obs) in L1; rt. break on 2R-1 (obs) in O. F larva. Same A3 x as Ipa(2R)15 and others. 62i.
Ipe(2) 29. #287. N-I. Lt. break on 2L-1 (inf) in L1; rt. break on 2R-1 (obs) in K. M larva. A3 x. 62g.
Ipe(2) 30. #290. N-I. Lt. break on 2L-1 (obs) in R1; rt. break on 2R-1 (obs) in Q. F larva. A3 x. 62g.
Ipe(2) 31. #310. R. Lt. break on 2L (obs) in hc; rt. break on 2R (obs) in E. A3 x. 62g.
Ipe(2) 32. #320. N-I. Lt. break on 2L (obs) in B; rt. break on 2R (obs) in hc. M larva. A7 x (ST_y female x male whose mother was from a non-inducer population, father from a ST_y substrain). 62h.
Ipe(2) 33. #334. N-I. Lt. break on 2L-1 (obs) in P; rt. break on 2R-1 (obs) in I. M larva. A3 x. 62h.
Ipe(2) 34. #343. N-I. Lt. break on 2L (obs) in H; rt. break on 2R (obs) in G. M larva. C2 x. 62h.
Ipe(2) 35. #364. R. Lt. break on 2L (obs) in I; rt. break on 2R (obs) in D. A1(N.C.) and H3 x's. First seen 62i.
Ipe(2) 36. #392. N-I. Lt. break on 2L-1 (inf) in hc; rt. break on 2R-1 (obs) in L. F larva. A1(N.C.) x. 62j.
Ipe(2) 37. #397. R. Lt. break on 2L (obs) in L2; rt. break on 2R (obs) in B. A1(O.) x's. First seen 62k.
Ipe(2) 38. #406. N-I. Lt. break on 2L-3 (obs) in K; rt. break on 2R (obs) in D. M larva. A1(N.C.) x. 62k.
Ipe(2) 39. #409. N-I. Lt. break on 2L-1 (obs) in F; rt. break on 2R (obs) in D. F larva. C3 x. 62k.
Ipe(2) 40. #430. N-I. Lt. break on 2L-3 (inf) in H; rt. break on 2R (obs) in Q. M larva. A1(Va.) x. 62l.
Ipe(2) 41. #450. R. Lt. break on 2L (obs) in L1; rt. break on 2R (obs) in B. First seen in F1 x's; has persisted in ST_y substrains and outcross stocks over 21 years. First seen 62l.
Ipe(2) 42. #453. R. Lt. break on 2L (obs) in L1; rt. break on 2R (obs) in C. Same B1 and B3 x's as Ipe(2)43. First seen 62l.
Ipe(2) 43. #454. R. Lt. break on 2L (obs) in T; rt. break on 2R (obs) in F. Same B1 and B3 x's as Ipe(2)42. First seen 62l.
Ipe(2) 44. #455. N-I. Lt. break on 2L-2 (obs) in W; rt. break on 2R (obs) in E. M larva. A1(Va.) x. 62l.
Ipe(2) 45. #471. R. Lt. break on 2L (obs) in A; rt. break on 2R (obs) in M. In two male larvae. B1 x. 63b.
Ipe(2) 46. #472. N-I. Lt. break on 2L (obs) in J; rt. break on 2R (obs) in G. M larva. B1 x. 63b.
Ipe(2) 47. #474. R. Lt. break on 2L (obs) in U; rt. break on 2R (obs) in R2. B1 and B3 x's. First seen 63b.
Ipe(2) 48. #537. R. Lt. break on 2L-1 (obs) in M; rt. break on 2R-1 (obs) in P. In two C2 x's, both 63c.
Ipe(2) 49. #540. N-I. Lt. break on 2L-1 (inf) in T; rt. break on 2R-1 (inf) in H. F larva. Same C2 x as Ipa(XL)18 and a T(2R:3L) (Levitan 1964). 63c.
Ipe(2) 50. #545. N-I. Lt. break on 2L-3 (obs) in K; rt. break on 2R (inf) in D. F larva. C2 x. 63c.

Pericentric Inversions: Chromosome 3

lpe(3) 1. #59. N-l. Lt. break in G; rt. break on 3R (obs) in hc. M larva. C2 x. 62c.
lpe(3) 2. #106. N-l. Lt. break in F; rt. break on 3R (obs) in E. F larva. A3 x. 62f.
lpe(3) 3. #241. R. Lt. break in D; rt. break on 3R (obs) in C. A3 x. 62g.
lpe(3) 4. #252. N-l. Lt. break in F; rt. break on 3R-1 (obs) in G. F larva. A3 x. 62g.
lpe(3) 5. #289. N-l. Lt. break in hc; rt. break on 3R (obs) in F1. M larva. Same A3 x as lpe(2)29. 62g.
lpe(3) 6. #362. N-l. Lt. break in C; rt. break on 3R (obs) in G. M larva. A1(O.) x. 62i.
lpe(3) 7. #449. N-l. Lt. break in L; rt. break on 3R (obs) in G. F larva. Same A1(Va.) x as lpa(XL)15 (Levitan 1964). 62l.
lpe(3) 8. #511. N-l. Lt. break in hc; rt. break on 3R (obs) in G. M larva. B1 x. 63c.

Lopez, M.M. University of Mar del Plata, Argentina. **Drosophila subobscura** has been found in the Atlantic coast of Argentina.

D.subobscura, a typical palearctic species, was found in South America in 1978 in Chile (Brncic et al. 1981) and in 1981 in the western region of Argentina (Prevosti 1983) around the Nahuel Huapi lake. This lake is part of a lacustral system which is a natural Andean pass. No **D.subobscura** were found by the same author in the east of the country (near the city of Buenos Aires).

During 1984, we took samples of Drosophila near Mar del Plata, a coastal city situated 400 Km south from Buenos Aires. In our captures, out of 1300 individuals, 26 were **D.subobscura** (i.e., about 2%). We found a considerable seasonal variation, similar to that found in Chile (Budnik et al. 1982).

This finding would indicate that the "pampa" plain is not a geographic barrier as suggested by Prevosti (1983). The absence of **D.subobscura** in the sample obtained in 1981 could have been due to: (1) the season when it was taken (not mentioned), and/or (2) colonization after 1981.

References: Brncic et al. 1981, Genetica 56: 3-9; Prevosti 1983, DIS 59:103; Budnik & Brncic 1982, Actas V Congreso Latinoamericano de Genetica 177-188.

Manousis, T.H. Aristotelian University of Thessaloniki, Greece. Larval saliva in several Drosophila species.

Salivary glands as well as isolated saliva of some developmental stages of the late third instar larvae and early prepupae of several Drosophila species were analysed by urea-polyacrylamide gel electrophoresis and the main components of the saliva of each species were localized on the zymograms (Fig. 1). There was an attempt to find correlations between the polypeptide content and the hardness of the "glue" in the fixative, the background the larvae pupate on and the degree of their phylogenetic relationship.

The number of the different saliva polypeptidic components seems to have no effect on the pupating behavior of the animals. Larvae with hard and rich in proteins content, tend to pupate on the container

TABLE

Subgroup	Drosophila species	Collection area	Stock No	main components of glue	colour of glue in fixative	hardness of glue in fixative	pupariation on the:
ananassae	varians	Los Banos-Luson Philippines	3146.53 Texas	4	white	very hard	container
	bipectinata	Thailand	3256.4	3	white	hard	container
	malerkotliana	Philippines	3146.56 Texas	2	white	hard	food
obscura	pseudoobscura		3339.5 standard Texas	2	light blue	hard	container
montium	serrata	Queensland	2404.6 Texas	4	transparent	syrup	food
	auraria	Kirishima Japan	3040.11b Texas	2	light white	very soft	food
	quadraria	Chi-Tou Taiwan	3075.1 Texas	3	white	medium soft	food
	triauraria	Tokyo, Japan	1731.1	2	white	medium soft	food
Takahashii	takahassii	Tagaytay-Luson Philippines	Texas	2	white	hard	container
willistoni	equinoxialis	Teffe Brazil	2533.3	5	white	hard	food & container

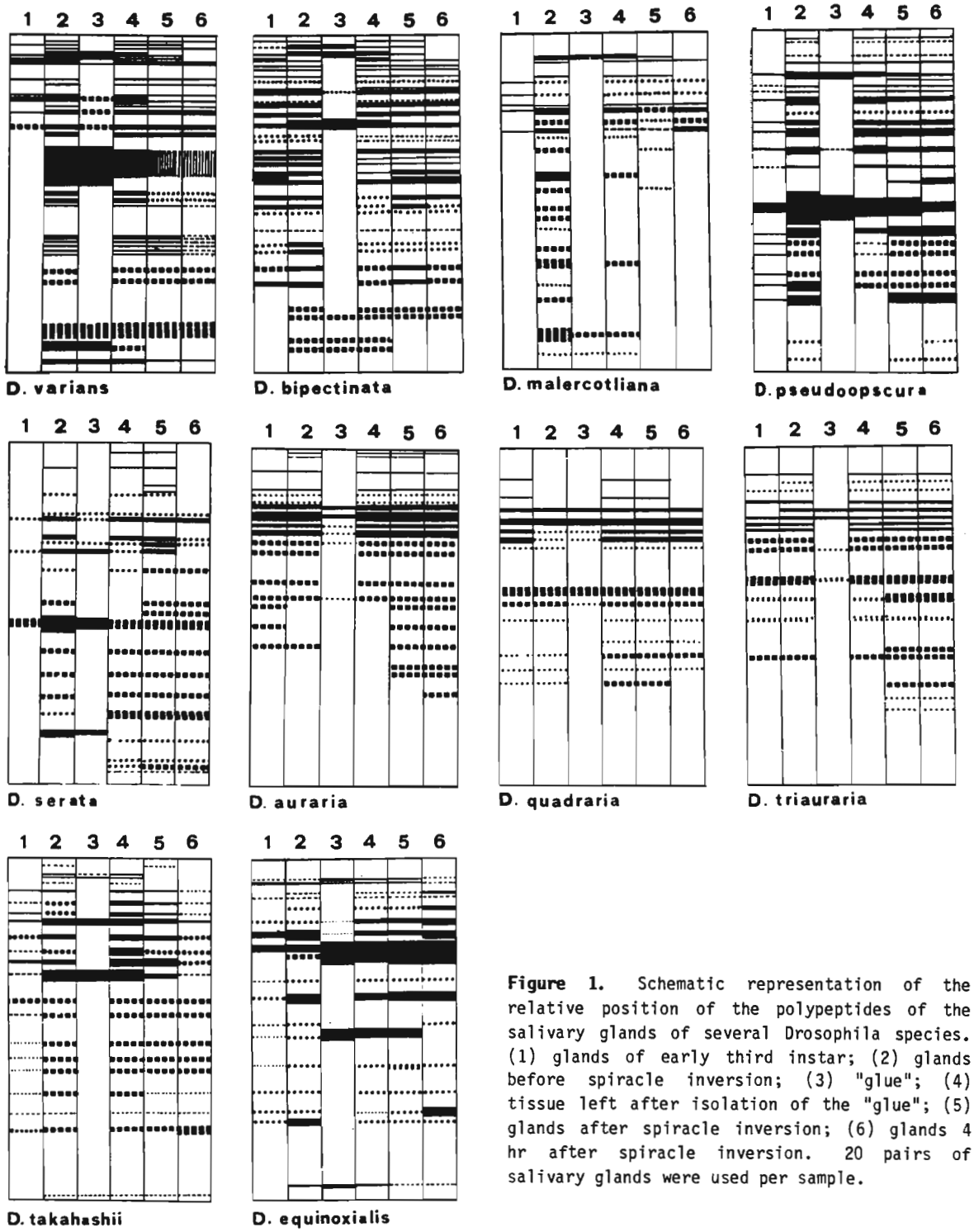


Figure 1. Schematic representation of the relative position of the polypeptides of the salivary glands of several Drosophila species. (1) glands of early third instar; (2) glands before spiracle inversion; (3) "glue"; (4) tissue left after isolation of the "glue"; (5) glands after spiracle inversion; (6) glands 4 hr after spiracle inversion. 20 pairs of salivary glands were used per sample.

and not on the food as larvae with soft or sirup-like saliva (Table 1). It seems that closely related species have similar hardness and richness of "glue" proteins and pupate on the same background. It should be noted though that in some cases there is still much saliva present in the glands even quite a few hours after formation of the puparium and even a second secretion into the lumen can be observed in the same species. This is evidence of an additional function of saliva other than the fixation of puparium on the substrate.

Acknowledgements: Supported by a grant from Volkswagenwerk Stiftung to Prof. Kostas D. Kastritsis.

References: Ashburner, M. 1970, Chromosoma 31: 356-376; Grossbach, U. 1969, Chromosoma (Berl) 28: 136-187; Korge, G. 1977, Devel. Biol. 8:339-355; Thomopoulos, G.N. & C.D. Kastritsis 1979, Wilhelm Roux's Arch. 187:329-354.

Markow, T.A. and N. Maveety. Arizona State University, Tempe, Arizona USNA. More character displacement for reproductive isolation in the Mulleri complex.

Wasserman & Koepfer (1977) described character displacement for reproductive isolation between two sibling species, **D.mojavensis** and **D.arizonensis** of the mulleri complex of the repleta group. Zouros & d'Entremont (1980) demonstrated that the presence of **D.arizonensis** in the Sonoran part of the range of **D.mojavensis** is the cause of reproductive isolation between the two geographic races of **D.mojavensis** (race A in Sonora and race B in Baja California).

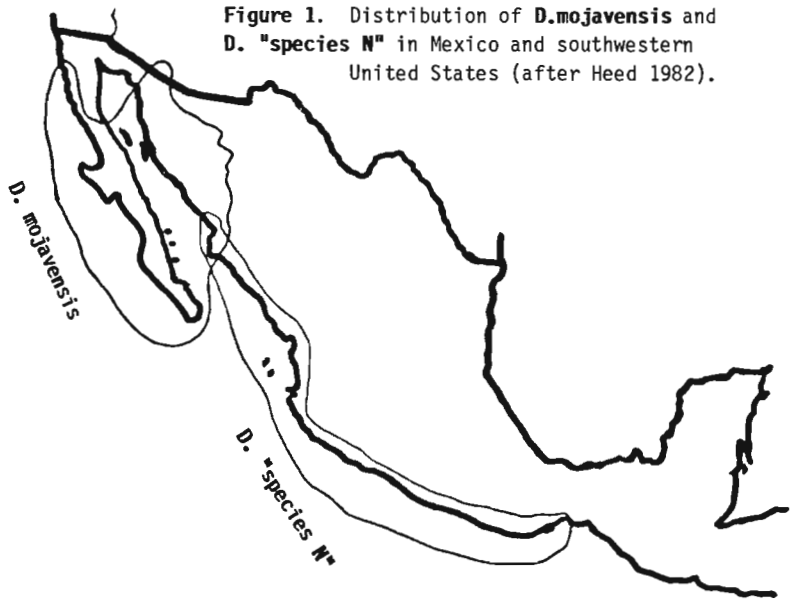


Figure 1. Distribution of **D.mojavensis** and **D. "species N"** in Mexico and southwestern United States (after Heed 1982).

Table 1. Indices of reproductive isolation for sympatric and allopatric populations of **D. "species N"** and **D.mojavensis.**

	Localities*			
D. "species N"	X	**D.mojavensis**	I + SE (n)	
ALLOPATRIC	X	ALLOPATRIC		
1. Nahuapa	X	Vallecito (California)	0.567 ± 0.075	(120)
2. Nahuapa	X	San Telmo (Baja)	0.591 ± 0.086	(88)
3. Nahuapa	X	Catavena (Baja)	0.571 ± 0.109	(56)
4. Nahuapa	X	Pt. Onah (Sonora)	0.960 ± 0.033	(60)
5. Nahuapa	X	San Carlos (Sonora)	1.0	(56)
ALLOPATRIC	X	SYMPATRIC		
1. Nahuapa	X	So. of Navajoa	0.92 ± 0.003	(55)
SYMPATRIC	X	ALLOPATRIC		
1. Navojoa(E2.1)	X	Vallecito (California)	0.790 ±0.09	(48)
2. Navojoa(E2.1)	X	San Telmo (Baja)	0.944 ± 0.039	(72)
3. Navojoa(E2.1)	X	Pt. Onah (Sonora)	1.0	(49)
4. Navojoa(E2.1)	X	San Carlos (Sonora)	0.967 ± 0.033	(61)
SYMPATRIC	X	SYMPATRIC		
1. Navojoa(E2.1)	X	So. of Navojoa	1.0	(51)

*D. "species N" from Navojoa, Sonora, was obtained from the **Drosophila** species stock center. All others were provided by the University of Arizona.

The discovery of the third, undescribed sibling species, **D. "species" N"** ("from Navojoa", Mexico) raises the question of additional character displacement for sexual behavior in areas where this species is sympatric with the others. Figure 1 shows the distribution of the three species in Mexico and the United States; **D. "species N"** is sympatric with **D.mojavensis** in southern Sonora and northern Sinaloa. There is no gene flow between peninsular and mainland **D.mojavensis.** We tested for intensity of sexual isolation between **D.mojavensis** and **D. "species" N"** from sympatric and allopatric strains of both species. Experiments were conducted using procedures reported by Markow (1981) and Markow et al. (1983) in which 10 pairs from 2 different species are placed in an observation chamber for 1 hr. Results are shown in Table 1. When either **D.mojavensis** or **D. "species N"** is from a sympatric collection, complete or nearly complete isolation is observed. In fact, the same degree of isolation is observed with **D.mojavensis** strains from allopatric strains in Sonora. This is probably best explained by gene flow among mainland populations of **D.mojavensis.** When both are allopatric, isolation indices are between .50 and .60. These data show that character displacement for sexual isolation exists in sympatric populations of these two species. The pattern is similar to that described above for **D.mojavensis** and **D.arizonensis.** There is evidently enough genetic variability in mate recognition systems to allow a degree of "fine-tuning" to evolve in areas where two species coexist. This variability should be very useful in studying the evolution of new mate recognition systems during speciation.

References: Wasserman, M. & H.R. Koepfer 1977, Evolution 31: 812-823; Zouros, E. & C.J. d'Entremont 1980, Evolution 34: 421-430; Markow, T.A. 1981, Evolution 35: 1022-1027; Markow, T.A. et al. 1983, Evolution 37: 699-652.

Martínez-Sebastián, M.J. and J.L. Ménsua. University of Valencia, Spain. Abdominal bristle number and sex-dimorphism in **D.subobscura.**

Mather (1941) observed enormous variation in abdominal bristle number, particularly in the ratio of number in the two sexes, among several species of Drosophila. On the other hand, differential response of the two sexes to artificial selection is a common feature of the selection experiments on abdominal bristles with **D.melanogaster** (Harrison 1953; Clayton & Robertson 1957; Sheldon 1963).

In a laboratory population of **D.subobscura**, the sex-dimorphism ratio oscillated between 1 and 1.02. These values indicate that there is no sex-dimorphism in **D.subobscura** in respect of abdominal bristle number, in contrast to other species of Drosophila (Yoo, Moth & Barker 1981).

Two replicate selection lines for abdominal bristle number in both high (H1 and H2) and low (L1 and L2) directions were established from the laboratory population. The response pattern was very similar between the sexes within a line. ANCOVA analysis shows significant differences between sexes only in L2 line ($F = 4.35^*$, $p=0.05$).

Table 1. Mean values ($\bar{X}$) of the crosses between high (H1 and H2) and low (L1 and L2) selected lines.

		Generation		
		P	F1	F2
♂H1 x ♀L1	$\bar{X}$♀	11.78±0.23	34.13±0.30	30.07±0.67
	$\bar{X}$♂	74.55±0.62	28.70±0.29	34.63±0.87
	$\bar{X}$	43.17±2.90	31.42±0.27	32.35±0.57
♂L1 x ♀H1	$\bar{X}$♀	74.48±0.66	33.87±0.29	39.14±0.81
	$\bar{X}$♂	13.47±0.33	41.41±0.28	35.93±0.71
	$\bar{X}$	43.98±2.82	37.64±0.32	37.54±0.55
♂H2 x ♀L2	$\bar{X}$♀	12.25±0.71	30.62±0.67	30.86±0.62
	$\bar{X}$♂	73.17±0.75	30.73±0.50	35.71±0.71
	$\bar{X}$	42.71±2.84	30.68±0.42	33.28±0.50
♂L2 x ♀H2	$\bar{X}$♀	72.40±0.64	38.69±0.25	40.28±0.65
	$\bar{X}$♂	15.77±0.53	45.13±0.35	38.02±0.73
	$\bar{X}$	44.08±2.63	41.91±0.30	39.15±0.49

Reciprocal crosses between lines were made when selection finished (Table 1). Differences between males and females from F1 indicate the existence of factors in chromosome X. Moreover, mean value of males and females from F2 of each cross increased or decreased in respect of F1 values, as was expected in agreement with chromosome X segregation.

Thus, the lack of sex-dimorphism in the laboratory population of **D.subobscura** and the agreement in the response of the two sexes, must be due to the presence of whole dosage compensation for abdominal bristle number.

References: Clayton, G.A. & A. Robertson 1957, J. Genet. 55:131-151; Harrison, B.J. 1953, Heredity 7:153-161; Mather,K. 1941, J.Genet. 41:159-193; Yoo, B.H., J.J. Moth & J.S.F. Barker 1981, DIS 56:163-164.

Mather, W.B. and R. Casu. University of Queensland, St. Lucia, Australia. Inversions from Phuket, Thailand. 5th Report.

In July 1983 sixty-two isolines of **D.s.albostrigata** and eight isolines of **D.albomicans** were established from Phuket, Thailand. Inversions in these species were last reported on from Phuket in January 1983 (Mather & Pope, DIS 60:142).

(a) **D.s.albostrigata.** Eight simple inversions were detected (Table 1). All inversions had previously been detected from Southeast Asia, but B_5, C_1, P_5, W_2, and Z_2 were new to Phuket. The heterozygosity frequency of all inversions detected is given in the Table.

Table 1.

Inversion	Chromosome	Het.Freq.%
A_5	II L	30.6
C_5	II R	77.4
B_5	III	3.2
C_1	III	30.6
F_3	III	17.7
P_5	III	11.3
W_2	III	11.3
Z_2	III	8.1

Table 2.

Inversion	Chromosome	Simple	Complex
E^1	II L	X	
B^1	III	X	
B_6	III	X	
C_1	III	X	
E_6	III		X
L_3	III	X	

(b) **D.albomicans.** Five simple and one complex inversion were detected (Table 2). All inversions had previously been detected from Southeast Asia, but B and C were new to Phuket.

The material was collected and the isolines established by W.B.M. The laboratory work was carried out by R.C.

Mather, W.B. and A.K. Pope. University of Queensland, St. Lucia, Australia. Inversions from Chiang Mai, Thailand. 5th Report.

In July 1984 thirty-eight isolines of **D.s.albostrigata** and seven isolines of **D.albomicans** were established from Chiang Mai, Thailand. Inversions in these species were last reported on from Chiang Mai in November 1983 (Mather & Tam, DIS 61: this issue).

(a) **D.s.albostrigata.** Six simple and two complex inversion were detected. All inversions had previously been detected in Southeast Asia, but F_3 was new to Chiang Mai. A photograph of the new inversion C_7 is presented and breakpoints assigned in relation to the standard photographic map (Thongmeearkom 1977, DIS 52:154). The heterozygosity frequency of all inversions is given in Table 1.

(b). **D.albomicans.** Five simple and one complex inversions were detected (Table 2). All had previously been detected in Southeast Asia but L_3 was new to Chiang Mai.

The material was collected and the isolines established by W.B.M. The laboratory work was carried out by A.K.P.

Table 1.

Inversion	Chromosome	Simple	Complex	Het. Freq.%	Breakpoints
A_5	II L	X		44.7	
D_5	II L		X	2.6	
E	II L	X		13.2	
C_7	II L		X	2.6	3.2-21.2
C_5	II R	X		10.5	
Y_6	III	X		2.6	
B_5	III	X		13.2	
F_3	III	X		2.6	

Table 2.

Inversion	Chromosome	Simple	Complex
R_5	I	X	
E^1	II L	X	
A_7	II L	X	
C_1	III	X	
E_6	III		X
L_3	III	X	

Figure 1. The free end of the chromosome is in the centre of the photograph.

Mather, W.B. and A.K. Pope. University of Queensland, St. Lucia, Australia. Inversions from Phuket, Thailand. 6th Report.

In January 1984 thirty isolines of **D.s.albostrigata,** twelve isolines of **D.albomicans** and two isolines of **D.kohkoa** were established from Phuket, Thailand. Inversions in **nasuta** group species were last reported on from Phuket in July 1983 (Mather & Casu, DIS 61: this issue).

(a) **D.s.albostrigata.** Eight simple and one complex inversion were detected (Table1). All inversions had previously been detected from Phuket. The heterozygosity frequency of all inversions detected is given in the Table.

Table 1.

Inversion	Chromosome	Simple	Complex	Het.Freq.%
A_5	II L	X		16.7
D_5	II L		X	46.7
I_2	II L	X		3.3
E	II L	X		33.3
C_5	II R	X		73.3
C_1	III	X		46.7
W_2	III	X		13.3
F_3	III	X		13.3
P_5	III	X		6.7

Table 2.

Inversion	Chromosome	Simple	Complex	Het.Freq.%
R_5	I	X		25.0
E^1	II L	X		66.7
C_1	III	X		58.3
L_3	III	X		16.7
E_6	III		X	66.7
B_6	III	X		33.3
Z_6	III	X		16.7
B^1	III	X		8.3

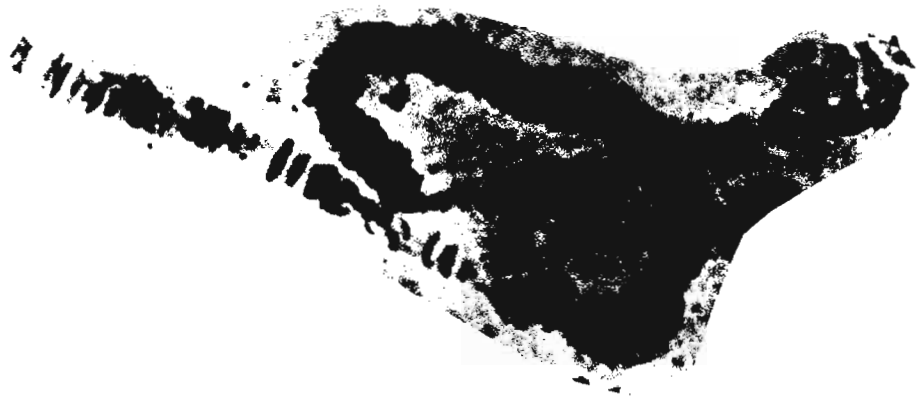


Table 3.

Inversion	Chromosome	Breakpoints
E^1	II L	
Y	III	
K_6	III	
L	III	
B_7	III	13.2-19.3

Figure 1. The free end of the chromosome is to the right.

(b) **D.albomicans.** Seven simple and one complex inversion were detected (Table 2). All inversions had previously been detected from Southeast Asia, but R_5 and Z_6 were new to Phuket.

(c) **D.kohkoa.** Five simple inversions were detected (Table 3). Four of the five inversions had previously been detected in Southeast Asia, but of these E^1, K_6, and L are new to Phuket. A photograph of the new inversion B_7 is presented and breakpoints assigned in relation to the standard photographic map (Mather, W.B. & P. Thongmeearkom 1978, DIS 53:150).

The material was collected and the isolines established by W.B.M. The laboratory work was carried out by A.K.P.

Mather, W.B. and A.K. Pope. University of Queensland, St. Lucia, Australia. Inversions from Phuket, Thailand. 7th Report.

In July 1984 twenty-eight isolines of **D.s.albostrigata** and ten isolines of **D.albomicans** were established from Phuket, Thailand. Inversions in these species were last reported on from Phuket in January 1984 (Mather & Pope, DIS 61: this issue).

(a) **D.s.albostrigata.** Seven simple and one complex inversion were detected (Table 1). All inversions had previously been detected from Phuket. The heterozygosity frequency of all inversions detected is given in Table 1.

(b) **D.albomicans.** Five simple and one complex inversions were detected (Table 2). All inversions had previously been detected from Phuket. The heterozygosity frequency of all inversions detected is given in Table 2.

The material was collected and the isoline established by W.B.M. The laboratory work was carried out by A.K.P.

Table 1.

Inversion	Chromosome	Simple	Complex	Het.Freq.%
A_5	II L	X		7.1
E	II L	X		14.2
D_5	II L		X	67.8
C_5	II R	X		64.2
C_1	III	X		42.8
P_5	III	X		3.5
W_2	III	X		28.5
F_3	III	X		10.7

Table 2.

Inversion	Chromosome	Simple	Complex	Het.Freq.%
R_5	I	X		10
E^1	II L	X		50
C_1	III	X		90
E_6	III		X	50
L_3	III	X		50
B_6	IIi	X		20

Mather, W.B. and K.S. Tam. University of Queensland, Brisbane, Australia. Inversions from Chiang Mai, Thailand. 3rd Report.

TABLE

Inversion	Chromosome	Simple	Complex	Het. Freq. %
A_5	II L	X		30.2
E	II L	X		22.2
C_5	II R	X		11.1
B_5	III	X		12.7
C_1	III	X		3.2
D_5	II L		X	1.6

In July 1983 sixty-three isolines of **D.s.albostrigata** and six isolines of **D.albomicans** were established from Chiang Mai, Thailand. Inversions in these species were last reported on from Chiang Mai in November 1982 (Mather & Pope, DIS 60:141).

(a) D.s.albostrigata. Five simple and one complex inversion were detected. All inversions had previously been detected in Southeast Asia but D_5 was new to Chiang Mai. Heterozygosity frequency of all inversions detected is given in the Table.

(b) D.albomicans. One simple and one complex inversion were detected. E' (simple) had previously been detected from Chiang Mai but J_8 (complex) although recorded from South East Asia was new to Chiang Mai.

The material was collected and the isolines established by W.B.M. The laboratory work was carried out by K.S.T.

Mather, W.B. and K.S. Tam. University of Queensland, St. Lucia, Australia. Inversions from Chiang Mai, Thailand. 4th Report.

Table 1.

Inversion	Chromosome	Simple	Complex	Het.Freq.%
A_3	I	X		3.6
G	I	X		7.1
A_5	II L	X		71.4
E	II L	X		28.6
D_5	II L		X	17.9
C_5	II R	X		7.1
D_3	II R	X		3.6
B_5	III	X		25.0
C_1	III	X		3.6

In November 1983 twenty-eight isolines of **D.s.albostrigata** and one isoline of **D.albomicans** were established from Chiang Mai, Thailand. Inversions in these species were last reported on from Chiang Mai in July 1983 (Mather & Tam, DIS 61: this issue).

(a) **D.s.albostrigata.** Eight simple and one complex inversion were detected. All inversions had previously been detected in Southeast Asia, but D_3, G, and A_5 were new to Chiang Mai. The heterozygosity frequency of all inversions detected is given in the Table.

(b) **D.albomicans.** One simple inversion was detected (inversion E^1). This had previously been detected from Chiang Mai.

The material was collected and the isoline established by W.B.M. The laboratory work was carried out by K.S.T.

Matyunina, L.V. and T.I. Gerasimova. Inst. of General Genetics, USSR Academy of Sciences, 117908 Moscow, USSR. Study of spontaneous sex-linked lethal mutations in the unstable line ct^{MR2} of **Drosophila melanogaster.**

Earlier an unstable ct^{MR2} mutation was obtained at the locus cut (20.0; 7B 3-4) under conditions of P-M hybrid dysgenesis (Gerasimova 1981). This mutation is induced by a mobile dispersed gene MDG4 (Gerasimova 1984a). This line homozygous for X-chromosome is characterized by a high reversion frequency, segregation of novel ct mutations with a new phenotype and superunstable ct mutations, as well as by the formation of new visible mutations for other loci of the X-chromosome (Gerasimova 1981).

Phenotypical changes arising in the $ct^{MR}2$ line are the markers of so-called transpositional bursts, i.e., mass simultaneous transpositions of different mobile elements in the same germ cell (Gerasimova 1984b). The frequency of transpositional bursts in the ct^{MR2} line was about $1x10^{-3}$. Since transpositional bursts result in active insertion mutagenesis, the true frequency of "bursts" may be significantly higher because in this case lethal mutations should occur. Therefore the objective of the present work is the estimation of the lethal mutation frequency in the X-chromosome of the ct^{MR2} line and characterization of the distribution of the lethals.

Table 1. Location of lethal mutations in the 6C12-8A5 region of X-chromosome.

	Character of complementation					
	Dp(1;3) sn^{13a}	Df(1) ct^{J4}	Dp(1;2)$^{+72d}$	Df(1)^{4b1}	ct^{JA124}	Number of
Region	6C12-7C9	7A1-7C1	7A8-8A5	7B3-7C4	7B3-4	lethals
6C12-7A13	-	+	+	+	+	13
7A2-7A8	-	-	+	+	+	-
7A8-7B2	-	-	-	+	+	2
7B3-4	-	-	-	-	-	33
7B5-7C1	-	-	-	-	+	4
7C2-7C8	+	-	+	-	+	-
haplolethal region						
7C9-8A5	-	+	-	+	+	29
						81

"+" = a lethal complementary to Df or Dp; i.e., 1/Df females survive and +/Dp; 1/Y males do not. "-" = a lethal noncomplementary to Df or Dp; i.e., 1/Df females do not survive and +/Dp; 1/Y males do.

To estimate the frequency of lethal mutations in the X-chromosome, the following matings were made: ♀ct^{MR2}/ct^{MR2} x ♂FM4/Y → (F_1) ♀ct^{MR2}/FM4 x ♂FM4/Y → (F_2) ct^{MR2}/FM4, ct^{MR2}/Y, FM4/Y. 31742 ct^{MR2} chromosomes were examined in F_2 and 353 lethal mutations; 50 reversions and several visible mutations were discovered. The frequency of ct^{MR2} → ct^+ reversion in this experiment was 1.9×10^{-3}. The frequency of lethal sex-linked mutations was 1.1×10^{-2}. The same scheme was used in the control experiments in which females of the wild line Oregon R were used instead of ♀ct^{MR2}/ct^{MR2} ones. 5120 chromosomes were examined and not a single lethal or visible mutation was found. This means that the formation of lethal, visible mutations and reversions is induced by the unstable line ct^{MR2}.

Since in the "ct^{MR2} system" visible mutations occur most frequently at genes cm(6E), ct (7B) and sn (7D), it was of interest to study this region in detail with respect to lethal mutations. Therefore the lethal mutations obtained were analyzed for the presence in the 6C12-8A5 region of the X-chromosome. To this end, females carrying the lethal mutation 1/FM4 were mated to males containing various deficiencies and duplications of this region: Df(1)4b1, ct^{4b1}oc ptg/In(1)dl-49, y sc lz^5B; Dp(1;3)sn^{13a} [Df(1)4b1 deficiency involves the 7B3-C4 region, duplication Dp(1;3)sn^{13a} involves the 6C12-7C9 region]; ct^{JA124} ; Dp(1;2)sn^{+72d}/In(2LR)Gla [the lethal mutation ct^{JA124} at the ct locus induced by X-rays and pertaining to complementation group III of the locus cut]; Dp(1;2)sn^{+72d} involves the 7A8-8A5 region; Df(1)ct^{J4} deficiency involves the 7A1-7C1 region. The results of the complementation analysis are presented in Table 1.

As is seen from Table 1, 81 of the mutations analyzed are located in the region 6C12-8A5. However, this region comprises about 8% of the whole X-chromosome. Thus, a disproportionately large number of lethal mutations are located in the 6C12-8A5 region. 33 of them were located at the cut locus. The rest of the lethals were tested for allelism with mutations at the loci carmine and singed. However, all the lethals tested were complementary to cm and sn mutations. Thus, besides the locus cut the remaining mutations pertain to unknown, vitally important loci and are located at different sites of the 6C12-8A5 region. It is of interest that 7C2-7C8 bands contain a haplo-lethal region (Lefevre & Johnson 1973) in which it is impossible to detect lethal mutations with the help of X-rays. In the present work, no lethal mutations have been found in the haplo-lethal region 7C2-7C8, too.

An overwhelming majority of lethal mutations occurred at the background of the ct^{MR2} allele. Only 6 of 353 lethals were accompanied by reversals of ct^{MR2} to the wild type ct^+. As well as visible mutations, many lethal mutations appeared to be unstable. Most reversions involved only the lethal mutation and sometimes in double revertants of the 1^+ct^+ type. In some cases, both types of revertants ($ct^{MR3}1$ and ct^+1^+) also carried new visible mutations at different loci of the X-chromosome (y, w, cm, ct, sn, g, pn, m, v, r, B) and the processes of reversion and mutagenesis involved 2-5 loci simultaneously. Besides, mutational transitions accompanied by the reversion of some lethal mutations and formation of lethals at other loci were observed. Thus, the lethal apparently changed its location on the chromosome. This type of mutational transition could be determined only after repeated localization of lethal mutations. Repeated localization of lethals in the 6C12-8A5 region was carried out after a 1-year period. It appeared that 14 out of 81 lethal mutations had changed their location. 4 lethals "left" this region; i.e., the transition l(6C12-8A5) ct^{MR2} → $1^+ct^{MR2}1$ occurred [1 being out of the 6C12-8A5 region]; one lethal "transited" from the locus to the neighbouring region 7B5-7C1, i.e., ct^{13} → $ct^{MR2}1^3$; 9 lethals earlier located in the 6C12-7A1 region occurred in 7B3-4, i.e, in the region of the cut locus: 1 ct^{MR2} → 1^+ct^1.

The unstable and apparently insertional character of the lethal mutations obtained suggests that they, as well as visible mutations, must be the result of transpositional bursts. Individuals with lethal mutations will be eliminated in early ontogenesis, the result being a sharp reduction in the yield of viable mutants. This points out that the true frequency of transpositional bursts must be higher as compared to that of visible mutations at least by a magnitude of one order.

The authors are deeply indebted to Dr. T.K. Johnson for sending stocks Df(1)4b1, ct^{4b1} oc ptg/In(1)dl-49, y sc lz^{5}B; Dp(1;3)sn^{13a}; ct^{JA124} Dp(1;2)sn^{+72d}/In(2LR)Gla; and Df(1)ct^{J4}/ct^{+}.Y .

References: Gerasimova, T.I. 1981, Mol. Gen. Genet. 184:544-547; Gerasimova, T.I., Y.V. Ilyin, L.J. Mizrokhi, L.V. Semjonova & G.P. Georgiev 1984a, Mol. Gen. Genet. 193:488-492; Gerasimova, T.I., L.J. Mizrokhi & G.P. Georgiev 1984b, Nature 309:714-716; Lefevre, G. & T.K. Johnson 1973, Genetics 74:633-645.

Melzer, S. and K.H. Glätzer. Institut für Genetik, Düsseldorf, FR Germany. Localization of RNP antigens in primary spermatocytes of **Drosophila melanogaster** by indirect immunofluorescence and their correlation to fertility factors.

A subset of monoclonal antibodies raised against nuclear proteins of **D.melanogaster** cells are specific for ribonucleoprotein complexes (RNP) (Risau et al. 1983). It could be shown that some of these crossreact with polytene chromosomes of **D.hydei** (Saumweber et al. 1980). Surprisingly the respective antigens are concentrated on distinct Y chromosomal structures in primary spermatocytes of this species (Glaetzer 1984). Because the Y chromosome in Drosophila is indispensible for male fertility, similar functions may be reflected by a similar accumulation of RNP antigens on particular Y chromosomal formations. We therefore tested a number of monoclonal antibodies on cytological preparations of spermatocytes of **D.melanogaster**. In addition we mapped the labeled nuclear structures on the Y chromosome with the positively reacting antibodies. For this purpose we used translocation stocks of J.A. Kennison (1981) which he had kindly donated to Dr. U. Schaefer of our institute.

Out of six antibodies tested (P11, Q16, S5, T7, V4, X4), four (S5, X4, P11, Q16) showed a positive reaction with spermatocyte nuclei. The comparison between X0 cells (Fig. 1d, e) and cells carrying a Y chromosome (Fig. 1a, b) or a fragment of it (Fig. 1c,f) revealed that S5 and X4 antigens are concentrated on Y chromosomal chromatin (data with X4 antibody not shown). The other antibodies P11 and Q16 are associated with the presumed autosomes and the remaining nuclear compartment (data not shown).

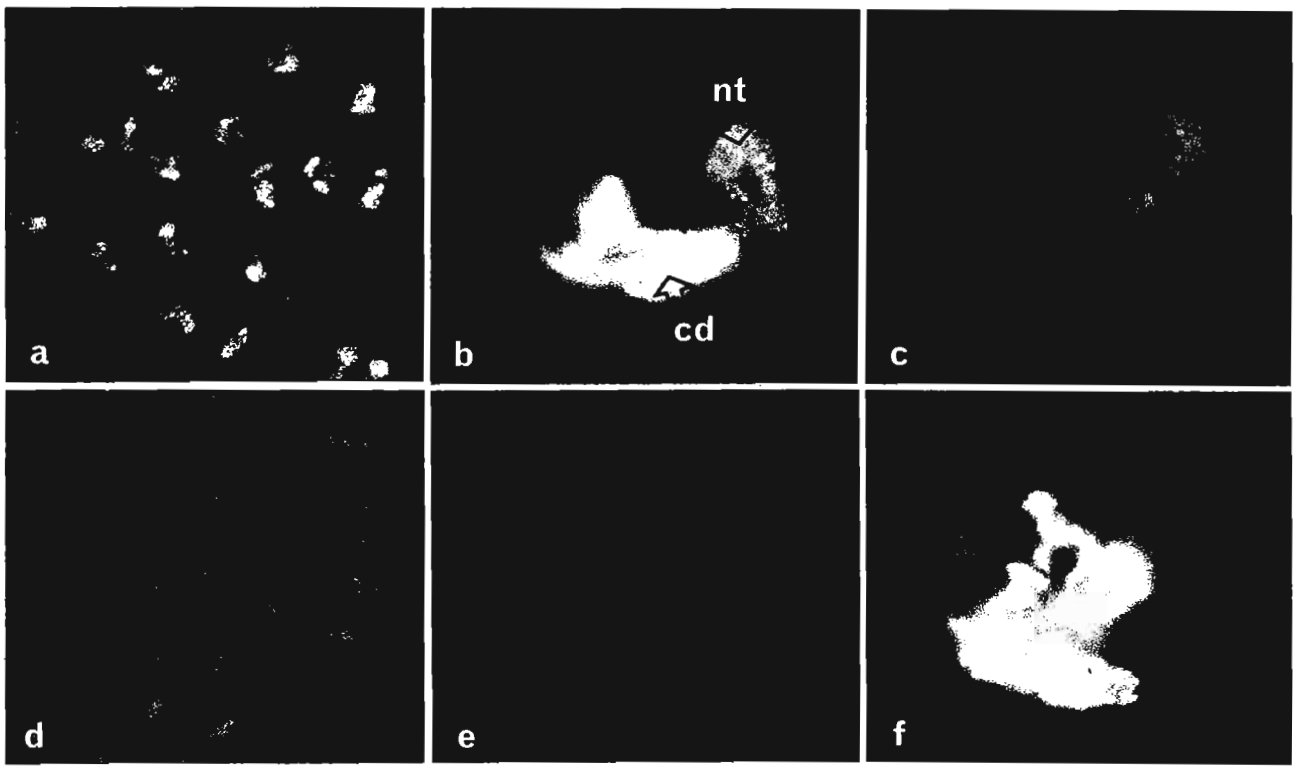


Figure 1. Localization of antigen S5 by indirect immunofluorescence. Staining pattern of: (a) X·$Y^{KL\cdot ks\text{-}1}$ V8-genotype; (b) X/Y-genotype; (c) X·Y^{KS} F12-genotype; (d,e) X0-genotype; (f) X/$Y^{kl\text{-}5}$ V24-genotype. **cd**: "clods"; **nt**: "net". Magnifications: a,d) x325; b,c) x1600; e) x1800; f) x2200.

From Fig. 1b it is evident that two Y chromosomal structures are decorated by S5 (and X4) antibody. It appears that the antigenic determinants of both antibodies are located on the same RNP structures. One structure is best described as coarse and lumpy; the other Y chromosomal formation consists of a fine granular network. Following the tradition of naming the Y chromatin in primary spermatocytes in accordance with its morphological characteristics, we propose to call them "clods" (cd) and "net" (nt), respectively. This is all the more justifiable as the "clods" correspond to the fertility factor kl-5 and the "net" to the functional unit ks-1. Neither structure, however, has an equivalent in the phase contrast microscope (Meyer et al. 1961).

This work was supported by DFG grant Gl 104/2-3.

References: Glaetzer, K.H. 1984, Mol. Gen. Genet. 196:236-243; Kennison, J.A. 1981, Genetics 98:529-548; Meyer, G.F., O. Hess & W. Beermann 1961, Chromosoma 12:676-716; Risau, W., P. Symmons, H. Saumweber & M. Frasch 1983, Cell 33:529-541; Saumweber, H., P. Symmons, R. Kabisch, H. Will & F. Bonhoeffer 1980, Chromosoma 80:253-275.

Miglani, G.S. and V. Mohindra. Punjab Agricultural University, Ludhiana, India. Detection of chromosomal aberrations in the progenies of EMS-induced recombinants in **D.melanogaster** males.

Table 1. Chromosomal aberrations observed in the larvae produced in the TC2 progenies of various TC1 recombinants induced with 0.75% EMS. Aberrations (1) and (2) were from two different larvae selected from TC2 progeny of the same TC1 recombinant; (i) and (ii) were detected in the same chromosome complement; (a) and (b) were detected in two different chromosome complements of larva (2).

Phenotype of TC1 recombinant		Nature of aberration	Chromosome	Breakage-union Points
+ b cn		Inversion	2R	57F-60C
+ b cn		Inversion	2L	25B-28A
+ b cn	(i)	Inversion	2L	24B-27C
	(ii)	Deficiency	2R	52E-52F
(1)+ b cn	(i)	Inversion	3R	94A-95F
	(ii)	Deficiency	2R	52E-52F
(2)+ b cn	(a)	Inversion	3L	70F-73C
	(b)	Inversion	2L	25B-28A

F_1 (Oregon-K) +/dumpy (dp) black (b) cinnabar (cn) **D.melanogaster** males were treated with 0.75% ethyl methanesulphonate (EMS), through feeding in the second one-third part of 96 hr larval life, at 25±1°C. Control experiments were also performed simultaneously. The untreated and EMS-treated F_1 males were crossed with dp b cn females and recombinants were recovered in the first test cross progeny (TC1). In EMS experiments, flies of phenotype + b cn appeared predominantly over their complementary class dp + +, suggesting induction of non-reciprocal recombination in dp-b region. The recombinant flies were again test crossed to obtain second test cross generation (TC2). Late third instar TC2 larvae were randomly selected and sacrificed for study of salivary chromosomes to determine whether any chromosomal aberration induced with EMS was associated with induction of a particular recombinant.

From each of 20 different TC2 progenies of TC1 male recombinants recovered in EMS experiments, salivary chromosomes of 4 to 6 larvae were examined. Chromosomes of all the 83 larvae studied from the control experiments were found to be free of chromosomal abnormalities. Out of 118 larvae studied from 28 progenies of TC1 male recombinants induced in EMS experiments, only four were found to carry a total of 8 chromosomal aberrations. Phenotype of the TC1 recombinants, nature of aberrations detected and the breakage-union points of aberration detected in a particular chromosome are given in Table 1. The salivary chromosomes revealed 4 different and 2 identical inversions and two identical deletions. Out of the 6 inversions detected, three overlap dp-p region. The remaining 3 inversions and 2 deletions are located outside the dp-b-cn region.

In the present study, progenies of only a few + b cn TC1 male recombinants were found to carry chromosomal abnormalities; no such abnormality was detected in the progenies of many other + b cn recombinant males. Majority of the + b cn TC1 recombinants produced recombinant type flies in TC2 below 15% and in certain cases it was as low as 0.38%. The probability of recombinant-type larva being sampled in TC2 progeny was thus very low. The examination of salivary chromosomes of a very large number of TC2 larvae per TC1 recombinant is desirable to have a better understanding about the possible association of chromosomal aberrations with induction of male recombination.

Montague, J.R. Barry University, Miami Shores, Florida USNA. Body size, reproductive biology, and dispersal behavior among artificial baits in **Drosophila falleni.**

In a recent note (Montague 1984), I reported the spatial and temporal dispersions of mushrooms and mycophagous drosophilids in a Central New York woods during 1980. The observed dispersions suggested seasonal variation in the densities and species diversity of mushrooms, as well as seasonal fluctuations in drosophilid densities. I present here analyses of the morphometric variation and dispersal behavior in the most abundant species from that study, **Drosophila falleni.**

The field site was a mixed Beech-Maple woods adjacent to a swampy area in Fayetteville, NY (Figure 1). Mycophagous drosophilids were initially collected from rotted commercial mushroom baits, marked with micro-fluorescent dusts, and released during five census periods: May 20-23, June 26-28, July 8-11, August 7-8, and August 25-26. After each release, adults were continuously aspirated from the baits every morning (7AM-10AM) until no marked adults were found, or until rain interrupted the recapture.

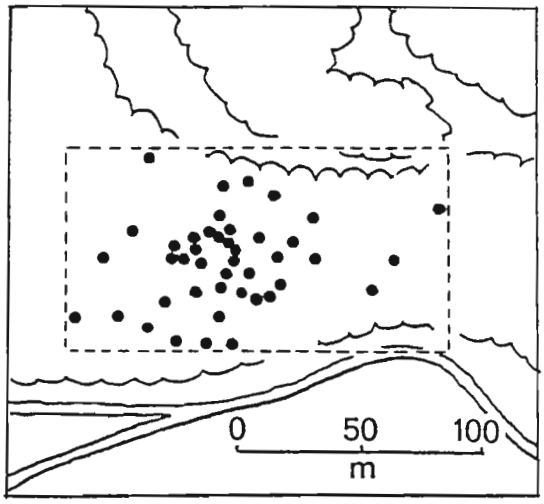


Figure 1. Field site in Fayetteville, NY. Circles are bait locations in 1980. Dashed lines enclose a 12,500 sq.m. area used in Fig. 2.

The mean dispersal distance per day was calculated as follows:

d_{ij} = distance flown by jth fly from release to ith bait,

$\bar{d}$ = mean dispersal distance per day,

$$= \left(\frac{\Sigma\ (d_{ij}/\#\text{days from release to capture of jth fly})}{(\text{total number of recaptured flies})} \right).$$

The mean dispersal distances per day for mycophagous drosophilids are shown in Table 1. These mean distances per day are less than the mean distance between baits (21 meters), and much less than mean distances between naturally occurring mushrooms (50-150 m: Montague 1984).

The distances travelled by marked **D.falleni** males and females during 1980 are summarized in Figure 2. Johnston & Heed (1976) noted that the choice of particular spatial patterns of collection baits strongly affects the measured dispersal rates during mark-recapture experiments. There should be a tendency for many flies to cluster among the closest baits, yet some flies may disperse great distances following the stresses of capture and marking; such behaviors would results in a leptokurtic (skewed) distribution

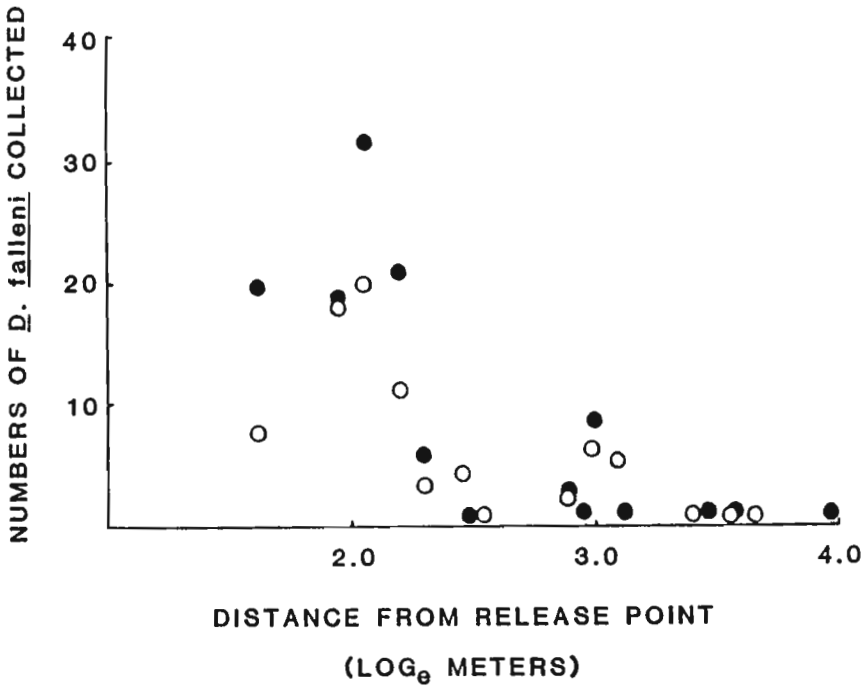


Table 1. Mean dispersal distances per day among artifical baits (all mycophagous species combined). The mean distance between baits is 21 m (Montague 1984).

Date	$\bar{d}$ (m)	S.D.	# released / # recaptured
5/20-23	12.13	10.59	200/57
6/26-28	15.10	13.10	420/65
7/ 8-11	16.54	18.43	270/13
8/ 7-8	9.54	3.71	175/13
8/25-26	16.43	11.39	70/7

Figure 2. Numbers of marked **D.falleni** adults (vertical axis) and dispersal distances (horizontal axis). Open circles are females; closed circles are males.

of dispersal distances (Dobzhansky & Powell 1974; Powell et al. 1976). The kurtosis values calculated for **D.falleni** females (3.33) and males (7.09) both represent significantly leptokurtic distributions (cf. Snedecor & Cochran 1967: p. 86-88).

The attractiveness of individual bait locations may vary during mark-recapture experiments. The total catch of mycophagous drosophilids during 1980 are summarized in Figure 3. Some bait locations that attracted large numbers of flies during May attracted proportionally lower numbers in June. This suggests either temporal variation in the spatial dispersions of the flies, or alternatively, temporal variation in adult preferences for particular bait locations.

The amount of morphometric variability within a drosophilid population can serve as an index of the degree of environmental selection (Roff 1977; Richmond 1978; Stalker 1980). Four collections of **D.falleni** females were examined in 1980: June 26-28, July 8-11, August 7-13, and August 26-27. Each female was examined with an ocular micrometer, then dissected to measure ovariole and egg development. The morphometric variables are summarized in Table 2. There were no significant differences among means for any of the variables during 1980. It is interesting to note, however, that the Coefficients of Variation (CV = S.D./$\bar{x}$) for the body size variables (e.g., thorax length, wing length, ovariole number) showed identical temporal patterns: an increase from late June through early August, then a decrease in late August. I showed (Montague 1984) that the early-summer population was expanding (correlated here with increased CV values for body size), but the August population was shrinking (correlated here with decreased CV values for body size). This seasonal pattern of variance in body size parameters suggests the presence of increased stabilizing selection during August, perhaps due to increased temperature-humidity stresses in the mid-summer.

On the other hand, the CV values for egg size (egg length and width) showed a different pattern: no change through June and July, but an increase in August. The increase in intra-specific variation in egg

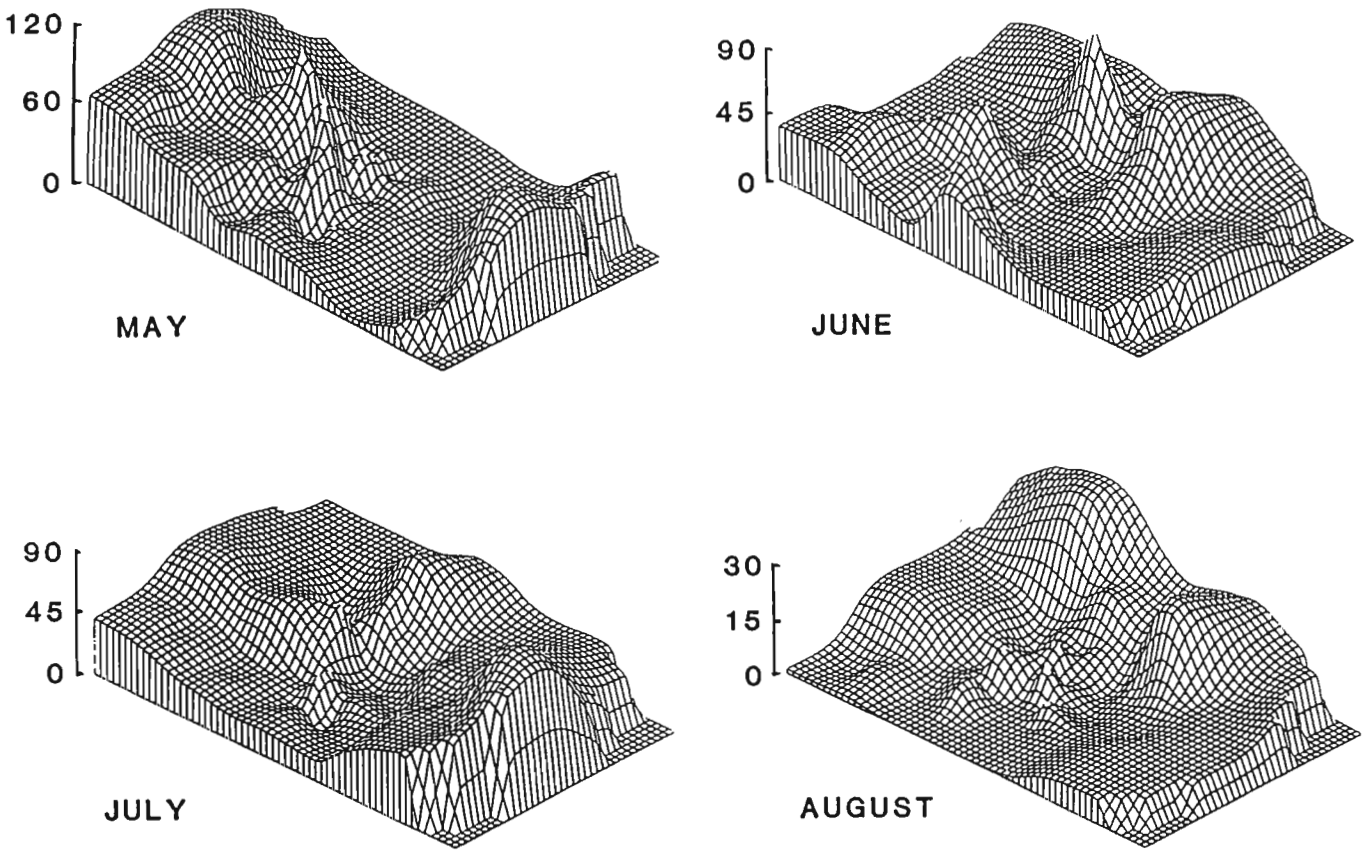


Figure 3. Monthly totals of mycophagous drosophilids collected within the 12,500 sq.m. rectanble in Fig. 1. The vertical axis (elevation) represents numbers of flies collected.

Table 2. Morphometric variables for **D.falleni** females during 1980.

Date	Thorax length (mm)	Wing length (mm)	Costal cell length (mm)	Cross vein length (mm)	CCL[a] CVL	Ovariole number	egg length (mm)	egg width (mm)
6/26-28:								
$\bar{x}$	1.15	2.90	0.46	0.28	1.65	26.38	0.54	0.19
S.D.	0.10	0.21	0.03	0.03	0.12	4.27	0.02	0.01
S.D./$\bar{x}$	0.087	0.072	0.065	0.107	0.073	0.162	0.037	0.053
n	29	29	29	29	29	8	8	8
7/8-11:								
$\bar{x}$	1.13	2.88	0.46	0.28	1.67	25.67	0.54	0.20
S.D.	0.11	0.24	0.04	0.04	0.14	5.45	0.02	0.00
S.D./$\bar{x}$	0.097	0.083	0.087	0.143	0.084	0.212	0.037	0.050
n	30	30	30	30	30	12	12	12
8/7-13:								
$\bar{x}$	1.11	2.82	0.45	0.25	1.79	25.00	0.54	0.19
S.D.	0.11	0.25	0.05	0.04	0.14	8.46	0.02	0.01
S.D./$\bar{x}$	0.099	0.089	0.111	0.160	0.078	0.338	0.037	0.053
n	23	23	23	23	23	4	4	4
8/26:								
$\bar{x}$	1.13	2.88	0.45	0.27	1.68	24.67	0.52	0.19
S.D.	0.08	0.16	0.03	0.03	0.13	5.20	0.03	0.02
S.D./$\bar{x}$	0.070	0.056	0.063	0.118	0.080	0.211	0.056	0.084
n	17	17	17	17	17	6	6	6

[a] ratio of costal cell length to cross vein length.

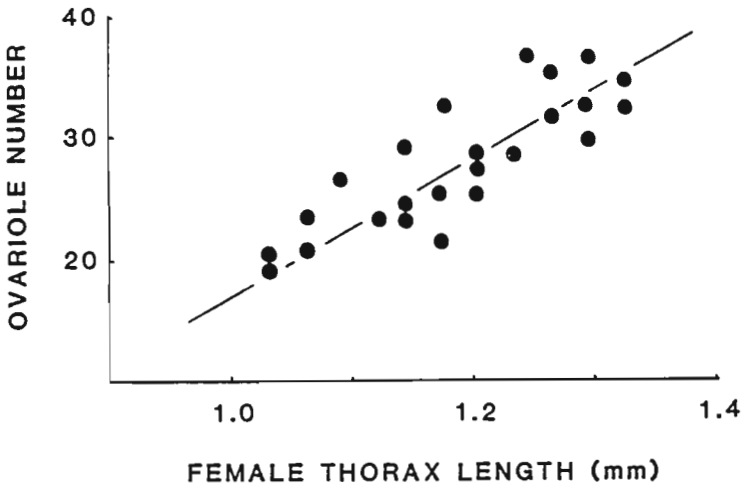


Figure 4. Female thorax length for **D.falleni** (horizontal axis) with ovariole number (vertical axis) for 24 adults (r^2 = 0.91).

size during August is difficult to assess in light of the decreased variation in body size parameters during the same period. Geisel et al (1982) reported similar ambiguities in correlations of bionomic variables for **D.melanogaster**, especially as regards environmental variation in temperature.

