DROSOPHILA INFORMATION SERVICE

82

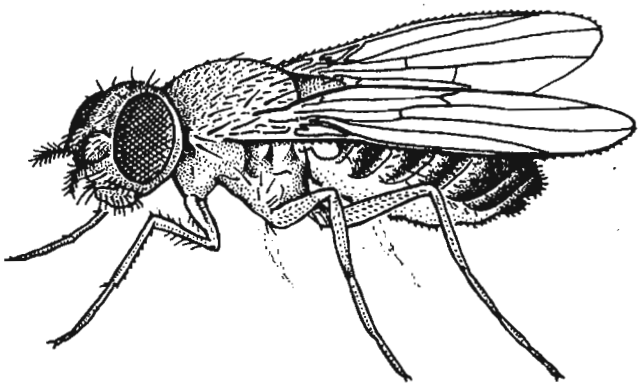
July 1999

Material contributed by
DROSOPHILA WORKERS

and arranged by
James N. Thompson jr.
and
Jenna J. Hellack

prepared at
Department of Zoology
University of Oklahoma
Norman, Oklahoma 73019

Drosophila Information Service



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Preface

Drosophila Information Service was first printed in March, 1934. Material contributed by *Drosophila* workers was arranged by C.B. Bridges and M. Demerec. As noted in its preface, which is reprinted in DIS 75 (1994), Drosophila Information Service was undertaken because, "An appreciable share of credit for the fine accomplishments in *Drosophila* genetics is due to the broadmindedness of the original *Drosophila* workers who established the policy of a free exchange of material and information among all actively interested in *Drosophila* research. This policy has proved to be a great stimulus for the use of *Drosophila* material in genetic research and is directly responsible for many important contributions." During the 65 years since that first issue, DIS has continued to promote open communication.

The production of DIS 82 could not have been completed without the generous efforts of many people. Diane Jackson, Robert Klitzman, Russell Fletcher, Stanton Gray, and other volunteers helped with manuscripts; Gloria Stephens, Robbie Stinchcomb, Diane Jackson, and Julie Rathbone maintained key records; and Coral McCallister advised on artwork and computer graphics.

For this issue, we owe a special acknowledgment to Michael Pence and his colleagues at the Space Station Biological Research Project, NASA Ames Research Center, for the prototype evaluation report included in this issue as a special report. We encourage other groups to use DIS as a venue to report technologies and techniques under development. Our hope is that ideas from the vast intellectual resources of the worldwide *Drosophila* genetics research community can benefit these landmark efforts.

We are also strong advocates of educational exchange and innovation. To help raise the visibility of earlier contributions to teaching, we reprint in this issue many of the teaching notes published in previous issues, most of which are now out-of-print. We have omitted those that dealt with specific computer programs, because of the rapid changes in that technology. Similarly, some mutant stocks mentioned in the reprinted articles may no longer be available in their original makeup. But we hope these notes at least stimulate ideas – and stimulate other contributions of teaching notes that can benefit genetic educators worldwide.

You will probably notice a change in the presentation format for individual DIS articles. For several years, the lead of each article was printed in two columns, with title and author information printed in the left-hand column and the text beginning in the right. But with increased interest in providing contact information like telephone, FAX, and e-mail addresses, this arrangement became difficult to work with. To minimize costs, the editors prepare each page in camera-ready copy, and the limitations presented by article size, table and figure placement, and other factors led us to try this new presentation format. We hope it will let you find articles more easily while giving us the greatest flexibility in designing each issue.

James N. Thompson, jr., Editor

Jenna J. Hellack, Associate Editor

Drosophila Information Service

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List of Contributions

Technique Notes

Mutation Notes: *Drosophila melanogaster*

Mutation Notes: Other Species

Teaching Notes: Reprinted from Previous Issues

Charge of the Flight Brigade

Half a vial, half a vial,
Half a vial downward,
All in the bottle of Death
Flew the six hundred.
'Forward, the Flight Brigade!
Charge for the cotton!' he said:
Into the bottle of Death
Flew the six hundred.

'Forward, the Flight Brigade!'
Was there a fly dismay'd?
Not tho' the larvae knew
Some one had blunder'd:
Their's not to make reply,
Their's not to reason why,
Their's but to breed and die:
Into the bottle of Death
Flew the six hundred.

Ether to right of them,
Ether to left of them,
Ether in front of them
Volatilized and slumber'd;
Pursued with swat and smell,
Boldly they flew and well,
Into the bottle of Death,
Into the mouth of Hell
Flew the six hundred.

Flash'd all their wings bare,
Flash'd as they turn'd in air
Surprising the PI there,
Charging the Academe, while
All the world wonder'd:
Plunged in the ether-smoke
Right thro' the cotton they broke;
Student and Postdoc
Reel'd from the ethers' choke
Shatter'd and sunder'd.
Then they flew away, but not
Not the six hundred.

Ether to right of them,
Ether to left of them,
Ether behind them
Volatilized and slumber'd;
Pursued with swat and smell,
white male and female fell,
They that had flown so well
Came thro' the jaws of Death,
Away from the bottle of Hell,
All that was left of them,
Left of six hundred.

When can their glory fade?
O the wild charge they made!
All the world wonder'd.
Honour the charge they made!
Honour the Flight Brigade,
Noble six hundred!

(with apologies to Tennyson)

Graham Thomas
Matthew Phillips

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Guide to Authors

Drosophila Information Service prints short research and technique articles, descriptions of new mutations, laboratory experiments and problem sets that will be helpful for teaching, stock lists, directory information, and other material of general interest to *Drosophila* researchers. Reports of Drosophila research contributions given at regional or national conferences are also welcome. The current publication schedule for regular issues is annually in late summer/early fall. To meet this target date, the deadline for submission of materials is typically 1 May. Later submissions can occasionally be accommodated by contacting the editor by email or telephone. Special issues will also be prepared on an irregular schedule.

Manuscripts, orders, and inquiries concerning the regular annual DIS issue should be sent to James Thompson, Department of Zoology, University of Oklahoma, Norman, OK 73019. Telephone (405)-325-4821; email jthompson@ou.edu; FAX (405)-325-7560.

Submission: Submissions are accepted at any time, but the deadline for the annual issue will be about 1 May or until the issue is full. To help minimize editorial costs, proofs will not be sent to authors unless there is some question that needs to be clarified or they are specifically requested by the authors at the time of submission. The editor reserves the right to make minor grammatical, spelling, and stylistic changes if necessary to conform to DIS format.

Manuscripts should be submitted in duplicate. If possible, a 3.5" diskette should also be sent with the manuscript in Microsoft Word, WordPerfect, or other common word-processing format. This improves the speed and accuracy of preparing manuscripts and is always greatly appreciated.

Citation of References: Citation should be by name and date in the text of an article (Smith, 1989; Jergen and Brown, 1990; Waters *et al.*, 1990). At the end of the article, references should be listed alphabetically by senior author, listing all authors with initials, date, journal, volume and page numbers. Article titles will not be included except for books, unpublished theses, and articles in press. An example format is:

Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

Stock Lists, Specialized Bibliographies, and Long Technical Articles: Long or complex material can generally not be accepted until it is submitted on diskette, with a printed copy for editorial guidance. We encourage submission of lists and other documentary material to complement presentations in other journals that might have more restrictive space limits or costs. Special justification will, however, be needed for material like bibliographic lists that are now often readily available by other means. Inquiries about formats for this kind of submission are welcomed.

Figures and Tables: Both line drawings and half-tone illustrations will be accepted, but half-tones should be provided as high contrast black and white prints. We are currently unable to publish figures in color. All tables are retyped by us to fit a uniform style, and it is critical that all numbers and symbols be clearly arranged and legible.

Research Notes

Accumulation of P elements on In(3L)P chromosome by P-M system of *Drosophila melanogaster* in Korean wild population.

Kim, Dae Il. Dept of Biology Mok-Won University, Taejon 301-729, S. Korea. E-mail: dikim@mwus.mokwon.ac.kr.

Most strains in TP and TY populations with In(3L)P were determined with Q and M′ strains by the P-M system. Q strain frequency of the flies with In(3L)P was tested more than M′ strain in all populations. The mean copy numbers for all TEs (P elements) pooled are higher on the whole arm of In(3L)P chromosomes than on the 3L standard chromosomes. Copy numbers of P elements in Q strains of TP and TY populations from 96/97 were distributed with more copy numbers than M′ strains, concentrated highly at loci 61C-D, 64B, 71C, and 79C on 3L chromosome location with In(3L)P. But M′ strains of TP and TY populations from 96/97 were distributed highly at loci 61C, 64A, and 72C on 3L chromosomes with In(3L)P.

Materials and Methods: *Drosophila melanogaster* from two Taejon regions were collected at Pan-am-dong (vineyard) and Yu-song (apple orchards) between early September and early November, 1996 and 1997. The collection of 598 inseminated females was transferred in vials containing cornmeal-molasses-yeast-agar medium and 0.5% propionic acid. The cultures were kept at room temperature, 23±1°C and humidity, 65±1%.

The smears of salivary gland chromosomes were prepared from F_1 third instar larvae which were selected randomly from each isofemale line. The salivary chromosome smears of the F_1 larvae from each isofemale line were made by the lactic-acetic-orcein method using siliconized slides. The salivary chromosome smears were observed with a BH2 Olympus microscope for the presence of heterozygous inversion. The standard chromosomal map of Bridges (1935) and revised map of Lefevre (1976) were employed to identify the breakage points of the chromosomal inversion.

Table 1. Phenotypes for GD sterility of various categories of strains

strain type	cross A % GD sterility	cross A° %GD sterility
M (true)	0	100
M'(pseudo-M)	0 - ?	0 – 100
Q (weak P)	0 - 10	0 – 10
P (moderate)	11 – 80	0 – 10
P (strong)	81 – 100	0 – 10

Two tester strains, a strong P strain (π_2) and the standard M strain (Canton-S), were used to assay the GD sterility of the wild strains.

Cross A was carried out using two females of Canton-S with one wild-type unknown male. Cross A° was carried out using one wild unknown female with two males of strong P factor (π_2). The vials with these flies were kept for a week at 29°C for the cross and then the parents were discarded. The F_1 flies emerged by the 11th day were transferred to fresh vials with medium at 25°C. After the flies had matured for four additional days, 24 F_1 females per line were screened for gonadal sterility by dissecting to detect whether rudimentary ovaries have one or two. The females with two dysgenic ovaries were classified as sterile. According to Kidwell's criteria (1983, 1986), the strains were identified as P, Q, M′, and M limiting a cut off point at 10% (Table 1).

In Situ Hybridization: Salivary gland preparations were made using F_1 larvae with In(3L)P chromosomes of isofemale lines. Pπ25.1 plasmid was offered by Prof. Kim,wook (Dan Kook Univ.) and from rapid, small-scale isolation of plasmid into *E. coli* Dh5α. The pπ25.1 was labeled with dig-11-dUTP. The hybridization solution contained 10:1 of 20X SSC (3M sodium chloride, 0.3M sodium citrate, adjusted to pH7 with 10N NaOH to 1 liter), 8:1 of 50% (wt/vol) dextran sulfate, 25:1 of formamide, 5:1 of probe DNA, and 2:1 of D.W. for a total of 50:1. This solution was heated to 95°C in boiling water for 5 minutes and quickly cooled on ice for 5 minutes just before use. Salivary gland preparation was denatured in an alkaline solution (0.07M NaOH) for 1 minute 55 seconds. Hybridization solution (20:1) was done using dig-Nucleic Acid Detection

Kit. The detection of *in situ* hybridization was observed with BH2-PC-/BH2-PCD Phase Contrast Attachment of BH2 Olympus microscope and photographed.

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

strains tested	cross A No. ovaries per females				cross A° No. ovaries per females			
	2	1	0	GD	2	1	0	GD
TP(96)	99.1033	0.0854	0.8113	0.8967	73.3802	0.0728	26.5470	26.6198
TP(97)	99.7655	0.0391	0.1954	0.2354	81.0511	0.0236	18.9253	18.9489
TY(96)	98.9145	0.1316	0.9540	1.0855	77.4548	0.2687	22.2765	22.5452
TY(97)	99.7040	0.1480	0.1480	0.2960	74.5715	0.0996	25.3288	25.4284
means	99.3718	0.1010	0.5272	0.6284	76.6144	0.1162	23.2694	23.3856

TP= Taejon Panam dong populations and TY = Taejon Yu-song populations

Table 3. The relative frequencies of strains for GD sterility of flies without In(3L)P and flies with In(3L)P

sites	N	N(In)	M°		M		Q		P	
			n	In	n	In	n	In	n	In
TP(96)	137	18(0.1314)	2(0.0146)	0(0.0000)	46(0.3358)	5(0.0365)	69(0.5036)	13(0.5036)	2(0.0146)	0(0.0000)
TP(97)	154	7(0.0455)	4(0.0260)	0(0.0000)	36(0.2338)	2(0.0130)	107(0.6948)	5(0.0325)	0(0.0000)	0(0.0000)
TY(96)	146	13(0.0890)	4(0.0274)	0(0.0000)	45(0.3082)	3(0.0205)	82(0.5616)	10(0.0685)	2(0.0137)	0(0.0000)
TY(97)	161	24(0.1491)	3(0.0186)	1(0.0062)	52(0.3230)	8(0.0497)	81(0.5031)	16(0.0994)	0(0.0000)	0(0.0000)
means	598	(0.1038)	(0.0216)	(0.0016)	(0.3002)	(0.0299)	(0.5658)	(0.0738)	(0.0071)	(0.0000)

N = tested individuals, In = frequencies of flies with In(3L)P, n = frequencies of flies without In(3L)P, TP = Taejon Panam dong populations and TY = Taejon Yu-song populations.

Results: The overall mean frequencies in cross A showed 0.6284 from the cross of F_1 males with Canton-S females by P-M system. In the cross A°, 291 TP and 307 TY isofemale lines were tested, and the mean sterility frequencies of both populations were 22.78 and 23.99%, respectively (Table 2). Each strain was tested with cross A and cross A° according to Kidwell's criteria (1986).

Distribution of most strains in these populations was determined with Q and M′ strains, but true M strains were in low frequency in all populations and P(M) strains were observed but only in one or two flies. M′ strains were lower in frequency than Q strains in all populations. The mean frequencies of M′ and Q strains of flies with In(3L)P were 0.0299 and 0.0738, respectively. True M strain frequency was observed with 0.0016 on the flies with In(3L)P in all populations. P activity of flies with In(3L)P was investigated to be concentrated completely to M′ and Q strains. The mean frequency of P(M) strain was only observed with

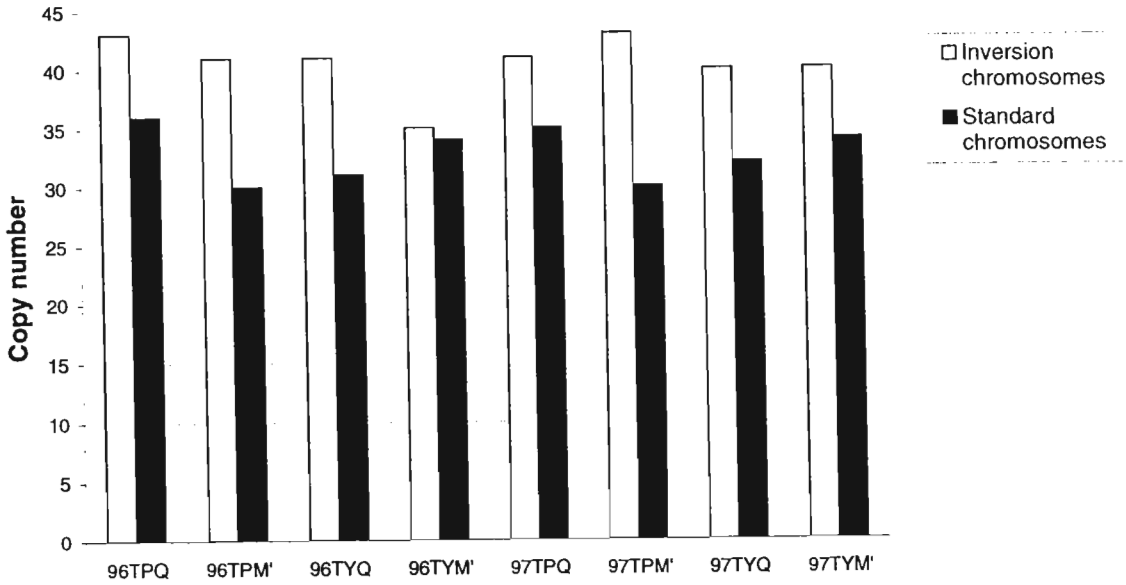

Figure 1. Overall comparison of copy numbers between five In(3L)P chromosome and standard 3L chromosomes.

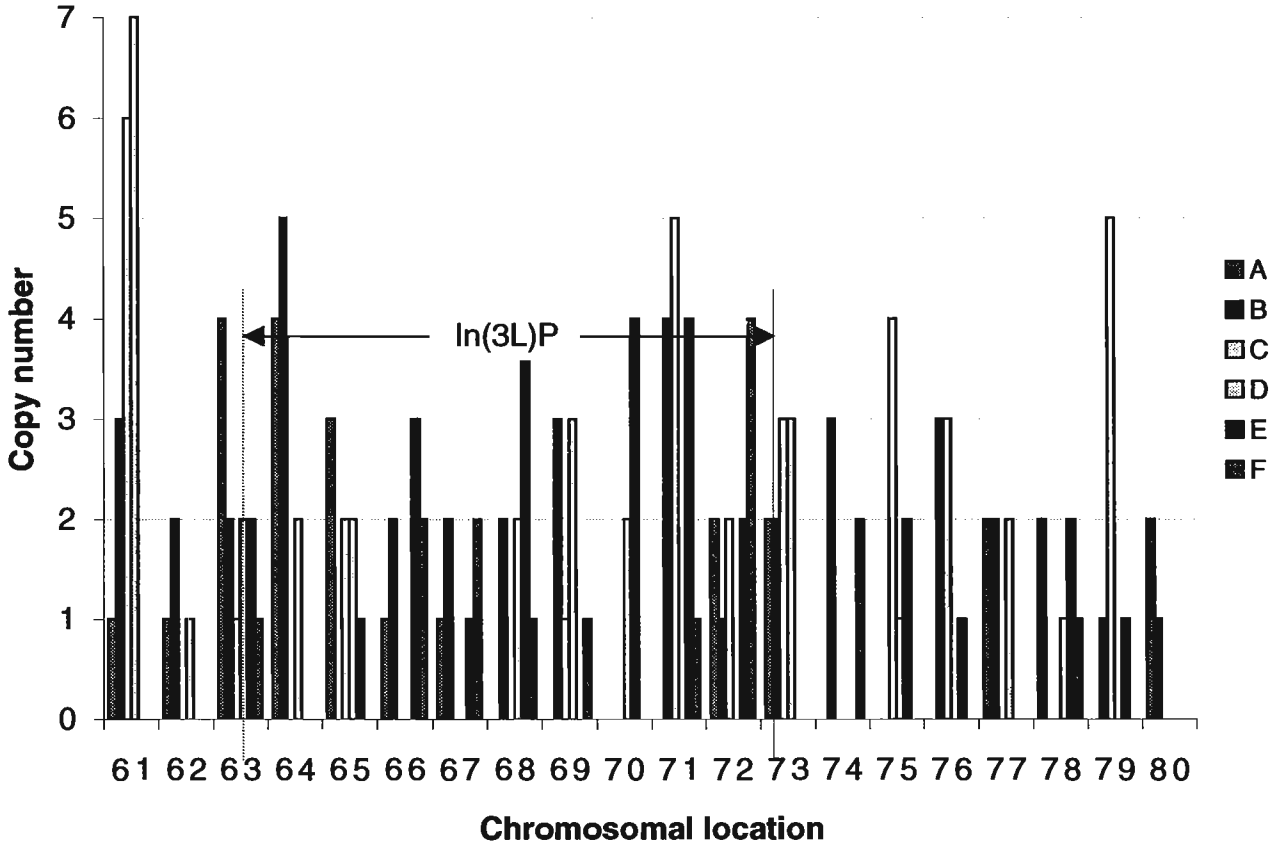

Figure 2. In(3L)P Q types 96,97.

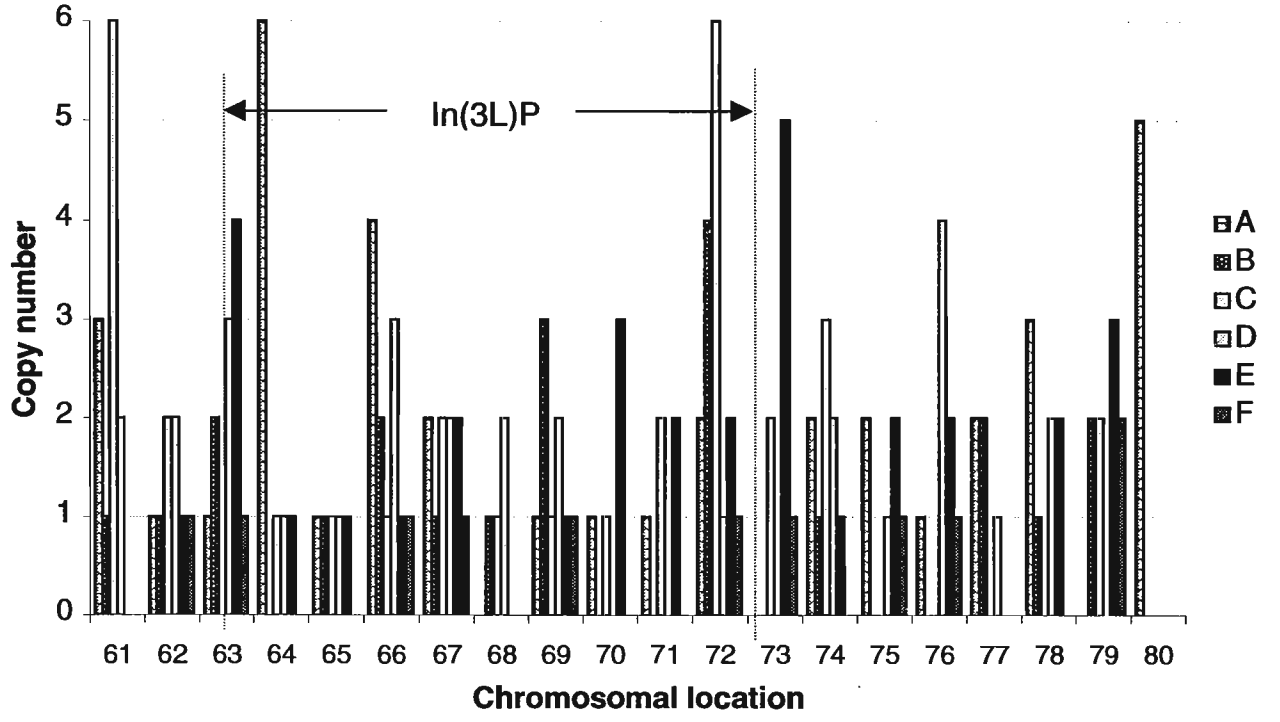

Figure 3. In(3L)P M' types 96,97.

Table 4. Copy number from 3L chromosomal location with In(3L)P Q strains 96,97.

	A				B				C				D				E				F				total
	96		97		96		97		96		97		96		97		96		97		96		97		
	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	
61	1	0	0	0	1	1	2	0	1	1	1	2	3	0	0	2	0	1	0	0	0	0	0	0	16
62	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	5
63	1	0	1	1	0	0	0	1	1	0	0	0	0	0	2	0	0	0	0	1	1	0	0	0	9
64	2	0	0	1	0	2	3	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	11
65	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	6
66	1	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	8
67	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	2	3	0	0	10
68	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	2	1	0	0	0	6
69	0	1	0	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	8
70	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	6
71	0	0	0	0	1	2	3	0	3	0	0	1	0	0	0	0	0	2	2	1	0	1	0	0	16
72	0	0	0	0	1	0	0	1	2	2	0	0	0	0	0	0	0	0	2	0	2	1	0	1	12
73	0	0	0	1	1	3	1	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	10
74	0	0	0	0	2	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2	0	8
75	0	0	0	0	0	1	0	0	0	0	0	2	0	0	1	0	0	1	0	1	0	0	0	0	6
76	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	2	0	0	0	0	0	0	7
77	0	1	0	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	6
78	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	6
79	0	0	1	0	0	0	0	0	1	1	0	2	0	1	0	0	0	0	1	0	0	0	0	0	7
80	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Total	6	6	5	7	11	11	16	6	12	5	2	9	5	5	7	8	3	6	7	8	7	8	3	2	165

Table 5. Copy number from 3L chromosomal location with In(3L)P M' strains 96,97.

	A				B				C				D				E				F				total
	96		97		96		97		96		97		96		97		96		97		96		97		
	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	TP	TY	
61	1	0	0	2	1	0	0	0	0	3	1	2	1	0	1	0	0	0	0	0	0	0	0	0	12
62	0	1	0	0	0	0	1	0	1	1	0	0	1	0	1	0	0	0	1	1	0	0	0	0	8
63	1	0	0	0	1	1	0	0	0	0	0	0	1	0	2	0	0	2	0	2	0	0	1	0	11
64	2	0	2	2	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	9
65	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	4
66	1	0	3	0	0	0	1	1	1	0	0	0	0	2	0	1	1	0	0	0	0	0	1	0	12
67	0	1	0	1	0	0	0	1	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	9
68	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	4
69	0	1	0	0	2	0	1	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	2	0	10
70	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	0	5
71	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	5
72	0	0	0	2	0	3	0	1	2	2	1	1	0	0	0	1	0	0	2	0	1	0	0	0	16
73	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	2	1	1	1	0	0	8
74	0	1	0	1	1	0	0	0	1	1	1	0	2	0	0	0	0	1	0	0	0	0	0	0	9
75	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	1	0	0	6
76	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0	7
77	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	5
78	0	1	0	2	0	1	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	8
79	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	1	2	0	0	0	0	2	0	9
80	1	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Total	7	8	8	15	6	6	6	3	9	9	7	6	10	4	7	7	6	9	11	6	3	2	6	2	161

0.0071 on 3L chromosome of the flies, but not on In(3L)P. Q strain frequency of the flies with In(3L)P was tested more than M′ strain in all populations (Table 3).

The total copy number of the P elements was determined by using each five lines of M′ and Q strains randomly from both TP and TY populations (96/97). Copy numbers of P elements on 3L chromosome with In(3L)P and 3L standard chromosome were tabled (Figure 1). All mean frequency of cosmopolitan inversion, In(3L)P, of *Drosophila melanogaster* is 11.18%. The mean copy numbers for all P elements pooled are higher on In(3L)P chromosomes, compared with 3L standard chromosomes. TEs (P) are more abundant within inversions in all cases. M′ and Q strains of *Drosophila melanogaster* from TP and TY populations were

analyzed with P-M hybrid dysgenesis. The occupied sites of P elements were detected by *in situ* hybridization on the salivary gland chromosome sampled from each five M′ and Q strains of TP and TY populations. These were investigated with copy numbers of P elements on 3L chromosome location with In(3L)P. Q strains have more copy numbers of P elements than M′ strains, concentrated highly at loci 61C-D, 64B, 71C, and 79C on 3L chromosome locations with In(3L)P, and M′ strains distributed highly at loci 61C, 64A, and 72C (Figures 2 and 3). Distribution for copy numbers of P elements in Q strains 96/97 located mainly at loci 61,64,71, and 79 on 3L chromosome with In(3L)P, M′ strains distributed highly at loci 61, 64, and 72 (Tables 4 and 5).

Discussion: TP 291 and TY 307 isofemale lines were tested in the cross A°. The mean sterility frequencies of both populations were 22.78 and 23.99%, respectively. Kim(1994) reported the mean sterility frequencies of both populations, 261 TP and 280 TY isofemale lines, were 39.36% and 35.61%, respectively, in the cross A°. These differences were from the mean sterility frequencies of both TP and TY populations. The mean frequencies of M′ and Q strains of flies with In(3L)P were 0.0299 and 0.0738, respectively. True M strain frequency was observed with 0.0016 on the flies with In(3L)P in all populations. P activity of flies with In(3L)P was investigated to be concentrated completely to M′ and Q strains. Q strain frequency of the flies with In(3L)P was tested more than M strain in all populations. Kim(1994) reported sterility frequency of flies with In(3L)P was observed to be concentrated completely in M′ and Q strains. The Q strain with In(3L)P was observed with higher frequency than M′ strain in these populations except for TP (Kim, 1994). Copy numbers of P elements on 3L chromosome with In(3L)P and 3L standard chromosome were tabled (Figure 1). The mean copy numbers for all TEs (P element) pooled are higher on the whole arm of In(3L)P chromosomes, compared with 3L standard chromosomes. The total copy number of the P elements was determined by using each five lines of M′ and Q strains randomly from both TP and TY populations (96/97). Paul D. Sniegowski and Brian Charlesworth (1994) reported five of the 10 TE families are more abundant on inversion chromosomes. The occupied sites of P elements were detected by *in situ* hybridization on the salivary gland chromosome sampled from each five M′ and Q strains of TP and TY populations. Koryakov and Zhimulev (1996) reported the most active in chromosome rearrangement formation are the following regions: 61C, 62A, 64CDE, 66ABC, 67DE, 70C, 75C, and 80AC in the 3L. Q strains have more copy numbers of P elements than M′ strain, concentrated highly at loci 61C,D, 64B, 71C and 79C on 3L chromosome location with In(3L)P, and M′ strains distributed highly at loci 61C, 64A, and 72C (Figures 2 and 3).

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Sexual dimorphism apparent in size-related wing asymmetry.

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Abstract: Sexual dimorphism in size-related wing asymmetry was examined in *Drosophila buzzatii*. Wing asymmetry in laboratory-reared flies was negatively correlated with distal wing length in females but not in males. These results are consistent with previous studies where distal wing length was uncorrelated with the level of asymmetry in wild-reared males, but suggest a relationship between wing size and asymmetry in females.

Introduction: In bilaterally symmetrical organisms, the absolute value of side-wise random deviations from perfect bilateral symmetry may be, at least for many size-related traits, negatively correlated with trait size (Moller, 1996; Rowe *et al.*, 1997).

Moreover, several fitness components may be negatively correlated with such deviations from symmetry, but male's mating success is the one more often studied. The relatively common finding that

asymmetry increases as trait size (or other measure of individual condition) decreases, has led to the view that fluctuating asymmetry (FA) plays a significant role in the evolution of sexual signals (but see Rowe *et al.*, 1997). However, the evidence that mating success is biased towards more symmetrical males remains unclear in *Drosophila* (Markov and Ricker, 1992). In a recent study in the cactophilic fly *D. buzzatii*, we observed an apparent negative correlation between the level of asymmetry in distal wing length and male's mating success in a caged experiment with wild-reared flies (Norry *et al.*, 1998). However, there was no evidence of correlations between the level of asymmetry and trait size in wild males (Norry *et al.*, 1998). Here, we report data about an apparent sexual dimorphism in the correlation between distal wing length and its level of asymmetry.

Material and Methods: A population breeding on *Opuntia vulgaris* at Arroyo Escobar (34°4' S; 58°7' W), Buenos Aires (Argentina), was sampled in mid-March 1993. Wild females collected over banana baits were individually kept in 95 × 20-mm shell vials containing 5 ml of David's (1962) yeast-killed medium (YKM). A total of 70 isofemale lines derived from wild-inseminated females were thus obtained. Seven mating groups were established. Each group was obtained by releasing one male and one virgin female from the G_1 of each isofemale line (140 flies) into a plastic chamber (100 × 200 × 300 mm) containing two 75-mm-diameter petri dishes with an egg-laying medium (15 g agar, 75 ml 95% ethanol, 15 ml glacial acetic acid in 1,500 ml water). After 48-72 hr, samples of 30 eggs were collected from each chamber and transferred to 95 × 20-mm shell vials with 5 ml of YKM.

Table 1. Means and SDs of distal wing length and its level of asymmetry (FA) are given for each sex in laboratory-reared *D. buzzatii* derived from the Arroyo Escobar population. FA values are in mm x 10^3; wing length (WL) is in mm. Spearman rank-correlation between FA and WL is listed as R_S. N is the sample size.

Statistics	Males	Females
FA		
N	105	105
Mean	4.02	4.09
SD	6.92	6.07
Wing length		
N	105	105
Mean	1.47	1.61
SD	0.04	0.05
Correlation		
R_S	0.07	-.33*

*P < 0.05.

The offspring eclosing from these vials were collected, and 105 randomly chosen flies of each sex were scored for distal wing length (the distance from anterior crossvein to distal tip of vein III; see Figure 1 in Norry *et al.*, 1997). Both wings were measured on a microscope slide at 1× magnification, using a Wild M-20 compound microscope. The unsigned left-minus-right values were used as asymmetry scores.

Results and Conclusions: Summary statistics for wing length and its asymmetry are given for each sex in Table 1. No sexual dimorphism in absolute asymmetry was detected (MEAN $RANK_{males}$ = 127, MEAN $RANK_{females}$ = 123; P = 0.90, Mann-Whitney test). However, the correlation between wing length and its asymmetry was negative in females but positive and nonsignificant in males (Table 1). These results suggest sexual dimorphism in size-related asymmetry, at least in laboratory conditions.

Sexual selection against wing asymmetry was apparent in a recent study with wild-reared males from the same population examined here (Norry *et al.*, 1998). In that study, wing asymmetry was uncorrelated with body size in wild males, which were collected on their natural substrates immediately after their ecdysis (Norry *et al.*, 1998). Thus, the present results in laboratory-reared flies are consistent with those in wild flies in that wing asymmetry and size are uncorrelated in males from the Arroyo Escobar population, but suggest a relationship in females.

X-linked effects in the apparent relationship between developmental stability and size might be an important factor influencing the evolution of sexual dimorphism in body size. However, the genetic basis of developmental stability (as indexed in terms of fluctuating asymmetry) is often population specific (Clarke 1998). Therefore, the present results should not be trusted as evidence that a X-linked effect is the cause of an apparent sexual dimorphism in size-related wing asymmetry. This apparent dimorphism might be population specific. Clearly, a comparative study of different populations is necessary to clarify this point.

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Met, a mutation involved in juvenile hormone action, does not prevent changes in the hormone metabolism of *Drosophila* under stress.

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As evidenced by the large body of data in the literature, the juvenile hormone (JH) is important in the stress reaction of insects. It has been shown that stress results in an increase in the level of JH as a result of both an elevation of its synthesis and a lowering of its degradation (see reviews: Rauschenbach, 1991; Cymborowski, 1991). We have previously demonstrated that in females of *Drosophila* (*D. virilis* and *D. melanogaster*) the level of JH-hydrolyzing activity sharply falls after the effect of a stressor (Rauschenbach *et al.*, 1995). We ask here whether a mutation impairing the action of JH will affect hormone metabolism in flies that are placed under stress. Here we present the results of studies showing the response of JH metabolism to stress in three strains of *D. melanogaster*. Strain *v Met*27 is a null allele of the *Methoprene-tolerant* gene that shows resistance to the toxic effects of the JH analog, methoprene (Wilson and Fabian, 1986; Wilson and Ashok, 1998). Both the *vermilion* (*v*) strain from which the *v Met*27 strain was derived and laboratory balancer strain First Multiple Seven (FM7) are sensitive to the effect of methoprene.

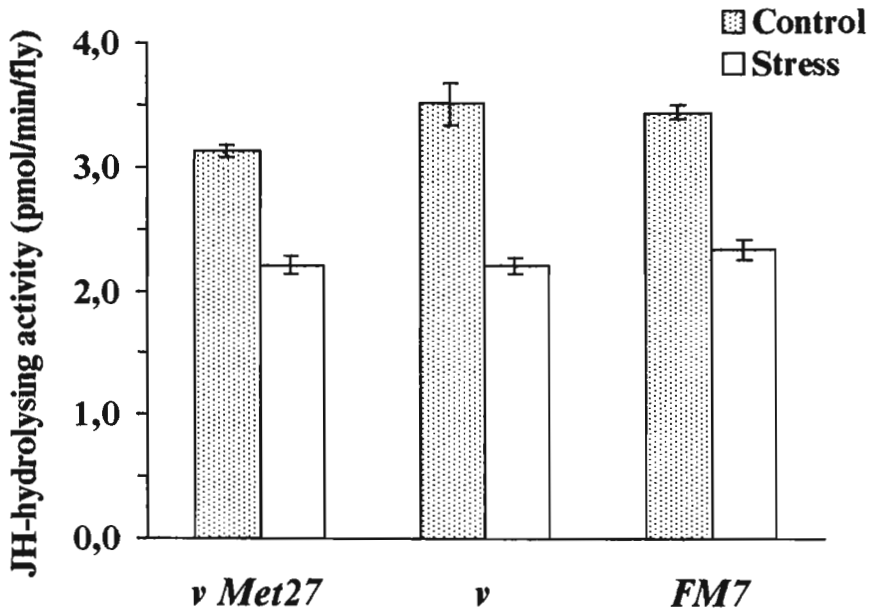

Figure 1. Level of JH-hydrolyzing activity in 24 h old females of strains *v Met*27, *v*, and FM7 under normal and stress (38°C, 2 h) conditions.

Cultures were raised on standard medium at 25°C, and adults were synchronized by eclosion. Adults (24 h old) were subjected to stress by placing them at 38°C for 2 h. Thereafter, they were frozen in liquid nitrogen and stored at -20°C. JH-hydrolyzing activity was measured according to Hammock and Sparks (1977).

The results of measurements of JH hydrolysis levels in females of strains *v Met*27, *v* and FM7 demonstrated that exposure to stress evoked in females of all three strains a significant ($P < 0.001$) decrease in JH-hydrolyzing activity compared to control females kept at 25°C (Figure 1). However, the *Met* flies showed a response that did not differ from the two Met^+ strains.

We have demonstrated that JH degradation in *v Met*27 females responds to a stressful agent by a sharp reduction in JH-hydrolyzing activity. It may be concluded that any impairment of JH action in this strain does not result in any perturbance in the hormonal stress reaction we studied.

Acknowledgments: This study was supported by a grant from the Russia fund of fundamental research and by a grant from the Siberian Branch of Russian Academy of Sciences for support of young prominent scientists.

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M.L. Filipenko 1995, J. Insect Physiol. 41: 179-189; Wilson, T.G., and M. Ashok 1998, Proc. Natl. Acad. Sci. USA 95: 14040-14044; Wilson, T.G., and J. Fabian 1986, Dev. Biol. 118: 190-201.

Variation in abdominal pigmentation pattern of *Drosophila immigrans* from Kumaun, India.

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Since the discovery of Sturtevant (1919) that *Drosophila melanogaster* has a closely resembling sibling species *Drosophila simulans*, both species are cosmopolitan and coexistent (Lachaise, *et al.*, 1988). The two species are mainly distinguished by checking the male offspring of isofemale lines because of different genital arches (Coyne, 1983; Shorrocks, 1972). Based on measurements of eye sizes of *D. melanogaster* and *D. simulans*, it is possible to make a distinction between the females (Burla, 1951; Gallo, 1973; McNamee and Dytham, 1993), but it is a painstaking job where large numbers of flies have to be examined. A high number of misqualifications have been reported, based on eye size definitions. Based on a paper by Eisses and Santos (1997), we decided to examine the abdominal pigmentation pattern of *D. immigrans* Sturtevant which is a very common species found in this region.

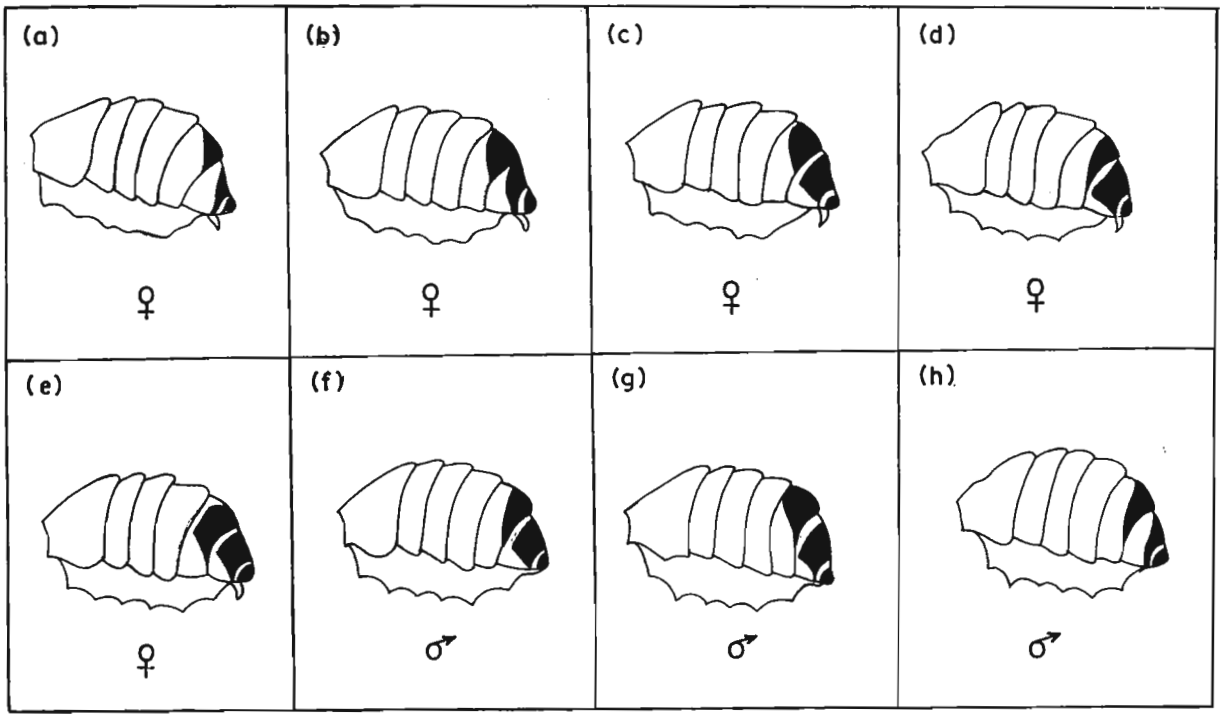

Figure 1. Pigmentation pattern of the 5th, 6th, and 7th tergites of *D. immigrans* Sturtevant. (a-e) females and (f-h) males.