The relationship between **D.falleni** female size and fecundity is shown in Figure 4. Larger females carry more ovarioles, indicating higher potential fecundity. Roff (1977, 1981) suggested that within a drosophilid species, larger body sizes were adaptive in increasing flight musculatures and flying efficiencies, as well as increasing potential fecundity. However, there was no statistical correlation between female thorax length and dispersal distance for **D.falleni** in 1980. The farthest bait was only 90 m from the release point; perhaps this was to short a distance to accurately discriminate size and flight efficiency differences within the **D.falleni** population.

Five additional samples of **D.falleni** females were collected during 1980: May 29-June 15, June 25-27, July 8-11, August 7-13, and August 25-26. These were dissected to determine the reproductive condition during each time period, and the results are shown in Table 3. During late May and early June, most females carried mature eggs. Since **D.falleni** adults first appeared on May 15 (Montague 1984), the mature females in early June were probably those initial arrivals. In late June, however, most of the females in the population were sexually immature; these were probably the first pulse of offspring produced by the May population. The proportion of immature females remained high for the rest of the summer.

During 1979 and 1980, I maintained milk-bottle cultures of **D.falleni** at Syracuse University. The culture medium was a mixture of Instant Drosophila Medium (Carolina Biological Supply) and mashed commercial mushrooms. I observed an approximate 14 day pre-adult period (egg to adult emergence @ 20°C), and an approximate 10-14 day perido for sexual maturity (adult emergence to first mature egg @ 20°C). These values suggest an approximate 24-28 day egg-to-egg generation time for **D.falleni.**

It is possible that **D.falleni** females over-winter in the adult stage. Toda (1979) recorded 24 species of hibernating adult drosophilds collected from winter samples in Southern Japan, including eight species that over-wintered as sexually mature females (the most abundant species from Toda's study included **Scaptomyza elmoi, Sc.pallida, Drosophila immigrans, D.lutescens,** and **D.suzukii**).

Summary. The 1980 observations indicate: (1) the mean dispersal distances per day of mycophagous drosophilds among artificial baits was less than the mean distance between baits, and much less than the mean distance between naturally occurring mushrooms; (2) the distribution of dispersal distances for **D.falleni** among baits was strongly leptokurtic (skewed), i.e., many marked adults clustered among the closest baits, while some marked adults dispersed great distances following release; (3) there was temporal variation in the attractivities of individual bait locations; (4) variation in body size parameters was correlated with population density of **D.falleni**; (5) there was no correlation between **D.falleni** body size and dispersal distance (less than 100 m); and (6) **D.falleni** females from the early summer population were mostly mature, while females from late June through late August populations were mostly immature adults.

Acknowledgements: I thank Tom Starmer, Robert Lacy and John Jaenike for advise and suggestions. Ronald Kalinowski (Syracuse University Computer Center) provided guidance with the Surface-II Graphics Program shown in Figure 3. Deborah Montague completed the figures.

Table 3. Seasonal variation in reproductive condition for **D.falleni** The proportion of females for each stage of maturity are listed for each time interval.

Stage of reproductive maturity (females)	5/29-6/15	6/25-27	7/8-11	8/7-13	8/25
Immature ovarioles	0.12	0.63	0.57	0.70	0.59
Mature ovarioles, immature eggs	0.12	0.07	0.03	0.13	0.06
Mature ovarioles, mature eggs	0.76	0.30	0.40	0.17	0.35
	n=26	n=30	n=30	n-23	n=17

References: Dobzhansky, T. & J. Powell 1974, Proc. Royal Soc. London Biol. Sci. 187: 281-198; Geisel, J., P. Murphy & M. Manlove 1982, Am. Nat. 119: 464-479; Johnston, J. & W. Heed 1976, Am. Nat. 110: 629-651; Montague, J. 1984, DIS 60: 149-152; Powell, J., T. Dobzhansky, J. Hook & H. Wistrand 1976, Genetics 82: 483-506; Richmond, R. 1978, Ecological Genetics (P. Brussard, ed.) Springer-Verlag, N.Y.: 127-144; Roff, D. 1977, J. Anim. Ecol. 46: 443-456; Roff, D. 1981, Am. Nat. 118: 405-422; Snedecor, G. & W. Cochran 1967, Statistical Methods, Iowa State Univ. Press, Ames, IA; Stalker, H. 1980, Genetics 95: 211-223; Toda, M. 1979, Low Temp. Sci., Ser. B 37: 39-45.

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Response of dysgenic and non-dysgenic populations to malathion exposure.

The rapid-invasion hypothesis explains the presence of P-elements in recently sampled **D.melanogaster** strains and their absence in older laboratory strains by proposing that transposable elements of the P-family have recently invaded **D.melanogaster** populations and rapidly increased in frequency (Kidwell 1983). Although positive selection for individuals containing P-elements is not necessary to explain their rapid increase in frequency (Hickey 1982), it is possible that they create an advantage to their host in a manner similar to that caused by selectable genes on a bacterial plasmid.

In particular, Bregliano & Kidwell (1983) suggested that transposable elements may have been involved in the recent increase in the insecticide resistance of Drosophila and other insects. We tested this idea by exposing dysgenic and non-dysgenic laboratory populations of **D.melanogaster** to malathion, a commonly used organophosphorus insecticide. A lab population synthesized from recently (1976) caught flies developed polygenic resistance to malathion when selected under similar conditions (Singh & Morton 1981). The two populations for the present experiment were maintained according to a scheme of reciprocal matings suggested by D. Hickey in which the genetic backgrounds would be similar, except that dysgenesis was induced in one case but not in the other (Figure 1). The P-strain (Harwich) and the M-strain (Canton S) were provided by M. Kidwell, and the experimental populations (in 2 replicates) were grown in 8 oz bottles on a banana food at 24°C (12 hr day - 12 hr night).

The malathion LC_{50} (adults, 24 hr feeding; see Holwerda & Morton 1983) of the Harwich strain (9.7±1.5 μM) was somewhat greater than that of the Canton S strain (6.0±0.6 μM). The LC_{50} of both experimental populations increased during the first 3 generations of equilibration without malathion exposure to values greater than either parental strain (Figure 2). Selection for malathion resistance was begun (generation 0, Figure 2) by splitting the populations in half and including 1 μM malathion in the food of the "selected" replicates. The malathion concentration was increased in 1 μM steps (Figure 2, top) as resistance developed, until by the 8th generation the dysgenic and non-dysgenic populations had diverged, and only the concentration for the non-dysgenic population could be increased. No progeny were obtained at 4 μM from the dysgenic, selected population. The experiment was continued for 2 more generations at different malathion concentrations, then terminated as it was obvious that resistance was increasing more rapidly in the non-dysgenic, selected population. At the 9th generation, samples of flies from each of the

Non-dysgenic population

M♂♂ F1 ♀♀ F2 ♂♂
x → x → x → etc.
P♀♀ M♂♂ P♀♀

Dysgenic population

P♂♂ F1 ♂♂ F2 ♂♂
x → x → x → etc.
M♀♀ P♀♀ M♀♀

Figure 1. Crosses used to produce a population in which P-element induced transpositions were active every other generation (dysgenic, bottom) and another with the same genetic background in which they were not induced (non-dysgenic, top).

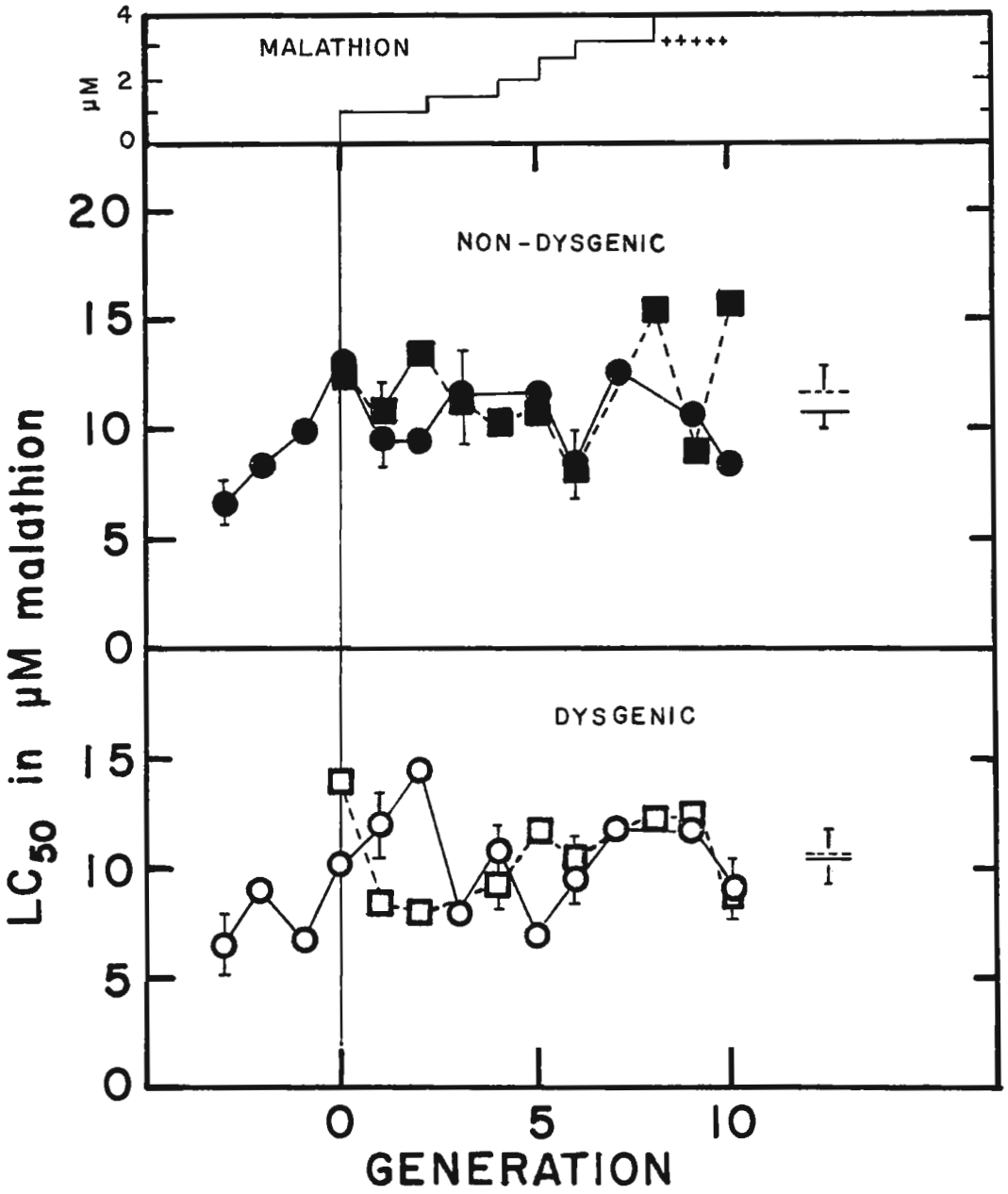

Figure 2. Adult malathion resistance of non-dysgenic (solid) and dysgenic (open) populations. Each generation consisted of one in the series of crosses given in Figure 1. Selection was begun at generation 0, and the initial malathion concentration is indicated at the top. At generation 8 the two populations diverged; +++ indicates that the concentration remained at 3 μM in the dysgenic population. The resistance of the selected part of each population is indicated by the squares and dotted lines, the unexposed part by the circles and solid lines. The lines and standard error bars at the right side of the figure indicate the average LC_{50} of each population over the course of the experiment.

Table 1. Malathion resistance of populations after 9 generations.

Population	Tested gener.[a]	Egg-to-pupa-survival (μM) 0	1	2	3	Adult LC_{50} (μM)[b]
Non-dysgenic Control	2(1C)	0.88	0.44	0.01	<0.002	10.8 (±1.0)
Non-dysgenic Selected	1(0)	0.17	0.07	0.33	0.21	Not Done
	2(1C)	0.86	0.46	0.36	0.19	11.5 (±0.8)
	2(1M)	0.63	0.64	0.48	0.27	>25
Dysgenic Control	2(1C)	0.91	0.20	<0.01	<0.002	14.1 (±0.8)
Dysgenic Selected	1(0)	0.51	0.61	0.38	0.02	Not Done
	2(1C)	0.73	0.62	0.32	0.08	12.9 (±1.0)
	2(1M)	-----Not Done -----				14.1 (±1.0)

a=After 9 generations (see Fig. 1) flies were allowed to mate among themselves and progeny tested after the number of generations indicated. Thus (0)=selected flies which were directly exposed to insecticide (4 μM for non-dysgenic selected, 3 μM for dysgenic selected) laid eggs onto banana food; (1C)=second generation tested after growth for one generation on banana food; (1M)=2nd generation tested from survivors after growth for one generation on banana food containing 3 μM malathion.
b=Standard error is bracketed.

4 populations and 2 replicates were removed from selection and allowed to mate among themselves for 2 additional generations (grown on either malathion-containing or normal banana food). Eggs and adults were tested for malathion resistance (Table 1).

The following points can be made from this data: (1) the selection populations were polymorphic for resistance as expected since they were being mated each generation to susceptible, unexposed flies. (2) Egg-to-pupa survival increased more rapidly than adult survival (changes in the latter were not significant). (3) Eggs laid by selected flies which had been reared on malathion-containing food survived poorly, even though they were not subsequently exposed to malathion. Egg viability was lower for the non-dysgenic, selected population (17%) than for the dysgenic, selected population (51%), perhaps because the former had been exposed to the greater malathion concentration (4 μM vs 3 μM). Paradoxically, egg survival from non-dysgenic flies was better on 3 or 4 μM malathion than on 0 or 1 μM. (4) After growth on normal food for one generation, egg survival (3 μM) for the non-dysgenic population was greater than that of the dysgenic. (5) LC_{50} values for adults two generations removed from selection were not significantly different (selected vs non-selected); however, if a generation of selection intervened, the non-dysgenic, selected flies were more resistant. These results indicate that the non-dysgenic population responded more rapidly to malathion selection than the dysgenic. Why this should be so is not clear, but the outcome was inconsistent with the hypothesis that insecticide resistance will increase more rapidly for a population in which P-element transposition is active.

References: Kidwell, M.G. 1983, Proc. Nat. Acad. Sci. USA 80:1655-1659; Hickey, D.A. 1982, Genetics 101:519-531; Bregliano, J-C. & M.G. Kidwell 1983, in:Mobile Genetic Elements (Shapiro, ed.), Academic Press, New York, p363-410; Singh, R.S. & R.A. Morton 1981, Can. J. Genet. Cytol. 23:355-368; Holwerda, B.C. & R.A. Morton 1983, Pest. Biochem. Physiol. 20:151-160.

Mrcarica, E. University of Nis, Yugloslavia.
The influence of parental age to sex ratio on their **Drosophila melanogaster** progenies.

In experimental populations of **D.melanogaster** the influence of parental age to the secondary sex ratio of their progenies has been studied.

In all experiments the males have been individually crossed to a number of virgin females. After 48 hr they were removed and each female was separated to a 20 cc vial where progenies were grown under noncompetitive conditions (13% yeast medium plus agar and sugar, 25°C). Four types of crosses have been made (Table 1). At the beginning of the experiment, young parents were 1-3 days and old ones 21-23 days of age. There were ten replications, but only those where males have inseminated four or more females (4-8) were taken in account. The sex ratios among the progenies of individual females were quite variable. This implies that both sexes are contributing equally to the sex ratio of their progenies.

The influence of parental age has been studied in all crosses (n=142) which have been divided into three groups: (1) Young males were crossed to young (A) and afterwards to old females (B). (2) Young males were crossed to young females (A), and aged together with other females, to be crossed (when more than 20 days old) to young virgin females (C). (3) A separate group of males were aged (together with females) and crossed (when 21-23 days old) to separately aged virgin females (D).

Table 1. Parent age and sex ratio of the progenies.

No. of crosses	Parents	Progenies N	%males	X^2	p
A 54	Young males vs. young females	2171	53.80	12.54	< .01
B 26	Young males vs. old females	1284	47.43	3.39	> .05
C 22	Old males vs. young females	2261	47.50	5.65	< .05
D 40	Old males vs. old females	2945	47.40	7.95	< .01

Table 1 shows that there is a significant excess of male progenies (53.8 ± 1.1%) from the crosses of young males and young females (X^2=12.54; p < 0.01). Among the crosses where males or females, or both, were aged, an excess of female progenies has been found (47.4 ± 0.6%).

The results obtained are showing that parental aging is significantly influencing the sex ratio of their progenies, leading to a significant decrease of male progenies.

Acknowledgement: The help of Prof. Dr. Dragoslav Marinkovic is kindly acknowledged.

References: Szilard,L. 1959, Proc. U.S. Nat. Acad. Sci. 45:30-45.

Nájera, C. University of Valencia, Spain.

Proportion of **D.melanogaster-D.simulans** in natural populations.

Flies from the sibling species **D.melanogaster** and **D.simulans** were captured in three different niches: a cellar in Requena (Valencia), a vineyard at 4 Kms from the cellar, and a pine-wood in La Canada (Valencia) at 70 Kms from the former two, at two different times of the year: sping and autumn.

Males were identified by their genitalia (Sturtevant 1919) while females were identified by the genital differences of their male progeny.

In the collections made inside the cellar at the two times of the year, not one individual of **D.simulans**, neither male nor female, was found. The population was exclusively of **D.melanogaster**. So the cellar provides this last species a system largely free of interactions with its sibling species.

The number of collected flies was 350 females (61.18%) and 187 males (34.82%) in the autumn population and 89 females (54.60%) and 74 males (45.40%) in the spring one. There is therefore a higher number of individuals in autumn (time of the vintage) although the cellar had a more suitable temperature in spring.

As regards the sex ratio, it is always less than one, indicating an excess of females. This excess was particularly marked in autumn (X^2=48.80; P<0.01), since in spring the sex ratio did not differ significantly from unity (X^2=1.2 ns).

In the vineyard and pine-wood populations there is a higher proportion of **D.simulans** than **D.melanogaster**, principally in autumn.

Table 1 shows the number of males and females collected from each of the two sibling species in the four populations as well as the percentage for each sex and the total percentage of each species. The frequencies of inseminated fertile females is also indicated, since it is an important component of the population structure. The percentage of inseminated females of **D.melanogaster** was higher (90.46%) than that of **D.simulans** (78.65%).

With regard to the sex ratio there are significant differences in the autumn vineyard population (X^2=87.34; P<0.01), autumn pine-wood (X^2=8.42; P<0.01), spring vineyard (X^2=3.92; P<0.05), and spring pine-wood (X^2=7.38; P<0.01) for **D.simulans**. For **D.melanogaster**, there are significant differences in the autumn vineyard population (X^2=44.8; P<0.01) and spring vineyard (X^2=6.18; P<0.05), while the differences are not significant in the spring and autumn pine-wood populations (X^2=0.72, X^2=0.06).

Table 1. Number of collected males and females; percentage of inseminated fertile females; total percentage of each species.

	D.SIMULANS						**D.MELANOGASTER**					
	Males		Females		insem.	% **simu-**	Males		Females		insem.	**melano.**
	No.	%	No.	%	% fem.	**lans**	No.	%	No.	%	% fem.	%
Autumn Vineyard	974	61.80	602	38.20	78.05	88.24	154	73.33	56	26.67	91.07	11.76
Autumn Pine-Wood	391	55.54	313	44.46	81.02	86.27	61	54.46	51	45.54	88.23	13.73
Spring Vineyard	61	41.50	86	58.50	79.27	53.65	49	38.58	78	61.42	89.74	46.35
Spirng Pine-Wood	191	43.41	249	56.59	76.27	75.86	68	48.57	72	51.43	93.06	24.14

In both species there is a disequilibrium in favour of males in autumn, both in the vineyard and in the pine-wood, while in spring the disequilibrium is in the opposite sense; differences are more acute in **D.simulans.**

Reference: Sturtevant, A.H. 1919, Psyche 26:153-155.

Nájera, C. University of Valencia, Spain. Study of eye colour mutant variability in natural populations of **D.melanogaster**. II. Vineyard.

51 and 70 females from two collections captured in a vineyard 4 Kms from the cellar of the preceding work (Ná jera & Mensua 1985, this issue, I. Cellar), at the same times of the year (autumn and spring), were analyzed. The purpose was the same: to search for eye colour mutations by inbreeding through F_1 pair matings from the females collected.

The number of females which were heterozygotic for eye colour mutations was 13 (25.49%) in autumn and 23 (32.85%) in spring. The number of mutations per fly was 0.25 and 0.39. These percentages seem to differ, being higher in spring than in autumn. When compared by means of a t test, no significant differences either with regaard to the heterozygotic females (t = -0.88 ns) or the number of mutations (t = -1.53 ns) can be observed. The distribution of mutations was:

	Autumn	Spring
females with 1 mutation	13	19
females with 2 mutations	0	4

Both fit a Poisson distribution (X^2=0.194 ns, and X^2=0.024 ns).

The percentage of heterozygotic loci for eye colour mutants was 9.8 (autumn population) and 16.07 (spring population).

The overall frequency of allelism was 7.1±4.9 (2/28) for the autumn population, 16.3±2.6 (30/184) for the spring population, and 11.5±2.6 (18/156) interpopulational. Alleles are distributed at random in both populations.

Compared with the cellar populations, the percentage of mutations in heterozygosis was rather smaller in the vineyard. As in the cellar, the frequency of allelism was greater in spring than in autumn, the interpopulational frequency being intermediate.

Nájera,C. and M.C. González-Bosch. University of Valencia, Spain. The maintenance of variability in artificial populations. III. Frequency of ADH alleles.

In a previous work the behaviour of four eye colour **D.melanogaster** mutants from a cellar was studied, compared with their wild allele from the same cellar, in artificial populations, and comparing two culture mediums, one supplemented with alcohol at 10% and the other without alcohol. The four mutants (sepia, safranin, cardinal and a multichromosomal strain which segregated cardinal and cinnabar mutants) attained different gene frequencies at equilibrium: 0.32, 0.27, 0.15 and 0.08 approximately (Najera & Mensua 1983).

In the eight populations there was a higher frequency of heterozygotes than could be expected (Najera 1984), which cannot be explained by the maintenance of inversions in heterozygosis (Najera & de Frutos 1984).

A study of the ADH frequencies was made in the artificial populations as well as in the five strains which gave rise to these populations. Table 1 shows the frequencies for the five strains. It can be observed that all the strains are homozygous: the wild strain and three of the four mutants (sepia, safranin and cardinal) for the F allele and the multichromosomal for the S.

As regards the populations, all the strains maintained in the standard culture medium appeared with polymorphism while the strains maintained in 10% ethanol appeared homozygous for the F allele (Table 2).

Although the initial allelic constitutions of the strains which give origin to the populations is not known, it seems probably that the strains were initially polymorphic, at least the wild strain which is the origin of all the populations, and that in the laboratory they changed to monomorphic through the loss of one of the two alleles.

It is noticeable that in all the populations maintained in the standard culture medium there is polymorphism, while in all the populations maintained with ethanol the F allele has been fixed.

It seems probable that in the populations supplemented with ethanol medium there is a directional selection against the S allele.

Table 1. Frequencies of ADH alleles in strains.

Strains	No.indiv. analyzed	% FF	% FS	% SS
wild	84	100	--	--
sepia	96	100	--	--
safranin	150	100	--	--
cardinal	95	100	--	--
multichromosomal	150	--	--	100

Table 2. Frequencies of ADH alleles in populations.

Populations	No.indiv. analyzed	% FF	% FS	% SS
wild/sepia w/alcoh.	106	100	--	--
wild/sepia w/o alcoh.	87	45	40	15
wild/safr. w/alcoh.	86	100	--	--
wild/safr. w/o alcoh.	80	40	47	13
wild/card. w/alcoh.	85	100	--	--
wild/card. w/o alcoh.	78	32	44	24
wild/multichr. w/alcoh.	141	100	--	--
wild/multichr. w/o alcoh.	96	19	43	38

The four polymorphic populations are in Hardy-Weinberg equilibrium and there is no excess of either homozygotes or heterozygotes in any of them.

In the multichromosomal strain where the In(2L)t was fixed (Najera & de Frutos 1984), the S allele for the ADH is also fixed, therefore there seems to be a linkage disequilibrium between them.

References: Najera, C. & J.L. Mensua 1983, DIS 59:94-95; Najera, C. 1984, DIS 60:154-156; Najera, Ca. & R. deFrutos 1984, DIS 60:156-157.

Nájera, C. and J.L. Ménsua. University of Valencia, Spain. Study of eye colour mutant variability in natural populations of **D.melanogaster**. I. Cellar.

Two samples of **D.melanogaster** were captured at two different times: autumn and spring. The place was a wine cellar in Requena (Valencia). 68 and 80 females, respectively, were analyzed from each collection for the purpose of searching for eye colour mutants. The F_2 of eight pairs from the F_1 generation of each wild female was analyzed.

The number of heterozygotic females for eye colour mutations was 36 (in autumn) and 42 (in spring); so 52.94% and 52.90% of the female populations were carriers of one eye colour mutation in heterozygosis.

The number of total mutations was 42 (in autumn) and 52 (in spring), that is to say 0.61 and 0.65 mutations per fly. Adding the results of both captures, 52.70% of the females were heterozygotic and there were 0.63 mutations per fly in the cellar.

The distribution of mutations inside the populations was as follows:

	Autumn	Spring
females with 1 mutation	28	35
females with 2 mutations	7	4
females with 3 mutations	0	3

both fit a Poisson distribution (X^2=1.935 ns; X^2=4.117 ns) although there is a non-significant lack of individuals without mutations.

Table 1. Frequencies of intra- and interpopulational alleles. No. of alleles in each population and total types of mutations.

	Autumn	Spring	Aut.-Spr.
Analyzed mutations	42	52	
No. of crosses completed	409	497	902
No. of allelic crosses	29	66	90
Freq. of allelic crosses	[7.6±1.4%]	[13.3±1.4%]	[10.0±1.0%]
1 allele	22	12	
2 alleles	5	6	
3 alleles	1	3	
4 alleles	-	1	
7 alleles	1	-	
10 alleles	-	1	
Types of mutations	29	28	

Considering that the loci number reported for eye colour mutations at the moment is about 112, the percentage of heterozygotic loci for eye colour mutants in these populations will be 25.89 (for the autumn population and 25.00 (for the spring population).

Intra e interpopulational allelism tests were carried out. Table 1 shows the results.

The distribution of alleles in both populations was random (X^2=1.800 ns; X^2=4.075 ns) although the dispersion coefficients were too high and there was a tendency to find an excess of lack of alleles on one hand and excessively high number of alleles on the other.

Ostrega, M.S. Roosevelt University, Chicago, Illinois USNA. Restriction endonuclease analysis of the relatedness of **D.montana** and **D.virilis** lines.

Mitochondrial DNA (mtDNA) from representative lines of geographically diverse populations of **D.montana** and **D.virilis** (Table 1) was analyzed with ten Class II restriction endonucleases (Bam HI, Bgl II, Cla I, Eco RI, Hae III, Hind III, Kpn I, Pst I, Sal I, and Taq I). With the exception of Bam HI and Cla I, the three **D.montana** lines showed identical mtDNA restriction fragment patterns. Bam HI sites occurred once in Kawasaki mtDNA and once in Moosonee mtDNA but not in Gothic A mtDNA, and there were three sites for Cla I in the mtDNA of Gothic A and Moosonee but four sites in Kawasaki mtDNA. This extra site in Kawasaki mtDNA probably evolved in the second restriction fragment because the summed molecular weight of the second and third fragments of Kawasaki mtDNA is approximately equal to the second fragment of Gothic A and Moosonee mtDNAs (the largest and smallest restriction fragments are nearly the same size in all three lines). Both **D.virilis** lines showed identical mtDNA digestion profiles with all but one of the ten enzymes used. Hind III sites occurred at four positions in the Carolina line but only three in Texmelucan. Three enzymes (Kpn I, Pst I, and Sal I) produced patterns indistinguishable in each of the five lines: there were no sites for Kpn I or Sal I and only one site for Pst I in the mtDNA of each of the five lines.

The estimated mean molecular weight of **D.montana** mtDNA was $10.19 \pm 0.03 \times 10^6$ daltons (N=20), while that for the Carolina line of **D.virilis** was $10.24 \pm 0.07 \times 10^6$ daltons (N=7). Neither differs significantly from earlier estimates for both species by Fauron & Wolstenholme (1976) and Shah & Langley (1977, 1979). The Eco RI, Hae III, and Hind III fragment patterns from the Carolina line correspond to the patterns reported by Shah & Langley (1979). There is evidence that Texmelucan mtDNA ($10.43 \pm 0.04 \times 10^6$ daltons; N=7) is larger than the Carolina mtDNA ($t_s = 2.357$; $P = 0.036$) as well as the previously reported **D.virilis** mtDNA estimates (Table 2).

Table 3 shows estimates of intra- and interspecific variation obtained from analysis of restriction fragment patterns by the method of Ewens et al. (1981). In estimating genetic variability using a pairwise comparison of homologous DNA sequences, i.e., n=2, the estimate of heterozygosity, Θ, has the same meaning as nucleotide diversity (Engels 1981). The estimated number of base substitutions per nucleotide site equalled zero when the fragment patterns of two lines compared were identical. Only cleavage of mtDNA from **D.montana** lines with Bam HI and Cla I and mtDNA from both **D.virilis** lines with Hind III produced patterns which gave values for Θ other than zero when compared intraspecifically. In interspecific comparisons, cleavage with Kpn I, Pst I, and Sal I produced identical fragment patterns in each of the five lines making Θ equal zero. Cleavage with Taq I produced patterns with many low molecular weight bands which could not be further resolved thus making any interspecific comparison unreliable. Cleavage with Bam HI, Bgl II, Cla I, Eco RI, Hae III, and Hind III produced patterns which better elucidated the interspecific relatedness of these organisms. Except for Hind III, similar values of Θ were obtained with each enzyme whether the pattern of each **D.montana** line was compared to the Carolina or Texmelucan line of **D.virilis.**

Composite estimates of intra- and interspecific variation were also calculated utilizing data obtained from the battery of restriction enzymes used in this assay (Table 4). Intraspecific comparison of **D.montana** lines revealed the largest number of base substitions to be between Gothic A and Kawasaki lines, the least between Kawasaki and Moosonee lines, and an intermediate value between Gothic A and Moosonee lines. This suggests a central evolutionary role for the Moosonee line but the large standard deviation associated with each Θ precludes any definite conclusions. Intraspecific comparison of the **D.virilis** lines revealed Θ to be slightly higher than that of the Kawasaki/Moosonee comparison. Interspecific comparisons resulted in a seven- to ten-fold increase of Θ over that of intraspecific comparisons. Average values of Θ from comparisons of **D.montana** lines to the Carolina line (0.721 ± 0.0020; N=3) and the Texmelucan line (0.0890 ± 0.0017; N=3) were significantly different ($t_s = 10.879$; $P < 0.001$).

Table 1. Geographic origin of lines surveyed.

Species	Line	Origin
D.montana	Gothic A	Colorado, USA
	Kawasaki	Japan
	Moosonee	Ontario, Canada
D.virilis	Carolina	Unknown*
	Texmelucan	Mexico

* Obtained from Carolina Biological Supply Company, North Carolina, USA.

Table 2. Texmelucan t-Tests.

Comparison	t_s	Critical t-value (P = 0.05)
Texmelucan/F & W*	5.024	2.213
Texmelucan/S & L**	4.480 (a)	2.306
Texmelucan/S & L**	2.060 (b)	2.040

* Fauron & Wolstenholme 1976, Measurements of mtDNA by electron microscopy.

**Shah & Langley 1977, Measurements of mtDNA by (a) agarose gel electrophoresis and (b) electron microscopy.

Table 3. Intra- and interspecific estimates of variation.

I. Intraspecific

Species	Enzyme	Comparison	θ
D.montana	Bam HI	Cothic A/Moosonee	0.120
		Gothic A/Kawasaki	0.120
		Kawasaki/Moosonee	0
	Cla I	Gothic A/Moosonee	0.030
		Gothic A/Kawasaki	0.030
		Kawasaki/Moosonee	0
D.virilis	Hind III	Carolina/Texmelucan	0.030

II. Interspecific

Enzyme	Comparison	θ
Bam HI	Gothic A/Carolina or Texmelucan	0.120
	Kawasaki/Carolina or Texmelucan	0
	Moosonee/Carolina or Texmelucan	0
Bgl II	Gothic A/Carolina or Texmelucan	0.120
	Kawasaki/Carolina or Texmelucan	0.120
	Moosonee/Carolina or Texmelucan	0.120
Cla I	Gothic A/Carolina or Texmelucan	0.040
	Kawasaki/Carolina or Texmelucan	0.060
	Moosonee/Carolina or Texmelucan	0.040
Eco RI	Gothic A/Carolina or Texmelucan	0.060
	Kawasaki/Carolina or Texmelucan	0.060
	Moosonee/Carolina or Texmelucan	0.060
Hae III	Gothic A/Carolina or Texmelucan	0.180
	Kawasaki/Carolina or Texmelucan	0.180
	Moosonee/Carolina or Texmelucan	0.180
Hind III	Gothic A/Carolina	0.048
	Kawasaki/Carolina	0.048
	Moosonee/Carolina	0.048
	Gothic A/Texmelucan	0.120
	Kawasaki/Texmelucan	0.120
	Moosonee/Texmelucan	0.120

Table 4. Composite estimates of intra- and interspecific variation.

Species	Comparison	θ ± SEM*
I. Intraspecific		
D.montana	Gothic A/Moosonee	0.0106 ± 0.0053
	Gothic A/Kawasaki	0.0152 ± 0.0062
	Kawasaki/Moosonee	0.0053 ± 0.0037
D.virilis	Carolina/Texmelucan	0.0082 ± 0.0058
II. Interspecific		
	Gothic A/Carolina	0.0759 ± 0.0134
	Kawasaki/Carolina	0.0712 ± 0.0130
	Moosonee/Carolina	0.0692 ± 0.0131
	Gothic A/Texmelucan	0.0924 ± 0.0143
	Kawasaki/Texmelucan	0.0880 ± 0.0139
	Moosonee/Texmelucan	0.0867 ± 0.0141

* Standard error of the mean; N = 9.

The rate of base substitution in mammalian mtDNA has been estimated to be 1% of the mitochondrial genome per 10^6 years (Brown et al. 1979). If this estimate can be applied to Drosophila mtDNA evolution, the time of divergence of **D.montana** and **D.virilis** can be estimated as occurring approximately 7-9 x 10^6 years ago. This estimate agrees well with Throckmorton's (1982) estimate of divergence time of these two species as occurring somewhere between 5 to 33 x 10^6 years ago.

Because the amount of genetic variation among the **D.montana** lines is small and does not include intrapopulational variation, this work sheds little light on the historical relationships of **D.montana** populations. However, detailed analysis of Cla I fragment patterns hints at a central evolutionary role for the Moosonee population. Analysis of the two **D.virilis** lines has shown the Texmelucan mtDNA to be larger in size than the mtDNA of the Carolina line and two other strains of this same species. Further analysis of more lines of both species using a more diverse battery of restriction enzymes will better elucidate the relationships of the organisms of this group.

References: Brown, W.M., M. George Jr & A.C. Wilson 1979, Proc. Natl. Acad. Sci. USA 76:1967-1971; Engels, W.R. 1981, Proc. Nat. Acad. Sci. USA 78:6329-6333; Ewens, W.J., R.S. Spielman & H. Harris 1981, Proc. Natl. Acad. Sci. USA 78:3748-3750; Fauron, C.M.-R. & D.R. Wolstenholme 1976, Proc. Natl. Acad. Sci. USA 73:3623-3627; Shah, D.M. & C.H. Langley 1977, Nucl. Acids Res. 4:2949-2960; _____ 1979, Nature 281:696-699; Throckmorton, L.H. 1982, in: The Genetics and Biology of Drosophila, v.3b (Ashburner et al., eds.), Academic Press, London.

Pascual, L. and R. de Frutos. University of Valencia, Spain. "In vitro puffs" after heat shocks at two different temperatures in **Drosophila subobscura.**

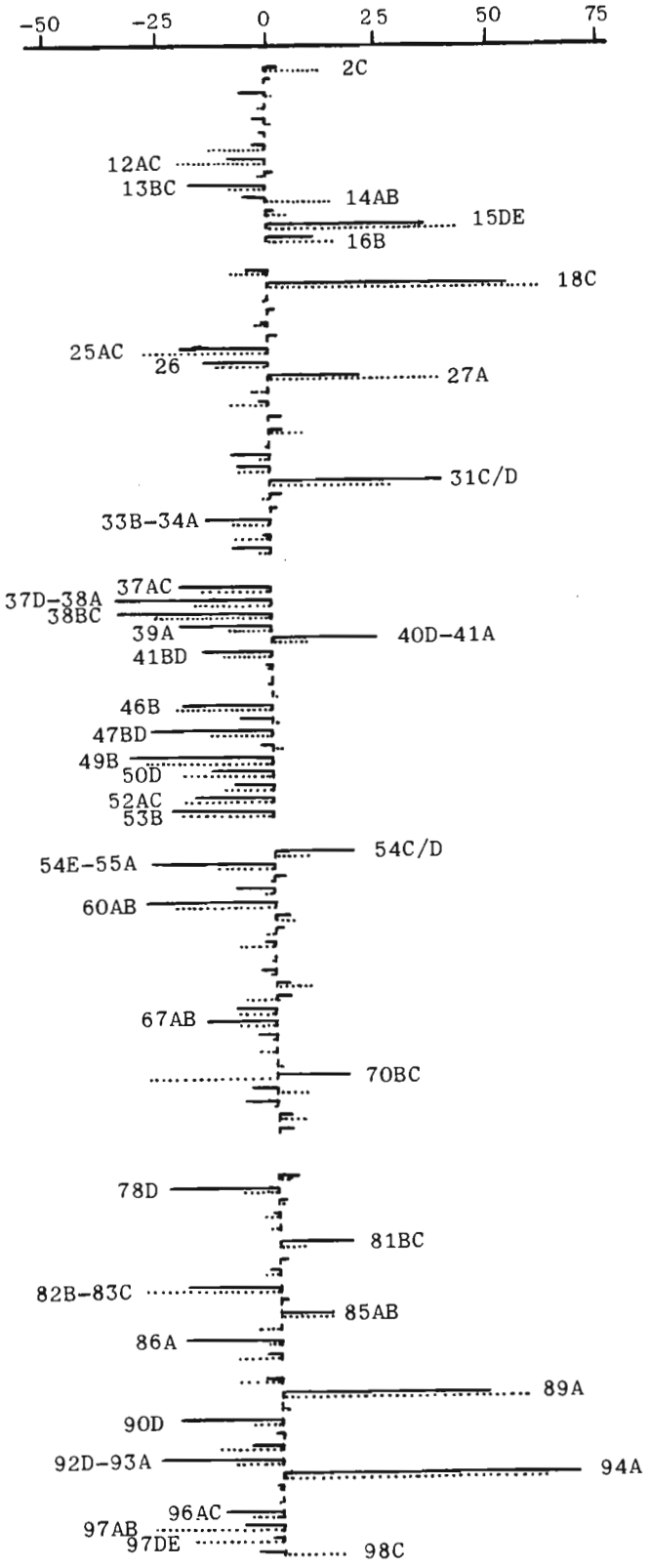

Figure 1. Increase or decrease of the genetic activity (puffing frequency in treated minus puffing frequency in control glands) after heat shock either at 31°C (continuous line) or 37°C (dotted line) referred to the control.

Heat shock has been used as a strong genetic activity inducer practically in every organism for some years (Ashburner et al. 1979; Schlesinger et al. 1982; Neidhardt et al. 1984).

When **D.subobscura** larvae, cultured at 19°C, are subjected to heat shock, the puffing pattern obtained depends, to a great extent, on the stringency of the treatment (Pascual et al., in prep.). "In vitro" heat shocks allow us to subject each gland from the same larva to different temperatures and so we can observe how the same "genetic material" responds to different treatments.

Figure 1 shows the changes (increase or decrease) of the genetic activity (frequency of puffing activity) when salivary glands are subjected to 31° or 37°C maintaining the contralateral ones at 19°C (20 min in Drosophila Ringer solution). A total of 15 loci increase their activity after heat shock: 2C, 14AB, 15DE, 16B, 18C, 27A, 31C/D, 40D-41A, 54C/D, 70BC, 81BC, 85AB, 89A, 94A and 98C. The remaining loci show either similar or less genetic activity than the control after treatment. This, in general, supports the results obtained after "in vivo" treatments (Pascual et al. 1983; Pascual et al., in prep.) although the loci 81BC and 70BC (only after heat shock at 31°C) increase their activity only after "in vitro" treatments.

There is a different response for the two heat shock temperatures. Some puffs are preferably active either after heat shock at 31°C (40D-41A, 54C/D, 70BC, 81BC) or 37°C (2C, 14AB, 27A, 98C).

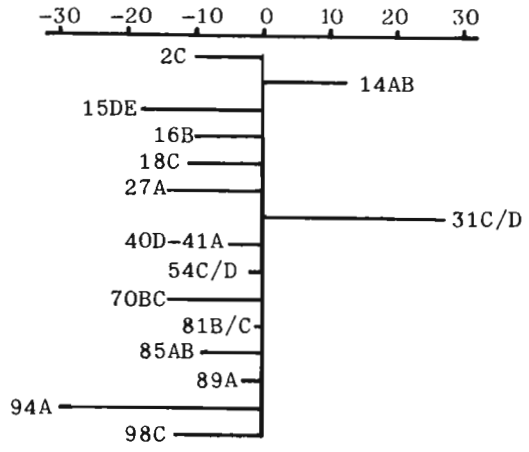

Figure 2. Increase or decrease of the genetic activity after heat shock at 37°C referred to that after heat shock at 31°C.

Moreover, there is a stronger decrease in the genetic activity of the non-induced puffs after shock at 31°C.

To observe the effect of both treatments on the same "genetic material", we can shock sister glands at 31° and 37°C, respectively. In Figure 2 we can see the result of this treatment on the 15 loci previously cited. Only loci 14AB and 31C/D are more active after heat shock at 37°C. The remaining loci show less activity in glands subjected to 37°C treatment.

References: Ashburner, M. & J.J. Bonner 1979, Cell 17:241-54; Pascual, L. & R. de Frutos 1984, DIS 60:158-59; Neidhardt, F.C., R.A. VanBogelen & V. Vaughn 1984, Ann. Rev. Genet. 18:295-329; Schlesinger, M.J., M. Ashburner & A. Tissieres 1982, Heat Shock from Bacteria to Man, Cold Spring Harbor, NY, 440 pp.

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Is abdomen pigmentation polymorphism in **D.erecta** selectively neutral?

Abdomen pigmentation polymorphism has been reported in many Drosophila species. In **D.erecta** there are only two morphs, light and dark. The fitnesses of the different genotypes at the abdomen pigmentation locus was investigated to test whether selection or neutrality is likely to be responsible for the maintenance of this polymorphism.

Material and Methods: Results of crossing in **D.erecta** were consistent with diallelism at the abdomen pigmentation locus, X linkage, dark dominant to light (which confirms results obtained by Ashburner & David, unpubl.). Gene expression is limited to females and is not influenced by temperature. Although no investigation of this polymorphism in nature (Africa) has been carried out, the ecology of the species could easily lead to loss of the polymorphism: population expansion occurs during the maturity of **Pandanus spp** fruits and lasts about two months. During the ten other months of the year, the population size is extremely small (Rio et al. 1983). The persistence of this polymorphism in the wild suggests that selection could be responsible for its continued existence. The light and dark homozygous strains used here were extacted from a population collected from the Ivory Coast and maintained since 1980 by serial transfer.

Population Cage Experiments: Eleven populations were studied at four temperatures (20, 22, 25 and 28°C). The starting gene frequency of the light allele was 0.10 (except at 20°C), 0.50 or 0.90. 1600 inseminated females formed the founding population for each cage. The technique described by Anxolabehere (1976) was used for maintaining the cages. Genotypes of dark flies were determined after test-crosses. The history of light allele frequency in the cages is shown in Figure 1. No frequency equilibrium was reached after 30 generations. Comparison with Hardy-Weinberg proportions displayed no larval or sexual selection. No difference in gene frequency was found between the sexes.

Developmental Time: The embryo duration of each genotype was estimated as the mean time between egg-laying and hatching, larvo-pupal duration as the mean time between hatching and emergence. Developmental durations of the genotypes were compared with t-tests, none of which were significant: we can conclude that developmental times of the genotypes are not different.

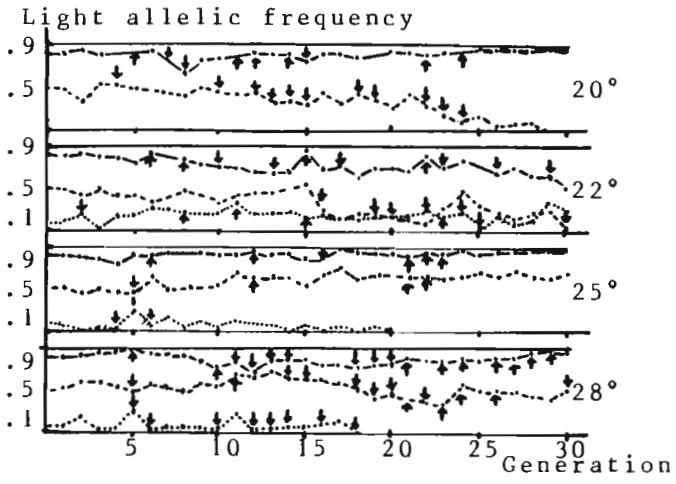

Figure 1. Gene frequency changes over generations in population cages at 20, 22, 25 or 28°C.

Sexual Selection: One hundred 5-day old virgin females were kept with 100 males for 1h45 in a bottle containing corn medium at 22°. Light allele frequency in both sexes was 0.10, 0.30, 0.50, 0.70, or 0.90. Females were then isolated in vials and the genotype of the mating male was inferred from the progeny. Replicates at each frequency were carried out until at least 500 matings had been scored. Comparison with random mating (tested with Chi-square) showed (Table 1) no sexual selection in males. In females the Chi-squares were significant for allelic frequencies of 0.50 and 0.70. Mating success estimated with a K coefficient (Petit 1958) indicated an advantage of light homozygous (C/C) over heterozygous (F/C) females. However, if two of the seven replicates are disregarded, the significant effect disappears: it is possible that the significant result is due to sampling error. The significant sexual advantage of homozygous dark (F/F) over F/C females when the allelic frequency was 0.90 may also have been caused

Table 1. Sexual selection estimated with Petit's K coefficient. P_c= allelic frequency; N = observed matings; N_t = theoretical matings; Σ = total number of matings; X^2_r = Chi-square between replicates.

	Competiting genotypes:					Mating genotypes:						Σ	X^2 ♂ pan-mixia	X^2 ♀ pan-mixia	♂ sexual selection coeff.	♀C/C-F/F selection coeff.	♀C/C-F/C selection coeff.	♀F/F-F/C selection coeff.
	C/C	F/C	F/F	C/Y	F/Y	C/C C/Y	C/C F/Y	F/C C/Y	F/C F/Y	F/F C/Y	F/F F/Y							
P_c = 0.10																		
N	16	288	1296	160	1440	2	5	11	86	45	381	530	0.52	0.61	1.11±0.15	1.29±0.51	1.33±0.51	0.97±0.11
N_t						0.5	4.8	9.5	85.9	45.9	386.4		(1ddl)	(2ddl)				
X^2_r													5.75	3.13				
													(8ddl)	(8ddl)				
P_c = 0.30																		
N	108	504	588	360	840	16	34	73	148	67	182	520	0	0.38	1.00±0.09	1.05±0.17	1.10±0.17	0.95±0.09
N_t						14.0	32.8	65.5	152.9	76.4	178.4		(1ddl)	(2ddl)				
X^2_r													1.70	5.68				
													(6ddl)	(12ddl)				
P_c = 0.50																		
N	300	600	300	600	600	80	77	129	113	59	73	531	0.05	6.51*	1.02±0.08	1.30±0.13*	1.18±0.14	1.10±0.12
N_t						66.4	66.4	132.7	132.7	66.4	66.4		(1ddl)	(2ddl)				
X^2_r													3.98	4.50				
													(6ddl)	(12ddl)				
P_c = 0.70																		
N	686	588	126	980	420	201	81	134	58	25	19	518	0.16	6.18*	0.97±0.10	1.25±0.12*	1.17±0.15	1.06±0.11
N_t						177.7	76.2	152.3	65.3	32.6	14.0		(1ddl)	(2ddl)				
X^2_r													3.33	9.71				
													(7ddl)	(14ddl)				
P_c = 0.90																		
N	972	216	12	1080	120	469	51	99	12	11	1	643	0	5.02	1.01±0.14	1.04±0.11	0.53±0.15*	1.96±0.59
N_t						468.8	52.1	104.1	11.6	5.8	0.6		(1ddl)	(2ddl)				
X^2_r													7.98	2.68				
													(6ddl)	(6ddl)				

by such sampling error: in each of the six replicates the two F/F females were inseminated, but the small number of F/F females involved suggests that little confidence can be placed in this result. It seems most likely that there was no sexual selection revealed in this experiment.

Discussion: No detectable difference in fitness at the abdomen pigmentation locus in **D.erecta** was found in these experiments. The results presented here are therefore consistent with the hypothesis that weak selection or selective neutrality at this locus is responsible for the maintenance of the polymorphism. Further results will be published elsewhere.

References: Anxolabehere 1976, Evolution 30:523-534; Petit 1958, Bull. Biol. Fr. Belg. 92:248-329; Rio 1983, Ann. Soc. ent. Fr. 19:235-248.

Perez Alonso, M. and R. de Frutos. University of Valencia, Spain. Electron microscopic analysis of the E polytene chromosome of **Drosophila subobscura.** Division 54.

The ultrastructure of the proximal regions of **Drosophila subobscura** E polytene chromosome has been analyzed under the electron microscope. We have followed the squash thin sectioning technique developed by Sorsa for **Drosophila melanogaster** (Sorsa 1983). Salivary glands from third instar larvae were fixed in acetic methanol. The slides were stained in heamalum Mayer for 10 min in order to select the chromosomes with the light microscope (Berendes 1968). After separating the polymerized material from the slide, the chromosomes were identified under the light microscope. The squash was cut into small pieces and each one adhered to a Durcupan block with a cyanocrylic glue. Thin sections of about 90-120 nm thickness were collected on 200 mesh copper grids. The grids were observed in a JEOL JEM-100 S electron microscope at initial magnifications from x2.000 to 5.000.

Figure 1 presents division 54 of the E chromosome of **D.subobscura** following the map of Kunze-Muehl (Kunze-Muehl & Mueller 1958). The centromere is at the tip of this chromosome so that the 54 region may be affected by the Beta-heterochromatin (Beerman 1972). This is why the banding pattern of the proximal part of this division is difficult to define and many more photographs will be necessary to make a detailed amp.

In a first approximation, the electron microscope seems to resolve some extra bands which cannot be seen with the light microscope. This agrees with the findings of Sorsa on **Drosophila melanogaster** (see, for example, Sorsa et al. 1984). Possible new bands are indicated by arrow heads. The asterisk indicates two bands which had been defined as a single one in the original light microscopic map.

References: Beerman, W. 1972, in: Results and Problems in Cell Diff. (Springer-Verlag):1; Berendes, H.D. 1968, DIS 43:115; Kunze-Muehl, E. & E. Mueller 1958, Chromosoma 9:559; Sorsa, V. 1983, Cell Differ. 12:137; Sorsa, V. et al. 1984, Chromosoma 90:177.

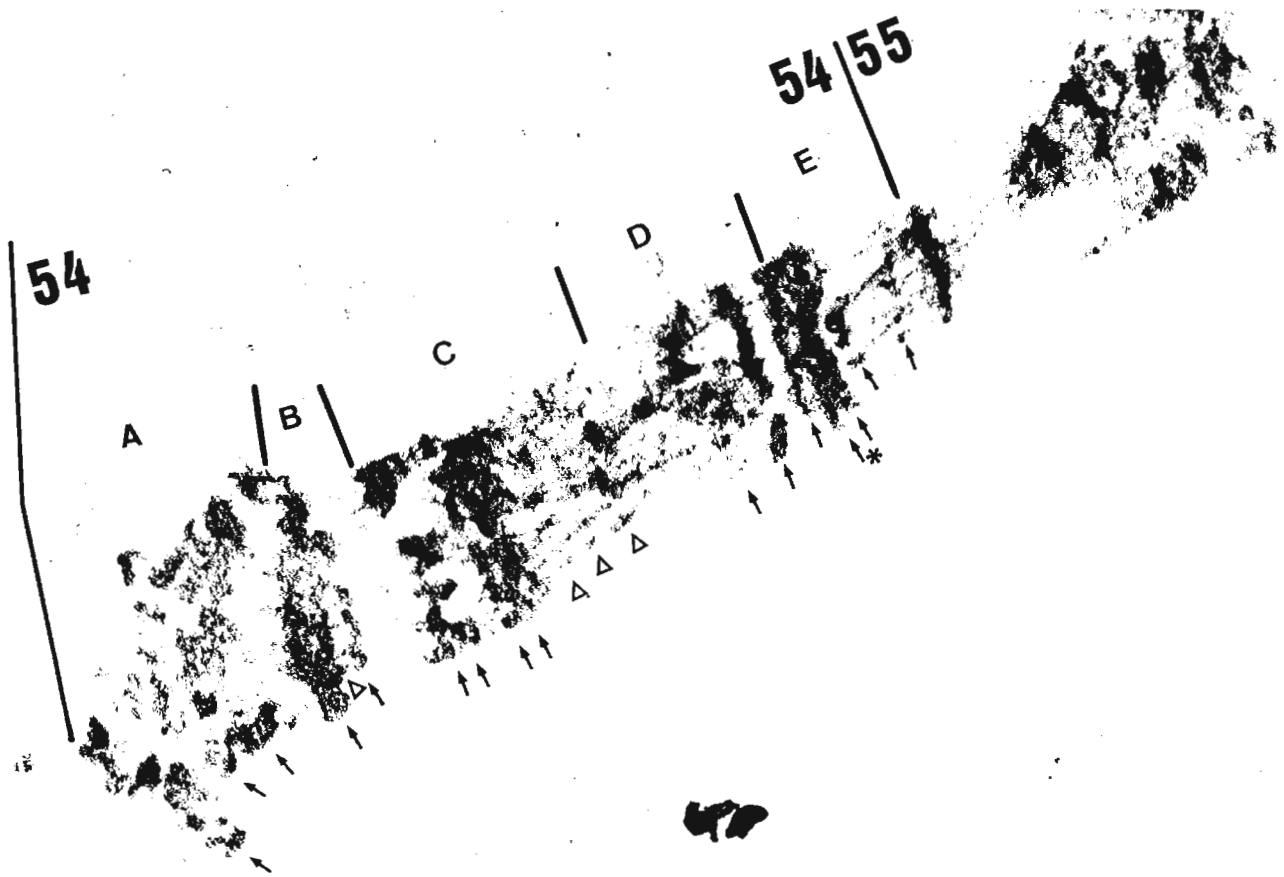

Figure 1. Division 54 of the E chromosome. The centromere is at the left. Magnif. ca. x15,000.

Poole, J.H. and L.K. Dixon. University of Colorado, Denver, USNA. Drosophila peroxidases: IV. Heritability (H) estimates for the three major isozymes.

In this report, we present our findings on quantitative variability of the three major peroxidase (PO) isozymes in **Drosophila melanogaster**, with estimates of the coefficient of genetic determination for each isozyme's activity. The study utilized 3-week old male adults from a wildtype population and 13 derived inbred strains.

METHODS: All stocks were maintained on cornmeal-molasses-agar-yeast medium in half-pint bottles (200-400 flies per bottle), with a diurnal cycle of 12 hr in the light at 25°C and 12 hr in the dark at 18°C. The wildtype sample was obtained from the National Center for Atmospheric Research, Boulder, Colorado (Dr. Edward Martell). Thirteen inbred strains were derived by 25 generations of full-sib mating. Stable populations of these strains were then built up by 2 months of free mating within each strain, during which all transfers and collections were carried out without the use of ether. Imagoes were collected at eclosure and transferred weekly to fresh medium, thereby maintaining distinct age-cohorts.

For assays, 3-week old (±3.5 days) imagoes were frozen, sexed, weighed, homogenized in buffer, centrifuged and photometrically assayed with PDA/H_2O_2 as previously described (Poole & Dixon 1984). Ten males were pooled for each extraction and assay. From each inbred strain and the wildtype population, four independent extractions and assays were performed for each of the PO isozymes (acid-PO, neutral-PO, alkaline-PO). PO activity is reported in picokatals (picomoles H_2O_2 reduced per second) at V_{max} per mg body mass (for conversion factors, see Poole & Dixon 1984). One-way analyses of variance were used to test the significance of inter-strain differences, for each isozyme's activity.

Heritability (H) was estimated from total and residual variances, by calculation of the adjusted coefficient of determination:

$$\text{(eq. 1)} \qquad H = \bar{R}^2 = 1 - (V_{resid}/V_{total}) \ ,$$

and the standard error of the coefficient was estimated as:

$$\text{(eq. 2)} \qquad S_{\bar{R}2} \simeq \left[2 \frac{V^2_{resid}\, a^2\, (b-1)(a+b-4)}{V^2_{total}\, b^2\, (a-3)^2\, (a-5)} \right]^{\frac{1}{2}} \ ,$$

where a=51 = total degrees of freedom, and b=39 = residual degrees of freedom (Cavalli-Sforza & Bodmer 1971).

Table 1. Peroxidase isozyme activities of inbred strains. Each data point is based on 49 males, age 3-weeks. ANOVA on inter-strain differences (inbreds only):
- acid-PO, $F(12,39) = 17.5$ ($p<0.0005$)
- neutral-PO, $F(12,39)=8.58$ ($p<0.0005$)
- alkaline-PO, $F(12,39)=7.17$ ($p<0.0005$)

	Peroxidase activity (pkat/mg tissue)					
	acid-PO		neut-PO		alk-PO	
Strain	mean	± SE	mean	± SE	mean	± SE
1	144	18.0	90.8	26.8	124	9.0
2	59	8.2	42.2	3.0	84	5.2
3	46	8.7	6.9	4.0	92	4.9
4	61	7.1	58.2	6.7	88	2.1
5	26	5.1	68.6	2.76	91	8.9
6	37	7.2	71.0	7.2	71	2.3
7	47	6.7	41.8	3.12	82	6.7
8	176	21.5	85.6	5.4	72	4.1
9	93	13.9	23.9	3.4	89	1.2
10	69	12.8	18.6	2.52	92	3.1
11	97	29.2	22.8	0.96	62	7.5
12	229	15.9	67.4	1.44	86	11.8
13	67	11.8	25.6	2.40	57	4.4
wildtype	96	7.2	77.5	5.4	72	1.6

RESULTS: Table 1 lists mean activities of the three PO isozymes in males of each strain. Large inter-strain differences were found for each isozyme. These differences are highly significant ($p<0.0005$ for each isozyme) and account for a large proportion of the overall observed variability in each isozyme's activity ($\eta^2=0.84$ for acid-PO, $\eta^2=0.73$ for neutral-PO, $\eta^2=0.69$ for alkaline-PO).

Table 2 lists Heritability (H) estimates for each isozyme's activity in males. The calculated value of H (eq. 1) is equivalent to $V_{between\ strains}/V_{total}$. If it is assumed that environmental variability is equally distributed over all strains and that there are no genotype-environment interactions, then H is an estimate of the proportion of variance in PO activity that is due to genetic variability in the overall population. It should be noted that H is a measure of heritability in the broad sense, which does not differentiate among additive, dominance and epistasis components of genetic determination. The results of this study indicate that in a heterogenous population, under standard laboratory conditions, about 60-80% of the variance in PO activity is under genetic control.

Heritable differences in the activity of a given PO isozyme may be due to any of the following types of genetic variability: allelic differences in the PO structural gene, allelic differences in PO regulatory

Table 2. Heritability estimates for peroxidase isozyme activities in males. H and its standard error were calculated using equations 1 and 2.

Isozyme	H
Acid-PO	0.79 ± 0.07
Neutral-PO	0.64 ± 0.12
Alkaline-PO	0.59 ± 0.13

loci (affecting quantity or developmental timing of PO synthesis), and allelic differences in substances that directly modulate PO enzyme function. We are currently engaged in a number of studies to examine these alternatives. First, if heritable differences in enzyme structure are present, such polymorphisms should be demonstrable by electrophoretic procedures we are applying to the Drosophila peroxidase system (viz., Lichtenstein et al. 1984). Second, segregation analyses on the offspring of inter-strain matings may allow us to differentiate the additive, dominance and epistasis components of PO heritability, and to estimate the total number of genetic loci involved in regulation of each PO isozyme's activity. Finally, we are engaged in a number of studies to determine the role of each PO isozyme in Drosophila metabolism--and the possible functional significance of heritable differences in each isozyme's activity.

References: Cavalli-Sforza, L.L. & W.F. Bodmer 1971, in:Genetics of Human Populations, Freeman & Co., San Francisco, p574; Lichtenstein, P.S., M. Emmett, L.K. Dixon & A.J. Crowle 1984, DIS 60:138-140; Poole, J.H. & L.K. Dixon 1984, DIS 60:165-168.

Ramachandra, N.B. and H.A. Ranganath.
University of Mysore, India. Further studies on B-chromosomes in **D.nasuta albomicana.**

Supernumerary or B-chromosomes have been reported in over 1000 plant species and 260 animal species (Jones & Rees 1982). In Drosophila, the occurrence of B-chromosomes is rare. The presence of such chromosomes were first detected by Kitagawa (pers. comm.) in **D.n.albomicana.** Recently we have reported the preliminary cytology of B-chromosomes in a Thailand strain of **D.n.albomicana** (Ramachandra & Ranganath 1984, 1985). After this preliminary screening that is in 1983, the Thailand strain was maintained under optimal conditions in the laboratory at 22°C for over two years and again the karyotypic composition of the individuals of this strain was analyzed. The important observations are as follows:

(a) Six different types of individuals with different number of chromosomes were recorded. They are without B's, with one, two, three, four or five B-chromosomes.