Drosophila immigrans Sturtevant is a member of the *nasuta* subgroup of the *immigrans* species group of the subgenus *Drosophila* of the genus *Drosophila*. The Kumaun region is so far represented by two species of the *immigrans* species group, viz. *D. immigrans* Sturtevant and *D. sulfurigaster* Duda. D. immigrans is fairly distributed throughout the Kumaun region, while *D. sulfurigaster* has been recorded only from a few localities. These two species are morphologically very similar and for the identification of these species one has to depend on the genital structures of the male. During the present investigations, an attempt has been made to examine the abdominal pigmentation pattern of *D. immigrans* collected from different geographical localities of this region.

A total of about 2,500 males and females were examined for their 5th, 6th, and 7th abdominal pigmentation pattern and the observations are shown in Figure 1 (a-e) females and (f-h) males. The difference in the pigmentation pattern of the 6th and 7th tergites of females and males are prominent. Five types of pigmentation pattern have been noticed in females and three types in males. Our study will be extended to the pigmentation pattern of *D. sulfurigaster*. A comparative study of the abdominal pigmentation pattern of these two species will be very helpful for the identification of the females of these two species.

Acknowledgments: Thanks are due to the Council of Science and Technology, U.P., for sanctioning a major research project to B.K. Singh to study the Drosophilidae of Kumaun region, India.

References: Burla, H., 1951, Rev. Suisse Zool. 58: 23-175; Coyne, J.A., 1983, Evolution 37: 1101-1117; Eisses, K. Th., and M. Santos 1997, Dros. Inf. Serv. 80: 87; Gallo, A.J., 1973, Ciencia and Cultura 25: 341-345; McNamee, S., and C. Dytham 1993, Syst. Entomol. 18: 231-236; Shorrocks, B., 1972, *Drosophila*. Ginn and Company Limited, London; Sturtevant, A.H., 1919, Psyche 26: 153-156.

Detailed description of puffing patterns in the salivary gland chromosomes of normally developing larvae and prepupae of *Drosophila melanogaster*.

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Puffing of the salivary gland polytene chromosomes in different *Drosophila melanogaster* mutants is an object of investigations in our laboratory for more than 25 years (Zhimulev, 1974; Belyaeva, 1982). We found that the puffs are highly reproducible in different stocks with normal development (Oregon-R, Canton-S, Batumi-L, *yellow*) either in sequences of changes of properly puffing patterns or Puff Stages (PS) (see for review Ashburner and Berendes, 1978), in schedule of appearance of the individual puffs or their sizes. Constant puffs (these are, as a rule, small ones), the regions of decondensed chromatin, with diameter not exceeding considerably the diameter of chromosome, are invariable as well.

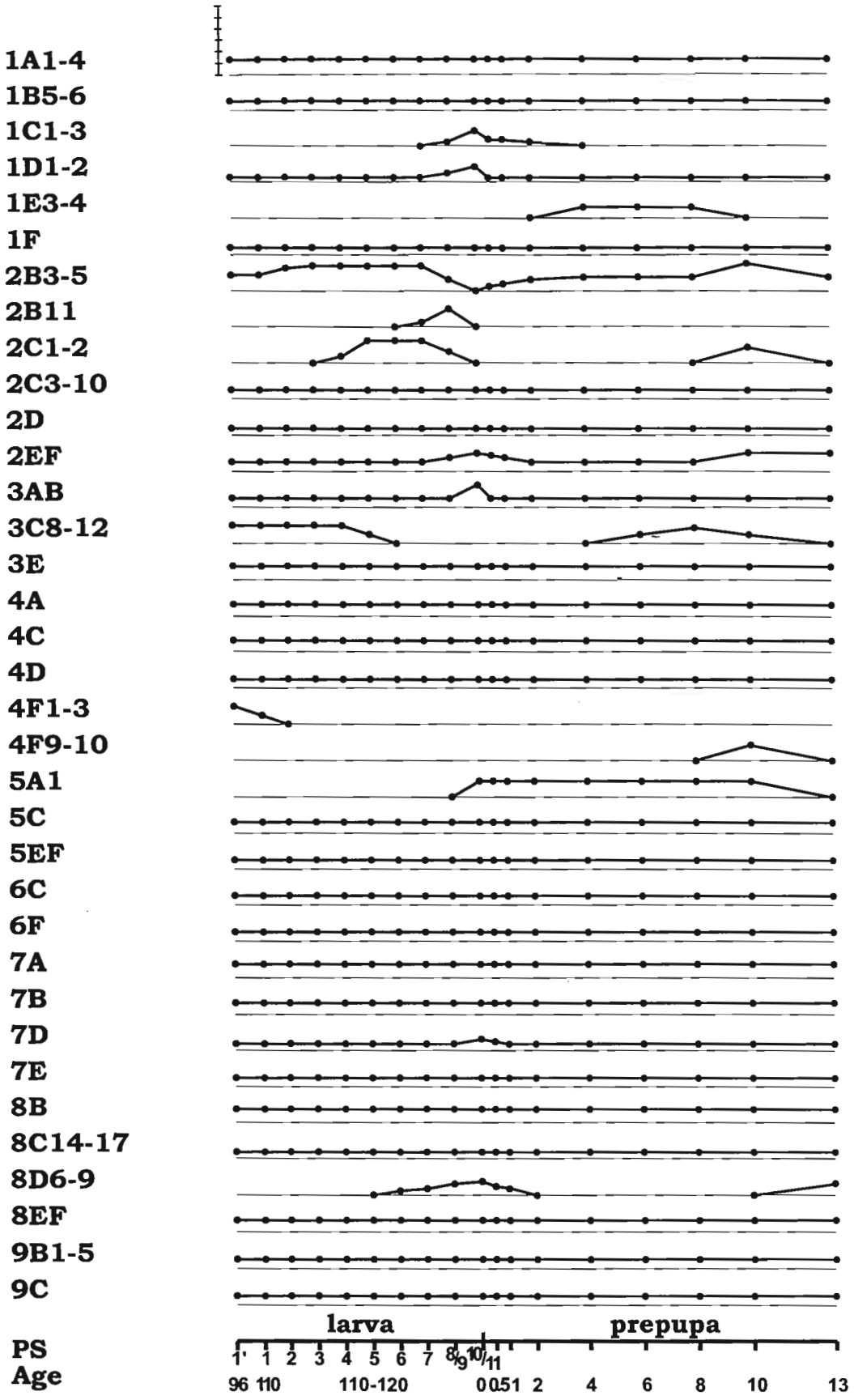
The diagrams, showing behavior of all visually revealed puffs during larval and prepupal development are given below (Figure 1). They seem to be useful for other researchers dealing with polytene chromosomes. The puff sizes are estimated using a 6-point scale (Zhimulev, 1974; Belyaeva, 1982) in larvae from 96 hr of development to 13 hr prepupae, *i.e.*, in the period when polytene chromosomes are accessible for analysis.

For puffing chronology in prepupae we used hours after formation of 0 h prepupae. This developmental stage is marked by spiracle eversion. For determination of developmental stages of larvae, morphological characteristics of the salivary glands were used (Figure 2).

Acknowledgment: The work was partly supported by grants: INTAS grant 96-1339, Russian Fund of Basic Research (RFBR) 96-15-97749 and Russian Frontiers Program in Genetics.

References: Ashburner, M., and H.D. Berendes 1978, In: (M. Ashburner and T.R.F. Wright, eds.), *The Genetics and Biology of* Drosophila, 2b: 316-395, Academic Press, London; Belyaeva, E.S., 1982, Doctor of Sciences Thesis. Institute of Cytology and Genetics, Novosibirsk; Zhimulev, I.F., 1974, Chromosoma 46: 59-76; Zhimulev, I.F., 1999, Advances in Genetics, 39: 1-550; Zhimulev, I.F., M.L. Izquierdo, M. Lewis, and M. Ashburner 1981, W. Roux Arch. Developm. Biol. 190: 351-357.

Figure 1 (following ten pages). Changes of puffing patterns during last 24 hours of larval and 13 h of prepupal development (25°C).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2D
2EF
3AB
3C8-12
3E
4A
4C
4D
4F1-3
4F9-10
5A1
5C
5EF
6C
6F
7A
7B
7D
7E
8B
8C14-17
8D6-9
8EF
9B1-5
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larva
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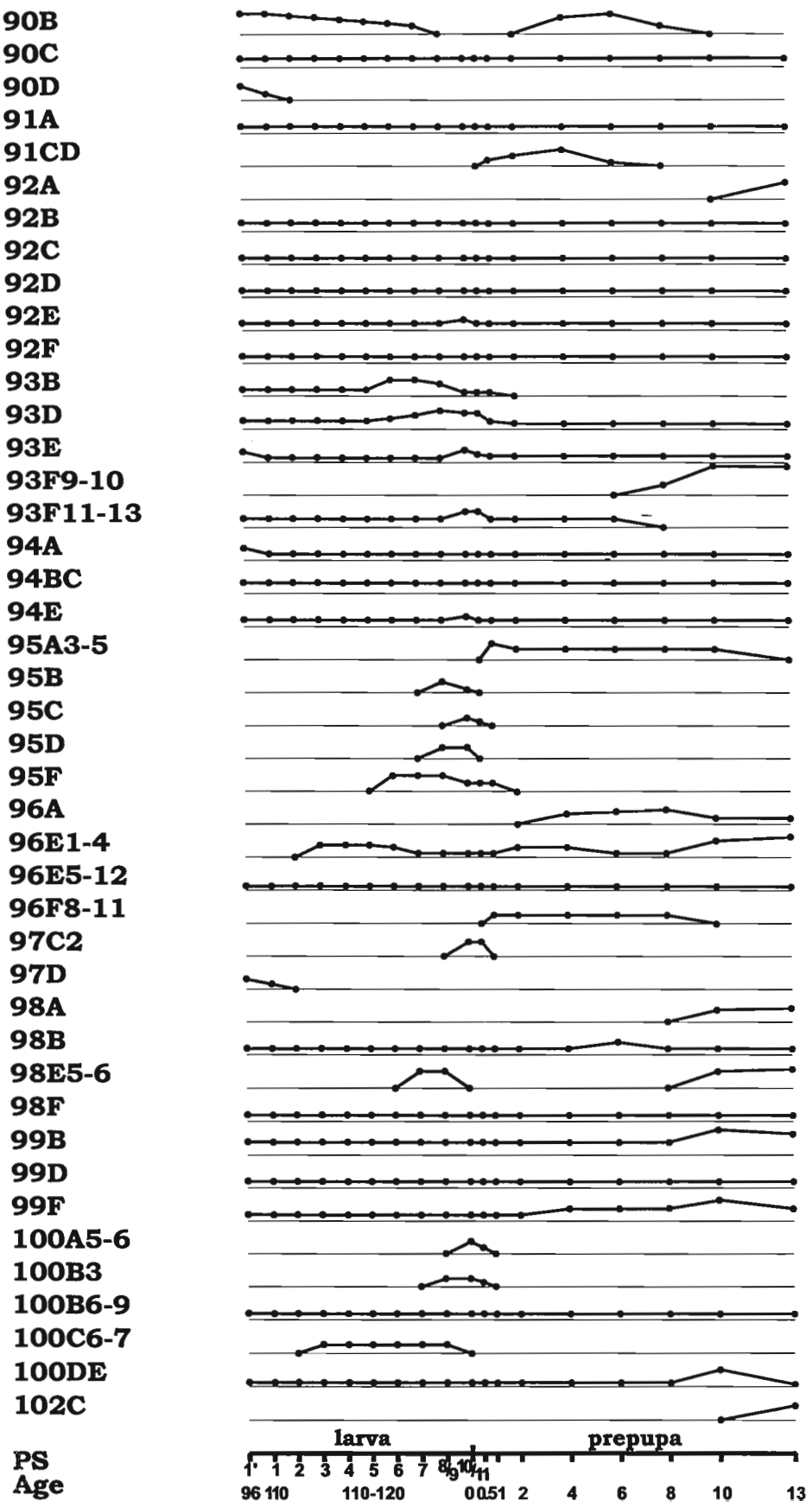
96 110 110-120 0 0.5 1 2 4 6 8 10 13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94A
94BC
94E
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95B
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95D
95F
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99D
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100B6-9
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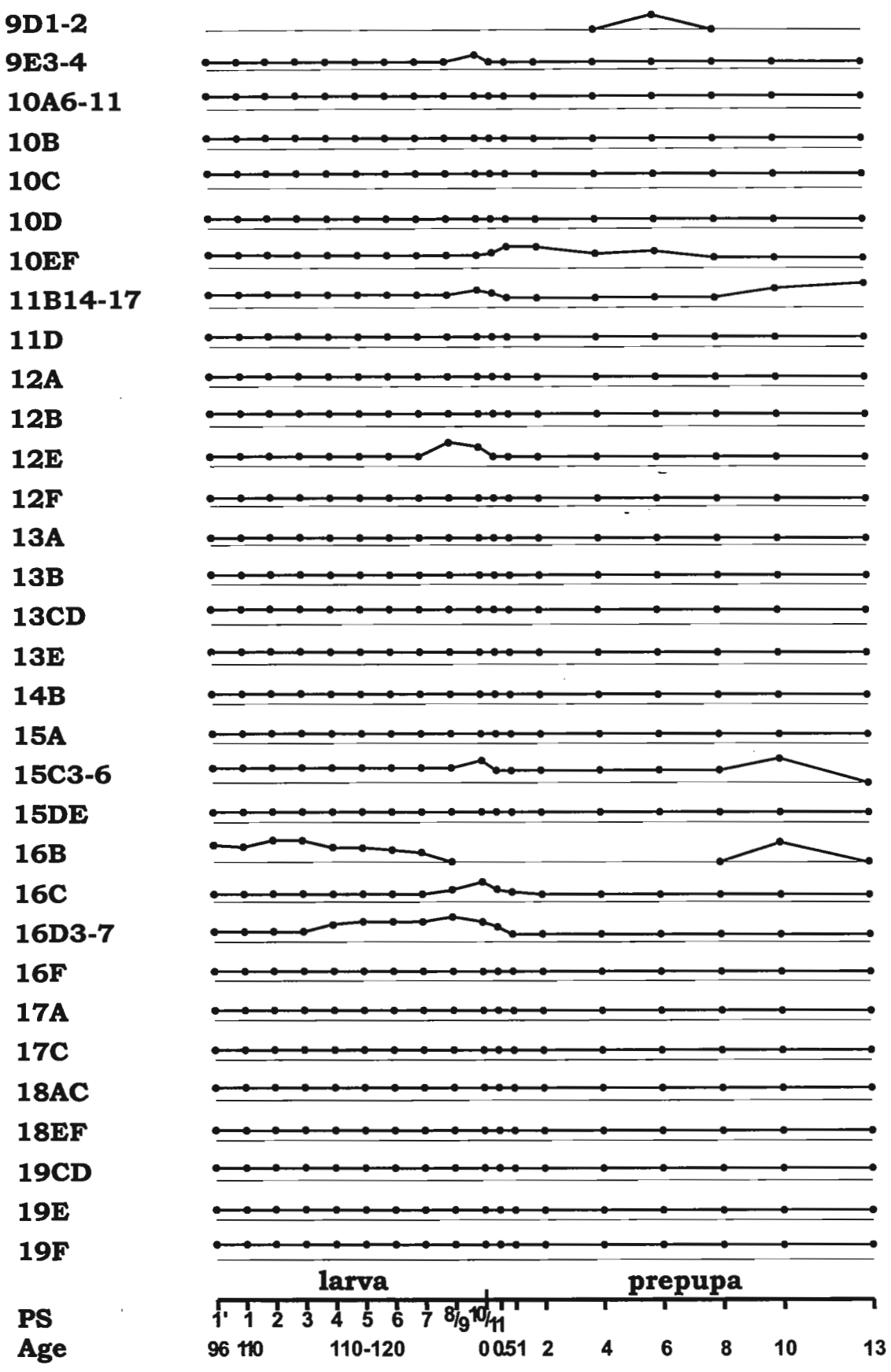
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15A
15C3-6
15DE
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Age
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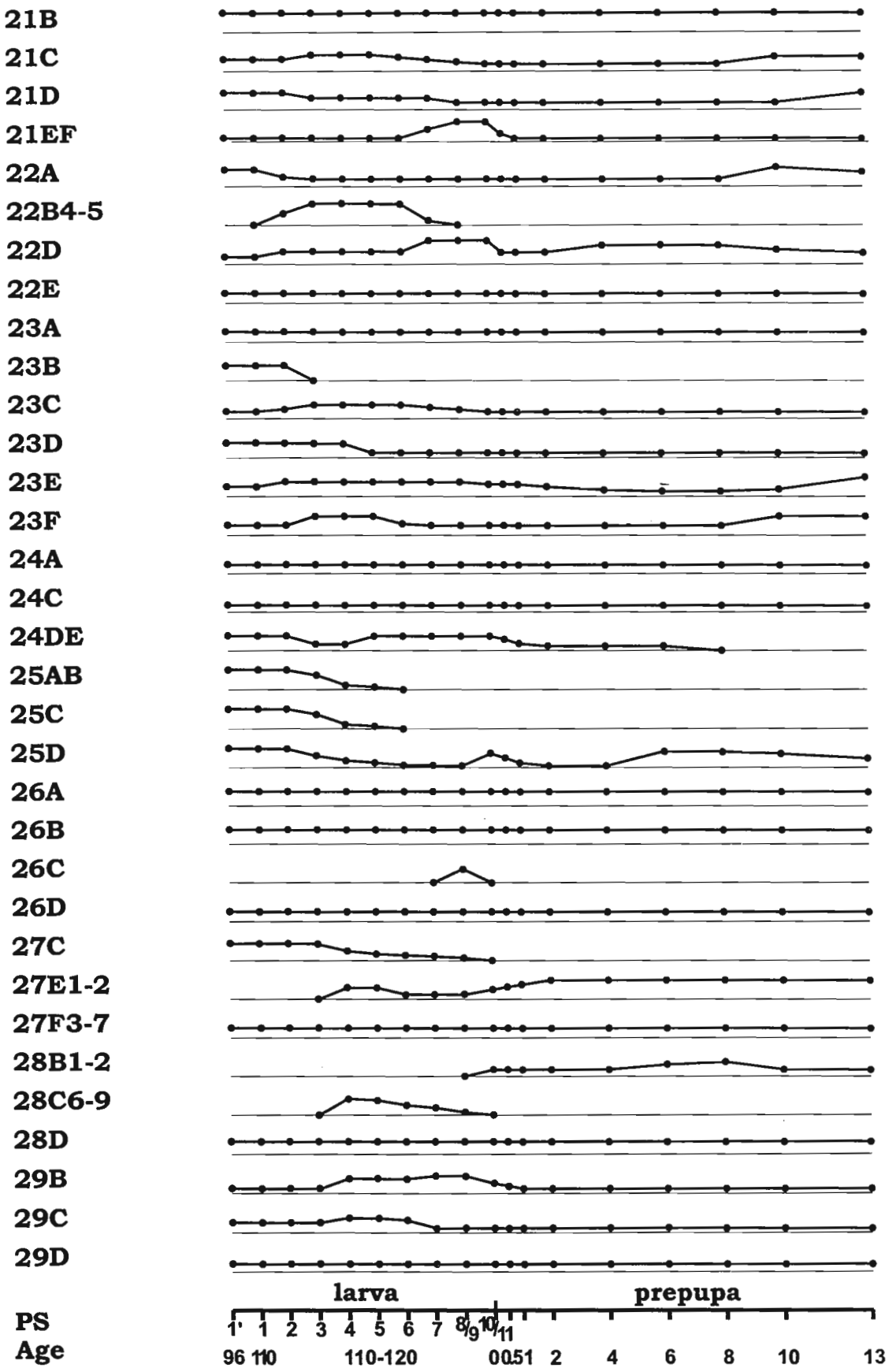
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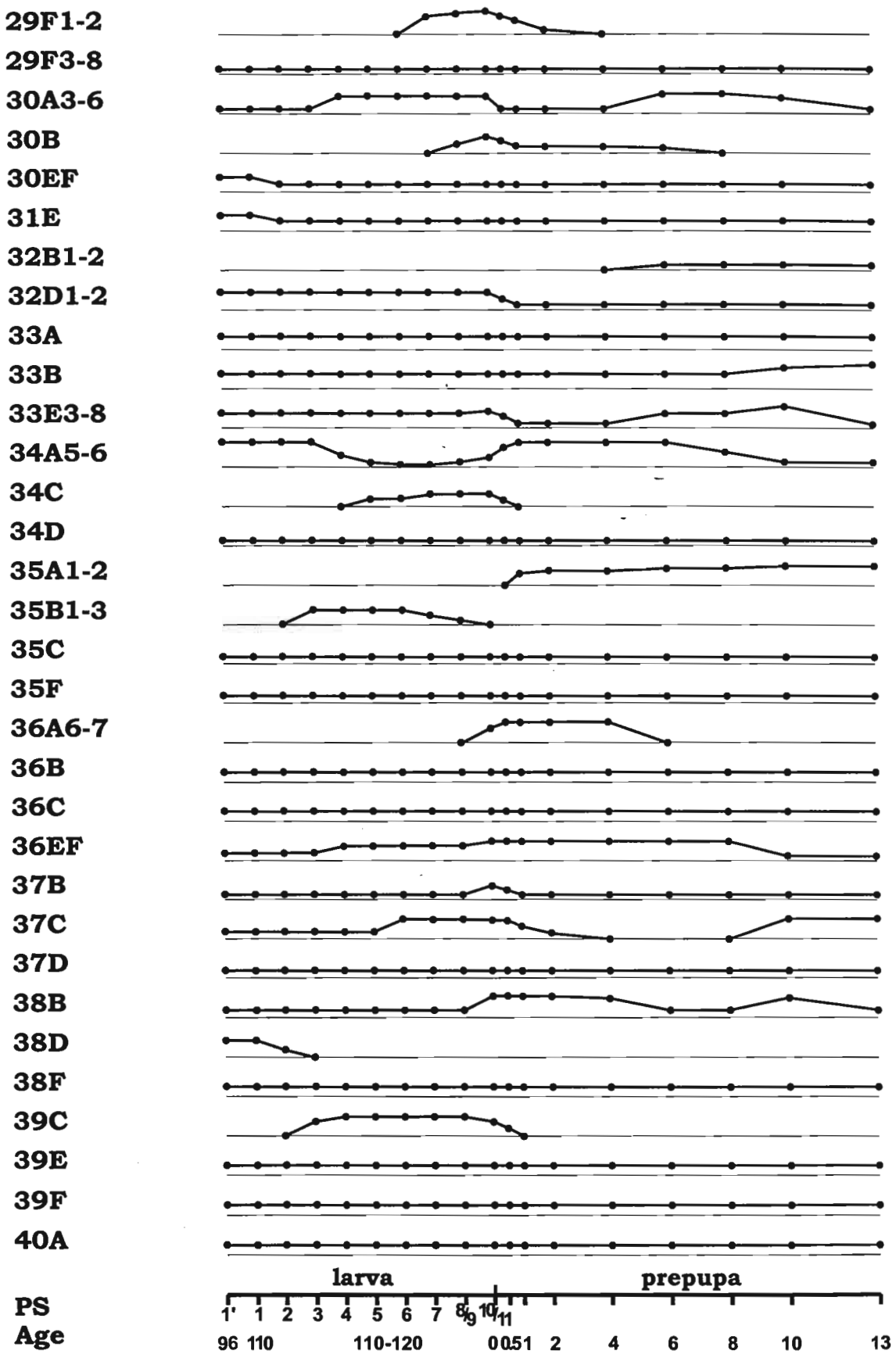
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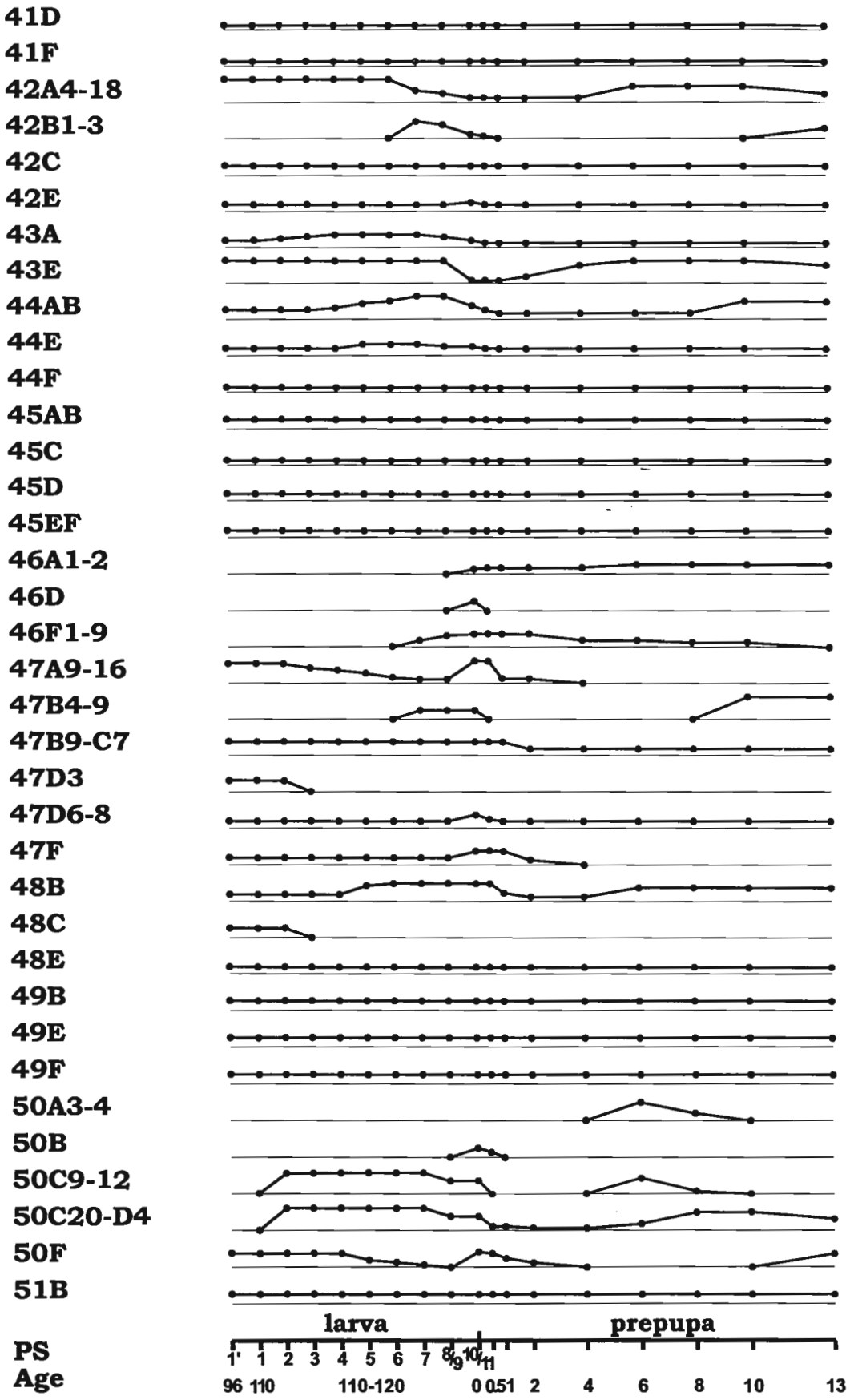
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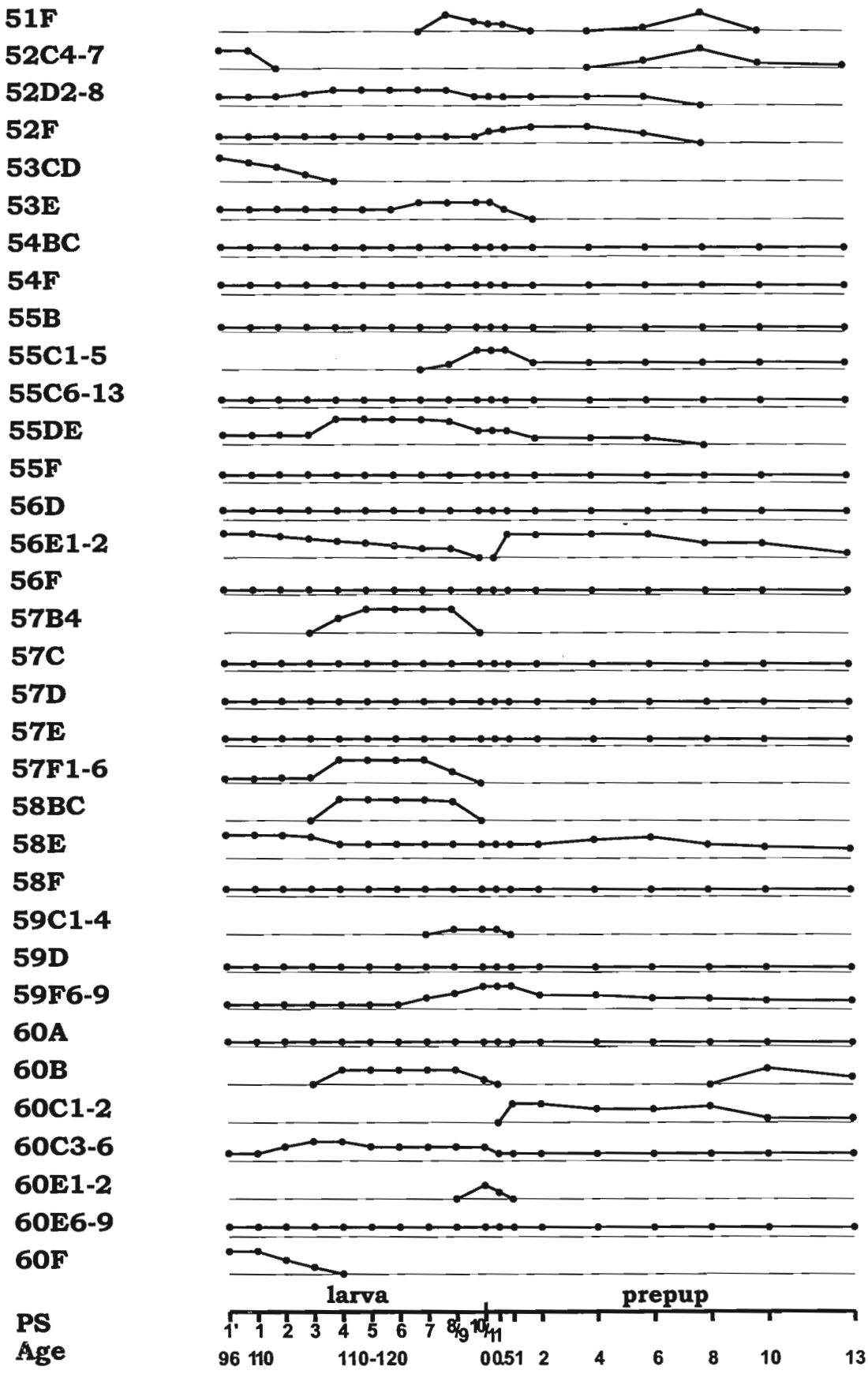
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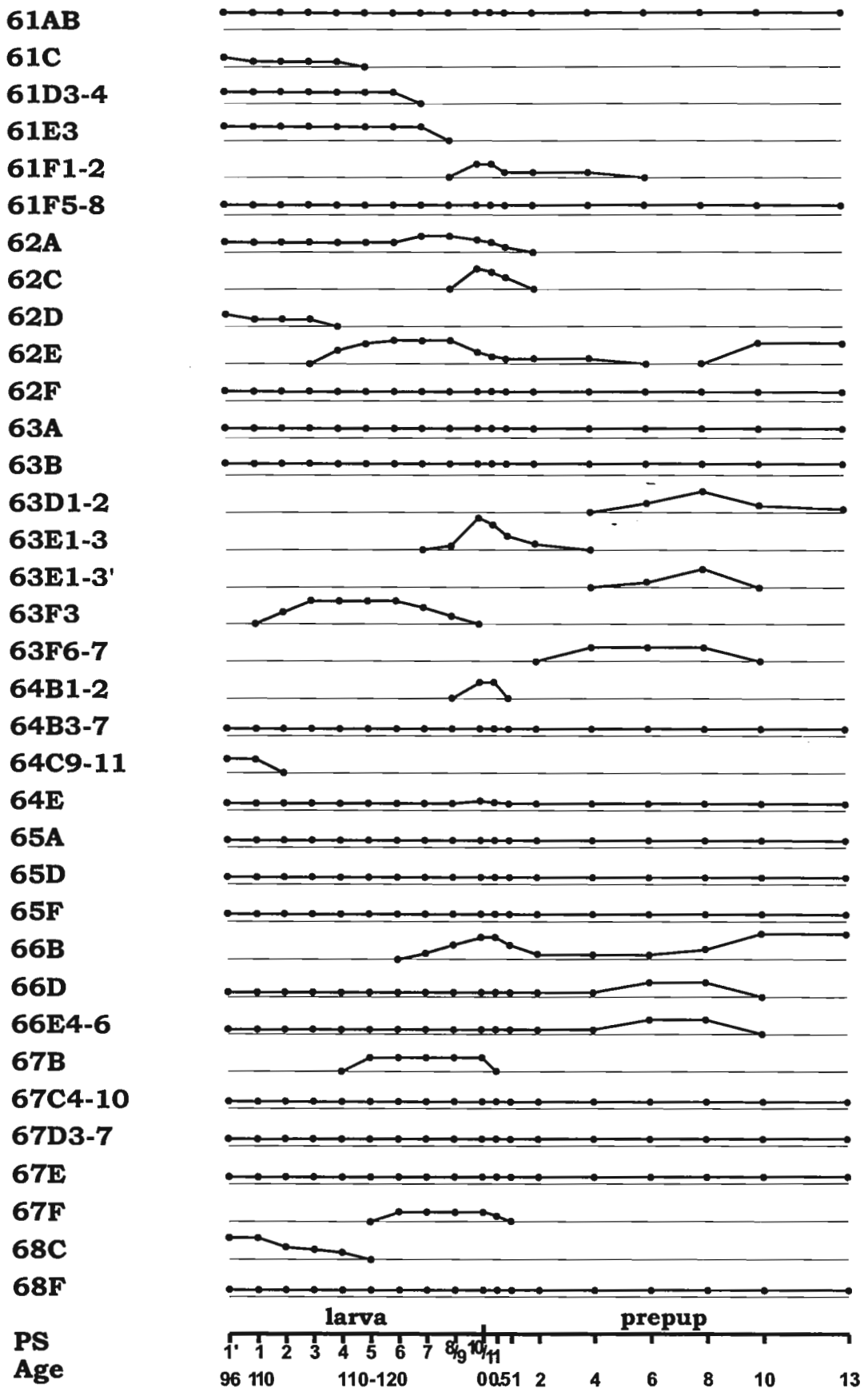
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65D
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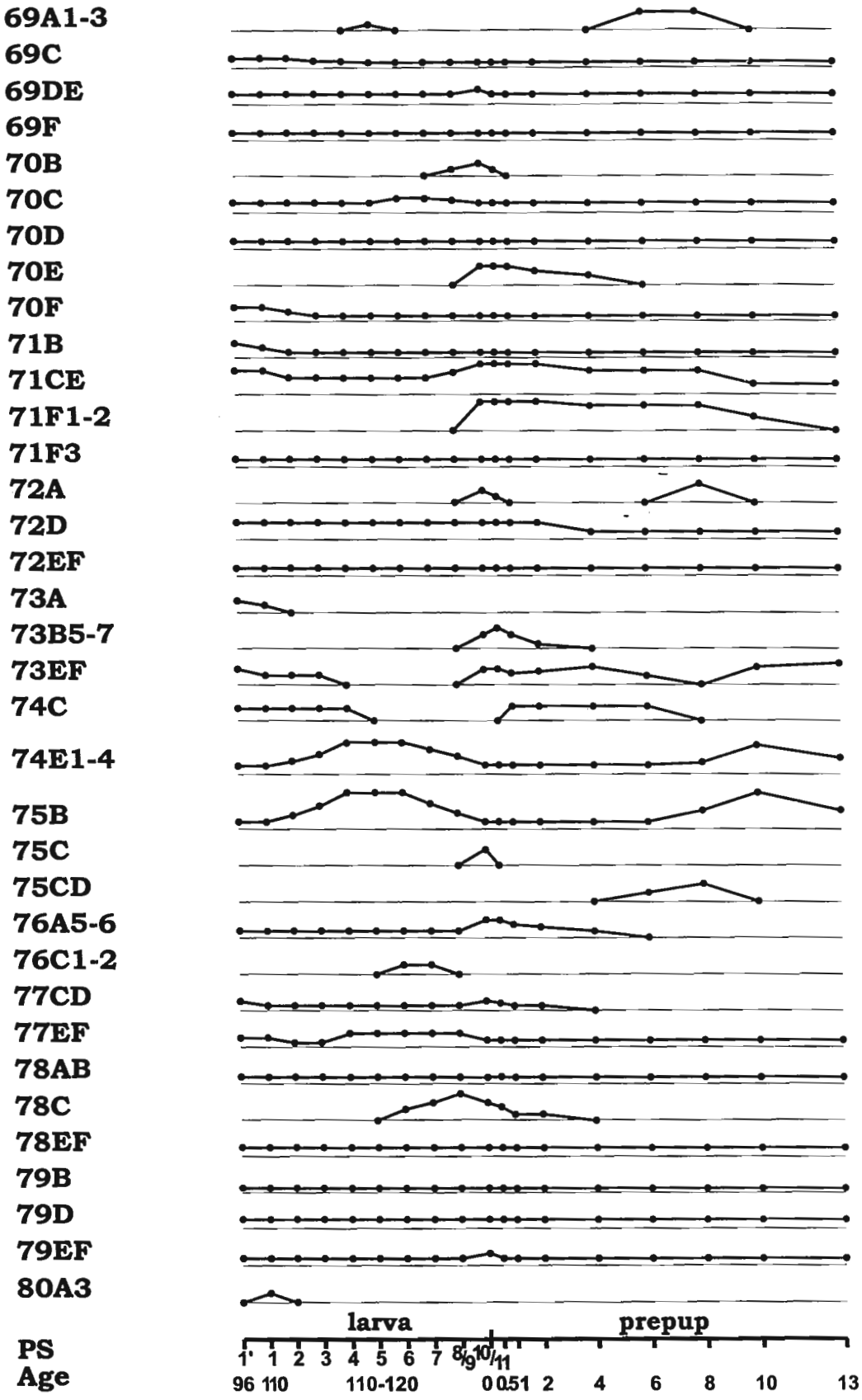
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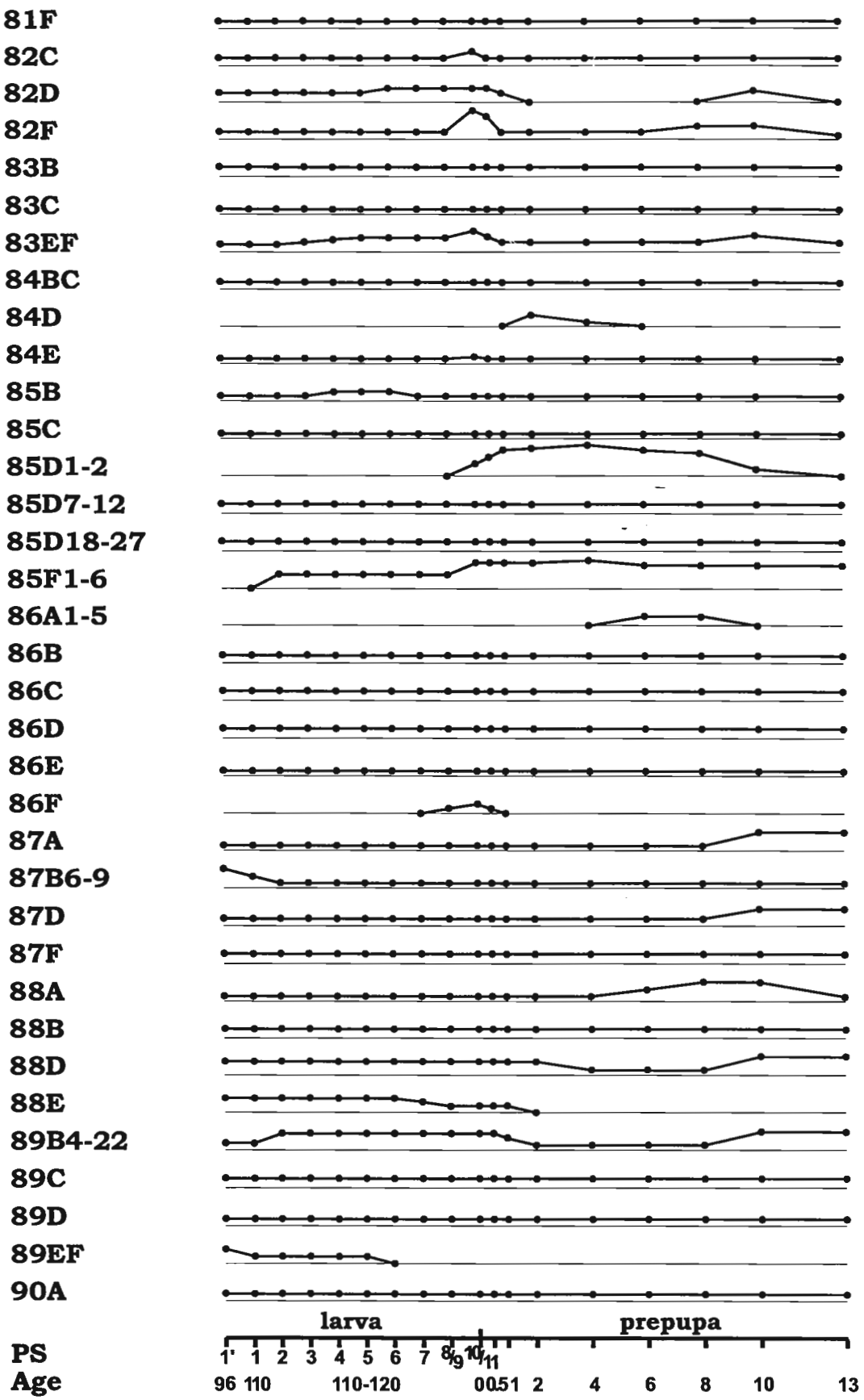
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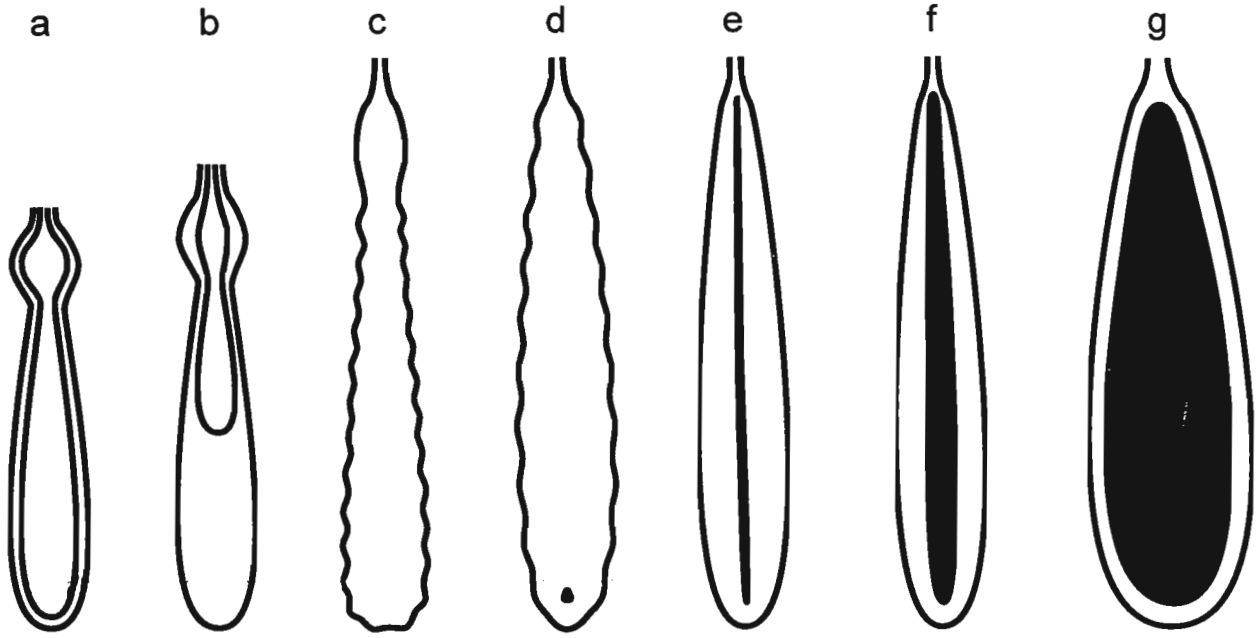