(b) Individuals with four and five supernumeraries were not recorded earlier. The meterphases with these B-chromosomes are presented in Figures 1a and 1b.

(c) The comparative account of the frequencies of different individuals with different B-chromosomes in the same strain of **D.n.albomicana** during 1983 and 1985 is given in Table 1. There is a significant decline in the incidence of individuals without B-chromosomes. The frequency of

Table 1. Relative frequencies (%) of individuals with different number of B-chromosomes in **D.nasuta albomicana** during 1983 and 1985 under laboratory conditions.

Years	0B	+1B	+2B	+3B	+4B	+5B
1983	33	36	26	05	--	--
1985	05	32	38	21	03	01

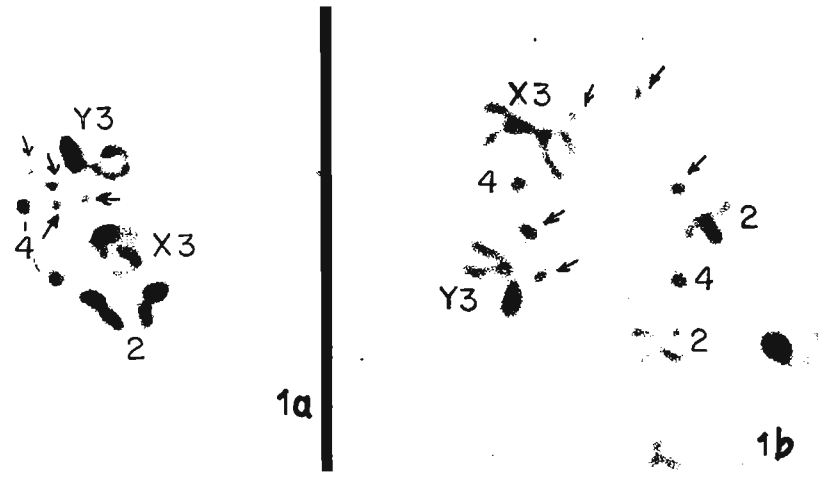

Figure 1a-1b: Karyotype of **D.n.albomicana** with 4 (1a) and 5 (1b) B-chromosomes. Arrows indicate B-chromosomes.

individuals with B's has a steep rise from 67% to 95%.

In view of these, we feel that some type of "B-chromosome accumulating" mechanism is operating in this system.

Acknowledgements: We wish to acknowledge our gratitude to Prof. N.B. Krishnamurthy for his help and encouragement; Prof. O. Kitagawa for sending flies; Mr. M.G. Vasudeva Rao for preparing photographs and to the University Grants Commission, New Delhi, and to the Indian National Science Academy, New Delhi, for financial support.

References: Jones & Rees 1982, B-chromosomes, Academic Press; Ramachandra, N.B. & H.A. Ranganath 1984, VII All India Cell Biol. Congr., Hyderabad (Abstract); _____ 1985, Experientia (in press).

Real, M.D. and J. Ferré. University of Valencia, Spain. Chemical synthesis and the "in vivo" formation of xanthurenic acid 8-0-β-D-glucoside in **Drosophila melanogaster.**

Recently, it has been shown that some eye colour mutants of **Drosophila melanogaster** accumulate a blue fluorescent compound not detected in chromatograms of the wild type. This compound has been identified as xanthurenic acid 8-0-β-D-glucoside (Ferre & Mensua 1983; Ferre et al. 1985). In this work we confirm the above structure by chemical synthesis, and propose a pathway for the biosynthesis of this compound.

The chemical synthesis was carried out following Butenandt et al.'s (1963) procedure for the synthesis of rodhommatin (a xanthommatin glucoside). A solution of xanthurenic acid and α-acetobromoglucose at pH 10.5 was stirred for several hours. The acetilated derivative was hydrolysed in strong alkaline medium to give the free glucoside. The purification of the final product was carried out by ion exchange chromatography.

The synthetic and natural compounds showed identical chromatographic behavior in thin-layer chromatography using different solvents. The UV spectra at different pH values, the excitation and emission fluorescent spectra and the IR spectrum, also showed that both compounds were the same chemical substance.

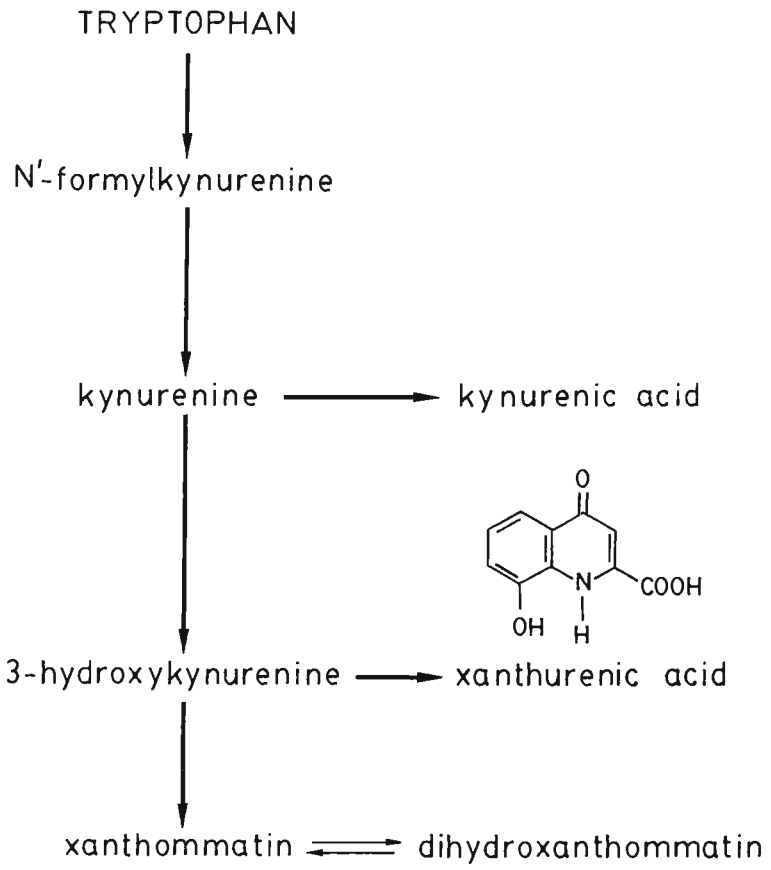

Table 1. Thin-layer chromatographic (TLC) analysis of head extracts from the vermilion purple mutant raised in differently supplemented media.

Supplemented metabolite	Compound after TLC separation* xanthurenic acid	xanth. acid 8-glucoside
none	-	-
3-hydroxykynurenine	+	+
xanthurenic acid	+	-

* TLC was carried out on cellulose plates using two-dimensional separation. First solvent: isopropanol/2% ammonium acetate (1:1); second solvent: 3% ammonium chloride.

Figure 1. Biosynthesis of "xanthommatins" in **Drosophila melanogaster.**

Because this newly found metabolite is a xanthurenic acid derivative, we looked for possible metabolic relationships with the biosynthetic pathway of xanthommatin (brown pigment of Drosophila eyes), from which xanthurenic acid is a side metabolite (Figure 1).

In Drosophila, the vermilion mutant (v) cannot convert tryptophan into N-formylkynurenine; the purple gene (pr) provokes, besides some other effects, the accumulation of xanthurenic acid 8-glucoside. Double mutant (v pr) larvae were fed with a medium supplemented either with 3-hydroxykynurenine or with xanthurenic acid. Adult flies were analysed for the accumulation of fluorescent metabolites and the results are shown in Table 1. The results suggest that 3-hydroxykynurenine is the "in vivo" precursor of both xanthurenic acid and its glucoside, but the former is not a precursor of the latter. It is likely that the biosynthesis of xanthurenic acid 8-glucoside from 3-hydroxykynurenine involves 3-hydroxykynurenine 0-glucoside as an intermediate. This proposed intermediate has never been found in Drosophila. The characterization of this biosynthetic pathway is currently under progress in our laboratory.

References: Butenandt, A., E. Biekert, H. Kubler, B. Linzen & P. Traub 1963, Hoppe-Seyler's Z. Physiol. Chem. 334:71-83; Ferre, J. & J.L. Mensua 1983, DIS 59:35-36; Ferre, J., M.D. Real, J.L. Mensua & K.B. Jacobson 1985, J. Biol. Chem. (in press).

Ricker, J.P. and J. Hirsch. University of Illinois, Urbana-Champaign, Illinois USNA. Evolutionary changes in laboratory populations selected for geotaxis.

Geotaxis (orientation and movement with respect to gravity) is measured in **D.melanogaster** with a multiple-unit maze (Hirsch 1959). Lines described here have experienced divergent selection for geotaxis since 1958 resulting in changes in genetic homeostasis (Lerner 1970). The negative (high) and positive (low) geotaxis lines have been selected in 292 and 294 of 550 generations, respectively, making this one of the longest selection experiments on record.

Selection was started from a line established by mixing three different stocks (Erlenmeyer-Kimling et al. 1962). Two-hundred flies of each sex were tested in the maze and the 60 most negative and most positive flies of each sex were bred for the high and low lines, respectively. After this time, the number tested in each line was usually between 100 and 200 of each sex; and the number selected was usually between 25 and 60 pair. Geotaxis scores range 0-15, the score of a fly indicating the number of up choices in the geotaxis maze.

Figure 1 presents the response to selection of males in the high and low lines. Female results are not presented but are similar to those of males. Selection data from generations 242 through 251 are missing but are presented graphically in Yeatman & Hirsch (1971). Selection response in the first 100 generations is gradual, there being a more rapid response initially in the low line, but then in the high line after generation 15. Excepting occasional losses associated with relaxed selection, gains from selection continue until around generations 90 and 162 in the high and low lines, respectively. The high line reached the upper limit of the measurement scale in generation 90 but the low line became more extreme in later generations, particularly after generation 514.

In several instances before generation 450, relaxed selection resulted in a regression of mean geotactic score indicating its association with reproductive fitness (Dobzhansky & Spassky 1969). The most striking instances of this are in generations 148, 219, 313, and 415 of the high line, and in generations 235, 313, and 415 of the low line. In generation 514, however, we observed that 26 generations of relaxed selection had resulted in no loss of selection gains--in fact, the high line had become more extreme. To test whether this phenotypic stability was due to the lines having become homozygous, reverse selection from the high and low lines was begun in generations 515 and 519, respectively. The high-reverse (HR) and low-reverse (LR) lines respond to reverse selection indicating that both lines are not homozygous.

Figure 2 presents the selection response of LR. When selection was relaxed in LR for 8 generations beginning in generation 25, its mean geotactic score regressed towards that of the first generation of reverse selection: Genetic homeostasis in the low line has changed to such an extent that changes away from an extreme expression of geotaxis are now resisted by effects of natural selection. When selection was relaxed for 8 generations beginning in generation 32, HR showed no change in mean geotactic score: the high line does not resist changes away from extreme expression of geotaxis. However, because the high line does not regress when selection is relaxed, it also shows effects of changes in genetic homeostasis.

The present study shows that long-term selection can result in development of a new genetic equilibrium and that a long period of directional selection with relatively small population sizes does not exhaust additive genetic variance. The lines described here may prove useful in studying the evolution of behavior.

References: Dobzhansky, T. & B. Spassky 1969, Proc. Natl. Acad. Sci. USA 62:75-80; Erlenmeyer-Kimling, L., J. Hirsch & J. Weiss 1962, Jrl. Comp. & Physiol. Psych. 55:722-731; Hirsch, J. 1959, Jrl. Comp. & Physiol. Psych. 52:304-308; Lerner, I.M. 1970, Genetic Homeostasis, Dover Publ. Inc.; Yeatman, F. & J. Hirsch 1971, Anim. Behav. 19:454-462.

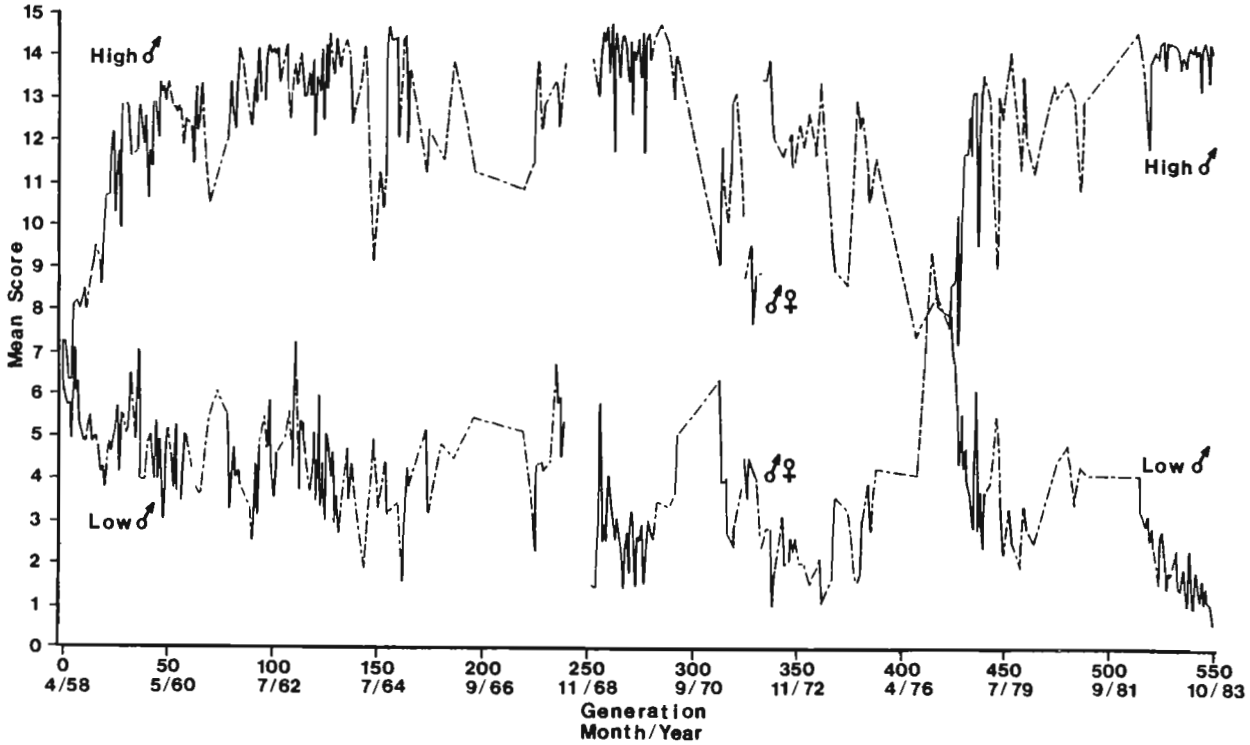

Figure 1. Response to selection of males in the high and low lines. Solid and broken lines indicate forward and relaxed selection, respectively. Males and females were not distinguished in generations 325-332 (indicated by a detached selection curve labelled with both a male and female symbol).

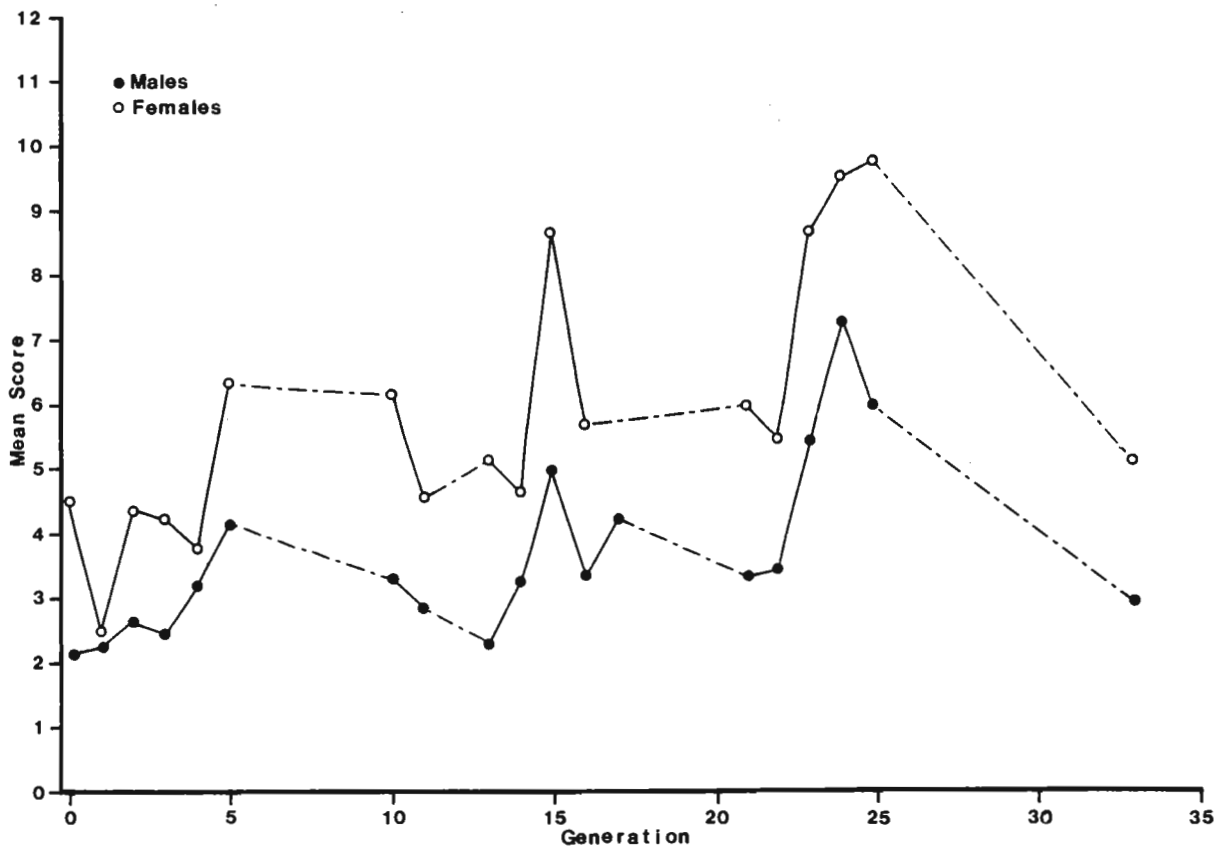

Figure 1. Response to selection of males in the high and low lines. Solid and broken lines indicate forward and relaxed selection, respectively. Males and females were not distinguished in generations 325-332 (indicated by a detached selection curve labelled with both a male and female symbol).

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Choline uptake in **Drosophila melanogaster** is linked to acetylcholine synthesis.

Radioactive choline, when injected into the haemolymph of living flies accumulates in specific regions of the brain (Buchner & Rodrigues 1983). The uptake is energy dependent and sensitive to the choline analogue, hemicholinium-3. The metabolism of acetylcholine following uptake of choline has been examined in **Drosophila** larval brains (Wu et al. 1983). Such studies in adult brains have proved difficult because of the high activity here of the acetylcholine hydrolytic enzyme, acetylcholinesterase. Much of the enzyme is present in a form which is easily rendered soluble, leading to the breakdown of acetylcholine during extraction procedures. We have overcome this problem by the use of tetraisopropyl-pyrophosphoramide (iso-OMPA) which is about 1000 fold more effective at inhibiting cholinesterases than eserine sulfate (Zingde et al. 1983).

20 µCi [methyl-^{3}H] Choline chloride (Sp. Act 15 Ci/mmol; Amersham) was injected into the haemolymph of female **Drosophila.** After incubation, under various conditions, at room temperature, the brains were rapidly dissected out and each placed in 15 µl extraction buffer (0.47M formic acid; 1.4M acetic acid; 10 mM iso-OMPA). The samples were freeze-thawed (6 times) and homogenized. The homogenate was spun at 15000g for 15 mins and the supernate lyophilized to 1 µl. Samples were spotted on a HPTLC cellulose plate (Merck 5787) and developed in a n-butanol: acetic acid: water: ethanol system (16:2:6:4). Radioactive spots were visualised by autoradiography on [^{3}H]-sensitive film (LKB, Sweden), (Fig. 1).

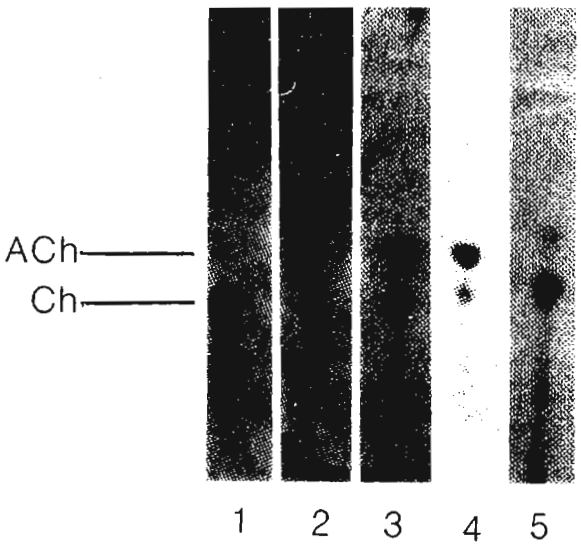

Figure 1. Detection of acetylcholine synthesis after [^{3}H] choline uptake in the brain of **Drosophila melanogaster.**

[^{3}H] Choline chloride (lane 1) and [^{3}H] acetylcholine chloride (lane 2) were run alongside as standards. After 30 mins of incubation the radioactivity was distributed approximately equally between choline and acetylcholine (lane 3). This includes the label present in both the intracellular and extracellular compartments of the brain. Incorporation of label into choline containing lipids was not observed in our experiments. Longer incubation times led to an increased conversion of choline to acetylcholine (60 mins in lane 4).

The extract in lane 5 is from a fly which was injected with 2nMoles of hemicholinum chloride together with [^{3}H] choline. The synthesis of acetylcholine is markedly inhibited. The radioactive spot could represent the choline in the extra-cellular spaces since previous studies have shown that hemicholinium-3 inhibits the specific uptake of choline.

References: Buchner, E. & V. Rodrigues 1983, Neuroscience Letters 42: 25-31; Wu, C.E. et al. 1983, J. Neurochem. 40: 1386-1396; Zingde, S. et al. 1983, J. Neurochem. 41: 1243-1252.

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Map expansion around ts-mutations in genes controlling cAMP metabolism in **D.melanogaster.**

Two ts-mutations in genes controlling cAMP metabolism were obtained following treatment with EMS and screening for ts-lethality on media supplemented with beta-adrenoblockator propranolol and phosphodiesterase (PDE) inhibitor theophylline (Savvateeva & Kamyshev 1981). Mutation ts155 leads to propranolol-dependent ts-lethality during postembryonic development at 29°C and to the increase in both cAMP content and adenylate cyclase (AC) activity. Mutation ts398 leads to inhibitor-independent ts-lethality and to increased PDE activity. Since in ts398 mutants (1) AC activity is higher than normal and is readily activated at 29°C; (2) activity of PDE-I assayed in heat-pretreated homogenates is higher than normal, and (3) boiled extracts of ts398 are potent activators of the wild type and its own PDE-I, the mutation is presumed to affect calmodulin, which is known to be stable at boiling and capable of activating both AC and PDE-I (Cheung 1980). Data on Ca^{2+} and EGTA effects suggest that ts398 presumably increases Ca^{2+}-binding activity of calmodulin (Savvateeva et al. in press).

To map both mutations in X-chromosome mutant males were mated to yctvf females, F_1 progeny was reared at 25°C and F_b at 29°C (in case of ts155 on media supplemented with propranolol), ts-lethality being the character to be mapped. Crossovers were scored in the survived male progeny. The obtained results (Table1) revealed quite unexpected facts: a region containing a ts-mutation exceeded the standard map distance approximately on 4.7 map units, while the distance between y-f in both cases fitted well the expected 56.7.

The suggestion that the observed map expansion resulted from the experimental procedure when F_b had to be reared at 29°C could be considered only while analyzing each case separately but not while comparing them. However, the necessity of mapping the mutations left the revealed peculiarity as a mere curiosity.

The next step in mapping was delimiting the interval around ts-mutations by the most proximate markers. Subtraction of the minimal distance for ts155--7.7 map units--from the map position of ct (1-20.0) localized the mutation in the vicinity of cv (1-13.7). When cv ct was used as a marker stock for mapping ts155 (Table 2), the distance between cv and ct appeared to be 27.5 map units instead of the expected 6.3. Considering this overwhelming result as a distortion due to a small sample size, we repeated mapping using y cv ct f stock (Table 2). The result appeared to be the same: while the distance between cv and ct was again 27.2, the distance between y and f did not differ significantly from the standard due to some reduction in recombination in the regions y-cv and ct-f. According to the data presented in Table 1, the map position of ts398 could be determined as 5.9 map units to the right from v, i.e., 1-38.9. Delimitation of the region by the markers dy (1-36.2) and wy (1-41.9) revealed the map expansion, which was evident in comparison with both the standard map and the results obtained while crossing Canton-S males to dy wy females, the increment being the same as in Table 1. At this stage it became evident that the map expansion around the ts-mutations deserved a special attention.

As to map position of ts398 the subtraction of the minimal distance

Table 1. Genetic mapping of ts155 and ts398.
(Exchanges around ts-mutations are underlined.)

y 1 2 ct 3 v 4 f
x x x x
ts155

No. of exchange, crossover phenotype	No. of flies	%
1 ct v f	130	11.32
2 y	49	4.26
3 y ct	112	9.75
4 y ct v	185	16.11
5 normal	14	1.21*
1-3 ct	3	0.26
1-4 ct v	26	2.26
2-3 y v f	-	
2-4 y f	6	0.52
3-4 y ct f	23	2.00
1-2-3 v f	6	0.52
1-2-4 f	13	1.13
2-3-4 y v	-	
1-3-4 ct f	1	0.08
1-2-3-4 v	1	0.08
P-type y ct v f	579	
Total No.	1148	

y_16.9_ts_7.7_ct_12.7_v_22.2_f
24.6 34.9
37.3
59.5

y 1 ct 2 v 3 4 f
x x x x
ts398

No. of exchange crossover phenotype	No. of flies	%
1 ct v f		11.77
2 v f	53	7.69
3 f	34	4.93
4 y ct v	103	14.95
1-2 y v f	2	0.29
1-3 y f	4	0.58
1-4 ct v	34	4.93
2-3 y ct f	-	
2-4 v	15	2.18
3-4 normal	3	0.43
1-2-3 ct f	-	
1-2-4 y v	-	
2-3-4 y ct	-	
1-3-4 y	-	
1-2-3-4 ct	-	
P-type y ct v f	364	
Total No.	689	

y_17.0_ct_10.2_v_5.9_ts_22.5_f
27.2 28.4
33.1
55.6

* coincidence 2.6

Table 2. Genetic mapping of ts155 and ts398, using proximate flanking markers.

cv 1 2 ct
x x
ts155

No. of exchange phenotype	No. of flies	%
1 ct	26	4.8
2 cv	88	16.1
1-2normal	18	3.3*
P-type cv ct	414	
Total No.	546	

cv 8.1 ts 19.4 ct
27.5
* coincidence 4.3

dy 1 2 wy
x x
ts398

1 wy	72	6.7
2 dy	23	2.1
1-2 normal	8	0.8*
P-type dy wy	965	
Total No.	1068	

dy 7.5 ts 2.9 wy
10.4
* coincidence 5.2

Canton-S/dy wy		
wy	76	2.1
dy	106	2.7
P-type normal	1869	
dy wy	1643	dy 4.9 wy
Total No.	3964	

y 1 cv 2 3 ct 4 f
x x x x
ts155

No. of exchange phenotype	No. of flies	%
1 cv ct f	66	6.4
2 ct f	13	1.3
3 y cv	59	5.7
4 y cv ct	299	28.9
1-2 y ct f	1	0.1
1-3 cv	1	0.1
1-4 cv ct	14	1.3
2-3 normal	96	9.3*
2-4 ct	2	0.2
3-4 y cv f	8	0.8
1-2-3 y	-	
1-2-4 y ct	-	
2-3-4 f	2	0.2
1-3-4 cv f	-	
1-2-3-4 yf	-	
P-type y cv ct f	472	
Total No.	1033	

y 7.9 cv 11.1 ts 16.1 ct 31.4 f
19.0
27.2
58.6
* coincidence 125.5

Table 3. Percent of recombination in different temperature regimes experienced in divelopment of F_1.

y 1 w 2 ct 3 4
x x x x
ts398 wy

No. of exchange crossover phenotype	Temperature regimes: 25° constant	29° embryo I instar	29° pupae	29° constant
1. w ct	0.2	0.3	0.5	0.4
2. ct	14.4	13.9	15.0	16.1
3. normal	21.3	21.4	20.2	21.8
4. y w ct wy	3.9	2.4	2.4	3.1
1-2. y ct				0.05
1-3. y	0.05			0.1
1-4. w ct wy				
2-3. y w	0.9	1.2	1.2	1.8
2-4. ct wy	0.2	0.2	0.4	0.6
3-4. wy	0.4		0.3	0.4
1-2-3. w	0.05			
1-2-4. y ct wy				
2-3-4. y w wy	0.2			0.05
1-3-4. y wy				
1-2-3-4 w wy				
No. of flies	2431	2264	1712	2561
Distances				
ct-ts	22.9	22.6	21.7	24.2
ts-wy	4.7	2.6	3.1	4.1
y-ts	38.9	38.2	38.8	43.3
ct-wy	27.6	25.2	24.8	28.3
y-wy	43.6	40.8	41.9	47.4

2.9 from the position of wy gave 39.0 which did not differ from the above 38.9, i.e., the mutation could be localized independently from the marker stock used and from the map expansion observed. Using Df(1)KA10 (11A1; 11A7) and Df(1)v^{65b} (9F12/13; 11A8-9), it became possible to place ts398 to the right of 11A8-9.

In the case of ts155 the map expansion which increased with the shortening of the distance between the flanking markers did not allow the localization of the mutation on the genetic map. The usage of Df(1)N73 (5C2; 5D5-6) and Df(1)C149 (5A8-9; 5C5-6) placed ts155 between 5C5-6 - 5D5-6.

What are the reasons for map expansion around EMS-induced ts-mutations, which are traditionally presumed to be point mutations, i.e., in any case affecting the structure of a gene rather than that of a chromosome? If we address Tables 1 and 2 once more, we can note that (1) double exchanges in the region containing ts-mutation occur easier than those that involve one site near ts-mutation and the other in the different region (for example 3-4 vs. 2-3 for ts398, 1-2 vs. 2-3 for ts155); (2) among the triple crossovers are more frequent the ones that involve two exchanges around ts-mutations; (3) on short, approximately equal distances (6.3 map units between cv and ct; 5.7 between dy and wy) double exchanges occur easily, and the occurrence of single exchanges is also greater; the coincidence being significantly greater than 1; (4) the magnitude of map expansion around ts155 is significantly less in y-ct region than in cv-ct one, that hardly could be observed provided the mutation resulted from cytologically-visible rearrangement.

It could be suggested that the elevated temperature known to increase recombination would have been the major factor responsible for the observed map expansion. However, since only the F_b progeny was reared at 29°C, the temperature might have affected the viability of crossovers but not the frequency of recombination. As seen from Table 1, the preferential survival of crossovers could not explain the region-specific map expansion around each mutation, since the same

marker stocks experienced the same temperature regimes. It is the position of ts-mutation that determines the crossover frequency: y ct is abundant in the case of ts155, since it results from a single exchange, but is absent in the case of ts398, where it would have emerged only after a triple exchange.

The data on temperature effects on the recombination itself was obtained using the "pupal system" of Grell (1973) which allows the registration of the consequences of heat-shocks experienced by the synchronous population of F_1 oocytes. According to the method y w ct females were mated to ts398 wy males and allowed to lay eggs for 4 hr and the egg samples were either set for development at constant temperatures 25°C and 29°C or given 24 hr heat-shocks at 29°C. The 1st shock covered the end of embryonic stage--the beginning of the 1st larval instar, the 2nd given between 120-144 hr after egg-laying, coincided with the premeiotic synthesis of DNA (Grell 1973). The development of F_b took place at 29°C. The obtained results (Table 3) have shown that in all variants except the permanent development at 29°C ts398 maps in the same position, while the maximal temperature-sensitivity shows the region ct-wy, which contains the ts-mutation, due to increase or decrease in double exchanges. When in the same experimental design ts398 wy is substituted for dy wy, neither dy-wy region, nor ct-wy region deviate from the standard map distances and no map expansion is observable (data to be published in detail elsewhere). Thus the phenomenon of map expansion seems to deserve more attention than a mere by-product of mapping experiments, since in the similar experiments in Kiger's group (Salz et al. 1982), they found the reduced recombination around the EMS-induced muation dunce^{M14}, which lead to the decreased activity of PDE-II. In the case of ts398, provided the mutation is in the structural gene coding for calmodulin, the enlarged recombination might have been the result of the high degree of internal homology revealed in the amino acid sequence of calmodulin (Cheung 1980). A gene coding for such a protein could facilitate conjugational conformations for an unequal crossing over. The structure of the gene affected by ts155 may be suspected to have high internal homology as well. The recombinational properties of dunce^{M14} and ts398, which affect learning ability, and of ts155, affecting locomotor activity, make the system of gene control of cAMP metabolism rather promising for further studies on genetic control of the second messengers functions important for cell regulation and neural plasticity.

References: Cheung, W.J. 1980, Science 207: 19-28; Grell, R.F. 1973, Genetics 73: 25-30; Salz, H.K., R. Davis & J. Kiger 1982, Genetics 100: 587-596; Savvateeva, E.V., I.V. Peresleny, V.A. Ivanushina & L.I. Korochkin 1984, Devel. Genet. in press.

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Serotonin affects locomotor activity in **Drosophila** via cAMP system.

Serotonin injected into 3-day old virgin Drosophila females was shown to produce the pronounced dosage-dependent increase in locomotor activity, the effect being maximal at the 3rd hour after the injection (Kamyshev etal. 1983). The study of the fate of H^3-serotonin in the Drosophila organism leads to the conclusion that the increase in locomotor activity results from the stimulatory action of serotonin itself, while the rather long latency is likely to be related to N-acetylserotonin effects. The latter metabolite was shown to be the only product of H^3-serotonin conversion, its production being mostly intensive immediately after H^3-serotonin injection and its excretion being rapid enough to make the substance undetectable by the end of the second hour when about 50% of injected serotonin was still present in Drosophila tissues.

It is well known that in many cases serotonin produces its effects via cyclic AMP system: the serotonin-sensitive adenylate cyclase is found in nervous tissue of various insects and its pharmacological properties are similar to those of serotonin receptors in mammals and molluscs (Evans 1980). This work was designed to test the possibility that the effects of serotonin on locomotor activity are mediated via cAMP.

cAMP content was measured in virgin 3-day old females (10 flies per sample) of wild-type strain Canton-S using standard cAMP determination kit (Amersham, England). Serotonin creatinine sulfate (Reanal, Hungary, 20 ng of serotonin-base in 0.2 µl of saline) was injected using the previously described technique (Kamyshev et al. 1983).

The dynamics of the increase in cAMP content following serotonin injection (Fig. 1) resembles the dynamics of the development of its effects on locomotor activity (Kamyshev et al. 1983), i.e., the pronounced effect becomes evident only after a rather long latency of about 2 hr. Thus, it seems likely that the changes in locomotor activity level result from the changes in cAMP content and this is in accordance with the data on positive correlation between cAMP content and locomotor activity in Drosophila ts-mutants with impaired cAMP metabolism (Savvateeva & Kamyshev 1981). The more complicated question is why both effects of serotonin have such a long latency. The intensive production of N-acetylserotonin following the injection of H^3-serotonin might have been responsible for the delay in the manifestation

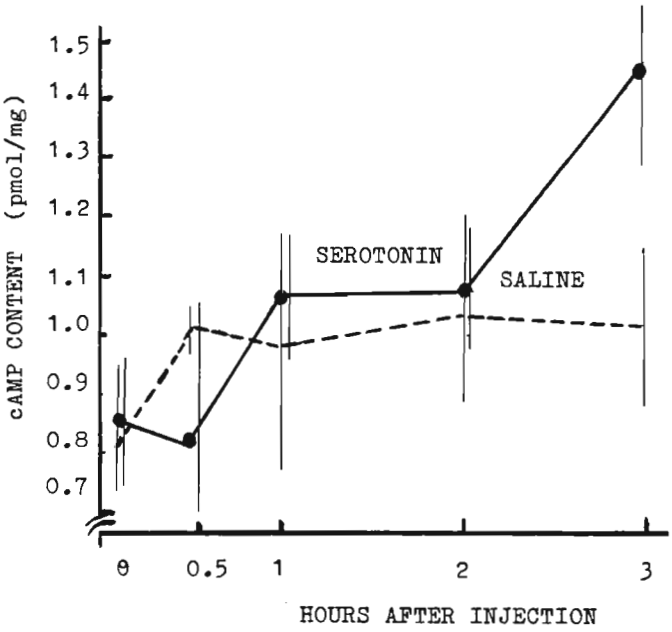

Figure 1. The dynamics of the increase in cAMP content following serotonin injection.

of serotonin effects on both the locomotor activity and cAMP content. N-acetylserotonin could either compete with serotonin for binding with the same receptor of serotonin-sensitive adenylate cyclase or affect any other factors involved into regulation of cAMP content. It is also possible that in our experimental design, when serotonin is injected into Drosophila abdomen, its effects could be amenable for registration only after 2 hr, since the substance has to be transported to the proper sites of its primary action.

References: Kamyshev, N., G. Smirnova, E. Savvateeva, A. Medvedeva & V. Ponomarenko 1983, Pharm. Biochem. Behav. 18: 677-681; Evans, P.D. 1980, Insect Physiol. 15: 317-473; Savvateeva, E. & N. Kamyshev 1981, Pharm. Biochem. Behav. 14: 603-611.

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Apparent neutrality of amylase in **Drosophila pseudoobscura** grown on starch and maltose media.

In order to further understand the forces which affect genetic variability in natural populations, we have studied the amylase locus in **Drosophila pseudoobscura.** Amylase hydrolyzes starch to maltose. There are two common alleles at this locus in natural poulations, $Amy^{1.00}$ and $Amy^{.84}$. We previously found that the $Amy^{1.00}/Amy^{1.00}$ homozygote is significantly more viable when in direct competition with the other two genotypes under stressful conditions (25°C, low amount of starch) (Seager & Anderson, submitted to Evolution).

We used the same 40 lines with which we measured viability (20 independently derived lines of the two homozygotes, all homokaryotypic for the Arrowhead inversion) to establish two starch and two maltose population cages. For each set of cages initial frequencies of $Amy^{1.00}$ were near .90 or .15. In order to approximate the conditions under which viability differences had been found, the cages were kept at 25°C and we attempted to keep both starch and yeast amounts relatively low. The food contained 6% starch or maltose, 2% killed brewers yeast, 1.5% agar, and propionic acid.

The generations in the cages were discrete. Each generation we obtained adult and zygotic allele frequencies by electrophoresing 200 adult males and 200 adult females from the cages, and an equivalent number of adults raised under nearly optimal conditions from egg samples. The cages were continued for 10 generations with each generation lasting about a month. After generation 8 in the maltose cages and 7 in the starch cages, zygotic samples were no longer obtained. The starch cages were increasingly difficult to maintain and at generation 8 the population in starch cage I decreased substantially.

Table 1. Frequency of $Amy^{1.00}$ in population cages of **D.pseudoobscura** maintained on starch or maltose medium. The other allele present was $Amy^{.84}$. 800 alleles were sampled at each life stage in each generation. Z = zygotic frequency and A = adult frequency.

Generation	Maltose I		Maltose II		Starch I		Starch II	
	Z	A	Z	A	Z	A	Z	A
0		87.9		16.7		87.5		15.8
1	91.1	96.3	25.3	22.5	77.2	82.5	33.1	32.2
2	98.3	95.6	24.8	32.4	78.6	81.0	33.7	30.6
3	95.9	97.1	29.0	24.0	80.0	83.2	22.6	26.3
4	95.1	97.6	24.1	25.7	82.2	83.2	34.2	29.7
5	97.0	90.3	29.6	28.0	82.7	85.7	28.2	26.8
6	96.3	93.8	30.1	31.2	83.2	83.6	29.1	25.2
7	97.4	93.8	32.0	31.0	87.5	83.4	24.3	22.5
8	95.5	94.3	33.1	31.7		92.3*		24.7
9		92.9		33.0		93.8		15.7
10		91.5		38.3		96.7		21.6

* population size drastically reduced.

The results are shown in Table 1. There is little consistent allele frequency change nor any evidence of selection in any of the cages. In particular the starch cages do not show any more change than the maltose cages. No evidence of selection has been detected using an experimental system designed to maximize the chance that selection will occur. Results from other experiments have been contradictory. Some have also failed to detect selection involving amylase (Yardley et al. 1977, Powell & Amato 1984) while others have seen selection (Anderson et al. 1979, Powell & Andjelkovic 1983). Taken together, these experiments emphasize how complex selection can be and show that the question of whether variation is selected or neutral may have no easy answers.

References: Anderson, Salceda & Turner 1979, Genetics 91:s2; Powell & Amato 1984, Genetics 106:625-629; Powell & Andjelković 1983, Genetics 103:675-689; Yardley, Anderson & Schaffer 1977, Genetics 87:357-369.

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EM mapping of rearrangements in the 24-25 sections of **D.melanogaster** 2L chromosomes.

Three series of chromosome rearrangements designated as dp^{hx}, sc^{19-x} and cl^{hx} were induced by X-ray irradiation (4000 R) of Oregon R and $T(1;2)sc^{19}$ males and then isolated by means of Dp(2;2)B3 de dp cl duplication; the first two series as well as Df(2L)M11 and Df(2L)M-z^B were balanced over Dp(2;1)B19 (Reuter & Szidonya 1983; a detailed description rearrangements and induction and their genetic analysis will be given elsewhere). Heterozygotes for Batumi L wild stock were used in electron microscopic (EM) mapping of deficiencies. Techniques of squashed chromosome preparations for EM have been described earlier (Semeshin et al. 1979). Revised Bridges' maps (Lindsley & Grell 1968) and EM data obtained on Batumi L were used for determination of rearrangement limits.

In the 24AF region Bridges described 30 bands including 8 doublets (Figure 1a). Some of the doublets, such as 24A1-2, D1-2, D5-6, F1-2, F6-7 and the 24C9, D3 and F3 thin bands were described by Saura (1980). However, in our EM studies all the doublets are visible as single bands; the 24B3, C6, C9, D3, F3, F5 and F8 thin bands were not detected (Figure 1b-e). Contradicting to Saura's data we could not visualize 4 bands in the 24F3-8 interval. In this region there are 2 zones with the diffused, slightly puffing material which are adjacent to the 24F1-2 and 25A1-4 thick bands (Figure 1f) and actively incorporates ^{3}H-uridine (Zhimulev & Belyaeva 1974). We observed the 24E2' thin band which was not described earlier (Figure 1e,f,g). Altogether, 24 bands were found in the 24 section of 2L chromosome.

The 25AC region was mapped earlier (Semeshin et al. 1985). As for the EM map of the 25DF region, it coincides with that of Bridges as well as with Saura's data, if Bridges' doublets are considered as single bands; the 25D1-2 "doublet" consisting of two separate bands is an exception (Figure 1f). The complete EM map of the 24 and 25 sections of the 2L chromosome is presented in Figure 6.

Df(2L)dp^{h19}. As is seen from Figure 1g the deficiency presumably deletes the 24F1-2 to 24F6-7 region. We also can not exclude the possibility that the proximal and distal breakpoints are located in the left edge of 25A1-4 complex and in the 24F1-2 band, respectively, and the remaining parts of these bands are fused.

Df(2L)M-z^B. According to Bridges' data this deficiency deletes the 24E2-F1 to 25A1-2 region (Lindsley & Grell 1968). However, Duttagupta & Dutta Roy (1984) consider that the 25A1-2 band remains intact and is not affected by the deficiency. EM data clearly show that the Df(2L)M-z^B deletes the 24E1-2 to 24F6-7 region (Figure 1h), but its proximal breakpoint can not be determined accurately. Because of the underreplication and the break in normal chromosomes the appearance of the 25A1-4 complex is rather variable (Figure 1b-f; see also Zhimulev et al. 1982) which often looks like a wedge. Similar pictures are observed in the chromosomes with deficiency (Figure 1h). However, we can not exclude that the left part of the 25A1-4 complex is removed and therefore attribute it to the uncertainty of mapping.

Df(2L)dp^{h28} deletes the 24D8 to 24F6-7 region (Figure 2a). We are not sure whether the 24D7 thin band is retained and the part of the 25A1-4 band is deleted; this material is attributed to the uncertainty of mapping.

Df(2L)M11 clearly deletes a part of the 25A1-4 complex because of the different thickness of the band in normal and deleted homologues (Figure 2b). The distal breakpoint of the deficiency is located just to the right of the 24D5-6 band (Figure 2c). Thus, the 24D8 to 24F6-7 region and the left part of the 25A1-4 band are included in the deleted material.

Df(2L)ed dp^{h1}. This deficiency also removes the left part of the 25A1-4 band (Figure 2d). Figure 2e shows clearly that the 24C3 band is preserved. Therefore, the deficiency deletes the region from the 24C5 band to the left part of the 25A1-4 complex. The 24C4 thin band is attributed to the uncertainty of mapping.

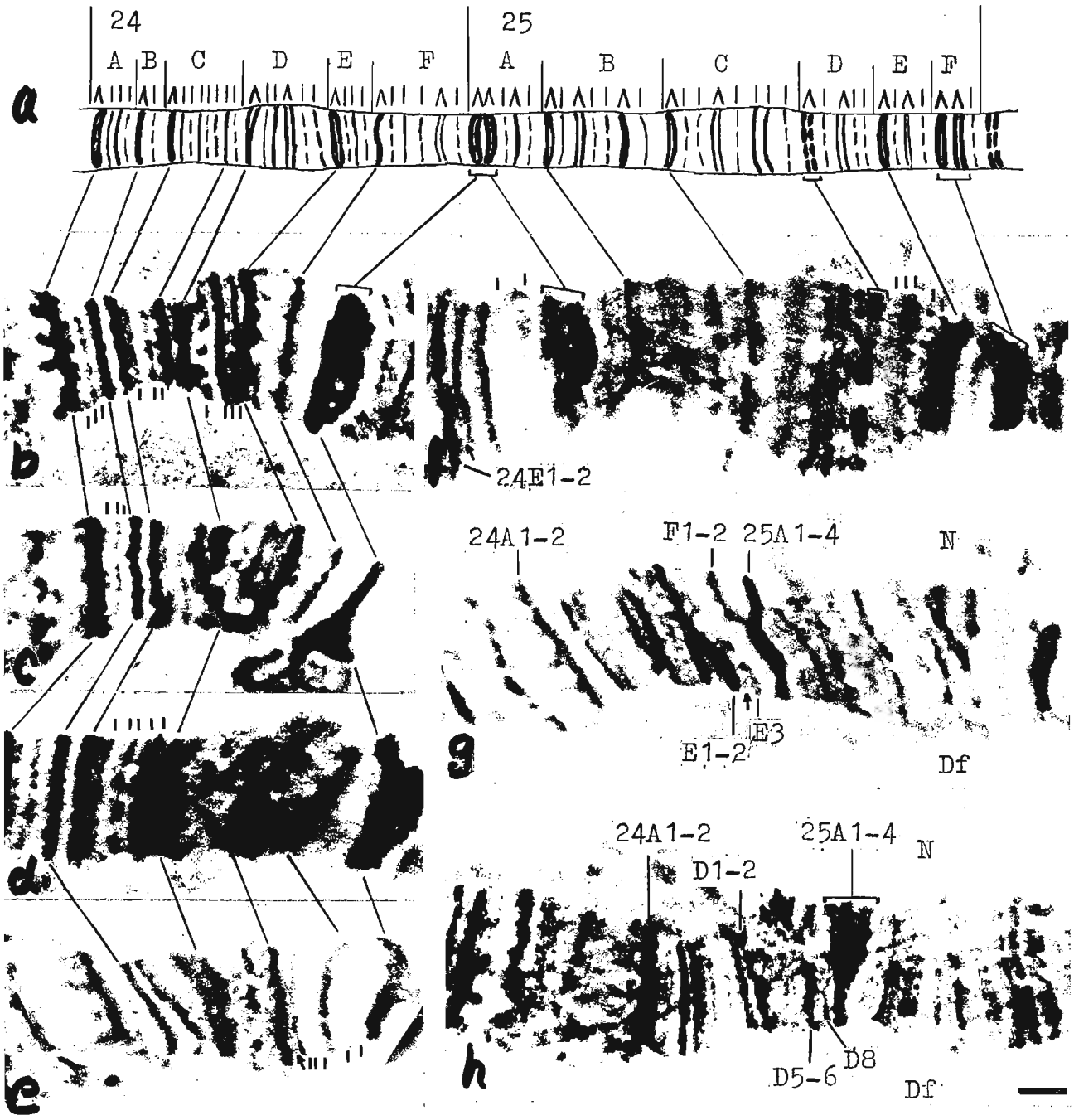

Figure 1. Banding pattern in the 24 (**b-e**) and 25 (**f**) sections of the 2L chromosome in the Batumi L stock and in heterozygotes for Df(2L)dp^{h19}/+ (**g**) and Df(2L)M-z^{B}/+ (**h**) deficiencies; (**a**) Bridges' map; (**b,c,d,e**) thin bands in the 24D, 24A, 24BC, 24EF subdivisions, respectively; the arrow (**e,f,g**) points to the new band 24E2'. The bar corresponds to 1μ.

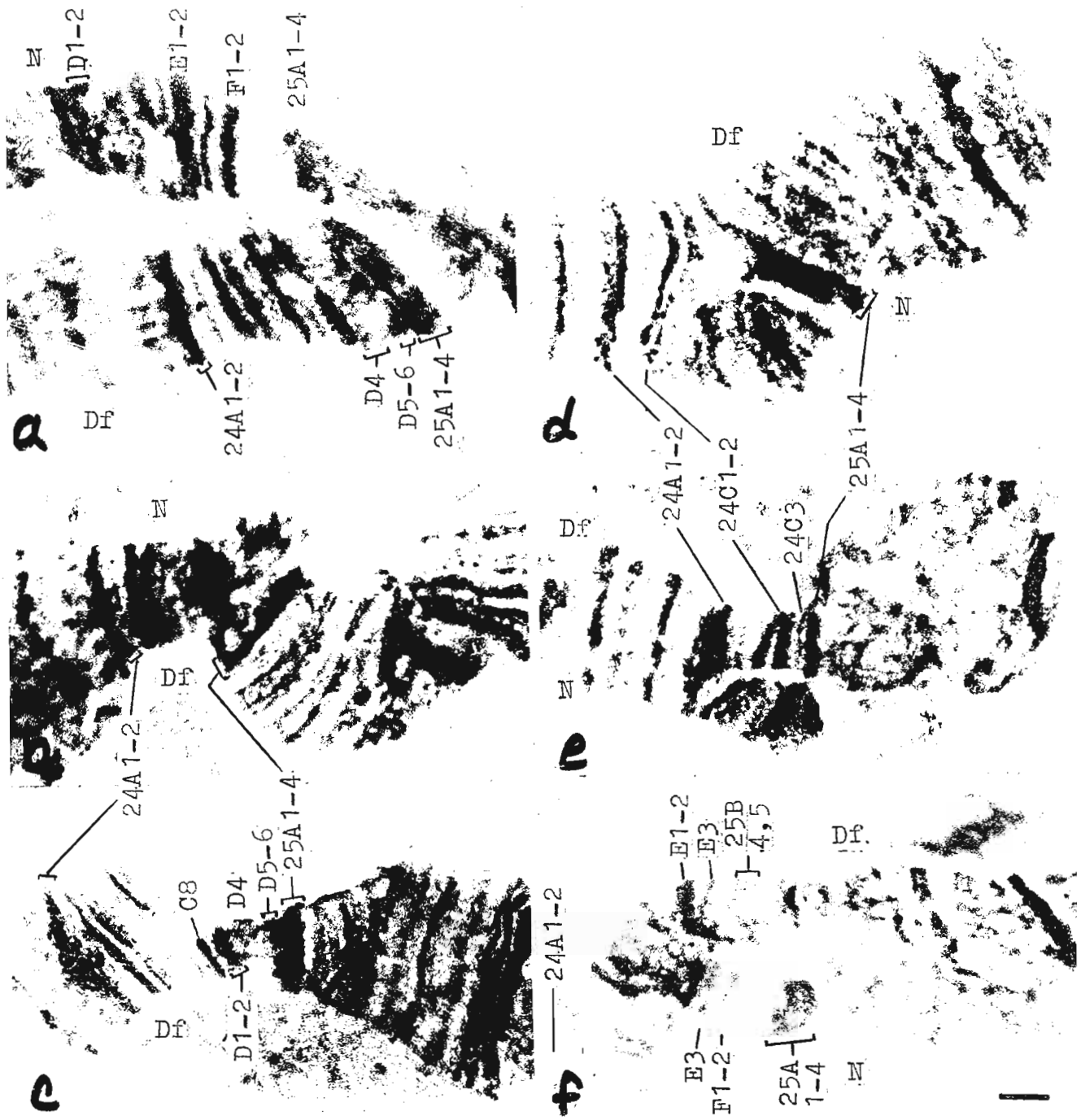

Figure 2. Deficiencies in the 24-25 regions of the 2L chromosome: (**a**) Df(2L)dp^{h28}/+; (**b,c**) Df(2L)M11/+; (**d,e**) Df(2L)ed dp^{h1}/+; (**f**) Df(2L)dp^{h25}/+.

Df(2L)dp^{h25}. The EM pictures may be interpreted in two ways: the deficiency deletes either the 24E4-25B2 region or 24F1-2 to 25B3′ one (Figure 2f). The 24E4,5 and 25B3,3′ bands are very thin and morphologically similar. So, it is impossible to determine exactly which pair of the bands is preserved.

Df(2L)sc$^{19-3}$. The deficiency deletes the 24E3 to 25A6-7 region. It is observed both in the heterozygote (Figure 3a) and in homozygote balanced over a duplication of deleted region (Figure 3b). The 24E2′ and 25A8,8′ thin bands are not identified on the EM photographs and so attributed to the uncertainty of mapping.

Figure 3. Deficiencies induced by irradiation in the T(1;2)sc^{19} stock: (**a**) Df(2L)sc$^{19-3}$/+; (**b**) Df(2L)sc$^{19-3}$ is balanced over Dp(2;1)B19; (**c**) Df(2L)sc$^{19-1}$/+; (**d**) Df(2L)sc$^{19-6}$ is balanced over Dp(2;1)B19; (**e**) Df(2L)sc$^{19-4}$/+.

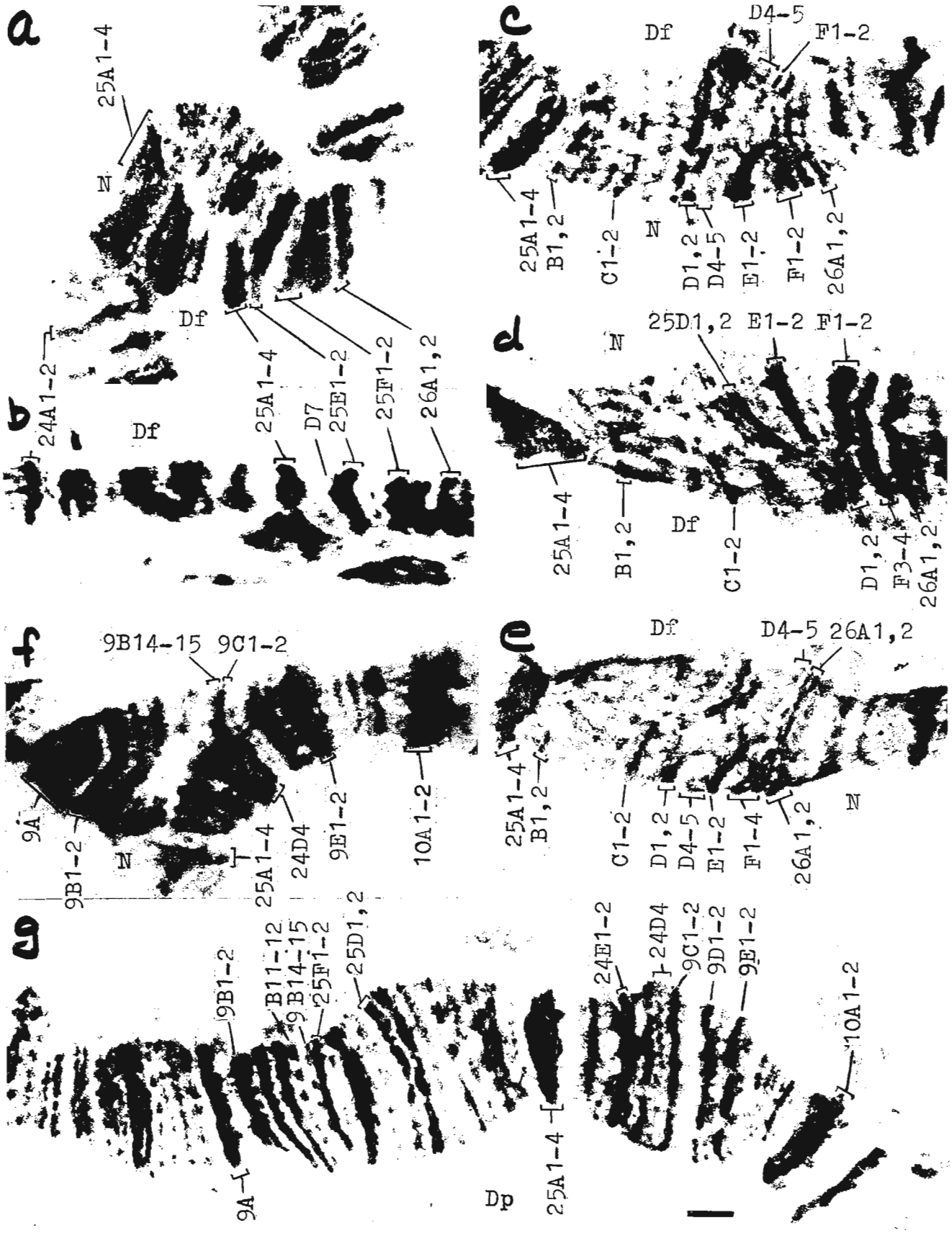

Figure legend is on the next page → → →

Figure 4 [previous page]. Deficiencies (**a-c**) and duplication (**f,g**) of the 24-25 region: (**a**) Df(2L)sc$^{19-5}$/+; (**b**) homologue with the same deficiency as (**a**) is slightly stretched; (**c**) Df(2L)cl^{h2}/+; (**d**) Df(2L)cl^{h1}/+; (**e**) Df(2L)cl^{h4}/+; (**f**) and (**g**) heteroand homozygote for duplication Dp(2;1)B19, respectively.

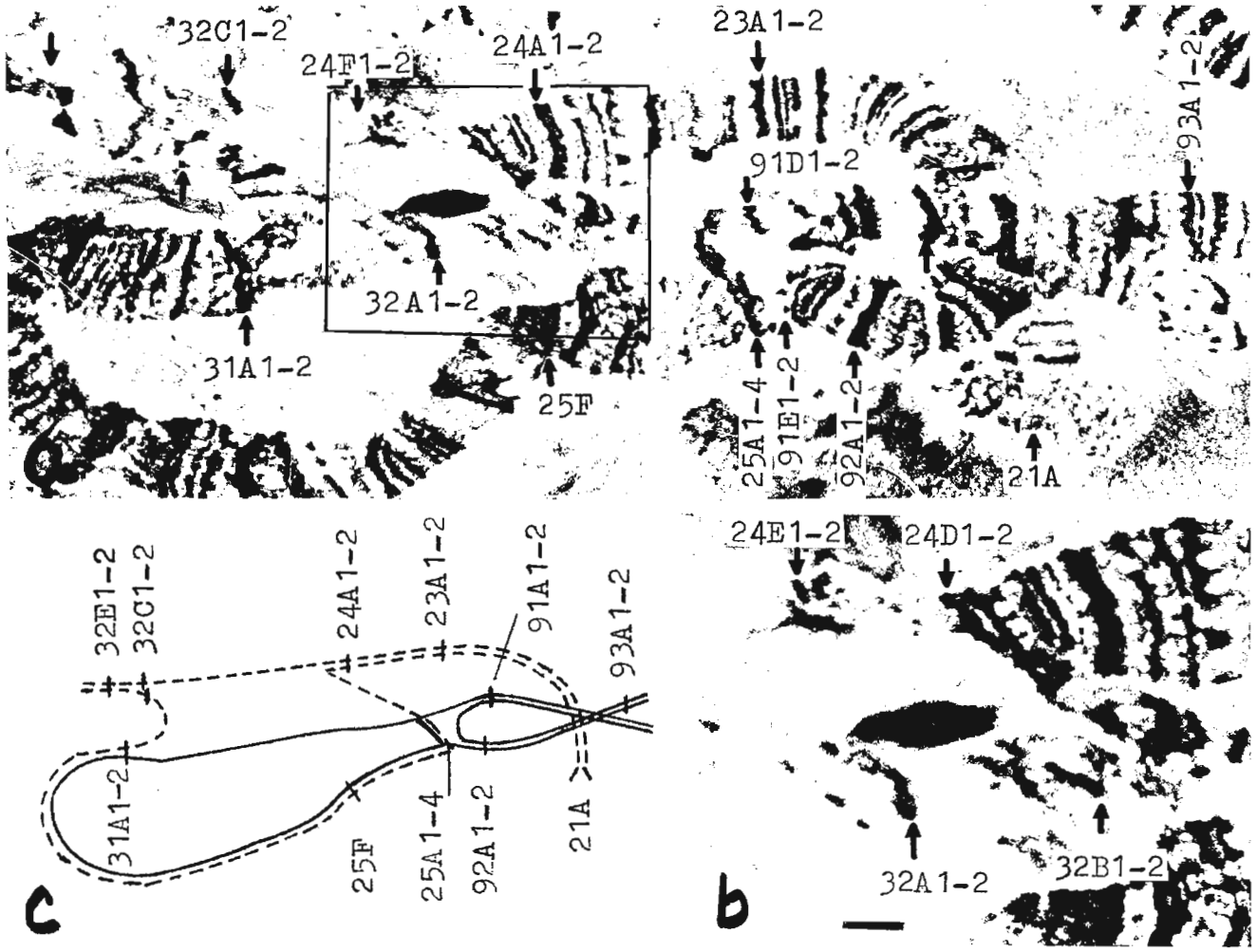

Figure 5. Translocation T(2;3)dp^{h27}/+ (**a**); (**b**) magnified fragment from (**a**) showing breakpoints in the 2L chromosome; (**c**) scheme of translocation: Dotted line - 2L; solid line - 3R chromosome.