Figure 2. Changes of salivary gland morphology in the second half of third larval instar of *Drosophila melanogaster* (according to G. Richards, 1979, personal communication; Zhimulev *et al.*, 1981; Zhimulev, 1999).
a and *b* – the middle of third instar, *a* - the salivary gland cavity is open completely; *b* – the cells close up beginning from distal part, the cavity is seen only in proximal part of the gland, the cells do not contain glycoprotein granules.
c – the cells close up along the whole gland and they are filled with glycoprotein secretion granules, the puff pattern in chromosomes corresponds to PS1 stage.
d – a small cavity appears in the most distal part of gland at the expense of glycoproteins secretion, the set of ecdysone-induced puffs corresponds to the PS2-3 stage;
e – a thin bundle of secretion fills the whole gland, PS4-5;
f – the thickness of secretion bundle is about one third of gland thickness, PS6-7;
g – the gland looks like swollen bag filled by secretion, the developmental stage is the one just before extrusion of secretion in surroundings and spiracle eversion, PS10-11.

Detection of homokaryotype selection, especially for SC/SC (Santa Cruz) and rigid chromosomal pattern in marginal ecological conditions for the third chromosome rearrangements in the *Drosophila pseudoobscura* populations from the Colombian high plateau Andes.

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Five *Drosophila pseudoobscura* populations from the Colombia Cundiboyacense high plateau in the Andes (Torobarroso, Susa, Sutatausa, Potosí and Santillana) were systematically studied each week during a year (1997), to analyze comparatively the thrid chromosome rearrangements among these Colombian populations. The first chromosomal study of this species in Colombia, which had been discovered a few years earlier, was carried out by Dobzhansky *et al.* (1963). Only two different chromosomal rearrangements, Santa Cruz (SC) and Tree Line (TL), were found in that study. As in this previous work, the SC and TL were the predominant rearrangements in the Colombian populations studied by us. Nevertheless, for first time, other

chromosomal rearrangements with low frequencies like Standard (ST), Olympic (OL), Cuernavaca (CU) and one highly similar to the Mexican endemism Amecameca (AM) were also detected. However, the first aim of the present study was the following: Can be detected any selective pressure in Colombia, like those reported in USA and Mexico that were associated with systematic changes in the chromosomal rearrangement frequencies during the year? Dobzhansky (1943, 1947a,b) determined that the natural selection was present in, at least, two of the three populations studied in St. Jacinto (California), because of the changes in chromosomal rearrangements detected month to month (the studies of Dubinin and Tiniakov, 1945, 1946 with *D. funebris* were also determinant). Lately, other studies also reported these cyclic changes in the following years (Dobzhansky *et al.*, 1964; Epling *et al.*, 1957). These changes cannot be caused by genetic drift, because they were cyclic and followed the seasonal changes every year. There were two demographic maximums in a year, for example in Piñon Flats or Andreas canyon, one in early spring, and other in autumn, exactly when the ST (Standard) frequencies were, with a high difference, the most elevated. In the hotter months (spring's end and almost summer) there was a demographic depression with an increasing of the AR (Arrowhead) and CH (Chiricaua) frequencies. In Mexico something similar was detected with the rearrangements CU and TL (Amecameca population). The former is more frequent in spring and autumn, while the latter is in summer. On the contrary, this last rearrangement (TL) did not show important changes with the season changes in the Californian populations (Levine *et al.*, 1995). However, the frequency variations are generally lighter in Mexico than in many of the reported Californian populations. Nevertheless, other Californian and North American populations did not show these annual cyclic changes (Powell, 1992). At 15 miles from the populations refereed, there is Keen Camp, with a different rain level but without cyclic variations during the year (Dobzhansky, 1943, 1947b; Epling *et al.*, 1957). For example, Crumpacker and Williams (1974) studied two groups of populations at North and South of Denver (Colorado). In the first one, the frequencies were constant during the year, while in the second one, the rearrangement frequencies were similar to the first, but they had seasonal cycles, with AR reaching high values on spring and early summer, with the lowest value finishing this season. In autumn, it reached a new maximum. This fact led us to formulate the following question: Have the Colombian populations a flexible genetic system like many of the American ones? Or contrarily, have they a rigid system? What kind of selection, if any, is present in Colombia?

Material and Methods

To establish the population dynamic of the two predominant rearrangements (SC and TL), the application of various population genetics procedures were performed. (1) To detect any bias of the Hardy-Weinberg equilibrium, the Wright'S F (1965), with the Rasmussen (1964)'s variance, and the Robertson and Hill's f (1984) statistics were used. The statistical significance of the hierarchical F statistics was obtained by means of the application of 500 jackknife permutations. In this way, confidence intervals (95 and 99 %) were generated for the three F statistics. (2) A correspondence factorial analysis (CFA) was performed to analyze the relationships between the populations and the chromosomal rearrangements studied simultaneously. The coordinate matrix from the factorial matrix, the absolute contribution matrix and the squared-correlation matrix were obtained both for the populations and for the chromosomal rearrangements. (3) To ratify, or not, the relationships found with the previous analysis, the Nei's (1978) and the Prevosti's (1974) genetic distance matrices among population-pairs were obtained. Two hierarchical tree algorithms were applied to these genetic distance matrices (UPGMA, Sokal and Michener, 1958; and WPGMC, Lance and Williams, 1967). (4) The last analysis used was a Mantel's (1967) test normalized with the Smouse *et al.*,'s (1986) procedure. The statistical significance of these results was carried out with a Monte Carlo simulation with 2000 permutations. The geographic distances between the populations were calculated both with the "Great Circle" method (Spuhler, 1972) and with aereal distances.

Results

The principal results obtained were the following: (1) The SC frequencies were very similar in the five populations studied (Table 1), being, in general, the SC frequencies slightly more elavated than those reported in past decades. This affirmation was specially significant for the Potosí population (0.782). (2) The

five populations studied were not in Hardy-Weinberg equilibrium, individually and as a unique set, by an elevated excess of homokaryotypes, and the consequent defect, in proof, of heterokaryotypes (F = 0.94-0.99) (Table 2). (3) The genetic heterogeneity between the five populations studied was not significant (F_{ST} = 0.008), which puts in evidence a high genetic homogeneity at the chromosomal rearrangement level in Colombia, this being very different to those found in North America. (4) Changes in the chromosomal composition were not observed throughout a year in each one of the populations analyzed, although some week samples were little and they could be submitted to a strong sample error. (5) The WPGMC tree with the Nei's genetic distance (Figure 1) clearly showed that the Potosi population was the most differentiated of the five populations studied. (6) The Mantel's test results, with the Great Circle and with the aerial distances, showed in all cases non-significant negative correlations, which indicates that there was not a conspicuous relationship between the genetic distances and the geographical distances among the populations analyzed.

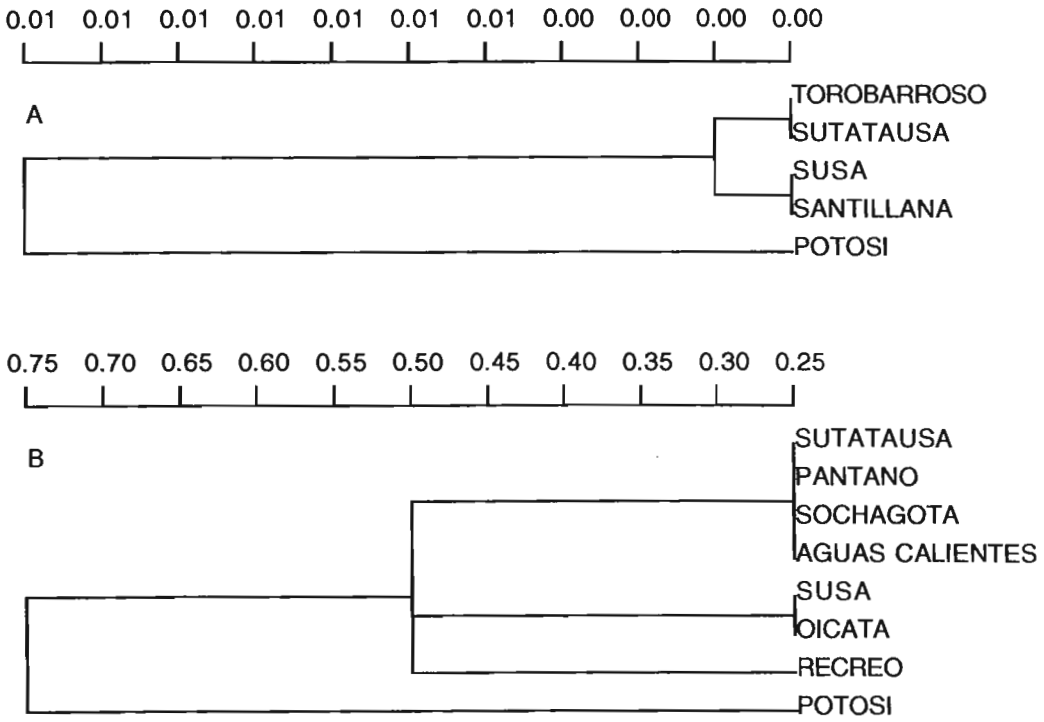

Figure 1. A, WPGMC tree with the Nei's genetic distance for the five Colombian *Drosophila pseudoobscura* populations studied for the third chromosomal rearrangement frequencies. B, Strict consensus tree using UPGMA and Single algorithms and the Manhattan and Canberra distances for eight Colombian *Drosophila pseudoobscura* populations sudied for 15 meristic morphological variables. See the strong difference of the Potosí population versus the other Colombian populations for both types of variables.

Discussion

Dobzhansky *et al.* (1963) showed that the *D. pseudoobscura* populations in Colombia were in contradiction with the Carson's theory, because a special heterotic system (excess of heterokaryotypes) was found in this population, isolated from the distribution central range in North America. In the 80's, Cárdenas (1988) found that this species in Colombia was basically in Hardy-Weinberg equilibrium for the third chromosomal rearrangements. In both studies it was affirmed that this species, although geographically restricted, was locally very common. On the contrary, this species was very scarce during our 1997 sampling. Only 96 isolines were established and 1672 chromosomes were analyzed. Our results are totally in contradiction with the results of previous authors and are in agreement with the Carson's theory. In all the

populations studied the excess of homokarotypes was nearly to the fixation. This new result shows that certain selective parameters in favour of the homokaryotypes (specially for SC) are acting on these Colombian populations during the last years. These new changes could be related to the deforestation, the expansion of cattle fields and habitat destruction by the human beings and/or due to climatic changes motivated by "El niño" phenomenon that was very strong in the Neotropical area in 1997. Moreover, the genetic heterogeneity degree between the Colombian populations was non-significant (F_{ST} = 0.008), which puts in evidence the non-significant importance of genetic drift, inbreeding and isolation-by-distance for these chromosomal rearrangements. A lot of the results obtained are opposed to those found in the North American *D. pseudoobscura* populations, showing that the current Colombian population has a rigid genetic system. Another interesting difference between the central range and the Colombian populations was that in the latter the rearrangement frequencies were usually constant during the whole year. In this study, as in Cárdenas (1988), SC was more frequent than TL. Only in some few registers TL frequency was approximately equal to the SC frequency, but the oscillations were not substantial. On the other way, in the USA populations there were reported important frequency changes between seasons in a year, and between the following years. As it was commented before, Dobzhansky (1943) found in Piñon Flats that the ST rearrangement had its lower frequency on May-June, while AR had the higher, but the situation was the opposite on September-November when the temperature decreased. Strickberger and Wills (1966) reported an inverse situation in a population near to Berkeley. The ST frequency reached its maximum in summer, and its minimum in winter. CH showed the opposite behavior, while AR did not change. A similar phenomenon, but with other rearrangements, has been reported in Mexico. In Amecameca, CU decreased between May and August, while TL clearly increased and EP (Estes Park) had a slight enlargement during these months (Levine *et al.*, 1995). Because there are not seasons, at least temperature-related, in the Colombian plateau, it could exist a uniform selective pressure constancy during the whole year for many ecological conditions. This could explain why the rearrangement frequencies did not change in all the time that this study took.

As we commented before, the studied populations showed an important homokaryotype excess. We propose that these populations are affected by stabilizing selection favoring the homokaryotypes. In general, the homokaryotype with a higher adaptability would be SC/SC in the major part of the year. TL/TL would have a W (fitness) lower than SC/SC, while the heterokaryotype (SC/TL) would have the smallest W. With some non-temperature related microclimatic changes (wetness and the rain regimes or other variables, like food resources and the application of insecticides), TL/TL could increase its fitness to values near to unity, sometimes. This could allow the coexistence of both arrangements with an homozygous excess, and with the higher frequency for SC/SC. It could be argued that the homokaryotype excess could be caused by some stochastic process (genetic drift and founder effects), that usually are associated to consanguinity. If the sub-populations considered have a low effective number (Crawford, 1984), the endogamy should be favored, because of a limited availability of sexual partners. However, the presence of inbreeding and/or genetic drift in these populations could be discarded easily when this kind of marker is studied. If genetic drift was present, we should not find a low F_{ST} value (0.008) without statistical significance between the subpopulations. This genetic homogeneity, also detected with the genetic distances, shows that a process such as stabilizing selection is more probable than genetic drift. But this does not means that effective numbers of these populations are not low. At this moment, two of the authors (D. A. and M. R.-G.) are studying the same populations using molecular markers (nuclear DNA STRP's and mtDNA RFLP's). If using these markers, the differences between populations were higher, this could probe the action of stabilizing selection on the third chromosome rearrangements. Effectively, in our studies with five microsatellite loci (DPSX001, DPS2001, DPS3001, DPS3002 and DPS4001) with the Colombian *D. pseudoobscura* populations studied here, an F_{ST} of 0.042 was obtained (Alvarez *et al.*, 1999). This value is five times greater than that obtained here (0.008). Moreover, in one microsatellite locus like DPS4001, the F_{ST} value was 0.081 among the Colombian populations, that is, 10 times greater than the value for this statistic on the thrid chromosome rearrangements. Additionally, the major part of these microsatellite loci were in Hardy-Weinberg equilibrium in the analyzed populations. These data indirectly support the presence of some stabilizing selective agent currently acting on the third chromosome in the Colombian populations. Our explanation is in total agreement with Crumpacker *et al.* (1974). They showed that a bottle-neck occurred in the Rist Canyon population in 1967. There were a

high AR (the most frequent) decrease and an increase of locally rare rearrangements such as TL, EP and ST. The frequency change of these arrangements is very difficult to explain by genetic drift. The presence of a strong selective pressure during an environmental stress period that caused a reduction in the population would be, as in our case, a good and parsimonious explanation. On the other hand, Strickberger and Wills (1966) in Berkeley sustained that CH was favored by high rain levels, while ST by an arid environment. Dobzhansky (1971) also showed the presence of increasing and decreasing in the ST and AT rearrangements during many years in Mather (California), related with the succession of wet and dry years, although in a lot of years the correlation was nonexistent. This could be a selective possibility in Colombia if the El Niño phenomenon has ecological consequences. However, in the Colombian plateau, contrary to the temperature ones, there are seasonal changes in the rain levels, although we did not find oscillations in the rearrangement frequencies associated to this fact.

The dispersion rates, at least, in the central distribution range are high. It has been detected values near to 170 m in the first day after reliberation (Crumpacker and Williams, 1973). Powell *et al.* (1976) concluded that the *D. pseudoobscura* dispersion is so elevated that they could homogenize populations in about one kilometer. However, the populations studied here were separated by more than these distances (more than 160 Km among the most distant populations). Gene flow seems to be a less parsimonious explanation than stabilizing selection for the chromosomal homogeneity found between the Colombian populations, because, moreover, the populations are not in Hardy-Weinberg equilibrium for these markers.

The fact that we could not detect clines in the Colombian plateau could suggest that there were no environmental gradients, which originated any significant spatial structure in the distribution of rearrangement frequencies (Endler, 1973, 1977). For this reason, we can sustain that in Colombia, there were not populations with flexible systems, nor separation zones among them like those found in North America, and there were not populations with rigid systems at a microgeographic level, like those found in Colorado by Crumpacker and Williams (1974), either. These authors detected two kind of patterns in Colorado, one population at Northern Denver, which lived in a place with wide macro and microflora, and had a rigid system of chromosomal polymorphisms, while another in Southern Denver, in a place with a poor flora, had a flexible pattern. They suggested that it would also occur in St. Jacinto, where there was a population with a rigid polymorphism scheme, that lived in a mature wood. In Colombia, this rule was broken, because there were populations with a constant chromosomal pattern, but in a possible marginal ecological situation. In USA some *D. pseudoobscura* populations with this last feature (rigid pattern in marginal conditions) have also been detected in the Yosemite region (Jacksonville).

We cannot discard either the possibility that insecticides, or another contaminant used, could have some selective impact on the chromosomal rearrangements. In fact, some of the Colombian populations studied are located close to flower farms where the use of these chemicals is very common (Potosí). Dobzhansky (1958) postulated that some Californian areas, where changes in chromosomal rearrangements were detected, have been submitted to the action of DDT and DDD. The PP appearance in California concurs with the use of DDT at high scale. This made some authors to think that PP was related with the use of this chemical, but during the 70's when DDT was still in use, the PP frequency began to decrease and the TL one to increase in some western USA areas (Anderson *et al.*, 1975). A population located near to the culture Riverside area was used to test if there was any relation between the insecticide use and the chromosomal anomalies in that region. No-anomalies were detected in that population (Dobzhansky *et al.*, 1964). Some authors, like L. Cory (in Dobzhansky, 1971), postulated that insecticides cannot be discarded as selective agents and experiments should be made in larvae and not in adults. It was sustained that some correlations, between the rearrangement changes and the place where the insecticides were used, could exist. However, the situation became complex when Anderson *et al.* (1968) introduced little DDT and dieldrine amounts in experimental populations with 4-5 arrangements. In the New York samples, there was not any fitness difference for the rearrangements in the colonies treated and untreated with insecticides. On the contrary, there were fitness differences between the two groups in the Japanese samples. The ST/ST homokaryotype had a better fitness than the ST/AR heterokaryotype in the insecticide presence, while in U. S. colonies, ST/AR always had a higher fitness independently of the environment. That means that little micro-habitat variances can be the cause of the differences in the susceptibility to the insecticide action as selective agent in *D. pseudoobscura*. These environmental particularities can be very diverse between populations and could

make difficult the finding of general rules. In other species, such *D. willistoni*, differences have been found in some morphological traits between the flies that inhabit urban and rural zones in the Brazilian population of Porto Allegre (Lucchese *et al.*, 1994). In the urban areas, there were flies with higher thorax size, wing length and SO_2, Cd and Pb concentrations than in the rural zones. Related to this, Valente *et al.* (1989) and Regner and Valente (1993) showed that in this *Drosophila* species there was an increase in the average number of chromosome inversions in females and the number of inversions in the chromosome III was higher in the city center than in the peripheral populations. In *D. nebulosa*, this kind of clinal structure depending of the urban degree was restricted to the chromosome III. It cannot discarded that, for example, the Potosí population was the most differentiated because any influence of this nature.

Table 1. Santa Cruz (SC) and Tree Line (TL) rearrangement frequencies and standard deviations in five Colombian *Drosophila pseudoobscura* populations.

	Frequencies	
Populations	SC	TL
TOROBARROSO	0.712 ± 0.027	0.287 ± 0.027
SUSA	0.685 ± 0.026	0.314 ± 0.026
SUTATAUSA	0.721 ± 0.019	0.278 ± 0.025
POTOSI	0.781 ± 0.019	0.218 ± 0.019
SANTILLANA	0.676 ± 0.026	0.323 ± 0.026
TOTAL	0.720 ± 0.010	0.279 ± 0.010

Table 2. Wright F endogamy statistic and variance for each one of the five Colombian *Droosphila pseudoobscura* populations studied. d. f. = degree freedom.

Populations	Wright F	Variance	$\chi 2$	d. f.
TOROBARROSO	0.9649	0.0006	129.413 *	1
SUSA	0.9833	0.0003	144.065*	1
SUTATAUSA	0.9846	0.0002	146.398*	1
POTOSI	0.9366	0.0008	214.061*	1
SANTILLANA	0.9462	0.0007	152.215*	1

*P < 0.0001

The comparison between the results of three of the populations studied here (Torobarroso, Potosí and Santillana) and also analyzed by Cárdenas (1988) shows that the differentiation dynamics are not the same in all of them. The SC frequency range between 0.60 and 0.80 (0.20 and 0.40 for TL) could be basically produced by stabilizing selection (Kimura, 1986). The Torobarroso population did not show significant differences in, at least, the last 220 generations (12 years). That means that the presence of genetic drift in this population has not been important, probably because it has maintained a big effective number in the last 12 years and the selective pressures had been constant. Moreover, during the sampling process this was the population where the capture of *D. pseudoobscura* was easier. On the contrary, the population from Potosí would be the one with the lower effective number, or where the selective pressures has changed more drastically. This fact is reinforced when we compared the results from Ruiz-Garcia and Alvarez (1997), who studied different morphological meristic features in eight Colombian and three American populations. Some of the populations analyzed here were included in that study (Potosí, Susa and Sutatausa) and they showed a very similar relationship in the dendrograms and in a principal coordinate analysis than the one presented here. Potosí was also the most divergent population (see Figure 2). This made us think that some morphological variations that have preferentially diverged in this population could be linked to some rearrangements of the third chromosome. That is, the genes (or polygenes) that codify for this kind of morphological variation (microchetae number in different structures) could be located on the third chromosome (Alvarez and Ruiz-Garcia, 1995).

The morphological, chromosomal, and molecular studies that are being currently made in some Colombian plateau *D. pseudoobscura* populations can profile, with a better precision, some of the evolutionary factors that rule the genetic structure of this insect.

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Molecular characterization of a point mutation in the *Drosophila inscuteable* gene.

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Asymmetric cell divisions play a crucial role in establishing different cell fates in various tissues, reviewed *e.g.* in Chia *et al.* (1997), Knoblich (1997), Lin and Schagat (1997), Jan and Jan (1998), Lu *et al.* (1998), Paululat *et al.* (1999). During embryonic development the specification of muscle progenitors and neuroblasts depends on the asymmetric distribution of intrinsic cell factors (Ruiz Gomez and Bate, 1997; Carmena *et al.*, 1998). Several proteins involved in this process were identified in *Drosophila*, including Inscuteable (Kraut and Campos-Ortega, 1996; Knirr *et al.*, 1997), Numb (Uemura *et al.*, 1989; Rhyu *et al.*, 1994; Knoblich *et al.*, 1995), Prospero (Hirata, *et al.*, 1995; Knoblich, Jan *et al.*, 1995; Spana and Doe, 1995), Miranda (Ikeshima-Kataoka *et al.*, 1997; Shen *et al.*, 1997), Sanpodo (Dye *et al.*, 1998; Park *et al.*, 1998; Skeath and Doe 1998), Bazooka (Kuchinke *et al.*, 1998) and Staufen (Li *et al.*, 1997; Shen *et al.*, 1998). Inscuteable (Insc) is one of the key components responsible for the asymmetric localization and distribution of *e.g.* Numb and contains a number of conserved motifs, some of which were functionally analyzed recently (Tio *et al.*, 1999). *Insc* mutants show severe defects in cell fate determination of muscle progenitors and neuroblasts (Burchard *et al.*, 1995; Kraut and Campos-Ortega, 1996; Kraut *et al.*, 1996; Knirr, Breuer *et al.*, 1997).

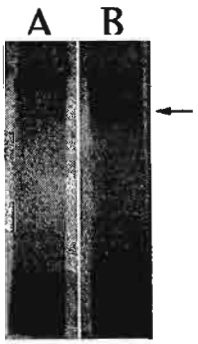

Figure 1. SSCP analysis. PCR amplification products from heterozygous *insc*^{22} flies (A) show conformational changes (arrow) when compared to a control (B, isogenic strain, except for the *insc* locus).

Here we show the molecular analysis of the EMS-induced *insc*^{22} allele (previously described as *nem*^{22}; (Burchard, Paululat *et al.*, 1995) that reveals a C to T transition resulting in a truncated protein of 82 aa, respectively.

For the sequence analysis we isolated genomic DNA from an embryonic collection that was enriched for *insc*^{22} homozygous embryos (heterozygous individuals hatch as larvae and were removed prior to DNA preparation). Based on the known *insc* sequence, primer pairs were designed that allowed the amplification of overlapping fragments of 200 to 300 base pairs in length, encompassing the entire coding region, the second and the third intron (Knirr, Breuer *et al.*, 1997). The first intron of about 10 kb in length and the untranslated regions of *insc* were not tested. PCR amplification products were applied to 10% polyacrylamide gels and used for a single strand conformation analysis (SSCP) (Savov *et al.*, 1992; Hongyo *et al.*, 1993).

A primer pair used for the amplification of 247 bp including the 3'end of the second exon and the 5' end of the second intron generates a fragment that reveals conformational polymorphism in comparison to a wildtype control (Figure 1).

For the sequence analysis, a PCR reaction was performed with the same primers as mentioned above using enriched *insc*^{22} DNA as a template. The amplification products were subcloned into the pPCR-Script vector (Stratagene) and six independent clones were sequenced. Two clones turned out to be wild type while four clones show an identical point mutation.

The sequence analysis of the DNA from homozygous *insc*^{22} embryos revealed a C to T transition 250 bp downstream of the ATG codon of the *insc* cDNA as a result of the EMS mutagenesis (Figure 2A, B).

The CAG in the wild type, respectively the TAG in *insc*^{22}, is the last codon of the second exon. Exon/intron boundaries of the second *insc* intron show highly conserved sequences that match the consensus splicing sites (Sharp, 1987; Mount *et al.*, 1992; Wassarman and Steitz, 1992). Thus 5'splicing of the second *insc* intron occurs downstream of the CAG (Gln) codon (Figure 3). Therefore in the *insc*^{22} allele the C to T

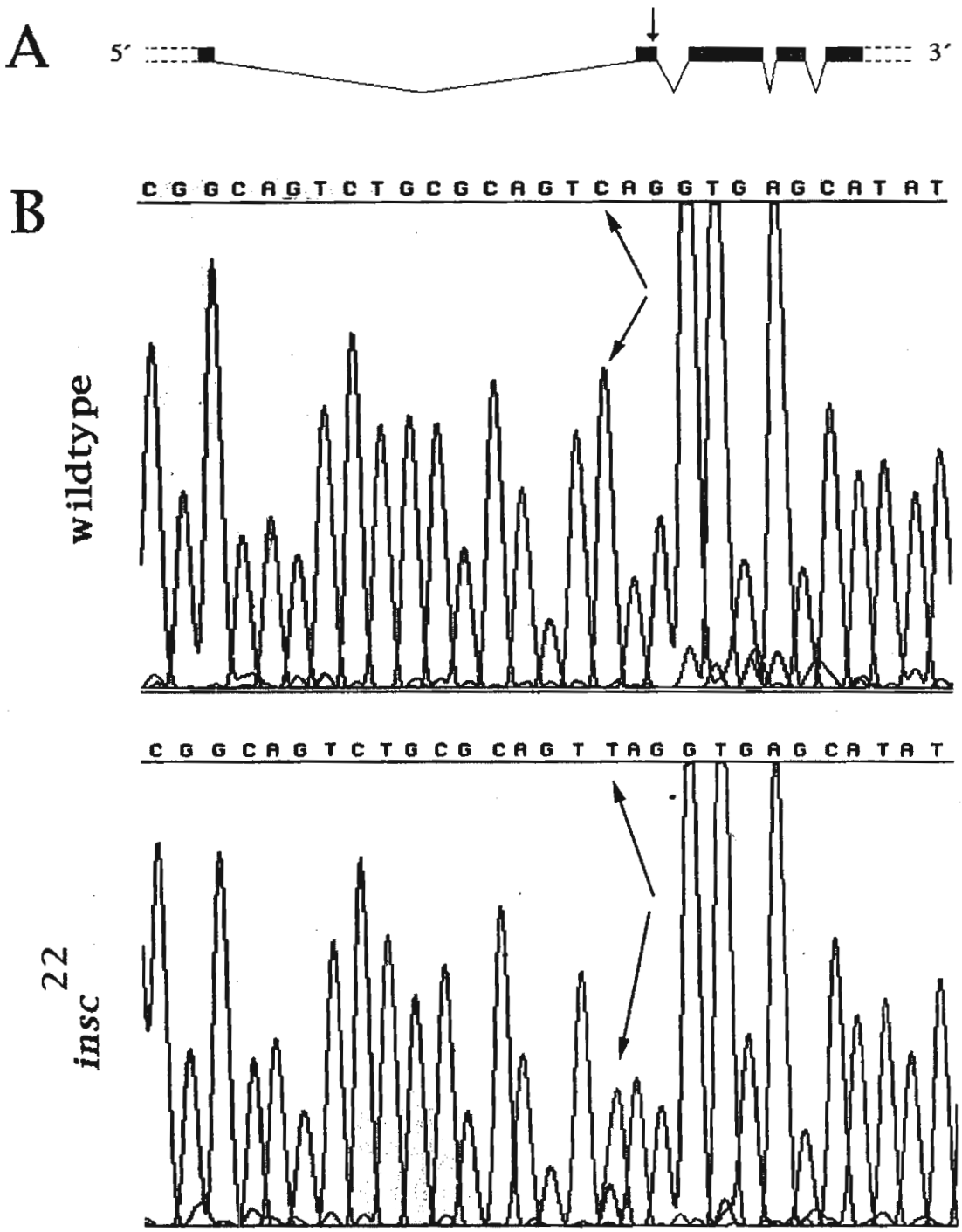

Figure 2. (A) shows the exon/intron structure of the *inscuteable* gene. The point mutation in *insc*^{22} is indicated by an arrow. Sequence analysis (B) of *insc*^{22}-DNA reveals a C to T transition (arrows) when compared to wildtype DNA.

transition in this codon likely creates an artificial stop codon resulting in a truncated Insc protein of 82 amino acids instead of 860 amino acids in the wild type.

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wildtype sequence

CTG	CGC	AGT	CAG	GTGAG -//- TTTG CAG	GAT	TCG
Leu	Arg	Ser	Gln	intron (247 bp)	Asp	Ser

insc^{22} sequence

CTG	CGC	AGT	TAG	GTGAG -//- TTTG CAG	GAT	TCG
Leu	Arg	Ser	**Stop**	intron (247 bp)	Asp	Ser

Figure 3. The second *insc* intron (boxed) shows highly conserved splice site sequences (see text for references). The C to T transition in *insc*^{22} occurred in the last CAG codon of the second exon, coding for Gln.

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Alteration of biogenic amines in *Drosophila virilis* under stress.

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We present here the results of studies of the content of noradrenaline (NA), octopamine (GA), and their precursors tyrosine (Tyr), tyramine (TA) and DL-B-(3,4-dihydroxyphenyl) alanine (DOPA) in individuals of line 101 and 147 of *D. virilis* under normal and stressful conditions, using a simple method based on high-performance liquid chromatography with electrochemical detection that obviates the necessity of previous sample purification. In individuals of the resistant line (101) contents of OA, Tyr, NA, and DOPA were lower than in the flies of the sensitive one (147) under normal conditions. Heat stress caused an increase in the content of GA, Tyr, NA, and DOPA in the resistant line whereas those titers in the sensitive line did not change. The lines did not differ in the level of TA under normal conditions and the amine content did not change under stress.

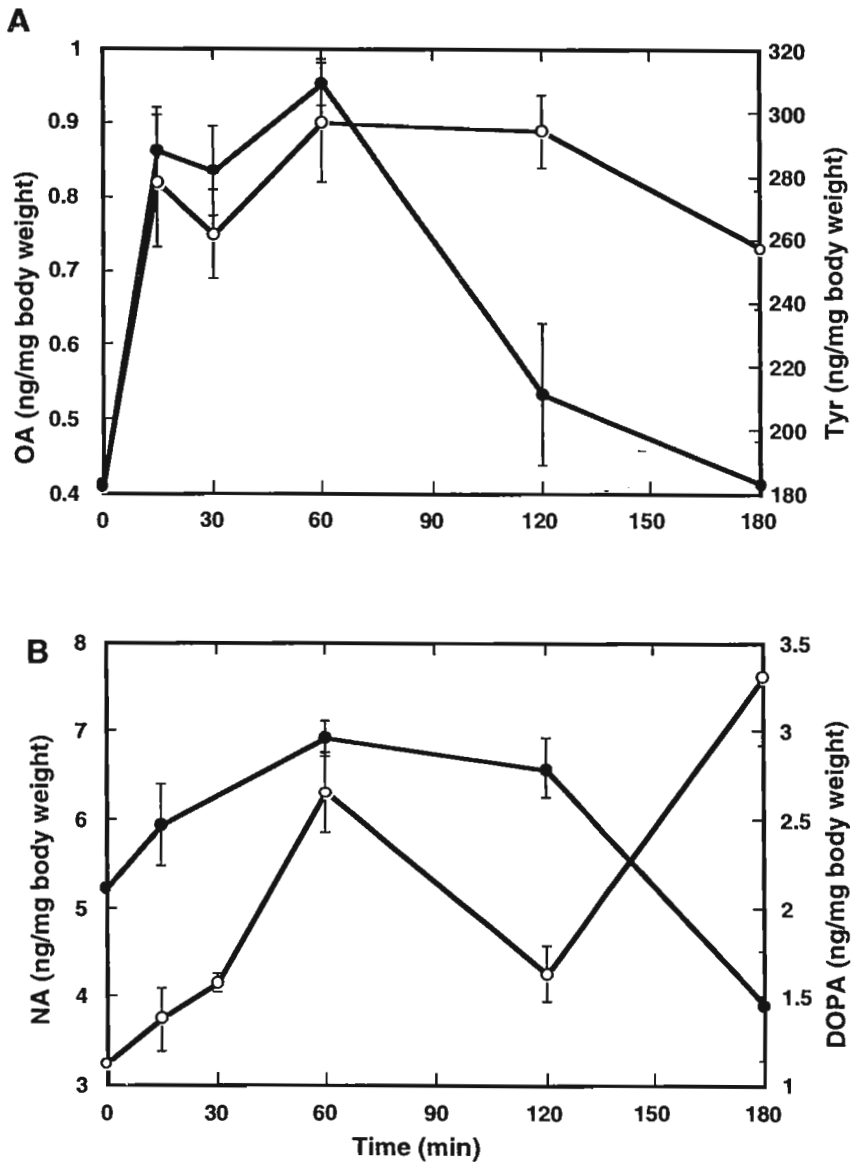

Figure 1. Time course of changes in A) OA (o) and Tyr (•), and B) NA (o) and DOPA (•) content in the *D. virilis* 101 line.

Dynamics of the contents of Tyr, OA, NA, and DOPA in females of line 101 are shown in Figure 1. It is well seen that the contents of Tyr, OA, NA, and DOPA sharply increase already after 15 min at 38°C and start to decrease after 1 h for Tyr and NA, and after 2 h of stressor action for OA and DOPA. DOPA content increases up to 1 h of stressing and after longer stress (2 h) the content of NA rises again.

It has been shown earlier that the activities of the enzymes of biogenic amines synthesis sharply decrease in individuals of the line 101 of *D. virilis* already after 30 min of stressing (Rauschenbach *et al.*, 1995; Sukhanova *et al.*, 1997). It has been also demonstrated that OA is synthesized in the insect's nervous tissue and released from the *corpus cardiacum* and from certain nerve terminals in the flight

muscles (Orchard, 1982) into hemolymph (Davenport and Evans, 1984: Hirashima and Eto, 1993). Bearing in mind these data and present results (the increase of Tyr. OA, NA, and DOPA contents after 15 min of stressor action) the most likely explanation of the observed changes in the levels of biogenic amines and their precursors in *D. virilis* under stress is as follows. A sharp increase in the contents of DA and OA results from their release from depot. The increase of product content may decrease the activity of the synthesizing enzymes, tyrosine hydroxylase (TH) and tyrosine decarboxylase (TDC), via a feedback mechanism, and we have demonstrated precisely this (Rauschenbach *et al.*, 1995: Sukhanova *et al.*, 1997). A sharp decrease in TH and TDC activities may, in turn, lead to the increase of Tyr pool, and this, again, we have precisely shown (Figure 1A). An increase in Tyr content would decrease the activity of alkaline phosphatase (APh), the Tyr-controlling enzyme. Indeed, it has been demonstrated that the activity of APh decreases under stress (Sukhanova *et al.*, 1996).