Df(2L)sc$^{19-1}$. The deficiency deletes either the 24D5-6 to 25C8 or the 24D8-25C9 region (Figure 3c). The 24D5-6 and 25C9 bands are morphologically very similar large singlets, so it is impossible to determine which of them is deleted.

Df(2L)sc$^{19-6}$. Three interpretations of the EM pictures of this deficiency (Figure 3d) are possible: (1) either the 24F1-2 to 25B10 region is deleted; (2) the 24F4 to 25C1-2 region is removed; or (3) the deletion covers the region from the middle part of the 24F1-2 band to middle part of the 25C1-2 band with remaining parts of both of the band fused. These variants can not be distinguished cytologically.

Df(2L)sc$^{19-4}$ deletes either the 25A5 to 25E4-5 region or the 25A6-25E6 one (Figure 3e). The 25A5 and 25E6 bands are very thin and were not distinguished in EM picutres.

Df(2L)sc$^{19-5}$. The deficiency probably deletes the 25A5-25D6 region (Figure 4a); the 25D7 thin band is preserved (Figure 4b); there are no signs of the presence of the 25A5 and D6 thin bands.

Df(2L)cl^{h2} deletes the 25D6 to 25E4-5 region (Figure 4c). We attribute the 25D6,7 and 25E6 thin bands to the uncertainty of mapping.

Df(2L)cl^{h1} deletes bands in the 25D4-5 to 25F1-2 interval (Figure 4d).

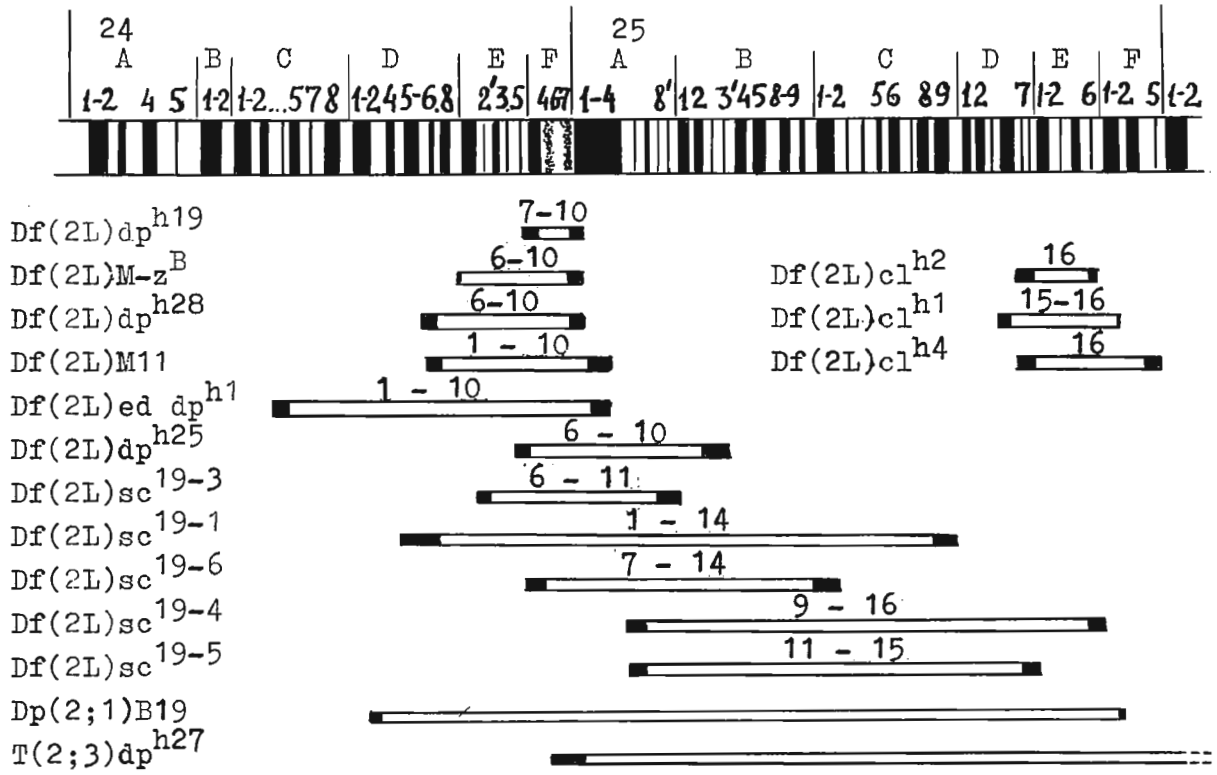

Figure 6. EM map of the 24 and 25 sections of the 2L chromosome and cytological limits of the chromosome rearrangements analyzed. Shaded areas represent the uncertainty of mapping. Numbers indicate mutations (complementation groups) localized in the deleted regions according to Szidonya's data (unpubl.): 1 - G-ft complex; 2 - Sz5-1; 3 - Sz49-1; 4 - ed; 5 - M(2)LS2; 6 - dw-24F; 7 - Sz3-1; 8 - h26-1; 9 - dp-complex; 10 - M(2)z; 11 - Sz64-1; 12 - Sz59-1; 13 - DTS; 14 - M(2)S1; 15 - tkv; 16 - cl.

Df(2L)cl^{h4} deletes the 25D6 to 25F3-4 region (Figure 4e). The 25D6,7 and 25F5 thin bands we attributed to the uncertainty of mapping.

Dp(2;1)B19. The duplication limits are 9B14-15/25F1-2 to 24D4/9C1-2 (Figure 4g). Figure 4f shows that the 9C1-2 band is located to the right of the duplication.

T(2;3)dp^{h27} was induced together with the series of deficiencies dp^{hx}. According to the EM data (Figure 5a-c), the breakpoints of translocation are: 91D4-5/24F4-7 to 32B1-2/91E1-2. We also asssume that the break may be located in the left part of the 25A1-4 band.

Figure 6 summarizes the data of EM mapping. The genetic analysis of the deficiences described permitted to find 10 complementation groups to the left of the 25A1-4 complex (Szidonya & Reuter, unpubl.) which are included in the Df(2L)M11 (the 24D8 to 25A1-4 region). The comparison of these data with cytological ones leads to the conclusion that 4 complementation groups, Sz3-1, h261, dp and M(2)z, are located in the 24F1-2 to 25A1-4 interval; the remaining 6 groups are situated in the 24D7-24E5 interval. In the 25 section the tkv mutation can be localized in the 25D4-5 band and cl in the 25E1-5 region.

Acknowledgements: We thank Dr. I.F. Zhimulev for useful discussions and E.M. Baricheva for help in this work.

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Cytogenetics of suppressor of black.

Five recessive, suppressor of black alleles (su(b)) have been mapped to the distal tip of the X and, on the basis of non-suppression in Df(1)260-1/su(b) females, the suppressor locus was placed proximal to 1B4-6 (Sherald 1981). However, since this deficiency is male lethal (Lindsley & Grell 1968) and FM4 contains su(b)$^+$, some ambiguity was indicated by the sporadic appearance of non-black males and Bar, yellow, black females from the cross; Df(1)260-1/FM4; b x su(b)/Y; b. The Df(1)260-1/FM4 stock was analyzed, and the exceptional progeny were determined to result from nondisjunction caused by the presence of a Y chromosome in some of the females: Single pair matings of Df(1)260-1/FM4 x Df(1)y^{RT10}/y^{2}scY were used to generate Df(1)260-1/y^{2}scY males which were crossed individually to C(1)DX/y^{2}scY females. In four out of 12 crosses, some y^1 female progeny (C(1)DX/Y) were found, indicating an unmarked Y derived from the grandparental females. While this analysis was performed for the stock obtained from Bowling Green, other crosses with the same stock from the Pasadena collection have also produced similar results suggestive of a free Y. The crossing scheme above was also used to isolate three stocks in which Df(1)260-1 was carried in males with only the y^{2}sc marked Y, and each stock was used to construct Df(1)260-1/su(b); +/b females for the test cross (Table 1, top). No black males were found in the progeny of these crosses, and the recovery of black females again confirms that Df(1)260-1 contains su(b)$^+$.

Table 1. Non-black and black progeny from the cross: Df/su(b); b/+ X su(b)/Y; b for ten terminal X deficiencies.

		Males	Females	
Deficiency	Breakpoint*	Non-black	Non-black	Black
Df(1)260-1	1B4-6			
stock a		203	430	145
stock b		198	337	127
stock c		133	277	75
Df(1)y^{RT8}	-**	379	565	0
Df(1)y^{RT10}	1B7-10	223	447	0
Df(1)y^{RT12}	1B7-9	87	151	0
Df(1)y^{RT18}	1B7-10	430	712	0
Df(1)y^{RT19}	1B7-10	410	844	0
Df(1)y^{RT20}	-**	321	497	0
Df(1)y^{RT21}	1B10-12	258	498	0
Df(1)y^{RT30}	1B3-8	586	832	0
Df(1)svr	1B10-13	414	752	0

* Breakpoints for Df(1)svr and Df(1)260-1 are from Lindsley & Grell (1968); other breakpoint determinations were by Drs. G.Lefevre, J. Lim, E. Strobel and/or H. Gyurkovics. For explanation of the three Df(1)260-1 stocks, see text.

**Breakpoints for these deficiences have not been cytologically determined; genetically they are y$^-$ sc$^-$ 1(1)EC7$^+$ su(s)$^+$.

To determine the cytogenetic position of the suppressor locus more precisely, nine terminal, male lethal deficiencies were tested by crossing Df/su(b); +/b females to su(b)/Y; b males (Table 1, bottom). The same suppressor allele, su(b)^{31}, was used for all tests and, except for Df(1)y^{RT12}, the progeny were obtained from duplicate crosses. While black homozygotes should constitute approximately half the progeny, none of the males displayed a black phenotype because all viable male zygotes were hemizygous for su(b). The absence of black female progeny indicated that all nine of the deficiencies uncovered the su(b) locus which must, therefore, lie at least die distal to 1B8. Taken together with the data from Df(1)260-1, these data indicate that su(b) lies between 1B4-6 and 1B8. This provides cytological confirmation for the previously reported nonallelism between su(b) and suppressor of sable, since su(s) is proximal to 1B10-13 (Lindsley & Grell 1968). Assuming that the su(b) alleles represent reisolations of the lost su(b) mutation reported by Plough (1927), this finding is at variance with the arbitrary cytogenetic map in Lindsley & Grell (1968); the black suppressor locus should be distal to su(s).

The locus for the silver mutation may also fall within this region, either in 1B7-8 or slightly to either side (Lefevre 1981). However, no evidence has been obtained for allelism between svr and su(b). None of the su(b) alleles have a visible phenotype, and svr/su(b)^{31} heterozygotes were found to be wild type, not silver. Nor was black suppression observed for svr, either as a hemizygote or in combination with su(b)^{31}.

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Silva, F.J. and J.L. Ménsua. Universidad de Valencia, Spain. Synergistic effects between eye-color genes of **Drosophila melanogaster.**

Double mutants have been commonly used as a means of studying synergistic effects between genes. The pigmentary pattern of a double eye-color mutant is generally the sum of the effects of each mutant; however, in some cases, a more extreme interaction appears. This might happen when two mutants are defective for the same mechanism. For this reason paired combinations of eye-color mutants were synthesized in order to find out between which mutants this kind of interaction occurs. The pigmentary pattern of the double mutants was studied by separation on thin-layer chromatography and quantification of the fluorescent spots as described by Ferre et al. (1983).

Table 1. Percentages of eye-pigments and related metabolites (Or-R has aribtrarily received the values of 100). NDP (neodrosopterin), DP (drosopterin), IDP (isodrosopterin), ADP (aurodrosopterin), ADHP (6-acetyl-dihydrohomopterin), SP (sepiapterin), H_2BP (dihydrobiopterin), BP (biopterin), PTE (pterin) and IXP (isoxanthopterin). Xanthurenic acid and other metabolites were not detected in any of the double mutants. ND (not detected).

Strain	NDP	DP	IDP	ADP	ADHP	SP	H_2BP	BP	PTE	IXP
g ltd	TRACE	TRACE	ND	ND	ND	ND	ND	ND	ND	ND
rb ltd	TRACE	TRACE	TRACE	ND	ND	ND	ND	ND	ND	ND
ltd rs^2	5±3	3±1	3±1	6±1	TRACE	52±7	37±6	101±13	ND	13±1
ltd ca	TRACE	2±0	2±0	5±1	TRACE	53±15	11±2	75±15	TRACE	1±1
rb p	13±5	5±1	3±0	9±1	48±10	74±5	65±2	248±49	TRACE	24±0
ltd p	8±3	5±0	3±1	7±1	TRACE	56±8	40±5	75±9	ND	22±0
ltd st	21±5	18±4	16±4	24±4	128±12	165±17	41±2	237±31	11±1	32±3
g st	58±30	5±1	3±0	9±0	16±9	116±2	81±34	109±13	ND	44±9
ca	5±0	5±0	7±0	17±0	90±16	113±4	47±8	180±36	11±4	50±6
g	17±2	19±1	19±1	42±3	52±9	206±10	53±3	178±9	24±3	54±2
ltd	43±1	36±3	35±4	63±2	203±8	203±33	95±6	165±31	26±7	33±8
p	18±3	20±4	19±3	43±5	86±18	98±14	72±7	184±28	30±6	59±6
rb	34±2	25±3	36±3	55±3	94±12	162±7	50±5	149±14	37±4	29±2
rs^2	43±15	50±18	44±13	100±10	129±23	144±23	91±6	154±22	52±7	89±7
st	80±2	93±6	88±8	95±2	116±37	98±7	94±4	78±5	60±5	69±1

Quantification of the pteridines of some of the double mutants studied is shown in Table 1 in percentages of wild type. All of them present lower quantities of pteridines than expected (see in Table 1 the pigmentary pattern of the simple mutants) but the more drastic interactions occur between the mutant lightoid (ltd) and the mutants garnet (g), ruby (rb) and rose^2 (rs^2). The double mutants g ltd and rb ltd have an almost completely white eye-color due to the absence of pteridines and brown pigment. This result contrasts with the higher expected quantities of pteridines for these double mutants. Then it seems that the gene ltd is acting synergistically with the genes g, rb and rs^2 to produce the wild type pigmentary pattern. It is likely that these genes are required for the normal transport of eye-pigments precursors since some researchers have found that the mutants ltd and g have defects in the transport of brown pigment precursors (Sullivan & Sullivan 1975; Howells et al. 1977), although they did not test pteridine precursors.

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Simmons, M.J., J.D. Raymond, M.J. Boedigheimer, E.A. Drier, G.J. Kocur, R.J. Morrison and J.R. Zunt. University of Minnesota, St. Paul, USNA.

Stablization of unstable X chromosomes.

Lim (1979, 1981) has described the behavior of an unstable X chromosome, called Uc, which was derived from an EMS mutagenesis experiment. The instability of this chromosome was evident from its high rate of lethal mutation and from its propensity to accumulate rearrangements, including deficiencies, duplications, inversions and combinations of these. Detailed genetic and cytological analysis of mutant and rearranged chromosomes established that there was a hot spot for mutation between 6F and 7C on the polytene chromosome map and that there was a corresponding hot spot for breakage in the 6F1-2 doublet. A later study by Lim et al. (1983) showed that a derivative of Uc could impart all these properties to a stable chromosome by co-existing with it in females for one generation. However, this phenomenon of homologue destablization did not involve recombination between Uc and its partner; rather, the stable partner of Uc acquired its Uc-like instability, including the mutational and breakage specificity, without any detectable recombination. All of these properties suggested the activity of a transposable element, designated the L factor, which resided in Uc.

This note reports experiments which show that derivatives of Uc which had previously been unstable have now stabilized. The method in each experiment was to measure the rate of occurrence of recessive X-linked lethal mutations. Other chromosomes unrelated to Uc were also tested to serve as controls. In every case, the lethal mutation rate was low, implying that the agent which previously had caused many lethals had been inactivated or lost. Further tests established that a high lethal mutation rate could not be restored by outcrossing Uc-bearing males to an unrelated stock, and that Uc could no longer destabilize another X chromosome in heterozygous females.

The genotype of the Uc chromosome is y^{59b} z w^{i} ct^{6} f; we studied three derivatives with this genotype (referred to as H3, H4, and H7) and one derivative in which the ct mutation had reverted. The latter chromosome is called Uc-ct^{r82}. The chromosomes denoted as H3, H4, and H7 were used in the experiments reported by Lim et al. (1983) and were collectively referred to in that paper simply as Uc-l^{r}. All three of these X chromosomes were descended from a single X, which, in turn, was derived from the original Uc. The name Uc-l^{r} was used by Lim et al. (1983) because the progenitor of H3, H4, and H7 carried a reversion of a lethal mutation which had occurred on Uc (see Laverty & Lim 1982).

The experiments reported by Lim et al. (1983) were performed in 1981 and 1982. Since the beginning of those experiments, two independent sets of stocks with the chromosomes H3, H4 and H7 have been maintained, one set in our laboratory and the other in the laboratory of J.K. Lim. We shall refer to the set maintained by Dr. Lim with the cognate labels H30, H40 and H70.

A stock carrying Uc-ct^{r82} was established by Dr. Lim in 1982 from ct^{+} males which appeared in a culture of one of the many Uc derivatives he maintains. This ct reversion did not appear in any of the stocks of H30, H40 or H70. Our ct^{r82} stock was derived from Lim's stock in 1984. Throughout their history, all the Uc derivatives discussed in this report have been maintained in the male line by using C(1)DX, y f/Y females.

The procedure for detecting newly arisen recessive X-linked lethal mutations made use of the FM7, y^{31d} sc^{8} sn^{x2} B balancer chromosome. The males whose mutation rate was to be measured were mated individually to FM7/sc^{7} females at 21°; the sc^{7} chromosome in these females has a recessive lethal mutation that was induced with EMS (see Simmons et al. 1980). Then single FM7/"X" daughters which had been able to mate with their FM7 brothers were placed individually in culture tubes to produce the next generation. The culture methods of Simmons et al. (1980) were used. The flies emerging from these cultures were scored for "X"/Y males, where the "X" chromosome is the one under test. The absence of these males indicated that a recessive X-linked lethal mutation had occurred. All cases of suspected lethals were retested to confirm the initial result. A chromosome was classified as lethal if the frequency of the indicator males was less than 0.025 in the retest. For convenience, we staggered the tests at weekly intervals. Therefore, in each experiment measurements were made on males of two age classes: young males, mated within two days of eclosion, and old males, mated exactly one week later. This permitted a check for any possible effects of age on the X-linked lethal mutation rate. Since no such effects were found, the data from the two age classes have been pooled in the results that follow.

The first set of experiments involved males collected directly from the various Uc stocks and from unrelated stocks that served as controls. The latter included a stock homozygous for the X chromosome In(1)Δ49, pn v B^{M1}, abbreviated simply as Δ49, and two stocks homozygous for the m mutation. One of these had the background of the Canton S wild-type strain and was therefore called m-CS, while the other was obtained from Dr. Lim and was therefore called m-Lim. This latter stock was the one used by Lim et al. (1983) in their homologue destablization experiments; their results showed that in the absence of any association with Uc, the m-Lim chromosome had a very low mutation rate and therefore could be regarded as intrinsically stable.

Table 1. X-linked lethal mutation rates by strain.

Experiment	No.males tested	Chromosomes tested, No.	No.indep. events	No. lethals	Mutation rate (%)
Uc derived:					
H3	309	3,105	2	2	0.06
H4	331	3,357	1	1	0.03
H7	426	3,628	0	0	--
H30	312	2,493	0	0	--
H40	306	2,605	1	1	0.04
H70	349	3,502	0	0	--
ct^{r82}	126	970	0	0	--
Controls:					
Δ 49	304	2,568	0	0	--
m-CS	263	2,148	2	2	0.09
m-Lim	287	2,482	1	1	0.04

Table 2. X-linked lethal mutation rate produced by outcrossing.

Experiment	No.males tested	Chromosomes tested, No.	No.indep. events	No. lethals	Mutation rate (%)
H3	433	4,268	3	3	0.07
H4	454	4,570	2	2	0.04
H7	458	4,652	4	4	0.09
m-CS	297	3,263	0	0	--
m-Lim	304	3,269	0	0	--

Table 3. X-linked lethal mutation rates from induction experiments.

Experiment	No.males tested	Chromosomes tested, No.	No.indep. events	No. lethals	Mutation rate (%)
Series A:					
m-CS	324	3,566	0	0	--
H7(CS)	360	2,539	1	2	0.08
m-Lim	209	2,019	5	10	0.49
H7(Lim)	364	2,619	4	4	0.15
Series B:					
m-CS	414	3,968	5	6	0.15
m-Lim	417	4,141	0	0	--
Series C:					
m-CS	399	4,033	3	3	0.07
m-Lim	210	2,186	2	2	0.09

Table 4. X-linked lethal mutation rates by line from experiments with derivatives of Uc-l^r (Lim et al. 1983). Mutation rates were calculated using the unweighted procedure of Engels (1979).

Uc-line	No.males tested	Chromosomes tested, No.	No.indep. events	No. lethals	Mutation rate ± s.e. (%)
H3-9	68	2,051	15	48	2.29 ± 0.64
H4-21	70	2,064	7	13	0.57 ± 0.24
H4-24	70	2,186	2	2	0.08 ± 0.06
H4-27	67	2,013	14	71	3.83 ± 1.20
H4-39	68	1,962	25	118	5.96 ± 1.70
H4-40	70	2,204	23	116	4.84 ± 1.25
H7-65	70	2,086	14	43	2.33 ± 0.72
H7-66	70	2,054	9	29	1.57 ± 0.61
H7-72	70	1,974	17	90	4.79 ± 1.60
H7-72	70	2,125	16	55	2.60 ± 1.03

The results of X-linked lethal tests with males taken from these various Uc and control stocks are given in Table 1. The mutation rates are uniformly low so there is no evidence for any mutational instability.

The second set of experiments involved males collected from various outcrosses. Males from a particular stock were crossed at 25° to C(1)DX, y f/Y females from an unrelated stock and their sons were used in the X-linked lethal tests. The purpose was to see if outcrossing induced X chromosome instability. The results of these experiments are given in Table 2. Again, the mutation rates are low so there is no evidence that this outcrossing procedure induces chromosome instability.

The last set of experiments involved males collected from various crosses between the H7 stock and the two m stocks. Two of these crosses were between m males (either m-CS or m-Lim) and C(1)DX, y f/Y females from the H7 stock; these produced m sons whose X-linked lethal mutation rates were estimated. We refer to these males as belonging to the "B" series of experiments. The purpose of this series was to see if any autosomally or maternally transmitted factor from the H7 stock could cause instability of the m chromosome. As is evident from the data given in Table 3, no instability was detected. Therefore, we conclude that no such autosomal or maternal factor is present in the H7 stock.

There were two other series of experiments in this final set. All were derived from crosses between H7 males and m/m females, which in turn came from either the m-CS or m-Lim stocks. One series of experiments made use of the m sons derived from these matings; in Table 3 these are referred to as the "C" series. Series "C" was carried out to see of some paternal contribution from the H7 stock could induce instability in either of the m chromosomes. Another series involved sons derived from the daughters of these matings. Since the daughters are genotypically Uc/m, they would be expected to produce many types of recombinants. We mated these females to their m brothers and collected only two types of sons (Uc or m), which were then tested for X-linked lethal production. The tests of these males comprise the "A" series of experiments. The purpose of this series was to see if the H7 chromosome became unstable in H7/m females and, further, to see if H7 could destabilize either of the m chromosomes without recombining with them.

The results of series "A" and "C" are given in Table 3, along with those from series "B". In no case was there any indication that the X-linked lethal mutation rate was high. Therefore we conclude that the H7 chromosome is not destabilized in females carrying one of the m chromosomes, and furthermore, that in such females, H7 does not destabilize either of these m chromosomes.

All of these results demonstrate that the previously unstable chromosomes H3, H4 and H7 have stabilized. In addition, Uc-ct^{r82}, although derived from an unstable chromosome, is now stable. This extensive evidence for the loss of instability was foreshadowed by some of the data collected by Lim et al. (1983), who measured the X-linked lethal mutation rates of seven genotypic classes of males derived from Uc/m females, as in series "A" above. See their paper for the details of the genotypes. Here we simply note that the Uc chromosome which produced these males came from either the H3, H4 or H7 stocks. An analysis of the mutation rates of the ten sublines which Lim et al. (1983) used is given in Table 4. While eight of the ten lines had X-linked lethal mutation rates greater than 1.5%, two of the lines had conspicuously lower rates. These were both derived from stock H4, suggesting that the agent responsible for the high mutability of that stock had disappeared or been inactivated in these two lines.

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Drosophilidae of Uttarakhand, U.P., India.

The Uttarakhand region includes eight border districts of Uttar Pradesh, viz. Dehra Dun, Tehri-Garhwal, Uttarkashi, Pauri-Garhwal, Chamoli, Nainital, Almora and Pithoragarh. Uttarakhand region is peculiar in its animal as well as plant fauna.

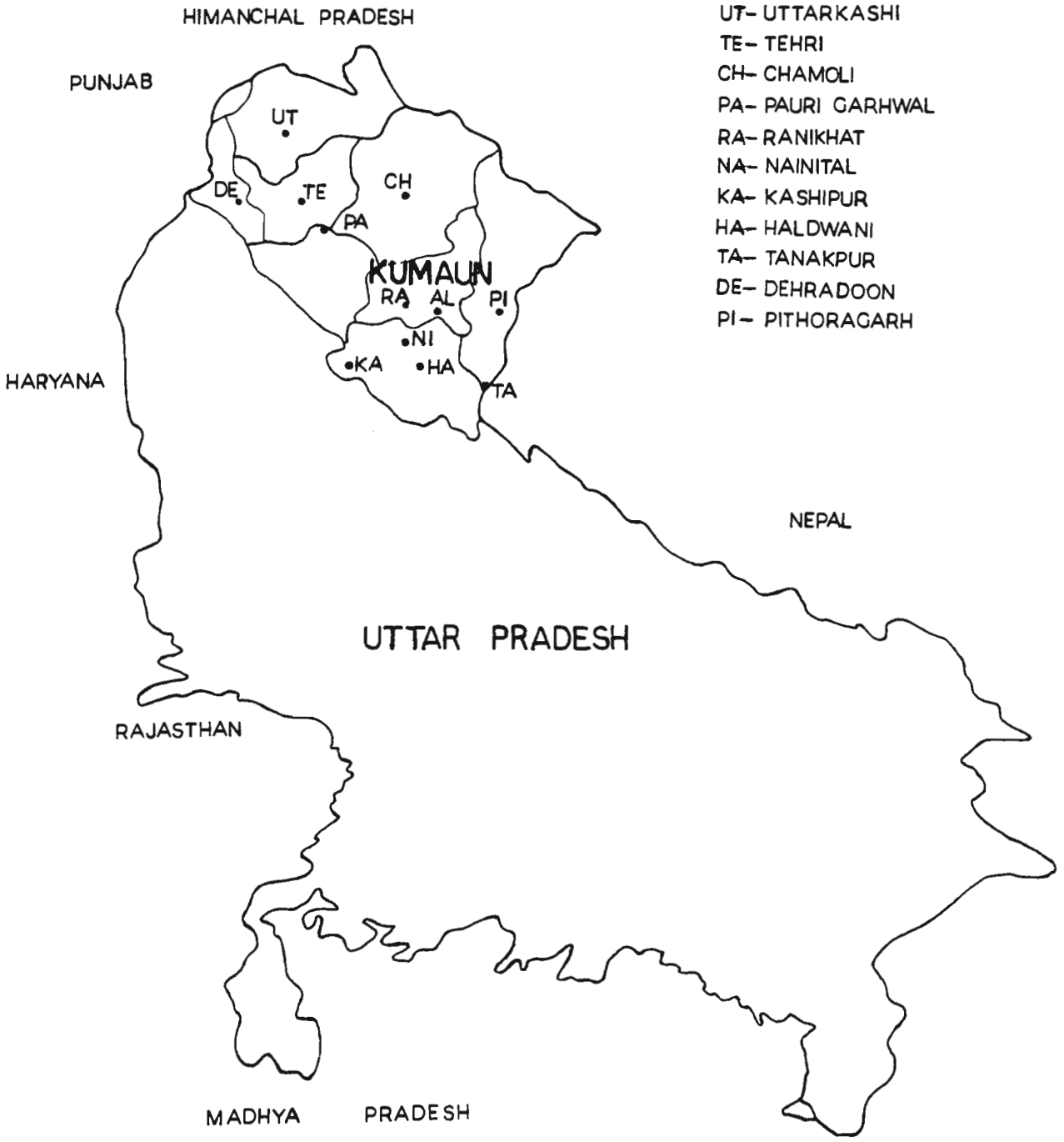
Despite the fact that the family Drosophilidae and that the genus Drosophila occupy a very important position among the organisms which are used as a material for genetic studies, very little has been known so far about the Drosophilid fauna of India. About 170 species belonging to different genera have been discovered so far by different workers in this country (Parshad & Paika 1964; Gupta 1969, 1970, 1971, 1972, 1973, 1974a,b; Singh & Gupta 1974, 1977a, b,c, 1979). During the present studies a thorough survey of Uttarakhand region, which is a completely virgin field from the above viewpoint, was undertaken. A preliminary survey of this region has yielded some interesting results regarding the distribution of different Drosophilid species.

A total of 2689 species were collected. The name of the species, their number and collection locality is shown in Table 1 and Figure 1. From our collection data we desire to point out that some of the species, viz. **D.immigrans, D.kikkawai, D.lacertosa, D.melanogaster, D.jambulina, D.nepalensis** and **D.malerkotliana**, were collected in large number while some species, viz. **D.ananassae, D.bipectinata** and **D.nasuta** which are very common in other parts of the country, were completely absent.

Table 1. Species name, number and collection locality.

Species	No. flies	Collection locality
D.immigrans	402	Pithoragarh, Nainital, Maikoti (Chamoli), Rampur
D.buski	110	Pithoragarh, Nainital, Maikoti (Chamoli)
D.kikkawai	220	Nainital, Pithoragarh, Thalasu (Chamoli)
D.repleta	52	Nainital, Pithoragarh
D.lacertosa	215	Nainital, Pithoragarh, Thalasu (Chamoli)
D.melanogaster	405	Nainital, Pithoragarh, Satarakhal (Chamoli)
D.jambulina	327	Nainital, Almora, Maikoti (Chamoli)
D.nepalensis	510	Nainital, Tanakpur, Chamoli
D.malerkotliana	206	Nainital, Pithoragarh, Rampur
D.takahashi	120	Nainital, Pithoragarh, Almora
Leucophenga interrupta	10	Pauna (Chamoli)
* **D.** sp.	35	Chamoli
* **D.** sp.	11	Tanakpur
* **D.** sp.	21	Tanakpur
* **D.** sp.	16	Tanakpur
* **D.** sp.	2	Chamoli
* **D.** sp.	25	Chamoli
* **Mycodrosophila** sp.	2	Chamoli
Total	2689	

* species not identified; supposed to be a new species.



Map of Uttar Pradesh, India [B.K. Singh et al.]

References: Gupta, J.P. 1969, Proc. Zool. Soc. (Calcutta) 22:53-61; _____ 1970, Proc. Ind. Nat. Sci.Acad. (B)36:62-70; _____ 1971, Amer. Midl. Natur. 86(2):493-496; _____ 1972, Orient. Insects 6(4):491-494; _____ 1973, DIS 50:112; _____ 1974a, Indian Biologist V(3):7-30; _____ 1974b, J. Ent. (B) 43(2):209-215; Parshad, R. & I.J. Paika 1964, Res. Bull. Punjab Univ. 15:225-252; Singh, B.K. & J.P. Gupta 1974, Ind. J. Zool. 15(1):23-26; _____ 1977a, Orient. Insects 11(2):237-241; _____ 1977b, Ent. Month. Mag. Oxford 113:71-78; _____ 1977c, Proc. Zool. Soc. 30:31-38; _____ 1979, Entomon 4(2):167-172.

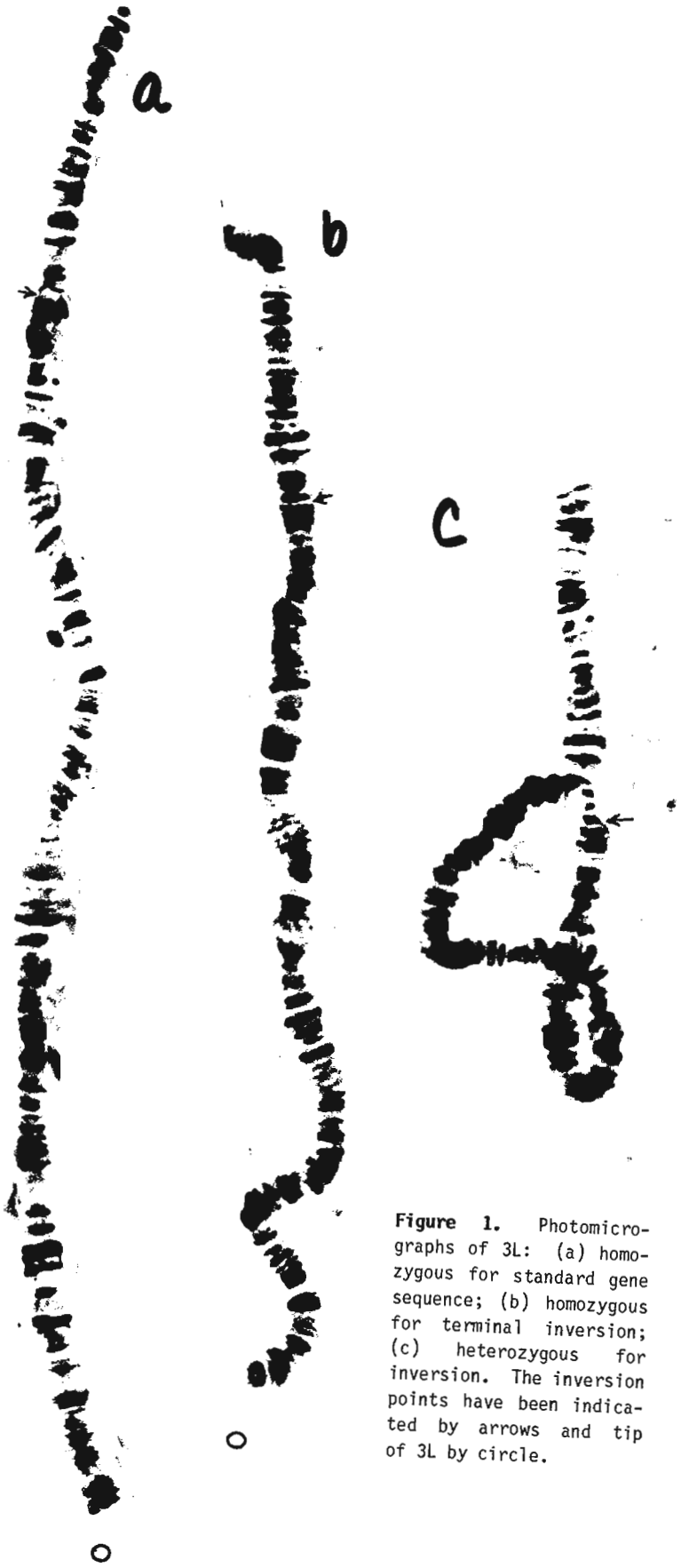


Figure 1. Photomicrographs of 3L: (a) homozygous for standard gene sequence; (b) homozygous for terminal inversion; (c) heterozygous for inversion. The inversion points have been indicated by arrows and tip of 3L by circle.

Singh, B.N. Banaras Hindu University, Varanasi, India. On the terminal inversion in **Drosophila ananassae.**

The terminal inversion in the left arm of the third chromosome of **D.ananassae,** first described by Kaufmann (1936) is coextensive with the species (Shirai & Moriwaki 1952; Futch 1966; Singh 1970). On the basis of differences in the banding patterns of free ends of homologous chromosomes in the heterozygous state, Kaufmann (1936) suggested that the inversion is terminal. However, complete pairing is not evident in the figures shown by Kaufmann (1936) and the banding pattern has not been compared in homozygous karyotypes. The break points of inversion have been located in the reference map of polytene chromosomes of **D.ananassae** constructed by different investigators (Seecof 1957; Futch 1966; Ray-Chaudhuri & Jha 1966; Hinton & Downs 1975).

Ray-Chaudhuri & Jha (1966) suggested that this inversion is not terminal but subterminal on the basis of similarities in the banding pattern of free ends in heterozygous karyotypes. However, the banding pattern of tips was not compared between standard and inversion homokaryotypes by these authors. Due to the occurrence of terminal inversion in 3L, three karyotypes--standard homozygote, heterozygote and inversion homozygote--are distinguishable. In the present analysis, the banding pattern was compared in three karyotypes in order to determine whether the inversion is terminal or subterminal because of theoretical implications regarding the origin of such chromosomal rearrangements.

The photomicrographs of different 3L karyotypes are given in Figure 1. 3L homozygous for standard gene arrangement is shown in Fig. 1a. Fig. 1b shows 3L homozygous for inversion. Comparison of the banding patterns of the tips between standard and inversion homozygotes clearly shows that they are completely different to the last visible band. The photomicrograph of 3L heterozygous for inversion presented in Fig. 1c shows that both ends are paired to the last visible band. Thus complete pairing is evident in the heterozygous karyotype and no free ends are seen. On the basis

of the Figure presented here, the inversion extends from the tip of the arm to the end of the 75A section in the reference map constructed by Hinton & Downs (1975).

The complete pairing of both ends to the last visible bands in the heterozygote and the differences in banding patterns of tips in standard and inversion homozygotes suggest that the inversion is completely terminal.

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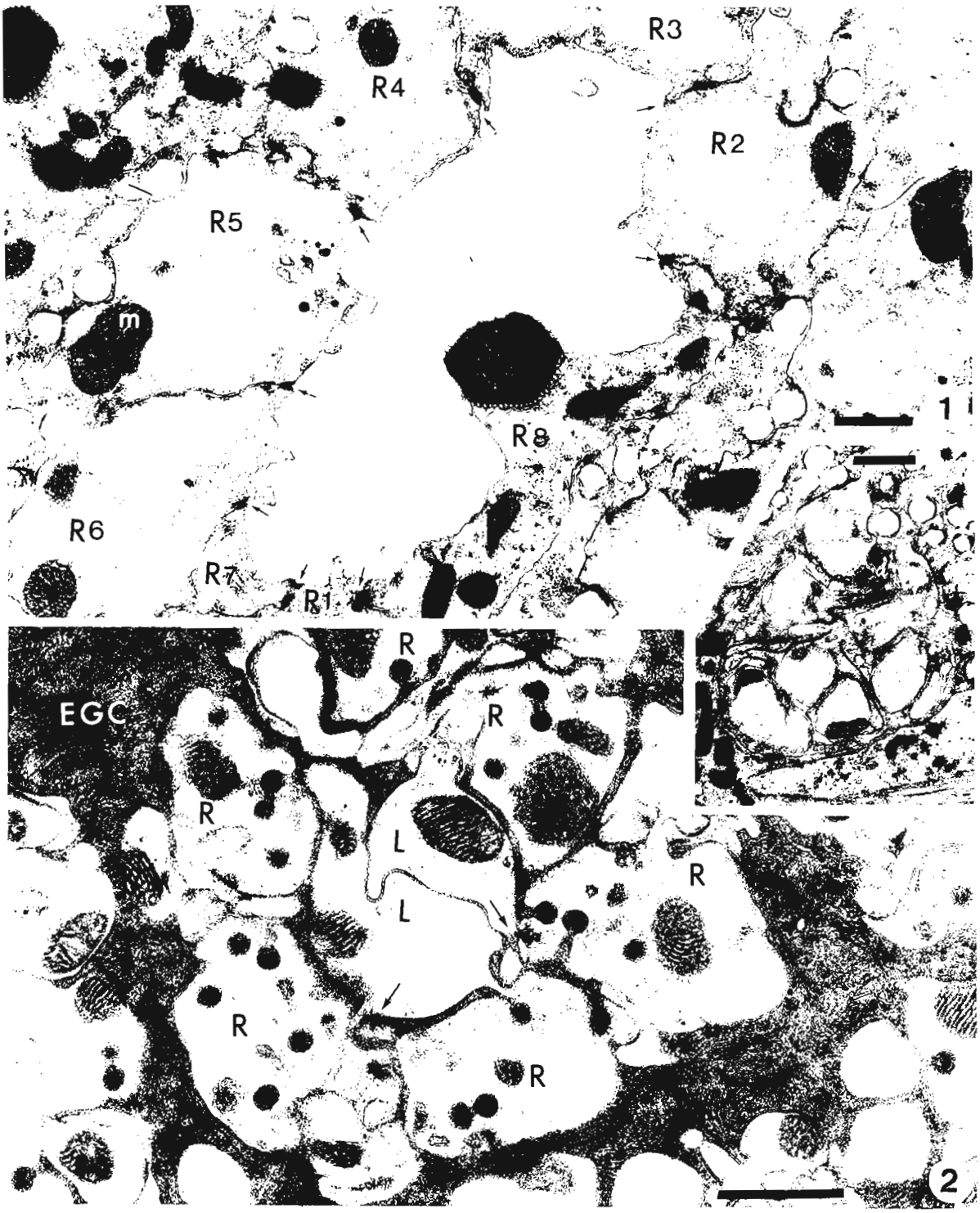
Stark, W.S. and S.D. Carlson.* University of Missouri, Columbia, USNA. *University of Wisconsin, Madison, USNA. Retinal degeneration in rdgB^{KS222} is blocked by ora^{JK84} which lacks photoreceptor organelles.

Harris et al. (1976) characterized a number of mutants with defects in the visual receptors which have since been used for studies of photoreceptor development, function and input. In the rdgB^{KS222} mutant, isolated by Hotta & Benzer (1970), the R1-6 receptors of the compound eye degenerate after exposure to light while the other types, R7 and R8, are spared (Harris et al. 1976; Harris & Stark 1977). This mutant has been utilized in over a score of studies (see Stark & Carlson 1982; Stark et al. 1983; Chen & Stark 1983 for references). Another mutant, ora^{JK84}, isolated by Koenig & Merriam (1977), eliminates the most of the photopigment containing organelle (rhabdomere) in each R1-6 cell (Harris et al. 1976). The latter strain has proven equally useful in vision research (see Stark & Carlson 1983 for references).

In a study of the mechanism of light induced degeneration in rdgB flies, rdgB was combined with ora (Harris & Stark 1977). The experimental strategy, after creating this double mutant, was to deprive rdgB flies of photic stimulation by ridding them of their photoreceptive organelle (and thus their capacity to effectively absorb photons). Earlier, Harris & Stark (1977) presented electron micrographs suggesting that ora protected against degeneration in rdgB but not completely. However, those micrographs were technically less than what is possible nearly a decade later. Furthermore, the literature now contains a wealth of electron micrographs which suggest, among other things, that fixation of the fly compound eye is not always achieved successfully. The purpose of this study is to reexamine the protection of rdgB offered by ora now that better EM technique is available. Our work was prompted in part by our extensive and recent ultrastructural examinations of rdgB (Stark & Carlson 1982) and of ora (Stark & Carlson 1983). In addition to the receptor somata, we examined the receptor axon terminals in the optic cartridges of the lamina ganglionaris, the first optic neuropile. This synaptic region is an important area to study because the R1-6 terminals in rdgB are especially sensitive to the deliterious effects of light (Stark & Carlson 1982; Carlson et al. 1984). An abstract of our findings is available (Carlson & Stark 1985).

We isolated rdgB;ora flies upon eclosion and then fixed them either promptly or after 1, 2 or 3 weeks of aging at 23°C on a 12 hr on, 12 hr off cycle of fluorescent laboratory illumination. Our micrograph are from the High Voltage Electron Microscope Laboratory, an NIH Biotechnology Resource, at the University of Wisconsin, Madison. Details of our techniques are given elsewhere (e.g., Stark & Carlson 1983).

Our results are presented in the accompanying plate. All scale bars = 1 µm. Figure 1 is a cross section through one ommatidium in the proximal part of the peripheral retina of an rdgB;ora fly aged 2 weeks. At this level R8 is the only cell possessing a rhabdomere, while the R1-6 receptors (except at the most distal level) lack this organelle. R7's short rhabdomere is distal and in tandem to that of R8. Thus one sees only the R7 axon in this plane. All R cells are joined to each other by belt desmosomes (arrows). The R1-6 cells are ultrastructually normal, i.e., they look precisely like R1-6 cells from flies with only the ora mutation, without signs of degeneration. That diagnosis is backed by checking the mitochondria (m) in this field; they are normal appearing,numerous and particularly conspicuous. Attenuated processes of pigmented glial cells (secondary pigment cells) spatially isolate the ommatidia. "Holes" in these cells represent sites where pigment granules were apparently extracted during the process of fixation. The insert shows the fasicle of 8 axons from one ommatidium just beneath the basement membrane which separates the retina from the lamina. This bundle (pseudocartridge) looks completely normal, similar or identical to that in wild type and ora flies. Figure 2 shows one cross sectioned optic cartridge in the lamina. Six R1-6 terminals make synapses mainly onto the processes of several laminar monopolar neurons. The most conspicuous of these are the pair of electron lucent L1 and L2 cells located in the core region surrounded by the six photoreceptor (R) terminals. The electron dense surround is made up of processes from 3 so called "epithelial" glial cells (EGC). These glial cells insert capitate projections (seen as small, dark spheres or mushroom-like structures) into the R1-6 terminals. Occasionally, T-bar synapses are seen and noted (arrows). The axonal projection to and termination of R1-6 axons in this area look completely normal and identical to that found



Stark & Carlson, "Retinal degeneration ...."

Figure 1. Cross-section through one ommatidium in the proximal part of the peripheral retina of an rdgB;ora fly aged 2 weeks.

Figure 2. One cross-sectioned optic cartridge in the lamina.

in wild type, a situation which is also the case for non-rdgB ora flies. Thus the R1-6 terminals, which are an especially sensitive indicator of degeneration in rdgB flies, survive well in rdgB; ora flies. We observed no differences in this regard with aging under the cyclic illumination, i.e., among flies fixed when newly emerged or after being aged 1, 2 or 3 weeks. To ascertain whether the stock we studied retained rdgB in combination with ora, we did a genetic cross to separate rdgB from ora. Optical examinations of the progeny verified that degeneration still occurred meaning rdgB, still present, had been uncovered from ora's protection. In summary, we conclude that ora affords fairly complete protection against degeneration in rdgB flies, even more protection than suggested by Harris & Stark (1977). In functional terms, genetic elimination of the photopigment and its organelle prevents light-induced degeneration by depriving rdgB flies of photic stimulation.

Supported in part by the High Voltage Electron Microscope (HVEM) Laboratory, and Faculty Development and Graduate Research Council and NSF (BNS 84 11103) grants to WSS.

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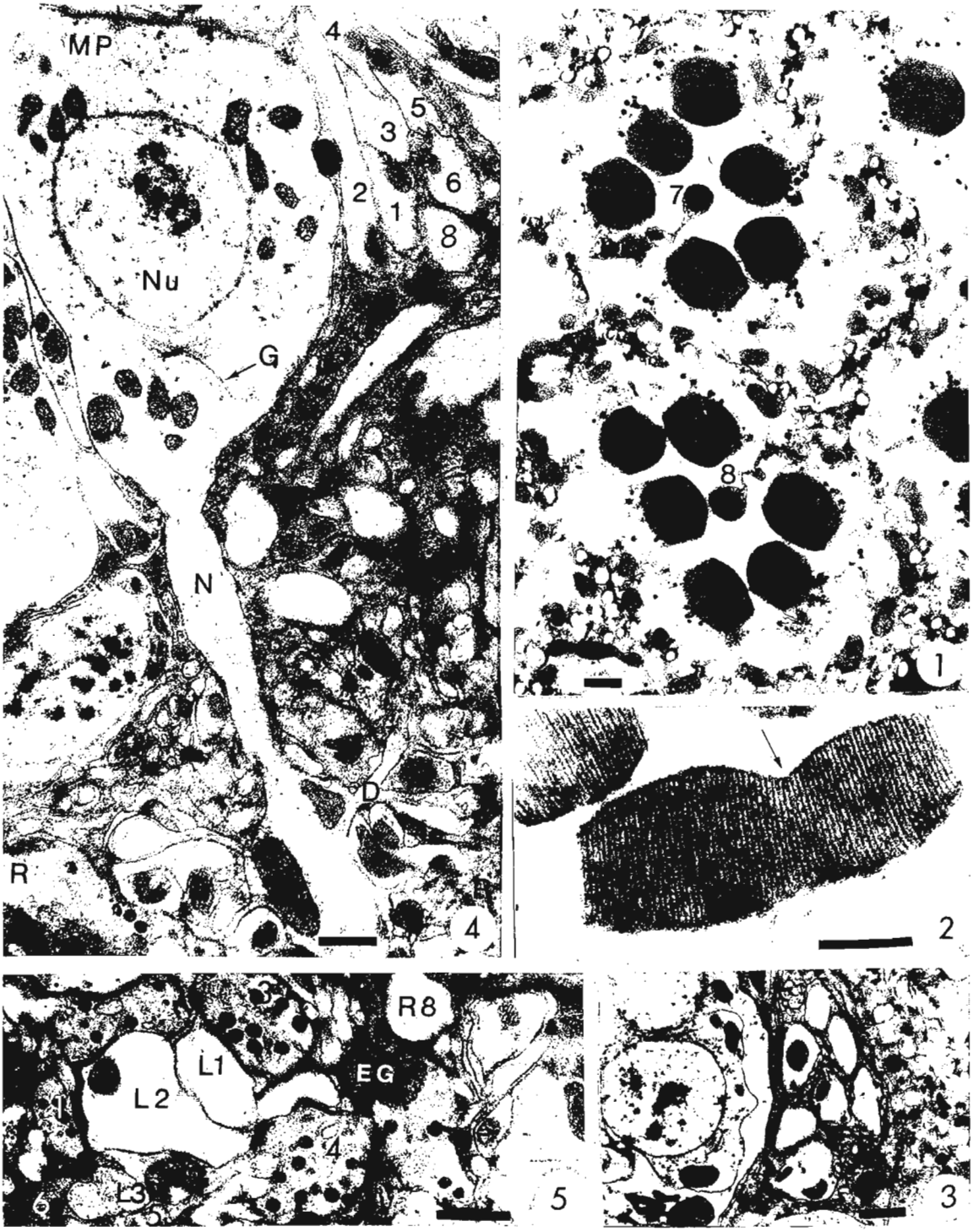
Stark, W.S. and S.D. Carlson.* University of Missouri, Columbia, USNA. *University of Wisconsin, Madison, USNA. Ultrastructure of the compound eye and first order neuropile in the sevenless (sev) mutant of **D.melanogaster.**

The sevenless (sev) mutant has been extremely useful in the analysis of function and development of photoreceptors in the Drosophila visual system (Harris et al. 1976; Stark et al. 1976, 1979; Heisenberg & Buchner 1977; Hu & Stark 1977, 1980; Jacob et al. 1977; Stark 1977; Labhart 1977; Fischbach & Reichart 1978; Hu et al. 1978; Campos-Ortega et al. 1979; Fischbach 1979; Willmund 1979; Stark & Johnson 1980; Miller et al. 1981; Broda & Willmund 1981; Coombe 1984). In the compound eye of a nonmutant fly, each ommatidium contains 8 receptor cells of 3 types: R1-6 has photoreceptive rhabdomeres peripherally oriented around the central axis of the ommatidium while R7 and R8 have distal and proximal central rhabdomeres, respectively. The rhabdomere of each photoreceptor cell is normally never fused to that of its neighboring cell in the open rhabdomere configuration of the fly retina. In the sev mutant the R7 cell does not form (Campos-Ortega et al. 1979) and thus it lacks R7 function (e.g., Harris et al. 1976). Recently, we initiated a program of ultrastructural research on Drosophila visual mutants, sponsored in part by the High Voltage Electron Microscope (HVEM) Laboratory, an NIH Biotechnology Resource, at The University of Wisconsin, Madison. We reexamined sev and a white-eyed strain (w sev) because there was so little ultrastructural data extant on that mutant. Our preliminary observations are now presented which include the premier micrographs from its lamina ganglionaris (first optic neuropile and thus the first synaptic relay station of the retinal projection).

In general, distal sections through the peripheral retina showed 6 rhabdomeres (R1-6) while proximal sections showed 7 (R1-6 and R8) as expected. A rare exception to this generalization is shown in Fig. 1 (bar = 1 μm). Here a distal ommatidium is cross sectioned to reveal the trapezoidal arrangement of photoreception cells, and we suggest that the central cell is R7 as labeled. The neighboring ommatidium is at a more proximal level, and from its shape and orientation it is proposed that the central cell is R8. It is possible that the designated R7 cell is really an R8 cell in the R7 position (see Campos-Ortega et al. 1979) but our observations suggest that a few isolated ommatidia near the equator may actually have R7. The electron dense small spheres in close apposition to the rhabdomeric microvilli are the ommochrome pigments of the retinula cells which migrate during light and dark adaptation in the red eyed fly. Very deep in the retina, there are quite few ommatidia which apparently lack some of the 7 expected rhabdomeres. On closer examination (Fig. 2, sev, bar = 1 μm), it is shown that rhabdomeres of adjacent R1-6 cells occasionally fuse (arrow). It should also be noted that rhabdomeric fusions are found in which the two sets of conjoined microvilli are at very different angles. At this proximal level, intraretinular pigment granules, which are typically concentrated distally, are not seen, as expected, even though this section (Fig. 2) is from a red eyed fly.

Beneath the basement membrane, the axons of each ommatidium are bundled into pseudocartridges (Fig. 3, w sev, bar = 1 μm). As expected, most of these fascicles have 7 axons. Fig. 4 (w sev, bar = 1 μm) shows a longitudinal section through the distal level of the first optic neuropile. A type 1 monopolar interneuron (L1 or L2) is revealed for a considerable length: through the perikaryon (MP), nucleus (Nu), neck (N) and up to a point where dendrites (D) project laterally to retinular cell terminals (R). The L cell soma shows numerous mitochondria as well as a Golgi body (G). The R cell terminal is identified by capitate projections. Another pseudocartridge, obliquely sectioned, lies just outside the L cell's perikaryon



Stark & Carlson, "Ultrastructure of the compound eye ....": **Figures 1 through 5.**

at the upper right of this figure. Axon numbering in this pseudocartridge is arbitrary. The perikaryon of a satellite glial cell is positioned to the left of the monopolar's neck. We have on rare occasions observed a pseudocartridge with 8 axons, confirming our contention that a few ommatidium may have 8 photoreceptor cells, including R7. Fig. 5 (sev, bar = 1 µm) shows a cross sectioned optic cartridge with its centrally localized L1, L2 and L3 interneurons surrounded by R1-6 axon terminals. To the right, and surrounded by an electron dense epithelial glial cell (EG) is the R8 axon without its R7 counterpart which is normally contiguous to R8. The closely paired R7 and R8 axons normally pass through the lamina without synapse on their way to terminations in the second neuropile, the medulla.

In summary, our micrographs show that, for the most part, the compound eye lacks the R7 cell. In addition, we have depicted the occasional fused condition of rhabdomeres in the peripheral retina of the compound eye. The structure of the lamina ganglionaris is unaffected by the loss of R7 except that R7's axon, which normally traverses the lamina, is lacking.

Supported in part by the High Voltage Electron Microscope (HVEM) Laboratory, and by Faculty Development and Graduate Research Council and NSF (BNS 84 11103) grants to WSS.

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Stocker, R.F. and M. Schorderet. University of Fribourg, Switzerland. Sensory projections of homoeotically transformed eyes in **D.melanogaster.**

Homoeotic mutants transform particular body parts into others and thereby create displaced sensory neurons, whose axons reach the central nervous system (CNS) at abnormal sites. This allows one to study how the position of sensory neurons affects the specificity of their central connections (Palka & Ghysen 1982; Stocker 1982). The pattern of sensory projections from all types of homoeotic structures tested so far are remarkably constant, i.e., terminals occupy their normal projection area or centers of serially homologous structures. Similar observations have been made in the pattern of afferents from surgically generated ectopic appendages (Stocker & Schmid, in prep.). These data suggest that sensory axons are able to recognize specific structures in the CNS, even if they arrive through an ectopic nerve.

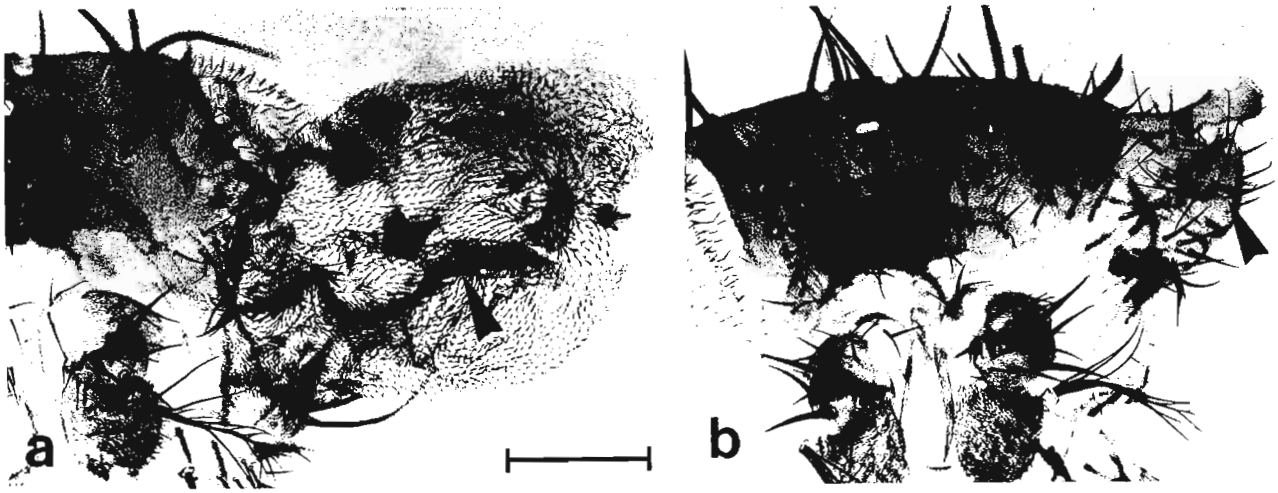

Figure 1. Homoeotically transformed eyes in ey^{opt} (**a**) and tuh (**b**). The wing tissue present in ey^{opt} is characterized by sensilla of the triple row (arrowhead). In tuh transformation leads to sensory bristles typical of abdominal tergites (arrowhead). Bar 200 µm.

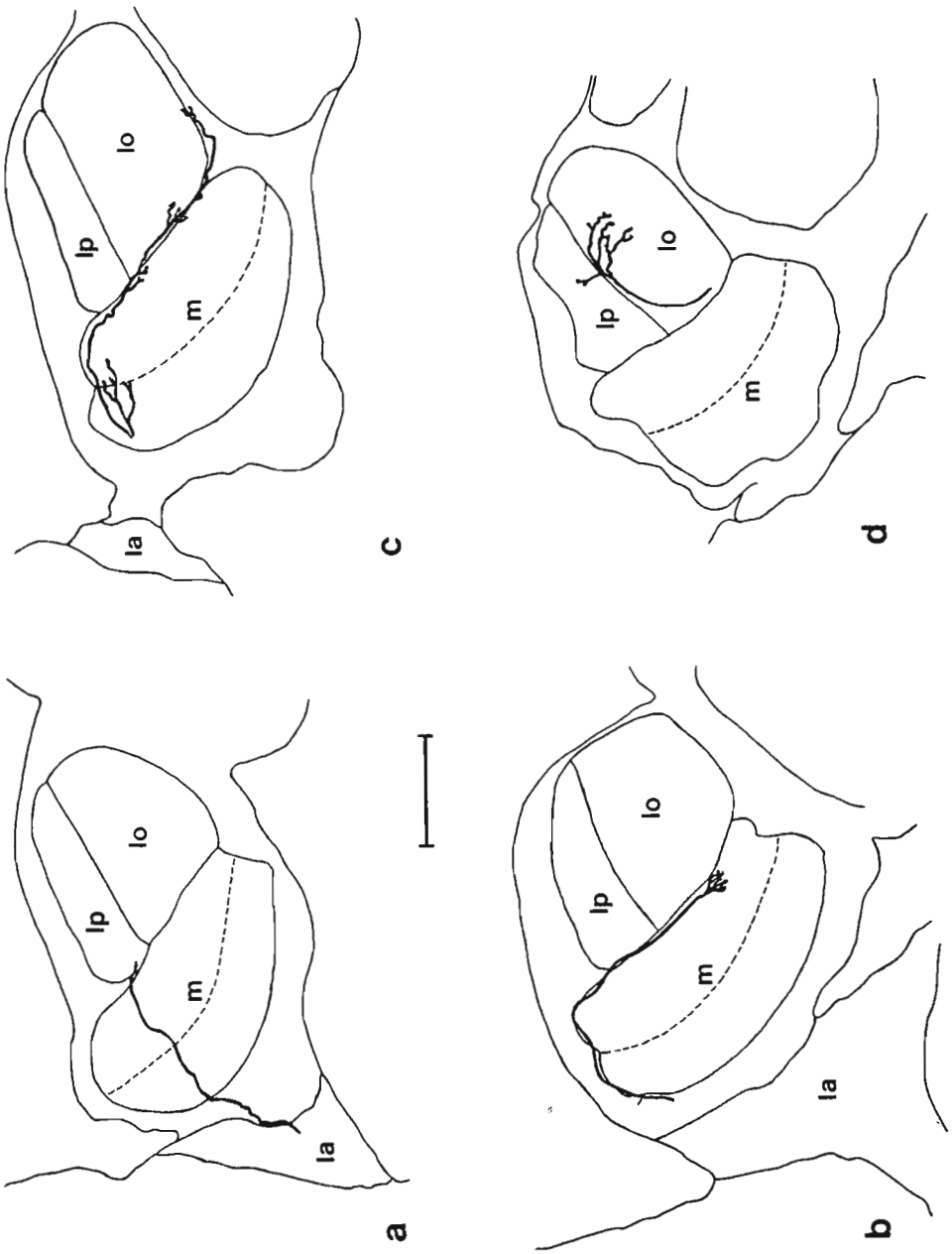

Figure 2. Camera lucida drawings of sensory projections from transformed eyes in the visual ganglia. **a**: A single axon in tuh extends through the medulla (m) into the lobula/lobula plate region (lo/lp). **b, c**: Other tuh fibers by-pass the medulla neuropil at its posterior border and project along the line which divides the medulla and the lobula. **d**: A single axon in ey^{opt} terminates in both lobula and lobula plate. la: lamina. posterior is on top. Bar 50 µm.