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Regulation of the expression of tissue-specific esterase in *Drosophila*.

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The esterase genes family was described in *Drosophila virilis* and *D. melanogaster* (Korochkin, 1980). The deduced amino acid sequences of tissue specific esterase S *D. virilis* and esterase 6 *D. melanogaster* reveals 50% homology. The exon-intron structure of both genes is similar (Sergeev *et al.*, 1995). The *est6* gene of *D. melanogaster* has only one promoter while gene *estS* of *D. virilis* has two.

Drosophila melanogaster was transformed with the esteraseS gene from *D. virilis*. This gene is strongly activated in ejaculatory bulbs in mature males of *D. virilis*. The closely related gene from *D. melanogaster* is activated in ejaculatory ducts. A genomic copy of this gene including 400 bp of 5' regulatory region was integrated into the genome of *Drosophila melanogaster* (Figure 1). The tissue- and stage-specific expression of genomic copy of the esterase S gene integrated into the *D. melanogaster* genome is the same as in *D. virilis*. The products of the transferred genes were detected in ejaculatory bulbs of transgenic flies (Korochkin *et al.*, 1995). The results suggest that this specificity is evidently determined by the regulatory region of the esterase S gene and controlled by cis mechanism and at the transcriptional level: *estS* gene of *D. virilis* is transcriptionally active only in ejaculatory bulbs of transgenic flies (Figure 2). "Transgenic product" could be transferred from male ejaculatory bulbs of transgenic flies into female genitals upon copulation, with subsequent degradation there. The using of lacZ reporter gene shows that tissue and stage specificity depends upon a relatively small 5' regulatory region of the esterase S gene. A 396 bp fragment of the regulatory region of the *estS* gene upstream to the major transcription initiation site can switch on the *estS* gene in many places of the developing animal, but DNA upstream from this fragment inhibits this gene activity in all organs excluding the ejaculatory bulb. Removal of different parts in 5' and 3' regions of the *estS* gene shows that deletions in 5' regulatory region of the *estS* gene up to 115 bp to the major transcription initiation site can reduce the transcription *in vitro* four-fold. The DNA fragments with a 5' region containing 830, 750, 450, 390 bp to the major transcription initiation site were also used in such experiments. The efficiency of *in vitro* transcription of these deletion constructions was practically the same. Computer analysis of the region from -390 to -60 to the major transcription initiation site shows that this region contains many sequences similar to

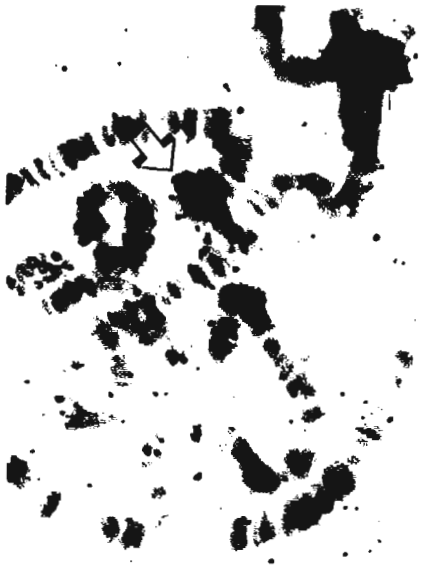

Figure 1. Cytological hybridization with polytene chromosomes from transformed flies using ^{3}H-labeled DNA probe.

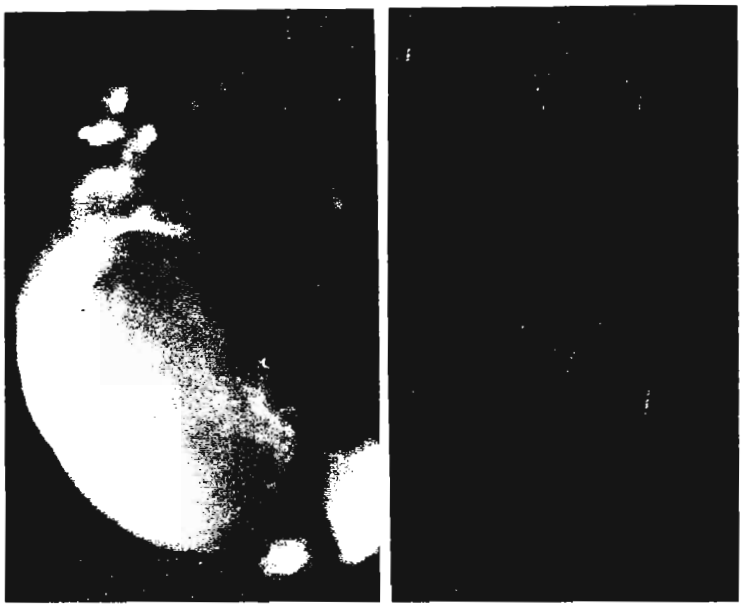

Figure 2. Results of *in situ* hybridization on total preparations of *D. melanogaster* reproductive system with DNA probe containing the *estS* gene of *D. virilis*. In transgenic animals (left) fluorescence is observed only in ejaculatory bulbs. In control (right) fluorescence is absent. Fluorescent method with digoxygenin was used.

the most common enhancer elements of eukaryotic genes. They are important for the high efficiency of *estS* gene *in vitro* transcription. We were interested in characterizing the regulatory elements of the *estS* gene of *D. virilis* which are responsible for its tissue specific expression. To localize these regulatory regions, several deletion constructs of upstream fragment of genomic copy of *estS* were made. One fragment, which includes DNA sequence from -3430 bp to +570 bp relative to the start of transcription, was subcloned first from plasmid pBR322 with previously cloned genomic sequence of *estS* into pBluescript II SK+ for making deletions. Then the obtained DNA fragments of *estS* were subcloned into pCaSper -augbgal for fly

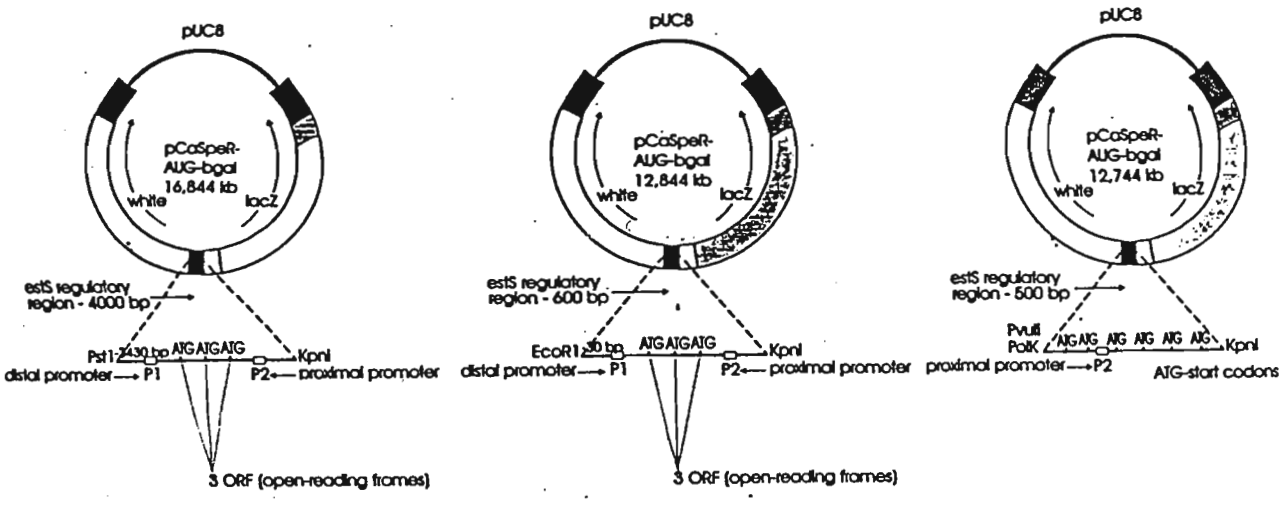

Figure 3. The constructs which were used for the analysis of the function of *estS* regulatory region.

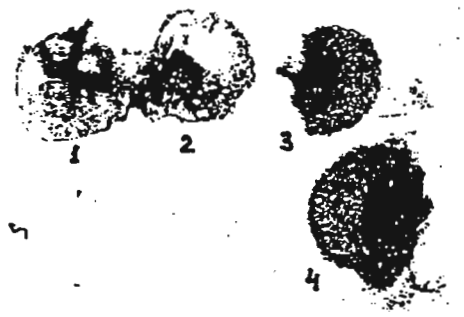

Figure 4. Ejaculatory bulbs of transformed flies. 1,2 – organs were isolated from males containing a small fragment of regulatory region; 3,4 – organs were isolated from males containing a long fragment of regulatory region.

transformation. The first construct contained 4 kb *Hind*III-*Cla*I fragment (from -3430 to +570 bp), the second one – 600 bp sequence (from -30 to +570), and the third one – 500 bp (+500 bp). The first two constructs included both presumed promoters – P1 (distal) and P2 (proximal) – with three small ORFs in between. The third construct included just P2 promoter (Figure 3). Downstream region between the P2 promoter and the *Cla*I site contained 5' universal ATG codons. All plasmid constructs were grown in *E. coli*, isolated by the usual alkaline lysis procedure, and purified by ultracentrifugation in CsCl. Five transgenic fly lines were produced with these constructs. To follow the insertion we used genetic markers *Cy*, *L*, *D*, and *Sb*. The integration of construct into the genome was confirmed first by Southern blotting, and then localized on polytene chromosomes by *in situ* hybridization with [^{3}H]-labeled DNA probe. The construct insertion was mapped to the 3rd chromosome in four transgenic fly lines, and to the X chromosome in one line. Efficiency of transformation was ~ 5% from surviving flies. To characterize specificity and activity of regulatory sequences of interest, the β-gal activity was analyzed in 20-30 flies of both sexes in three independent experiments. The whole flies were punctured with a dissecting needle in regions of the abdomen and thorax and then stained in buffer containing X-gal. The staining of flies transformed with the third construct containing only one promoter revealed β-gal expression in a wide range of organs: in esophagus, intestines, in some parts of muscles, legs, proboscis, and Malpighian tubules. However, the expression in ejaculatory bulbs was law (Figure 4). The second construct with two promoters and short upstream region has also revealed non-specific expression in many organs, though the level of expression was significantly lower than that of the third construct, and weak staining of ejaculatory bulbs was detected.

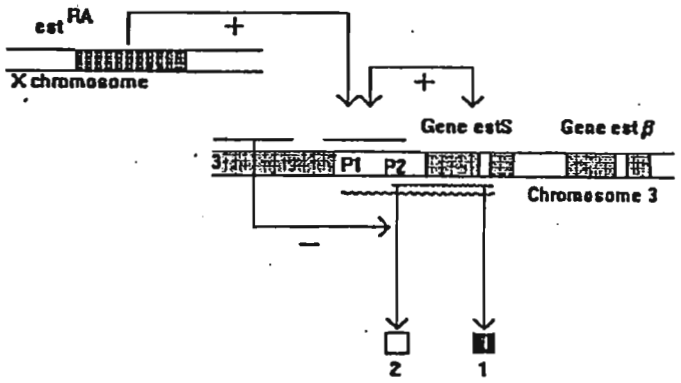

Figure 5. Scheme of the regulation of the tissue-specific expression of *estS* gene in ejaculatory bulbs of *Drosophila virilis*.

The expression level of the first construct with both promoters and big upstream region (3.4 kb) was higher in ejaculatory bulbs than the expression of the second construct (however, not very significantly). The staining of other organs in this case was substantially reduced and varied from weak to non-detectable depending on the animal (Figure 4). Transcription of gene *estS* regulating esterase enzymatic activity is initiated with P1 promoter in different organs including ejaculatory bulbs. There is a regulatory region located upstream of P1 which suppresses *estS* expression everywhere but ejaculatory bulbs. Thus, both positively and negatively regulating elements participate in determining the organ-specific pattern of *estS* gene expression (Figure 5).

References: Korochkin, L., 1980, In: *Isozymes: Current Topics in Biological and Medical Research.* 4: 159-202; Sergeev, S., V. Panin *et al.*, 1995, FEBS-letters 360: 194-196; Korochkin, L., V. Panin, G. Pavlova *et al.*, 1995, 213: 302-310.

The effect of relative humidity and genetic strain on the interaction between *Drosophila hydei* and *D. melanogaster* in continuous culture.

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Introduction

Hodge and Mitchell (1998) demonstrated that atmospheric humidity and the water content of the resource could affect the interaction between *D. melanogaster* and *D. hydei* in single generation laboratory experiments. Previously, Arthur (1986) had shown that the interaction between these two species in continuous culture could be affected by the amount of resource, the mechanism of this effect being linked to smaller quantities of resource having relatively greater surface area to volume ratios and therefore being more prone to desiccation. Thus, the hydrological aspects of the environment appear to have an important role in determining how these flies interact.

The aim of this series of experiments was to further examine the effect of relative humidity on the interaction between *D. hydei* and *D. melanogaster* under conditions of continuous culture. In order to examine how general the effect of humidity is in affecting population performance and interactive outcomes, two different strains of *D. melanogaster* have been used.

Methods

Population cages consisted of clear plastic rectangular boxes (17 × 11 × 6cm) with six glass bottles (30ml) screwed into the underside. The two oldest bottles of medium were replaced each week, so each bottle of medium remained available to the flies for three weeks. Each glass bottle initially contained 1.5g of Instant *Drosophila* Medium (IDM; Blades Biological, Edenbridge, Kent) hydrated with 6ml of distilled water.

All experiments were carried out at 25±1°C and a light regime of 16:8 hours light:dark. Each experiment consisted of monoculture cages for each species and a mixed culture treatment. Populations were initiated using 50 males and 50 females. Cages at high humidity (45-50%) were maintained in an insect room. Cages designated to a low humidity (25-35%) were kept in an incubator, using trays of silica gel to maintain the low humidity. There were at least three replicates of each treatment.

At either 1-week or 2-week intervals, the whole adult population was anaesthetized using carbon dioxide and counted by hand. Every 8 weeks, the bottles of resource and adult flies were transferred to a clean cage. Populations were counted until at least week 12 or until two successive counts contained no adults.

Two different strains of *D. melanogaster* were used: one with a white eye mutation (*w*) and one carrying an ebony body (*e*) genetic marker. Both stocks were obtained from Phillip Harris Educational, Staffordshire, England, in 1993. The *D. hydei* stock was a wild-type strain derived from flies captured in Britain.

If a population became extinct, the equilibrium population size was designated as being zero. To estimate equilibrium values of extant populations, use was made of the negative relationship between growth rate and population size, characteristic of an S-shaped, logistic, growth pattern (Varley *et al.*, 1973). The *per capita* growth (PCG = $(N_{t+1} - N_t) / N_t$) was calculated for every two-week period, corresponding approximately to the generation time of the flies, and plotted against population size (N_t) (or the logarithm of population size, as the relationship was often curvi-linear)(Gilpin and Justice, 1972; Turchin, 1991). The regression equation produced was used to determine the population size at which *per capita* growth was equal to zero, *i.e.*, when the population was at a theoretical equilibrium. In most cases this method worked well, providing sensible values when comparing them to graphical representations of population time series. Data were analyzed using GLM, with relative humidity and presence/absence of the second species as factors.

Results

The average equilibrium population size for *D. hydei* was significantly greater at the higher humidity than at the lower ($P < 0.001$; Table 1), with populations becoming extinct in half of the cages maintained under low humidity conditions. The performance of *D. hydei* was not affected by either strain of *D. melanogaster* at either of the humidities used.

Table 1. Equilibrium population levels for *D. hydei*, *D. melanogaster w* and *D. melanogaster e*. (mean ± SE). (*- indicates statistically significant difference from appropriate monoculture.)

	D. hydei			*D. mel w*		*D. mel e*	
Humidity	mono	mix (w)	mix (e)	mono	mix	mono	mix
Low	90 ± 54	0 ± 0	99 ± 96	230 ± 42	0 ± 0*	317 ± 161	473 ± 84
High	313 ± 41	263 ± 40	274 ± 47	164 ± 14	1 ± 1*	326 ± 7	0 ± 0*

Table 2. Summary of the interactions between different strains of *D. melanogaster* and *D. hydei* at different atmospheric humidities. (The symbol on the right represents the effect of *D. hydei* on *D. melanogaster*.)

		Strain of *D. melanogaster*	
		white eye	ebony body
Humidity	Low (25-30%)	(0, -)	(0, 0)
	High (45 - 50%)	(0, -)	(0, -)

D. melanogaster w performed similarly at each humidity (Table 1). *D. hydei* caused a significant reduction in *D. melanogaster w* population size at both humidities ($P < 0.001$), with *D. melanogaster w* being excluded in all but one mixed culture cage.

The equilibrium population size of *D. melanogaster e* responded significantly to the interaction between relative humidity and the presence of *D. hydei* ($P < 0.02$; Table 1). At low humidity, *D. melanogaster e* was able to tolerate the presence of *D. hydei* and showed no difference in equilibrium population size. However, at high humidity the population size of *D. melanogaster e* was significantly reduced when *D. hydei* was present.

The pairwise interactions between *D. hydei* and *D. melanogaster* are summarized in Table 2. The interactions were predominantly amensal, with *D. hydei* being the dominant species. However, the interaction between *D. hydei* and *D. melanogaster e* at low humidity was neutral, the *D. melanogaster e* being able to coexist with *D. hydei* under these conditions. This implies that the interaction may or may not be perceived to change under different atmospheric conditions, depending on which strain of *D. melanogaster* is considered. Similarly, genetic strain may or may not influence the interactive outcome, dependent upon the humidity at which the experiment is performed.

Discussion

Relative humidity affects the interaction between *D. hydei* and *D. melanogaster* primarily through alterations in the performance of *D. hydei* (see Hodge, 1995; Hodge and Mitchell, 1998). The extinction of *D. hydei* in some low humidity cages was due to failure of the larvae rather than desiccation stress in the adult flies. Larval survival in *D. hydei* drops off rapidly if the resource is too dry (Hodge and Wilson, 1997) and resources which tend to dehydrate quickly can do so before the slow-developing *D. hydei* larvae complete their development (Arthur, 1986). At high humidity the resource remains fluid long enough to enable *D. hydei* larvae to grow and pupate. The rate of desiccation of the medium is an important factor in the success of *D. hydei* populations, both in monoculture and by affecting its competitive prowess in mixed cultures.

Differences found in the results of different experimenters working with *Drosophila* have sometimes been attributed to differences in the strain of flies used (*e.g.*, Robertson and Sang, 1944) and *D. melanogaster* is known to possess different intra- and inter-strain competitive abilities (Lewontin, 1955; Bakker, 1961). Strains where adult flies have more pigmentation have been suggested as being more tolerant of dry atmospheres than paler flies (Kalmus, 1941) and this may explain why the white eye strain of *D. melanogaster* was unable to coexist with *D. hydei* in mixed cages at low humidity whereas the ebony body strain of *D. melanogaster* was able to persist.

The results of this experiment highlight once more that the environmental conditions under which populations are maintained in the laboratory can influence how they will interact in mixed cultures. The results also reinforce the need to take into account the genetic makeup of the populations used when considering the general applicability of results to other laboratory investigation and, ultimately, to a field situation.

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The number and distribution of eggs laid by *D. melanogaster* and *D. hydei* is not influenced by the presence of the other species.

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Introduction

In nature, the interspecific interactions which take place within guilds of drosophilids are thought to be moderated by chance avoidance of species due to the independent aggregation of eggs between patches of resource (*e.g.*, Atkinson and Shorrocks, 1984). *D. melanogaster* and *D. hydei* have been found to interact in various ways when maintained in laboratory systems (*e.g.*, Arthur, 1986; Hodge and Mitchell, 1998; Hodge, 1999) but these previous experiments have generally been in the form of single generation experiments in glass tubes or have examined populations maintained in restricted culture cages. In the field, interactions between larvae may be abated or accentuated if females of one species tend to avoid or select sites occupied by the eggs of another. However, it is believed that the aggregated distribution of eggs produced by drosophilid females is produced independently of other species present in the guild (Rosewell *et al.*, 1990; Shorrocks *et al.*, 1990). This experiment examined how *D. melanogaster* and *D. hydei* interact, with respect to the number, distribution and success of eggs laid over an array of resource patches.

Methods

Experiments were carried out in 1.0m × 0.5m × 0.5m nylon mesh cages, maintained in an insect room at a temperature of 25°C, a light:dark cycle of 16:8 hours and a relative humidity of 35-45%. A 6 × 8 grid of small glass pots (30mm in diameter) containing food medium was placed in the centre of the cages on a plastic tray 35cm × 45cm. This gave a resource density of approximately 305 patches per m^2. Each pot contained 1.5g of Instant *Drosophila* Medium (IDM; Blades Biological, Edenbridge, Kent, UK) hydrated with 6.0ml of distilled water.

The populations used in the experiment were a white eye mutation of *D. melanogaster* Meigen and a wild-type strain of *D. hydei* Sturtevant. Single species cages were set up using 250 male and 250 female flies. Mixed culture cages used 250 male and 250 female flies of each species. Four replicates of each species and of the mixed culture treatment were set up.

The glass pots were removed from the cages after 18 hours, so the eggs could be counted before those of *D. melanogaster* started to hatch (the eggs of *D. melanogaster* and *D. hydei* are easily distinguished by the number of filaments). The degree of aggregation of eggs was estimated by calculating the variance / mean ratio and a value of k for a negative binomial distribution. The goodness-of-fit of the data to a negative binomial distribution was confirmed using a G-test.

The pots were then placed into individual plastic cups (9cm tall; 6cm diameter) with plastic screw lids. For ventilation, nine holes (4mm diameter) had been placed into the lids and then covered with nylon mesh. These cups were placed in an incubator set at 25°C, 16:8 hour light:dark cycle and a relative humidity of 35-45%. The cups were checked daily and any emerged adult flies removed.

Results

The values for mean number of eggs per pot, the variance / mean ratio, the value of *k* for the distribution of eggs, the proportion of patches containing eggs and the hatch-rate of the eggs showed no difference between mixed species cultures compared to those in the appropriate single species culture (Table 1; $P > 0.05$, ANOVA and Mann-Whitney tests). This indicates there was no effect of either species on the egg laying behaviour of the other.

Table 1. Summary of egg laying performance in single and mixed species cultures (mean ± SE; N = 4)

	D. mel	*D. mel* mixed	*D. hydei*	*D. hydei* mixed
Eggs per pot	15.9 ± 3.3	19.4 ± 5.3	14.8 ± 5.4	24.2 ± 8.6
variance / mean	24.1 ± 8.4	20.9 ± 7.7	41.1 ± 4.1	42.7 ± 15.5
k of egg distribution	0.573 ± 0.047	0.673 ± 0.223	0.137 ± 0.054	0.239 ± 0.141
Occupied patches (%)	79.7 ± 1.3	75.0 ± 10.9	37.5 ± 9.2	45.8 ± 15.8
Egg hatch rate (%)	57.55 ± 5.82	58.53 ± 5.25	33.63 ± 9.44	39.70 ± 10.60

To assess whether there was any association between the species in mixed cultures, χ^2 tests were used to examine the presence or absence of eggs within each patch (Table 2). No significant negative or positive associations between the eggs of the two species were found.

Table 2. Association of *D. hydei* and *D. melanogaster* eggs in mixed culture cages.

	Number of patches with eggs of:					
	D. mel only	*D. hyd* only	Both species	Neither species	χ 2	P
Replicate 1	19	1	2	26	0.682	>0.30
Replicate 2	18	4	19	7	0.762	>0.30
Replicate 3	21	2	21	4	0.584	>0.30
Replicate 4	8	3	36	1	0.180	>0.50

Discussion

Although the larvae of these two species interact in various ways and with varying intensity when restricted to smaller, less complex, laboratory environments, in this experiment the adults did not modify any of the parameters measured in the other species' performance. Even at these artificially high densities of adults flies, the pattern of egg laying of one species was not affected by the presence of the other. The eggs of both species tended to be highly aggregated and showed no association with those of the other species. These results agree with similar studies investigating the distribution and independence of egg laying in *Drosophila* (Rosewell *et al.*, 1990; Shorrocks *et al.*, 1990; but see Worthen and McGuire, 1988).

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Is *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) currently colonizing the Neotropical region?

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On March, 20th, 1999, while walking in an orchard belonging to a ranch named *Chácara Santa Mônica*, which stands 11 km NE of Santa Isabel, in the metropolitan area of São Paulo City, state of São Paulo, Brazil, I spotted some overripe and partially eaten persimmon fruits still attached to a persimmon tree (*Diospyrus kaki* L.; Ebenaceae). Inside a large hole of one fruit, about half of its diameter and probably made by birds, I noticed, among some large beetles and wasps, several small flies moving their probosces back and forth very rapidly, apparently devouring the wet pulp. Two or three of these flies especially attracted my attention for they had a pair of white and conspicuous stripes along the submedian area of the dorsal surface of the head and thorax, an unnusual feature for the neotropic drosophilids. Immediately, I remembered that only

in the museum's collections I had seen similar specimens, which I supposed, at first sight, to belong to a species of the genus *Zaprionus*, described in 1902 (cited in several papers as 1901) by Coquillett. Unfortunately, I had no vials and entomological net, so I was unable to capture the flies.

One week later, on March 27th, early in the morning, I returned to the same place with the collecting kit. However, there were no fruits of a similar stage of ripening at a workable height. Therefore, I looked on the ground for the remains of some still wet, fallen persimmon fruits and I could see, among several drosophilids, some white-striped flies, apparently feeding and also performing courtship movements.

Among one hundred and sixty-eight drosophilids aspirated (Table 1) on three fragmented fruits, from 10 to 11 a.m., I identified sixty-six (forty-five males and twenty-one females) specimens as belonging to *Zaprionus indianus* Gupta, 1970, a species of Afrotropical origin, which is currently widespread in three other biogeographical regions. One male of the latter flies was dissected and its terminalia compared with the drawings presented by Tsacas (1980) for the five African sibling species belonging to the *Z. vittiger* complex, which was later diagnosed and renamed as *Z. vittiger* subgroup (currently comprising 12 species) by Chassagnard and Tsacas (1993). Complex nomenclatural problems involving the several binomials under which this widespread biological species, currently recognized as *Zaprionus indianus*, has been identified were the subject of a paper by Tsacas (1985). The genus *Zaprionus*, which currently comprises 52 described species (Chassagnard and Kraaijeveld, 1991; Chassagnard and Tsacas, 1993) inhabiting the Australian, Afrotropical, Oriental and Palearctic regions, and the species *Zaprionus indianus* are recorded for the first time from the American continent. In an attempt to produce isofemale lines, twenty out of the 21 aspirated females were kept, each with a male, individually in vials with banana-agar medium, under controlled temperature (22 ± 1°C), and all of them yielded offspring. After the third day, the larvae were given, once a day, fresh Baker's yeast and *ca.* 18 days later the imagines of the first generation emerged in large numbers.

To check if the flies were also using persimmon fruits as breeding sites, fragments of five wet fallen fruits were also collected on March, 27th, and kept individually in ¼ liter vials containing a layer of wet sand, under controlled temperature (22 ± 1°C) and photoperiod (13 h : 11 h; L : D). The emerged flies were daily aspirated and are also listed in Table 1. A total of 399 imagines emerged, of which 44.1 % belong to *Zaprionus indianus* and 45.1% to *Drosophila malerkotliana*. It should be pointed out that both drosophilid species are introduced to the neotropics and together they represented most (89.2%) of the flies that emerged from the fruits of persimmon tree, also an introduced species. Voucher specimens of the isofemale lines and of both aspirated and emerged flies will be housed in the *Museu de Zoologia, Universidade de São Paulo,* São Paulo City, Brazil.

As far as I know, no strain of any species belonging to the genus *Zaprionus* has ever been maintained, either in the *Laboratório de drosofilídeos, Departamento de Biologia, Universidade de São Paulo*, São Paulo City, since its establishment in 1943 under the leadership of Prof. Th. Dobzhansky, or in any of the several laboratories dedicated to the study of drosophilids that had been set up subsequently in other cities of the state of São Paulo and in other states of Brazil. The collections made since 1943 in several localities of the country apparently have never sampled any specimen of any species belonging to the genus *Zaprionus*. This fact seems to indicate that this afrotropical species has very recently been introduced in Brazil. Whether or not it will be able to spread its distribution through the several ecosystems of the American continent in competition with the local species is unpredictable.

It should be pointed out that strains of many species of *Zaprionus* had been maintained for decades in the *Drosophila* Species Resource Center at Austin, Texas, USA, which is currently at Bowling Green, Ohio. So, there is a remote possibility that specimens could have escaped from the laboratory in Austin, reached the wild, spread southwards through Mexico and Central America and finally reached South America. If this was the case, the species would likely have been collected somewhere on its route southwards.

Another hypothesis is that it could have been introduced into the state of São Paulo, directly from some area out of the three biogeographical regions where it currently is a common species. As the surveyed orchard and the International Airport of São Paulo at Guarulhos stand, in straight line, only *ca.* 33 km apart from each other, there is a great possibility that the flies could inadvertently have been introduced by means of airplanes, either as adults, due to the increasingly number of flights from several African and Asian countries to São Paulo City, or as larvae and/or eggs eventually present in meals (fruits for instance) offered to

passengers. If this was the case, the African *Zaprionus indianus* could currently be gradually spreading its geographical distribution, in the Brazilian territory, from the putative area of introduction.

Table 1. Imagines of drosophilids aspirated from overripe, fallen persimmon fruits, on March 27th, 1999 at *Chácara Santa Mônica*, 11 km NE of Santa Isabel, state of São Paulo, Brazil or emerged from the remains of five of them collected on the same day on the ground and brought to the laboratory

Species	aspirated		emerged	
	Males	Females	Males	Females
Drosophila bandeirantorum	1	0	0	0
Drosophila caponei	0	2	0	0
Drosophila capricorni	1	0	0	0
Drosophila malerkotliana	15	8	90	90
Drosophila medioimpressa ?	0	2	0	0
Drosophila pallidipennis	1	1	1	0
Drosophila paulistorum	4	3	0	0
Drosophila polymorpha	1	1	1	1
Drosophila simulans	1	1	11	11
Drosophila sturtevanti	5	2	0	0
Drosophila willistoni	28	25	10	7
Drosophila zottii	0	0	0	1
Zaprionus indianus	45	21	82	94
TOTAL	102	66	195	204

It should be pointed out that on April, 10th I noticed, at the same surveyed ranch cited above, several white-striped imagines (not collected) probably belonging to *Z. indianus* feeding on a perforated overripe orange fruit, which was still attached to an orange tree (*Citrus sinensis* (L.) Osbeck; Rutaceae), thus indicating that the introduced flies are not exclusively associated to the persimmon fruits in that area. It's worthwhile to note that Lachaise and Tsacas (1984) reported 73 kinds of fruits belonging to 31 families of plants, both native and introduced to Africa, as breeding sites for the fruit breeder *Z. indianus*.

According to Tsacas (1985) and Chassagnard and Kraaijeveld (1991), *Zaprionus indianus* is an invading fly of Afrotropical origin, where it is the most common species of its genus, and is currently widespread in India and several islands of the Indian Ocean. It should be pointed out that it was also reported to occur in the Atlantic Saint Helena Island, which stands midway between Africa and meridional South America. Therefore, this island could also have been a step in its movements, probably mediated by humans beings, from one or several African countries to Brazil.

Being a invading and generalist species regarding its breeding sites (Chassagnard and Kraaijeveld, 1991), it is likely that given opportunities it could become, very rapidly, a semicosmopolitan species widespread in the tropical zone of the world. The monitoring of its route in the Neotropical region, including the Caribbean islands, and even in the southern Nearctic region is desirable. It would also be interesting to check if it will be able to breed in native and not commercialized neotropical fruits and to analyze the results of its likely competition with the local species.

Acknowledgments: I would like to thank Dr. Ann J. Stocker for her comments on the manuscript. I am also indebted to FAPESP, CAPES, CNPq and FINEP (66.93.0618.00) for financial support.

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New insertions of the A^R4-24 element with variegated *white* gene expression.

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As a rule position effect variegation (PEV) is a consequence of the placement of a euchromatic gene into vicinity of the centromeric heterochromatin. With such rearrangements, the undamaged translocated locus is inactivated in some, but not all cells, thereby giving rise to a mosaic phenotype. Euchromatic genes show PEV when moved into the heterochromatin not only by chromosomal rearrangements but when inserted into the heterochromatin by P element transposition (for review see Zhimulev, 1997). The expression of the *Drosophila white* gene is necessary for deposition of pigments in the eye, the ocelli, the larval and adult Malpighian tubules, and the adult testes. In the case of the eye, the gene is expressed in all ommatidia, resulting in uniform pigmentation of the eye as a whole. Since *white* is expressed cell-autonomously, it can serve as a reporter gene for the detection of the genomic position effects. For induction of position effect variegation of the *white* gene we induced transposition of the A^R4-24 *P[white, rosy]* element, contained *white* and *rosy* genes, from the 24D1-2 region of the polytene chromosome, using *delta2-3* P element (Robertson *et al.*, 1988). The phenotype A^R4-24 insertion (Figure 1) suggests that *white* gene expression is being repressed in

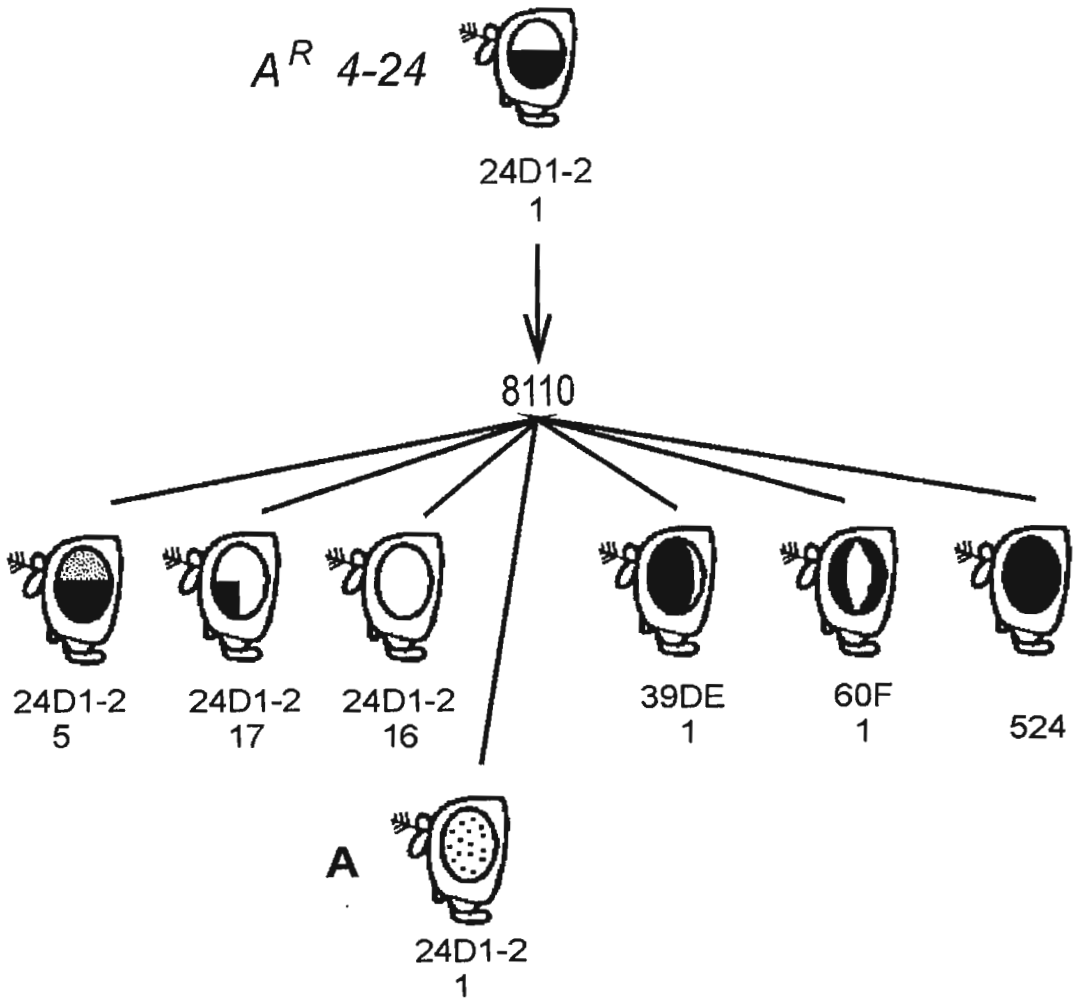

Figure 1. Phenotypes of eyes result from retransposition of the A^R4-24. The cytological position and the number of the lines are shown under the pigment patterns. The mosaic line A was used for generating new transpositions.

Table 1. List of mosaic insertions of the transposon $A^R4\text{-}24$.

Line number	Localization	Comments
A	second chromosome	"salt and pepper"
1	"	"
2	"	"
3	40C	close to wild type
4	second chromosome	"salt and pepper"
5	"	"
6	"	close to wild type
8	"	"salt and pepper"
9	"	"salt and pepper"
11	"	close to wild type
12	"	"salt and pepper"
13	Y-chromosome	"colored sectors"
1-14	tip of the IV-chromosome	"salt and pepper"
15	base of 2L-chromosome	"
16	25F	"
18	102AB	"
19	second chromosome	"salt and pepper" one or two colored facets per eye
20	"	"salt and pepper"
21	"	only ocelli are colored
22	third chromosome	only ocelli are colored
24	second chromosome	"salt and pepper"
25	"	"
26	"	"salt and pepper" one or two colored facets per eye
27	"	"
28	Y-chromosome	colored sectors
1-D	second chromosome	"salt and pepper"
1-B	"	"
29-2	"	"salt and pepper" one or two colored facets per eye
29	Y-chromosome	colored sectors
31	second chromosome	"salt and pepper"
34	second chromosome	close to wild type
35	"	"salt and pepper" one or two colored facets per eye
36	"	"salt and pepper"
38	tip of 2R-chromosome	only ocelli are colored
39	second chromosome	"salt and pepper"
41	"	"
42	"	"
44	"	"salt and pepper", lethal
45	"	closed to wild type
47	"	"
49	"	"salt and pepper", lethal
51	"	close to wild type
52	"	"salt and pepper"
53	40DF	close to wild type
55	75D	"salt and pepper"
56	39A	"
60	second chromosome	"
61	41AB	"
63	second chromosome	"
XX	41F1-2	close to wild type
65	second chromosome	"salt and pepper"
66	"	only ocelli are colored
70	tip of 2R-chromosome	"
71	second chromosome	"salt and pepper"
2-A	"	"
1-F	102A	close to wild type

Figure 2. "Salt and pepper" pigment pattern of the $A^R4\text{-}24$ in the 24D1-2 region (line A).

the dorsal part of the eye rather than being activated in the ventral eye, since the $A^R4\text{-}24$ element contains *white* regulatory sequences rendering it competent to be expressed in all regions of the eye (Levis *et al.*, 1985). This unique dorso–ventral pattern of the *white* expression depends on pairing of a flanking DNA regulatory elements (Hazelrigg and Petersen, 1992), so any removing the $A^R4\text{-}24$ from flanking DNA should change pattern of the eye pigmentation.