Most of the transformed appendages can be homologized with the original appendage on morphological criteria, such as wings and halteres, or legs and antennae. Moreover, the sensory projection centers involved in these systems reflect the metameric organization (though in the head ganglia individual neuromeres are less obvious than in the thoracic or abdominal CNS). One might therefore argue that the observed terminal patterns are due to structural homologies between the peripheral tissues on the one hand and their projection centers on the other. Here we are studying a system in which particular epidermal structures are replaced by completely different structures, and we allow the sensory axons to grow into a CNS region of distinct architecture. This is the case in the mutants eyeless-ophthalmoptera (ey^{opt}) and tumorous-head (tuh), which transform the eye partially into wing or abdominal tergite structures, respectively (Fig. 1; Postlethwait et al. 1972; Postlethwait 1974). Will the sensory fibers from these transformed tissues, which are now confronted with the highly ordered lattice of the visual ganglia, still project in a specific manner?

Females of the genotypes ey^{opt} (4-2.0) and tuh-1;tuh-3 (1-64.5;3-58.5) were used. For light microscopic examination of the transformed structures, heads were treated with 5% KOH and mounted in Faure's solution. The projection patterns were observed by filling the sensory axons with horseradish peroxidase (HRP, Sigma type VI, 10% w/v, or type II, 20%) after amputation of the transformed tissues. The HRP marker was visualized according to the standard DAB technique (Coggshall 1978). Heads were embedded in soft Epon and serially sectioned at 20 µm.

The phenotype of the transformations varies in both mutants. We followed projections only from appendages with well discernible sensory structures, i.e., double or triple row bristles in ey^{opt} and tergite bristles in tuh (Fig. 1). Surprisingly, only 11% of the HRP-fillings in ey^{opt} and 7% in tuh yielded labelled sensory axons in the brain. This low success rate is unlikely to be due to incomplete diffusion of the tracer molecule, since filling from another transformed region in tuh, the leg-like antenna, resulted in 68% of labelled preparations. We rather suspect that in the majority of cases afferent connections are not formed at all, probably because structures essential for guiding sensory axons to the optic ganglia are missing. The presence of such cues could depend on the precise localization of the transformed tissue in the eye, although there is apparently no simple correlation between the two phenomena. A complete lack of afferent projections is reported of "extra" eyes in the mutant two-faced (Kankel 1984).

Retinula cells R1-6 of normal eye ommatidia send their axons into the first order visual neuropil, the lamina; fibers from R7 and R8 pass through the lamina and terminate at a particular depth in the second neuropil, the medulla (Fischbach 1983). Both types of axons are characterized by peculiar terminal swellings. The fibers observed in fillings from transformed eyes (8 fillings in ey^{opt} and 5 in tuh) are of different shape. In both mutants they appear to pass through the lamina and then to choose one of two pathways: some of them project into the medulla and from there further into the third visual neuropil, the lobula/lobula plate complex (Fig. 2a). Others by-pass the medulla neuropil at its ventral border to reach the lobula/lobula plate complex from posterior (Fig. 2b,c). We never saw axon branching in the lamina, and only rarely in the medulla. In contrast, in the lobula and lobula plate the fibers arborize extensively, but the patterns produced are quite variable. In ey^{opt} mutants fibers extend mostly along the bundle which divides lobula and lobula plate and send off terminals into both of these neuropils (Fig.2d). Axons passing along the medulla/lobula border have been found in tuh; they branch at the anterior end of this region (Fig. 2b,c). Other fibers in tuh project into the lobula and lobula plate like those in ey^{opt}. In addition to these patterns, two completely different types of projections were observed. In ey^{opt} a single axon was seen to enter the brain via the antennal nerve and to terminate without branching in the mechanosensory antennal center. In the periphery this axon followed the "nervus tegumentalis" (Hertweck 1931) which innervates the dorsal wall of the head close to the eye margin. In another case in tuh, axons originating in the eye region reached the suboesophageal ganglion via a side branch of the labial nerve and arborized immediately after arriving in the CNS, below the center of normal proboscis fibers present in the same nerve. Thus, the terminals appear to avoid the center of a heterologous structure.

In fills from transformed eye tissue, labelled axons similar in shape and distribution to those of retinula cells R7-8 were occasionally present. These fibers might originate in normal ommatidia from the vicinity of the homoeotic structures and have been accidentally filled because of damage during the amputation of the transformed tissue. Alternatively, they might stem from the homoeotic structures, but have retained their original identity (cf. Palka & Ghysen 1982).

Our data show that sensory axons from eyes transformed into wing or tergite structures arrive at different sites in the CNS of the head. Moreover, their terminal pattern is most variable. We conclude that sensory axons may choose any available peripheral nerve as guidance cue to reach the CNS (cf. Ghysen & Deak 1978). The fact that terminals of transformed eye structures are not restricted to the normal eye projection regions lamina and medulla, and the absence of a reproducible pattern suggest that the fibers are unable to recognize specific structures in the CNS. These data seem to contradict the pattern specificity observed in other homoeotic systems or in surgical transplants. The specificity of the projections has been explained by the existence of a surface marker common to all projection centers of homologous appendages (Stocker 1982; Stocker & Schmid, in prep.). However, it is conceivable that the visual ganglia possess the same general marker as wing or tergite projection centers, but that retinula fibers relate to additional cues specific for lamina and medulla which homoeotically transformed axons are unable to read. In a comparable situation, sensory terminals from leg-like antennae in aristapedia mutants distribute randomly in the antennal lobe; this is quite in contrast to the well-patterned wildtype antennal terminals (Stocker & Lawrence 1981). We conclude that sensory axons depend on different mechanisms for tracing particular projection centers and for establishing their terminal arborization pattern.

We thank Dr. J.A. Campos-Ortega (Cologne) for discussion and Dr. R. Foelix (Fribourg) for critical comments.

References: Coggshall, J.C. 1978, J. Comp. Neurol. 177:707; Fischbach, K.F. 1983, Habil. Thesis, Wurzburg, F.R.G.; Ghysen, A. & I.I. Deak 1978, Roux' Arch. 184:273; Hertweck, H. 1931, Z. wiss. Zool. 139:559; Kankel, D.R. 1984, Proc. XV Int. Congr. Genet. 3:205; Palka, J. & A. Ghysen 1982, TINS 5:382; Postlethwait,J.H. 1974, Devl. Biol. 36:212; Postlethwait, J.H. et al. 1972, Devl. Biol. 29:337; Stocker, R.F. 1982, Devl. Biol. 94:31; Stocker, R.F. & P.A. Lawrence 1981, Devl. Biol. 82:224.

Takada, H. Sapporo University, Japan.
Curtonotidae and Drosophilidae from Ussuriysk, U.S.S.R.

Faunistic and taxonomic data of Curtonotidae (Diptera) and Drosophilidae from Dr. A.L. Ozerov, the Zoological Museum, Moscow Lomonosov State University's material collected in Ussuriysk, Ussuri, U.S.S.R., from June 30 to August 31, 1984; of the 8 species of drosophilids flies are new to fauna of Eastern Siberia.

Family Curtonotidae
Genus Curtonotum Macquart
Curtonotum anus (Meigen), 1830.
female, July 12, 1984 (by Dr. Ozerov).

Family Drosophilidae
Genus Amiota Loew
Subgenus Amiota Loew
Amiota (Amiota) **rufescens** (Oldenberg), 1914
male, August 11, 1984 (by Dr. Ozerov).

Subgenus Phortica Shiner
Amiota (Phortica) **conifera takadai** Okada, 1977
male, July 7 and female, August 21, 1984
(by Dr. Ozerov).

Genus Leucophenga Mik
Subgenus Neoleucophenga Oldenberg
Leucophenga (Neoleucophenga)
quinquemaculipennis Okada, 1956.
male, July 9, 1984 (by Dr. Ozerov).

Genus Drosophila Fallen
Subgenus Scaptodrosophila Duda
Drosophila (Scaptodrosophila) **coracina**
Kikkawa & Peng, 1938.
9 females and 7 males, June 30 to August 31, 1984
(by Dr. Ozerov).

Subgenus Hirtodrosophila Duda
Drosophila (Hirtodrosophila) **confusa** Staeger, 1844
2 females, July 2 and a male, July 7, 1984
(by Dr. Ozerov).

Subgenus Sophophora Sturtevant
Drosophila (Sophophora) **auraria** Peng, 1937.
male, August 10, 1984 (by Dr. Ozerov).

Subgenus Drosophila Fallen
Drosophila (Drosophila) **testacea** von Roser, 1840
5 females and 5 males, July 5 to August 26, 1984
(by Dr. Ozerov).

Drosophila (Drosophila) **transversa** Fallen, 1823.
2 males, August 21 and 31, 1984 (by Dr. Ozerov).

Thompson, S.R. Ithaca College, New York USNA. The effect of density on death rates in Drosophila population cages.

Milkman (1975) demonstrated that flies in **Drosophila melanogaster** population cages will preferentially die in empty vials (food cups), if they are provided; such sites were termed "death vials." According to Milkman, one of the causes of emigration of flies to death vials could be the territorial behavior of flies within the cage. For example, flies could establish a particular space or "moving territory" around themselves from which they would keep other flies. The less successful flies would be driven away from desirable space and other resources, and could find themselves in the death vials, space which is not fought over. Flies found in the death vial could be of three types: (1) healthy flies who inadvertently find themselves within the vial and who can escape; (2) moribund flies, those who exhibit erratic, uncoordinated behavior and who cannot escape the death vial; and (3) dead flies. If territoriality plays a role in the movement of flies to the specific death vials, then increasing the cage density should cause an increase in the rate of emigration to death vials. This study examines the effect of increase in cage density on death rates.

Seven-day old adult Oregon-R, equal numbers of males and females, were inserted in population cages (lucite boxes 135 x 110 x 160 mm o.d., on 115 mm supports, screen vented at each end, and fitted with six standard 25 x 95 mm culture vials in two rows), at known densities. All but one of the culture vials contained about 10 ml of a standard cornmeal, molasses, Brewer's yeast, agar medium. The empty vial, which occupied a terminal position, served as a "death vial," and contained a 1 x 4 cm heavy paper strip to ease the departure of healthy flies from the vial. On start-up, flies were made to crawl from clean, empty vials into the cage so that no dead or moribund flies entered the cage. The numbers of dead and moribund flies were enumerated every day for a period of seven days, with a new, clean death vial inserted at each count. Before the death vials were removed for classification, the vials were repeatedly disturbed, "rattled," rotated, etc., to cause relatively healthy flies to leave the death vial. Those flies remaining in the death vial were anesthesized with ethyl ether and classified; moribund flies being those which did not leave the death vial, but which recovered from the ether treatment, and dead flies being those which either did not recover from the ether or which were obviously dead prior to treatment.

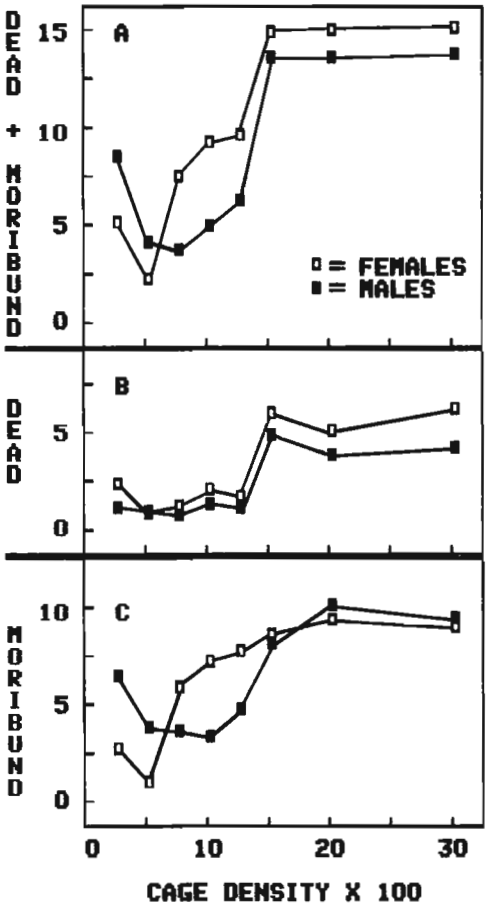

Figure 1. Components of death rate (average %).

The effect of varying cage density on the overall death rate (% dead + moribund) is shown in Figure 1A, where the individual points represent the average of three trials at each density. With the exception of the lowest cage density, 250 flies/cage, increasing density causes an increase in overall death rate up to 1500 flies/cage. At that density, and above, the overall death rate is constant. Females and males differ significantly in overall death rates from 750 through 1250 flies/cage, and at the lowest density, 250 flies/cage, where a very small numerical difference results in a large rate difference. Separation of the two components of death rate into the average % dead and the average % moribund revealed two things. (1) There are two percentage rates of dead flies, one below 1500 flies/cage where there is no significant difference between males (1.33% dead) and females (1.71% dead); and a second rate at cage densities of 1500 flies/cage and above where the average rate for males (4.17%) is significantly different from that of females (5.78%) (see Figure 1B). (2) The sex differences in the overall rate are due to differences in the average % moribund flies below cage densities of 1500 flies/cage (see Figure 1C).

With increasing cage density, two things appear to occur. First, an overall increase in the number of flies emigrating to the death vials at densities up to1500 flies/cage occurs. Secondly, at high densities, 1500 flies/cage or higher, the rate of emigration to the death vial remains constant, but a much higher percentage of dead flies is found in the death vial. This second event may in part be due to the marked increase in absolute numbers of flies in the death vial, a large increase in numbers could cause physical trampling of weaker individuals resulting in their death. The absolute number of flies in the death vials almost doubles between the densities of 1250 (112 flies in the death vial) and 1500 (212 flies in the death vial) and it is at this point that the % dead increases markedly.

From other studies, we know of several other things which will affect the rate at which flies emigrate to death vials. (1) Different mutants have different rates, mutants which confer poor optimotor behavior usually lead to a marked increase in rate, e.g., the mutant ebony (e) has a very high rate. (2) Locomotor activity of flies. Flies with high rates of activity are less likely to be found in the death vial than those with lower rates of activity. In part this accounts for the difference in the male-female emigration rate to the death vial at cage densities between 750 and 1500, as males are more active than females at seven days and apparently escape the death vial more readily. Activity rate may also explain why at low densities (500 or below) more males are found in the death vial; being more active, a male may find himself in such a space, while exploring the cage, and once there may not escape.

Reference: Milkman, Roger 1975, Biol. Bull. 148:274-285.

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A semibalancer system for detecting third chromosome two-arm synthetic lethals in **D.melanogaster.**

To facilitate the detection of two-arm synthetic lethal third chromosomes (synthetic lethals with components in each arm of the metacentric third chromosome), we have developed a "semibalancer" system. It utilizes the old-fashioned third chromosome balancer Me Sb [In(3L)P In(3R)C, Me Sb e I(3)e] in combination with structural heterozygosity for the first chromosome balancer Inscy. The left and right arm inversions suppress third chromosome intra-arm recombination while the first chromosome structural heterozygosity increases crossing over in the centromeric region that is free to recombine.

Table 1. Third chromosome recombination in females heterozygous for the Inscy and Me Sb chromosomes. Frequencies based on counts of 525 offspring for intervals to the left of th and counts of at least 196 offspring for intervals to the right of th. Male offspring hemizygous for Inscy were excluded from the counts.

Map position	0.2	20.0	26.5	43.2	50.0	58.2	62.0	79.1	91.1	100.7
Marker	ve	Me	h	th	cu	Sb	sr	bar-3	ro,tx	ca
% Recombination	0.0	0.0	0.0	10.2	4.1	0.5	0.0*	1.1	0.0	

* Estimated from the Sb-ca recombination value in conjunction with the other values given.

Table 2. The nature of lethality as revealed by classes of recombinant progeny (excluding individuals that exhibit Ser and thus carry TM3).

Recombinant progeny classes present	Nature of lethality
Me	left arm lethal
Sb	right arm lethal
Me & Sb	two-arm synthetic lethal*
neither	left and right arm lethals

*This pattern is also consistent with the presence of a lethal in the th-sr interval, so that conclusive evidence for existence of a two-arm synthetic lethal will require further analysis.

A perfect semibalancer chromosome system would exhibit no recombination within arms but free recombination between arms. Approximate third chromosome recombination frequencies for Incsy/+; Me Sb/-- females appear in Table 1. These frequencies are based on observed recombination with third chromosomes of genotypes ca, ve h th, bar-3 tx, ve h sr e^s ro ca, and ve h th cu sr e^s ro ca. The latter two chromosomes bear a recessive lethal. Note that recombination is very low in the right arm and absent in the left arm, but about normal in the th-Sb interval which contains the centromere (at map position 46.0).

The use of this system to detect two-arm synthetic lethals involves three steps: (1) the production of female Incsy/+; Me Sb/lethal double heterozygotes, (2) the crossing of these females to +/Y; TM3, Sb Ser/lethal males, and (3) the scoring of their offspring for the presence of Me, Sb and Ser. Excluding the Ser progeny (which carry the TM3 balancer), the absence of Me recombinants indicates a right arm lethal, the absence of Sb recombinants indicates a left arm lethal, and the absence of both indicates independent (non-synthetic) lethals in both arms (Table 2). The presence of both Me and Sb recombinants indicates a two-arm synthetic lethal (or a centromeric lethal). Because hemizygosity for the Incsy chromosome sometimes suppresses the expression of Ser, sc y male offspring must be omitted from the progeny counts (a nuisance that might be avoided by substituting TM6 for TM3). Using the system, the lethal associated with the multiply marked chromosomes ve h sr e^s ro ca and ve h th cu sr e^s ro ca proved to lie in the right arm to the right of Sb.

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Another new arrival to the Hawaiian Islands: **Drosophila bryani** Malloch.

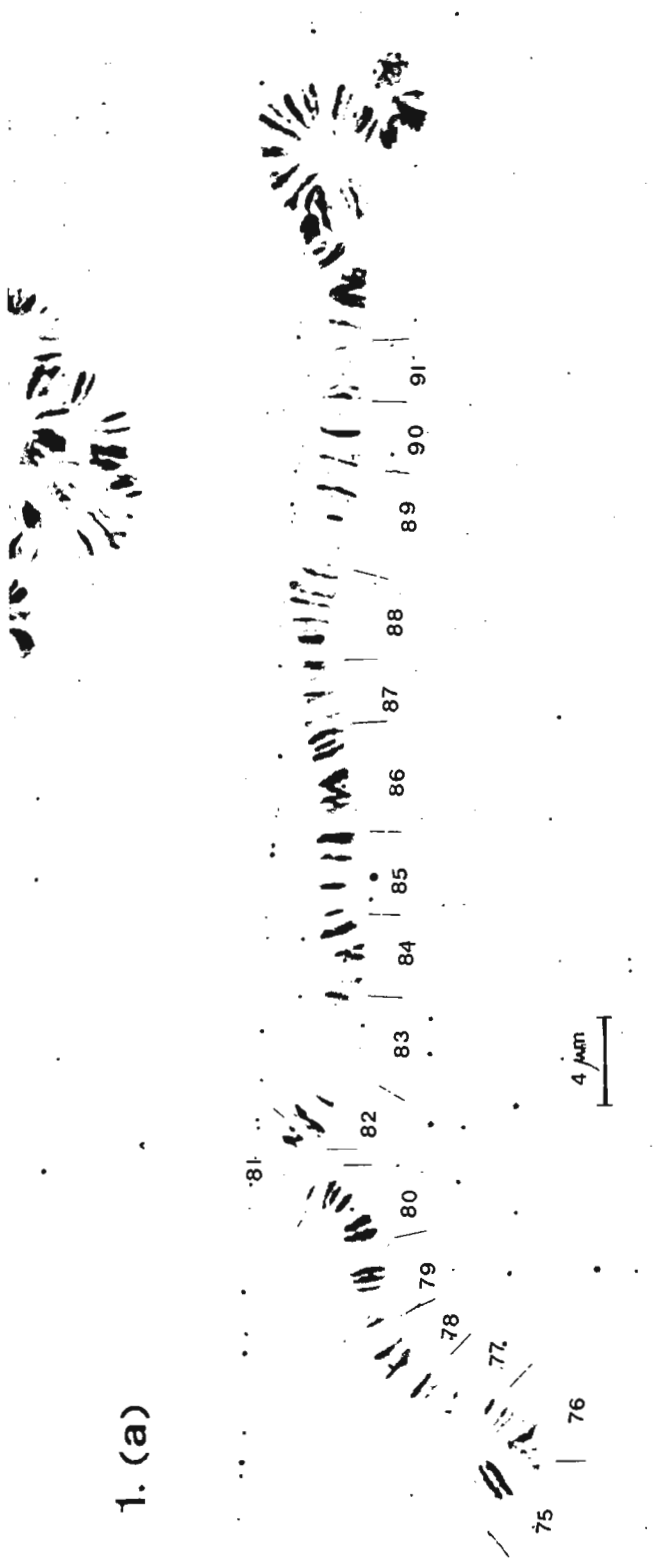
In December of 1984 a male Drosophila was collected in Honolulu behind the University of Hawaii in Manoa Valley. The collection site was under a Chinese banyan tree **Ficus microcarpa** L.S. situated immediately behind the Biomedical Sciences Building at the University. This fly was collected using banana bait in a ground-level container. The fly died shortly after collection. External characteristics indicated that it belongs to the subgenus **Scaptodrosophila.** These characteristics include one pair of enlarged prescutellar acrostichal bristles and a minute pair of second oral bristles. This fly was kindly confirmed for us to be of the species **bryani** by Dr. Ian R. Bock. **Drosophila bryani** has one sibling species, **dicrhomos** Bock, described from Queensland, Australia. It is distinguished from **bryani** by minute genitalial differences.

A female specimen was collected from the same site within two weeks of the male. This female died without laying eggs. Attempts to collect further specimens have not been successful.

The known distribution of **bryani** includes most of Micronesia from Saipan south to Guam, Yap, Palau, the Carolines, Ponape, Kusaie and the Marshall and Gilbert Islands. It is also found in the Philippines and Australia. **Drosophila bryani** has not previously been found in Hawaii and thus these two specimens represent a new record for the islands. This species, the first of the subgenus **Scaptodrosophila** to be recorded, brings the total number of exotic species of the family Drosophilidae in Hawaii to 26: 17 of these belong to the genus Drosophila.

References: Bock, I.R. 1976, Austr. J. Zool. Suppl. Serv. No. 40:1-105; Wheeler, M.R. & H. Takada 1964, Insects of Micronesia 14(6):163-242.

Torramilans, X. and E. Juan. Universidad de Barcelona, Spain. Surface spreading of **Drosophila subobscura** polytene chromosomes.



The technique of Kalisch (1981, 1983) was applied to spread the chromosomes of **D.subobscura**, but the pretreatment solution of propionic and citric acid in several proportions proved inadequate to allow the spreading of the chromosomes on the urea drop.

The spreading was obtained by pretreating the chromosomes with 50% acetic acid.

Eggs were placed in cornmeal agar medium (50 eggs/30 cm^3 medium) at 17°C until 2nd instar larvae developed. Then the cultures were transferred to 13°C.

Glands were excised in 50% acetic acid, mechanically destroyed with a drawn glass rod and pretreated in the same solution for 45 min.

Other parts of the process are the same as in Kalisch (1981).

Acknowledgements. We are indebted to Dr. E.W. Kalisch who has kindly helped us with this technique. Thanks are also given to Dr. Prevosti for his support and to M. Papaceit for the squash photomicrograph.

References: Kalisch, W.E. & K. Hagele 1981, Eur. J. Cell Biol. 23: 317-320; Kalisch, W.E. & T. Whitmore 1983, Cytobios 37: 37-43; Kunze-Muhl, E. & E. Muller 1958, Chromosoma (Berl.) 9: 559-570.

Figure 1(a). Squash preparation of **Drosophila subobscura** 0_{std} chromosome. Total magnification: X 2.880. Number of bands observed (for the equivalent region in Fig.1(b)): 133. Section(s) and numbers in both figures correspond to the published map of Künze-Mühl & Müller (1958).

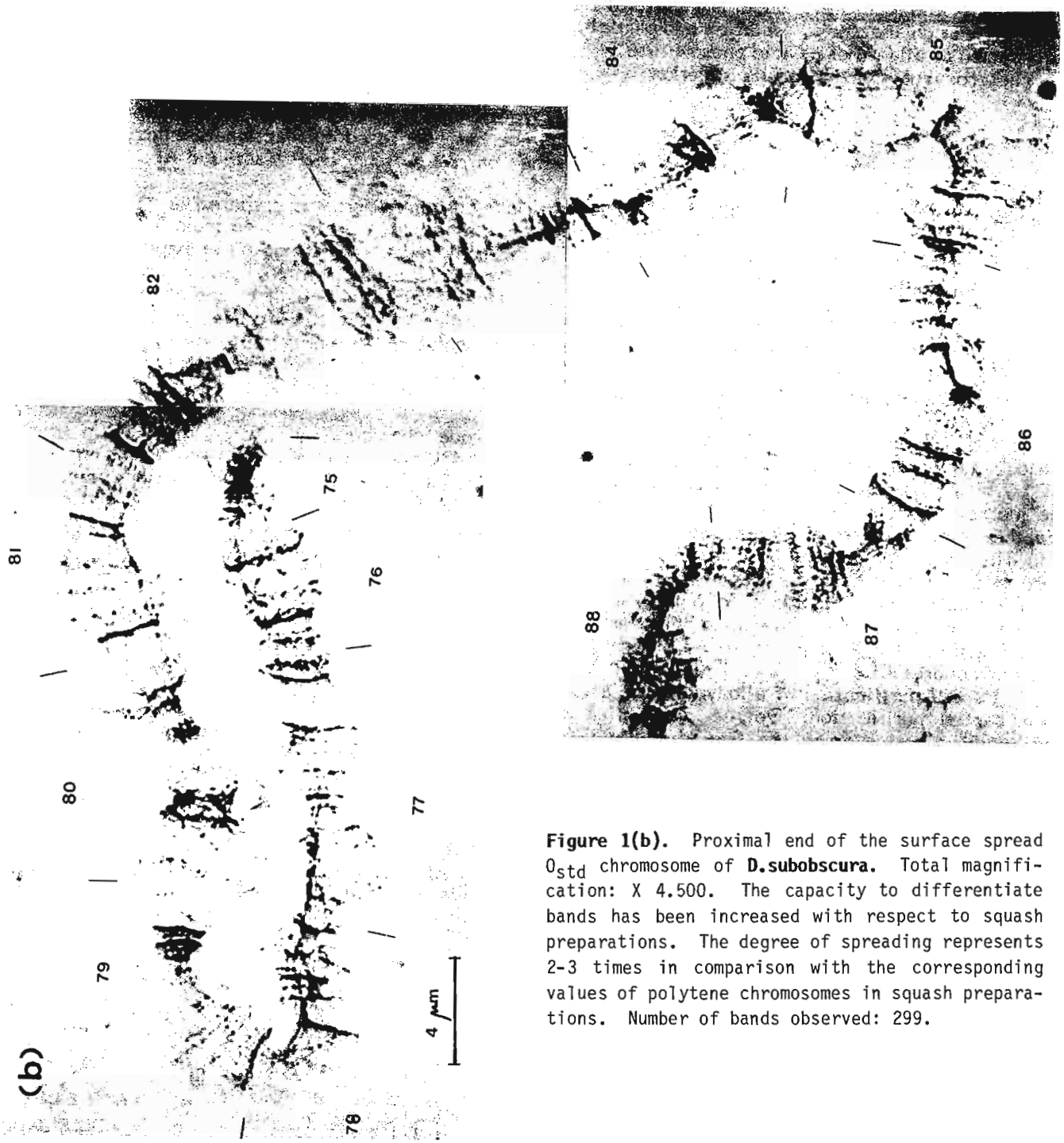


Figure 1(b). Proximal end of the surface spread O_{std} chromosome of **D.subobscura.** Total magnification: X 4.500. The capacity to differentiate bands has been increased with respect to squash preparations. The degree of spreading represents 2-3 times in comparison with the corresponding values of polytene chromosomes in squash preparations. Number of bands observed: 299.

Trehan, K.S. and K.S. Gill. Punjab Agricultural University, Ludhiana, India. Isolation and partial purification of allozymes of acid phosphatase from heterozygotes of **Drosophila melerkotliana.**

Isolation of allozymes: Characterisation of heteromultimers, produced in hybrids, is of prime importance to elucidate molecular basis of heterosis and developmental homeostasis. We have developed a simple starch gel electrophoretic technique to fractionate homomultimeric and heteromultimeric allozymes from heterozygotes. Presently this technique has been used to fractionate allozymes of acid phosphatase (a dimeric enzyme) synthesised under the control of Acph-$1^{1.05}$ (fast) and Acph-$1^{0.95}$ (slow) alleles.

Using Tris buffer (Poulik 1957), starch gel of 15% concentration was prepared in 17 cm x 14 cm x 1 cm glass tray (Trehan 1971). After the gel had set, glass strips were removed from 14 cm sides, and from each of these sides, a 2 cm wide gel was cut and removed. A Whatman filter paper (3 mm) strip (henceforth called isolation strip) measuring 6 cm x 1 cm was loaded with 0.1 ml of crude extract (1 g of flies crushed in 2 ml of glass-distilled water), and placed against the middle of one of the cut surfaces of the gel. Two strips (henceforth called marker strips) loaded with the same crude extract were placed one on each side of and at a distance of 1 cm from the isolation strip (Fig. 1). One cm wide gel, cut from one of 2 cm wide gels removed earlier, was placed against the loaded side of the gel. Sufficient care was taken to ensure that no air bubble was left in the interfaces between the strips and the gel surfaces.

The loaded gel tray was connected to the electrode chambers by 1.5 cm thick foam sponge, presoaked in borate buffer (Poulik 1957). The gel was run at 4°C for 4 hr at 300 volts and 30 mA current.

After completion of electrophoresis, the gel was cut longitudinally into three portions, separating the marker portions from the isolation portion (Fig. 2). The former were stained for acid phosphatase and then restored to their original positions to localise the position of three allozymes in the isolation gel. The three demarcated portions of the unstained isolation gel, each containing a different allozyme, were cut, adjusted to same weight, and stored in deep freezer. Ten different gels were subjected to the procedure described above to obtain sufficient amount for each of the fractionated extract. Gel portions containing the same allozyme were pooled. The frozen gels were thawed, homogenised and centrifuged at 10,000 x g for 20 min. The fractionated allozymes were tested for their homogeneity by electrophoresis.

Partial purification of allozymes: Isolation of allozymes, described above, simultaneously achieves their partial purification. Degree of purification was determined by estimating the protein contents and specific activities in the fractionated allozyme extracts and the diluted crude extract. Dilution factor in the allozyme gel portions was determined by taking a portion of the gel having a weight equal to that of the three allozyme gel portions and drying it. Distilled water was then added to the crude extract so that it was diluted to same extent as the allozyme extract. The data obtained for protein content and specific activity are given in Table 1.

The total protein in the three fractionated extracts is 48.52 ± 0.92 ug/ml. Fractionation by electrophoresis has, thus removed 329.27 ± 7.58 ug/ml protein and achieved 7.8 times purification in a single step. It may also be noted that different amounts of protein have been removed in different

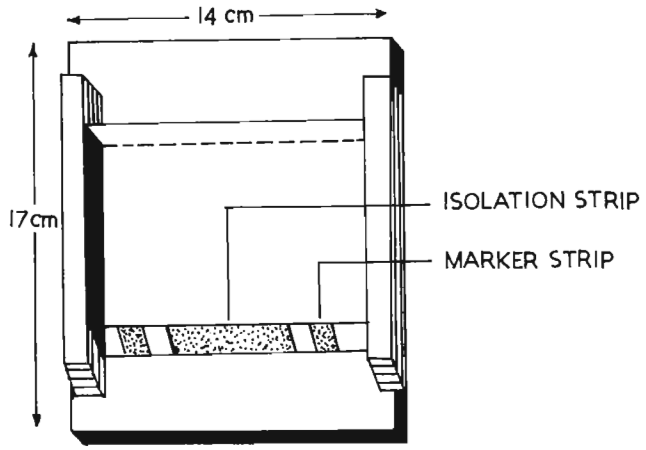


FIGURE 1.

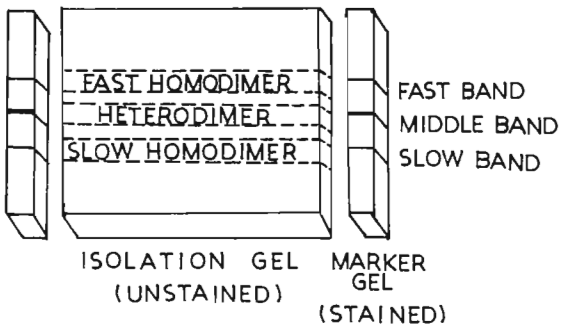


FIGURE 2.

Table 1. Protein contents and specific activities in crude extracts, fractionated allozyme extracts from heterozygotes and partially purified extracts from homozygotes.

	Heterozygotes		Fast homozygotes		Slow homozygotes	
Extract	Prot.cont.	Sp.Act.	Prot.cont.	Sp.Act.	Prot.cont.	Sp.Act.
Crude	379.50±12.60	12.01	379.50±12.60	5.54	379.50±12.60	8.94
Fractions						
Fast homodimer	12.87±0.29	26.83				
Slow homodimer	19.90±0.30	32.25				
Heterodimer	15.85±0.33	74.06				
Partially purified			23.29±0.56	73.17	30.75±0.48	113.72

fractions. The total specific activity in the fractionated extracts is 138.24 uM of napthol-released/min/mg protein and that in crude extract is 12.01. Thus partial purification has resulted in 11.50 times increase in specific activity.

Partial purification of allozymes from homozygotes: The technique described above is applicable and was used for achieving partial purification of allozymes from homozygotes. Partial purification of crude extract from fast homozygotes resulted in removal of 356.4 ± 12.04 ug/ml of protein and thus obtaining 16.29 fold purification for fast homodimer. However, in case of slow homozygotes, less protein was removed (348.75 ± 12.2 ug/ml) than that in fast homozygotes and 12.06 fold purification was observed for slow homodimer. It may be noted that the average protein content is same in the crude extracts from three genotypes, i.e., 379.50 ± 12.68.

References: Trehan, K.S. 1971, M.Sc. Thesis, Panjab Univ, Chandigarh; Poulik, M.D. 1957, Nature 180:1477.

Tsacas, L.* and F.P. Saitta.+ * - Laboratoire de Biologie et Génétique Evolutives, Gif-sur-Yvette, France. + - University of Swaziland, Kwaluseni. [Saitta-present address: 58 Butler St., Pittston, Pennsylvania 18640 USNA). Drosophilids in Swaziland, Southern Africa.

In the most recent publications dealing with the African Drosophilidae (Tsacas 1980 and Tsacas et al. 1981), there is no record of the presence of this family in Swaziland. In 1982, one of us (F.P.S.) made some collections in this country and 6 species were identified: **Drosophila buzzatii** Patterson & Wheeler, **D.hydei** Sturtevant, **D.** sp. of the **latifasciaeformis-finitima** complex, now under study, **Zaprionus collarti** Tsacas, **Z.ghesquierei** Collart. **D.buzzati** was collected from **Opuntia** pads, the two species of the **latifasciaeformia-finitima** complex from the fermenting fruits of **Ficus sansibarica** while the remaining species were collected from fermenting pineapple. Swaziland is the southernmost locality for **Z.ghesquierei.** Up to now, seven species are known from Mozambique and 39 from South Africa, the two neighbouring countries. A large number of species probably remains to be collected in Swaziland as well as in the two other countries.

Ushakumari, A., N.B. Ramachandra and H.A. Ranganath. University of Mysore, India. Relative performance of two strains of **D.nasuta nasuta** (wild and mutant) on different food media.

Drosophila species utilize a variety of sugar sources (Hassett 1948; Taylor & Condra 1983). Recently Ramachandra & Ranganath (1984) have recorded the existence of "subtle" differences between ecologically closely placed and between phylogenetically closely linked forms of Drosophila to the media with different types of sugars. Sang (1972) has reported that the mutant of Drosophila can be used to assess the significant role of nutritional resources on some aspects of the biology of Drosophila.

The present experiment involves two strains of **D.nasuta nasuta**, namely a wild strain and a mutant (white eye) strain. The adaptedness of these two strains has been measured in four different types of media containing either molasses or sucrose or fructose or glucose. The aim of this experiment is to assess and to quantify the ecological differences, if any, under laboratory conditions, between a wild and a mutant strain of **D.n.nasuta.**

These strains were maintained in four different media by adopting the serial transfer technique of Ayala (1965). Four replicates were made for each type of media. The populations were maintained at 21°C for 16 weeks. The mean values for population size, productivity, mortality and flies per bottle were calculated and the same is presented in Table 1. The mean square error from the test of analysis of variance was used to ascertain the inter strain differences in different media.

Table 1. Mean values (for 4 replicates) along with standard errors for population size, productivity, mortality, and flies per bottle in 4 different media for wild and mutant strains of **Drosophila nasuta nasuta.**

D.n.n.		SUGAR:			
Strain	Parameter	Molasses	Sucrose	Glucose	Fructose
Wild	Pop. size	211.30± 9.75	221.12±3.59	234.85±4.77	115.12±7.36
	Productivity	133.75± 3.49	111.32±3.33	119.01±3.91	54.74±4.72
	Mortality	118.73± 4.45	101.00±2.81	110.14±2.80	50.83±4.49
	Flies/bottle	58.27± 2.68	57.99±0.94	61.60±1.25	30.19±1.93
Mutant	Pop. size	125.16±10.52	66.98±1.64	31.26±3.68	37.86±4.74
	Productivity	71.09± 6.64	37.37±2.44	10.95±1.30	15.07±2.11
	Mortality	50.82± 7.54	30.77±1.79	12.46±0.92	11.68±1.31
	Flies/bottle	37.33± 3.70	20.04±0.59	9.79±0.89	11.02±1.38

Adaptedness refers to ability of the carriers of a genotype or a group of genotypes to survive and reproduce in a given environment (Dobzhansky 1968). This provides means for comparing the overall biological performance of one gene pool with another, where both are maintained under similar or defined environmental conditions. The statistical comparison of the performance of the strains under investigation reveals the following:

(a) The population size of wild strain in the media containing either molasses or sucrose or glucose is almost the same without significant differences, while in the media containing fructose, it has attained the least values for population size. Therefore, the relative performance of the wild strain is as follows: Glucose = Sucrose = Molasses > Fructose.

(b) On the other hand, there exists striking differences in the ability of the mutant strain of **D.n.nasuta** to exploit the media with different sugars. This can be represented as follows: Molasses > Sucrose > Fructose = Glucose.

(c) Interstrain comparison reveals, that the wild strain of **D.n.nasuta** has attained better adaptedness values than the mutant strain in all the four types of media under study.

Similarly, the relative viability of five mutant strains and a wild strain of **D.melanogaster** has been studied by Ribo & Prevosti (1969). Rudkin & Schultz (1949) have shown that mutants (yellow, white, Vermilion) of **D.melanogaster** survive better than the wild type on tryptophane diets. Gale (1964) has found that the mutant vestigial was more superior to dumpy mutant and dumpy is superior to Oregon K strain for their inter and intra specific competitive abilities.

Thus, the present investigation has revealed the differential ability of the wild and the mutant strains of **D.n.nasuta** to utilize the media containing different types of sugars.

Acknowledgements: Authors are grateful to Prof. N.B. Krishnamurthy, Head of the Dept. of Zoology, for his help and encouragement; to the University Grants Commission and to the Indian National Science Academy for financial assistance.

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Ushakumari, A. and H.A. Ranganath. University of Mysore, India. Importance of sugar and yeast in the nutrition of Drosophila.

Experiments have been undertaken to study some aspects of nutritional requirements of Drosophila under laboratory conditions. The present research note deals with four different types of wheat cream agar media. They are (a) media without yeast and sugar; (b) media with yeast and without sugar, (c) media with sugar and without yeast, and (d) media with yeast and sugar. Ten different strains of Drosophila were exposed to these media. The populations of these were maintained at 22°C for 16 weeks by adopting the serial transfer technique of Ayala (1965). Four replicates were maintained for each set up. Adaptedness evinced by different strains of Drosophila in these media has been used as a parameter to assess the impact of variations in the constitution of the food media over the reproductive performance of Drosophila. The components of adaptedness measured in the present study are: (a) population size, (b) productivity, (c) mortality, and (d) flies per bottle. The observations of the present study are as follows:

(1) All the strains fail to breed in the media without yeast and sugar.

(2) In the media with yeast and without sugar, it was interesting to note that only **D.sulfurigaster neonasuta** was able to survive and reproduce during the 16 weeks of the experimental period, while all the other strains did not succeed to reproduce in this media.

(3) Drosophila strains under study were able to exploit and reproduce in the other two types of media, namely the media with sugar and without yeast as well as the media with sugar and yeast.

The mean values for the parameters of adaptedness assessed are presented in Table 1 and 2. Almost all the strains of Drosophila under study have attained better adaptedness values in the media with yeast and sugar than in the media with sugar and without yeast.

Thus, the differential impact of different media over the population fitness of Drosophila strains under study is striking. Further experiments are in progress to evaluate the role of different types of sugars and different species of yeast on the biology of these Drosophila strains.

Table 1. Mean values (for four replicates) along with Standard Errors for Population size, Productivity, Mortality, and Flies per bottle in the media with sugar and without yeast for ten different strains of Drosophila.

STRAIN:		PARAMETERS: Population size	Productivity	Mortality	Flies/bottle
D.s.sulfurigaster	(3019.8)	41.77±2.71	22.98±2.01	20.09±1.37	12.85±0.71
D.s.sulfurigaster	(P-11)	45.98±5.73	32.75±5.12	27.98±3.27	14.15±1.77
D.s.albostrigata	(W-3)	75.38±4.03	50.53±4.56	39.77±2.26	23.19±1.24
D.s.albostrigata	(S-11)	34.96±3.30	26.33±2.88	24.23±0.96	10.76±1.02
D.s.bilimbata	(Gum-8)	44.57±3.63	18.80±1.98	14.02±2.91	13.71±1.12
D.s.bilimbata	(HNL-111)	38.23±2.59	23.08±0.58	16.50±0.73	11.77±0.79
D.s.neonasuta	(Polymorphic)	109.40±3.29	63.50±1.72	49.02±3.41	33.36±1.01
D.s.neonasuta	(Monomorphic)	48.25±1.79	24.03±2.24	17.93±2.24	14.85±0.55
D.pulaua	(V-6)	62.75±5.25	46.38±4.36	35.52±3.94	19.31±1.61
D.pulaua	(S-18)	47.98±2.32	32.63±3.24	25.21±2.45	14.72±0.72

Table 2. Mean values (for four replicates) along with Standard Errors for Population size, Productivity, Mortality, and Flies per bottle in the media with sugar and yeast for ten different strains of Drosophila.

STRAIN:		PARAMETERS: Population size	Productivity	Mortality	Flies/bottle
D.s.sulfurigaster	(3019.8)	112.95±22.75	81.36±13.83	59.77±12.90	26.35±8.52
D.s.sulfurigaster	(P-11)	126.44±16.07	82.58±12.15	64.70± 9.03	36.78±4.68
D.s.albostrigata	(W-3)	167.64± 9.47	110.46±11.60	88.97± 7.19	48.77±2.76
D.s.albostrigata	(S-11)	132.68±19.09	106.95±13.52	86.98±12.65	38.55±5.59
D.s.bilimbata	(Gum-8)	123.43±19.19	77.78±14.87	62.78±11.30	35.94±5.55
D.s.bilimbata	(HNL-111)	114.86± 3.01	71.61± 4.15	53.87± 2.35	33.39±0.87
D.s.neonasuta	(Polymorphic)	243.29±11.37	146.38± 6.49	115.35± 5.10	70.75±3.33
D.s.neonasuta	(Monomorphic)	172.79± 5.85	96.16± 7.20	77.41± 5.03	50.20±1.68
D.pulaua	(V-6)	133.87± 6.06	109.95± 8.82	82.37± 7.47	39.58±1.31
D.pulaua	(S-18)	159.93±16.24	120.27±11.55	102.90±13.06	46.52±4.75

Acknowledgements: Authors are grateful to Prof. N.B. Krishnamurthy, Head of the Dept. of Zoology, for help and encouragement; to Indian National Science Academy, New Delhi, for awarding research grants to HAR; to Prof. O. Kitagawa for sending flies.

Reference:

Ayala, F.J. 1965, Genetics 51:527-544.

Van Delden, W. and A. Kamping. University of Groningen, Haren, Netherlands. The influence of yeast amount on body weight and ADH activity in **D.melanogaster** strains selected for tolerance to hexanol.

It has been found in **Drosophila melanogaster** that the amount of alcohol dehydrogenase (ADH) protein and ADH activity per unit body weight increases with increasing body weight (Kamping & Van Delden 1978; Clarke et al. 1979; Van Dijk 1981). Clarke et al. (1979) further showed that increasing the quantity of yeast in the food medium resulted (up to a certain limit) in increased body weight and quantity of ADH. In selecting for increased tolerance to hexanol-1 by exposing monomorphic Adh^S and Adh^F strains to hexanol-1 for 90 generations, Van Delden & Kamping (1983) found an increase in tolerance, both in the egg-to-adult and adult life stages. The increased resistance to hexanol in the tolerant strains (SSH and FFH) was accompanied by an increase both in body weight and ADH activity of adults (with the exception of SSH males) compared with the control strains (SSC and FFC) which were continuously kept on regular food.

To examine the effects of yeast content on tolerance to hexanol and other alcohols in relation to body weight and ADH activity, first experiments were started in which the yeast content in the medium was varied. In addition to regular food, consisting of 1000 ml water, 19 g agar, 54 g sucrose, 32 g dead yeast and 13 ml nipagin solution (10 g nipagin dissolved in 100 ml 96% ethanol), food was prepared with various amounts of dead yeast (0, 4, 8, 16, 64 and 96 g per liter medium). Eggs of each of the SSH, FFH, SSC and FFC strains were collected and transferred to each of the seven media with different yeast amounts. The number of eggs per bottle was such that the larvae grew up under uncrowded conditions. When the flies were hatched ADH activity of whole fly homogenate of 6-day old males was assayed as described by Van Delden et al. (1975). ADH activity was determined in batches of 25 males (2 replicas per strain) and expressed in Δ E $mg^{-1}min^{-1} \times 10^3$. Body weight was determined in batches of 10 males (5 replicates per strain).

Table 1. Mean weights (in batches of 10 males) and mean ADH activities (in batches of 25 males) of H and C strains, cultured on medium with various amounts of yeast.

Strain	Amt of yeast g/l medium	Mean weight mg (s.d.)	Mean ADH activity Δ E $mg^{-1}min^{-1}x10^3$ (s.d.)
SSC	96	7.94 (0.13)	64.9 (1.3)
	64	7.98 (0.19)	62.1 (1.8)
	32	7.88 (0.13)	65.5 (1.0)
	16	6.86 (0.18)	60.0 (4.1)
	8	5.32 (0.59)	51.5 (1.5)
	4	4.03 (0.21)	40.4 (0.5)
SSH	96	7.96 (0.09)	61.0 (1.3)
	64	8.10 (0.10)	59.2 (0.8)
	32	8.12 (0.11)	61.9 (0.6)
	16	6.86 (0.20)	57.2 (1.2)
	8	5.38 (0.19)	53.4 (0.5)
	4	4.24 (0.18)	35.9 (1.9)
FFC	96	7.84 (0.13)	275.0 (5.9)
	64	7.72 (0.15)	268.9 (1.2)
	32	8.00 (0.19)	277.0 (7.4)
	16	6.92 (0.13)	227.6(17.5)
	8	6.10 (0.14)	202.0 (4.7)
	4	4.66 (0.15)	160.5(10.0)
FFH	96	8.98 (0.08)	343.0 (4.0)
	64	8.94 (0.09)	334.7(12.0)
	32	8.80 (0.16)	333.0 (7.4)
	16	7.48 (0.23)	273.8 (7.6)
	8	6.04 (0.09)	201.4 (5.6)
	4	4.55 (0.24)	156.0 (3.1)

The results are shown in Table 1; no data are given for the 0 g yeast level, as no flies were obtained. It is clear that increasing the amount of yeast in excess of the amount present in regular medium (32 g per liter medium) does not result in higher body weight or ADH activity. At the lower yeast amounts applied in this study, however, both body weight and ADH activity decrease. In agreement with the results of Van Delden & Kamping (1983) there are no differences between the males of the SSC and SSH strains, neither for body weight nor for ADH activity at none of the yeast levels. Also in agreement with previous results are the higher body weights and ADH activities found in FFH males compared with FFC males. However, it is clear that this phenomenon only occurs at the higher yeast levels where, as shown, both body weight and ADH activity are maximized. At the 16 g yeast level (half of the content of regular medium) and at the lower levels, the differences between FFH and FFC disappear. It thus appears that the rise in body weight and ADH activity observed in FFH males is conditional on the yeast amount in the medium.

References: Clarke, B., R.G. Camfield, A.M. Galvin & C.R. Pitts 1979, Nature 280:517-518; Kamping, A. & W. van Delden 1978, Biochem. Genet. 16:541-551; Van Delden, W. & A. Kamping 1983, Ent. exp. & appl. 33:97-102; Van Delden, W., A. Kamping & H. van Dijk 1975, Experientia 31:418-419; Van Dijk, H. 1981, DIS 56:150-151.

Van den Berg, M.J. University of Groningen, Haren, Netherlands. The influence of isolation during rearing on male vigor in **D.melanogaster.**

In the last decade much attention has been paid to the role female pheromones play in the mating behavior of **Drosophila melanogaster** (see Jallon 1984 or Tompkins 1984 for some recent papers). Venard & Jallon (1980) and Antony & Jallon (1982) developed a simple and straightforward bioassay, to test whether certain compounds can act as sex attractants. In their assay an adult male Drosophila is offered a dead male conspecific, which is first deprived of his own odour and then covered with the compound to be tested. The male will often show courtship behavior towards the decoy, provided that a sex attractant is applied. On using this technique I covered decoys with raw hexane soluble extracts from females, but was unable to provoke sufficient sexual behavior. The extract was made in a similar way as described by Antony & Jallon (1982), who showed that this extract functioned as sex attractant. Antony & Jallon, however, stored the males between eclosion and day of test individually, while in our experiments they were stored in groups of 25.

To test whether this difference in method was responsible for the failure to replicate the bioassay, two groups of males were tested using a decoy covered with raw female extract (the amount was equivalent with approximately 1 female). Males of one group (isolates) were stored individually in shell vials (diam. 2.3 cm, height 7.8 cm), while in the other group (socialites) the males were stored in similar vials but in lots of 25 individuals. Males of both groups had been reared as pre-adults under identical, uncrowded conditions at 25°C and were tested individually 3-5 days after eclosion. The test was done in a small mating chamber at 25°C (see Van den Berg et al. 1984 for further details). The behavior was observed continuously for 10 min using a binocular microscope (10 x magnification) and recorded on an OS3 event recorder. The behavior elements noted were: Orientation, Wing vibration, Licking and Attempting to copulate (see Bastock & Manning 1955 for detailed description).

Table 1. Mean frequencies and mean durations of the different behavior elements of the two groups of flies.

Behavior element	Isolites (n = 14)	Socialites (n = 13)	Mann Whitney U
Orientation			
frequency*	21.43	3.23	48.5 ($P < 0.05$)
duration**	70.84	13.41	50.0 ($P < 0.05$)
Wing vibration			
frequency	21.36	3.15	45.5 ($P < 0.05$)
duration	59.15	11.47	48.5 ($P < 0.05$)
Licking			
frequency	4.86	0.69	68.5 (N.S.)
duration	5.47	0.95	68.5 (N.S.)
Attempting to copulate			
frequency	0.50	0.00	71.5 (N.S.)
duration	1.00	0.00	71.5 (N.S.)

*mean total frequency. **mean duration (seconds)

Table 1 shows clearly that the isolates are much more vigorous in courting than the socialites. This explains the original failure to promote sexual behavior using Venard & Jallon's bioassay. The results presented here show clearly that attention has to be paid to possible effects on behavior caused by isolated or social storage conditions. This is particularly true when flies are to be used for single or mass mating experiments, where phenomena such as female choice or male vigor are tested, because these differences in courtship behavior are likely to affect mating success.

Several possibilities exist as to the causes for this effect. It is possible that the chemoreceptors of the males become adapted to the cuticular hydrocarbons. In this case this would have to be a process of cross adaptation, because in **Drosophila melanogaster**, male hydrocarbons differ from female hydrocarbons. Therefore adaptation is not likely to be the cause underlying the effect found here (see Van den Berg et al. 1984 for more details).

Drosophila males sometimes court each other upon encountering, but will soon stop if the partner proves to be inappropriate. In the vials with 25 males, these encounters will be numerous during the 3-5 days of storage. It is therefore possible that a negative conditioning occurs, which could result in a lower male vigor to the offered decoy during the test. It would be very interesting to test this last hypothesis further.

References: Antony, C. & J.M. Jallon 1982, J. Insect. Physiol. 28:873-880; Bastock,M. & A. Manning 1955, Behaviour 8:85-111; Jallon, J.M. 1984, Behav. Genet. 14:441-478; Tompkins,L. 1984, Behav. Genet. 14:411-440; Van den Berg, M.J., G. Thomas, H. Hendriks & W. Van Delden 1984, Behav. Genet. 14:45-61; Venard, R. & J.M. Jallon 1980, Experientia 36:211-213.

Van Herrewege, J.*and J.R. David.+ *-Université Claude Bernard, Villeurbanne, France. +-C.N.R.S., Gif-sur-Yvette, France. Ethanol tolerance in **D.melanogaster:** parallel variations in larvae and adults from natural populations.

Drosophila melanogaster is known for its high ethanol tolerance, which in turn is related to a high alcohol dehydrogenase (ADH) activity. This physiological property correlates with the ecological niche of the species: adults are abundant in wine cellars, and larvae thrive in fermenting jars or grape residues, in which the ethanol concentration may exceed 10%.

There are genetic variations between geographic populations, both as to frequencies of ADH alleles and as to ethanol tolerance. Tolerance data, however, are greatly influenced by the technical procedure used to estimate tolerance since ethanol is highly volatile. For example, when alcohol is incorporated into a food medium, on which the adults oviposit, the alcohol concentration steadily decreases, becoming almost negligible after 10 days, i.e., at the end of the development.

Many published data obtained with this procedure are difficult to compare, since they depend on the rate of alcohol evaporation. To overcome this technical imprecision, we decided, some years ago (David et al. 1974) to use adults for toxicity tests, putting them in air tight-vials in the presence of various concentrations of ethanol. This procedure has given a large amount of reproducible results, showing in particular that the adults of European populations are highly tolerant to alcohol, having an LC 50 (lethal concentration that kills 50% of the flies) above 16%, while Afrotropical flies, which correspond to the ancestral populations of the species, are much more sensitive (L.C.50 about 7%) (David & Bocquet 1975).

Recently, a comparative study (David & Van Herrewege 1983) of numerous **Drosophila** species showed that the ethanol tolerance of adults correlated with the amount of alcohol in larval breeding sites: the adults of species breeding in non-fermenting resources, such as fungi

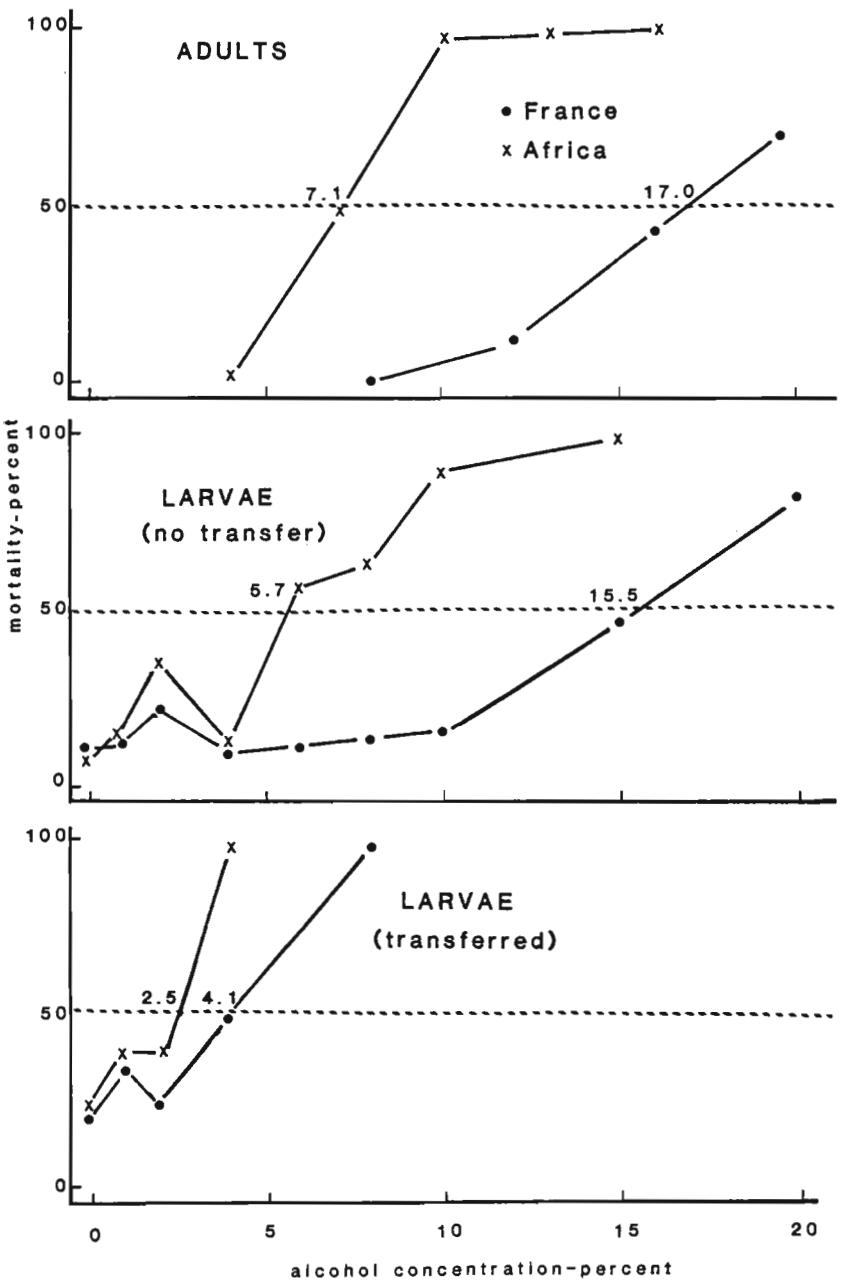

Figure 1. Influence of ethanol concentration upon **D.melanogaster** mortality: comparison of an Afrotropical and a French strain. **TOP:** mortality of adults after two days of ethanol treatment in air tight vials. **MIDDLE:** egg to adult mortality when development took place on the same medium without any additions and with a naturally declining ethanol concentration. **BOTTOM:** egg to adult mortality when developmental stages, larvae or pupae, were transferred every day to fresh medium in order to keep the ethanol concentration constant. [The numerical values on each curve indicate the LC 50 in percent of ethanol.]

or flowers, were found to be very sensitive to alcohol, whereas species breeding in sweet, fermenting fruits were found to be more tolerant. This suggests that, if environmental ethanol is really a selective factor it is more likely to act upon larvae than on adults. If that is the case, ecological genetics studies should preferably be carried out on larvae rather than on adults.

Of course, a possibility remained that larval and adult tolerances were highly correlated, so that adult tolerance would be mainly a by-product of larval adaptation.

To test this last hypothesis, we have compared larval and adult ethanol sensitivies in European and Afrotropical strains of **D.melanogaster.** Because of the difficulty arising from ethanol evaporation, we tried to keep constant the alcohol concentration during whole development. To this end we worked out a powdered killed yeast medium (formula to be published elsewhere) which can be prepared with cold water. Every day the larvae were sieved out of the medium and transferred to a fresh one containing the appropriate ethanol concentration. The results obtained for the transferred larvae, for adults of the same strains, and also for larvae kept in the same medium without any transfer during their whole development are shown in Figure 1.

When the alcohol concentration during the development was kept constant (i.e., when larvae were transferred every day) the LC 50 values were quite low, being 2.5 and 4.1% ethanol for African and French flies, respectively. But when the larvae were kept in the same medium, from which the alcohol progressively evaporated, the LC 50 values were much higher (5.7 and 15.5%). Interestingly, these latter values were very close to those (7.1 and 17.0) found for adults of the same strains.

From these observations we can conclude that, at least in **D.melanogaster**, variations of larval and adult tolerance are highly correlated. As had been assumed previously (David & Bocquet 1975), the divergence between European and Afrotropical populations may be attributable to different amounts of alcohol in the resources.