To generate transpositions, *CyO/Sp;*ry^{506}*P[*ry^+*delta2-3]*99B,*Sb/TM6* males were crossed to homozygous w^{1118}, $A^R4\text{-}24$ females. Single male progeny of the genotype w^{1118}*/Y;* A^R4*-24/CyO;* ry^{506}*P[*ry^+*delta2-3]*99B, *Sb/+* was then crossed to w^{1118}/w^{1118} virgin females and mosaic non *Cy* and *Sb* males were selected. These males were crossed singly to w^{1118}/w^{1118}*;CyO/+* virgin females and then homozygous stocks were set up by crossing *Curly* progeny. 524 wild-type revertants were isolated from 8110 screened males. 40 extreme derivatives of $A^R4\text{-}24$ were also isolated. They were similar to those obtained by X-ray mutagenesis of the $A^R4\text{-}24$ chromosome (Hazelrigg and Petersen, 1992). All extreme derivatives had reduced pigmentation of the eye in ventral regions as well as dorsally. Most of them were localized in the polytene band 24D1-2 (Figure 1) as a parental insertion $A^R4\text{-}24$ and demonstrated pairing-

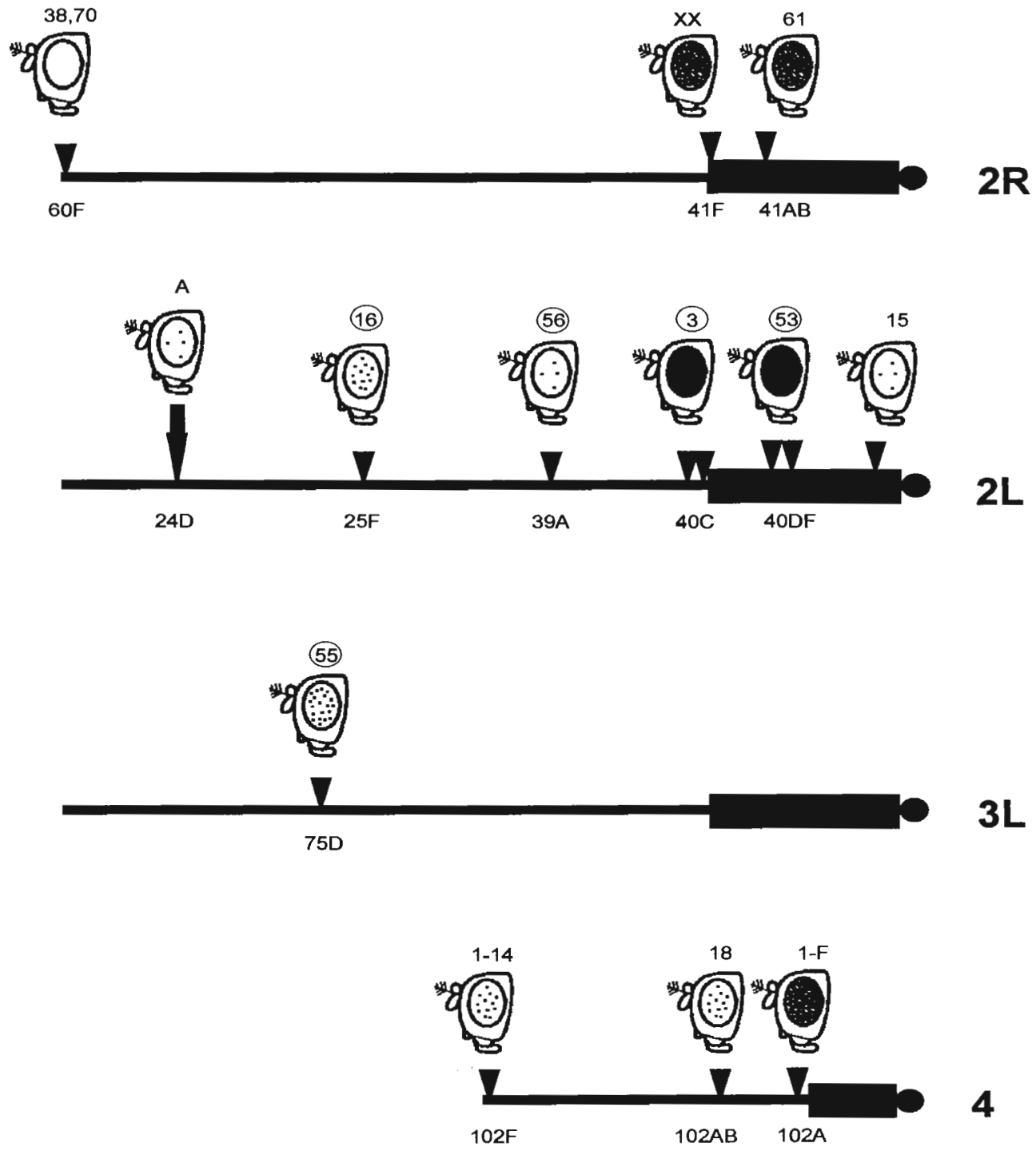

Figure 3. The scheme of *in situ* localization some of the A^R*4-24* derivatives. The euchromatic portions of the chromosome arms are represented by horizontal lines, the heterochromatic - black bars. New sites are marked along the lines with black triangles. The cytological position of each insertion is noted under chromosomes. The original location of the A^R*4-24* is indicated by the heavy arrow. The pigment patterns and line numbers are shown above the chromosomes. Encircled lines contain more than one insertion.

dependence *white* expression as was described by Hazelrigg and Petersen (1992). So, we concluded that the same regulatory flanking elements could be responsible for *white* repression in our case. The only new insertion (A) had the classical "salt and pepper" phenotype (Figure 2), modified by lower temperature and removing Y chromosome such as a classical heterochromatin-induced PEV. Its cytological position is 24D1-2. To be sure that "salt and pepper" phenotype in 24D1-2 region is not a result of *white* gene damage or mutation we have generated new transpositions of the A^R*4-24* element from 24D1-2, using this mosaic line, by analogy with outline described before. Relocating the gene should result in a wild-type eye color at most new positions if its mutant phenotype is due to a position effect, but not if it is due to a mutation intrinsic to the gene. 44280 males were screened, 2323 wild-type revertants, 2 stable repression of *white* expression were registered and 54 "salt and pepper" lines with different extent of mosaicism were isolated (Table 1). Thus, position effect in the 24D1-2 regions is very probably caused by adjacent to A^R*4-24* element genomic DNA, since transposon is placed in a distance of 15 cytological division from nearest heterochromatin and there are no visible reasons for *cis-* or *trans-* interactions (Figure 3).

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Proportions of *Drosophila melanogaster* and *D. simulans* in eastern Australian populations.

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Conventional wisdom among drosophilists states that typical relative abundances of *Drosophila melanogaster* and *D. simulans* vary with latitude and with indoor vs outdoor feeding/breeding site, as well as with season and temperature. In March and April of 1997, I collected *Drosophila* flies at 40 localities near the coast of eastern Australia, in New South Wales, Victoria and Tasmania. Data were recorded on the numbers of both sexes of *D. melanogaster* and *D. simulans* captured at each site, and on characteristics of the site itself. The data and some analyses are presented here to contribute to the literature on the ecology of these species.

The collections were intended to sample *D. melanogaster* populations in order to determine the state of the previously described clinal pattern in P-M hybrid dysgenesis (Boussy, 1987; Boussy and Kidwell, 1987; Boussy *et al.*, 1988). Latitudes of collection sites were determined using a hand-held global positioning satellite receiver (Garmin GPS XL 45). Flies were collected by sweeping at sites attractive to *Drosophila* flies (*e.g.*, discarded fruit in orchards or vineyards, waste bins in fruit-processing sheds, displays and discard bins in fruit and vegetable shops, pomace heaps at wineries, a home compost heap), or from buckets containing chopped bananas and live yeast as bait. I used an insect net with a tubular trap in the bottom ("Drosophila Net," Wards, cat. No. 10W0495; modified by stitching the trap smaller to snugly fit a 25 mm vial). The net was fitted to a standard insect net frame ("Collapsible Net," BioQuip, cat. No. 7115CP), used with a 24 inch extension handle. Flies were swept over sites or bait buckets, and collected from the net into 25 mm vials containing previously prepared standard yeast-agar-treacle-maize meal *Drosophila* food. The vials were kept cool until sorting (within a few hours) in a *ca.* 0.1 m^3 styrofoam container with ice or cold water in containers. Flies were anesthetized with ether and

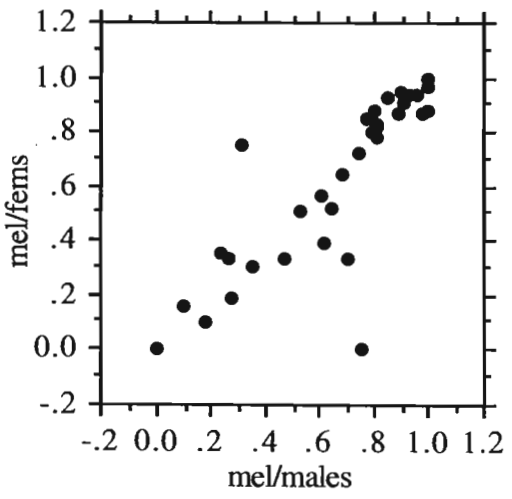

Figure 1. Proportion of *D. melanogaster* in samples of males *vs.* samples of females.

Table 1. *D. melanogaster* and *D. simulans* in eastern Australian collections.

Collection locality	in/out	°SLat	mel males	sim males	mel females	sim females	mel/ males	mel/ females	mel/ both
Coffs Harbour, NSW	o	30.244	54	48	29	28	.529	.509	.522
Nambucca Heads, NSW	i	30.641	43	10	18	4	.811	.818	.813
Kempsey Farmers' Market, NSW	i	31.081	15	4	16	4	.789	.800	.795
Hasting River Winery, NSW	o	31.300	57	102	40	91	.358	.305	.334
Wauchope, NSW	o	31.536	23	205	33	179	.101	.156	.127
Laurieton, NSW	i	31.648	60	14	45	9	.811	.833	.820
Taree, NSW	i/o	31.913	9	5	12	11	.643	.522	.568
Forster, NSW	i	32.179	54	16	41	7	.771	.854	.805
Wootton, NSW	o	32.263	•	•	400	44	•	.901	.901
Bennett's Green, NSW	i	32.998	56	13	40	11	.812	.784	.800
Kanwal, NSW	i	33.264	28	32	11	22	.467	.333	.419
Berkeley Vale, NSW	o	33.325	•	•	73	3	•	.961	.961
Woy Woy, NSW	i	33.488	31	20	16	12	.608	.571	.595
Glebe, NSW	o	33.875	•	•	3	67	•	.043	.043
Berry, NSW	i	34.775	36	4	36	2	.900	.947	.923
Bomaderry, NSW	o	34.846	9	42	4	35	.176	.103	.144
Ulladulla, NSW	i	35.364	57	7	13	2	.891	.867	.886
Batehaven, NSW	i	35.733	23	0	8	0	1.000	1.000	1.000
Malua Bay, NSW	i	35.792	16	10	20	31	.615	.392	.468
Moruya, NSW	i	35.910	11	2	12	1	.846	.923	.885
Tuross Head, NSW	i	36.060	23	8	16	6	.742	.727	.736
Nageela Orchard (in), NSW	i	36.160	23	59	9	38	.280	.191	.248
Nageela Orchard (out), NSW	o	36.160	26	12	25	14	.684	.641	.662
Narooma, NSW	i	36.214	8	0	6	0	1.000	1.000	1.000
Fairhaven, NSW	o	36.389	1	180	1	190	.006	.005	.005
Cobargo, NSW	o	36.427	0	0	2	2	•	.500	.500
Bega, NSW	i	36.676	44	0	34	1	1.000	.971	.987
Tathra, NSW	i	36.730	7	3	1	2	.700	.333	.615
Merimbula, NSW	i	36.889	12	0	7	1	1.000	.875	.950
Pambula, NSW	o	36.931	5	16	6	11	.238	.353	.289
Eden, NSW	i	37.065	33	8	15	2	.805	.882	.828
Nicholson River, Vic	o	37.796	29	79	30	60	.269	.333	.298
Johnsonville, Vic	i	37.816	27	2	29	2	.931	.935	.933
Bunyip, Vic	i	38.076	21	1	15	1	.955	.938	.947
Grovedale, Vic	i/o	38.207	3	1	0	2	.750	.000	.500
Lorne, Vic	o	38.534	5	11	6	2	.313	.750	.458
Rosevear, Tas	i	41.350	3	87	•	•	.033	•	.033
Hillwood, Tas	i	41.244	30	3	29	3	.909	.906	.908
Grove, Tas	i	42.989	100	0	22	0	1.000	1.000	1.000
Trial Bay Orchards, Tas	i	43.138	44	1	27	4	.978	.871	.934
Cygnet, Tas	i	43.161	29	0	33	0	1.000	1.000	1.000

NSW: New South Wales; Vic: Victoria; Tas: Tasmania; in/out: inside a building or shelter (i), or outside (o), or mixed (i/o); °Slat: degrees south latitude. mel males: number of *D. melanogaster* males; sim males: number of *D. simulans* males; mel females: number of *D. melanogaster* females; sim females: number of *D. simulans* females; mel/males: proportion of *D. melanogaster* among males; mel/females: proportion of *D. melanogaster* among females; mel/both: proportion of *D. melanogaster* among both sexes (pooled).

sorted under a stereo microscope to discard species other than *D. melanogaster*. Sites with fewer flies received much more collection effort in order to collect reasonable numbers of flies, so the data cannot be used to infer latitudinal or other patterns of absolute abundances. Some collections were made at outdoor sites, whereas others were made inside open buildings or sheds. Since *D. simulans* is thought to not enter buildings, sites were documented as indoors (i) or outdoors (o) or a mix (i/o) to determine if the species' proportions differed between these categories.

Counts were kept of *D. melanogaster* and *D. simulans* male and female numbers. The males of *D. melanogaster* and *D. simulans* can be easily discriminated by the shape of the genital arch of the male (Coyne, 1983; Shorrocks, 1972). The females can usually be discriminated by the pattern of dark pigmentation on the sixth and seventh abdominal tergites (Thompson *et al.*, 1979; Eisses and Santos, 1997; A. Hoffmann, personal communication). Female flies whose tergites had pigment extending to the lateral edges were scored as *D. melanogaster*; those with the pigment band stopping before the posterior-lateral corner of the tergite were scored as *D. simulans*. All males were discarded after scoring. Individual *D. melanogaster* females were put into fresh food vials with a sprinkle of live baker's yeast, and allowed to lay eggs in order to establish isofemale lines; these lines were later checked to be certain that no *D. simulans* had been included by examining the genitalia of offspring males. Fewer than 5% of these lines were *D. simulans*, indicating that my ability to rapidly sort females was good (though not perfect).

Flies were very numerous at some localities, so the flies counted are only a sample from those populations. For expediency in the field, one sex was not scored from some collections; hence there are some missing data. The data are presented in Table 1.

Figure 1 shows the proportion of *D. melanogaster* determined from females plotted against that determined from males for each collection. As is apparent, there is a very good correlation between the two (Spearman Rank Correlation, Rho (corrected for ties) = .907), and the Wilcoxon Signed Rank test indicated that the proportions did not differ significantly between the two sexes (Z-value = -1.384, P = .1664). The data for the two sexes were thus combined. The three outliers (Tathra, Grovedale and Lorne) are based on very small samples (n = 13, 6 and 24, respectively).

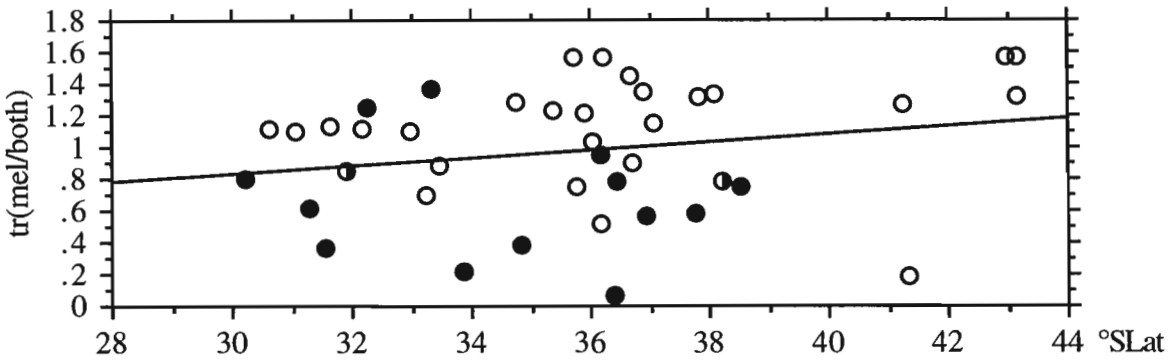

Figure 2. Regression of the proportion of *D. melanogaster* against latitude. Each site was scored as indoors (open circles), mixed (half-dark circles) or outdoors (dark circles). The ratios of pooled data for both sexes were arc-sine square-root transformed before analysis. The regression line has the formula tr(mel/both) = 0.114 + 0.24 × °S lat, $r^2 = 0.04$. The ANOVA for the effect of the slope yields F = 1.68, P = 0.203.

Figure 2 shows the proportion of *D. melanogaster* (both sexes combined, arc-sine square-root transformed data) plotted against degrees south latitude. The unweighted regression analysis showed no significant clinal pattern over latitude in that proportion, although there was a slight (non-significant; P = 0.203) increase towards the south. Visual inspection of Figure 2 leads to the same conclusions: there was great variability between sites, but latitude was not a strong determinant of species proportion.

The data in Figure 2 are shown as indoors (open circles), mixed (half-dark circles) or outdoors (dark circles). A Kruskal-Wallis test of the significance of these groupings on the proportions of *D. melanogaster* yielded H (corrected for ties) = 11.53, P = 0.003, indicating that these three categories of sites differed

significantly in their proportions of the two species. While it is obviously not a strict rule, a higher proportion of *D. melanogaster* was found in indoor sites, and the reverse was true for *D. simulans*.

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The effect of *Drosophila* larvae on the pH of their resource.

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Introduction

A number of studies have examined the relationship between *Drosophila* performance and resource acidity. These include investigations into the success of *Drosophila* larvae (Burdick and Bell, 1954; Posch, 1971; Hodge *et al.*, 1996), developmental stability (Goldat and Beliaieva, 1935; Gordon and Sang, 1941), and the responses of adult *Drosophila* to acidic media (*e.g.*, Fluegel, 1981).

A point which is often overlooked is that the pH of the resource may change with time. Thus, the correlation of responses in *Drosophila* performance with the initial pH of the resource may be erroneous, as this is unrepresentative of the pH the animals actually encounter (see Hodge and Caslaw, 1998). This aspect of the system may have further significance as the changes in resource pH may be caused by the *Drosophila* themselves (Pearl and Penniman, 1926; Bridges and Darby, 1933); the common reference to *Drosophila* as 'vinegar flies' has long been testimony to their association with the acidification of fermenting substances (see Unwin, 1907). Interactions between *Drosophila* have sometimes been ascribed to modifications in the environment caused by larvae ('resource conditioning') (Weisbrot, 1966; Budnik and Brncic, 1975; Dolan and Robertson, 1975). Modification of resource pH is a potential mechanism via which the effects of conditioning may become manifest.

This paper describes the changes which occurred in the pH of *Drosophila* resources and established how these changes were affected by *Drosophila* larvae. The pH changes in artificial and natural resources were examined and the influence of the initial pH on subsequent pH modification was investigated.

Methods

General methods

Two species of wild-type *Drosophila* were used in this study: *D. melanogaster* ('Kaduna') Meigen and *D. hydei* Sturtevant. All experiments were carried out using standard glass vials (75mm × 25mm diameter), plugged with polyurethane foam bungs, as the experimental vessel. Instant *Drosophila* Medium (IDM; Blades Biological, Edenbridge, Kent) was used as the laboratory rearing resource. The pH of the resource was determined using an electronic pH meter [Jenway 3015, Jenway Ltd., Essex, England].

The effect of larval density on induced pH changes

Vials of resource were set up using 1.0g of IDM and 4.0ml of distilled water and the initial pH measured. Four replicates of seven densities of first instar *D. melanogaster* larvae (0, 2, 4, 8, 16, 32, 64) were then added to these vials. The pH of the resource was measured again when pupation of the larvae had ceased.

The effect of initial resource acidity on the extent of Drosophila-*induced pH changes*

A range of initial resource pH values was obtained by hydrating IDM with various concentrations of hydrochloric, citric and acetic acids (see Results for actual pH values). Distilled water (resource pH ≈ 6.2) was used as a control. To make up the media, 1.0g of IDM was hydrated with 4.0ml of liquid. For the HCl, 6 replicates of each acid concentration were set up for both *D. hydei* and *D. melanogaster* (35 1st instar larvae) and the controls. For acetic and citric acid, only 3 replicates were set up and only *D. melanogaster* larvae were used (25 1st instar larvae). The vials were placed in an incubator maintained at 25±1°C, relative humidity 60%. The pH of the medium was measured again when pupation had ceased.

The effect of Drosophila *larvae on pH changes in natural resources*

Ten types of fresh fruit (orange, lemon, grapefruit, banana, cucumber, melon, tomato, avocado, pear and apple) were peeled, chopped and puréed using a pestle and mortar. 5.0g of purée was placed into vials, with each fruit replicated 12 times. The pH was measured and 35 first instar *D. melanogaster* larvae introduced into six vials in each fruit treatment. The vials were maintained in an incubator at 26±1°C, relative humidity 45±5% and a light:dark cycle of 16:8 hours. After 20 days (corresponding to the time of last adult emergence) the pH of the fruit was measured a second time.

Results

The effect of larval density on induced pH changes

The presence of *D. melanogaster* larvae caused a significant reduction in the pH of the medium to around 4 compared to the pH of the control medium (with no larvae) which remained close to the initial value of 6.2 (Table 1; $F_{6,21} = 686.1$, $P < 0.0001$). There was no clear relationship with larval density and pH change and the presence of larvae appeared all that was required to lower the pH.

The effect of initial resource acidity on the extent of Drosophila-*induced pH changes*

For each combination of acid and *Drosophila* species, there was a significant interaction between the initial pH of the resource and the presence of larvae on the extent of the pH change (Table 2; interaction terms GLMs, all $P < 0.001$). The pattern of pH change was similar for larvae of *D. hydei* and *D. melanogaster* and for *D. melanogaster* larvae on different acids. When the initial pH of the resource was greater than 5.0, the presence of larvae caused the pH to drop to between 4 and 4.5, whereas the pH of IDM without larvae tended not to change. At low pH (< 4.5) the final pH of the resource tended to remain unchanged whether *Drosophila* larvae were present or not. The exception to this occurred when *D. melanogaster* larvae where introduced onto medium hydrated with citric acid, the larvae causing a slight increase in pH (Table 2d).

The effect of Drosophila *larvae on pH changes in natural resources*

The initial pH values of the fruit were significantly different ($F_{9,110} = 4764.7$, $P < 0.001$) and provided a good range of values (3.5 - 7) (Table 3). The extent of the pH change - usually an increase - differed between fruits and could be further modified by the presence of *D. melanogaster* larvae (Table 3; interaction term GLM, $F_{9,99} = 7.1$, $P < 0.001$). The pH of the resources modified by larvae could be higher, lower or the same as the controls and there was no obvious relationship between pH change and the initial pH of the resource.

Discussion

Pearl and Penniman (1926) and Bridges and Darby (1933) found that the addition of *Drosophila* larvae to different types of culture medium brought about a reduction in pH to values varying between 3.8 and 4.8, depending on which culture media was used. In the current investigation, the experiments using artificial media extended those results by demonstrating an even greater generality; the convergence of pH being apparent when larvae of different species were used, when different acids were used to alter the initial pH of the resource, and being independent of the original pH of the resource and larval density. The consistency of

Table 1. The effect of density of *D. melanogaster* larvae on reduction in pH of artificial culture media (initial pH was 6.2) (mean standard error was 0.025).

Density of larvae	0	2	4	8	16	32	64
Final pH of resource	6.2	3.8	3.8	4.2	4.1	4.1	4.2

Table 2. The change in the pH of artificial resource caused by *Drosophila* larvae. (a) *D. melanogaster* with HCl, (b) *D. hydei* larvae with HCl, (c) *D. melanogaster* with acetic acid and (d) *D. melanogaster* with citric acid (mean SE ≈ 0.02).

(a)

Original pH	6.2	5.2	3.7	2.8	2.4
No larvae	6.1	5.4	3.9	3.3	2.8
Larvae	4.4	4.4	3.9	3.4	2.9

(b)

Original pH	6.2	5.2	3.8	2.9	2.4
No larvae	6.3	5.4	3.6	2.9	2.4
Larvae	4.4	4.5	3.7	2.7	2.0

(c)

Original pH	6.2	5.4	4.5	4.0
No larvae	5.8	5.3	4.4	3.7
Larvae	4.2	4.2	4.4	3.7

(d)

Original pH	6.2	5.2	4.3	3.5
No larvae	5.8	5.2	4.4	3.5
Larvae	4.2	4.2	4.5	4.1

Table 3. The change in pH of natural resources after 20 days with and without the addition of *D. melanogaster* larvae (mean ± SE).

Resource	Initial pH	Final pH no larvae	Final pH with larvae
Lemon	2.4 ± 0.0	5.8 ± 0.7	4.1 ± 0.5
Grapefruit	3.0 ± 0.0	6.3 ± 0.2	7.3 ± 0.2
Orange	3.5 ± 0.0	6.6 ± 0.5	5.0 ± 0.1
Apple	3.7 ± 0.0	5.9 ± 0.7	2.8 ± 0.1
Tomato	4.1 ± 0.0	9.3 ± 0.3	9.5 ± 0.1
Banana	4.7 ± 0.1	7.6 ± 0.1	4.6 ± 0.7
Pear	4.8 ± 0.0	3.6 ± 0.1	4.4 ± 0.1
Cucumber	5.3 ± 0.0	9.5 ± 0.0	9.5 ± 0.1
Melon	5.9 ± 0.0	9.6 ± 0.0	7.5 ± 0.5
Avocado	6.8 ± 0.1	9.0 ± 0.3	8.7 ± 0.1

the final pH in the laboratory culturing media may be a result of the buffering qualities of this resource when confronted with a small quantity of weak acid.

A pH of around 4 is considered good for yeast populations (Darby, 1930) and it can be speculated that the *Drosophila* larvae benefited themselves by promoting the growth of their primary food source. It has previously been suggested that the positive effects caused by the 'conditioning' of resources by dipteran larvae may be due to increased levels of micro-organisms (*e.g.*, Weisbrot, 1966; Dolan and Robertson, 1975) and this lowering of resource pH could therefore influence the quality of the larval diet.

The mechanism by which the pH drop was effected remains unclear. Excretion is one mechanism by which pH change of a dipteran resource can occur. However, uric acid would tend to be too insoluble to cause a pH shift, urea tends to be pH neutral and the excretion of free ammonia into the resource would tend to cause a pH increase rather than drop. Only the presence of a few larvae were required to lower pH, suggesting that pH was altered by micro-organisms transferred to the resource via the larvae, rather than by the larvae themselves. Pearl and Penniman (1926) and Bridges and Darby (1933) both demonstrated that the addition of yeast to culture media effected a reduction in pH without the presence of *Drosophila* larvae, though both papers also reported that the further addition of *Drosophila* larvae accelerated this change, implying there was an interaction between the yeast and larvae. The lack of effect of the larvae at low pH may be due to the larvae - and the microorganisms forming their diet - being inhibited in acidic media (see Posch, 1971; Hodge *et al.*, 1996).

In natural resources the presence of larvae modified how the pH changed over time but did not consistently bring about an increase or decrease nor was there a convergence to a particular pH value. This result again suggests that an interaction between the *Drosophila* larvae, the different microorganisms communities present on different fruits and other resource variables (such as protein and sugar content) may be determining the final pH.

In conclusion, the addition of *Drosophila* larvae to laboratory culture media brought about a convergence in pH, to a value which could potentially facilitate the growth of their primary food resource. However, the clear patterns observed using artificial culture media were no longer found when natural resources were used. pH modification would appear to be a product of the interaction between the *Drosophila*

larvae, microorganisms and various properties of the resource. The precise mechanism of pH modification remains unclear.

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A parthenogenetic strain of *D. pallidosa*-like in the *D. ananassae* complex.

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In the *D. ananassae* complex, parthenogenetic strains of *D. pallidosa* and *D. ananassae* were already reported by Futch (1972). According to Futch (1973, 1979) this trait is genetically controlled and the mechanism of parthenogenesis of *pallidosa* and *ananassae* seems to be pronuclear duplication and partly terminal fusion. Now, we found parthenogenetic females from an iso-female strain (LAE 345) of *D. pallidosa*-like collected at Lae, Papua New Guinea in 1981, and established a new parthenogenetically reproducing strain. *D. pallidosa*-like distributing in Papua New Guinea was described by Tomimura *et al.* (1993). In addition to a "impaternate" strain of LAE 345, we established a bisexual "bridge" strain of LAE 345. Because F_1 virgin females between LAE 345-Im females and e^D/Sb *ananassae* males have any parthenogenetic ability (Table 1), genes controlling the parthenogenesis might be recessive. Dr. Futch kindly gave us a parthenogenetic strain marked with *yellow* of *ananassae*, and we made crosses between *ananassae* impaternate females and males from the "bridge" strain of LAE 345 of *pallidosa*-like. Because F_1 virgin females have also parthenogenetic ability the same as parental parthenogenetic strains (Table 1), the parthenogenetic ability of the two species might be controlled by the same genetic factors.

Futch (1972) showed that parthenogenetic females of *ananassae* and *pallidosa* were found in only South Pacific Island. Now we found the parthenogenetic strain of *pallidosa*-like in Papua New Guinea. But, the distribution of parthenogenetic strains was still restricted in the South Pacific Islands including Papua New Guinea in *ananassae* complex (Table 2, and Futch 1972). Some genetic factors controlling parthenogenesis might incorporate into the gene pool of *pallidosa*-like in Papua New Guinea from *pallidosa* and/or *ananassae* from South Pacific Islands by hybridization in nature as already suggested by Tomimura *et al.* (1993) based upon the components of chromosome rearrangements among *ananassae* complex.

Table 1. Parthenogenesis ability and productivity of impaternate adults.

Strains (range)	No. of mothers tested	No. of mothers produced adults	% of mothers produced adults	Impaternates / mother
pallidosa-like (LAE 345-Im)	61	59	96.7	12.6 (1-28)
ananassae - Im[y]	43	35	81.4	8.1 (1-28)
F_1 (LAE345-Im/*ana*[e^D])	75	0	0.0	0
F_1 (*ana*-Im/LAE345-Br)	13	13	100.0	13.7 (2-19)

Im: "impaternate" strain. Br: "bridge" strain.

F_1: (female parents / male parents)

References: Futch, D., 1972, Dros. Inf. Serv. 48: 78; Futch, D., 1973, Genetics 74: s86-s87; Futch, F., 1979, Genetics 91:

s36-s37; Tomimura, Y., M. Matsuda, and Y.N. Tobari 1993, In: Drosophila ananassae. *Genetical and Biological Aspects*, (Tobari, Y.N., ed.), pp.139-151.

Table 2. Number of strains with impaternate females in various species of the *ananassae* complex.

Species	Locality	No. of tested strains (No. of females tested)	No. of strains with impaternate females
ananassae			
	Nairobi, Kenya (L)	1 (17)	0
	Kandy, Sri Lanka (C)	2 (61)	0
	Coinbatore, India (D)	2 (59)	0
	Hyderabad, India (HYD)	1 (21)	0
	Bukit Timer, Singapore (W)	2 (73)	0
	Chiang Mai, Thailand (B)	1 (27)	0
	Kuala Lumpur, Malaysia (X)	2 (23)	0
	Sandakan, Malaysia (S)	1 (10)	0
	Palawa, Philippines (R)	1 (16)	0
	Los Banos, Philippines (Q)	3 (77)	0
	Australia (AUS)	1 (25)	0
	Guam (GUM)	2 (45)	0
	Lae, Papua New Guinea (LAE)	1 (20)	0
	Port Moresby, Papua New Guinea (POM)	2 (101)	0
	Ponape, Caroline Islands (PNI)	2 (111)	0
	Tongatapu, Tonga	1 (10)	0
	Vava'u, Tonga (VAV)	1 (15)	0
	Pago Pago, Samoa (PPG)	1 (41)	0
pallidosa-like			
	Wau, Papua New Guinea	2 (78)	0
	Lae, Papua New Guinea	3 (94)	1
pallidosa			
	Lautoka, Fiji (NAN)	4 (162)	0
Taxon K			
	Kotakinabalu, Malaysia	2 (69)	0
papuensis-like			
	Wau, Papua New Guinea	2 (78)	0
	Lae, Papua New Guinea	2 (43)	0

Strains, species, and symbol of locality were described in detail by Tomimura *et al.* (1993)

Distribution of *Drosophila* in Okinawa and Sakishima Islands, Japan.

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Distribution of *Drosophila* flies in six islands of Okinawa prefecture of Japan had been surveyed in 1980's and 1990's from ecological and biogeographical viewpoints. Flies were collected using banana bait traps, within seven days after trap setting. At an exceptional site in Nago, Okinawa, flies were collected by sweeping over the garbages around pineapple yard. Figure 1 shows the collection sites of Okinawa and Sakishima islands (Miyakojima, Ishigakijima, Iriomotejima, Haterumajima and Yonagunijima). Flies were classified into one *Phorticella* and 21 *Drosophila* species according to Okada (1987). In his paper,

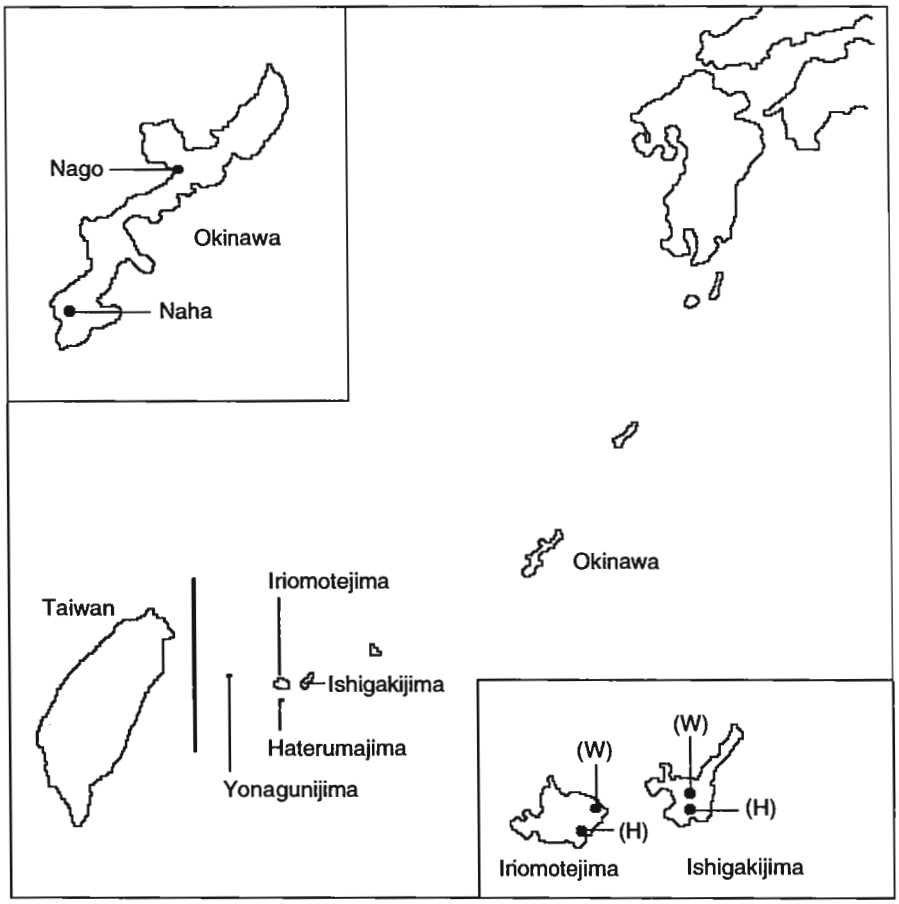

Figure 1. Collection sites of *Drosophila* in Okinawa and Sakishima islands (Miyakojima, Ishigakijima, Iriomotejima, Haterumajima and Yonaguni-jima).(H) and (W) show the sites of human habitation and wild forest, respectively.

Scaptodrosophila is categorized as the subgenus of *Drosophila*. But now it is in an independent genus (Grimardi, 1990). Two *Scaptodrosophila* species from Iriomotejima are not described in Okada (1987).

Table 1 shows the results of fly collection near human habitation of Okinawa and Miyakojima in 1982 and 1983. In a total of 1928 flies, 11 species were found in the Okinawa and Miyakojima. The most abundant species was *D. bipectinata*, followed by *D. melanogaster*, *D. takahashii* and *D. ananassae*. The collection of *D. simulans* and *D. triauraria* is a new record of distribution in Okinawa prefecture, although the colonization of *D. simulans* in Miyakojima had already reported in Watada *et al.* (1986). The new distributional record of *D. triauraria* is biogeographically important, since the closely related species, *D. quadraria* is reported from Taiwan. They have neither significant genetic divergence at the protein level nor reproductive isolation (Ohnishi *et al.*, 1983; Kimura, 1987) The new record of *D. triauraria* in Okinawa island supports the idea that *D. quadraria* may be a geographical race of *D. triauraria* (Kimura, 1987). Additionally, *D. quadraria* might be a founder of *D. triauraria*, because *D. quadraria* had never been collected after a single female collection in Taiwan.

A further survey of *Drosophila* had been made in Okinawa and five Sakisihima islands from 1996 to 1999. In Ishigakijima, Iriomotejima and Haterumajima, flies were collected in human habitation (H) and natural forest (W). Table 2 shows collection sites, date and the results of fly number in Okinawa and five Sakishima islands. A total of 11252 flies were classified as one *Phorticella flavipenis* and 20 *Drosophila* species. The most abundant species was *D. takahashii* and followed by *D. bipectinata*, *D. albomicans*, *D. dorsocentralis*, *D. ananassae*, *D. melanogaster* and *D. daruma*. In spring, *D. takahashii* was the most abundant in human habitation of Ishigakigjima and Iriomotejima. This species was also dominant in the wild forests of the islands. A similar distribution pattern was found in *D. albomicans*, *D. dorsocentralis*, and *D. ananassae*. However they were abundant in fall rather than in spring. *D. bipectinata* and *D. melanogaster* were

Table 2. Number of *Drosophila* flies collected in six islands of Okinawa prefecture.

	Naha	Nago	Miyako*	Ishigakijima			Iriomotejima						Haterumajima		Yonagunijima
	(H)	(P)	(H)	(H)	(W)		(H)			(W)			(H)	(W)	(H)
	96/11	96/11	99/3	98/3	98/3	98/11	96/11	98/3	98/11	96/11	98/3	98/11	98/11	98/11	98/12
P. flavipennis	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-
bryani	1	-	-	-	-	-	97	-	21	-	1	-	71	-	11
coracina	-	-	-	-	3	15	4	36	1	2	8	6	-	-	8
dorsocentralis	9	5	2	48	47	61	10	59	219	98	1	244	81	13	27
*Scapt. sp.*A	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
*Scapt. sp.*B	-	-	-	-	-	-	-	-	-	2	-	2	-	-	-
takahashii	34	6	160	1461	149	6	1	1444	43	5	69	37	2	1	-
melanogaster	-	71	61	20	-	-	7	104	94	-	-	-	174	-	199
simulans	-	73	8	-	-	-	-	-	-	-	-	-	-	-	-
ficusphila	-	-	6	4	-	-	3	7	1	-	1	-	7	1	1
ananassae	1	-	5	25	-	12	-	-	174	-	-	382	122	68	2
bipectinata	492	521	120	166	-	13	12	23	714	16	-	20	198	-	70
bocki	-	-	-	-	-	32	5	-	-	6	-	20	2	4	1
kikkawai	-	-	273	13	-	-	-	-	-	-	-	-	-	-	-
lacteicornis	-	-	-	-	10	15	-	-	-	17	9	2	-	-	-
daruma	-	-	-	-	-	-	-	-	-	338	300	43	-	-	-
bizonata	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-
albomicans	146	5	243	55	48	350	10	95	22	130	11	115	5	60	10
formosana	-	-	-	-	4	10	-	-	-	27	2	10	-	-	-
immigrans	-	-	106	26	-	-	-	-	-	-	-	-	-	-	-
quadrilineata	-	-	-	4	-	1	-	-	1	4	-	23	-	-	1
Total	683	681	986	1822	262	517	149	1768	1290	646	404	904	662	148	330

Naha and Nago are collection site of Okinawa island, and Miyako* means Miyakojima. (H), (P) and (W) show collection sites of human habitation, pineapple yard and wild forest, respectively.

mainly collected in human habitation. On the other hand, *D. daruma* was found only in the wild forest of Iriomotejima. This species is usually rare and collected in riversides of southern Japan. *D. lacteicornis* and *D. formosana* were collected only in wild forests of Ishigakigjima and Iriomotejima although they were not dominant in the islands.

The collection of *D. bocki* in Ishigakijima, Iriomotejima, Haterumajima and Yonagunijima is a new record of the distribution in Japan. *D. bocki* is a closely related species to *D. kikkawai* which is a domestic and world wide species. The first collection of Japanese *D. bocki* was in Iriomotejima in 1979 by the one of the authors (M.W.). Since morphological classification of the species was actually difficult at that time, it was identified as *D. bocki* by mating experiments, two-dimensional electrophoresis and allozyme electrophoresis (Ohnishi *et al.*, 1983). The present study shows that *D. bocki* dwells in the four Sakishima islands and prefers the wild environment rather than human habitation. *D. bocki* may not be a recent colonizer in Japan. A difficult identification might have missed the species as in the case in Taiwan (Baimai, 1979; Baimai *et al.*, 1980).

D. simulans was a colonizing species in mainlands of Japan (Honsyu, Kyusyu, Shikoku and Hokkaido), and had never been found in Okinawa and Sakishima islands (Watanabe and Kawanishi, 1978). Many *D. simulans* were once collected in Miyakojima in 1982. However, this species seems to be suffering from settlement there. Only 8 flies are *D. simulans* in a total of 986 *Drosophila* flies in spring of 1999. New colonization of *D. simulans* was found in Nago (Okinawa island) by sweeping. But the species was not collected in Naha. A further and precise survey is needed for the study of colonization of *D. simulans* in Okinawa and Sakishima islands.

Table 1. Number of *Drosophila* collected in human habitation of Okinawa (Naha and Nago) and Miyakojima in 1982 and 1983.

	Nana (H)	Nago (P)	Miyakojima (H)	
	82/10	82/10	82/10	83/8
bryani	0	6	18	79
dorsocentralis	1	3	31	10
takahashii	119	6	69	19
melanogaster	1	341	7	5
simulans	0	0	137	5
ficusphila	3	0	22	11
ananassae	2	168	2	21
bipectinata	3	0	508	199
kikkawai	23	32	6	8
triauraria	2	0	0	0
albomicans	7	6	7	0
Total	161	562	807	356

(H) and (P) show collection sites of human habitation and pineapple yard, respectively.

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Why is *mama* not *adipose*?

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I reported earlier (Doane, 1996) that *fs(2)lto5DF6*, a recessive mutation in the gene *female sterile (2) late oogenesis5*, does not complement adp^{fs}, a female sterility mutation previously believed to be an allele of *adipose* (*adp*), the first obesity gene of *Drosophila melanogaster* to be described. The former was isolated in a second chromosome saturation screen for EMS-induced female-steriles (Schupbach and Wieschaus, 1989, 1991; T. Schupbach *in* Lindsley and Zimm, 1992, page 237). The latter was described nearly 40 years ago as a spontaneous mutation derived from a natural population in Kaduna, Nigeria (Doane, 1960a, b).[1] It became apparent that *fs(2)lto5DF6* and adp^{fs} are recessive alleles of the same gene based on genetic location, failure to complement one another, and similarities in their female sterility phenotypes (Doane, 1996). I have named this gene *maternal metaphase arrest* (genetic symbol, *mama*). Its name reflects the maternal effect lethality of eggs laid by females homozygous for either of these female sterility mutations and the meiotic or mitotic arrest

associated with the first of them to be described. Late stages of oogenesis are affected by both mutations (see Lindsley and Zimm, 1992).

The *mama* gene has three known alleles to which I have assigned the following genetic symbols: $mama^{+}$ for its wild-type allele; $mama^{1}$ for the spontaneous mutation formerly called adp^{fs} (synonym, *fs(2)adp*); and $mama^{lto5}$ for the EMS-induced allele previously known as *fs(2)lto5DF6*. These symbols conform to the guidelines of *FlyBase* (M. Ashburner, personal communication) and will be used throughout the remainder of this report. The primary question addressed here is: Are *mama* and *adp* the same or different genes? The facts that $mama^{lto5}$ mutants do not express an obese phenotype and that the obese adp^{60} mutants are fertile suggest that they are discrete (Doane, 1996). If true, *adipose* has only two known alleles, adp^{+} and adp^{60}, and it can be assumed that adp^{60} was linked to $mama^{1}$ when the latter was first isolated from the Kaduna population. Evidence for this already exists (Doane, 1961).

The relationship between *mama* and *adp* is clarified in this report by a series of relatively large-scale, classical recombination experiments. The goals were to determine (1) whether or not *mama* and *adp* are separate genes, (2) what the genetic distance between them might be if they are different genes, and (3) what their gene order might be relative to closely linked markers. First, a pilot experiment was conducted to test the hypothesis that *adp* and *mama* are separate genes. Heterozygous females of the genotype $cn^{1}\ adp^{+}\ mama^{lto5}\ bw^{1}/cn^{+}\ adp^{60}\ mama^{+}\ bw^{+}$ were mated to males homozygous for $cn^{1}\ adp^{60}\ mama^{1}\ bw^{1}$. The progeny from this test cross numbered 1,235 flies of both sexes. These were scored for eye color, but only females displaying brown eyes (115) were scored for *mama* and *adp* mutant phenotypes to avoid the tedium of aging and testing all flies individually for these traits. The results were consistent with crossing-over having occurred between *adp* and *mama* at an estimated frequency of ~0.73%, but this was based on only two *adp-mama* recombinants and double crossover events could not be scored.

The second experiment was a 3-point recombination analysis that did score for double crossover progeny. Here $adp^{60}\ mama^{1}\ bw^{1}/adp^{+}\ mama^{+}\ bw^{+}$ females were crossed to homozygous $adp^{60}\ mama^{1}\ bw^{1}$ males, and the female test cross progeny were counted (N = 1955) and classified for eye color and fertility. To correct for flies that might express sterility unrelated to *mama*, only fertile females were tested for *adp* phenotypes. These numbered 1,031 and included: 10 single crossovers between *adp* and *mama*; 196 singles crossovers between *mama* and *bw*; and one double crossover between *cn* and *bw* (Interference, 53.8%). The results confirmed that *adp* and *mama* are separate genes and established the gene order as *adp - mama - bw*, with *adp* centromere-proximal to *mama*. Also, a better estimate of the map distance between *adp* and *mama* was calculated, namely ~1.07 cM.

Table 1. Recombination analyses performed to map *adp* relative to *stau* and *Pcl* and to determine the map distances between them.

Test Cross	Test Cross Progeny: Phenotypic Classes	Number	% Crossing-over
A: $c^{+}\ stau^{ry9}\ adp^{+}$ / cl $stau^{+}\ adp^{60}$ female	Parental : wild-type	1,781	—
x	Single Crossovers: c-stau	99	5.23
$c^{1}\ stau^{+}\ adp^{60}$ / $c^{1}\ stau^{+}\ adp^{60}$ male	Single Crossovers: stau-adp	12	0.63
	Total number scored:	1,892	
B: $c^{+}\ Pcl^{Pl}\ adp^{+}$ / cl $Pcl^{+}\ adp^{60}$ female	Parental: wild-type	1,418	—
x	Single Crossovers: c-Pcl	101	6.61
$c^{1}\ Pcl^{+}\ adp^{60}$ / $c^{1}\ Pcl^{+}\ adp^{60}$ male	Single Crossovers: Pcl-adp	10	0.65
	Total number scored:	1,529	

Two additional 3-point analyses were performed (Table 1, test crosses A and B). All gene loci involved were located within a span of 10 cM, thus eliminating double crossover classes and simplifying genetic calculations. The *adp* gene had been located on the genetic map at 2-83.4, *i.e.*, 7.9 map units distal to *curved* (*c*, 2-75.5) (see Appendix *in* Doane, 1969). Because there is a paucity of genetically well defined, visible markers in this region of the genome (*FlyBase*, 1999), stable *P* element-induced *staufen* (*stau*, 2-83.5) and *Polycomblike* (*Pcl*, 2-84) mutations were used as markers. The mutant alleles employed were $stau^{ry9}$ and Pcl^{Pl}. These are associated with the transposon *P{ry11}* (St. Johnston *et al.*, 1991; Lonie *et al.*, 1994) which carries a wild-type *rosy* gene (ry^{+}). Test crosses were set up so that all flies were homozygous for $ry^{506}\ e^{1}$.

This permitted heterozygous progeny carrying a single copy of *stau*^{ry9} or *Pcl*^{P1} to be detected by their wild-type eye color. Flies without these mutations had rosy-colored eyes.

Only one-half of the progeny from each test cross in Table 1 were counted since no double crossing-over was anticipated. Reciprocal classes were discarded. This greatly simplified the tasks of aging the flies one week and classifying their fat body phenotypes. In test cross A, only flies with wild-type wings were counted because curved-wing flies tended to become stuck in the food medium and die during the required 1-week aging period. For test cross B, special care was taken to prevent this from happening so both wild-type and curved-wing flies were scored. To half the number of progeny scored, only males were scored because the *adp* phenotype can be determined with better accuracy in males than females.

The molecular studies of Lonie *et al.* (1994) indicate that *stau* and *Pcl* are separated by about 8 kb of DNA and that *stau* is located proximal to *Pcl*, which is consistent with the genetic map positions given above. Taking this and the results of both test crosses in Table 1 into account, it now appears that the *adp* gene lies about 0.6 cM distal to the *stau-Pcl* region on the genetic map and that the combined gene order is *c - stau - Pcl - adp*. Despite the limitations of gene mapping based on recombination data, the percentages of crossing-over between *stau* and *adp* and between *Pcl* and *adp* listed in Table 1 (0.63 % and 0.65%, respectively) are remarkably similar. The crossing-over distances between *c* and *adp* derived from test crosses A and B were calculated to be 5.86 and 7.26 map units, respectively. Both of these distances are less than the 7.9 cM reported earlier (Doane, 1969), but only by ~0.6 cM in the latter case.

The recessive *mama*^{1} and *adp*^{60} phenotypes are both expressed in deficiency heterozygotes carrying *Df(2R)PC4, Df(2R)Pcl-W5, Df(2R)Pcl7B, Df(2R)Pcl11B* and *Df(2R)Pcl*^{XM82}, but not *Df(2R)PC29, Df(2R)P34* or *Df(2R)PC66* (Doane and Dumapias, 1987; Doane, 1994, 1996 and unpublished data). They also fail to complement a deletion that apparently exists at the left breakpoint in the inversion chromosome *In(2R)Pcl*^{11} (Doane, 1994). This deletion also fails to complement *stau* and *Pcl*, but it does complement *thr* (Doane, 1994 and unpublished data), which is in region 55A1 of the polytene chromosome map (*FlyBase*, 1999). This implies that the left breakpoint of the deletion is between 55A1 and 55A4, wherein *stau* is located (*FlyBase*, 1999). Clearly, the deletion in the *In(2R)Pcl*^{11} chromosome is much larger than originally supposed since it spans the region containing *stau* and *mama*, which are ~1.6 cM apart on the genetic map. The latter suggests that *mama* may be cytogenetically located in 55B and not 55A, wherein *adp* is still likely to be found. Furthermore, earlier speculation that *PpY-55A* and *adp* might be the same gene (Foehr and Doane, 1994) is negated by the fact that *PpY-55A* is in 55A1-3, which is proximal to *stau*, while *adp* is distal to *stau* and *Pcl*.

I have knowingly omitted reference to the report by Tearle (1996) that is archived in *FlyBase* in its original, unedited draft form. The EMS-induced lethal and female-sterile mutations isolated by Tearle should eventually prove very useful for analyzing the part of the genome spanned by *Df(2R)PC4*, which includes the *mama* and *adp* genes. However, attempts to interpret this report with regard to these two genes proved fruitless at this time.

Conclusions: The following conclusions may be drawn from the data presented here: (1) *mama* and *adp* are different genes with a genetic distance of ~1 cM between them; (2) *adp* is located ~0.6 cM distal to the *stau-Pcl* region on the genetic map; (3) the gene order for the loci included in this study is *c - stau - Pcl - adp - mama*; (4) *adp* is probably located cytogenetically toward the distal end of 55A, while *mama* is likely in regions 55B; and (5) *PpY-55* and *adp* are clearly different genes.

Acknowledgments: The source of the *mama*^{hto5} and *stau*^{ry9} mutations was the Bloomington, Indiana Stock Center. *Pcl*^{P1} was provided by the laboratory of R. Saint, University of Adelaide, Australia. Other mutations used in test crosses, including *adp*^{60} and *mama*^{1}, have been maintained in my laboratory for many years. Two different stocks containing *In(2R)Pcl*^{11} were tested with the same results; one came from the Bloomington Stock Center and the other was provided by Daniel Moore while at the Whitehead Institute for Biomedical Research, Cambridge, MA.

References: Counce, S.J., 1960, Dros. Inf. Serv. 34:48; Doane, W.W., 1960a, J. Exp. Zool. 145:1-21; Doane, W.W., 1960b, J. Exp. Zool. 145:23-42; Doane, W.W., 1961a, Dros. Inf. Serv. 35:45; Doane, W.W., 1961b, Dros. Inf. Serv. 35:78; Doane, W.W., 1963, Dros. Inf. Serv. 37:47; Doane, W.W., 1994, Dros. Inf. Serv. 75:168; Doane, W.W., 1996, Dros. Inf. Serv. 77:78-79; Doane, W.W., and F.E. Dumapias 1987, Dros. Inf. Serv. 66:49; FlyBase 1999, A Database of the Drosophila Genome, Available through the FTP server at flybase.bio.indiana.edu; Foehr, E.D., and W.W. Doane 1994, Dros. Inf. Serv. 75:168-169; Lindsley, D.L.,

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[1]*Errata*: S. J. Counce gave me the adp^{fs} mutation (syn. *fs(2)adp*) in 1956. My original name for it was *female-sterile(2)adipose* but the gene locus was renamed *adipose* after my isolation of the fertile adp^{60} mutation (Doane, 1963). Counce has been credited with discovery of adp^{fs} (Lindsley and Grell, 1968; Lindsley and Zimm, 1992), but she recently pointed out that C. Auerbach had isolated it from the Kaduna wild population maintained at the University of Edinburgh (S. J. Counce, personal communication). To further complicate its history, my first description of *fs(2)adp* as a new mutant appeared in *Dros. Inf. Serv.* as the "Report of S. Counce" (see Counce, 1960). This error was corrected a year later when the same description appeared in a report under my own name (Doane, 1961). An editorial note accompanying it stated: "This report supersedes that in *Dros. Inf. Serv.*-34 inadvertently attributed to S. Counce." Unfortunately, *FlyBase* (1999) has perpetuated the prior error in authorship by including reference FBrf0094540 in its bibliography without comment or correction.

Drosophila hormone receptor 38: phenotypic analysis of mutations generated by P-element excision.

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DHR38 is a member of the steroid receptor superfamily in *Drosophila* sharing homology with vertebrate NGF1-B-type orphan receptors. As a monomer, DHR38 interacts with the USP component of the ecdysone receptor complex *in vitro* in yeast and in *Drosophila* cell line, suggesting that DHR38 might modulate ecdysone triggered signals.

Fine functional and structural analyses of this gene were carried out as described in Sutherland *et al.* (1995), Fisk and Thummel (1995), and Kozlova *et al.* (1998). The gene spans more than 40kb in length, has a complex genomic organization and produces multiple mRNA species developmentally regulated. Four mutations in this gene are known: one P(ry+, lacZ) insertion (named *l(2)02306*) and three EMS induced. All mutations result in local fragility of the adult cuticle: the cuticle in the leg joints is ruptured by mechanical stress, this leads to melanization of the damaged spots. Subsequently flies die as pharate adult or within a few hours after eclosion. The phenotypic abnormalities and effective lethal phase suggest an important role of DHR38 in late stage of epidermal metamorphosis.

It is known that the *Dhr38* gene expresses during most *Drosophila* developmental stages, suggesting that this gene may perform a critical function during another stage (more early). To test this hypothesis we induced the new mutations by imprecise removal of the P element.

To generate mutations in the *Dhr38* gene P(ry+, lacZ) transpozon related with *l(2)02306* mutation localized 34 bp upstream of cTK61 isoform (Figure 1, from Kozlova *et al.*, 1998) was mobilized according to standard genetic scheme, using Δ2,3 (ry+) on the third chromosome as a source of transposase. ry-excisions were checked for viability in combinations with Df(2)DS9, Df(2)KetelRX32 or *l(2)02306*. From more than 10,000 analyzed flies we obtained 106 ry-excisions and 18 of them were lethal during pharate adult/adult stages when hemizygous with deletions. The lethal phenotypes exhibited by new mutations in hemizygous condition are similar to the ones described earlier.

Determination of the lethal phases for 14 selected lines shows that most of them are adult lethals like the original P insertion (Table 1), but some are derivatives with more severe phenotypes- larval, prepupal or pupal lethals. v9 and v27 homozygotes show delayed pupariation up to four days. It is interesting to note that

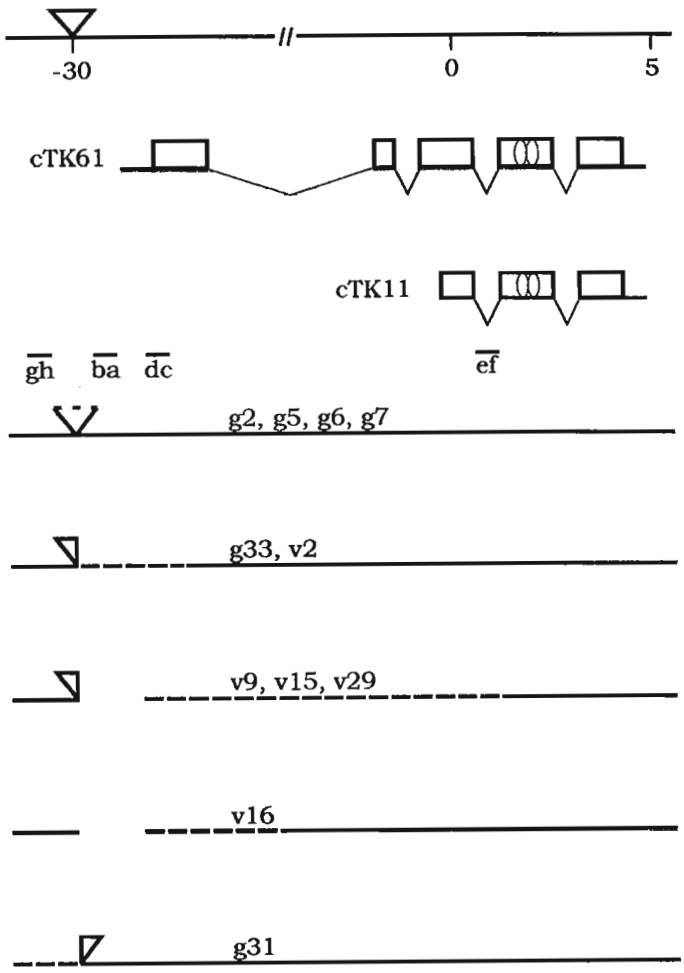

Figure 1. Molecular mapping of the new excision alleles. Molecular map of the *Dhr38* gene is adapted from Kozlova *et al.* (1998) and positions of exons and introns are indicated. Protein coding regions are represented by open rectangles, ovals show the position of Zn fingers. Untranslated sequences are shown by solid lines. Solid triangle represents insertion site of *l(2)02306* P-element insertion line. Fragments amplified by the primers used for molecular mapping of the rearrangements are shown below molecular map (gh, ba, dc, ef). Genomic sequences still present in new rearrangements are shown by solid lines, dashed areas represent an uncertainty of mapping. Part of the P-element construct retained in rearrangements (if any) is shown by an open triangle.

many new mutations such as g6, v15, v29, g33 behave as earlier lethals when homozygous rather than in combination with deficiency. For example, 64% of hemizygous Df(2)/g6 flies survive until the adult stage and die displaying melanization in the leg joints. However the majority of g6 homozygous flies (91%) die at prepupal and early pupal stages with only 1% surviving until adulthood. A few excision mutations such as v16 behave as hypomorphic, showing 86% survival to adulthood when homozygous and lethality throughout larval and pupal stages with only 8% of adults eclosing when hemizygous.

PCR analysis of selected lethal ry- chromosomes was performed on DNA templates prepared from heterozygous and homozygous flies using several sets of primers based on genomic and cDNA sequences (Figure 1) as well as a primer corresponding to the terminal repeats of the P element. In most of the excisions part of the P-element construct is still present (v9, v15, v29, g33, v2, g31). In others, both P element ends and adjacent genomic sequences are intact (g2, g5, g6, g7), suggesting that ry- phenotype is caused simultaneously by internal rearrangement in the P(ry+, lacZ), and possibly by inversion or duplication, frequently generated in P mutagenesis (Zhang and Spradling, 1993; Dorer and Henikoff, 1994).

We identified four excisions (v9, v15, v29, v16) removing the first exon of the *Dhr38* gene, containing 5 UTR sequences and part of the A/B domain present in cTK61 isoform (Figure 1). None of these eliminated the DNA-binding and ligand-binding sequences of *Dhr38*. Since the distance between the insertion site of P element and 5' end of the cTK61 is only 34 bp, all the excisions generated probably affect only this cDNA isoform and therefore are not complete loss-of-function alleles of *Dhr38*. Our lethal phase analysis and molecular mapping suggest that excision alleles retaining part of the P element construct (in contrast to the original P element insertion) show dominant phenotype manifested in earlier larval and pupal lethality, but

Table 1. Viability of mutants at different developmental stages.

Genotype	Larvae %	Pupae %	Pharate adults %	Eclosed adults %	Lph
P/CyO, y+	100	98	96	96	—
P/P	100	96	94	89	A
v2/v2	100	95	60	34	P, A
v2Df	100	74	70	64	A
v8/v8	100	97	78	62	A
v9/v9	100	62	0	0	L, P
v15/Df	100	64	62	59	L,A
v15/v15	100	97	0	0	P
v16/v16	100	86	86	86	A
v16/Df	100	44	44	8	L, P, A
v27/v27	100	60	0	0	L, P
v29/v29	100	26	0	0	L, P
v29/DF	100	60	53	53	L, A
g2/g2	100	90	86	86	A
g5/g5	100	95	92	92	A
g6/g6	100	92	1	1	P
g6/Df	100	66	66	64	L, A
g7/g7	100	94	94	94	A
g31/g31	100	95	33	0	P
g31/Df	100	50	50	46	L, A
g33/g33	100	96	34	0	P
g33/Df	100	68	68	64	L,A
g36/g36	100	92	0	0	P

The respective genotypes of animals homozygous or hemizygous in combination with the deficiency Df(2)KetelRX32 or Df(3L) DS9 are shown in the left column; P represents the insertion allele l(2)02306, and P/CyO, y+ is the control. Homozygous or hemizygous mutant first instar larvae, pupal cases, pharate adults (stage P15, Bainbridge and Bownes, 1981) and eclosed adults were scored. All eclosed adults with the exception of controls in P/CyO, y+ died within a few hours after eclosion displaying melanization in appendage joints. Lethal Phase (LPh) of corresponding genotypes is summarized in the right column: L-larval, P-pupal and pharate adults, A-eclosed adults.

also represent loss-of-function alleles at later pharate adult/adult stage when *Dhr38* function is required (Kozlova *et al.*, 1998). We believe that these ry- derivatives may be considered as gain-of-function alleles at larval and pupal stages due to the activity of the remaining P-element promoter sequences which probably cause ectopic *Dhr38* transcription. This suggestion is in agreement with earlier observations that *Dhr38* is transcribed at a very low level at larval stages and overexpression of *Dhr38* under hsp70 promoter at this stage causes ectopic lethality (Kozlova *et al.*, 1998; Kozlova, T.Yu., unpublished).

In conclusion, we described here several new excision alleles of *Dhr38*, some of which represent true hypomorphs (v16), and others (g6, v15, v29) posses both gain-of–function and loss-of-function characteristics.

Acknowledgments: The work was supported in part by grant of the Program "Frontier Program of Genetics" and RFBR grants 96-15-97749 and 99-04-49270.

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The mutation impairing the heat-shock response does not prevent the development of the stress-reaction in *Drosophila*.

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It is known that unfavorable effects produce in insects an unspecific adaptive response both on the level of the cell (the heat shock response) and the whole organism (the neurohormonal stress reaction) (see reviews: Rauschenbach, 1991; Cymborowski, 1991; Lindquist, 1993). There are many studies concerned with each of these adaptive mechanisms conducted worldwide. However, the interrelations of the two systems remain unestablished. The open questions are whether the heat shock response arising during the first minutes of the onset of the effect of a stressor is the trigger link for the development of the stress reaction or do these systems respond independently to the stressor, each providing adaptation on its own level? We present here the results of studies of the response of two central links on the stress reaction (the systems of the juvenile hormone and octopamine metabolism) to stress in line ts403 of *D. melanogaster* carrying the recessive temperature-sensitive lethal mutation *l(1)ts403* in the X chromosome (Arking, 1975). When temperature is raised, HSP83 and HSP35 are not synthesized and the synthesis of a number of proteins of the HSP70 group is suppressed in individuals of this line (Evgen'ev *et al.*, 1990).

We have previously demonstrated that the changes in the activities of JH-hydrolyzing enzymes and of the first enzyme involved in the pathway for OA synthesis, tyrosine decarboxylase (TDC), can serve as indicators of the development of the stress reaction in *Drosophila* (Rauschenbach *et al.*, 1995; Sukhanova *et al.*, 1997). TDC activity was determined using a radioisotopic method of McCaman and co-authors (1972). Measurement of hydrolysis of radioactive JH were carried out by the method of Hammock and Sparks (1977).

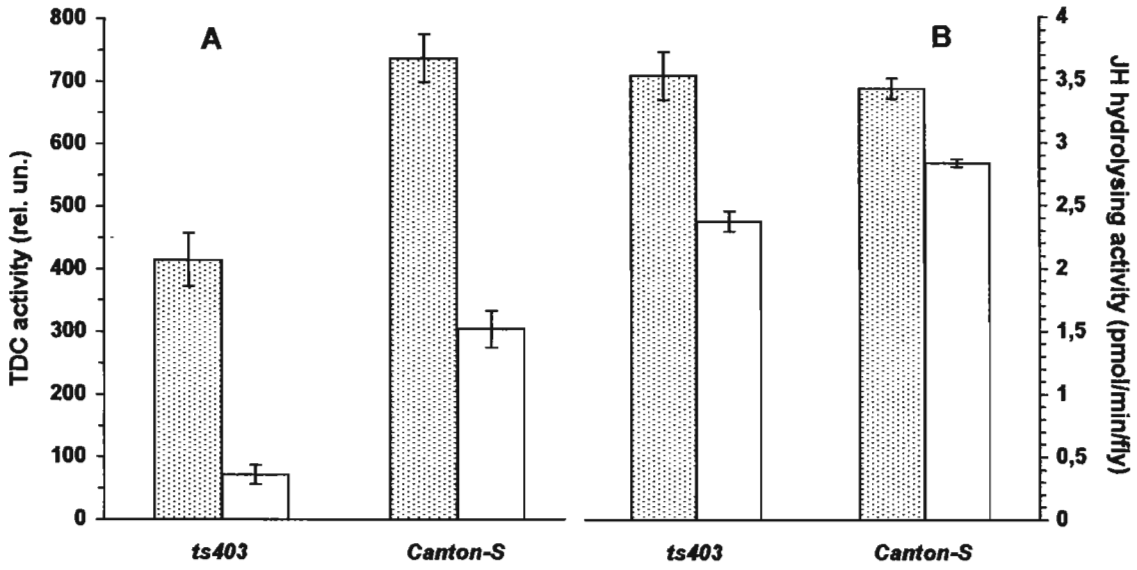

Figure 1. TDC activity (A) and level of JH-hydrolyzing activity (B) in 5-day-old females of lines ts403 and Canton-S under normal and stressful conditions (38°C, A - 60 min; B - 120 min).

The results of TDC activity measurements in individuals of line ts403 and of wild-type line Canton-S (the latter was used as control) demonstrated that exposure to stress evoked in line ts403 individuals, as a Canton-S flies, a significant ($P < 0.001$) decrease in enzyme activity compared to control (Figure 1).

The level of JH-hydrolyzing activity also considerably decreased under the effect of the stressor in females of line ts403 (Figure 1), as well as in wild-type Canton-S females (the differences from the control are significant in both lines, $P < 0.001$).

Thus, we demonstrated that the system of JH degradation in flies of line ts403 responds to a stressful agent by a sharp reduction in JH-hydrolyzing activity. The response of the system of OA metabolism to the action of the stressor was also strongly manifested in individuals of line ts403. It may be concluded that impairment of the synthesis of the HSPs in this line does not result in perturbance in any one of the links of the hormonal stress reaction we studied. It may be inferred that the heat shock response presumably is not a trigger link of the stress reaction and that the two adaptive mechanisms to unfavorable conditions in insects are triggered independently or in parallel, being under the common central control of a, so far, unknown factor.

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Frugivorous *Drosophila simulans* begins to exploit *Opuntia ficus-indica* cladodes.

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The introduction of exotic plants creates potential new niches for endemic animals. European tradespeople brought the cactus *Opuntia ficus-indica* in the 16th century from Mexico to Europe to serve as substrate for the cochineal insect (*Dactylopius coccus*), which is used for the production of red dye. Cosmopolitan frugivorous *Drosophila* species as *D. melanogaster*, *D. simulans*, and *D. hydei* are known to use the fruits of this exotic plant (Carson, 1965; Haouas *et al.*, 1984, Santos *et al.*, 1999). Whereas the cactophilic species *Drosophila buzzatii* is not known to breed outside its plant-specific niche, *i.e.* decaying cladodes and cactus fruits (Carson, 1965), we show that *D. simulans* made a start to adapt to the non-fruit part of *O. ficus-indica*. **(A)** Naturally rotting *O. ficus-indica* pads were collected in a semi-abandoned *O. ficus-indica* plantation in southern Spain, 3 km north of Carboneras (37 01'N, 1 52'W) (Eisses and Santos, 1997). These pads yielded low but increasing numbers of *D. simulans* in two successive years (Table 1). In a few cases (3/10), the frugivorous *D. simulans* outnumbered the cactophilic *D. buzzatii*. Inherently to cactophilic *Drosophila*, *D. buzzatii* emerged from rotting pads in much higher numbers, *e.g.* in September 1993 and 1994 up to 1109, and 1861, respectively, per cladode. **(B)** The non-accidental occurrence of *D. simulans* in cladodes was tested by placing baits in the field in June 1995 (Table 1). Fresh *O. ficus-indica* cladodes were cut into discs (7 cm diameter) and placed on moist vermiculite in plastic plant-breeding trays. After about 100 h in the field, the cladode-discs were brought back to Barcelona, and checked for emerging flies (Table 1). All 39 *D. simulans* emerged from 6/12 discs placed near a flowering *Agave ricana* and a solitary *O. ficus-indica* at one of the six sites. Only one of these discs produced both *D. buzzatii* and *D. simulans*. The mature *D. simulans* and *D. buzzatii* were very small compared with those emerging from standard laboratory medium or *O. ficus-indica* fruits. In contrast with the normal situation, some *D. simulans* males were bigger than females. Emerging *D. simulans* males and females were paired and placed on standard laboratory medium to check their fertility: all pairs produced offspring. The fresh cladode discs yielded only a total of 33 adult *D. buzzatii*. **(C)** Discs, which did not yield flies but continued to decay, were used for additional experiments in the

laboratory. These experiments showed that *D. simulans* was able to develop in one-third of rotting cladode-discs (Tables 1 and 2). Several hundreds of *D. buzzatii*, *D. simulans*, and *D. melanogaster* were put into a population cage (25 × 25 × 35 cm) together with the rotten discs for one day. *Drosophila simulans* and *D. melanogaster* made up about one-quarter of the total number of flies. All experimental flies descended from flies emerging from cactus fruits (prickly pears) collected in Carboneras in September 1993 (A. Galiana, Universitat Autònoma de Barcelona). A second experiment was performed with 24 more or less rotten cladode discs, which had been in the field for 6¾ days in September 1995. These discs did not produce any insects within 2 months of being brought into the laboratory. During 96 h three cages contained approximately 350 *D. buzzatii* and *D. simulans* (10:1) (progeny of flies that emerged from prickly pears collected in September 1995, Santos *et al.*, 1999). *D. simulans* outnumbered or equalled *D. buzzatii* in three discs, and developed in 8/21 (Table 2). The correlation coefficient between the total number of emerged flies per patch and the weight of the 18 **rotten** cladode-discs at the start of the experiment was 0.785 ($F_{(1,16)} = 25.75$, $P < 0.0001$), whereas the correlation coefficient with the original fresh weights in September was -0.212 ($F_{(1,16)} = 0.75$, NS). The average residual weight, expressed as percentage of fresh weight remaining after 2 months were: green discs: 71.3 ± 3.8%, rotting discs 55.3 ± 8.5%. After larval growth the averages had dropped to 41.5 ± 15.4% (green cladode-discs), and to 20.3 ± 6.9% (rotten discs). Table 3 shows the average ambient temperatures and the averages of the mean developmental times in the three experiments with cladode discs. Egg-to-adult development of *D. buzzatii* varied from 14 to 43 days, whereas *D. simulans* developed in 12 to 25 days. Long developmental times were not due to second generation flies.

Table 1. Number of emerged cactophilic *D. buzzatii* and other *Drosophila* species from a number (n) of natural rotting cladodes of *O. ficus-indica* or cladode-discs. Collections were made in different years.

Date	n	*D. buzzatii*	Other species
Natural rotting cladodes			
March 1978*	34	325	0
June 1981**	40/400↔	666	0
September 1993*	34	5,897	1 *D. simulans*
September 1994	52/91↔	13,915	15 *D. simulans* (1/3)+
November 1994	42/63↔	4,421	24 *D. simulans* (2/7)+
Cladode-discs in the field			
June 1995	12/144↔	33	39 *D. simulans* (5/6)+
Cage experiments			
1) July 1995	7/7↔	805	18 *D. simulans* (0/2)+
			3 *D. melanogaster* (0/3)
2) November 1995	21/24↔	600	22 *D. simulans* (3/8)+

*Unpublished data from M. Santos, A. Leibowitz, J. Quezada-Diaz and H. Laayouni.

**Data from Ruiz *et al.*, 1986.

+The number of cladodes in which *D. simulans* formed the majority of the emerged *Drosophila* or equaled *D. buzzatii* over the number of cladodes or cladode-discs containing *D. simulans*.

↔Cladodes or cladode-discs with emerging *Drosophila* over the total number of collected cladodes or experimental cladode-discs, including those without any *Drosophila* emerging.

D. simulans emerging from *O. ficus-indica* cladode material proved to be not accidental, although this species is well known as frugivorous (Carson, 1965; Atkinson and Shorrocks, 1977), and has been reported as developing only on fruits of various *Opuntia* species (Carson, 1965; Atkinson and Shorrocks, 1984; Haouas *et al.*, 1984). The relative frequency of *D. simulans* in natural rots increased more than six-fold from September to November 1994, and the percentage of cladode-discs yielding *D. simulans* doubled. *D. simulans* appears to be able to use a broader range of yeast species than *D. melanogaster* (Parsons, 1975). Some of the yeasts associated with rotting cladodes are also found in *Opuntia* fruits, though three times less commonly (Ganter *et al.*, 1989). The low content of free sugars in cladodes compared with cactus fruits (Fogleman and Abril, 1990) accounts at least partly for the much slower decay (Starmer and Aberdeen, 1990). In fruits, fast-developing species such as *D. melanogaster* and *D. simulans* will have a selective advantage over slowly-developing species as *D. buzzatii* and *D. hydei* because of the relatively fast degradation of the fruits. However, in rotting cladodes, larvae can survive only if they can tolerate the slow release of nutrients the decay of cactus material

Table 2. Emergence and developmental times of the cactophilic species *D. buzzatii* and the frugivorous species *D. simulans*. Adult flies of both species were mixed and put in population cages from 16-20 November 1995, together with cladode-discs in various stages of decay.

D. simulans		*D. buzzatii*	
No. of flies	First and last day of emergence (mean)	No. of flies	First and last day of emergence (mean)
Green cladode-discs*			
-	-	8	27-29 (28.0)
-	-	18	32-40 (35.7)
-	-	17	23-33 (26.3)
3	12-14 (12.7)	82	14-30 (19.5)
1	13	70	23-43 (33.6)
Rotting cladode-discs*			
1	15	1	26
-	-	4	23-30 (25.0)
-	-	20	26-37 (30.9)
-	-	20	19-30 (24.6)
-	-	17	30-43 (34.8)
9	13-14 (13.7)	6	21-24 (22.0)
1	13	65	20-32 (25.9)
-	-	74	17-26 (21.8)
-	-	71	17-33 (24.9)
-	-	4	31-40 (35.8)
3	14-19 (16.0)	59	19-31 (23.7)
-	-	9	27-36 (31.0)
1	13	42	20-33 (26.0)
-	-	3	33-42 (36.0)
3	13-14 (13.3)	3	36-41 (38.0)
-	-	7	26-36 (31.6)

*One green and two rotting cladode-discs did not yield adult flies.

provides, which is one of the adaptations made by cactophilic *Drosophila* species. *D. simulans* from the Carboneras area showed a broad range of development times in fresh and rotting cladode patches. Comparative experiments with *D. simulans* strains from geographically separate areas would show whether these results apply only to adaptations of a local *race* of *D. simulans* or to *D. simulans* in general. The fact that *D. simulans* can develop into viable adults utilizing the resources in cladodes probably allows it to persist in the area between the successive fruiting seasons. Flies were trapped in the Carboneras area in April 1995, when no prickly pears were present: *D. simulans* was present in similar numbers to *D. buzzatii*. The beginning of a niche expansion by *D. simulans* may have been witnessed in the arid area of Carboneras.

Acknowledgments: We thank Andrew Davis (Department of Biology, University of Leeds) for confirming the identification of *D. simulans* emerging from a rotting cladode. This work was funded by Contract No. CHRX-CT92-0041 from the Commission of the European Communities, and grant PB-0843 from the DGICYT (Spain) to Professor Antonio Fontdevila.

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Table 3. The averages of the mean developmental times and standard deviation of *D. buzzatii* and *D. simulans* in cladode-discs under ambient laboratory conditions with average temperatures and standard deviations in different periods of the year 1995.

Period	Average temperature (°C)	Mean developmental times ± SD (days)		
		D. buzzatii	*D. simulans*	*D. melanogaster*
June 1995	25.2 ± 2.5	20.4 ± 3.6 (7)	16.6 ± 1.8 (6)	-
July 1995	26.5 ± 2.9	20.8 ± 4.5 (7)	15.2 ± 1.5 (2)	16.3 ± 1.5 (3)
November 1995	21.0 ± 2.7	28.6 ± 5.5 (21)	13.7 ± 1.2 (8)	-
Fast-cladode-discs		24.4 ± 2.4 (12)	13.9 ± 1.3 (6)	-
Slow-cladode-discs		34.1 ± 2.5 (9)	13.2 ± 0.2 (2)	-

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Cytological localization of the *Drosophila melanogaster Dhr38* gene.

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The *Dhr38* gene codes for a protein belonging to superfamily of steroid hormone receptors and plays an important role in regulation of metamorphosis processes at late developmental stages in *D. melanogaster*

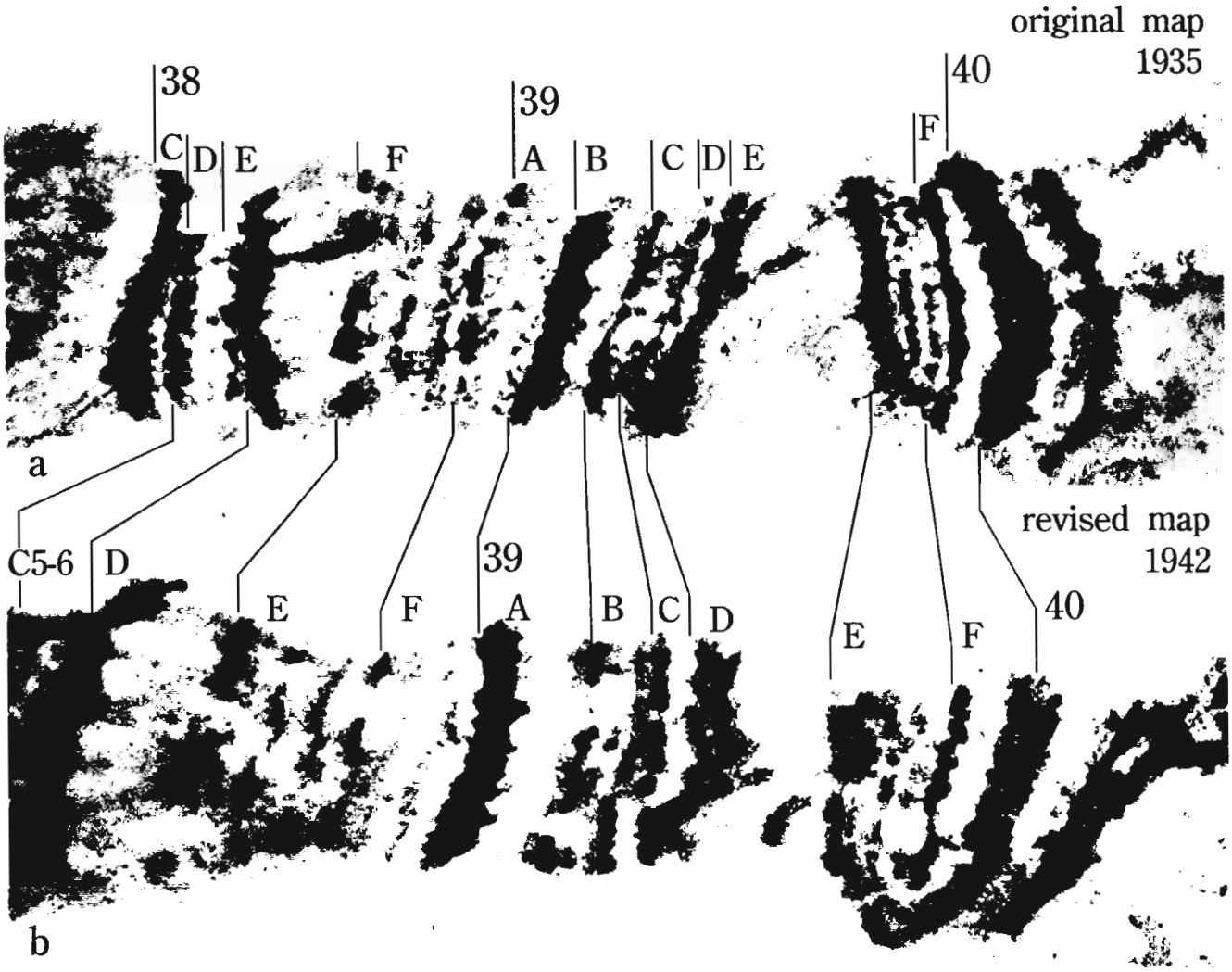

Figure 1. Electron microscopical map of 38C-39F region. Batumi L strain. Sections and subsections are designated according to C.B. Bridges, 1935(a) and P.N. Bridges, 1942(b).