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Van Zijll Langhout, B.W. and F.M.A. van Breugel. University of Leiden, Netherlands. Cytological localization of the Aldox gene of **Drosophila melanogaster** in the region 3R 89A1.2.

For successful microdissection of genes from salivary gland chromosomes, exact cytological localization of the gene in question is required. With the ultimate aim of cloning the well-studied and histochemically interesting Aldox gene, we tried to locate the gene as accurately as possible. So far Spillmann & Nothiger (1978) assigned the locus to the region 88F-89A1 to 89B1-4 on chromosome 3R. Dickson Burkhart (1984) recently isolated from a North Carolina population two Aldox-null alleles associated with inversion breakpoints close to the 89A bands. While studying $Aldox^{n1}$ heterozygotes with various wildtype 3R chromosomes, we discovered that the $Aldox^{n1}$ mutation, originally isolated from an Urbana-S wild type strain (Dickinson 1970), in fact might be a small one-band or intraband deficiency. F1 larvae from a cross Aldox sbd x wildtype (Leiden) consistently showed much less stainable material in 89A (Figure 1) on one of the two homologous chromosomes. More proximal and distal regions fitted exactly with the Bridges (1935) map. Our conclusion is that the Aldox gene must be located in the double band 89A1.2. Upon inspection of the paper of Ashburner (1967), we found a very similar situation of unequal banding on the two homologous chromosomes on at least one of his photographs (viz. Figure 14A) of the Oregon-R wildtype strain. This suggests, that this wildtype strain could have been polymorphic for the small Aldox deficiency we have described here.

References: Spillmann, E. & R. Nöthiger 1978, DIS 53:124; Dickson Burkhart, B. et al. 1984, Genetics 107:295-306; Dickinson, W.J. 1970, Genetics 66:487-496; Bridges, C.B. 1935, J. Heredity 26:60-64; Ashburner, M. 1967, Chromosoma 21:398-428.

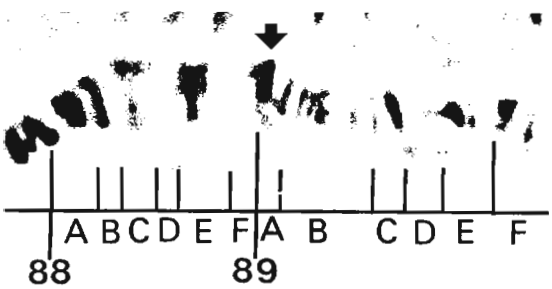

Figure 1. Part of chromosome 3R showing a deficiency for the Aldox locus in the lower part of band 89A.

Vouidibio, J.*, A. Bockatola*, L. Tsacas+ and J.R. David+. *-Université M. N'Gouabi, Brazzaville, Congo. +-C.N.R.S., Gif-sur-Yvette, France.

Drosophila polychaeta, Patterson & Wheeler 1942 (=**D.baole**, Burla 1954): another domestic Drosophila species to achieve cosmopolitan status.

The distribution and abundance of animal species are crucial information for understanding population biology and evolutionary processes. In the drosophilid family, much attention is paid to successful species, which have variously been called widespread, dominant, ubiquitous, generalist, colonizing and cosmopolitan. "Widespread" species were recently defined by Parsons & Stanley (1981) as those that are abundant over large geographic areas: examples of such species are **Drosophila subobscura** in Europe and **D.pseudoobscura** in America. In an examination of the distribution of species known to occur in several biogeographic regions, David & Tsacas (1981) defined as "cosmopolitan" those which were established in at least three non-adjacent regions. In all, 21 species were found to fulfil this criterion and in every case, recent introduction due to their accidental transportation by Man appeared responsible for the present geographic distribution. Obviously, widespread and abundant species are more likely to colonize new continents. There is, however, no absolute correlation between the breadth of the ecological niche and the cosmopolitan status: some species, belonging for example to the genera **Dettopsomya** and **Cacoxenus**, are neither abundant nor widespread but were transported by Man in spite of their ecological specialization.

David & Tsacas (1981) argued that involuntary transportations of **Drosophila** species were proceeding at an increasing rate and that several species, already established in two non-adjacent regions or in islands, could become cosmopolitan in the near future. Since that paper, some new establishments have been reported, the most spectacular being the spread of the European **D.subobscura** into Chile (Brncic et al. 1981) and the colonization of New Zealand by the American **D.pseudoobscura** (Lambert & McLea 1983).

Habitat selection and segregation play a significant role in species coexistence. Most **Drosophila** species are very reluctant to enter human constructions while others, which are really domestic, will concentrate in houses and other buildings, provided some resources are available there. It therefore seems possible to speak of a guild of domestic species (David et al. 1983), which, in the tropics, comprises mainly three cosmopolitan species, i.e., **D.melanogaster**, **D.ananassae** and **D.repleta**. During a survey of the domestic **Drosophila** community in Brazzaville (Congo), two more species have been found to make permanent populations in houses and their discovery is reported here. One of these is **D.virilis**, which had already been defined as cosmopolitan but had been known in Africa from only a single locality: one female collected in Johannesburg, South Africa, by Agnew (March 1973). There had been some doubt that a natural population of this species could still occur on the African continent, and the present observation confirms its establishment in a tropical country.

The second observation is more interesting, since it concerns a widespread American species, **D.polychaeta**. The discovery of a permanent population in Brazzaville extends its distribution and allows it to be classified as cosmopolitan. In fact, **D.polychaeta**, which seems to have a neotropical origin, was already known to have permanent populations in Hawaii and Micronesia and was also occasionally found in North America and Europe (Wheeler 1981). We do not know when **D.polychaeta** was successfully introduced to Africa. The lack of any previous report could suggest recent introduction. However, as pointed out by Tsacas et al. (1981), the Afrotropical fauna is far from being well known, and many new species from the Ivory Coast have been described by Burla (1954). We have examined these "African endemics" and found that **D.baole**, collected by Burla in 1951 but described in 1954, was in fact **D.polychaeta**, described by Patterson & Wheeler in 1942. The **Drosophila** strain from Brazzaville was crossed with a **D.polychaeta** strain from Guadeloupe and proved to be fully fertile. Morphologically, **D.baole** cannot be distinguished from **D.polychaeta** and must be considered identical. Thus the synonymy is established as follows: **Drosophila baole**, Burla (1954) = **Drosophila polychaeta** Patterson & Wheeler (1942). **D.polychaeta** is probably widespread in tropical Africa although it may be restricted to urban areas. We now know that at least 22 drosophilid species are cosmopolitan, 6 of which have an American origin.

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Watabe, H. Hokkaido University of Education, Sapporo, Japan. A preliminary note on the drosophilid flies collected at timberyards in northern Japan.

A number of drosophilids have been hitherto known to utilize tree sap fluxes and rotting barks as their feeding and breeding substrates. As often as I have opportunity, I have collected drosophilid flies at timberyards in northern Japan since 1979. Here, I preliminarily report the drosophilid fauna of five different timberyards. Flies were collected at a timberyard in Sakata (SK; 39°N, 140°E) and Kiyokawa (KY; 39°N, 140°E) in the Tohoku district, and Jyozankei (JY; 43°N, 141°E), Hobetsu (HB; 43°N, 142°E) and Ashoro (AS; 43°N, 143°E) in the Hokkaido district. Collections were made by net-sweeping and direct aspirating on logs and fallen barks. Fruit-traps were also used at SK and AS. Except for Sakata facing the Sea of Japan, all timberyards are in the mountainous region.

The common kind of logs accumulated at timberyards of Hokkaido are coniferous tree **Picea jezoensis, Abis sahalinensis,** larch **Larix leptolepsis, Kalopanax pictus,** linden tree **Tilia japonica,** oak **Quercus mongolia** var. **grosseserrata,** white birch **Betula platyphylla** and **Cercidiphyllum japonicum,** and those at KY are Japanese cedar **Cryptomeria japonica** and beech **Fagus crenata.** Although I could not identify the tree species at SK, most logs are coniferous trees, some of which have been transported from foreign countries, e.g., USSR.

A total of 1683 specimens were collected, belonging to 5 genera and 32 species. Table 1 shows the common species in each collection locality. The remaining species are as follows: **D.nigromaculata** at AS (1♂, 4♀♀), **D.brachynephros** at HB (1♂), **D.curvispina** at AS (1♀), **D.testacea** at JY (3♂♂, 3♀♀), HB (1♀) and AS (3♀♀), **D.hydei** at SK (1♀), **D.melanogaster** at SK (1♂), **D.subauraria** at AS (2♂♂, 1♀), **D.lutescens** at KY (1♀), **D.clarofinis** at AS (1♀), **D.makinoi** at JY (1♀), **D.alboralis** at JY (1♂), **D.busckii** at SK (1♀), KY (1♀) and HB (1♂), **D.collinella** at AS (1♀), **Scaptomyza pallida** at JY (2♂♂, 2♀♀), **Sc.consimilis** at JY (8♂♂, 7♀♀) and AS (1♀), **Amiota okadai** at JY (1♀), and **Leucophenga** sp. at SK (1♀). As breeding sites for drosophilid flies, four types of substrates, slime fluxes, fleshy fungi, fermenting fruits and decayed leaves, have been known in northern Japan (Kimura et al. 1977). Most species shown in Table 1, all members of the **virilis** and **robusta** groups, **D.bifasciata** and four species of **Chymomyza,** utilized decayed barks as their feeding and/or breeding sites. During the survey, the breedings were confirmed in **D.virilis** (SK), **D.kanekoi** (HB, AS), **D.lacertosa** (KY, JY), **D.moriwakii** (JY, AS) and **D.busckii** (KY). Many larvae, probably of **Chy. caudatula,** were also found in barks of relatively new logs at KY.

Table 1. Drosophilid flies collected at timberyards in northern Japan.
(* = numbers in parentheses represent number of males and females, respectively.)

Locality Date:	Sakata Jun 24-26	Kiyokawa Jun 27-29	Jyozankei May 5-Nov 3	Hobetsu Jul 27-29	Ashoro May 21-24 Jul 9-11
Genus **Drosophila**:					
the **virilis** sp.-group					
D.virilis	58(34,24)*	--	--	--	--
D.lummei	3(1,2)	--	--	--	--
D.kanekoi	2(1,1)	3(2,1)	--	6(3,3)	79(27,52)
D.ezoana	--	--	14(11,3)	10(4,6)	133(93,40)
the **robusta** sp.-group					
D.lacertosa	--	19(10,9)	2(2,0)	6(3,3)	40(24,16)
D.moriwakii	--	--	151(111,40)	--	163(79,84)
D.sordidula	--	--	2(2,0)	--	--
D.okadai	--	--	28(23,5)	--	7(4,3)
D.neokadai	--	--	7(3,4)	--	--
the **melanogaster** sp.-group					
D.auraria	10(3,7)	4(3,1)	--	10(6,4)	6(2,4)
the **obscura** sp.-group					
D.bifasciata	--	--	4(3,1)	--	14(5,9)
Genus **Chymomyza**:					
Chy. caudatula	--	60(30,30)	113(103,10)	251(205,46)	22(14,8)
Chy. costata	--	--	11(6,5)	31(23,8)	21(13,8)
Chy. fuscimana	--	--	110(95,15)	84(73,11)	81(71,10)
Chy. distincta	--	--	46(41,5)	17(16,1)	3(3,0)

It is difficult to compare quantitatively the samples between the timberyards, because the same sample unit has not been used for every collection. However, a few characterisitcs for the distribution of several species were noticeable. **D.virilis,** the well-known domestic species, has not been collected at any timberyards of Hokkaido, although its large populations have been found at breweries of Hokkaido.

This is probably due to low temperatures during the winter of Hokkaido. Conversely, **D.ezoana** has not been collected at the timberyards of Tohoku. **D.kanekoi,** a recently found species, is most widely distributed among the members

Table 2. Seasonal changes in the number of individuals collected at the Jyozankei timberyard in northern Japan.

Month	May	Jun	Jul	Aug	Sep	Oct
D.ezoana	13	1	--	--	--	--
D.moriwakii	138	12	1	--	--	--
D.okadai	25	3	--	--	--	--
Chy.caudatula	7	--	103	3	--	--
Chy.costata	--	--	4	4	3	--
Chy.fuscimana	--	--	74	27	8	1
Chy.distincta	--	--	42	4	--	--

of this group. **D.lummei** was described from Finland (Hackman 1972), who remarks that it has an extensive eastward distribution. To date in Japan, the collection records of **D.lummei** are almost restricted to timberyards in the western part of Tohoku, although the detailed information is still poor. Furthermore, as mentioned above, some logs at the SK timberyard are imported from USSR. These facts suggest that the Tohoku district might be a marginal zone for the distribution of **D.lummei**, or that its SK individuals might have colonized with logs from the Eurasian Continent. In the **robusta** group and the genus **Chymomyza**, **D.lacertosa** and **Chy. caudatula** were the most widely distributed species, respectively.

To study seasonal fluctuations of drosophilids at a timberyard, periodical collections were made at weekly intervals from May 5 to November 3 in 1979, at Jyozankei near Sapporo. The **virilis** group, the **robusta** group and **D.bifasciata** have been collected exclusively in spring (Table 2).

This is due to the dessication of logs during the summer, so that these flies would withdraw into surrounding forests in this season. Compared to these species, the adults of **Chymomyza**, except for **Chy. caudatula**, began to appear about two months later, which would depend upon differences in the life cycles between the species. Like a majority of drosophilids distributed in the Holoarctic region, the **virilis** group and the **robusta** group spend the winter in the adult stage, while **Chy. costata** and **distincta** are known to commit larval diapause (Watabe & Beppu 1977; Enomoto 1981; Lumme & Lakovaara 1983). Therefore, the adults of **Chymomyza** appear in late June to early July in Hokkaido. Judging from the phenological data, it seems that **Chy. fuscimana** would be also a larval diapause species.

At last, an excess of **Chymomyza** males in the samples taken is remarkable. This has relation to behavior of males. The males of **Chymomyza** appeared twice in a day, morning and evening, on the cut end of logs, and exhibited complex and species-specific courtships, e.g., active walking, wing scissoring and foreleg swinging. On the other hand, most females usually remained resting near the edge of logs. Therefore, the males could be captured more effectively in the collection using the insect net or an aspirator. Such a bias of the sex ratio is not true in the natural populations of **Chymomyza**, of course.

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Williamson, R.L. and A.D. Riggs. Beckman Research Institute (City of Hope), Duarte, California USNA. 5-Aza-2'-deoxycytidine does not cause recessive lethal mutations in **Drosophila melanogaster.**

In mammalian cells, 5-aza-2'-deoxycytidine (5-AzaCdR) at relatively low concentrations (less than 10 μM) will often efficiently cause somatically heritable changes in cellular phenotype (reviewed in Riggs & Jones 1983). This analog is thought to function by preventing the formation of 5-methylcytosine. Present evidence suggests that 5-AzaCdR is not a significant mutagen for mammalian cells in culture (Landolph et al. 1982; Jones 1984; Olsson & Forchhammer 1984; Kerbel et al. 1984; Delers et al. 1984), but nevertheless, may be a carcinogen (Carr et al. 1984). Drosophila has been reported not to contain detectable 5-methylcytosine (Urieli-Shoval et al. 1982), although a recent report indicates that Drosophila may contain this modified base at very low levels (Achwal et al. 1984). It is possible that only the germ cells of the adult contain 5-methylcytosine. For these reasons, we thought it would be interesting to see if 5-AzaCdR was toxic, mutagenic, or would affect fertility in Drosophila. Our test for the mutagenic effect of 5-AzaCdR was modified from the standard design of Muller (1928), but used a lethal-bearing FM6 chromosome, designated l(FM6), in place of ClB. Virgins for the cross were derived from Canton-S male and l(1)J1/l(FM6) parents.

Feeding experiments with 5-AzaCdR were unsuccessful; therefore, Canton males were injected with 0.1 μl of Drosophila Ringers solution (Ephrussi & Beadle 1936), either with or without 5 mg/ml 5-AzaCdR (Sigma). Solutions were made fresh prior to injection.

DADE 5-μl Accupettes that had been drawn to fine needles with a pipette puller were used for the injections. A graph-paper scale was glued to a short segment of fine polyethylene tubing and a lengthwise

wedge of the tubing was cut out so that it could be clipped onto the pipette and used as a movable scale to measure the volume of the injection. Injections were made in the right side of the ventral abdomen, medial to the 5th or 6th sternites. No leakage of fluid was observed after injection. No toxic effects were seen, and fertility was not obviously affected.

To ensure that all stages of meiosis were assessed, the treated males were placed with fresh +/I .(FM6) virgins (3 to 6 days old) on 3 successive occasions lasting 3 days each, and on a fourth occasion lasting 5 days. The females that had been mated were subsequently transferred to new bottles and allowed to lay additional eggs for a time equal to the times that they had been with the males. An exception was the second 5-AzaCdR-treated brood whose females were inadvertently discarded prior to the second egg lay.

Lethal mutations, detected by the absence of adult males, were subsequently made heterozygous with FM6 balancers and confirmed in the fourth generation.

Results: Lethal mutations/chromosomes tested

Broods	1	2	3	4	Total	%
Treatments:						
Control	0/311	0/371	1/233	0/227	1/1142	0.09
5-AzaCdR	0/431	0/260	0/229	1/233	1/1153	0.09

Our results indicate that injections that should lead to an initial concentration greater than 2 mM 5-AzaCdR in the haemolymph are relatively nontoxic and do not cause mutations in the germ line. This apparent lack of mutagenicity agrees with the studies on mammalian cell culture. Since our results are negative, they are not unambiguously interpretable. For example, the metabolism of 5-AzaCdR may be different in Drosophila than it is in mammalian cells.

We do not plan to pursue this work further at this time.

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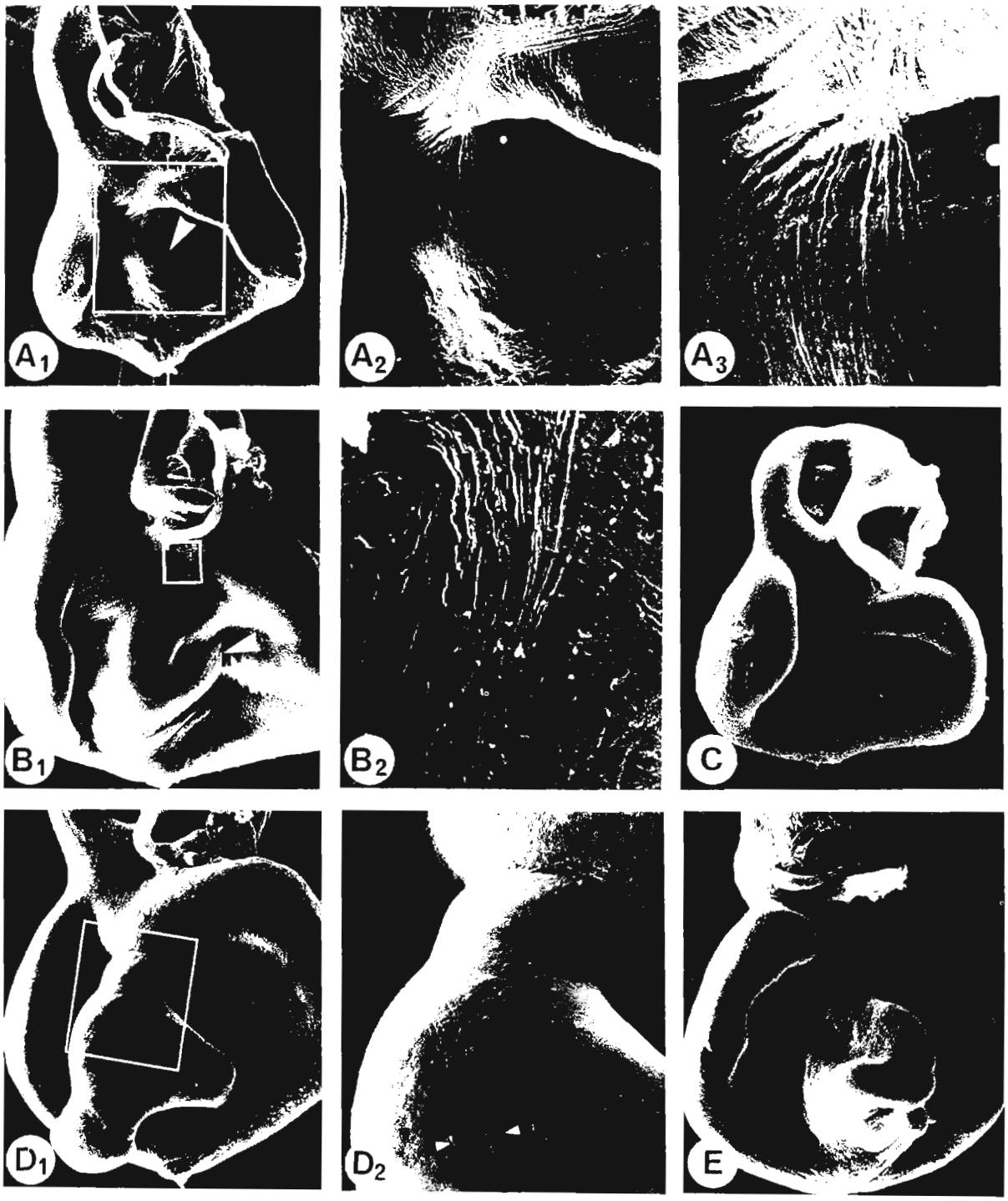
Zůst, B. and J. Wůest. Université de Geneve, Switzerland. The tumorous-head mutant of **Drosophila melanogaster:** examination by scanning electron microscopy of eye imaginal discs.

In flies of the homoeotic mutant tumorous-head (tuh) of **Drosophila melanogaster** head structures are partially replaced by abdominal, genital or undefined "amorphous" outgrowths (Newby 1949; Postlethwait et al. 1972; Kuhn et al. 1981). Kuhn and collaborators described the aldehyde oxidase (aldox)-staining patterns in the eye imaginal discs of tuh larvae (Kuhn & Cunningham 1976; Kuhn & Walker 1978) and found it likely that "the changes in aldox distribution correlate with the transformed portion of the disc affected by the homoeotic mutation". These transformed portions of tuh discs, isolated and cultured in vivo, differentiated autonomously (Kuhn et al. 1979). In this study tuh eye discs with visible abnormal regions were analysed by scanning electron microscopy (SEM).

Imaginal eye discs of third instar larvae of a tuh strain (Arizona State University strain; kindly provided by D.T. Kuhn) were dissected in Ringer's solution to which 2% glutaraldehyde in 0.1M cacodylate buffer (pH 7.3) was slowly added. They were fixed in pure 2% glutaraldehyde for 2h at room temperature, then washed, and in some cases stored up to 5 days in 0.1M cacodylate buffer (pH 7.3) at 4°C. Postfixation was done in 2% aqueous OsO_4 for 1h. The osmium-fixed discs were stored in 70% ethanol and later prepared for SEM. Eye discs of **D.melanogaster** Colmar strain served as controls.

Results and Discussion. 52 eye discs carrying transformed areas have been grouped according to size of their outgrowths: 14 small (27%; Fig. A: arrow), 14 medium (27%; Fig B1: arrow) and 24 large ones (46%; Fig. D1. Fig. E). Some of these areas had uniform shape (20/52 = 38%; Fig. A1), others appeared lobed (26/52 = 50%; Fig. D1), or twisted (6/52 = 12%; Fig. E). No correlation between size and shape has been found. Certain of these abnormal growths appeared clearly delimited from their unaltered supporting tissue (Fig. D1), while in others no clear limit could be detected (Fig. A1, B1). In the 52 discs analysed, 43 growths were located in the central region of the disc (region 2: giving normally rise to many of the eye facets), one in each lateral part of the disc (region 1 = ventral and 3 = dorsal) and in 7 cases the region could not be determined (for more details on the eye disc regions (see Kuhn & Walker 1978).



Figures: Imaginal eye discs of 3rd instar **D.melanogaster** larvae. C from Colmar control strain, A, B, D, E, from tuh mutant larvae with outgrowth in region 2. A1 disc with small outgrowth (arrow) of uniform shape, not clearly delimited from unaltered disc tissue, A2, A3 details with folds. B1 disc with medium sized outgrowth (arrow), slightly lobed, only in part delimited from unaltered disc tissue, B2 detail with folds. C control disc. D1 disc with large outgrowth, lobed, clearly delimited from unaltered tissue, D2 detail with folds (arrows). E disc with large outgrowth, twisted, only in part clearly delimited from unaltered disc tissue. Position of enlarged regions represented in Fig. A2, B2, D2 are indicated in Fig. A1, B1, D1. A1, B1, C, D1, E = 280x. A2, D2 = 700x. A3, B2 = 2100x.

A striking feature of these discs are folds at their surface which occur in the region of contact between the outgrowth and the underlying disc. Of the 52 discs studied, 39 (75%) showed such folds (Fig. A, B, D, E). In 31 discs these folds are oriented parallel to the anterior-posterior axis of the disc and they connect disc and transformed area (Fig. B2, D2); in 5 discs the folds ran parallel to the boundary between the disc and transformed area and in 3 cases they ran at an angle (Fig. A2). These folds are found in transformed regions of all sizes and shapes. Since the transformed areas of discs without visible connecting folds do not appear to be different from transformed areas having such folds, the role of these folds is not clear. Folds may be caused in part by mechnical stress, but not exclusively since in the disc represented in Fig. D2, for example, they run along a convex region of the abnormal outgrowth. The folds seem to comprise only the peripodial membrane. Occasionally such folds occur in areas other than the border region between disc and outgrowth.

The present study shows different connections between the eye disc and its transformed area in homoeotically transformed tuh mutant larvae. From these it appears likely that Kuhn et al. (1979) isolated and transplanted mainly those outgrowths (small, medium or large) which appear clearly delimited. Whether such a selection might influence the frequencies with which the various homoeotic differentiations occurred in these experiments is not known.

Kuhn & Walker (1978) also state that after dissection "aldox positive areas revealed, in each case, that a smooth boundary uniformly surrounds the entire area". That the transformed areas are functionally only little integrated into the normal part of the eye disc is also suggested by an observation by Stocker who could not show any nerves projecting into the transformed regions of the flies (pers. comm.).

It is interesting to compare the frequencies of abnormal outgrowths in eye discs of tuh larvae with the frequencies of abnormalities in the head of the tuh imago. Kuhn & Cunningham (1976) report that 80-90% of adult flies show head abnormalities (derived from eye and antennal discs). In 3rd instar larvae aldox staining revealed abnormalities in one or two eye discs in 63% of all animals. Since antennal discs stain positively in mutated and non-mutated larvae, their transformed areas cannot be recognized, which accounts for some of the differences between the frequency of the abnormalities in adults and larvae. In 84 3rd instar larvae analysed for visible abnormalities in their eye discs, 100% had them in one or two discs. Of 303 of their metamorphosed siblings, however, 11% did not show any visible eye abnormalities. These figures suggest on the one hand that aldox staining does not allow identification of all the transformed areas before their differentiation during metamorphosis, and on the other hand that either not all the abnormalities in tuh discs are caused by the genes responsible for the tuh trait or that a few transformations are not recognizable on unsectioned metamorphosed eyes.

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Zullo, S. Southern Illinois University, Carbondale, Illinois USNA. The influence of singed (sn^3) in **Drosophila melanogaster - D.simulans** hybrids.

The outcome of interspecific crosses between **Drosophila melanogaster** females and **D.simulans** males is well-known, producing sterile hybrid females (Sturtevant 1920). During investigations conducted with a field-collected strain of **D.simulans** (from Niles, Michigan USNA) (Figure 1), bristle abnormalities were noted in hybrids from matings with fertile, singed (sn^3) **D.melanogaster** females (Figure 2).

Six virgin y, sn^3, lz^{50e30}, v females were mated with 6 **D.simulans** males in each of 7 cultures (standard agar, yeast, molasses medium). Only 4 cultures produced progeny, 284 sterile hybrid females. One or more bristles of all the hybrids except one were misshapen (Figure 3). The posterior scutellars were most often affected. Occasionally the dorsocentrals, supra-alars, post-alars, and verticals also demonstrated a singed influence. The microchaetae showed a slight singed influence. The bristles appeared slightly thicker than in the hybrids with wild-type parents. The lone fly without a misshapen bristle did have thick bristles with a slight waviness. I noted no definite singed bristles in the hybrids.

While missing bristles were noted in hybrids from interspecific crosses between wild-type **D.melanogaster** females and these **D.simulans** males, as reported by others (Sturtevant 1920; Biddle 1932), no abnormal bristles were found on over 500 sterile hybrid females.

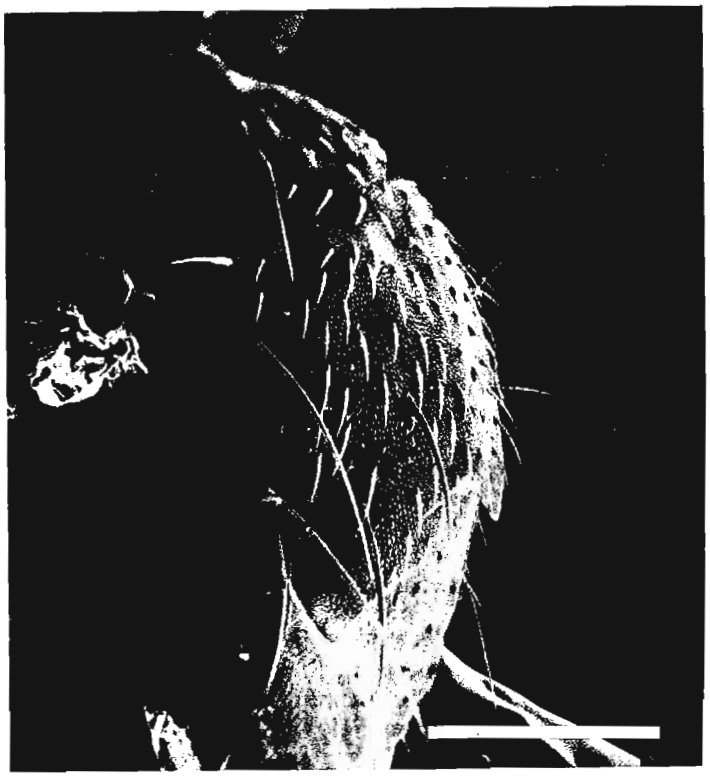

Figure 1. **D.simulans** bristle morphology. Bar represents 231 µm. Bar represents 231 µm.

Figure 2. **D.melanogaster** singed 3 bristle morphology. Bar represents 120 µm.

The salivary gland chromosome pattern of **D.melanogaster - D.simulans** hybrids is not irregular in the singed region (7D 1-2) though differences are present in other chromosomal regions. Indeed, the chromosome banding pattern of this **D.simulans** strain is different from that of a laboratory strain **D.simulans** (Zullo 1983).

It was unexpected that the single copy of the normally recessive sn^3 allele would produce an observable effect in the hybrids. A search for differential replication (and ultimately transcription) of the two species' DNA in the polytene chromosomes of the bristle-forming organs may prove significant. Differential replication has been invoked for asynapsis/desynapsis of polytene chromosomes (Roberts 1979). This **D.simulans** strain has been deposited in Mid-America Drosophila Stock Center in Bowling Green, Ohio.

I gratefully acknowledge the use of Prof. Harvey A. Bender's Lab at Notre Dame, the expert scanning electron microscopy work of the Center for Electron Microscopy of Southern Illinois University at Carbondale, and the secretarial assistance of Mona Sandefur.

References: Biddle, R.L. 1932, Genetics 17:153-174; Roberts, P.A. 1979, Genetics 92:861-878; Sturtevant, A.H. 1920, Genetics 5:488-500; Zullo, S.J. 1983, Trans. Ill. St. Acad. Sci. 76:103-110.

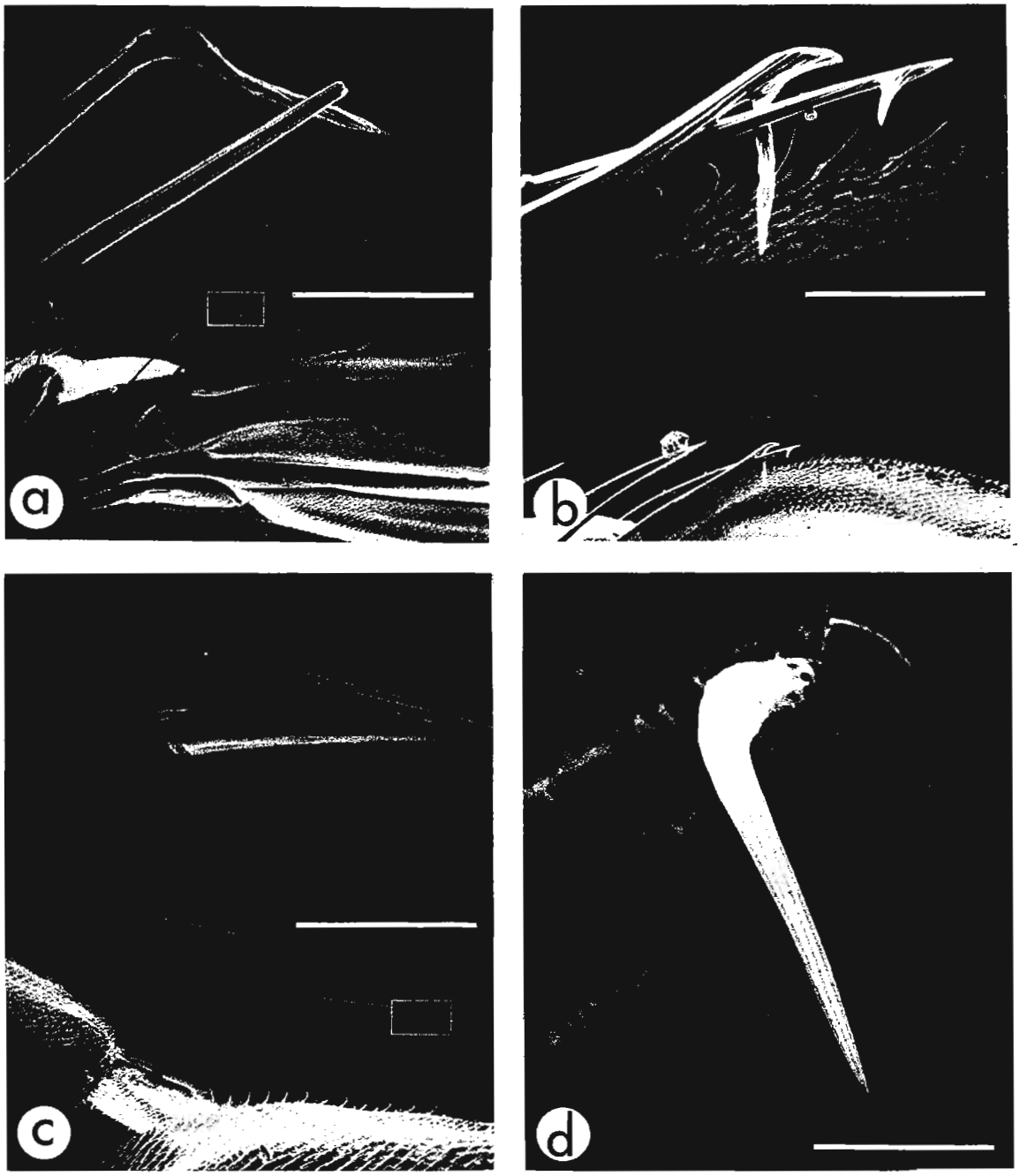

Figure 3. Hybrid bristle morphology. (a) Bar represents 500 µm. (b) Bar represents 270 µm. (c) Bar represents 150 µm. (d) Bar represents 23.1 µm.

Clark, A.G. and W. Gellman. Pennsylvania State University, University Park, USNA.

A rapid spectrophotometric assay of triglycerides in Drosophila.

For the purposes of a study of genetic variation in triglyceride storage, we needed a rapid assay sensitive enough to quantitate the triglycerides in a single fly. Most commonly the analysis of lipids begins with an extraction in organic solvents such as methanol-chloroform (Christie 1982). Solvent extraction is not practical for single fly measures, but the following protocol yields consistent and accurate determinations of triglycerides.

The procedure follows Bucolo & David (1973), and uses the Sigma Chemical Co. serum triglyceride kit 335. The reagent containts lipase to cleave the fatty acids from the triglycerides, glycerokinase and ATP to phosphorylate the resultant glycerol to glycerol-l-phosphate, pyruvate kinase and phosphoenol pyruvate to generate pyruvate, and finally it contains LDH and NADH to reduce the pyruvate to lactate. The end reaction is followed spectrophotometrically by measuring the change in absorbance at 340nm due to the oxidation of NADH to NAD+. Live flies are homogenized in 72-well tissue plates, with 200 ul distilled water per fly. The enzyme reagent is dissolved in 30 ml of distilled deionized water and 1.5ml is dispensed into a series of 10x75mm test tubes. To each tube is added 10ul of the homogenate, and the solution is briefly vortexed. The tubes are allowed to incubate for 1 hr at 25° and then the absorbance at 340nm is measured. Triglyceride standards containing 10, 25 and 50 ul of 1mg/ml solution are run in parallel. The effect of turbidity is negligible in such small samples, so clearing is not necessary.

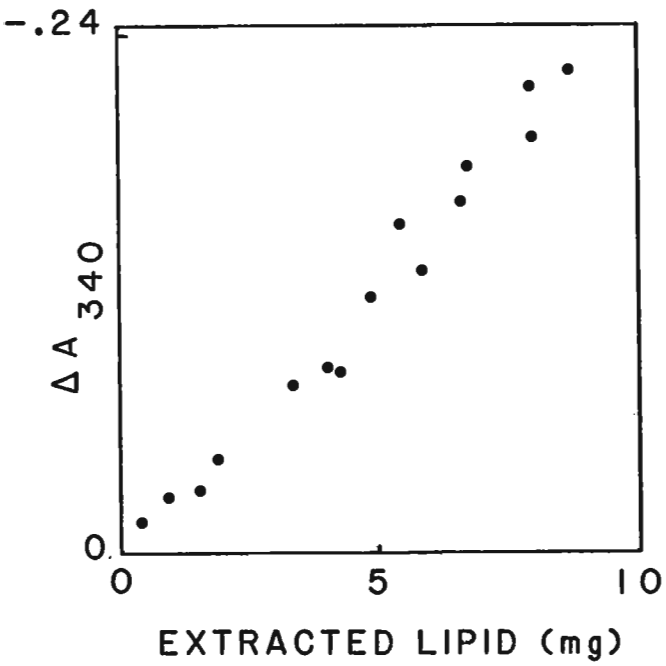

Figure 1. An analysis of lipid contents in 15 split samples of Drosophila by methanol-chloroform extraction and by an enzymatic procedure.

The triglyceride assay procedure was developed for serum rather than whole-organisms, and its accuracy must be tested in this context. Variation in quantitites of free glycerol, PEP, pyruvate or NADH would affect the apparent measure of triglycerides, so the accuracy of the method depends on the relative quantities of these residual substrates and triglycerides. A test of this was done by splitting a series of Drosophila samples and analyzing them both for triglycerides (using the above procedure) and for total lipids (using a methanol-chloroform extraction). Samples of 10 to 150 flies were used for the extractions, and lipids were quantified by the difference in dry weights before and after extraction. The extraction measures total lipids rather than just triglycerides, but in this homogeneous sample of flies, triglycerides represent a constant proportion of total lipids. Figure 1 clearly indicates that the two procedures give comparable results, and the correlation coefficient among these data is 0.97. The enzymatic test is very linear over the range from 0.5 to 40ug of triglyceride, with a coefficient of variation of 3.5%. An excellent correspondence is also seen between the enzymatic procedure and quantitation of triglycerides by TLC followed by sulfuric acid charring (Marsh & Weinstein 1966). The influence of endogenous NADH and substrates was tested by adding 10ul of fly homogenate to a series of buffers with OmM, 0.5mM, .25mM and .5mM NADH and following the change in absorbance. In no case was the change greater than 0.003, suggesting that the endogenous products do not grossly distort results. In conclusion, the enzymatic procedure appears to be a fast and accurate means of quantitating triglycerides in individual Drosophila.

References: Bucolo, G. & H. David 1973, Clin. Chem. 19:476; Christie, W.W. 1982, in: Lipid Analysis: Isolation, Separation Identification and Structural Analysis of Lipids, 2nd ed., Pergamon Press, Oxford; Marsh, J.B. & D.B. Weinstein 1966, J. Lipid Res. 7:574-576.

Curtsinger, J.W. University of Minnesota, St. Paul, USNA. Dot-matrix printer characters for geneticists.

This note describes procedures for redefining nine seldom used micro-computer keys as math symbols or as other symbols often needed for experimental reports. The procedure involves sending information to dot-matrix printer RAM via a simple BASIC program. The program contains DATA statements which encode the new dot patterns. After running the BASIC program, the printer will use the newly defined symbols instead of default symbols until the information in printer RAM is lost (either by turning off the printer or by sending a master reset code). The signal to print the new symbols can be sent to the printer from any source, including word processing programs. Monitor output will not be modified.

The following procedures apply to the Epson FX-80 printer and Microsoft BASIC-80 (CP/M-85). The DATA statements can be used with other programmable printers, but other BASIC statements may differ. The first step is to activate printer RAM by setting the DIP switch 1-4 to OFF (inside the printer; this should be the factory-set position). Next, enter and save "MATHFONT"; do not enter the comments on the DATA statements:

```
100    REM       MATHFONT
110    REM  THIS SECTION REDEFINES #, $, %, AND &
120    LPRINT CHR$(27)":"CHR$(0)CHR$(0)CHR$(0); 'Download font to RAM
130    LPRINT CHR$(27)"%"CHR$(1)CHR$(0); 'Activate RAM
140    LPRINT CHR$(27)"&"CHR$(0)"#&; ' Redefine # through &
150    FOR Y=1 TO 4:LPRINT CHR$(139); 'Attribute byte
160    FOR X=1 TO 11: READ C: LPRINT CHR$(C);: NEXT X
170    NEXT Y
180    REM THIS SECTION REDEFINES @, \, ¦, ~, AND !
190    FOR Y=1 TO 5
200    IF Y=1 THEN LPRINT CHR$(27)"&"CHR$(0)"@@"; ' Redefine @
210    IF Y=2 THEN LPRINT CHR$(27)"&"CHR$(0)"\\"; ' Redefine \
220    IF Y=3 THEN LPRINT CHR$(27)"&"CHR$(0)"¦¦";
230    IF Y=4 THEN LPRINT CHR$(27)"&"CHR$(0)"~~";
240    IF Y=5 THEN LPRINT CHR$(27)"&"CHR$(0)"!!";
250    LPRINT CHR$(139);                'Attribute byte
260    FOR X=1 TO 11:READ C:LPRINT CHR$(C);:NEXT X
270    NEXT Y
1000   DATA 129,66,129,36,129,24,129,0,129,0,0 'Define summation sign
1010   DATA 2,4,10,16,34,16,10,4,2,0,0         'Define delta
1020   DATA 1,0,1,2,60,64,128,0,128,0,0        'Define integral sign
1030   DATA 24,4,32,4,32,8,16,40,68,0,0        'Define alpha
1040   DATA 84,0,84,0,84,0,84,0,84,0,0         'Define "defined as" sign
1050   DATA 4,16,2,40,1,68,0,130,0,0,0         'Define less than or equal to
1060   DATA 130,0,68,1,40,2,16,4,0,0,0         'Define greater than or equal to
1070   DATA 128,0,128,0,128,0,128,0,128,0,0    'Define overline bar
1080   DATA 0,0,0,0,242,0,0,0,0,0,0            'Define exclamation
```

After running "MATHFONT" with printer on, eight keys will be redefined:

STANDARD:	!	@	#	$	%	&	~	¦	\
MATHFONT:	!	≡	Σ	Δ	∫	α	¯	≥	≤

For experimental reports, substitute the following DATA statements, again omitting the comments, and save a second program called "EXPFONT":

```
1000   DATA 56,4,64,4,64,184,0,128,0,128,0     'Define lower case sigma
1010   DATA 36,0,84,0,254,0,84,0,72,0,0        'Define dollar sign
1020   DATA 192,2,196,8,16,32,70,128,6,0,0     'Define percent sign
1030   DATA 24,4,32,4,32,8,16,40,68,0,0        'Define alpha
1040   DATA 63,64,16,68,16,68,40,0,0,0,0       'Define beta
1050   DATA 127,0,8,4,0,8,120,0,0,0,0          'Define mu
1060   DATA 24,0,36,0,164,24,192,0,224,0,0     'Define male sign
1070   DATA 0,0,64,160,0,160,64,0,0,0,0        'Define degree sign
1080   DATA 50,0,72,7,72,0,50,0,0,0,0          'Define female sign
```

After running "EXPFONT" with the printer on, seven keys will be redefined from the standard font:

STANDARD:	!	@	#	$	%	&	`	¦	\
EXPFONT:	♀	β	σ	$	%	α	°	σ	µ

DATA statements can be chosen so that any nine of the 14 non-standard characters defined here are available simultaneously. Key redefinition does not interefere with other signals from BASIC that control print pitch or quality.

Duttagupta, A.K., M. Das (Mutsuddi) and D. Mutsuddi. University of Calcutta, India. The maintenance of the sensitive Drosophila stocks in laboratory culture.

Perhaps all the Drosophila workers in the tropical countries, like India would agree with us that the transhipment of different Drosophila species/strains to such places is a quite difficult job. In India, the mild winter in our place, which stays from middle of November to early February, is only suitable for receiving stocks in healthy condition. Even within this period, the stocks very often arrive either dead or with a few larvae and pupae on decomposed culture medium, often infected with fungi. Furthermore, due to mite infection and elevated temperature during the transhipment, the newly arrived flies do not give enough progeny. For these reasons, we had to find out some means to overcome the problems.

Sensitive stocks like **D.miranda** are difficult to maintain. Due to its high sensitivity to temperature (it prefers temperature below 18°C), the maintenance of the stock was initially almost a failure. The flies, at that time, used to lay very few eggs on the standard Drosophila culture medium which invariably developed scums; with poor hatching of the larvae, most of the eggs were destroyed before hatching. We overcame this by adopting the following method:

1. The flies were first allowed to breed on a vial containing pasted banana. A few pieces of filter papers (with nepazine) were inserted leaving some portion outside the food. The flies were observed to lay eggs more in number. The eggs hatched successfully and the larvae were rather healthy.
2. At pupation (meanwhile the banana gradually became decomposed) the pupae were brought outside the vial and washed carefully in Drosophila Ringer (pH 7.2) to remove the decomposed food.
3. The pupae were dried on filter paper.
4. The empty vials were taken and their inner walls were smeared (with brush) with Drosophila culture medium to make the wall sticky. The pupae were applied to stick there by placing their ventral sides facing to the wall of the vials.
5. The newly emerged flies were transferred to the fresh culture medium.

By following the same method, we were also successful in removing the mites from Drosophila stocks. The pupae were brought outside the mite-infected culture medium and were washed in Ringer carefully and the above described process was followed to make mite-free stocks.

Hey, J. and D. Houle. University of New York, Stony Brook, USNA. Rearing **Drosophila athabasca.**

Drosophila athabasca is a complex of three semispecies (Miller & Westphal 1967), all of which are difficult to rear in the laboratory. We have developed a media and rearing protocol that works well for these flies and allows their use as a convenient experimental organism. The basic food recipe is a simple modification of the standard Drosophila media and consists of the following: 2900 ml of water; 116 gr of corn meal; 116 gr of dead brewer's yeast; 80 ml of molasses; 80 ml of light corn syrup; 24 gr of agar; 15 gr of peptone (SIGMA catalog no. P-7750); 15 gr of casein (SIGMA catalog no. C-0376); and 35 ml of 10% Tegosept in 95% ethanol. Whenever wild caught flies are used, we also add 0.6 gr of streptomycin sulphate and 0.2 gr of penicillin.

The ingredients could probably be combined in a variety of ways, though we prefer the following procedure: bring 2200 ml of water to a boil; mix the agar with 200 ml of water and quickly add it to the boiling water; bring the mixture to a boil while stirring; remove from heat and add the corn syrup and molasses; combine the remaining water with all of the remaining solid ingredients by stirring in a blender at high speed for several minutes; add this slurry to the water/agar/sugar mixture and stir to homogeneity;

the tegosept is added last. The food is pumped while hot in 7-8 ml aliquots into 8 dram vials, and is sufficient for nearly 500 vials. Great care must be taken to avoid the introduction of any molds or yeasts to the food, so we generally cover the vials with sterile cheese cloth while they cool before plugging with sterile cotton plugs. The critical way that this food differs from others appears to be the addition of the peptone and casein. It is quite possible that different sugars or different proportions of other ingredients may also be satisfactory.

For rearing, all flies are kept in the light at 19° and 80% humidity. A long photoperiod or constant light is important because these flies seem to go into diapause with a short photoperiod (pers. obs.) and will not mate in the dark (Curtright & Miller 1979). When the larvae in a vial begin to pupate, a small square of sterile tissue is placed inside the vial and dampened with a few drops of 1% propionic acid. If this tissue is not added as a pupation site, nearly all larvae will pupate in the food and die. If flies are young when they are put in a vial of new food, they often will not produce many eggs for several days. In this time the food may dry out or develop a yeast culture. We often find it preferable in these cases to simply transfer the flies to a vial of fresh food.

With these methods we have found **D.athabasca** to be quite tractable. Wild caught females successfully produce progeny greater than 90% of the time and any particular line can be maintained indefinitely if 3 or 4 vials are maintained in rotation. We have also found these methods suitable for other related species including: **D.algonquin, D.affinis, D.narragansett, D.pseudoobscura,** and **D.azteca.**

References: Miller, D.D. & N.J. Westphal 1967, Evolution 29:531-544; Curtwright, R.D. & D.D. Miller 1979, Anim. Behav. 27.

Marcus, C.H. Albert Einstein College of Medicine, Bronx, New York USNA.

Single fly DNA extraction procedure.

In order to obtain usable DNA from single flies, we developed the following straightforward procedure. There are two distinct features of this procedure. First, there are no precipitation steps. Second, homogenization occurs directly in the microfuge tube in which the fly is stored (see Figure for how to make homogenizer), thereby eliminating the need to transfer sample from a standard homogenizer (DNA sticks to glass), and the time consuming process of washing standard Dounce homogenizers after each extraction.

1. Homogenization. (a) Take frozen fly in microfuge tube and add 50 microliters of ice cold homogenization buffer (-SDS) (0.15 M NaCl; 0.015 M EDTA, pH 8.0; 0.05 M Tris, pH 8.0). Note: we like to keep frozen flies on dry ice up until homogenization buffer is added. (b) Using the pasteur pipet homogenizer (see Figure) grind fly for 30-45 seconds using a twisting motion (as opposed to a piston-like motion, to prevent possible shearing). Most fly parts should be homogenized with possible exception of wing and leg parts. Eye pigment if present is a useful indicator of complete homogenization. (c) With homogenizer still in microfuge tube rinse the homogenizer with 50 microliters of room temperature buffer (+SDS) (same buffer as above with 0.04% SDS). We feel that the SDS concentration is critical. Higher concentrations of SDS have proved to inhibit restrictability of DNA.

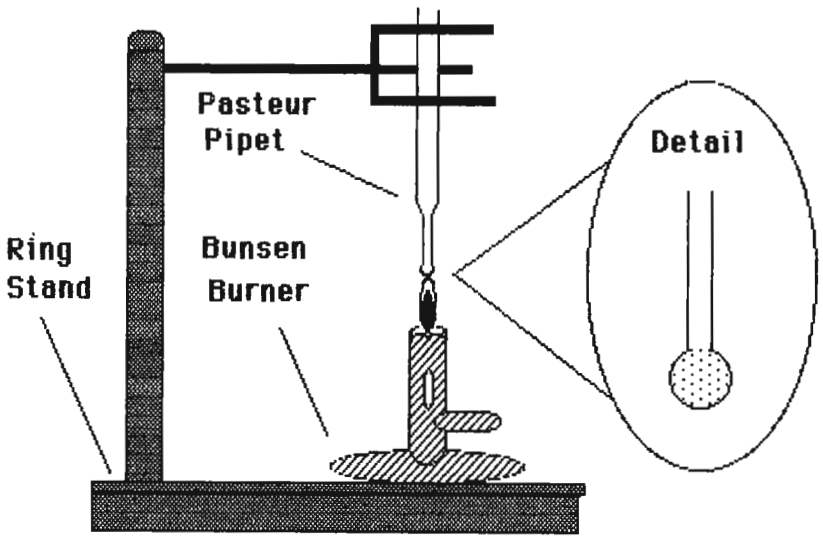

Figure. Making homogenizer: using above assembly, heat pipet until a solid ball of a diameter of approx. 3 mm is reached. Homogenizer should fit neatly into bottom of a microfuge tube.

2. Proteinase K digestion. Immediately add 5 microliters of ice cold Proteinase K (10 mg/ml) and place at 65°C for 30-60 min.

3. Phenol:chloroform extraction (2X). (a) Add 200 microliters of1:1 phenol:chloroform solution. Phenol is buffered with TE (10 mM Tris HCl, pH 8.0; 1 mM EDTA, pH 8.0). (b) Mix phases thoroughly by gently inverting tube 5 or 6 times. Microfuge for 3 min (first extraction), 2 min (second extraction). (c) Take top layer using a cut pipet tip. Use cut tips whenever handling genomic DNA to prevent shearing of DNA. (d) Repeat (a) - (c).

4. Isoamyl alcohol extraction. (a) Add 200 microliters of isoamyl alcohol. (b) Microfuge briefly (2-3 seconds). (c) Discard top layer, which is isoamyl alcohol.

5. Dialysis. (a) We use spectrapor membrane tubing 1.0 cm, molecular weight cutoff: 12,000-14,000. We boil tubing in approx. 5mM EDTA, pH 8.0 twice, and then store tubing in 50% ethanol. (b) Take sample (with cut pipet tip) and place in dialysis bag--seal bag. (c) Dialyze against TE. We use roughly 1500-2000 fold volume for 36-48 hr, changing solution four times. (d) Remove DNA samples.

Procedure can work just as well for ten flies using the same procedure, but raising volumes as follows: homogenization buffer -SDS: 200 microliters; homogenization buffer +SDS: 200 microliters; Proteinase K (10 mg/ml): 20 microliters; Phenol:chloroform: 800 microliters; Isoamyl alcohol: 800 microliters. Note: for multiple extractions a single homogenizer can be used. After each homogenization wipe homogenizer, dip homogenizer in pure ethanol, wipe, dip in distilled water, wipe.

McRobert, S.P. and L. Tompkins. Temple University, Philadelphia, Pennsylvania USNA.
A method for observing the behavior of groups of flies.

We have developed a simple procedure for observing the behavior of groups of flies. This technique has been used to study courtship, although it could be used to study any behavior that flies in a group perform.

The observation chamber is a square plastic petri dish (Falcon 1012, 100 x 100 x 15 mm) into which a thin layer of cornmeal-molasses-agar medium has been poured. After hardening, a small section of medium is removed from two opposing corners of the dish so that flies can be introduced without their sticking to the food. The flies are transferred to the chamber by aspirating them through small holes in the lid, which have been made with a soldering iron, that are over the corners from which medium has been removed. In our study the flies were anesthetized with CO_2, although it would be easy to introduce un-anesthetized flies into the chamber. The entrance holes are then covered with clear tape to prevent flies from escaping. A light is positioned over the chamber and the behavior of the flies is monitored by observation through the lid.

S.P.M. and L.T. were supported by N.I.H. grant GM33511.

Milner, M.J.. University of St. Andrews, Fife, Scotland. Culture medium parameters for the eversion and differentiation of **Drosophila melanogaster** imaginal discs in vitro.

We use Shields and Sang's M3 (Shields & Sang 1977) for the culture of imaginal discs in vitro. Originally, the 1977 formulation supplemented with 10% non-heat inactivated foetal bovine serum (FBS) was used, but more recently we have reduced the amount of FBS to 2%, as this yields better differentiation. This necessitates a non-serum formulation of the medium (Table 1) to compensate for the absence of various ions previously supplied via the higher level of serum (Shields & Sang's M3(NS) - Shields & Sang, pers. comm.). It should be noted that this formulation is also bicarbonate-free. The medium is made up as before except that the pH is raised directly to 6.8 by addition of 1% NaOH. A batch of medium may be used for up to 6 weeks after preparation, and a dilution series of 20-hydroxy ecdysone is best used within 10 days of preparation. As found for embryonic cell culture, optimal medium conditions are reached between the first and second week after serum addition (Shields & Sang 1977).

It may be desirable to culture discs in the absence of serum, either because of difficulty in obtaining a suitable batch of serum, or in order to culture discs in more rigorously defined medium conditions. To this end, I have tested a number of serum substitutes used in other tissue culture systems, at a range of concentrations, adding them directly to M3(NS) and assessing their ability to support eversion, differentiation and pigmentation of wing discs on a scale of 1 to 3. One represents poor differentiation, 3 good development and 2 an intermediate level. All additives were purchased from Sigma. The results

Table 1. Shields and Sang's medium M3(NS). (amounts in mg/100 mls). * = optional

Kcl	260	Threonine	50	Tyrosine	25
$MgSO_4 \cdot 7H_2O$	400	Serine	35	Phenylalanine	25
$CaCl_2 \cdot 6H_2O$	140	Asparagine	30	beta-Alanine	25
Na·glutamate	786	Glutamine	60	Histidine	55
$NaH_2PO_4 \cdot 2H_2O$	88	Proline	40	Tryptophan	10
glucose	1000	Glycine	50	Arginine	50
oxaloacetic acid	25	alpha-Alanine	150	Lysine HCl	85
BIS-TRIS	105	Valine	40	Cysteine HCl	20
T.C. Yeastolate (Difco)	100	Methionine	25	Choline HCl	5
Aspartic acid	30	Iso-leucine	25	Penicillin G.Na*	3
		Leucine	40	Streptomycin sulphate*	10

Table 2.

Additive to G3(NS)	Concentration mg/ml	Differentiation level	Additive to G3(NS)	Concentration mg/ml	Differentiation level
No additive		2-3	Polyvinyl Alcohol type 11	1	3*
2% Foetal Bovine Serum (average batch)		3	(B.D.Bavister 1981, J.Exp. Zool. 217:45-51)	0.1	2
	5	1	gamma-Globulins (bovine Cohn Fraction 11)	1	2
Bovine Albumin (fatty acid free)	1	3*			
	0.1	2	Chicken egg white globulins	5+	2
10	3*		Insulin (crystalline, from bovine pancreas)	0.1+	1
Bovine Albumin fraction V	1	3*			
	0.1	3*	(G.Mosna 1981, Experim. 37:466-467)	0.001	2

* indicates acceptable substitutes for 2% FBS. + used as saturated solution.

of these experiments are given in Table 2. A control with no additive gave a reasonable level of differentiation, compared to the control of 2% FBS. However, the discs are difficult to handle in medium alone as they stick to glass surfaces and cannot easily be moved through surface tension layers, so this cannot be recommended. Acceptable substitutes to FBS are indicated in Table 2; however, I consider that a good batch of FBS can give qualitatively better results than these. It is interesting to note that under these conditions insulin inhibited differentiation at a range of concentrations.

Reference: Shields, G. & J.H. Sang 1977, DIS 52:161.

Nichols, R. and W.L. Pak. Purdue University, West Lafayette, Indiana USNA.

A simple medium for vitamin A deprivation of **Drosophila melanogaster.**

One method of studying proteins that contain a vitamin A-derived group, such as rhodopsin, is to observe the effect of reducing vitamin A in the diet of the organism being studied. In studies today, vitamin A deprivation of Drosophila is accomlished by raising flies on one of several synthetic diets (Sang 1956; Falk & Nash 1974). These synthetic media have numerous disadvantages: (1) it is laborious to prepare the medium; (2) mold and bacterial growths are difficult to control; (3) the antibiotics and mold inhibitors included in the medium could have undesirable side effects on flies; (4) eggs or larvae often need to be sterilized before introducing them onto fresh medium at each generation; (5) the deprived flies are often unhealthy; (6) the degree of deprivation is inconsistent. We describe in this report a procedure for producing vitamin A deprived flies that circumvents many of these difficulties.

The medium we used is similar to that described for egg collection (Elgin & Miller 1978). Water (270 ml) and grape juice (Welch's; 230 ml) were heated to boiling. After removing the liquid from heat, the following ingredients were added while stirring: Bacto-agar (Difco), 11 g; glucose, 30 g; sucrose, 10 g; fructose, 5 g; yeast (Fleischmann's dry) 10 g. Yeast dissolved more readily if first mixed with approx. 25-50 ml of heated water-juice and then the paste is added to the mixture. The mixture was then autoclaved for 15 min. After cooling the mixture to approx. 60°C, the remaining ingredients were added while stirring; 1 M NaOH, 10 ml; propionic acid, 2 ml; phosphoric acid, 0.2 ml. The medium was poured into sterilized containers, covered with autoclaved tops, e.g., Kaputs (Carolina Biological Supplies), and stored at either room temperature or 4°C.

To produce vitamin A deprived flies, adult flies were introduced into vials containing the vitamin A deficient medium, the vials were cleared of the flies after 1-3 days, and the eggs laid on the deficient medium were allowed to develop into adults. Vitamin A deprivation can be maintained over many generations simply by repeating the above procedure at each generation, i.e., by allowing previously deprived adult flies to lay eggs on a fresh, deficient medium and letting the eggs develop into a new generation of deprived flies. We have maintained flies on this medium for over fifty generations with no obvious ill effects on flies.

Principal effect of vitamin A deprivation on flies is a drastic reduction in visual pigment concentration. There are at least two methods of determining the amount of visual pigment in intact, white-eyed flies: microspectrophotometry of the deep pseudopupil (Stavenga et al. 1973) and the electrophysiological measurement of the amplitude of the M-potential (Stephenson & Pak 1980). In addition, the amplitude and waveform of the prolonged depolarizing after potential (PDA) of the electroretinogram (ERG) give a semiquantitative measure of the amount of pigment (Stark & Zitzmann 1976; Larrivee et al. 1981), and the distinctness of the deep pseudopupil is in itself a qualitative measure of the amount of pigment. All these tests were carried out on the flies grown on the described medium. Results showed consistently that the visual pigment concentration of the deprived flies were less than a few % of undeprived flies.

References: Elgin, S.C.R. & D.W. Miller 1978, in:Genetics and Biology of Drosophila (Ashburner & Wright, eds.), Academic Press, v2a:112-121; Falk, D.R.& D. Nash 1974, Genetics 76:755-766; Larrivee, D.C., S.K. Conrad, R.S. Stephenson & W.L. Pak 1981, J. Gen. Physiol. 78:521-545; Sang, J.H. 1956, J. Exp. Biol. 33:45-72; Stark, W.S. & W.G. Zitzmann 1976, J. Comp. Physiol. 105:15-27; Stavenga, D.G., A. Zantema & J.W. Kuiper 1973, in:Biochemistry and Physiology of Visual Pigments (Langer, ed.) Springer Verlag, p175-180; Stephenson, R.S. & W.L. Pak 1980, J. Gen. Physiol. 75:353-379.

Ralchev, K.H. and N.T. Harisanova. University of Sofia, Bulgaria. A convenient method for mass production and harvesting of synchronous Drosophila embryos.

We have developed a method to collect large quantities of **Drosophila hydei** embryos that we used to prepare chromatin and histones. In order to obtain gram quantities of synchronous Drosophila embryos, we had to start with a large homogenous population of flies (Mitchel & Mitchel 1964; Comings et al. 1977).

Flies were reared in culture boxes (Fig. 1) on cornmeal-agar medium. About 30-40 mg of Drosophila embryos were uniformly spread with small volume of water on the food surface of each culture box. This assured optimal density of the developng larvae as well as a population of flies which is uniform in age within two or three days. We needed about 50 to 60 culture boxes to obtain a population of 50 to 70000 flies which were transferred into the population cage. Two cages containing 25 to 35000 flies each, maintained in a constant temperature and humidity room (25°C, 70%), provided an excellent egg supply within four weeks.

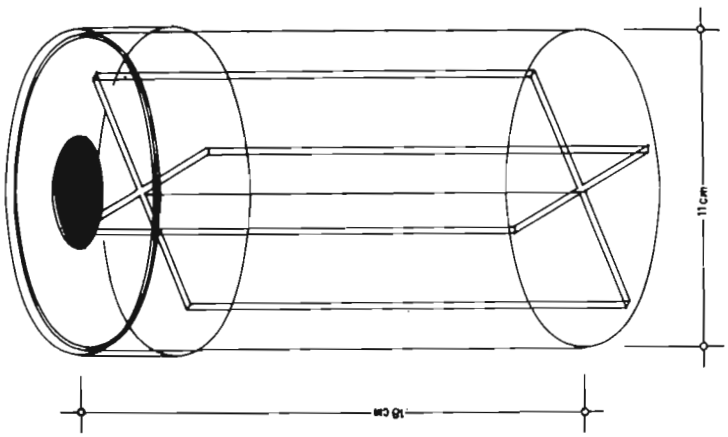

Figure 1. Drosophila culture box.

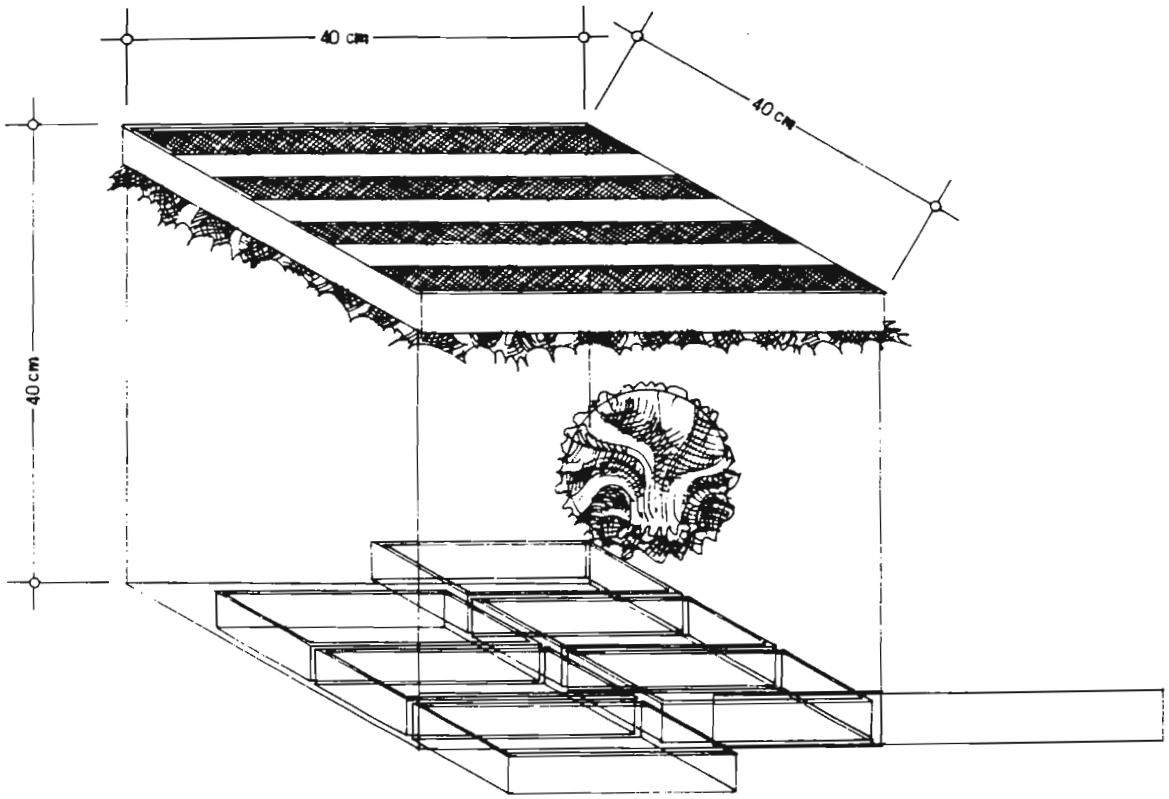

Figure 2.
Drosophila
population
cage.

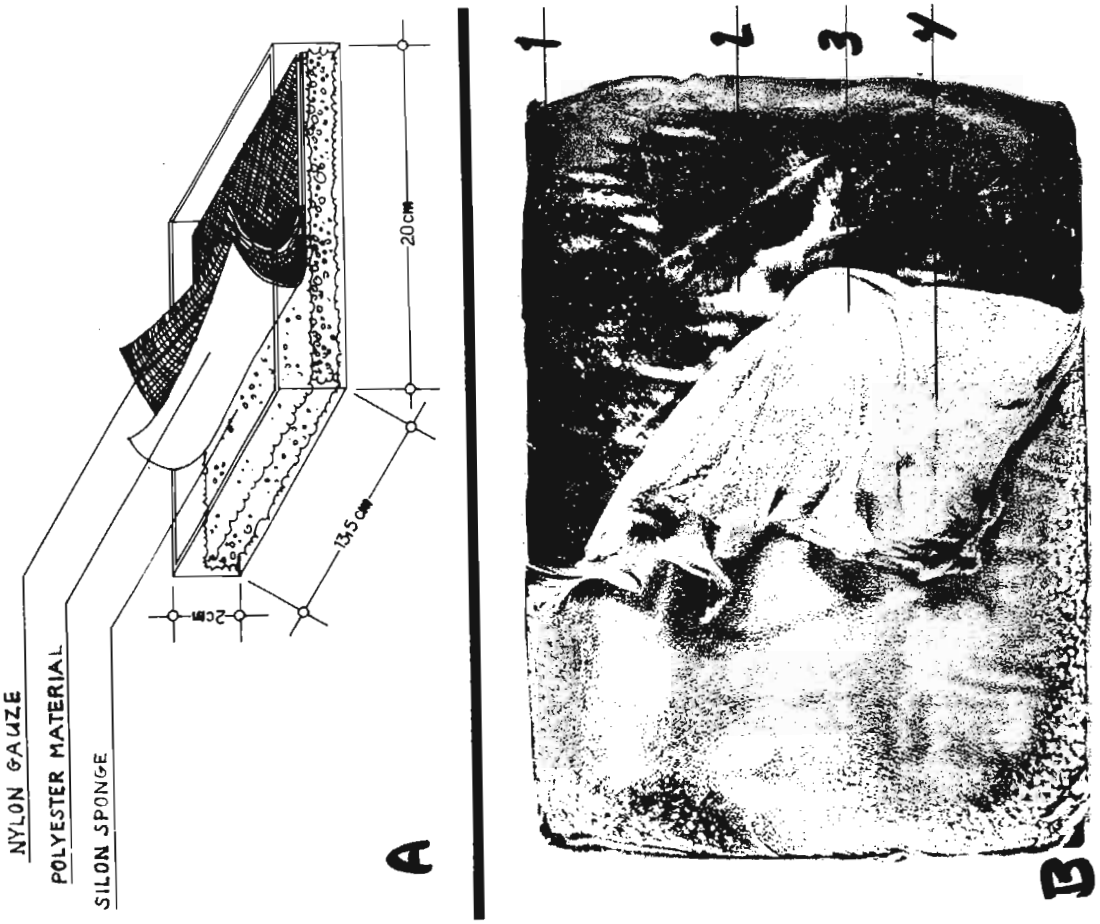

Figure 3.
Drosophila
embryo harvesting
tray: (a) scheme,
(b) photograph:
1: polyester
material;
2: yeast
suspension;
3: nylon gauze;
4: eggs.

Figure 2 shows the structure of a cage. The ceiling was wrapped with cheese-cloth to provide ventilation. The food was provided daily on trays (20 x 13.5 x 2 cm). New trays were placed through the openings on the bottom of the cage, pushing the old ones into an anaesthetizing box in order to collect flies from the medium surface. The anaesthetized flies were put back into the cage through the cheese-cloth sleeve of the side opening.

All equipment mentioned above was made of transparent plexiglass.

The embryo harvesting trays were placed and removed in the same way. Each harvesting tray (Fig. 3) consisted of a flat piece of silon sponge, a piece of polyester material and some fine nylon gauze. The sponge piece (20 x 13.5 x 1.5 cm) was wetted with a solution containing 1.5% acetic acid, 2.5% ethanol and covered with the polyester material whose colour resembled the food medium colour. A thick suspension of Baker's yeast was layered and the nylon gauze was placed over it. In this way the desired rough medium surface which is the preferable place of egg deposition was obtained (Spencer 1950; Elgin & Miller 1978). In addition the nylon gauze prevented flies from sticking to the yeast suspension. The embryos were collected within two hours during the oviposition activity of the flies.

Egg harvesting was carried out by washing the nylon gauze in water and filtering the washing water through a sintered glass funnel S_1.

The method described above allowed us to collect 10-15 g of synchronous Drosophila embryos for a laying period of two hours, employing a population of 50 to 70000 flies.

References: Comings, D.E., D.C. Harris, T.A. Okada & G. Holmquist 1977, Exp. Cell Res. 105: 349-365; Elgin, S.C.R. & D.W. Miller 1978, in: The Genetics and Biology of Drosophila, v2b, pp.112-120 (Ashburner et al., eds.), Academic Press, NY; Mitchell, H.K. & A.M. Mitchell 1964, DIS 38: 135-137; Spencer, W.R. 1950, in: Biology of Drosophila, pp.535-590 (M.Demerec, ed.), NY - L.

Real, M.D., J. Ferré and J.L. Ménsua. University of Valencia, Spain. Methods for the quantitative estimation of the red and brown pigments of **Drosophila melanogaster.**

Quantitative estimation of the red (drosopterins) and brown (xanthommatins) eye pigments of **Drosophila melanogaster** in eye color mutants has normally been carried out following Clancy's "double extraction" procedure (1942). This method is based in the extraction of the red pigment in AEA (30% ethyl alcohol acidified with HCl to pH 2.0) by placing the heads in this solvent for 24 hr. Then, the brown pigment is extracted by transferring the heads into a solution of AMA (absolute methyl alcohol containing 1% by volume of dry HCl).

Ephrussi & Herold (1944) systematically studied Clancy's procedure and found it inappropriate for the analysis of the brown pigment in mutants having also drosopterins. They reported that the "double extraction" had two main problems: the AEA seems to render insoluble a part of the brown pigment, and second, some brown pigment is extracted by the AEA.

We have revised the procedures reported in the literature for selective extraction of the eye pigments. In mutants containing both eye pigments, an accurate quantitative estimation can be made using the following methods:

Quantitation of the red pigment ("drosopterins"): Fly heads (around 40, 20 from males and 20 from females, nine-day-old adults) split longitudinally into halves, are placed in AEA (3 ml) for 24 hr (Ephrussi & Herold 1944). Then, the extract is filtered through an inert filter (glass fiber) and the absorbance measured at 480 nm. The absorbance is linear versus the number of heads at least up to values around 0.900.

In order to test the above procedure, the following mutants were chosen: scarlet (it does not have "xanthommatins"), sepia (it has no "drosopterins" and it is the mutant known to accumulate the highest amount of the yellow pigment sepiapterin) and brown (it only has "xanthommatins"). Table 1 shows that the extraction of the red pigment is higher when the heads are split longitudinally or when homogenized with AEA. Interferences due to the yellow and brown pigments were higher when heads are homogenized than when split into halves (Table 1). Only in the case of the sepia mutant was the yellow pigment contribution important. In all the other eye color mutants, the "drosopterins"/sepiapterin ratio is high enough to minimize sepiapterin contribution to "drosopterins" quantitative estimation.

The stability of the red pigment in AEA has been compared with its stability in 0.1 M NaOH and 0.1 M HCl. After 72 hr, the loss of absorbance at 480 nm of a homogenized extract of scarlet heads is: 2% in AEA, 9% in HCl and 90% in NaOH.

Table 1. Extraction of eye pigments using different procedures.

Mutant	Absorbance at 480 nm: 40 heads in 4 ml of AEA (after 24 h)	40 heads homogenized in 4 ml of AEA	40 heads split and placed in 4 ml of AEA (after 24 h)
scarlet	0.368	0.548	0.544
brown	0.006	0.042	0.002
sepia	0.018	0.050	0.023

Table 2. Extraction, purification and quantitation of the brown pigment ("xanthommatins") from **D.melanogaster**. w.t.= water treatment. v.o.l. = volume of the organic layer.

Strain*	Absorbance at 492 nm of organic layer throughout the purification procedure of dihydroxanthommatin: Before w.t. (v.o.l.=2.3ml)	After 1st w.t. (v.o.l.=1.3ml)	After 2nd w.t. (v.o.l.=1.0ml)
bw (150 heads)	0.249	0.328	0.402[a]
v (150 heads)	0.037	--	0.011
v (150 whole flies)	0.092	--	0.045
cn se (150 heads)	0.013	--	--
Hn^{r3} v (250 heads)	0.052	0.015	--

* bw = brown, v = vermilion, cn se = cinnabar sepia; Hn^{r3} v = Henna-recessive[3] vermilion.

[a]Considering the differences in volume between the taken and discarded aliquots of the organic layer after each treatment, the amount of dihydroxanthommatin of the bw mutant in the final solution was 70% that of the initial extract.

Quantitation of the brown pigment ("xanthommatins"): This is carried out following Butenandt et al.'s (1960) procedure for the isolation of dihydroxanthommatin, which has been modified to suit quantitative purposes. Fly heads (around 150, 75 from males and 75 from females, nine-day-old adults) are homogenized in 1.5 ml 2 M HCl. Sodium metabisulfite (10 mg) and n-butanol (2 ml) are added and the mixture is tumbled for 30 min. After centrifugation at 4000 g for 5 min, the organic layer is separated. An aliquot of 1.7 ml is mixed with 10 mg of sodium metabisulfite and 1.5 ml of water. The mixture is tumbled and centrifugated again. After repeating the procedure with 1.2 ml of the washed organic layer, its absorbance is measured at 492 nm. Values can be corrected in order to obtain the amount of dihydroxanthommatin in the initial extract, considering the differences in volume between the aliquot taken and the aliquot discarded of the organic layer after each treatment.

The following mutants were used to test the above procedure: brown, vermilion (no "xanthommatins"), cinnabar sepia (no "xanthommatins", no "drosopterins" and high levels of sepiapterin) and vermilion Henna-recessive[3] (no "xanthommatins", small amounts of "drosopterins" and high amounts of sepiapterin and acetyldihydrohomopterin, a minor pigment of the eyes). As shown in Table 2, fly heads are preferred to whole flies because interferences from body pigments can be avoided. After the second treatment of the butanolic layer with water, interferences from head pigments other than dihydroxanthommatin are considerably reduced. At the same time, dihydroxanthommatin is concentrated up to 160% of the initial concentration, making the quantitation more precise. We have found that the absorbance of dihydroxanthommatin versus the number of heads is linear at least up to 0.900.

References: Butenandt, A., E. Biekert, H. Kubler & B. Linzen 1960, Hoppe Seyler's Z. Physiol. Chem. 319:238-256; Clancy, C.W. 1942, Genetics 27:417-440; Ephrussi, B. & J.L. Herold 1944, Genetics 29:148-175.

Wallace, Bruce. VPI & SU, Blacksburg, Virgina USNA. A $100 incubator.

The material required for the construction of the incubator box includes two 4' x 8' sheets of 1/4" plywood finished on one side, 32 sq ft of 3/4" polystyrene insulating panels, 60 feet of 1" x 1" (actually 3/4" x 3/4") wood strips, hinges, and latches.

The plywood sheets are cut according to the pattern shown in Figure 1 (I had mine custom cut at the lumber yard). Wooden strips are glued and nailed carefully around the outside edge (rough side) of one sheet of each component (door, top, end, bottom, and back). The polystyrene sheets are cut to fit within the edging strips; scrap pieces can be used as well as larger ones because they won't be seen. The second panel of each component is glued and nailed (finished side out) over the strips and polystyrene insulation. (The pattern allows for saw cuts; notice, however, that the larger door components (D_2) can be lengthened, if necessary, so that the total length of the two door components is $35\frac{1}{2}$").

Figure 2 shows that, when assembled, the top rests on the ends, and that the ends overlap the bottom. The back overlaps the ends and bottom; the top rests on the back. Both glue and nails are used in assembling the different components. The incubator can then be stained and varnished.

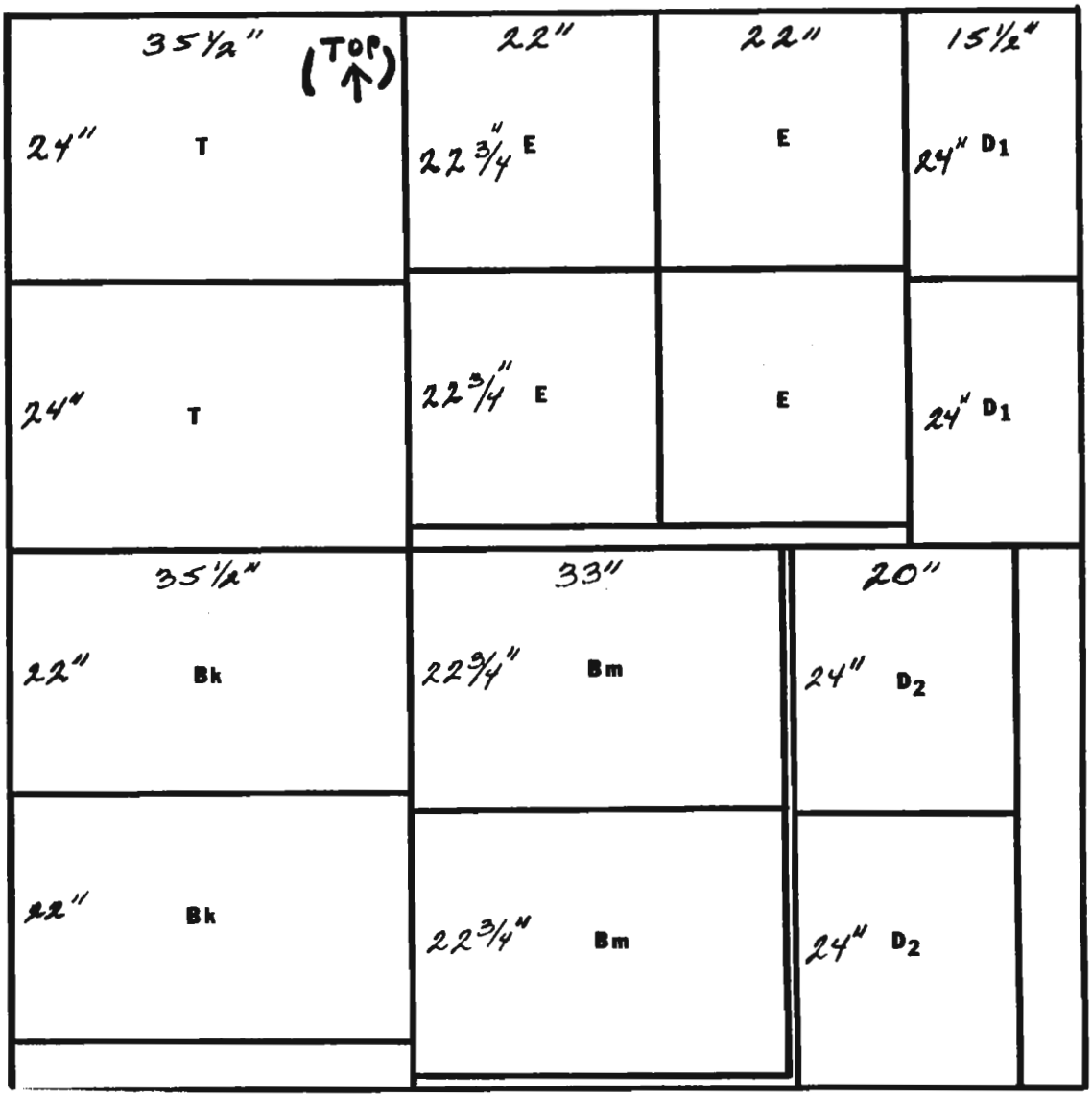

Figure 1. Cutting pattern for two 4' x 8' sheets of 1/4" plywood. T = top; E = end; D_1 and D_2 = door; Bk = back; and Bm = bottom. Two pieces are needed for each component because polystyrene paneling is sandwiched between for insulation. If, because of saw cuts, pieces D_1 are not 15½" wide, the width of pieces D_2 can be increased so that the total width of the two completed doors is 35½".

Details concerning the interior can probably vary--even be improved--according to different tastes. I had a spare shelf from a Fisher electric oven, 22½" deep and 28" long. That shelf, shown in Figure 2, determined the position of the divider that separates the heating element (two 40 watt bulbs) from the bottle compartment.

The divider is made of two pieces of 1/4" plywood 20-3/4" x 22-3/4" (not included in the two 4' x 8' sheets). Matching circular holes about 3" in diameter are cut in the upper front; matching rectangular holes about 3" x 12" are cut in the lower rear. A piece of hardware cloth covering the latter opening is sandwiched between the two pieces of plywood which are glued and held together by strategically located stove bolts. A small circulating fan is mounted on the circular hole; it blows warmed air from the heating compartment into the bottle compartment while cooler air returns through the lower, rectangular hole.

I bolted the Fisher oven shelf (made of expanded metal) to the divider before it, with its attached shelf, was guided into channels at the top and bottom of the incubator. The channels were formed by fastening strips of 1/2" quarter round molding in parallel, leaving a half-inch gap for holding the divider. The other end of the shelf was then bolted to the end of the incubator through pre-bored holes. I then closed the front of the heating chamber with a piece of plywood about 4-3/4" x 20-3/4" in size.

The two 40 watt bulbs that heat the incubator are mounted in ceramic sockets on the floor of the incubator within the heating chamber (Figure 2). I have arranged matters so that the fan is "on" only when the bulbs are "on" (the fan must be wired in parallel with the bulbs; it does not run if wired in series).

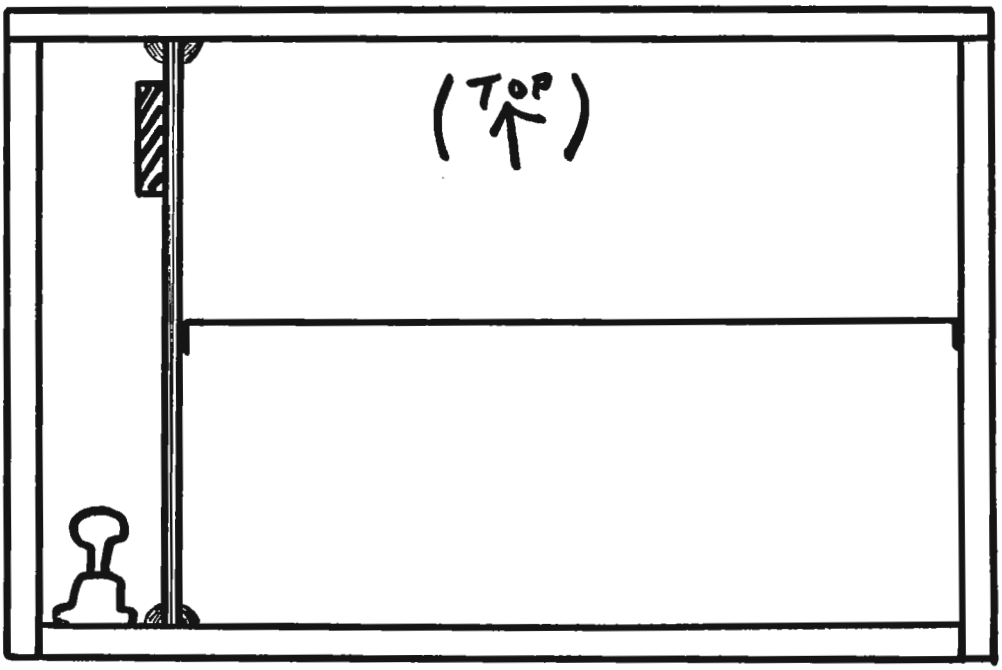

Figure 2. Scheme showing the assembly of the ends, bottom and top. The back overlaps the bottom and the ends; the top rests on the back and the two ends. The doors are mounted flush with the top and the ends; they overlap the bottom. Strips of felt may be needed on the front edges of the top and bottom to give the door a proper seal. The circulating fan is shown attached to the divider whose position, in my case, was determined by the length of the Fisher oven shelf.

The thermostat which controls the temperature of the incubator is mounted outside on the end opposite the heating chamber. The sensing bulb is passed through the end, low and in the rear; it is mounted on the back of the bottle compartment not far from the rectangular return opening in the divider. A one-inch hole was bored in the rear corner of the top at the end opposite the heating chamber. A thermometer, mounted in a rubber stopper, is placed in this hole. The thermometer bulb is protected from breakage by a small strip of hardware cloth that is fastened diagonally across that corner of the incubator, near the top. The thermometer extending above the incubator has been protected by attaching a jar lid (through which a circular hole 1" in diameter was punched) around the thermometer, and screwing the glass jar into the lid. My incubator is mounted on four 4" lengths of a square oak table leg that otherwise had been consigned to the scrap heap.

Aside from items that are available at most hardware stores (plywood, polystyrene panels, wire, light bulbs and ceramic sockets, wire, cabinet, hinges, latches, and Mortite caulking for plugging the holes that were drilled in the incubator walls for the thermometer, thermostat, and wiring, two items were purchased elsewhere: 3" square fan, D33,588 from Edmund Scientific Co., 101 East Gloucester Pike, Barrington, NJ 08007 ($20.00 including shipping costs); 800 watt thermostat, FT-7, from A.M. Leonard Inc., 6665 Spiken Road, Piqua, OH 45356 ($24.00, including shipping).

Whitmore, T., G. Schwitalla and W.-E. Kalisch.
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Incident light microscopy of SSP chromosomes.

For cytological mapping of polytene chromosomes, transmission light and electron microscopy have been used almost exclusively so far, because surface structure studies of native chromosomes have not been able to yield any detailed information concerning the banding patterns. This is the case even in scanning electron microscopic analyses (Iino & Nagura 1980). Using the surface spread polytene (SSP) chromosome preparation technique, however, where chromosomes are spread laterally and longitudinally, more structural details are depicted in transmission light (Kalisch 1982) and electron microscopy (Kalisch & Whitmore 1983) than can be seen in well-extended squash preparations. Through the spreading process the SSP chromosomes are flattened enough so that individual bands become distinguishable as surface structures due to their supercoiled DNA (in comparison with the uncoiled DNA of the interbands). We have shown previously with scanning electron microscopy that the surface pattern of SSP chromosomes is identical with the one which can be seen using transmission electron microscopy (Kalisch & Jacob 1983). In this preliminary note, we show that even incident light microscopy together with differential interference contrast (DIC) can be used for a detailed pattern analysis of SSP chromosomes in Drosophila.

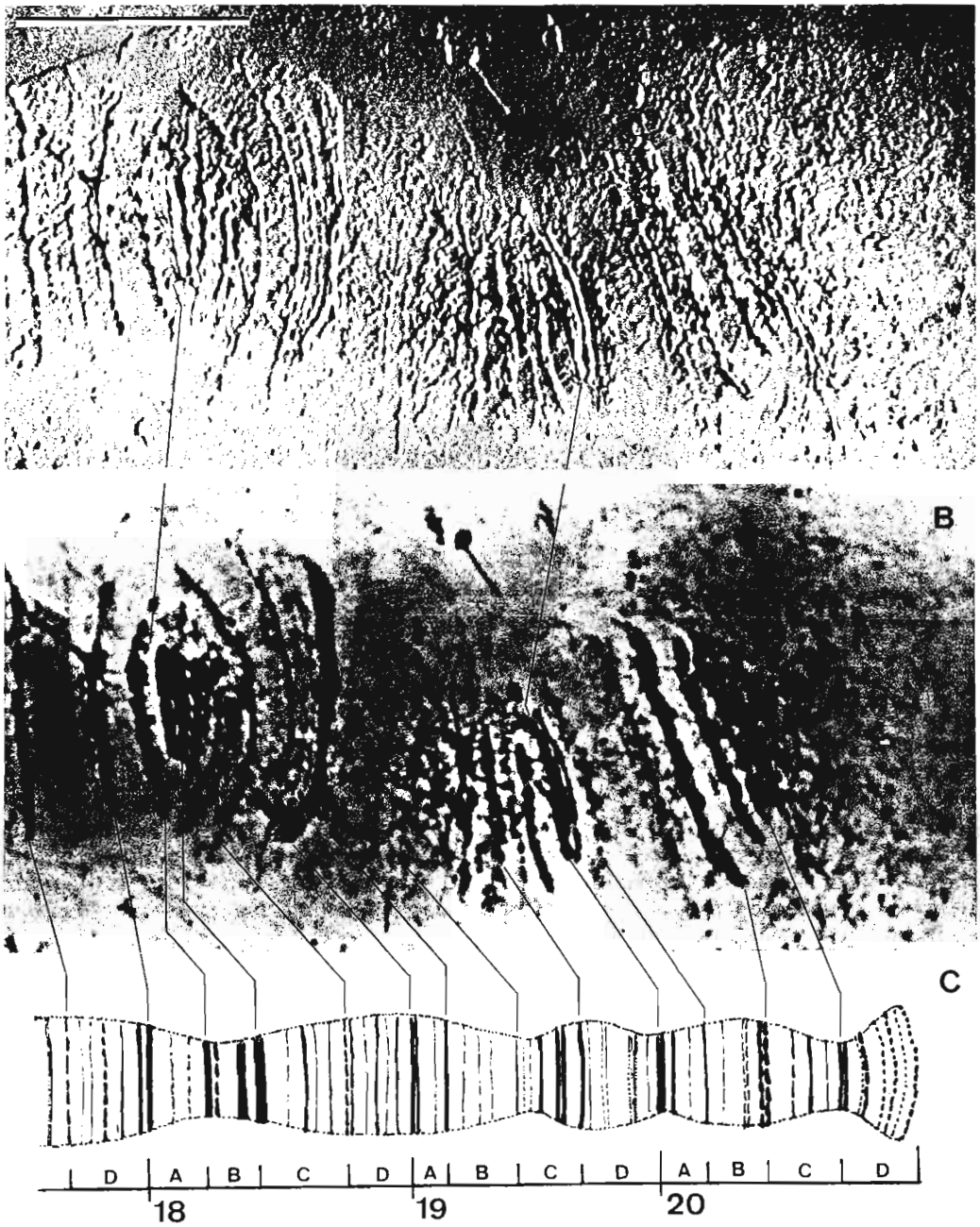

Figure 1. Region 17D-20D of a SSP chromosome-X from a late third instar larva of a **D.hydei** wildtype strain (Sao Paolo). **(A)** Incident light, differential interference contrast (Epiplan 80/0.95 Pol objective). **(B)** Transmission light, phase contrast (Planapo 63/1.4 Ph3 oil objective), green filter (11nm FWHM). **(C)** Chromosome map of Berendes (1963) based on transmission light microscopic analyses of many squash preparations. Micrographs were taken with ZEISS Universal Microscope on Agfapan 25 ASA film. Methodological details of SSP chromosome preparation, pattern analysis and incident light microscopy have already been published elsewhere (Kalisch & Whitmore 1983; Kalisch et al. 1985a and b). Bar = 20 µm.

In Figure 1 the same specimen is depicted with incident (A) and transmission light (B). In both cases patterns are identical, with the exception that in sections with a lower degree of spreading, some additional structures (faint chromosome bands and tight double bands) can be seen with incident light (compare structures labeled in A and B). Additional structures seen in (A) also coincide with the pattern seen by transmission electron microscopy (unpubl. data).

For incident light microscopy the chromosome preparation was sputtered with a 4nm gold layer (on top of the chromosomes for better reflection of the incident light). Even better results should be expected if microscope slides with a thicker gold or silver layer were to be used and the SSP chromosomes, in contrast, prepared on top of it. The phase contrast depiction in (B) is unstained. A subsequent staining with Orcein did not show an improvement in the finer details but rather only an overstaining of the prominent band groups.

The chromosome map in (C) shows the pattern known so far from many transmission light microscopical studies on well-extended squash preparations. The pattern given by this map can not be depicted,

however, from an individual squash preparation due to the thickness of polytene structures on the one hand and the juxtaposition of the individual bands on the other. A homologous chromosome-X region with a higher degree of longitudinal spreading has already been published (Kalisch 1982). Note that in comparison with the chromosome map (C), more structural details are to be seen in the depictions of both papers than in squash preparations (for example: 207) and that the telomeric section (20D) of chromosome-X is always flared in SSP chromosomes. Additionally, in Fig. 1 of this paper, a structural disorder is shown in 19C. This comes from ectopic pairing which is often found between 19C and 20A (unpubl. data).

The resolution of incident light microscopy can be improved further through the use of the ZEISS Laser Scanning Microscope, as could be shown recently with SSP chromosomes of Chironomus (Kalisch et al. 1985b). In that paper we demonstrated the methodological advantages and disadvantages of the technique in different aspects of polytene chromosome mapping.

The intention of this note is to demonstrate the possibility of also using incident light microscopy for the relatively low polytene chromosomes of Drosophila and to emphasize the following advantages of this technique: Use of unstained SSP chromosomes; surface depictions, which show the distribution of total chromosome DNA in individual bands; additional information about pattern details compared with light microscopic techniques used so far.

This work is financially supported by the Deutsche Forschungsgemeinschaft (Ka 309/7-1). We like to thank Dr.Oehlschlegel (RU-Bochum) for technical advice with the DIC microscopy.

References: Berendes, H.D. 1963, Chromosoma (Berl.) 29:118-206; Iino, A. & T. Naguro 1980, Cytobios 27:157-165; Kalisch , W.-E. 1982, DIS 58:85-87; Kalisch, W.-E. & H.J. Jacob 1983, Cytobios 36:39-43; Kalisch, W.-E. & T. Whitmore 1983, Cytobios 37:37-43; Kalisch, W.-E., T. Whitmore & H. Reiling 1985a, Cytobios 141:47-62; Kalisch, W.-E., T. Whitmore & A. Siegel 1985b, J. Microsc., in press.

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The Great Clone Mill Tramp Disaster

Three brothers who pooled one of the biggest ass-raising clone mills in North America on 5,000 acres in southwestern Montana were killed where they lay this morning by tramping clones. Here's the story:

Inside the clone mill, the gene vats were cooking. Sterile workers clocked in and out and tramps loitered in the crawl space, under the warming boilers, eating whatever fell through the safety net and the floor cracks, drinking seepage that killed them by the dozens and changed some into a broad mix of beastly forms.

It was mall of horror and a school of blood from which a few escaped to roam abroad as huge beavers, buttfish, goose-necked businessmen and oreodonts, preying on everything that wasn't them, sometimes cooking fat bits in brute lard brought to a slow boil.

The mill owners, I.P. Freely, Seymour Butts and Wazee Moose, like the Stooges, were sleeping together on the desert floor one evening when some goose-necked business types, cloned accidentally when a mill worker spat unchewed goose fat, passed by in company with a rabid brute and a numbskull. They asked the mill owners for the time, but the three merely wheezed and whimpered and turned over all at once. This angered the unnatural clones, and their greasy circuits screamed "Kill!"

At the clone mill, more tramps had come to drink the seepage. Thousands were metamorphosed, to parade abroad and launch satellites of the parent company, Hour of the Beast Ltd.

Services for the mill owners will be held at Lamanno Panno Fallo, a sunnyside stooge mortuary. Survivors are Clone Freely, Clone Butts and Clone Moose, all sons; Yellow Bleacher, founder of Mixmeat Pies, a close friend; a mutant shrew and a gaggle of beakwomen; and Minnie and Michael Rat, the prexy and his spouse, a Detroit squirrel.

SUBMITTED STOCK LISTS - **D. melanogaster**

UNIVERSITY OF CHILE. Dept. of Cell Biology and Genetics, Casilla 70061, Santiago (7), Chile.

Wild Stocks
Bellavista (Chile)
Copiapó (Chile)
Quilicura (Chile)
Boliche (Ecuador)
El Triunfo (Ecuador)
Piura (Perú)
Oregon R-C (U.S.A.)

X-Chromosome
pn^2
sc - cv
w
w^a
y
y^2, w^a

Chromosome 2
bw
Cy
dp
Po - vg
vg

Chromosome 3
e^{11}
e^{11} - se
tx

Chromosomes 1-2
y ; pr
y ; vg

Chromosomes 1-3
f ; ven

Chromosomes 2-3
dp ; e"

Balancers
Muller 5

KUMAUN UNIVERSITY. Department of Zoology, Naini Tal 263 002 India.

Wild type stock.

1. **D.melanogaster**

KYUSHU UNIVERSITY. Faculty of Agriculture, Department of Sericultural Science, Dr. Bungo Sakaguchi, Fukuoka 812, Japan.

Wild stocks
Florida 9
Wageningen

Inbred stocks
Oregon R
Sevelen

Chromosome 1
B
FM 7a, $y_{31d}\,sc^8w^av^o$ B
mal
w^a B
y w m f
y w r^{11} /C1B

Chromosome 2
vg
vg bw

Chromosome 3
ca
e
ry-1
se

UNIVERSITÄT MÜNSTER. Institut für Strahlenbiologie, Hittorfstr. 17, D-4400 Münster, FR Germany.

1. + (Berlin wild)
2. B
3. y
4. w
5. e^{11}
6. y sc^{S1} In49 sc^8; bw; st p^p
7. y f:= & y sc^{S1} B In49 sc^8 ("Binscy")
8. y & y/y^+Y
9. y f:=/y^+Y & y/y^+Y
10. $y/y/y^+Y$ & y/y^+Y ("Trisom")
11. C(2L)RM, b; C(2R)RM, vg

RESEARCH INSTITUTE OF MEDICAL RADIOLOGY. AMS USSR, Dr. I.D. Alexandrov,
Laboratory of Neutron Radiology, Komarova st., 4, 249020 Obninsk, USSR.

WILD-TYPE STOCKS

001 Algeria
002 Berlin wild
003 Boa Esperance, Minas Gerais, Brazil
004 Canton-S
005 Crimea
006 D-18
007 D-32
008 Formosa
009 Gruta, Argentina
010 Hikone-R
011 Inozemceva
012 Magarach
013 Oregon-R (Johannesburg)
014 Oregon-R (Umeå, Sweden)
015 Pacific-2
016 P-86
017 Sevelen
018 Stellenbosch (Johannesburg)
019 Ultuna

CHROMOSOME 1 STOCKS

1 Basc/rud
2 bo
3 C(1)DX, y f/Df(1)ac, y ac f
4 C(1)DX, y f/Df(1)w^{76k}, w^{76k}
5 C(1)DX, y f/Df(1)w^{78h}, w^{78h}
6 C(1)DX, y f/Df(1)w^{78k1}, w^{78k1}
7 C(1)DX, y f/Df(1)w^{79b2}, w^{79b2}
8 C(1)DX, y f/Df(1)w^{79b3}, w^{79b3}
9 C(1)DX, y f/Df(1)$y^{-75a22-1(2)}$ w MMS-10/Dp y^{2}Y67g19.1
10 C(1)DX, y f/Df(1)y^{78a}, y^{78a}
11 C(1)DX, y f/Df(1)y^{78a}, y^{78a}
12 C(1)DX, y f/Dp(1;1)w^{a}, (w^{a}/w^{a})
13 C(1)DX, y f/g^{2} mei-41^{A1} f
14 C(1)DX, y f/mei-9^{AT1}
15 C(1)DX, y f/mus 102^{A1} cv v f car
16 C(1)DX, y f/w^{71d31}
17 C(1)DX, y f/w^{74c30}
18 C(1)DX, y f/w^{74d10}
19 C(1)DX, y f/w^{75ks}
20 C(1)DX, y f/w^{77a63}
21 C(1)DX, y f/w^{79b13}
22 C(1)DX, y f/w^{79b14}
23 C(1)DX, y f/w^{83b32}
24 C(1)DX, y f/w mus 101^{A1}
25 C(1)DX, y f/y^{79d2}
26 C(1)DX, y f/y^{79d3}
27 C(1)DX, y f/y^{93fXL}
28 C(1)DX, y f/y^{84dS}
29 C(1)DX, y f/y^{84eS}
30 C(1)DX, y f/y^{84e61S}
31 C(1)DX, y f/y^{84f}
32 C(1)DX, y f/y ac sc pn w^{e59} spl ec ct
33 C(1)DX, y f/y mus 102^{A1} g^{2} mei-41^{A1} f
34 C(1)DX, y f/y sc z w^{a} ec
35 C(1)DX, y f/y sc z w^{74b}
36 C(1)DX, y f/y sc z w^{74b29}
37 C(1)DX, y f/y sc z w^{74c17}
38 C(1)DX, y f/y sc z w^{74c157}
39 C(1)DX, y f/y sc z w^{74d50}
40 C(1)DX, y f/y sc z w^{74d145}
41 C(1)DX, y f/y sc z w^{74j}
42 C(1)DX, y f/y sc z w^{75a}
43 C(1)DX, y f/y sc z w^{76a}
44 C(1)DX, y f/y sc z w^{76b2}
45 C(1)DX, y f/y sc z w^{76b98}
46 C(1)DX, y f/y sc z w^{76j2}
47 C(1)DX, y f/y sc z w^{76j10}
48 C(1)DX, y f/y sc z w^{77a112}
49 C(1)DX, y f/y sc z w^{79b6}
50 C(1)DX, y f/y sc z w^{79b8}
51 C(1)DX, y f/y^{2} w^{bf} spl ec ct
52 C(1)DX, y f/z Tr(w^{+})
53 C(1)RM, y v f mal^{2}/Df(1)mal^{5}, y^{2} ct f mal^{5}/y^{+}Ymal^{106}
54 cho^{2}
55 Df(1)w^{59k13} Dp(1;3)w^{co}
56 Df(1)w^{-67k30} lz ras v/FM7
57 Df(1)w^{72b}
58 Df(1)w^{74c}/FM4
59 Df(1)w^{76b55}, y sc z w^{76b55}
60 Df(1)w^{76j}
61 Df(1)w^{77a2}/FM4
62 Df(1)w^{79b4}
63 Df(1)w^{79b7}/FM4
64 Df(1)w^{79b10}
65 Df(1)w^{79d6}/FM4
66 Df(1)w^{79g}/FM4
67 Df(1)w^{81a}/FM4
68 Df(1)w^{81c44}
69 Df(1)w^{81k12}
70 Df(1)w^{83f49}/FM4
71 Df(1)y^{71k}
72 Df(1)y^{7111}
73 Df(1)y^{72d2}
74 Df(1)y^{74b151}
75 Df(1)y^{74d2}
76 Df(1)y^{74d30}
77 Df(1)y^{74d40}
78 Df(1)y^{74e2}
79 Df(1)y^{74k}
80 Df(1)y^{7513}
81 Df(1)y^{76i}
82 Df(1)y^{76j}
83 Df(1)y^{76k}
84 Df(1)y^{77a}
85 FM4,l(1)?^{ts}/l(1)mys
86 g^{2}
87 g^{76b}
88 In(1)AM/r^{39k} f B
89 In(1)w^{m51b19}
90 In(1)w^{mJ}, w^{m} v f car/y w f:=
91 In(1)y^{72d3}
92 In(1)y^{74b51}
93 In(1)y^{74c166}
94 In(1)y^{74e}
95 In(1)y^{76b94}
96 In(1)y^{79b21}
97 In(1)y^{79d2}
98 In(1)y^{83fXL}
99 l^{39}(Pgd^{-}) pn/FM4
100 l(1)w^{76b}/FM4
101 l(1)w^{76l}/FM4
102 l(1)w^{77d}/FM4
103 l(1)w^{79b1}/FM4
104 l(1)w^{79b5}/FM4
105 l(1)w^{79h2}/FM4
106 l(1)w^{81f3}/FM4
107 l(1)w^{83b21}/FM4
108 l(1)w^{83d13}/FM4
109 l(1)w^{83f40}/FM4
110 l(1)w^{83f58}/FM4
111 l(1)y^{78d}/FM4
112 l(1)y^{79b}/FM4
113 l(1)y^{79d1}/FM4
114 l(1)y^{79h2}/FM4
115 l(1)y^{82c38}/FM4
116 l(1)y^{83c}/FM4
117 M-5 (y sc^{8}Ins sc^{S1} w^{a})
118 mal/y w f:=
119 pn
120 pn^{MS2} l^{DES}/FM6/Pgd^{-} pn^{-} w^{+}Y
121 sc $z^{mottled}$
122 T(1;2)y^{76b37}
123 T(1;2)y^{79d}/FM4
124 T(1;3)y^{78j}/y f:=
125 T(1;4)y^{74c40}
126 w
127 w^{22cHI}
128 w^{56l12}
129 w^{66g}
130 w^{67a}
131 w^{67b}
132 w^{67d}
133 w^{67g}
134 w^{71k}
135 w^{72a2}
136 w^{72a29}
137 w^{72a189}
138 w^{72d75}
139 w^{74c68}

CHROMOSOME 1 STOCKS (contin.)

140 w^{74f}
141 w^{76b37}
142 w^{76b129}
143 w^{76j3}
144 w^{76l}
145 w^{78e}
146 w^{78f1}
147 w^{79b11}
148 w^{79b12}
149 w^{79b15}
150 w^{79b16}
151 w^{79d2}
152 w^{79d3}
153 w^{79d5}
154 w^{79f}
155 w^{79h3}
156 w^{79h4}
157 w^{79h5}
158 w^{81b}
159 w^{81c40}
160 w^{81f2}
161 w^{81k1}
162 w^{81k2}
163 w^{81k3}
164 w^{81k4}
165 w^{81k5}
166 w^{81k6}
167 w^{81k7}
168 w^{81k8}
169 w^{81k9}
170 $w^{81k10}/FM4$
171 $w^{81k11}/FM4$
172 w^{82c}
173 w^{83b12}
174 w^{83b38}
175 w^{83b49}
176 w^{83c12}
177 w^{83c19}
178 w^{83c20}
179 w^{83f10}
180 w^{83f29}
181 w^{83f42}
182 w^{83k}
183 w^{84f}
184 w^{84g}
185 $w^{84h}/FM4$
186 w^{a}
187 w^{a} su(f)
188 w^{Bwx}
189 w^{bl}
190 w^{ch} spl
191 w^{co} sn^{2}
192 w^{col}
193 w^{e}
194 w^{h}
195 w^{m4}
196 w^{sat}
197 w^{sp}
198 w^{sp2}
199 w^{spA}
200 X^{c2}, y v/y^{+} sc^{8}·Y
201 y
202 y^{td}
203 y^{2}
204 y^{2S}
205 y^{2} sc car·Dp(1)sc^{VI},y^{+}
206 y^{2} sc w^{-} spl
207 y^{2} sc w^{i} w^{ch}
208 y^{2}su(w^{a}) w^{a}
209 y^{2} w^{bf} spl sn^{3}
210 y^{2} w^{spA}
211 y^{31d} w^{74b166}
212 y^{31d} sc w^{78f2}
213 y^{31d} w^{79d1}
214 y^{34c}
215 y^{66c}
216 y^{71k}
217 y^{71l2}
218 y^{72a}
219 y^{72d1}
220 y^{72d4}
221 y^{74b46}
222 y^{74b121}
223 y^{74d1}
223a y^{74e3}
224 y^{75l2}
225 y^{76a110}
226 y^{76a123}
227 y^{77d}
228 y^{77f}
229 y^{79b18}
230 y^{79d3}
231 y^{79dsd}
232 y^{79g}
233 y^{79h1}
234 y^{81c1}
235 y^{81c2}
236 y^{81c3}
237 y^{81k29}
238 y^{82c1}
239 y^{82c2}
240 y^{82c3}
241 y^{83f12}
242 y^{83f26}
243 y^{83f58}
244 y ac z Dp(1;1)w^{60h30}/y w f:=
245 y Df(1)w^{258-45}/FM4
246 y ec ct v f
247 y w
248 z

CHROMOSOME 2 STOCKS

249 b^{66a}
250 b^{71k2}
251 b^{74b2}
252 b^{74b4}
253 b^{74b5}
254 b^{74c2}
255 b^{74c4}
256 b^{74c5}
257 b^{74d2}
258 b^{74d4}
259 b^{74d6}
260 b^{75a}
261 b^{76b1}
262 b^{76b2}
263 b^{76e1}
264 b^{76e2}
265 b^{76f3}
266 b^{76j1}
267 b^{76j2}
268 b^{76j3}
269 b^{76k1}
270 b^{76k2}
271 b^{77a1}
272 b^{77a2}
273 b^{77a3}
274 b^{77a4}
275 b^{77a5}
276 b^{77j}
277 b^{78a}
278 b^{78f1}
279 b^{78f2}
280 b^{78g}
281 b^{78k1}
282 b^{78k2}
283 b^{78k3}
284 b^{78k5}
285 b^{79a1}
286 b^{79a4}
287 b^{79b1}
288 b^{79b7}
289 b^{79d2}
290 b^{79d4}
291 b^{79d8}
292 b^{79d10}
293 b^{79d11}
294 b^{79d13}
295 b^{79f2}
296 b^{79g2}
297 b^{79h2}
298 b^{79h3}
299 b^{81a2}
300 b^{81f3}
301 b^{81c}
302 b^{81c2}
303 b^{81c17}
304 b^{81k}
305 b^{82c3}
306 b^{82c7}
307 b^{83c20}
308 b^{83c25}
309 b^{83c35b}
310 b^{83c36}
311 $b^{83c47}/SM5$

CHROMOSOME 2 STOCKS (contin.)

312 b^{83d29b}
313 b^{83d35}
314 b^{83d36}
315 b^{83f17}
316 b^{83f18}
317 b^{83f51}
318 b^{83f52}
319 b^{83fXD}
320 b^{831}
321 b^{84g}
322 b^{84h34}
323 b^{83b40} Bl/b^{83b40} l
324 b^{83c26} Bl/b^{83c26} l
325 b cn Pm/b lt l cn mi sp
326 b cn vg
327 b In(2)bw^{VDe1}/b lt l cn mi sp
328 b j
329 b Pm/In(2LR)Cy, net dp^{txl} Cy b pr Bl lt^3 cn^2 L^4 sp^2
330 b so^2 cn (?)
331 b vg
332 Bl/SM5
333 Bl cn^{79d2}/cn^{79d2} l
334 blo
335 bw
336 bw^D
337 cn^{67d}
338 cn^{72a2}
339 cn^{74b4}
340 cn^{74c2}
341 cn^{74c5}
342 cn^{74c7}
343 cn^{74c8}
344 cn^{74c9}
345 cn^{74c10}
346 cn^{74d1}
347 cn^{74d4}
348 cn^{74d5}
349 cn^{74d7}
350 cn^{74d8}
351 cn^{74a2}
352 cn^{76b}
353 cn^{76e2}
354 cn^{76k2}
355 cn^{77a2}
356 cn^{77a3}
357 cn^{77a4}
358 cn^{77a9}
359 cn^{77a10}
360 cn^{77c2}
361 cn^{78a}
362 cn^{78b1}
363 cn^{78g}
364 cn^{78j2}
365 cn^{78k1}/SM5
366 cn^{781}
367 cn^{79b5}
368 cn^{79b7}
369 cn^{79b15}
370 cn^{79c}
371 cn^{79d1}
372 cn^{79d3}
373 cn^{79b6}
374 cn^{79d12}
375 cn^{79d18}
376 cn^{79h1}
377 cn^{79h2}/SM5
378 cn^{79h3}/b Pm
379 cn^{79h6}
380 cn^{79h7}/SM5
381 cn^{80j}
382 cn^{81a1}
383 cn^{81c51}
384 cn^{81f1}
385 cn^{81k1}
386 cn^{81k2}
387 cn^{81k5}
388 cn^{811}/SM5
389 cn^{8114}
390 cn^{8115}/SM5
391 cn^{82c1}
392 cn^{83b31}
393 cn^{83c17}/SM5
394 cn^{83c58}
395 cn^{83d15}
396 cn^{83f16}
397 cn^{83f17}
398 cn^{83f50}
399 cn^{83f51}
400 cn^{84f76}
401 cn^{84h27}
402 cn vg
403 cn^{79d15} vg
404 cn^{81c21a} vg
405 cn^{81c32} vg
406 cn^{82c41} vg
407 cn^{82c63} vg
408 Df(2)b^{71k1}/SM5
409 Df(2)b^{89j}/SM5
410 Df(2)b^{78j}/SM5
411 Df(2)b^{79b3}/SM5
412 Df(2)b^{79b4}/SM5
413 Df(2)b^{79b8}/SM5
414 Df(2)b^{79h1}/SM5
415 Df(2)b^{80k}/SM5
416 Df(2)cn^{74c3}/SM5
417 Df(2)cn^{74c6}/SM5
418 Df(2)cn^{78j1}/SM5
419 Df(2)cn^{79b8}/b Pm
420 Df(2)cn^{79d9}/b Pm
421 Df(2)cn^{79d10}/SM5
422 Df(2R)vg^B, b cn vg^B/In(2L)NS In(2R)NS, px sp
423 Df(2R)vg^C, vg^C/In(2LR)Rev^B,Rev^B
424 l(2)b^{77c}/SM5
425 l(2)b^{79a3}/SM5
426 l(2)b^{79d5}/b Pm
427 l(2)b^{79d6}/SM5
428 l(2)b^{801}/SM5
429 l(2)b^{81a}/SM5
430 l(2)b^{81f1}/SM5
431 l(2)b^{81f2}/SM5
432 l(2)b^{8117}/SM5
433 l(2)b^{81140}/SM5
434 l(2)b^{81142}/b Pm
435 l(2)b^{82c16}/SM5
436 l(2)b^{82c44}/SM5
437 l(2)b^{82c54}/SM5
438 l(2)b^{83b11}/SM5
439 l(2)b^{83b22}/SM5
440 l(2)b^{83c35a}/SM5
441 l(2)b^{8311}/SM5
442 l(2)b^{8311}/SM5
443 l(2)b^{8312}/SM5
444 l(2)b^{84h14}/SM5
445 l(2)b^{84h40}/SM5
446 l(2)b^{84h50}/SM5
447 l(2)b^{84h70}/SM5
448 l(2)cn^{74b1}/SM5
449 l(2)cn^{74b2}/SM5
450 l(2)cn^{74b3}/b Pm
451 l(2)cn^{76c}/SM5
452 l(2)cn^{76i}/b Pm
453 l(2)cn^{76k1}/SM5
454 l(2)cn^{76k3}/b Pm
455 l(2)cn^{77a6}/SM5
456 l(2)cn^{78e}/SM5
457 l(2)cn^{78f}/SM5
458 l(2)cn^{78k5}/SM5
459 l(2)cn^{79b1}/SM5
460 l(2)cn^{79b9}/SM5
461 l(2)cn^{79b10}/b Pm
462 l(2)cn^{79b11}/b Pm
463 l(2)cn^{79b13}/SM5
464 l(2)cn^{79b14}/SM5
465 l(2)cn^{79b18}/b Pm
466 l(2)cn^{79h5}/SM5
467 l(2)cn^{81a2}/SM5
468 l(2)cn^{81f3}/SM5
469 l(2)cn^{81f6}/SM5
470 l(2)cn^{81k3}/SM5
471 l(2)cn^{81k4}/SM5
472 l(2)cn^{8111}/SM5
473 l(2)cn^{8112}/SM5
474 l(2)cn^{82c33}/b Pm
475 l(2)cn^{83b27}/SM5
476 l(2)cn^{83b33}/SM5
477 l(2)cn^{83c23}/SM5
478 l(2)cn^{83d21}/SM5
479 l(2)cn^{831}/SM5
480 l(2)cn^{84f37}/SM5
481 l(2)cn^{84h}/SM5
482 l(2)cn^{84h}/SM5
483 l(2)cn^{84h80}/SM5
484 l(2)vg^{67d1}/SM5
485 l(2)vg^{71a1}/b Pm
486 l(2)vg^{74b1}/SM5
487 l(2)vg^{76d1}/SM5
488 l(2)vg^{76i1}/SM5
489 l(2)vg^{76k2}/SM5

CHROMOSOME 2 STOCKS (contin.)

490 l(2)vg^{76k2}/SM5
491 l(2)vg^{77d1}/SM5
492 l(2)vg^{78b4}/SM5
493 l(2)vg^{78j1}/SM5
494 l(2)vg^{78j2}/SM5
495 l(2)vg^{78j3}/SM5
496 l(2)vg^{78k3}/SM5
497 l(2)vg^{79a}/b Pm
498 l(2)vg^{79b4}/SM5
499 l(2)vg^{79b4}/SM5
500 l(2)vg^{79b5}/b Pm
501 l(2)vg^{79b6}/SM5
502 l(2)vg^{79d2}/SM5
503 l(2)vg^{79d3}/b Pm
504 l(2)vg^{79f1}/SM5
505 l(2)vg^{79f1}/SM5
506 l(2)vg^{79h4}/b Pm
507 l(2)vg^{79h7}/b Pm
508 l(2)vg^{80l1}/SM5
509 l(2)vg^{81a}/SM5
510 l(2)vg^{81b1}/b Pm
511 l(2)vg^{81c}/SM5
512 l(2)vg^{81c41d}/SM5
513 l(2)vg^{81l11}/SM5
514 l(2)vg^{81l18}/SM5
515 l(2)vg^{82c61}/SM5
516 l(2)vg^{82c62}/SM5
517 l(2)vg^{83b}/SM5
518 l(2)vg^{83c31}/SM5
519 l(2)vg^{83f15}/SM5
520 l(2)vg^{83f36}/SM5
521 l(2)vg^{83f38}/SM5
522 l(2)vg^{83f52}/SM5
523 l(2)vg^{83f58}/SM5
524 l(2)vg^{83l2a}/SM5
525 l(2)vg^{83l2b}/SM5
526 l(2)vg^{84f}/SM5
527 l(2)vg^{84f65}/SM5
528 l(2)vg^{84h49}/SM5
529 nub b pr
530 or^{45a} sp^{2}
531 pu
532 rk cn bw
533 sca
534 sca l(2)C/SM5
535 so
536 T(2;Y)cn^{79b2}/b cn vg
537 T(2;Y)cn^{81f5}/b cn vg
538 T(2;Y)cn^{84h29}/SM5/b Pm
539 T(2;Y)vg^{76d2}/b cn vg
540 vg
541 vg^{67d2}/SM5
542 vg^{74c5}
543 vg^{76f}
544 vg^{76i2}
545 vg^{77a4}/SM5
546 vg^{78a2}/SM5
547 vg^{78b3}
548 vg^{78f2}/SM5
549 vg^{78k2}
550 vg^{79b1}
551 vg^{79h5}
552 vg^{81c13}
553 vg^{81f}
554 vg^{81l24}
555 vg^{82c13}
556 vg^{83c45}/SM5
557 vg^{N83l}
558 vg^{no78a1}/SM5
559 vg^{np}
560 vg^{np83c}/SM5
561 vg^{nw71k2}
562 vg^{nw74b2}
563 vg^{nw74c1}/b Pm
564 vg^{nw74c4}/b Pm
565 vg^{nw74c6}/b Pm
566 vg^{nw74c7}/SM5
567 vg^{nw75a}/SM5
568 vg^{nw78b1}/SM5
569 vg^{nw78b2}
570 vg^{nw79d4}/b Pm
571 vg^{nw79d6}/b Pm
572 vg^{nw79d7}/SM5
573 vg^{nw79f2}/b Pm
574 vg^{nw79h1}/SM5
575 vg^{nw79h6}/b Pm
576 vg^{nw80l2}/b Pm
577 vg^{nw81b2}
578 vg^{nw81c18}/SM5
579 vg^{nw81c28}/b Pm
580 vg^{nw81k1}/SM5
581 vg^{nw81l26}/SM5
582 vg^{nw82c14}/b Pm
583 vg^{nw83b22}/SM5
584 vg^{nw83b24}/SM5
585 vg^{nw83b27}
586 vg^{nw83b39}/SM5
587 vg^{nw83c3}/SM5
588 vg^{nw83c5}/SM5
589 vg^{nw83c7}/SM5
590 vg^{nw83c24}/SM5
591 vg^{nw83c43}/SM5
592 vg^{nw83d}/SM5
593 vg^{nw83d4}/SM5
594 vg^{nw83fXD}/b Pm
595 vg^{nw84f}/SM5
596 vg^{nw84h}/b Pm
597 vg^{st76j1}/b Pm
598 vg^{st77d2}/SM5
599 vg^{st79d5}
600 vg^{st81a}/SM5
601 vg^{st83c42}/SM5
602 vg^{st83l}/SM5
603 vg^{st84h}/SM5

CHROMOSOME 3 STOCKS

604 e^{11}
605 mus(3) 312^{D1}/TM3, y^{+} ri p^{p} sep bx^{34e} e^{s} Sb Ser
606 st
607 st c(3)G ca/Ubx^{130}, ri Ubx^{130} e^{s} ca
608 st mus(3) 301^{D1}/st mus(3) 301^{D1}
609 st mus(3) 302^{d2}/TM2, Ubx^{130} se e^{s}
610 st mus(3) 302^{D2}/TM3, y^{+} ri p^{p} sep bx^{34e} e^{s} Sb Ser
611 st mus(3) 304^{D1}/TM3, y^{+} ri p^{p} sep bx^{34e} e^{s} Sb Ser
612 st mus(3) 305^{D1}/TM3, y^{+} ri p^{p} sep bx^{34e} e^{s} Sb Ser
613 st mus(3) 310^{D1}/TM3, y^{+} ri p^{p} sep bx^{34e} e^{s} Sb Ser

CHROMOSOME 1 - Y

614 $\overline{XY}$, y B/Y & y f:=
615 Y·w^{+}/y w^{a}

CHROMOSOME 3 - Y

616 B^{S}Y^{y31d}; mus(3) 312/TM3,
y^{+} ri p^{p} sep bx^{34e} e^{s}Sb Ser

CHROMOSOME 1 - 2

617 C(1)DX,y f/Y; b & su(b)^{18}/Y; b
618 C(1)DX,y f/Y; b & su(b)^{31}/Y; b
619 Inscy; dp b cn bw
620 Inscyw^{a} (M-5); b cn vg

CHROMOSOME 1 - 3

621 fs(1)K 10 w/C1B; mwh se e
622 y w^{co}/y^{+}·Y; flr^{3} se/TM2, Ubx^{130} se e^{s}

CHROMOSOME 2 - 3

623 cn; e^{11}
624 cn bw; e^{11}

CHROMOSOME 1 - 2 - 3

625 B; Cy/Pm; D/Sb
626 Inscyw^{a} (M-5); bw; st p^{p}
627 Inscyw^{a} (M-5); cn vg; e^{11}
628 sc z +^{is}; Cy; Ubx^{130}/Xa
629 y sc^{S1} In49 sc^{8}; bw; st p^{p}
630 y^{2} sc w^{a} w^{ch} fa; Cy; Ubx/Xa

SUBMITTED STOCK LISTS - **Other Species**

UNIVERSITY OF CHILE. Dept. of Cell Biology and Genetics, Casilla 70061, Santiago (7), Chile.