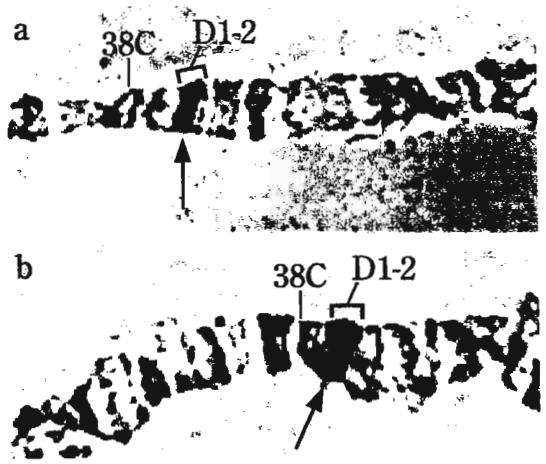

Figure 2. *In situ* hybridization of λTD31 DNA (a) and P-element DNA (b) labeled by biotin with polytene chromosome salivary glands of *l(2)02306* strain. Arrows indicate label localization.

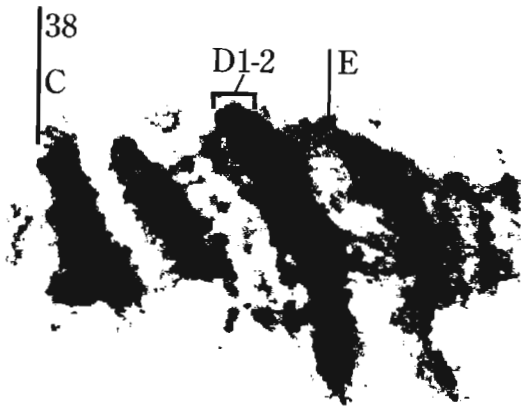

Figure 3. EM photography of the 38C-38E region in *l(2)02306* strain.

(Fisk and Thummel, 1995; Sutherland *et al.*, 1995; Kozlova *et al.*, 1998). According to *in situ* hybridization data, *Dhr38* is localized in the 38 region of 2L chromosome (Sutherland *et al.*, 1995). When mapping the 38-39 region some confusion takes place connected with divergences in subsection boundary designations in original (C.B. Bridges, 1935) and revised (P.N. Bridges, 1942) maps (see as well Lefevre, 1976; Lindsley and Zimm, 1992). In detail, this difference was described earlier (Saura and Sorsa, 1979) and is connected with shifts of subsection boundaries in the revised map of 1942, where subsections 38C and 38D (1935) are combined into a single one, 38C (1942), and on the contrary, subsection 39E (1935) is divided into two, 39D and 39E (1942).

To make the gene location clear, new electron microscopic (EM) mapping of the 38C-38F region was performed. The material and methods used were the same as previously described (Semeshin *et al.*, 1985a). Hereinafter we will use designations of the revised map of P.N. Bridges (1942) which were used earlier for EM mapping of this and other chromosome regions (Saura and Sorsa, 1979; Semeshin *et al.*, 1985b).

The data obtained by EM mapping of the region are given in Figure 1. The banding patterns found in EM studies correspond well to the original Bridges map (1935), if "doublets" in 38E, 39B and 39F sections, as well as "triplets" in 38C and 39E regions, are considered as single bands. Divergences of the revised Bridges map (1942) and previously obtained EM data (Saura and Sorsa, 1979; Saura, 1983) follow: the 38C5-6 band consists of two single bands; all other bands designated as double look like single ones, and fine bands, such as 38C4, 10, 38F2, 6, 39A6, 39C3, 4, 39D4, 5, 39E5 were not revealed in this study.

For localization of the *Dhr38* gene on the new EM map *in situ* hyridization of biotinylized DNA probe of λTD31 clone containing cDNA of this gene was carried out with polytene chromosomes. A signal was observed in the region of the large 38D1-2 band (Figure 2a). A similar pattern was obtained after *in situ* hybridization of P-element DNA with chromosomes of *l(2)02306* strain (Figure 2b), containing insertion of PZ construction in the TATA box region of the *Dhr38* regulatory zone (Kozlova *et al.*, 1998). PZ construction contains material of *lacZ* and *ry* genes and its length is about 17kb. That is why it could be proposed that insertion of such construction to *l(2)02306* strain close to the 38D1-2 band leads to formation of a new band, while its absence witness to joining of transposon material with the band as it was observed in analysis of other transposed DNA fragments (Semeshin *et al.*, 1989). As it is shown in Figure 3 EM analysis of the 38D region in *l(2)02306* strain does not reveal a new morphological structure (for comparison see Figure 1a). Insertion material probably joins to the 38D1-2 band and is incorporated into the band structure. From the data obtained we conclude that the *Dhr38* gene is localized in the dense single 38D1-2 band of the 2L chromosome.

Several deletions in the 38-39 region were mapped in the study. The new data on the break points of the deletions are given below.

Df(2) DS8: 39A6-7 – 39D3
Df(2) DS9: 38D1-2 – 39B
Df(2) TW1: 38B3 – 39D
Df(2) prA14: 37D – 39B2

Acknowledgments: The work was supported in part by a grant of the Program "Frontier Program of Genetics" and RFBR grants 96-15-97749 and 99-04-49270.

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Ontogenetic profiles of male accessory gland secretory proteins in a few species of *nasuta* subgroup of *Drosophila*.

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Seminal fluid molecules critical for many of the mating induced changes in *Drosophila* are produced in the male's accessory gland (Chen *et al.*, 1988; Kalb *et al.*, 1993; Harshman and Prout, 1994; Chapman *et al.*, 1995). These secretory proteins are sex specific, stage specific, and tissue specific (Chapman and Wolfner, 1988). Their transcripts and translational products can be detected right from the time of adult eclosion (Chen, 1985).

Stumm-Zollinger and Chen (1985) have shown that the accessory gland secretory proteins in *D. melanogaster* comprise 40 fractions in one-dimensional gels and 85 in two dimensional gels. It has been documented that in unmated males 7 days after eclosion, the secretory proteins that accumulated in the gland lumen amounts to nearly three quarters of the total soluble protein (Chen, 1991).

The *nasuta* subgroup of *Drosophila immigrans* group consists of a cluster of morphologically almost identical, related species having varying degrees of reproductive isolation (Wilson *et al.*, 1969). Shivanna and Ramesh (1995) working with the male accessory gland secretory proteins in two *nasuta* subgroup species, namely, *D. n. nasuta* and *D. s. neonasuta*, have shown that the patterns are much simpler and the accumulation of secretory proteins reaches maximum in about a week of post eclosion in the unmated males. However, the information pertaining to developmental changes in the male accessory gland secretions of both *D. melanogaster* and *nasuta* subgroup is confined to quantitative measurements. Thus the qualitative studies involving the developmental patterns of the secretory protein fractions are lacking. Present investigations were undertaken to study the pattern of accessory gland secretory protein profiles during the development of the imago in seven species of *D. nasuta* subgroup, to compare the extent of variation if any, in this tissue specific, sex specific, and stage specific protein among closely related species.

In the present study, we have used *D. nasuta nasuta* (201.001), *D. n. albomicans* (202.001), *D. n. kepulauana* (203.001), *D. kohkoa* (204.001), *D. sulfurigaster sulfurigaster* (205.001), *D. s. albostrigata* (207.001) and *D. s. neonasuta* (206.001). All these stocks were obtained from *Drosophila* stock centre, University of Mysore, Mysore, India. All these cultures were maintained at 22±1°C under uniform conditions. For the experimental purposes, synchronized eggs were collected following the modified method of Delcour (Romachandra and Ranganath, 1988). 50 eggs thus collected were allowed to develop in 3" × 1" vials containing equal quantities of food medium, maintained at a temperature of 22±1°C. The adult males, within 3 hours of their emergence from such cultures, were isolated from females and placed in fresh culture vials and were also maintained under the said environmental conditions.

Accessory glands were isolated by dissecting the males of specific age. The isolated glands were fixed in 95% ethanol to precipitate the secretions, which later were separated from the gland with the help of fine entomological needles. Samples were prepared from isolated secretions by putting them into 20µl of sample buffer (0.625 M Tris HCl pH 6.8, 2% SDS, 5% β-Mercaptoethanol and 10% Glycerol). Samples from 1 to 12 day old unmated males were prepared, processed separately and analyzed by way of SDS-polyacrylamide gels (T = 13.4%; C = 3.5%). These gels were then stained with Coomassie brilliant blue R-250 and the patterns were documented.

The major protein fractions of accessory gland secretions in various members of *nasuta* subgroup could be arbitrarily categorized into 3 groups, based on the intensity of CBB staining and homology of their SDS-PAGE mobility. When the ontogenetic patterns of accessory gland secretory proteins were analyzed, it was found that all the three major group fractions appear on day one and there is a progressive increase in their quantity from day one to day twelve (Figure 1). However, a minor fraction having an approximate molecular weight of 70 kD appears on day one and its quantity increases up to day eight and then gradually diminishes (Figure 1a). At present, the functional significance of this 70 kD fraction is not known. Further, it is observed that the rate of accumulation of all the three groups of fractions is not the same. It is observed that the group III fractions, the majority of which are produced by X-chromosomal genes (Ravi Ram and Ramesh – communicated for publication), accumulate to the maximum extent as evidenced by the thickening of bands (see Figure). Thus, the synthesis of this tissue specific and sex specific protein is characterized by progressive increase of all the secretory protein fractions, except the 70 kD fraction.

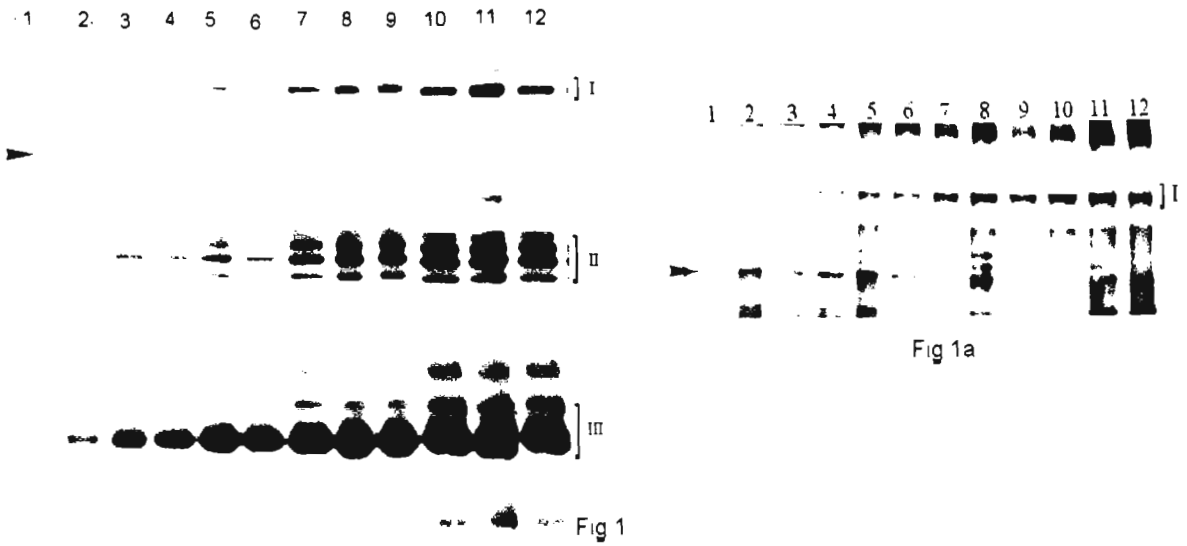

Figure 1. SDS-PAGE patterns of male accessory gland secretory proteins during development. 1-12: accessory gland samples from one to twelve day old unmated adult males (CBB R-250 staining). Figure 1a. Showing the disappearance of 70kD (arrow head) fraction (Silver staining).

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Studies on the genotoxicity of cypermethrin in *Drosophila melanogaster*.

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Cypermethrin (CAS No. 52315-07-8) is a synthetic pyrethroid insecticide used against a wide range of crop pests. It is reported to decrease the activities of acid and alkaline phosphatases in the liver and muscle of fishes (Bhatnagar *et al.*, 1995). It also depletes blood cholesterol, free fatty acids and mitochondrial enzymes in different tissues of fishes (Ghosh, 1990).

According to Pluijmen *et al.* (1984) and Klopman *et al.* (1985) this compound failed to induce gene mutations in *Salmonella typhimurium*. In *E. coli*, cypermethrin did not induce gene mutations (Brooks, 1976). Atale *et al.* (1993) observed a reduction in mitotic index and induction of chromosomal aberrations in *Capsicum annuum*. It also induced chromosomal aberrations in the spermatogonial cells of grasshopper *Poecilocerus pictus* (Shyin and Usha Rani, 1994). According to Amer and Aboul-ela (1985) this compound induced micronuclei in high frequencies in the bone marrow cells of mouse. The present communication describes the results obtained in the *Drosophila* wing spot as well as sex-linked recessive lethal tests following larval exposures.

Wing mosaic test is a fast one-generation test which assays several genetic end points induced by a mutagen in the wing primordial cells of *Drosophila*. The *mwh* +/+ *flr*^{3} trans-heterozygous larvae were obtained from the cross of high bioactive strains of *mwh* females and *flr*^{3}/TM3, *Ser* males. The allele *mwh* (*multiple wing hairs*, 3-0.3) and *flr*^{3} (*flare*, 3-38.8) are recessive genetic markers expressed autonomously as multiple trichomes or thick, misshapen trichomes on an otherwise normal adult wing. For details of the markers please refer to Lindsley and Zimm (1992). Third instar larvae were exposed to the LD_{50} (1×10^{-4} %) and lower doses of the test compound in instant food for the rest of the larval life, *i.e.*, for 48 h (Graf *et al.*, 1984). The wings of the eclosing flies were mounted and observed under a compound microscope to record the size and frequency of the mosaic spots (clones). Each experiment was repeated and the data were pooled and are represented in Table 1. For each experiment a concurrent control experiment was run where the larvae were exposed to the solvent (distilled water). The data were statistically evaluated following the conditional binomial test (Frei and Wurgler, 1988).

The sex-linked recessive lethal (SLRL) test, although time consuming as it involves more than one generation, is regarded as the best validated genotoxicity test in *Drosophila*. In this test, Oregon R larvae of same age were exposed to similar doses of cypermethrin as in the wing mosaic assay. The adult males, on eclosion, were crossed with 3 *Basc* homozygous females for 3 days. The resulting *Basc*/Y males were mated to their *Basc*/Ore-R sibs at a ratio of 1:1 in individual vials. The F_2 progeny were checked for the presence/absence of males with wild type eyes. The data on the frequency of lethal induction (Table 2) were evaluated statistically following Kastenbaum and Bowman (1970).

The frequency of small singles (*mwh* or *flr*^{3}) with 1-2 cells, large singles with 3 or more affected cells and twin (*mwh*/*flr*^{3}) spots were evaluated separately and the statistical outcomes were inconclusive for all types of spots. In this assay single spots originate due to the induction of gene mutations or gene conversions in the corresponding wild-type genes, deletion of chromosome parts carrying the wild-type alleles (Graf *et al.*, 1984) or induction of mitotic recombination in the chromosome region between the *mwh* and *flr*^{3} loci (Garcia-Bellido and Dapena, 1974). Twin spots with *mwh* and *flr*^{3} subclones, on the other hand, stem from the induction of mitotic recombination in the chromosome region between the *flr*^{3} locus and the centromere (Becker, 1976). In the present experiments, since the frequencies of different wing spots were not significantly higher than the control frequencies, it is concluded that cypermethrin is nongenotoxic in the wing primordial cells of *Drosophila*.

In the SLRL test, the frequency of lethals although higher than the control frequency was not significantly different from the control. Normally the sex-linked recessive lethals arise due to induction of

gene mutations, deletions of small chromosome parts and certain types of chromosomal aberrations (Lee *et al.*, 1983).

Cypermethrin, although it has been reported to induce sex chromosome loss in *Drosophila* (Marcos *et al.*, 1986), has failed to induce sex-linked recessive lethals in the present experiments. Thus it is concluded that this synthetic pyrethroid is nongenotoxic both in the somatic and germ line cells of *Drosophila melanogaster* following larval treatments.

Table 1. Summary of data obtained in the wing mosaic assay.

Treatment (hr)	Conc. (%)	No. of wings observed	Spots per wing (No. of spots) Small singles (s = 1-2) [m =2.0]	Large singles (s > 2) [m = 5.0]	Statistical Dignoses* Twins (t) [m = 5.0]	Total (T) [m = 2.0]
	Control	80	0.25(20)	0.04 (3)	0.00 (0)	0.29 (23)
	1×10^{-4}	80	0.36 (29) i	0.03 (2) -	0.01 (1) i	0.40 (32) i
48	5×10^{-5}	80	0.31 (25) i	0.04 (3) i	0.00 (0) i	0.35 (28) i
	2.5×10^{-5}	80	0.31 (25) i	0.01 (1) -	0.00 (0) i	0.33 (26) i

*Statistical diagnoses according to Frei and Wurgler (1988). + = positive, i = inconclusive, - = negative, m = multiplication factor. Probability levels: Alpha = Beta = 0.05. One-sided statistical tests.

Table 2. Summary of data obtained in the sex-linked recessive lethal test.

Treatment (hr)	Conc. (%)	Males tested	Tested chromosomes per male (Mean ± SD)	X chromosomes Total	Lethal	%	Conclusion*	Lethals per male 0	1	2
	Control	134	18.51 ± 3.08	2480	7	0.28		147	7	0
	1×10^{-4}	52	20.37 ± 1.63	1059	5	0.47	NS	47	5	0
48	5×10^{-5}	68	18.69 ± 2.41	1271	4	0.31	NS	64	4	0
	2.5×10^{-5}	64	20.16 ± 1.75	1290	4	0.31	NS	60	4	0

* Conclusion on the basis of Kastenbaum and Bowman (1970). NS = not significant, level of significance $P < 0.05$.

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The comparison of the induced and spontaneous frequencies of somatic mosaicism for the abdomen and the eye-antennal, prothoracal and wing imaginal disk derivatives in *Drosophila melanogaster*.

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The somatic recombination of the recessive on the homozygous X-chromosomal marker genes in *D. melanogaster* individuals is widely used in the test systems for chemical compounds mutagenic activity.

We studied the induced and spontaneous frequencies of the *y* and *sn* spots, appearing as a result of somatic mutations and recombinations in the *y* + +/+ *w sn*^{3} females' abdominal tergites and imaginal disk derivatives: head, notum, humerus and sternopleura. It is expected that the mosaic spots registration on the head, notum, humeras, sternopleura and on the abdomenal tergites will allow identification of mutagens, which require or do not require metabolic activation. The stable will affect primarily the abdominal cells, which start division during the prepuparitation, and less affect the imaginal disks cells, which start division earlier (in 15 - 17 hours after the larva hatching). The direct acting unstable mutagens (which do not need metabolic activation) will affect both the imaginal disks derivatives and the histoblasts nests derivatives in the abdomen.

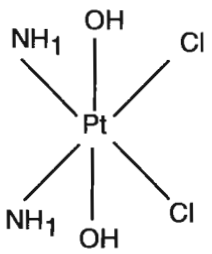

Figure 1. Structural formula of oxoplatin.

In the course of the experiment the *y* females × *w sn*^{3} males were crossed. The parents were placed into the standard vials with 10 ml of the agar-yeast nutrient medium for a period of 30 hours under a temperature of 25 °C. Upon this period the parents were taken out, and the newly hatched first instar larvae (F_1) were put to experimental use. In the case of the induced mosaicism the nutrient medium with the first instar larvae was treated by the 2 g/l oxoplatin solution (the anti-tumor drug, which is a strong mutagen of the platinum family). For its structural formula see Figure 1. The rate of induced mosaicism was compared to that of untreated control. For details see Reference 1.

The *y* + +/+ *w sn* ^{3} females from the F_1 were examined and the *y* and *sn* macrochaeta spots were registered. On the imaginal disks derivatives the 7 head macrochaetes, 2 humeral, 11 notal and 2 sternopleural ones on each side were taken to account (44 macrochaetes total). On the abdomen the 11 macrochaetes of the posterior row on each side on the segments II-VI were considered (110 macrochaetes total). The spot localization was presented by a small scheme. The results of the experiments are shown in Table 1.

Table 1. Induced and spontaneous mosaic spots frequencies on the imaginal disks derivatives and abdomen of the *y + + / + w sn*^{3} females.

	Spontaneous mosaicism			Induced mosaicism (oxoplatin, 2g/l)		
Females observed	4053			625		
Mosaic spots and their frequencies Spots localisation	y (p, %) [p_1, %]	sn (p, %) [p_1, %]	Σ spots (p, %) [p_1, %]	y (p, %) [p_1, %]	sn (p, %) [p_1, %]	Σ spots (p, %) [p_1, %]
Head, humeras, notum, sternopleura	12 (0.296)* [6.729×10^{-3}]	29 (0.716)* [1.626×10^{-2}]	41 (1.012)* [2.300×10^{-2}]	145 (23.200) * [0.527]!	203 (32.480)* [0.738]!	348 (55.680) * [1.265]!
Abdomen	49 (1.209) * [1.099×10^{-2}]	62 (1.530) * [1.391×10^{-2}]	111 (2.739) * [2.490×10^{-2}]	263 (42.080) * [0.383]!	278 (44.480) * [0.404]!	541 (86.560) * [0.787]!
Total	61 (1.505) * [9.773×10^{-3}]	91 (2.245) * [1.458×10^{-2}]	152 (3.750) * [2.435×10^{-2}]	408 (65.280) [0.424]	481 (76.960) [0.500]	889 (142.240) [0.924]

The differences between the pairs of frequencies within same column marked by asterisk (*) or by notes of exclamation (!) are significantly different in the Fisher's test at 1% significance level. p is the somatic mosaicism frequency per 1 female, %, p_1 is the somatic mosaicism frequency per 1 macrochaeta, %, p_1 = spots number/ (females number x macrochaetes number on the given structure), where macrochaetes number = 44 for head, humeras, notum and sternopleura taken together, 110 for abdomen and 154 for total flies.

Table 2. The χ^2 values at 1 degree of freedom for H_0 about 1:1 *y* and *sn* mosaic spots ratio on abdomen and on the head, notum, humeras, sternopleura in cases of spontaneous and induced mosaicism.

	Spontaneous mosaicism	Induced mosaicism
Imaginal disks derivatives	7.048 p< 0.01	9.666 p< 0.005
Abdomen	1.523 p> 0.10	0.416 p> 0.50

It was shown that:

1. The somatic mosaicism frequencies per 1 fly are significantly higher for abdomen than for other studied areas of the fly surface both in spontaneous and induced mosaicism cases (see Table 2). Therefore, observing of abdomen, which carries many more macrochaetes in comparison with other studied structures, considerably improves the test resolution. The increase in the number of bristles taken into account has the same effect as with increased number of flies examined.

2. The somatic mosaicism frequencies per 1 macrochaeta have no statistically significant differences for abdomen and imaginal disks derivatives both in case of induced and spontaneous mosaicism.

3. It's known that on the imaginal disks derivatives in $y + +/+ w\ sn^3$ heterozygotes the mosaic spot frequency *y* is higher than the mosaic spots frequency of *sn* (Stern, 1936). This regularity remains also for the imaginal disk derivatives in $y + +/+ w\ sn^3$ *vg/vg* heterozygotes both in the case of induced and spontaneous mosaicism. However, the *y* and *sn* mosaic spot frequencies obtained from the abdominal macrochaetes are related as 1:1 both in the case of induced and spontaneous mosaicism (see Table 2).

References: Ref. 1, 1982, Metodicheskie rekomendatsii po primeneniu somatichestogo mutagenesa u *Drosophila Melanogaster* v kachestve test-sistemi dlia uskorennogo opredelenia kanzerogenov, - M. MZ USSR, 1982; Stern, C., 1936, Genetics, 21: 625-730.

The behavior of *Drosophila pavani*, *Drosophila gaucha*, and their reciprocal hybrids in stressful environments.

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Drosophila pavani and *D. gaucha* are two sibling species that with another six constitute the *mesophragmatica* group; these species are endemic to South America (Val *et al.*, 1981). They are predominantly Andean in their distribution. In addition, *D. pavani* and *D. gaucha* may produce abundant interspecific hybrids under laboratory conditions; these hybrids are, however, sterile (Brncic and Koref-Santibañez, 1957). In the present study we compare the behavior of *D. pavani*, *D. gaucha* adult flies and their reciprocal hybrids in a reduced space in the presence and in the absence of food. The aim of this investigation is to inquire whether adults of these four types of genotypes exhibit a similar behavior in response to stress. It is important to investigate this type of problem because behavioral changes under stress conditions may reveal information on the role of behavior in determining patterns of geographical distribution (Hoffmann and Parsons, 1994). Under environmental stress the parental species may show a similar behavior, but the species hybrids may exhibit a different one. In this case we could infer that the species differ genetically in the control of the behaviors observed.

We used strains originated from adults collected in Chillán (*D. pavani*) and Buenos Aires (*D. gaucha*). Virgin flies of those strains were reciprocally crossed and the behavior of F_1 adults of the four types of genotypes (p × p, g × g, p × g, and g × p) was observed in vials of 18 and 36 cm^3 in volume. The flies used were grown individually in isolation vials filled with 4 cm^3 of culture medium. Once filled with the medium, the vials had 36 cm^3 of free space; they will henceforth be called "big space vials". In one experiment, adult flies of the Chillán and Buenos Aires strains were introduced individually in "virgin" big space vials and their behavior was observed for 2 min. In another experiment, the flies were introduced in vials with food where the available space was 18 cm^3 ("reduced space vials"), and their behavior recorded for 2 min. In a third experiment, flies of the mentioned strains were individually introduced in reduced space vials without food

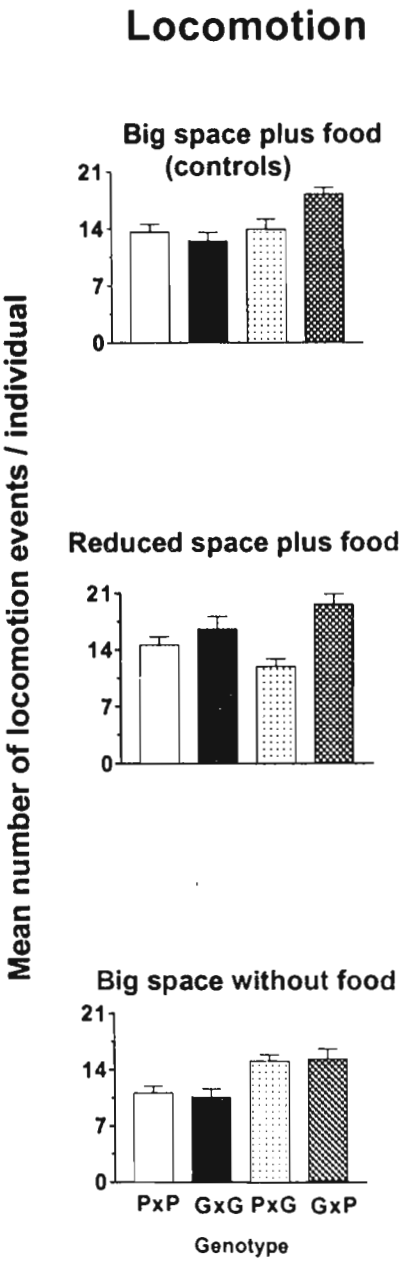

Figure 1. Locomotion rate of the p × p, g × g, p × g and g × p males in vials of 18 cm^3 (reduced space) and 36 cm^3 (big space) of free space, in the presence and in the absence of food.

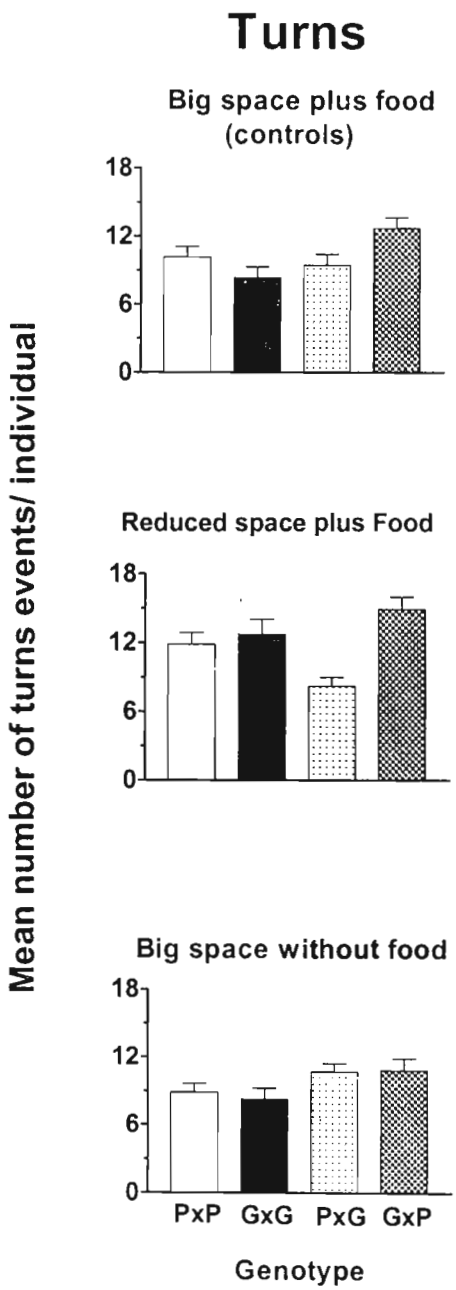

Figure 2. Turning behavior of the p × p, g × g, p × g and g × p males. Other details as in Figure 1.

and their behavior recorded for 2 min. In the three kinds of experiments we always used "virgin" vials for each fly. The behaviors recorded were: i) locomotion, ii) turning, iii) jumps and iv) grooming. Preening was exhibited while the flies remained without movement in the vials.

Figures 1 and 2 show the rates of locomotion and turning of males of the four types of genotypes in the environments. In big space vials the locomotion and turning rates of the g × p males statistically differ

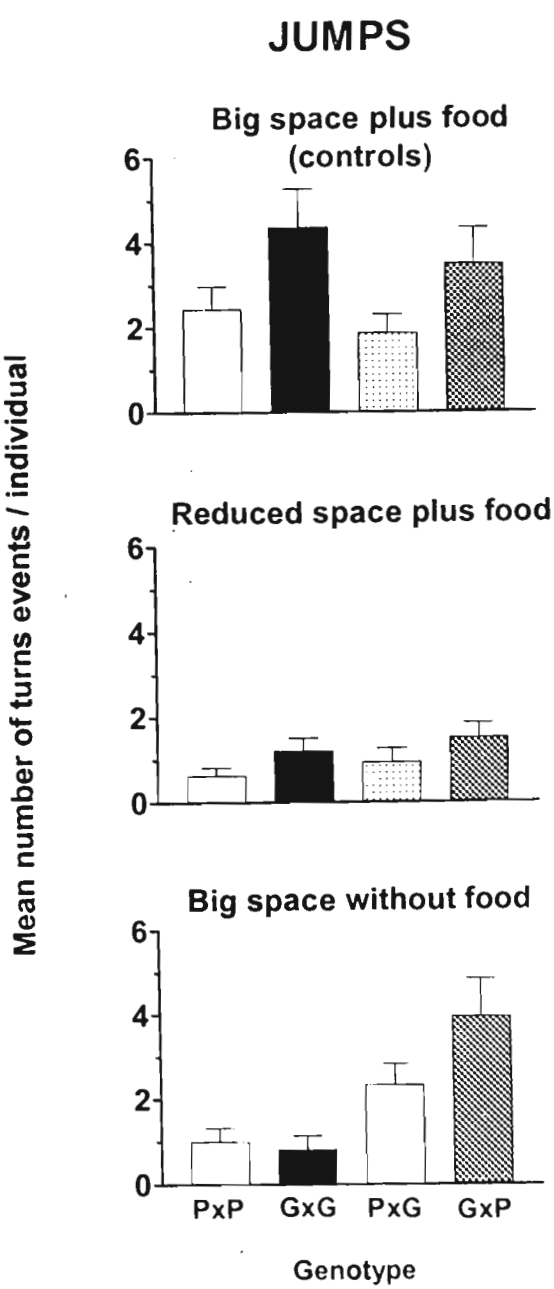

Figure 3. Jumping rate of the p × p, g × g, p × g and g × p males. Other details as in Figure 1.

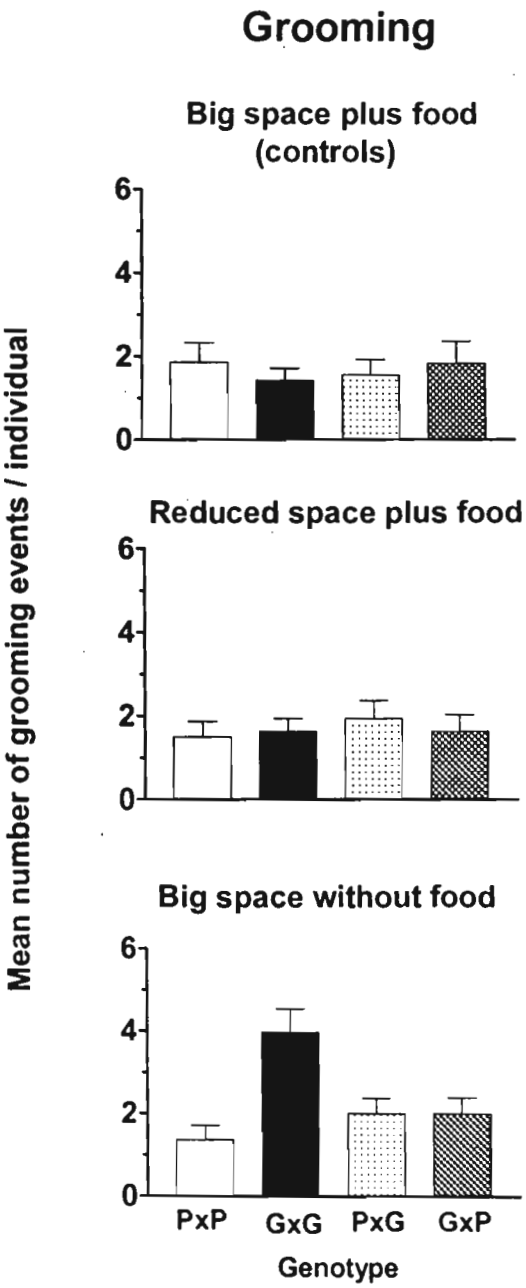

Figure 4. Grooming behavior of the p × p, g × g, p × g and g × p males. Other details as in Figure 1.

from the other three genotypes (p × p, g × g and p × g) (Figures 1 and 2) (ANOVA). However, in reduced space vials, the p × g hybrid males show a lower locomotion and number of changes in direction than p × p, g × g and g × p males (ANOVA). In big space vials without food, the p × p and g × g males reduced locomotion, but the turning rate is similar among the p × p, g × g, p × g and g × p males (ANOVA). Figure 3 shows the jumping rate of males of the four types of genotypes. In big space vials, the jumping rate of the g × g and p × g males is statistically different to that of the p × p and p × g males. In reduced space vials, the jumping rate decreased in the four types of males without statistical differences between them. By contrast, in

big space without food vials, the jumping rate of the species hybrids is greater than that of the parental species (ANOVA). Figure 4 shows the rate of grooming of the p × p, g × g, p × g and g × p genotypes. Statistically significant differences between the four genotypes were only found in big space vials without food, that is *D. gaucha* males substantially increase grooming in big space vials without food. On the other hand, in contrast with the males, females of the four types of genotypes show a similar behavior in the three environments where the male's behavior was studied (ANOVA) (data not shown).

Locomotion and turning are behavioral elements that configure patterns of movement in *Drosophila* adults and larvae (Godoy-Herrera *et al.*, 1997). Our results indicate that in the same environment, males of the four groups of genotypes may modify those behaviors in different ways. Thus, in big-space vials with food, p × p males decrease locomotion and turning rate while g × p males increase these behaviors, indicating there exists a genotype-environment interaction for their expression. These results seem to suggest that the g × p hybrid males could adjust in different ways to stressful environments. It is also interesting to note that the jumping rate decreases when the males are confined in reduced space. Jumping seems merely to represent aborted flight of the males in the vials. Given that in a relatively reduced space male jumping behavior equally decrease in the four groups of genotypes, we conclude that there is no genotype-environment interaction for the expression of such behavior. Grooming seems to increase in stressful circumstances (Hoffman and Parsons, 1994), and it is believed that it counteracts the effect of stress (Equibar and Moyaho, 1997). Our results show statistically significant differences for grooming rate between the four groups of genotypes when the males are confined in big vials without food and water. This kind of environment is probably very stressful for the flies. The results indicate that g × g males exhibit the greater rate of grooming, suggesting that this genotype could be more sensitive to the absence of food and water. In contrast with the males of the four groups of genotypes, the females did not show significant differences among them for the behaviors observed. We are planning further experiments to better understand these findings.

Acknowledgments: Supported by Fondecyt 1960727.

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Perturbation of sex determination in the strain *In(1)BM2(reinverted)*.

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The strain *In(1)BM2(reinverted)* of *Drosophila melanogaster*, shows a mutation in the structure of the polytene male X chromosome (Mazumdar *et al.*, 1978). In third instar male larvae reared at 18°C, about 25% of polytene nuclei reveal X chromosomes that appear twice as wide as the X chromosome of wild type larvae (Figure 1a). However, unlike polytene chromosome puffs, such puffy Xs do not manifest enhanced transcriptional activity (Kar and Pal, 1995). This characteristic thus classifies the puffy Xs as pompons (reviewed in Zhimulev, 1995). The absence of correlation between puffing and transcription suggests that the chromosomal rearrangement perturbs a pathway that controls the structure, but not the transcription, of the male X chromosome. The sex and chromosome specificity further suggests the possibility that the rearrangement affects the function of a regulator of the dosage compensation pathway.

The expression of the puffy Xs is controlled by the rearranged breakpoint at the 16A region of the polytene chromosome (Kar and Pal, 1995). In order to map the rearranged breakpoint, mutagenesis of segment 16A of *In(1)BM2(reinverted)* X chromosome has been initiated. Freshly eclosed *In(1)BM2(reinverted)* males were irradiated with 4000 rads of g-irradiation and mated to virgin *Df(1)B* females (*Df(1)15F9-16A1; 16A6-7*, Lindsley and Zimm, 1992). Flies emerging from such matings were scored for lethal or visible mutations, alteration in sex ratio and reversal of the puffy X phenotype.

In one of the matings, a single intersexual female was recovered. A stock was made from the females that were heterozygous for a balancer chromosome and the irradiated chromosome, and the line was designated as $B^{M2\neq1.36}$. Less than 5% of heterozygous females manifest transformation in sexual characteristics (Figure 1b). $B^{M2\neq1.36}$ males were viable. Sexually dimorphic characters of such males were unaffected, with the exception of one male where the orientation of the sex comb was altered. Cytological examination did not reveal the presence of any visible deletions at the 16A region of the X chromosome. Third instar male larvae of $B^{M2\neq1.36}$ however did not show any puffy X chromosomes when reared at 18°C. This indicated that reversal of the puffy X phenotype had been obtained. Mapping of the mutation in $B^{M2\neq1.36}$ is currently underway. Although there are three ealier reports of perturbation of the sexual phenotype in *In(1)BM2(reinverted)* (Kar and Pal, 1995; Chakraborti *et al.*, 1996; Mukherjee and Basu, 1997), it is not known whether the structural alteration of the X chromosome and the sexual transformation are brought about due to the perturbation in the function of the same gene or not.