D.brncici
Wild stock
Bogotá (Colombia)

D.buzzatii
Wild stocks
Copiapó (Chile)
Santiago (Chile)

D.busckii
Wild stock
La Campana (Chile)

D.funebris
Wild stocks
Chillán (Chile)
La Serena (Chile)
Puerto Montt (Chile)
Punta Arenas (Chile)
Osorno (Chile)
Tierra del Fuego (Chile)
Valdivia (Chile)
Valparaíso (Chile)

D.gaucha
Wild stocks
Buenos Aires (Argentina
San Luis (Argentina)
Cochabamba (Bolivia)
Campos do Jordan (Brazil)
Moitos Capoes (Brazil)
Tainhas (Brazil)

X-Chromosome
y
w

D.gasici
Wild stocks
Arica (Chile)
Bogotá (Colombia)
Cochabamba (Bolivia)

D.hydei
Wild stock
Chillán (Chile)

D.immigrans
Wild stocks
Concepción (Chile)
Punta Arenas (Chile)
Santiago (Chile)
Valdivia (Chile)
Valparaíso (Chile)

D.mesophragmatica
Wild stocks
Bogotá (Colombia)
La Paz (Bolivia)
Machu-Pichu (Perú)

D.nebulosa
Wild stock
Los Tintos (Ecuador)

D.pavani
Wild stocks
Algarrobo (Chile)
Bellavista (Stgo.-Chile)
Copiapó (Chile)
Mendoza (Argentina)
Olmué (Chile)
S. José de Maipo (Chile)
Vallenar (Chile)
Vizcachas (Chile)

X-Chromosome
m

D.pseudoobscura
Wild stock
Arizona (U.S.A.)

D.obscura
Wild stock
Cataluña (Spain)

D.repleta
Wild stock
Valparaíso (Chile)

D.simulans
Wild stocks
Las Palmas (Chile)
Quilicura (Chile)
Valdivia (Chile)

D.subobscura
Wild stocks
Almería (Spain)
Bariloche (Argentina)
Cinisi (Italy)
Coihaique (Chile)
La Campana (Chile)
La Florida (Stgo.-Chile)
Los Angeles (Chile)
Mallorca (Spain)
Pucón (Chile)
Puerto Montt (Chile)
Salto del Laja (Chile)
Tübingen (W. Germany)
Valdivia (Chile)

D.viracochi
Wild stock
Bogotá (Colombia)

D.virilis
Wild stock
Santiago (Chile)

KUMAUN UNIVERSITY. Department of Zoology, Naini Tal 263 002 India.

Wild stock
1. **D.jambulina** 2. **D.nepalensis** 3. **D.immigrans** 4. **D.lacertosa** 5. **D.malerkotliana**

KYUSHU UNIVERSITY. Faculty of Agriculture, Department of Sericultural Science, Dr. Bungo Sakaguchi, Fukuoka 812, Japan.

Subgenus Sophophora

D.pseudoobscura **D.equioxialis** **D.willistoni**

Report of M. Loukas and Y. Vergini. Agricultural College of Athens, Athens, Greece.

Linkage groups in **Drosophila** species of the **obscura** group
by using enzyme polymorphisms detected electrophoretically.

In the following Table we give the crosses performed to establish linkage groups in the species **Drosophila obscura** and **D.ambigua** (both belonging to the **obscura** group) by using electrophoretically detected enzyme polymorphisms. Abbreviations used: A for α-GPD locus; B for ADH; C for PEP-1;D for XDH; E for MDH; G for AO; H forLAP; I for ME; K forG-6-PD; L for the hypothesized homologous locus to EST-7 locus of **D.subobscura** and F and S for the Fast and Slow alleles of each locus, respectively.

	Loci tested	Cross: male		Cross: female	F_1		Are they linked?
D.obscura	α-GPD-ADH	$A^FA^SB^FB^S$	X	$A^FA^FB^FB^F$	$A^FA^FB^FB^S$	9	yes
					$A^FA^SB^FB^F$	1	
	MDH-ADH	$E^FE^SB^FB^S$	X	$E^FE^FB^FB^F$	$E^FE^FB^FB^F$	5	yes
					$E^FE^SB^FB^S$	4	
	PEP-1-XDH	$C^FC^SD^FD^S$	X	$C^SC^SD^FD^F$	$C^FC^SD^FD^F$	3	yes
					$C^SC^SD^FD^S$	7	
	LAP-AO	$H^FH^SG^FG^S$	X	$H^FH^FG^FG^F$	$H^FH^SG^FG^S$	4	yes
					$H^FH^FG^FG^F$	11	
	LAP-XDH	$H^FH^SD^FD^S$	X	$H^FH^FD^FD^F$	$H^FH^SD^FD^F$	8	yes
					$H^FH^FD^FD^S$	4	
	LAP-ME	$H^FH^SI^FI^S$	X	$H^FH^FI^FI^F$	$H^FH^SI^FI^F$	6	yes
					$H^FH^FI^FI^S$	9	
	LAP-ADH	$H^FH^SB^FB^S$	X	$H^FH^FB^SB^S$	$H^FH^FB^FB^S$	7	no
					$H^FH^SB^SB^S$	5	
					$H^FH^FB^SB^S$	2	
					$H^FH^SB^FB^S$	6	
D.ambigua	PEP-1-XDH	$C^FC^SD^FD^S$	X	$C^SC^SD^FD^F$	$C^FC^SD^FD^F$	5	yes
					$C^SC^SD^FD^S$	10	
	G-6-PD	K^F-	X	K^SK^S	♀♀ : all K^FK^S		sex-linked
					♂♂ : all $[K^S]$		
	LAP-AO-XDH	$H^FH^SG^FG^SD^FD^S$	X	$H^FH^FG^SG^SD^FD^F$	$H^FH^FG^SG^SD^FD^F$	9	yes
					$H^FH^SG^FG^SD^FD^S$	7	
	PEP-1-EST-7	$C^FC^SL^FL^S$	X	$C^SC^SL^FL^F$	$C^FC^SL^FL^F$	3	no
					$C^SC^SL^FL^S$	5	
					$C^FC^SL^FL^S$	10	
					$C^SC^SL^FL^F$	6	

From the Table it is apparent that for **D.obscura** the loci studied belong to two different linkage groups. One includes the loci α-GPD; ADH; and MDH and the other the loci PEP-1; XDH; LAP; AO and ME. For **D.ambigua** the six loci studied belong to three different linkage groups: the locus G-6-PD is sex-linked; the loci PEP-1, LAP, AO and XDH belong to the same linkage group; and finally the locus EST-7 belongs to another. These findings are in accordance with the corresponding linkage groups of **D.subobscura** (Loukas et al. 1979). Thus, since the loci α-GPD, ADH and MDH are located on the U chromosome of **D.subobscura**, we postulate that these loci are located on the B chromosome of **D.obscura** which is homologous to the U chromosome (Steinemann et al. 1984). Similarly, since the loci PEP-1, XDH, LAP, AO and ME are located on the O chromosome of **D.subobscura**, we postulate that these loci must be located on the E chromosome of **D.obscura** and on the U chromosome of **D.ambigua** (for the first four loci) which are homologous to the O chromosome (Steinemann et al. 1984). Finally, since the locus EST-7 is located on the J chromosome of **D.subobscura**, the hypothesized homologous locus studied here is most probably located on the JR chromosome of **D.ambigua** which is homologous to the J chromosome (Steinemann et al. 1984).

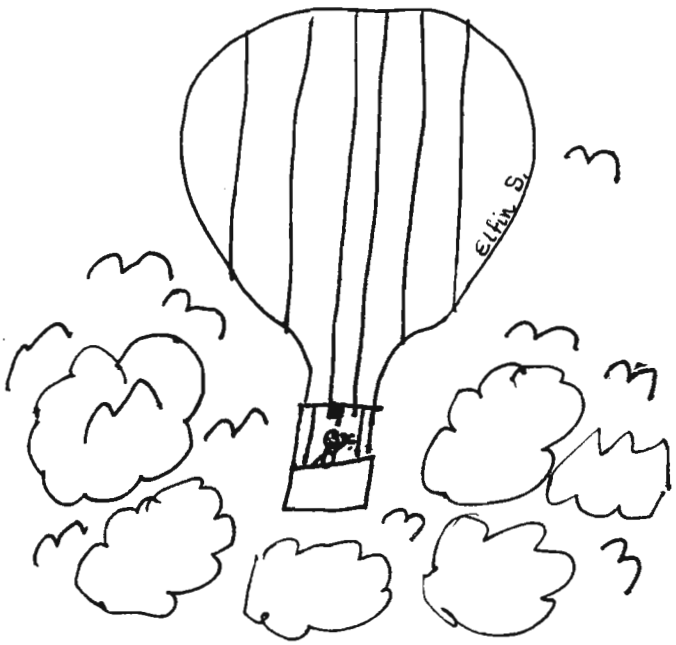
References: Loukas, M., C.B. Krimbas, P. Mavragani-Tsipidou & C.D. Kastritsis 1979, J. Heredity 70:17-26; Steinemann, M., W. Pinsker & D. Sperlich 1984, Chromosoma 91:46-53.

Report of Y. Maeda.

Hyogo Prefectural Nagata High School, Kobe, Japan.

The mutant unextended [uex: DIS 36:39 (1962); Jpn. J. Genet. 59:249-257 (1984)] is now shown to be allelic to rickets (rk, 2-48.2: for recent information, see Woodruff & Ashburner 1979, Genetics 92:117-132).

I thank Dr. M. Ashburner for pointing out the possibility and for providing the rk alleles.



Report of I.D. Alexandrov, M.A. Ankina and M.V. Alexandrova.
Research Institute of Medical Radiology, Obninsk, USSR.

Genetics and cytogenetics of the yellow mutations induced by gamma-rays, ^{252}Cf and fission neutrons.

The following list is a part of our general stocklist (see Stock List, this issue) and contains information on the transmissible yellow mutations which were discovered in various experiments designed for estimating intra-locus mutation rates (see Research Notes, this issue) in the different post-meiotic stages of the wild-type (D-32, D-18) or c(3)G males (the last column list) after low (gamma-rays of ^{60}Co)- and high (^{252}Cf, fission neutrons)-LET radiation (the fifth column). All the mutants were basically obtained by mating the irradiated males to Ins(1) sc^{SIL} sc^{8R}+ dl-49, y sc^{SI} sc^{8} w^{a}; b cn vg females (Alexandrov 1977).

In gamma-rays, ^{252}Cf, caffeine+ gamma-rays and actinomycin-D+gamma-rays series, a total of 4 subsequent broods of 1,1,1 and 2 days duration were obtained representing the mature sperm (MS), late (L), middle (M) and early (E) spermatids (Sd), respectively. In the caffein+ gamma-rays series, freshly emerged D-32 males were kept on tissue paper soaked with 0.2% caffeine and 10% honey in water for 3 days before and for 6 hr after irradiation. In the actinomycin-D + gamma-rays series, the males were fed on 100 µg/ml antibiotic in 10% sucrose for 42 hr prior to irradiation.

The mutants were named (first column) by the accepted alphanumeric code (Lindlesy & Grell 1968) in which the first numbers and letter indicate the year and month of discovery and the last number, the order of detection of the particular mutant in the same experiment. The second, third and fourth columns are respectively giving information on the phenotype, pattern of complementation with y^2 as well as cytology for each mutant. For cytological analysis a combination of slightly modified SSP chromosome preparation technique (Kalisch 1981) and routine squashing was used. Namely, salivary glands were quickly dissected in the solution of 66% propionic and 33% citric acid (1:1), fixed in the other drop of the same medium on a slide for 10 min, then touched up by the poured drop of 1% propiono-lactic orcein for 8 min and finally squashed by a cover-slide as usual. The fresh preparations were examined under the phase microscope.

As seen, 16 out of 56 transmissible and viable in homo- or hemizygotes, yellow visibles do not have the complex 1B1,2 band of X chromosome. Therefore, the locus in question appears to be located at the region of this salivary band, but not of 1A5,6 (see Lindsley & Grell 1968). This statement seems to be independently confirmed by the same location of the breakpoints established for inversions and translocations studied. 29 out of 56 viable yellow visibles have a normal picture of this band and can be accepted as true gene mutations, VV^{g} (Alexandrov 1984). The proportion of such mutations among all yellow visibles that have been discovered in the above experiments was estimated and the results are presented in the accompanying Research Note.

Designation of mutation	Phenotype	Compl. with y^2	Cytology	Modifier used radiation, dose	Genotype and male germ cells irradiated
y^{66c}	y*	-**	Normal	γ-rays, 40 Gy	D-32, MS***
y^{71k1}	as y^{c4}*	-	Normal	γ-rays, 40 Gy	D-32, MS
y^{71k2}	as y^{34c}*	-	Df(1)1B1,2±	γ-rays, 40 Gy	D-32, M Sd
y^{7111}	y	-	Df(1)1B1,2±	γ-rays, 40 Gy	D-18, M Sd
y^{7112}	y	-	Normal	γ-rays, 40 Gy	D-18, M Sd
y^{72a}	y	-	Normal	γ-rays, 40 Gy	D-18, MS
y^{72d1}	y	-	Normal	γ-rays, 40 Gy	D-18, MS
y^{72d2}	y	-	Df(1)1B1,2±	γ-rays, 40 Gy	D-18, L Sd
y^{72d3}	y	-	In(1)1B1,2;20A	γ-rays, 40 Gy	D-18, M Sd
y^{72d4}	y	-	Normal	γ-rays, 40 Gy	D-18, E Sd
y^{74b46}	y	-	Normal	caffeine +γ-rays, 40 Gy	D-32, MS
y^{74b51}	as y^{c4}	-	In(1)1B1,2;20D There are additional rearrangements between X and third chromosome.	caffeine +γ-rays, 40 Gy	D-32, M Sd
y^{74b121}	y	-	Normal	caffeine +γ-rays, 40 Gy	D-32, MS
y^{74b151}	y	-	Df(1)1B1,2±	caffeine +γ-rays, 40 Gy	D-32, MS
y^{74c40}	y	-	T(1;4)1B1,2;101F	caffeine +γ-rays, 40 Gy	D-32, MS
y^{74c166}	as y^{c4}	-	In(1)1B1,2;9D	caffeine +γ-rays, 40 Gy	D-32, MS
y^{74d1}	y	-	Normal	caffeine +γ-rays, 40 Gy	D-32, MS
y^{74d2}	y	-	Df(1)1B1,2±	γ-rays, 40 Gy	D-32, M Sd

y^{74d30}	y	-	Df(1)1B1,2±	caffeine + γ-rays, 40 Gy	D-32, L Sd
y^{74d40}	y	-	Df(1)1B1,2±	caffeine + γ-rays, 40 Gy	D-32, L Sd
y^{74e}	y	-	In(1)1B1,2;20D	γ-rays, 40 Gy	D-32, MS
y^{74e2}	y	-	Df(1)1B1,2±	γ-rays, 40 Gy	D-32, M Sd
y^{74e3}	y	-	Normal	Spontaneous	
y^{74k}	as y^{c4}	-	Df(1)1B1,2±	γ-rays, 40 Gy	c(3)G, MS
y^{7511}	y	-	Df(1)1B1,2±	γ-rays, 40 Gy	D-32, L Sd
y^{7512}	y	-	Normal	γ-rays, 40 Gy	D-32, L Sd
y^{7513}	y	-	Df(1)1B1,2±	γ-rays, 40 Gy	D-32, M Sd
y^{76a110}	as y^{39e*}	-	Normal	γ-rays, 40 Gy	D-18, M Sd
y^{76a123}	y		Normal	γ-rays, 40 Gy	D-18, M Sd
y^{76b37}	as y^{c4}	-	T(1;2)1B1,2;60F	γ-rays, 40 Gy	D-18, M Sd
y^{76b94}	as y^{td*}	-	In(1)1B1,2;16c8	γ-rays, 40 Gy	D-18, L Sd
y^{76i}	y	-	Df(1)1B1,2±	actinomycin-D + γ-rays, 40 Gy	D-32, MS
y^{76j}	as y^{c4}	-	Df(1)1B1,2±	actinomycin-D + γ-rays, 40 Gy	D-32, E Sd
y^{76k}	as y^{c4}	-	Df(1)1B1,2±	actinomycin-D + γ-rays, 40 Gy	D-32, E Sd
y^{77a}	y	-	Df(1)1B1,2±	actinomycin-D + γ-rays, 40 Gy	D-32, MS
y^{77d}	as y^{c4}	-	Normal	0.35MeV neutrons, 10 Gy	D-32, MS
y^{77f}	y	-	Normal	actinomycin-D + γ-rays, 40 Gy	c(3)G, M Sd
y^{78a}	y	-	Df(1)1b1,2±	actinomycin-D + γ-rays, 40 Gy	c(3)G, M Sd
y^{78d}	Lethal, males $y^{78d}/y^{+}\cdot Y$ are not viable	-	Df(1)1A3±;1B1,2	γ-rays, 40 Gy	c(3)G, L Sd
y^{78j}	y	-	T(1;3)1B1,2;82A1	γ-rays, 40 Gy	c(3)G, L Sd
y^{79b}	Lethal, males $y^{79b}/y^{+}\cdot Y$ are viable	-	Df(1)1A5,6;1B6±	0.85MeV neutrons, 10 Gy	D-32, MS
y^{79b18}	y	-	Normal	γ-rays, 40 Gy	D-32, L Sd
y^{79b21}	y	-	In(1)1B1,2;16D	γ-rays, 40 Gy	D-32, M Sd
y^{79d}	y	-	T(1;2)1A6-B2;23E5	0.85MeV neutrons, 10 Gy	D-32, MS
y^{79d1}	Lethal, males $y^{79d1}/y^{+}\cdot Y$ are not viable	-	Df(1)1A3±;1B1,2	γ-rays, 20 Gy	D-32, MS
y^{79d2}	y	-	In(1)1B1,2;20A,B	γ-rays, 20 Gy	D-32, MS
y^{79d3}	y	-	Normal	γ-rays, 10 Gy	D-32, MS
y^{79dsd}	♀ as y^{2*} ♂ as y^{c4}	-	Normal	0.85MeV neutrons, 10 Gy + γ-rays, 10 Gy	D-32, MS
y^{79g}	y	-	Normal	γ-rays, 40Gy	D-32, MS
y^{79h1}	y	-	Normal	γ-rays, 40 Gy	c(3)G, MS
y^{79h2}	Lethal, males $y^{79h2}/y^{+}\cdot Y$ are not viable	-	Df(1)1A3±;1B1,2	γ-rays, 40 Gy	c(3)G, E Sd
y^{81c1}	y	-	Normal	γ-rays, 40 Gy	D-32, E Sd
y^{81c2}	y	-	Normal	γ-rays, 40 Gy	D-32, MS
y^{81c3}	y	-	Normal	γ-rays, 40 Gy	D-32, M Sd
y^{81k29}	as y^{c4}	-	Normal	γ-rays, 30 Gy	D-32, MS
y^{82c1}	y	-	Normal	^{252}Cf, 14 Gy	D-32, MS
y^{82c2}	y	-	Normal	^{252}Cf, 14 Gy	D-32, MS
y^{82c3}	y	-	Normal	^{252}Cf, 14 Gy	D-32, L Sd
y^{82c38}	Lethal, males $y^{82c38}/y^{+}\cdot Y$ are viable	-	Df(1)1A6-B4	^{252}Cf, 14 Gy	D-32, L Sd
y^{83c}	Lethal, males $y^{83c}/y^{+}\cdot Y$ are not viable	-	Normal	γ-rays, 40 Gy	D-32, MS
y^{83f12}	y	-	Normal	γ-rays, 40 Gy	D-32, M Sd
y^{83f26}	y	-	Normal	γ-rays, 40 Gy	D-32, M Sd
y^{83f58}	as y^{2s*}	-	Normal	γ-rays, 40 Gy	D-32, L Sd
y^{83fXL}	y	-	In(1)1B1,2;20EF	X-rays, 40 Gy	c(3)G, L Sd
y^{84dS}	y	-	Normal	Spontaneous	In stock st c3G ca/ ri Ubx^{130} e^{s} ca
y^{84eS}	y	-	Normal	Spontaneous	In stock l(2) vg^{8312b}/Bl
y^{84e61S}	y	-	Normal	Spontaneous	In stock st c(3)G ca/ ri Ubx^{130} e^{s} ca

*see Lindsley & Grell 1968; **No complementation; ***see text.

Report of Ronald Konopka.
Clarkson University, Potsdam, New York USNA.

Three X-linked mutations have been isolated after EMS mutagenesis of **D.mauritiana:**

1. m (miniature wings); wings reduced in length. When crossed to miniature-winged **D.melanogaster**, progeny have wings of various lengths.
2. tbl (thin bristles); bristles present, but very thin. Not viable in females.
3. bey (bright eye); eyes brighter than normal. Produces orange eyes in combination with bg (Reference: DIS 55:217).

Report of J. Lawrence Marsh and Lynette Mock.
Developmental Biology Center, University of California, Irvine, Calif. 92717

The following lesions were recovered by irradiating adult Canton-S males with 4000rads of X-rays at 1000rads/minute using a 1mm A1 filter.

$Df(3L)st^{LM3}$	$bul^{-}st^{-}tra^{-}DTS5^{-}$
$Df(3L)st^{LM19}$	$bul^{-}st^{-}tra^{-}DTS5^{-}$
st^{LM2}	scarlet allele, homozygous viable $bul^{+}tra^{+}$
st^{LM10}	scarlet allele, homozygous viable $bul^{+}tra^{+}$
st^{LM54}	scarlet allele, rare homozygotes survive, $bul^{+}tra^{+}$

Blackoid: Bkd^{M}: Recovered from an Oregon-R chromosome treated with EMS and formaldehyde. It is dominant and is designated **Blackoid** on the basis of this phenotype (dark dusty black body color) in heterozygotes and its location between **cn** and **curved** on 2R. We do not know whether the mutant itself is homozygous viable since the lethality of the chromosome may be due to lesions at other loci.

Report of A.V. Medvedeva, E.Yu. Kupert and E.V. Savvateeva.
Pavlov Institute of Physiology, Academy of Sciences USSR, Leningrad USSR.
New X-ray induced inversions of **D.melanogaster.**

Three new inversions in X-chromosome, lethal in hemizygote, were obtained following X-ray irradiation (1000 r) of Canton-S males.

Inversion	Breakpoints	Recombination within the regions			
		y-cv	cv - ct	ct - f	y - f
In(1)MKS45	9A; 18A	9.1	4.5	16.5	30.1
In(1)MKS47	9A1; 20EF	5.1	1.7	3.4	10.2
In(1)MKS78	14B; 20CD	13.7	7.3	25.4	40.7

Report of F. Mestres.
University of Barcelona, Spain.
Description of a new mutation of **Drosophila subobscura.**

The new mutation, **af**, is characterized by the fusion of the first two proximal articles of the first pair of legs. It is found both in males and in females, although it is more conspicuous in males because of the reduction in the number of tarsal combs. The penetrance is complete, but the different degrees of fusion determine different expressivity values. Usually four articles are observed, but sometimes only three are apparent; the tarsal fusion phenomenon is also observed in the other pairs of legs, although less clearly. The trait shows an autosomic recessive inheritance.

The trait is always associated with other phenotypic characteristics: the presence of only two cephalic ocella (the most anterior one is missing) and the interruption of some longitudinal or transverse veins, although in this case the penetrance is not complete and the expressivity shows a high degree of variability. On the area where the ocella are located, a reduction in the number of macrochaetae is sometimes observed. In most cases, the last individuals born from the culture vials show very handicapped twisted legs (mainly the last posterior pair). Very often there is also a reduction in the number of legs; flies with 5,4,3 or even 2 legs can be found (the last case has been observed only twice). On two occasions, individuals with extra articles in one of the posterior legs were observed.

Finally, it is worth pointing out some other casual abnormalities which are sometimes apparent, as the 90 or 45 degrees twisting of the male genitalia, a missing external copulator apparatus (although it was possible to see the testes), an altered wing morphology, a reduction of scutellum size, few thoracic bristles and a deformed proboscis.

Mutant individuals have a viability and fertility not significantly different from the normal ones. The rank that best fits with the characteristics of the **af** mutant is the number 2.

Report of C. Nájera.
Department of Genetics, University of Valencia, Spain.

List of the different eye colour mutants of **Drosophila melanogaster** obtained in two different captures carried out in a vineyard from Requena, Valencia (Spain).

Localized mutants

se^{79i} (sepia-79)

v^{79i} (vermilion-79)

v^{81d} (vermilion-81)

g^{79i} (garnet-79)

pr^{81d} (purple-81)

cd^{81d} (cardinal-81)

$s1f^{79i}$ (safranin-79) - Two alleles at the same capture

sf^{81d} (safranin-81) - Eight alleles at the same capture

Non localized mutants

44.- Eye colour dark red (two alleles)
45,55.- Eye colour dark chestnut
46.- Eye colour soft dark brown
51,53.- Eye colour light brown
52.- Eye colour chestnut
54.- Eye colour like wild, darkening with age
56.- Eye colour light chocolate
57.- Eye colour dark brown (two alleles)
58.- Eye colour darkchestnut (two alleles)
59.- Eye colour dull red, darkening with age
47,60,63.- Eye colour ruby
61,62.- Eye colour spoony brown
48.- Eye colour scarlet
49.- Eye colour vermilion (two alleles)
50.- Eye colour lighter red than wild

Report of Y. Perez-Chiesa, I. Cintron and E. Morales.
University of Puerto Rico, Rio Piedras, Puerto Rico USNA.

fs(1)5e: female-sterile(1)5e (1-37.5)

EMS induced in an X-chromosome free of other fs mutations and isolated according to Mohler (1977, Genetics 85: 259-272). Males fertile; homozygous females are fecund but sterile at 19°C, 25°C, and 29°C. Temperature-sensitivity is observed in the hybrid. Approximately, 90% of heterozygous females are fertile at 25°C but only about 5% are fertile when grown at 29°C or after shifting for three days to 29°C. The eggs laid are permeable to neutral red and burst when dechorionated with a 3% sodium hypochlorite solution. This suggests a fragile vitelline membrane. External anatomy and viability of the flies is normal at 25°C.

Report of R.P. Sharma, P.R. Chitnis and J. Shyngle. Indian Agricultural Research Inst., New Delhi, India.

Spiny leg-bristle orientation reversal ($sple^{bor}$) - a new temperature sensitive allele of spiny leg in **D.melanogaster.**

A second chromosome mutation with highly deformed legs was isolated from a screen of 0.3% EMS mutagenized F_3 homozygous lines. Subsequent studies revealed that this mutant is endowed with disturbed/reversed bristle orientation on legs and is allelic to spiny leg (sple, 2-56). Hence it was designated as spiny leg-bristle orientation reversal mutation.

Unlike sple, the phenotype of $sple^{bor}$ was found to be temperature sensitive. The legs of $sple^{bor}$ flies raised at 19°C were highly condensed (Figure 1a) with incomplete joints, haphazard bristle pattern, reversal in orientation of many bristles, wildly multiplied sex comb teeth on prothoracic legs of males, occasional increase in the number of bristle rows and absence of some prominent markers. Flies with this phenotype, designated as low temperature phenotype (LTP), were unable to walk and therefore died soon after eclosion by drowning in the medium.

When the mutant flies were raised at 28°C, about 53% of legs, though apparently normal, had abnormalities such as swollen second, third and fourth tarsal segments, abnormal tarsal joints and reversal in bristle orientation in the middle of every segment (Figure 1b). Since this phenotype was observed only at higher temperature, it was designated as high temperature phenotype (HTP). The flies with HTP phenotype were able to walk and breed. About 30% of legs of flies raised at 28°C showed phenotype intermediate to HTP and LTP. Remaining legs (17%) showed LTP.

The mutant phenotype was expressed in the transformed cephalic legs of the homeotic mutations **Antennapedia** and **spineless-aristapedia** and in induced mitotic clones, suggesting thereby that the action of $sple^{bor}$ is tissue specific and also cell autonomous.

The evagination of leg imaginal disks in the mutant grown at low temperature was found to be defective. This observation coupled with the observations on LTP and HTP suggest that $sple^{bor}$ mutation is unique in the sense that it interferes with morphogenetic events including cell movement.

Figure 1a. Legs of the $sple^{bor}$ homozygous fly raised at 19°C showing low temperature phenotype (LTP).

Figure 1b. Portion of a leg of $sple^{bor}$ homozygous fly raised at 28°C showing high temperature phenotype (HTP). Notice the reversal of orientation of bristles in the femur and part of tibia.

Report of S. Zusman, D. Coulter and J.P. Gergen.
Princeton University, Princeton, New Jersey USNA.

Lethal mutations induced in the proximal X-chromosome of **Drosophila melanogaster** using P-M hybrid dysgenesis.

We have done a series of mutagenesis screens in order to recover lethal mutations induced by P-M hybrid dysgenesis in the proximal region of the X-chromosome. The specific goal of these experiments was to induce alleles of the runt and fog loci (see Wieschaus et al. 1984) which would allow us to clone these genes using the P-element transposon tagging strategy (for review, see Engels 1983).

The basic mating schemes used to induce and recover the mutations are outlined in Figure 1. Our approach was to screen for dysgenically induced mutations that fall within the limits of a deficiency that uncovers both runt and fog; Df(1)mal^{12} (see Schalet & LeFevre 1976; and Figure 2). Single X-chromosomes that had been exposed to dysgenesis were recovered in females and these females were then individually test mated to deficiency males [Df(1)mal^{12}/y$^+$Ymal$^+$]. Chromosomes with lethal and/or semi-lethal mutations in the deficiency were identified by their reduced viability over the deficiency and recovered from the male progeny of the test cross which survive because they carry the Y-chromosome duplication. The scheme is designed to recover mutations induced on the inverted X-chromosome, In(1)sc^8. This inversion has a breakpoint in the proximal X which essentially eliminates recombination in this region in heterozygous females. Therefore the DNA sequence organization of the mutant chromosomes can be compared to that of the homozygous In(1)sc^8 parental stock in order to identify alterations due to dysgenesis. This inversion also moves the region of interest away from the centromere, thus making it more amenable to cytological analysis.

We carried out pilot experiments in order to determine the best conditions for a large scale mutagenesis. In order to more reliably estimate the mutation rates in these experiments, we scored the test crosses not only for mutations in Df(1)mal^{12}, but also for other X-linked mutations. These give a reciprocal pattern of viability in the test cross. We found the mutation rate in chromosomes recovered from dysgenic females (131 lethals/1511 chromosomes = 8.7%) was higher than that from dysgenic males (22 lethals/883 chromosomes = 2.5%). We also found the dysgenically induced sterility to be less severe in the dysgenic females. We tested two different P strains (Harwich and TT2) and found no significant difference in the mutation rates although the dysgenic sterility was more severe in the crosses with Harwich. Based on these results, in the rest of our mutagenesis experiments, we used the TT2 P strain and recovered the mutagenized chromosomes from dysgenic females.

From a total of 10,500 chromosomes exposed to dysgenesis, we recovered 32 independently induced mutations in Df(1)mal^{12}. These are listed in Table 1. These mutations were characterized using a set of pre-existing chromosomal deficiencies and point mutations for this region of the X-chromosome (Figure 2). Although the region has been extensively characterized and is probably close to saturated for lethal complementation groups, we only tested the dysgenic mutations against those that were available for the regions near runt and fog. We recovered alleles of four known complementation groups in these regions including three independent hits in the runt locus. We did not recover point mutant alleles of fog. Mutations in the EC235 locus were recovered at a disproportionately high frequency (12 independent hits versus 3, 3 and 2 hits in the other loci). In addition to these apparent point mutations, we obtained four

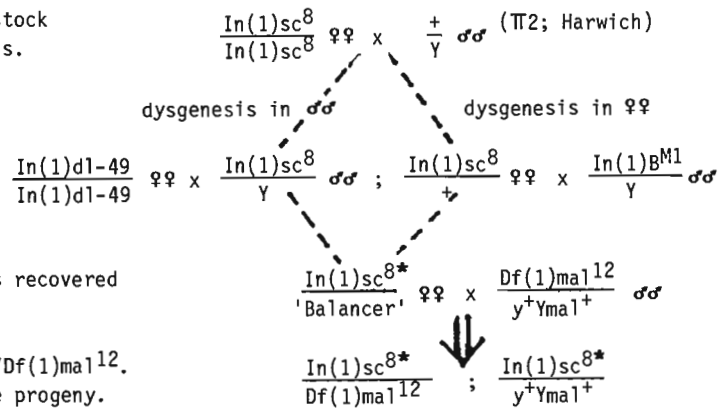
Figure 1: Mutagenesis scheme:

(1) Mate females from homozygous In(1)sc^8 stock to P strain males to induce hybrid dysgenesis.

(2) Hybrid dysgenic progeny mated to inverted "balancer" chromosomes.

(3) Single mutagenized In(1)sc^8 chromosomes recovered in females and test mated with deficiency.

(4) Tubes scored for viability of In(1)sc^{8*}/Df(1)mal^{12}. Lethal mutations recovered from sibling male progeny.



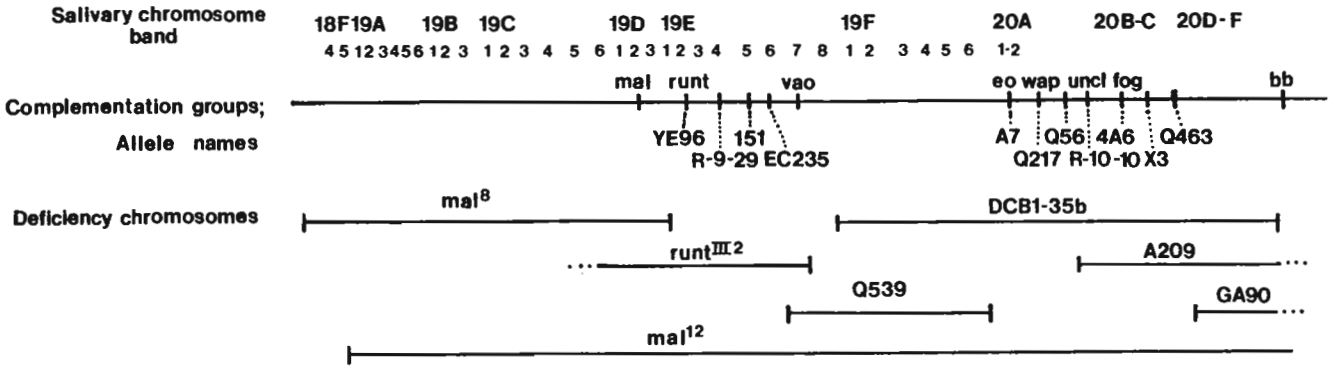


Figure 2.

Table 1. Mutations induced in the proximal X by hybrid dysgenesis.

Category	Allele Name	Complementation Pattern +	Complementation Pattern −
1) Known Complementation Groups:			
runt - YE96	PV1, 34A, 50-2	mal^8, R-9-29	YE96
- EC235	BH9, HT1, PC7, PG7, PUI, 5-7, 7-2, 24A(C), 26A, 31B(C), 40A, 48-1	151, Q539	EC235
eo - A7	4-2, 47-1(2), 51-1	Q539, Q217	A7
- X3	PH1, 30A	4A6, Q463	X3
2) Deficiencies:			
	PC1	R-10-10, Q463	4A6, X3
	26B	R-9-29	151, EC235, Q539
	46-1	R-10-10	4A6, X3, GA90
	48-2	A7, GA90	Q217, 4A6, Q463
3) Other Mutations:			
	6-5, 11-1	$runt^{III2}$, A209	Q539, DCB1-35b
	13-3	$runt^{III2}$, A209	Q539, DCB1-35b
	52-1(2)	$runt^{III2}$, A209	Q539, DCB1-35b
	59-1	Q539, A209	DCB1-35b
	3-5, HU1, HF1(2)	A209	bb^1

chromosomal deficiencies in the two regions of interest. The complementation patterns for these deficiencies are given in Table 1. We recovered 8 mutations which are not in the intervals near runt or fog. The complementation patterns of these mutations with the various deficiencies are also given in Table 1. Five lie between runt and fog and three are apparently semi-lethal alleles of bb.

This work was supported by an NSF Graduate Fellowship (S.Z.) and an NIH Postdoctoral Fellowship (D.C.). J.P.G. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research. This research was also supported by an NIH Grant to Eric Wieschaus in whose laboratory and with whose encouragement these experiments were carried out.

References: Engels, W.R. 1983, Ann. Rev. Genet. 17:315-344; Schalet, A. & G. LeFevre Jr. 1976, in:Genetics and Biology of Drosophila, vol 1b (Ashburner & Novitski, eds.), p847-902; Wieschaus, E., C. Nusslein-Volhard & G. Jurgens 1984, Wilhelm Roux's Arch. 193:296-307.

NOTE: DIS number 60 [1984] contains the last full directory listing, pp. 246-288.

ARGENTINA: Mar del Plata: University of Mar del Plata, Dpto de Biología, Fac de Cs. Exs. y Naturales, Funes 3250-7600. [NEW LISTING]
Lopez, Monica

AUSTRALIA: Armidale, N.S.W. 2351: Univ of New England, Animial Sci. Dept. [UPDATE]
add: Rintoul, G B BSc research fellow population genetics Delete: East, P D
add: Schafer, D J BSc research asst population genetics Delete: Soliman, M H

AUSTRALIA: St. Lucia, Queensland 4067: Univ of Queensland, Genetics Lab G19 Goddard Bldg. PO Box 107, [CHANGE ADDRESS FROM Brisbane TO St. Lucia] Tel (07) 377 2493
Casu, R grad student Pope, A K grad student
Mather, W B AssocProf Head of Lab chromosomal polymorphism, isolating mechanisms, speciation

BELGIUM: Namur B-5000: Fac Univ N.-D. de la Paix, Lab de Genetique, Rue de Bruxelles 61
add new names: C. Bierniaux, D. Dernoncourt-Sterpin, S. Wattiaux-DeConinck

BRASIL: Rio de Janeiro: UFRJ, Inst de Biologia, Dpto Genetica, CxP 68011, Ilha do Fundao
add: C.A.C. Andrade

BULGARIA: Sofia 1421: University of Sofia, Dept of Genetics, 8 Dragan Tsankov str. [NEW LISTING]
G.K. Genova, N.T. Harisanova, Dr. K.H. Ralchev

CHILE: Santiago 7: Univ of Chile, Dept of Cell Biology & Genetics, Fac of Medicine, Casilla 70061 [NEW LISTING]
Awad, E Mr technician Ponce, M M Mrs Secretary
Brncic, D Prof ecological genetics of native species Zarate, E Mr technician
Budnik, M Prof ecological genetics, species interactions
Godoy-Herrera, R PhD AsstProf behavior genetics, developmental behavior

CHINA, PEOPLE'S REPUBLIC OF: Haikou, Hainando, Guangdong: Hainan University [NEW LISTING]
Hu Kai Prof evolutionary genetics

CHINA, PEOPLE'S REPUBLIC OF: Xian: Northwestern University [UPDATE]
Delete: Hu Kai (moved to Haikou)

GERMANY, FR: Bochum D-4630 1: Ruhr-Univ, Abt f Naturw. Medizin, Inst f Genetik [UPDATE]
add: G. Schwitalla

GERMANY, FR: Düsseldorf D-400: Univ Düsseldorf, Inst f Genetik [UPDATE]
add: Bünemann, H [new telephone] 311-3421 Delete: Kunz, W
add: Hessová, Z DrAsst Y chromosome of D.hydei, Y-specific proteins, immunological methods 311-3423
add: Melzer, S Delete: Tischendorf, G

GERMANY, FR: Tübingen D-7400: Inst f Biol II, Lehrstuhl f Populationsgenetik [UPDATE]
Delete: Pfriem, P

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Garnett, David

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Collins, Mike Hewitt, J K

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Karess, Roger E Dr

GREAT BRITAIN: York YO1 5DD: Univ of York, Dept of Biology, Heslington [UPDATE]
add: Clements, J PhD lecturer chemical mutagenesis

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Louis, Christos

GREECE: Thessaloniki: Univ of Thessaloniki, Lab of General Biology, Tel 031-991 448 [NEW LISTING]
Dimitriadis, V K lecturer developmental and cell biology
Kastritsis, C D Dr Prof developmental and cell biology, evolution
Manousis, T C lecturer molecular biology of insect viruses
Mavragani-Tsipidou, P cytogenetics
Pentzos-Daponte, A AsstProf population genetics of D.subobscura
Scouras, Z G Dr cytogenetics and developmental
Thomopoulos, G AsstProf developmental and cell biology
Triantaphyllidis, C D AssocProf isoenzymes in natural population of Drosophila and mouse, nucleic acid hybridization in situ

INDIA: Ludhiana, Punjab: Punjab Agricultural University, Dept of Genetics [UPDATE]
Delete: Sharma, A K

INDIA: Nainital 263 002: Kumaun University, Dept of Zoology [NEW LISTING]
Bhatt, M research scholar systematics, cytogenetics and ecology of Drosophila
Negi, N S research scholar systematics and cytogenetics of Drosophila
Singh, B K Dr lecturer systematics, cytogentics and evolutionary biology of Drosophila

INDIA: Varanasi 221 005: Banaras Hindu Univ, Dept of Zoology, Genetics Lab [UPDATE]
Singh, B N PhD Reader population and behaviour genetics

INDONESIA: Bandung: Institut Teknologi Bandung, JL Ganesha 10, Jurusan Biologi [NEW LISTING]
Ramdani, Maelita PhD

JAPAN: Kobe 63: Hyogo Prefectural Nagata High School, Ikeda-tani machi, Nagata-ku [NEW LISTING]
Maeda, Y

JAPAN: Kobe 657: Kobe Univ, Fac of Science, Biology Dept, Nada-ku Tel 078-881-1212 x4457 [UPDATE]
add: Furukawa, E BS grad student developmental genetics, SR spiroplasmas
add: Furuta, C BS grad student developmental genetics

JAPAN: Mishima, Shizuoka-ken 411: Natl Inst of Genetics, Lab of Genetics, Lab of Cytogenetics (C), Phenogenetics (P), Evolutionary (E) and Developmental (D) Genetics and Genetic Stock Center (S), Tel 0559-75-0771
Funatsu, M Curator of Stocks (S) [REPLACE OLD LISTING]
Inoue, K MSci grad student developmental and molecular genetics (C)
Inoue, Y DrSci instructor population genetics, salivary (S)
Kawahara, M research asst (P)
Kim, B K grad student speciation (E)
Kiryu, K grad student protein evolution (E)
Kuroda, Y DrSci Prof developmental genetics, tissue culture (P)
Minato, K MSci instructor developmental genetics (P)
Murakami, A DrSci & Agr AssocProf mutagenesis and gametogenesis (P)
Nawa, S DrSci Prof transformation and DNA (D)
Suzuki, K research asst (C)
Syoji, E research asst (P)
Takada, Y research asst Curator of stocks (P)
Watanabe, T K DrSci AssocProf evolutionary biology, simulans (E)
Yamada, M A MSci instructor DNA, SR and differentiation (P)
Yamamoto, M PhD instructor cytogenetics (C)

JAPAN: Sapporo 064: Hokkaido Univ of Education, Biological Lab, South 22, West 12 [NEW LISTING]
Kobayashi, K Watabe, H

KOREA: Seoul: Ewha Womans Univ, Dept of Science Education, Genetics Lab Tel 02-362-6151 x516, 517, 709 8289, 8292 [REPLACE OLD LISTING]
Cho, C S MS Asst amylase activity, SD analysis, Curator
Cho (Shin), I S grad student milk phosphoglucomutase
Chung, Y J PhD Prof population genetics
Kang (Song), S J PhD Assoc Prof amylase polymorphism
Kim, M S grad student human cytogenetics
Lee (Ahn), M S MS Instructor human cytogenetics
Lee (Lee), Y R MS Instructor electrophoresis
Park, M K grad student human cytogenetics
Sun, M S grad student electrophoresis
Tai (Choi), H J MS Instructor SD analysis
Yang, I S grad student SD analysis

NETHERLANDS: Haren (Gn) 9751 NN: Univ of Groningen, Genetics Dept, Biological Centre, Kerklaan 30 [UPDATE]
Delete: M. Bos and J.W.M. Kerver Tel 050-115781
correct spelling: Hoeksema-du Pui, M L L Curator of Stocks [insted of Joeksema-du Pui]

NETHERLANDS: Leiden 2300 RA: State Univ, Biology Dept, Kaiserstraat 62, Tel 071-148333 [NEW LISTING]
Brandt, R Curator of stocks
Van Breugel, F M A Dr senior research scientist (head)
Spreij, Th E senior research scientist
Van Zijll Langhout, B research scientist

NETHERLANDS: Nijmegen 6536 AV: Katholieke Univ, Toernooiveld, Dept of Genetics, Tel 080-55 88 33 x2931/2926
add: Dijkhof, R technician [UPDATE]
add: Kremer, J M J Drs cytology & electron microscopy of spermatogenesis Delete: Knoppier, W
add: Lankenau, D-H Diplo-Biol nucleic acids Delete: Ruiters, M

POLAND: Warsaw: Institute of Biochemistry & Biophysics, Polish Academy of Sciences [NEW LISTING]
Nowak, J.
Piechowska, M.J.

SPAIN: Barcelona 28: Univ of Barcelona, Dpto de Genetica, Fac de Biologia [UPDATE]
add: Torramilans, X
add: Dr. F. Mestres

SPAIN: Burjassot (Valencia): Univ de Valencia, Dpto de Genetica, Dr. Moliner, 50, Tel (96) 3630011
Arbona, M grad student induced puffs in D.subobscura [REPLACE OLD LISTING]
Bel, Y grad student pteridin biosynthesis
Botella, L M grad student genetics & ecology of development
Calatayud, M T grad student eye colour mutants
Castro, J grad student Stockkeeper larval competition
Climent, M C grad student genetic effect of inversion in D.subobscura
de Frutos, R PhD AssocProf gene expression on polytene chromosomes of D.subobscura
Ferré, J PhD AsstProf genetics & biochemistry of eye pigments
Garcia, P grad student artificial selection
González Candelas, F grad student theoretical population genetics
González Garrido, A AsstProf lethal in natural populations
Latorre, A AsstProf developmental puffs in D.subobscura
Martínez-Sebastián, M J PhD AsstProf artificial selection
Ménsua, J L PhD Head of Dept population genetics
Molto, M D grad student puffing patterns of D.guanche
Moya,A PhD AsstProf theoretical population genetics
Nájera, C grad student natural selection
Pascual, L grad student temperature puffs in D.subobscura
Perez, M student salivary chromosomes of D.subobscura, electron microscopy
Real, M D grad student tryptophan metabolism
Silva, F grad student genetics and biochemistry of eye pigments

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Wüest, J
Züst, Brigitta

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Rubin, Jerry

USNA: Berkeley, California 94720: Univ of California, Dept of Genetics, Mulford Hall Tel 415-642-5405
Fristrom, Jim [NEW LISTING]

USNA: Bronx, New York 10461: Albert Einstein College of Medicine, Dept of Genetics [UPDATE]
add: Marcus, Craig

USNA: Cambridge, Massachusetts 02138: Harvard Univ, Museum of Comparative Zoology [UPDATE]
Delete: Brooks, L (moved to Raleigh, North Carolina--see new listing)

USNA: Carbondale, Illinois 62901: Southern Illinois Univ, Dept of Chemistry & Biochemistry [NEW LISTING]
Zullo, Steve

USNA: Carbondale, Illinois 62901: Southern Illinois Univ, Zoology Dept [UPDATE]
Delete: Benson, A J

USNA: Chicago, Illinois 60605: Roosevelt Univ, Biology Dept, 430 S. Michigan Ave. [UPDATE]
add: Ostrega, M S Dr

USNA: Cleveland, Ohio 44106: Case Western Reserve Univ, Dept of Developmental Genetics & Anatomy, Sch of Medicine
Jacobs-Lorena, Marcelo [NEW LISTING]

USNA: College Station, Texas 77843: Texas A & M Univ, Biology Dept Tel 713-845-7756 [NEW LISTING]
Rickoll, Wayne L AsstProf developmental genetics, molecular genetics

USNA: Duarte, California 91010: Beckman Research Inst of City of Hope, Tel 818-357-9711 [UPDATE]
add: Itoh, N Visiting Scientist (Div of Neurosciences) Delete: Crawfod, G
add: Riggs, A D (Div of Biology) Change: Williamson, R TO Div of Biology

USNA: Durham, North Carolina 27706: Duke Univ, Arts & Sciences, Zoology Dept, Tel 919-684- [NEW LISTING]
Ward, C L PhD Prof speciation, chromosomal polymorphism -3270

USNA: Fairfax, Virginia 22030: George Mason Univ, Biology Dept, 4400 University Drive [NEW LISTING]
Sherald, A F Voelker, R A

USNA: Geneva, New York 14456: Hobart & William Smith Colleges, Biology Dept [NEW LISTING]
Glover, T Prof Kaplan, H student

USNA: Honolulu, Hawaii 96822: Univ of Hawaii [UPDATE]
add: Titus, Elizabeth Wisotzkey, R G

USNA: Irvine, California 92717: Univ of California, Developmental Biology Center Tel 714-856- [NEW LISTING]
Erfle, Mary research asst -6677 Haymer, David postdoc -6677 Leeds, Carol research asst -6677
Eveleth, David grad stu -6677 Konrad, Ken postdoc -6677 Marsh, J Lawrence AsstProf -6677
Gibbs, P D L grad stu -6677

USNA: Ithaca, New York 14850: Ithaca College, Biology Dept Tel 607-274-3972 [NEW LISTING]
Thompson, S R PhD AssocProf general & population genetics, science education

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USNA: Logan, Utah 84322: Utah State Univ, Dept of Biology Tel 801-750-2485 [NEW LISTING]
Bowman, J T PhD Prof developmental genetics Simmons, J R PhD Prof biochemical genetics
Gardner, E J PhD Prof Emeritus developmental genetics

USNA: Los Angeles, California 90024: Univ of California, Biology Dept and Molecular Biology Institute [UPDATE]
Add: Ackerman, Susan grad student behavior -1417 Tel 213=825-
Add: Balderelli, R grad student molecular development On leave: Roark, M
Delete: Moscoso del Prado, J On leave: Underwood, E

USNA: Macomb, Illinois 6144: Western Illinois Univ, Biology Dept [DELETE THIS LISTING]
Stephen H. Bryant has moved to Pomona, California: Calif State Polytechnic Univ

USNA: Pomona, California 91768: California State Polytechnic Univ, Biology Dept, Tel 714-598-4444
Bryant, Stephen H PhD AssocProf population biology [NEW LISTING]

USNA: Potsdam, New York 13676: Clarkson Univ, Biology Dept Tel 315-268-6641 [REPLACE OLD LISTING]
Bedian, V AsstProf development, monoclonal antibodies
Hotchkiss, S K AsstProf aging, biological rhythms
Konopka, R J AssocProf biological rhythms, neurogenetics

USNA: Princeton, New Jersey 08544: Princeton University, Biology Dept [NEW LISTING]
Gergen, J Peter Coulter, D Zusman, S

USNA: Providence, Rhode Island 02912: Brown Univ, Div of Biology & Medicine Tel 401-863- [UPDATE]
change: Kidwell, M G -2808 (new telephone) Delete: Kidwell, J F

USNA: Raleigh, North Carolina 27695: North Carolina State Univ, Statistics Dept, Box 8203 [NEW LISTING]
Brooks, Lisa D [moved from Harvard Univ, Cambridge, Massachusetts]

USNA: Rio Piedras, Puerto Rico 00931: Univ of Puerto Rico, Biology Dept Tel 714-787-5903 [REPLACE OLD LISTING]
Ajenjo, I BS technician
Bruck, D PhD AssocProf ecological genetics
Cintrón, C N BS grad student
Colón, V BS grad student
Dávila, I BS grad student
Delgado, V M BS grad student
Flores, M S BS grad student
Pérez-Chiesa, Y PhD Prof devel. genetics, mutagenesis

USNA: San Francisco, California 94117: Univ of San Francisco, Biology Dept, Harney Science Center [UPDATE]
add: Andrews, Kevin Dr
add: Culich, J J
add: Del Puerto, Gus

USNA: Stony Brook, New York 11794: State Univ of New York [NEW LISTING]
Dept of Biochemistry: Paul M. Bingham, C.H. Chapman
Dept. of Ecology & Evolution: Jody Hey, D. Houle

USNA: Storrs, Connecticut 06268: Univ of Connecticut, Biological Sciences Group, Molecular Genetics and Cell Biology Section Tel 203-486- [REPLACE OLD LISTING]
Chovnick, A PhD Prof -3810/3043
Clark, S PhD research asst Prof -2257/3043
Dutton, F L PhD postdoc fellow -2266
Krider, H PhD AssocProf -4860
McCarron, M PhD research assoc -2266
Strausbaugh, L AsstProf -2693

USNA: Wayne, New Jersey 07470: William Peterson College, Dept of Biology [NEW LISTING]
Weisbrot, David

USNA: West Lafayette, Indiana 47907: Purdue Univ, Dept of Biological Sciences [NEW LISTING]
Nichols, Ruthann
Pak, W L

YUGOSLAVIA: Nis 180 000: Bioloski Institut, Medicinski Fakultet, Brace Taskovica 81 [NEW LISTING]
Mrcarica, Estera Dr

ALPHABETICAL DIRECTORY - Updates and New Listings

DELETE THE FOLLOWING NAMES:

Alegre, J.J. Burjassot, Spain
Benson, A.J. Carbondale, USNA IL
Bos, M. Haren, Netherlands
Chang, H.W. Seoul, Korea
Couper, D.G. Lewiston, USNA ME
Crawford, G. Duarte, USNA CA
East, P.D. Armidale, Australia
Gonzalez Bosch, M.C. Burjassot, Spain
Kawanishi, M. Mishima, Japan
Kerver, J.W.M. Haren, Netherlands
Kidwell, J.F. Providence, USNA RI
Knoppier, W. Nijmegen, Netherlands
Kunz, W. Düsseldorf, FR Germany
Llop, P. Burjassot, Spain
Lopez, B. Burjassot, Spain
Moscoso del P., J. Los Angl., USNA CA
Ohnishi, S. Mishima, Japan
Oshima, C. Mishima, Japan
Otake, K. Mishima, Japan
Park, E.K. Seoul, Korea
Pfriem, P. Tübingen, FR Germany
Ruiters, M. Nijmegen, Netherlands
Sharma, A.K. Ludhiana, India
Soliman, M.H. Armidale, Australia
Tischendorf, G. Düsseldorf, FR Germany

CHANGES IN PREVIOUS LISTINGS:

Brooks, L.D. Raleigh, USNA NC
(old: Cambridge, USNA MA)
Bryant, S.H. Pomona, USNA CA
(old: Macomb, USNA IL)
Casu, R. St. Lucia, Australia
(old: Brisbane, Austr.)
Hoeksema-du Pui, M.L.L. Haren, Neth.
(old spelling: Joeksem-du Pui)
Hu Kai. Haikou, China-PRC
(old: Xian, China-PRC)
Mather, W.B. St. Lucia, Australia
(old: Brisbane, Austr.)
Pope, A.K. St. Lucia, Australia
(old: Brisbane, Austr.)

ADD THE FOLLOWING NEW NAMES:

Ackerman, S. Los Angeles, USNA CA
Ajenjo, I. Rio Piedras, USNA PR
Andrade, C.A.C. Rio de Janeiro, Brazil
Andrews, K. San Francisco, USNA CA
Arbona, M. Burjassot, Spain
Awad, E. Santiago, Chile
Balderelli, R. Los Angeles, USNA CA
Bedian, V. Potsdam, USNA NY
Bel, Y. Burjassot, Spain
Bhatt, M. Nainital, India
Bingham, P.M. Stony Brook, USNA NY
Bierniaux, C. Namur, Belgium
Bowman, J.T. Logan, USNA UT
Brandt, R. Leiden, Netherlands
Brncic, D. Santiago, Chile
Bruck, D. Rio Piedras, USNA PR
Budnik, M. Santiago, Chile
Bünemann, H. Düsseldorf, FR Germany
Calatayud, M.T. Burjassot, Spain
Chapman, C.H. Stony Brook, USNA NY
Cintrón, C.N. Rio Piedras, USNA NJ
Clements, J. York, GB-England

Add the following new names: (contin.)

Climent, M.C. Burjassot, Spain
Collins, M. Birmingham, GB-England
Colón, V. Rio Piedras, USNA PR
Coulter, D. Princeton, USNA NJ
Culich, J.J. San Francisco, USNA CA
Dávila, I. Rio Piedras, USNA PR
Delgado, V.M. Rio Piedras, USNA NJ
Del Puerto, G. San Francisco, USNA CA
Dernoncourt-Sterpin, D. Namur, Belgium
Dijkhoff, R. Nijmegen, Netherlands
Dimitriadis, V.K. Thessaloniki, Greece

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Eveleth, D. Irvine, USNA CA
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Funuatsu, M. Mishima, Japan
Furukawa, E. Kobe, Japan
Furuta, C. Kobe, Japan
Gardner, E.J. Logan, USNA UT
Garnett, D. Aberstwyth, GB-Wales
Genova, G.K. Sofia, Bulgaria
Gergen, J.P. Princeton, USNA NJ
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Hey, J. Stony Brook, USNA NY
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Karess, R.E. London, GB-England
Kastritsis, C.D. Thessaloniki, Greece
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Kim, B. Mishima, Japan
Kim, M.S. Seoul, Korea
Kiryu, K. Mishima, Japan
Kobayashi, K. Sapporo, Japan
Konopka, R.J. Potsdam, USNA NY
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Mavragani-Tsipidou, P. Thessaloniki, Greece
Melzer, S. Düsseldorf, FR Germany
Mestres, F. Barcelona, Spain
Mrcarica, E. Nis, Yugoslavia
Negi, N.S. Nainital, India
Nichols, R. West Lafayette, USNA IN
Ostrega, M.S. Chicago, USNA IL

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Ralchev, K.H. Sofia, Bulgaria
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Rintoul, G.B. Armidale, Australia
Rubin, J. Berkeley, USNA CA
Schafer, D.J. Armidale, Australia
Schwitalla, G. Bochum, FR Germany
Scouras, Z.G. Thessaloniki, Greece
Sherald, A.F. Fairfax, USNA VA
Simmons, J.R. Logan, USNA UT
Singh, B.K. Nainital, India
Singh, B.N. Varanasi, India
Spreij, Th.E. Leiden, Netherlands
Strausbaugh, L. Storrs, USNA CT
Sun, M.S. Seoul, Korea
Suzuki, K. Mishima, Japan

Thomopoulos, G. Thessaloniki, Greece
Thompson, S.R. Ithaca, USNA NY
Titus, E. Honolulu, USNA HI
Torramilans, X. Barcelona, Spain
Triantaphyllidis, C.D. Thessaloniki, Greece
Van Breugel, F.M.A. Leiden, Netherlands
Van Zijll Langhout, B. Leiden, Netherlands
Voelker, R.A. Fairfax, USNA VA
Ward, C.L. Durham, USNA NC
Wattiaux-DeConinck, S. Namur, Belgium
Wisotzkey, R.G. Honolulu, USNA HI
Wüest, J. Geneva, Switzerland
Yang, I.S. Seoul, Korea
Zarate, E. Santiago, Chile
Züst, B. Geneva, Switzerland
Zullo,S. Carbondale, USNA IL
Zusman, S. Princeton, USNA NJ