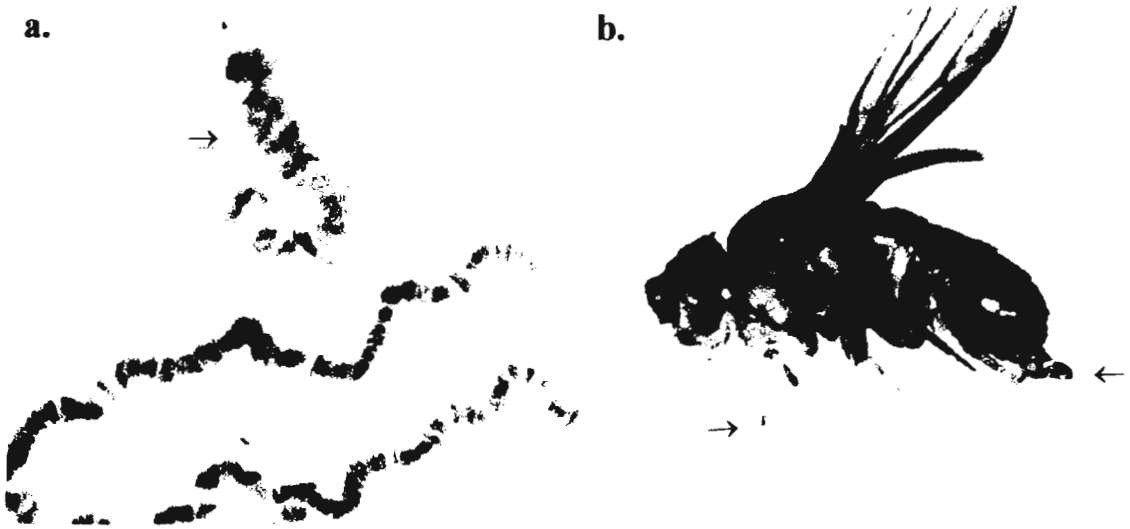

Figure 1. (a), Puffy male X chromosome (→) of the strain $In(1)B^{M2}$ *(reinverted);* (b), Phenotype of intersexual $B^{M2\neq1.36}$ / FM7 female. ← indicates transformed genitalia and → indicates sex combs on prothoracic leg.

References : Chakraborti, D., *et al.,* 1995, Genome 38: 105-109; Kar, A., and J.K. Pal 1995, J. Genet. 74: 47-59; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster; Academic Press Inc., New York; Majumdar, D., *et al.,* 1978, Cell Chromosome News Lett. 1: 8-12; Mukherjee, A. S., and S. Basu 1997, Indian J. Exp. Biol. 35: 203-211; Zhimulev, I.F., 1995, Adv. Genet. 34: 1-497.

Effects of insulin, wortmannin, LY294002, and rapamycin on protein phosphorylation in the *Drosophila* ovary.

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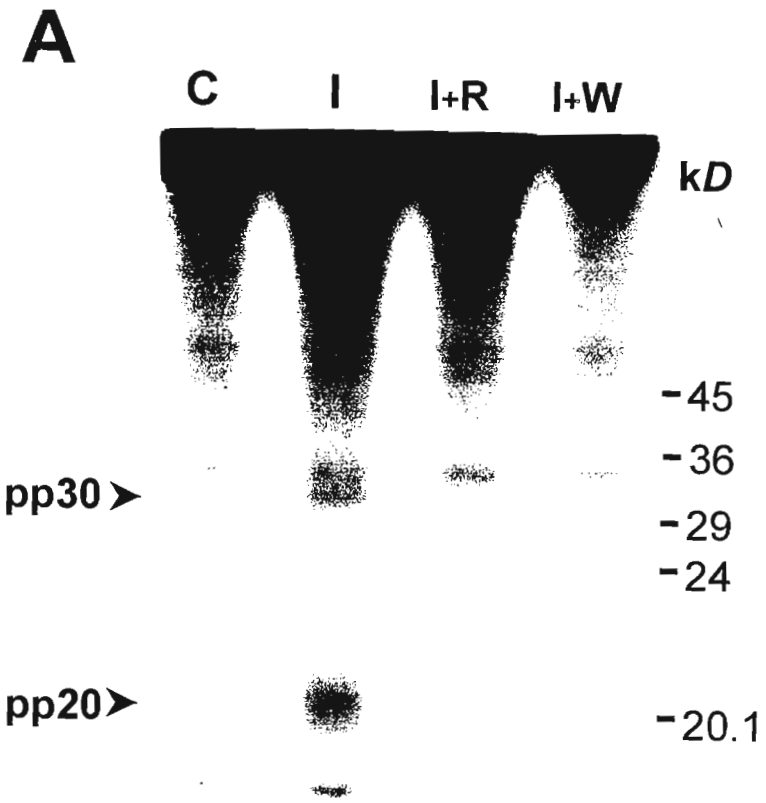
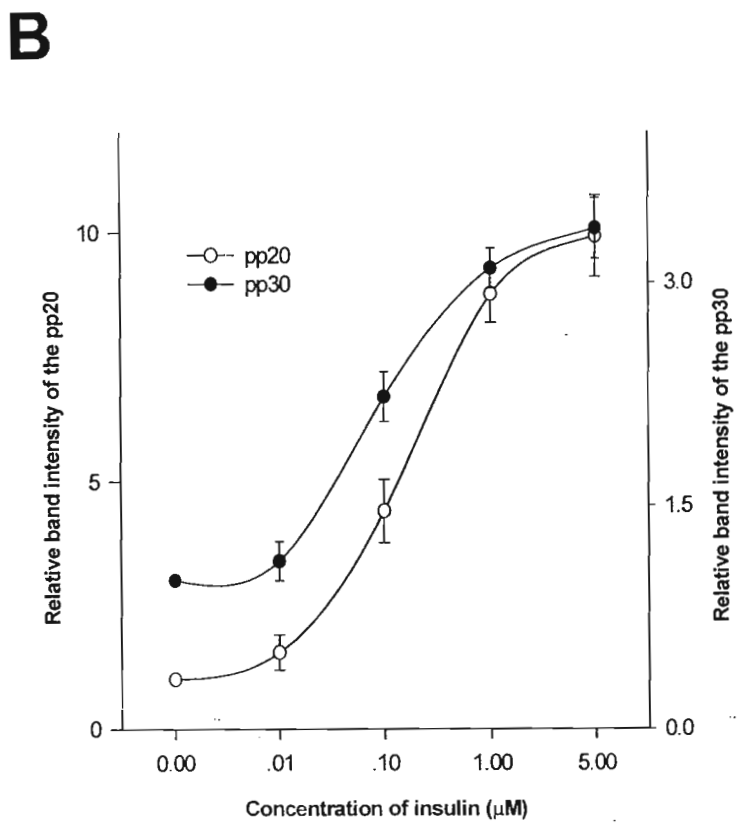
Abstract: Although *Drosophila* insulin-like peptide and insulin receptor have been isolated and characterized, the downstream signal of insulin has not been described well in *Drosophila*. To examine the regulatory mechanism of insulin in the ovary, we investigated the protein phosphorylation induced by insulin. Two proteins (appropriate *M*r-20,000 protein and a *M*r-30,000 protein) were identified as insulin-induced phosphoproteins at low molecular weight range (70,000 – 14,000 Dalton). As in vertebrates, these insulin-





induced protein phosphorylations were inhibited by wortmannin, LY294002, and rapamycin which are known to be inhibitors of elongation initiation factor 4E binding protein (4E-BP) and ribosomal protein S6 phosphorylation. These results imply that the downstream signal of insulin might be well-conserved in *Drosophila.*

Introduction:

Recent studies in vertebrates have suggested that insulin is essential for normal development including oocyte maturation as well as for proper signal pathways (James and John, 1981; Chung *et al.*, 1992; Jefferies *et al.*, 1994). Insulin has been proposed to be a *Drosophila* hormone, although it is originally identified as a pancreatic hormone of vertebrates. Primary embryonic cells treated with high concentration of insulin were induced to differentiate (Seecof and Dewhurst, 1974). Moreover, mutations in the *Drosophila* insulin receptors result in abnormal development of both neurons and glia (Fern<ndez *et al.*, 1995).

The use of specific inhibitors wortmannin, LY294002, and rapamycin facilitated the studies on the insulin pathways. The rapamycin

Figure 1. Effects of insulin, rapamycin, and wortmannin on the phosphorylation of the pp20 and the pp30 (A) C, control; I, insulin (1 μM); R, insulin (1 μM) after treatment of rapamycin (1 μM); W, insulin (1 μM) after treatment of wortmannin (1 μM). (B) The results represent relative band intensity of the pp20 (open circle) and the pp30 (closed circle). Its band intensity was measured as described under "Materials and Methods". The values are means ± S.E. (n = 5).

is an immunosuppressant that specifically inhibits activity of the mammalian target of rapamycin (Chung *et al.*, 1992; Price *et al.*, 1992). Treatment of mammalian cells with rapamycin results in blocking of the phosphorylation of elongation initiation factor 4E binding protein (4E-BP) (von Manteuffel *et al.*, 1996; Brunn *et al.*, 1997) and ribosomal protein S6, by blocking stimulation of p70 S6 kinase (Chung *et al.*, 1992). Wortmannin and LY294002, PI3-kinase inhitors, also exert the same effects on these molecules (Chung *et al.*, 1994; Vlahos *et al.*, 1994; Brunn *et al.*, 1996). These inhibitions repress translation of mRNAs having the polypyrimidine tract motif (Jefferies *et al.*, 1994).

The insulin has been well described as a potential mechanism for regulation of cellular function in the ovary of vertebrates; however, insulin signal has been rarely explored in the *Drosophila* ovary. To investigate the downstream of insulin pathway in the ovary of *Drosophila*, we examined the effects of insulin, rapamycin, wortmannin, and LY294002 on ovary protein phosphorylation.

Table 1. Effects of LY294002 on the insulin-induced phosphorylation of the pp20 and the pp30. The results represent relative band intensity of the pp20 and the pp30. The values obtained were normalized to that obtained in the absence of LY294002 (1) and are means ± S.E. (n =3).

	Insulin (1 μM)	LY294002 (5 μM) + Insulin (1 μM)	LY294002 (10 μM) + Insulin (1 μM)
pp20	1	0.93 ± 0.03	0.19 ± 0.02
pp30	1	0.65 ± 0.12	0.34 ± 0.12

Materials and Methods:

Materials: Porcine insulin and LY294002 were purchased from Sigma Chemical Co. Rapamycin and wortmannin were from Research Biochemicals International Co. Ortho-$^{32}PO_4$ (10 mCi/ml) was from Amersham Co.

Phosphorylation of ovary proteins: A pair of ovary was dissected from adult fly (5-day old) and incubated in phosphate-free Grace's medium for 15 min. The sample was incubated for another 15 min in the absence or the presence of rapamycin, wortmannin, or LY294002 and then porcine insulin was added. After 15 min incubation, ortho-$^{32}PO_4$ (0.5 mCi/ml) was added to the media. After 90 min labeling, the tissue was sonicated, and the extract was separated by SDS-PAGE (15%). The gel was dried and autoradiographed. In order to determine the level of protein phosphorylation, the autoradiograph was scanned and analyzed with SigmaGel (ver 1.0).

Results and Discussion:

From phosphorylation assay with intact ovaries, at least two prominent proteins (one is a *Mr*-30,000 protein and the other is about *Mr*-20,000 protein) were found to be phosphorylated by insulin. These were referred to as phosphoprotein 20 (pp20) and phosphoprotein 30 (pp30), respectively (Figure 1). Insulin significantly increased the levels of the phosphorylation with half-maximal phosphorylation at 0.21 μM (pp20) and 0.09 μM (pp30) (Figures 2A and B). At concentrations above 1 μM, incubation of the ovary with insulin resulted in about 10-fold (pp20) and 3-fold (pp30) increases. From their size and effects of specific inhibitors described below, these proteins can be inferred as the *Drosophila* homologue of 4E-BP (apparently molecular mass of rat counterpart is 22 kDa; Diggle *et al.*, 1995) and *Drosophila* ribosomal protein S6 (31 kDa; Spencer and Mackie, 1993). It was reported that 4E-BP from most mammalian cells appears as several bands in SDS-PAGE (Lin *et al.* 1994), because of decreased electrophoretic mobility following the phosphorylation levels, and of the existence of isoforms. Consistent with this, pp20 showed a broad band pattern, and appeared as 3 bands sometimes (data not shown).

As in vertebrates, rapamycin, a mTOR inhibitor, attenuates the effect of insulin in *Drosophila*. The insulin-stimulated protein phosphorylations were blocked by rapamycin in a dose-dependent manner and with half-maximal inhibition (I_{50}) at each 0.5 μM (pp20) and 0.8 μM (pp30), respectively (Figure 3A). Wortmannin, a PI3-kinase inhibitor, also inhibits the phosphorylation of the proteins. I_{50} of wortmannin on the pp20 and the pp30 were 0.05 and 0.06 μM, respectively (Figure 3B). LY294002, another PI3-kinase inhibitor that is more specific than wortmannin (Vlahos *et al.*, 1994), inhibited the insulin-induced phosphorylation on the concentration of 10 μM (Table 1). These results suggest that the pp20 and the pp30 might be the components of the pathway regulated by PI3-kinase and mTOR.

So far, there is no evidence for the *Drosophila* homologue of mTOR (DTOR). However, evidence for the existence of the substrates of the DTOR has been accumulated. It was reported that the S6 phosphorylation and *Drosophila* p70 S6K activity are sensitive to rapamycin (Fern<ndez *et al.*, 1995; Stewart *et al.*, 1996).

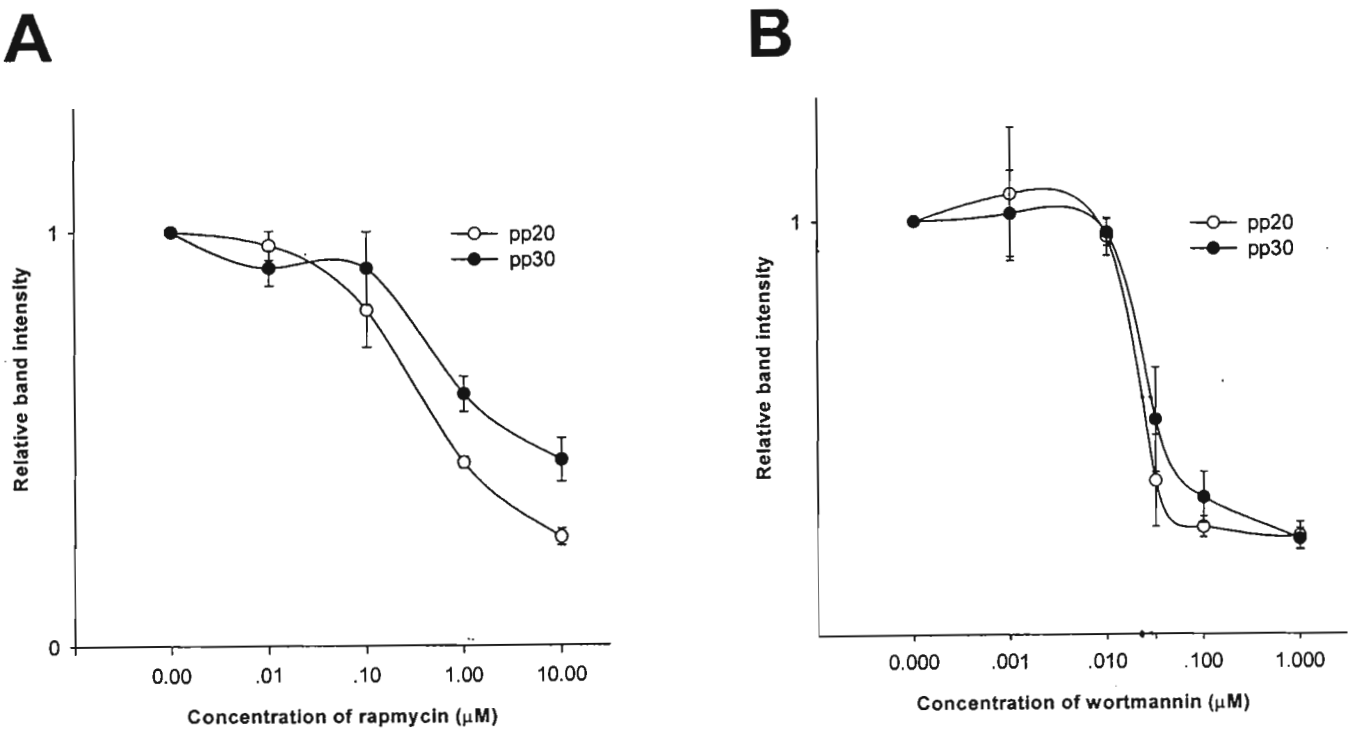


Figure 2. Effects of rapamycin and wortmannin on the insulin-induced phosphorylation of the pp20 and the pp30. The results represent relative band intensity of the pp20 (open circles) and the pp30 (closed circles). The values obtained were normalized to that obtained in the absence of rapamycin and wortmannin (1) and are means ± S.E. (n = 5). (A) Effects of rapamycin on the insulin-induced phosphorylations. (B) Effects of wortmannin on the insulin-induced phosphorylations.

Another potential substrate of the DTOR, *Drosophila* homologue of 4E-BP, has recently been cloned and characterized (Miron *et al.*, 1997). Taken together, our results suggest that the insulin pathway might be well conserved in *Drosophila*, and that the insulin might play an important role in the cellular process of the *Drosophila* ovary.

Acknowledgments: This study was supported by grants from Korea Science and Engineering Foundation (KOSEF) through the Research Center for Cell Differentiation at Seoul National University and from the Basic Science Research Program (Ministry of Education, Korea).

References: Brunn, G.J., *et al.*, 1996, EMBO J. 15: 5256-5267; Brunn, G.J., *et al.*, 1997, Science 277: 99-101; Chung, J., *et al.*, 1992, Cell 69: 1227-1236; Chung, J., *et al.*, 1994, Nature 370: 71-75; Diggle, T.A., *et al.*, 1995, Biochem J. 306: 135-139; Fern<ndez, A.R., *et al.*, 1995, EMBO J. 14: 3373-3384; James, L.M., and W.K. John 1981, Dev Biol. 85: 309-316; Jefferies, H.B., *et al.*, 1994, Proc. Natl Acad. Sci. USA 91: 4441-4445; Lin, T.A., *et al.*, 1994, Science 266: 653-656; Miron, M., *et al.*, 1997, A. Conf. Dros. Res. 38: 282B; Price, D.J., *et al.*, 1992, Science 257: 973-977; Seecof, R.L., and S. Dewhurst 1974, Cell Differ. 3: 63-70; Spencer, T.A., and G.A. Mackie 1993, Biochim Biophys Acta 1172: 332-334; Stewart, M.J., *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93: 10791-10896; Vlahos, C.J., *et al.*, 1994, J. Biol. Chem. 269: 5241-5248; von Manteuffel, S.R., *et al.*, 1996, Proc. Natl. Acad. Sci. USA 93: 4076-4080.

Developmental expression of the rp49 gene in *Drosophila melanogaster* and *D. simulans*.

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The rp49 gene is often used in *Drosophila* as a reference to estimate the amount of RNA loaded in northern blot experiments. However, although its expression pattern was once reported in a *Drosophila melanogaster* strain (O'Connell and Rosbash, 1984), no detailed study concerning its quantitative variation along development is available. We have investigated the variations of rp49 expression in two populations of *Drosophila melanogaster* and five populations of *D. simulans*.

Total RNA was extracted by the guanidium chloride method (Cox, 1968) from second and third instar larvae, pupae, and adult males and females. RNA was size-fractionated on agarose gel, BET-stained,

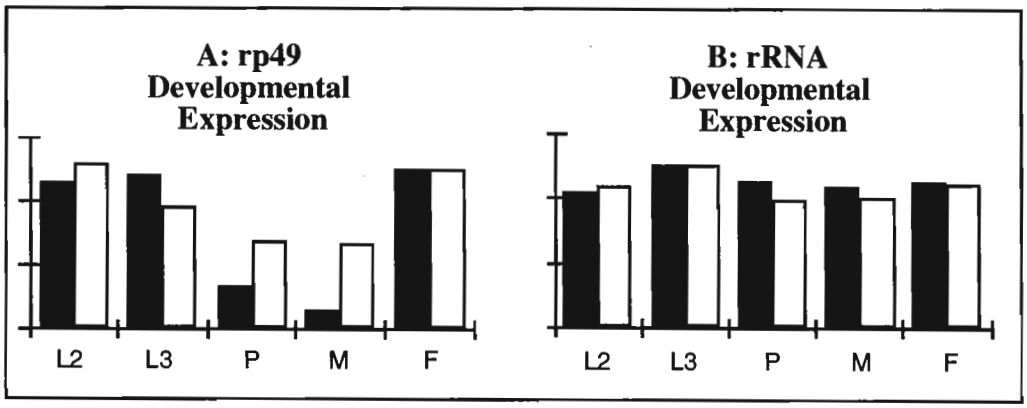

Figure 1: Expression patterns of rp49 (A) and rRNA (B) genes for different developmental stages: second instar larvae (L2), third instar larvae (L3), pupae (P), adult males (M) and females (F). Data correspond to mean values. (■) *D. melanogaster*, (□) *D. simulans*.

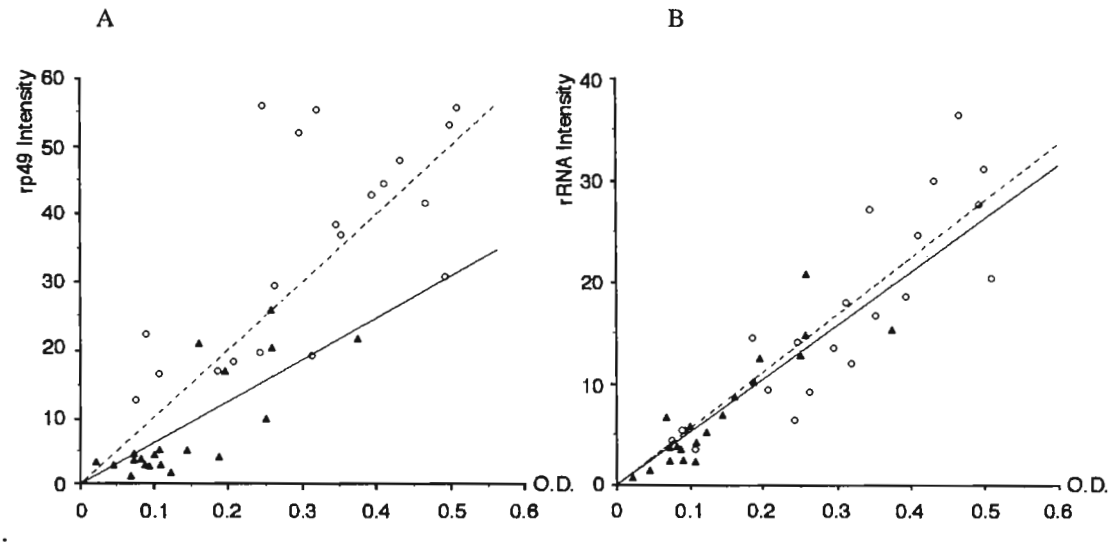

Figure 2: Relationship between rp49 (A) and rRNA (B) spot intensity and optical density (O.D.) for males (▲) and females (O). Data from the two species are pooled.

transferred onto nylon membrane, and hybridized with a rp49 radiolabelled probe. Image analyses of rp49 autoradiographic spots and rRNA BET-stained spots were performed as in Loevenbruck *et al.* (1991). RNA amounts were estimated in three ways: 1) by measurement of Optical Density (O.D.) at 260 nm for one µl of extract, 2) by quantitation of rp49 spots, and 3) by quantitation of rRNA spots. For a comparison of the three sets of data, the last two values were divided by the volume loaded so as to give an intensity estimated for one µl of extract.

Figure 1 shows the expression of rp49 (Figure 1A) and rRNA genes (Figure 1B) for different developmental stages in the populations of *D. melanogaster* and *D. simulans*. Only one 600 bp long transcript was observed for rp49, as in O'Connell and Rosbash (1984). The two rp49 and rRNA genes did not present the same expression pattern, although they were expressed at all stages of development. As seen in the figures, rRNA expression remained constant along development while rp49 expression varied greatly. The rp49 expression was lower in pupae than in larvae and much greater in females than in males. Both rp49 and rRNA expression patterns were conserved between the two *Drosophila* species.

A statistical analysis was performed on data from males and females. Figure 2 shows that although the expected linear relationship between spot intensity and O.D. was highly significant for both rp49 ($r^2_m = 0.823$, $r^2_f = 0.907$) and rRNA ($r^2_m = 0.930$, $r^2_f = 0.941$), the slopes differed between males and females for rp49 but not for rRNA. These results show that the rp49 gene of *Drosophila* can be used for comparing gene expression of different samples at the same developmental stage. This gene should not be used, however, when different stages are considered.

References: Cox, R.A., 1968, Methods Enzymol. 12: 120-129; Loevenbruck, C., C. Biémont, and C. Arnault 1991, Fingerprint News 3: 8-10; O'Connell, P., and M. Rosbasch 1984, Nucl. Acid Res. 12: 5495-5503.

Molecular characterization of the insertion site in eight P-insertion lines from the Kiss Collection.

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As part of the genetics laboratory at Denison University my undergraduate students and I have both meiotically mapped the P{w+} inserts (data not shown) and molecularly characterized the insertion sites of eight P-insertion lines from the Kiss Collection (Torok *et al.,* 1993) available through the Bloomington Stock Center. We carried out plasmid rescue of 3' flanking DNA after digesting genomic DNA with *Eco*RI (Bier *et al.*, 1989). This rescued 3' flanking DNA was sequenced using a primer that recognizes the P-element's inverted repeat, yielding 600 – 900 nucleotides of sequence. These genomic sequences were used in BLAST searches during the week of 4-29-99 against the Berkeley Drosophila Genome Project database (www.fruitfly.org).

Two of these lines' (P539, P996) 3' flanking DNA had already been characterized, and so served as positive controls for the plasmid rescue technique, the sequencing and the database searches (Table 1).

Table 1. Matches to 3' plasmid rescue sequences previously characterized.

Bloomington Stock[a]	3' Flanking DNA match[b]
P539	AQ034143; bases
l(2)k04203	503-764
	(1.6e – 52)
P996	AQ025938; bases
l(2)k10609	122-183
	(7.4e-3)

Two of these lines (P174, P1112) had significant matches to both genomic clones and cDNAs or ESTs in the database, leading us to conclude that they are new alleles of previously identified genes (Table 2). Both lines' genomic localization corresponded to the transposon insertion site as determined by *in situ* mapping. Line P174 is likely to be an allele of burgundy (*bur*). Line P1112 is likely to be an allele of downstream of receptor kinase (*drk*). Line P1112's mutant phenotype in wing imaginal discs has been recently determined (Roch *et al.*, 1998).

Two of these lines (P420, P539) had significant matches to an EST, but not to a genomic clone (Table 2). Line P420 likely represents a unique allele of the gene known only by the EST GH16502, while line P539 likely represents a unique allele of the gene known by the EST GH20022.

Table 2. Results of BLAST searches.

Bloomington Stock[a]	Genomic DNA match[b]	cDNA or EST match[c]	Allele of[d]:
P174 l(2)10523 39B01-02	AC006574; bases 96,803-97,675 39A03-39B01 (7.5e-186)	LD17122; bases 1-537 (1.0e-113)	burgundy (bur)
P1112 l(2)k13809 50A12-14	AC005652; bases 67,877-68,654 50A (1.3e-184)	GH14963; bases 1-668 (2.7e-143)	downstream of receptor kinase (drk)
P420 l(2)00628 60A08-09	Not identified	GH16502; bases 61- 629 (3.3e-116)	
P539 l(2)k04203 33C04-05	Not identified	GH20022; bases 132-398 (1.3e-53)	
P381 l(2)10642 26B08-09	AC004758; bases 38-883 26A05-26B05 (4.4e-163)	Not identified	
P918 l(2)k09801 58D04-05	AC005714; bases 57,281-58,136 58D04-58E02 (3.9e-183)	Not identified	
P996 l(2)k1060 28B01-02	AC005834;bases 130,658-131,521 28B01-28B04 (5.1e-172)	Not identified	
P447 l(2)02516 48C01-02	Not identified	Not identified	

a. The Bloomington stock number, the Kiss isolation number, and the insertion site as determined by in situ hybridization are given.

b. The NCBI accession identifier, bases matched, the physical map placement and the smallest sum probability statistic are given.

c. The Berkeley Drosophila Genome EST identifier, bases matched, and the smallest sum probability statistic are given.

d. Alleles of previously identified genes are indicated.

Three of these lines (P381, P918, P996) had significant matches to genomic clones, but not to cDNAs or ESTs in the database (Table 2). All three lines' genomic localization corresponded closely to the transposon insertion site as determined by in situ mapping, although line P381's site was found to be slightly more distal. It is possible that these transposons are interrupting genes not yet characterized molecularly. Alternatively, exon sequence may lie beyond the ~900 base pair limit of our BLAST search on the 3' end, or may lie to the 5' side of the transposon.

One line (P447) did not match anything in the database (Table 2). Thus it is likely that line P447 falls in an as of yet uncharacterized region of the second chromosome.

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Drosophila female receptivity to males with different sound parameters values.

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Drosophila males from the various species usually have specific displays during courtship (Spieth, 1974; Ewing and Benet-Clark, 1968); these, when detected by the females should stimulate them until they are physiologically ready to mate. Generally, potentially receptive females wait at least one complete courtship sequence before adopting an acceptance behavior. The most conspicuous courtship behavior is some form of wing movement that produces a specific acoustic stimulus (Ewing and Bennet-Clark, 1968; Spieth, 1974; Ewing, 1979). The importance of this sound stimulus for conspecific identification and successful mating has been shown, experimentally, using different methodologies for each of the several *Drosophila* species studied (Manning, 1967; Bennet-Clark and Ewing, 1967; Spieth, 1974; Schilcher, 1976; Ewing, 1978; Kyriacou and Hall, 1982; Ikeda *et al.*, 1981; Liimatainen *et al.*, 1992). The interpulse interval (IPI) and the fundamental frequency (FF) are the sound stimulus parameters considered most appropriate for identification, as they vary among the species but are characteristic to each one (Bennet-Clark and Ewing, 1969; Chang and Miller, 1978; Tomaru and Oguma, 1994).

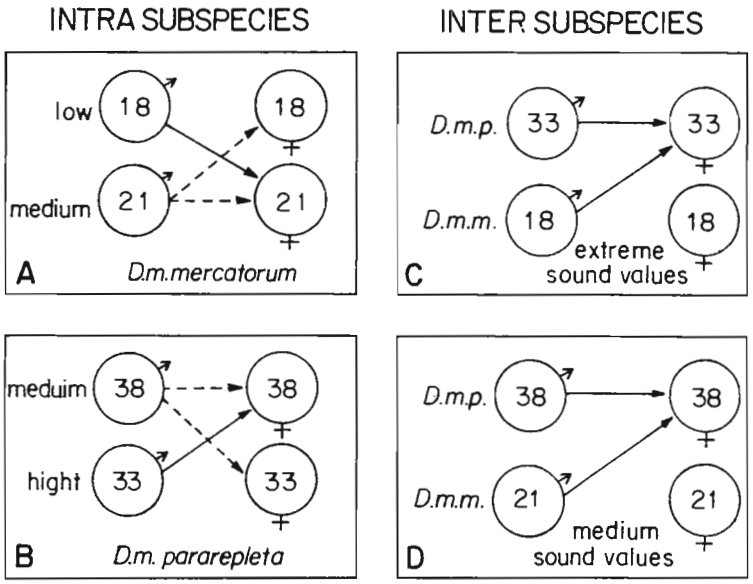

Figure 1. Graphical representation of *Drosophila mercatorum* female receptivity to males with different sound parameter values. A and B – crosses among lineages of the same subspecies with different values of sound parameters (Low, Medium and High) C and D – crosses among lineages of the two subspecies. The arrows indicate the results of crosses that were significantly more frequent. The numbers inside the symbols refer to the codes of the lineages.

—— – non casual mating, -------- - casual mating.

Drosophila mercatorum

Drosophila mercatorum (Paterson and Wheller, 1942) belongs to the *mercatorum* subgroup of the *repleta* group. The populations of this species are divided into two subspecies, typical

and *D. m. pararepleta,* based on karyotype, chromosome inversions and geographic distribution (Dobzhansky and Pavan, 1943; Wharton, 1944).

The sound pattern of *D. mercatorum mercatorum* was initially described by Ikeda *et al.* (1980), who identified two types of sounds, A and B, which play a critical role in the female' sexual acceptance (Ikeda *et al.*, 1981). These sounds occur at different moments of courtship, vary in oscillogram, and it was suggested (Ewing and Myian, 1986) that sound A is related to species identification while sound B is the sexual stimulus. Manfrin *et al.* (1997) described the sound of the two subspecies and found that *D. m. pararepleta* have IPI and FF values for sound A significantly higher than *D. m. mercatorum.* Analysis of sound B proved to be complex but no significant difference was detected between subspecies.

Since the male courtship must be recognized by the female and her acceptance plays an important part in the evolution of the behavior of the male, the development of a specific pattern should depend on the variability of the male signals and on the females capacity to discriminate them. Thus, this work investigates if the variation in some parameters of sound A, observed among subspecies and geographic populations of *D. mercatorum*, interferes on the male's capacity to be accepted by the females.

Materials and Methods

Two isofemale lines of *D. m. pararepleta* (D33F43 - Cristalina, GO, BRAZIL and D38N62 -Luiz Antonio, SP, BRAZIL) and two lines of *D. m. mercatorum* (1521.18 - Rochester, NY and 1521.21 - Pupukea, Oahu, HI), which differ in their IPI and FF, were used (Table 1).

Experiments of the mating choice were carried out using one male and two females. The tests were done with virgin females, six to seven days old. During sexual maturation, the males were kept individually isolated, in 80 ml flasks, and the females were kept together in 105 ml flasks, both with banana culture medium. Each line was marked with a color of Radiant Color Powder Paint to differentiate the females, 24 hours before the experiment was carried out. To conduct the experiment, two females from different isolines and a male from one of the isolines were aspirated into an 80 ml glass flask containing culture medium. The three flies were observed for 60 minutes, or until copulation took place.

Three types of pairing were carried out using lines with values of IPI and FF statistically tested at level of 0.05 (Manfrin *et al.*, 1997).

1) between lines of the two subspecies, with different extreme values (33×18);
2) between lines of the two subspecies, with similar values (38×21);
3) between lines of the same subspecies: 33×38 (*D. m. pararepleta*) and 18×21 (*D. m. mercatorum*).

The Stalker index (Stalker, 1942) was used to determine the degree of isolation; it was calculated from the data by taking the difference between homogamic (among individuals of the same lineage) and heterogamic (among individuals of different lineage) matings and dividing by the total matings. Its significance (consistency) was calculated by the proportions method (Woolf, 1968, Ohta, 1978).

Results

During the experiments, homogamic and heterogamic crosses were observed, intra as well as inter subspecies (Table 2). When the two subspecies were compared, we could see that the *D. m. pararepleta* females are more receptive to the males of both subspecies than the *D. m. mercatorum* females. Further, asymmetry was also observed among lines of the same subspecies, since males with extreme IPI and FF values crossed preferentially with females from lines with average values for these parameters (Figure 1).

Discussion

The crossings observed in this work, intra as well as inter subspecies, show that, in general, the receptivity of the females was not casual. The data obtained up to now, however, do not show whether the crosses pattern represented in Figure 1 is a direct consequence of the sound differences among the lineages.

Ikeda *et al.* (1980) observed significant differences in the sound A IPI values in *D. m. mercatorum* lines three times smaller than the ones that were used in this report. In crosses involving those lines, Ikeda *et*

Table 1. Interpulse interval (IPI) and fundamental frequency (FF) characteristics of the lines of *D. mercatorum* used in the present study.

Lines	Codes	Subspecies	IPI (ms)	FF (Hz)
D33F43	33	*D. m. pararepleta*	10.8	419
D38N62	38	*D. m. pararepleta*	10.0	405
1521.21	21	*D. m. mercatorum*	10.3	359
1521.18	18	*D. m. mercatorum*	9.3	374

al. (1980) observed asymmetric matings and suggested that these patterns could be a consequence of the difference in IPI of the sound A.

Tomaru *et al.* (1998) studied the courtship behavior and the effects of courtship song in inter- and intraspecific crosses in the four sympatric species of the *D. auraria* complex and suggested that courtship songs are of great importance in mate discrimination and the sexual isolation between the species of this complex.

The asymmetric matings observed could also be a consequence of the intraspecific variation in the pattern of the female receptivity and male mating success that are not directly related to differences in sound IPI and FF values.

Some studies suggest that other courtship parameters must be acting on the establishment of the female *Drosophila* sexual preferences, for example, anal droplets (Ritchie and Kyriacou, 1994) and chemical signs (Scott, 1994; Liimatainen and Hoikkala, 1998). According to Scott (op. cit.) besides the sound parameters could be involved the speed and frequency of the copulations. In the *Ceratitis capitata* fly, mating success depends on the variation of the sexual activity among males (number of courtships, copulation attempts) and on the courted female (Whitties *et al.*, 1994).

In the crosses involving different *D. mercatorum* subspecies, the females were more receptive to *D. m. pararepleta* males, regardless of their IPI and FF values (Table 2). This suggests that characteristics possessed by *D. m. pararepleta* males may be more attractive for females than those of the *D. m. mercatorum* males. Besides, *D. m. pararepleta* females were more receptive to any males than *D. m. mercatorum* females. The observed result may indicate divergence in relation to sexual behavior among subspecies, as happens with other characteristics.

Considering that *D. m. mercatorum* originated from marginal *D. m. pararepleta* populations (Wasserman, 1982; Sene, 1986), the asymmetry observed in the crosses involving these subspecies would not be expected according to Kaneshiro's hypothesis (Kaneshiro, 1976). According to this hypothesis, derived

Table 2. Results of crosses between lines of the two *D. mercatorum* subspecies with known IPI and FF values.

Different subspecies, with extreme values of IPI and FF										
Male	IPI and FF	Females	N	NC	%NC	HOM	HET	I	C	TYPE
33 Dmp	high	33 + 18	65	23	35	31	11	0.38	3.80	homogametic
18Dmm	low	33 + 18	124	86	69	9	29	-0.53	5.79	heterogametic
Different subspecies, with similar values of IPI and FF										
Male	IPI and FF	Females	N	NC	%NC	HOM	HET	I	C	TYPE
38 Dmp	medium	38 + 21	45	14	31	21	10	0.35	2.41	homogametic
21 Dmm	medium	38 + 21	64	34	53	5	25	0.67	5.28	heterogametic
Same subspecies, with extreme values of IPI and FF										
Male	IPI and FF	Females	N	NC	%NC	HOM	HET	I	C	TYPE
33 Dmp	high	33 + 38	36	19	53	8	22	-0.46	2.52	heterogametic
38 Dmp	medium	33 + 38	33	3	9	18	12	0.20	0.57	random
21 Dmm	medium	18 + 21	31	8	25	13	18	-0.16	0.89	random
18 Dmm	low	18 + 21	31	-	-	7	24	-0.55	3.0	heterogametic

SPP = subspecies, N = number of crosses, NC = number of crosses without mating, I = isolation index (Stalker 1942), C = consistency.

populations simplify their behavior in courtship as a result of founder effects. Thus, females in the ancestral population discriminated against males from the derived population, while females from the derived population not only accepted courtship with males from the ancestral population, but sometimes even preferred them over males from their own population. Similar results for *D. mercatorum* subspecies were observed by Koeper and Fensten (1991), who found asymmetric mating between different geographic strains of *D. mercatorum*. However, their results did not allow to accurately infer direction of evolution between *D. mercatorum* subspecies.

The asymmetries observed in the intrasubspecific crosses show that males from lines with extreme IPI and FF values are accepted preferentially by females from lines with medium values for these parameters. This suggests a stabilizing selection on sound A. This fact agrees with the prediction made by Ewing and Myian (1986) after analyzing the sound of the species from the *repleta* group. According to these authors, sound A may be considered an isolating or identifying signal in the species that takes place at the beginning of courtship. It is unambiguous and subject to stabilizing selection.

Our results show that there is variation in the sexual behavior of *D. mercatorum* and it could not be explained by an exclusive hypothesis, instead it should be a byproduct of the modifications of the evolutionary process involving this species.

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The attractiveness of *Drosophila* bait to arachnid predators and hymenopteran parasitoids.

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Introduction

When trapping drosophilids in the field it is not unusual to collect a number of hymenopteran parasitoids in the same traps. The parasitoids are attracted to olfactory cues from their host's resource in order to locate hosts for their larvae (*e.g.*, Carton *et al.*, 1986; Vet *et al.*, 1984; van Alphen *et al.*, 1991).

During a survey of drosophilids in New Zealand orchards, it was noticed that spiders and harvestmen were often found in the fruit-baited traps. *Drosophila* are often used as food for spiders maintained in

laboratory cultures and appear suitable prey for a wide variety of spider families (*e.g.*, Linyphiidae - Turnbull, 1962; Lycosidae - Hardman and Turnbull, 1974; Salticidae - Edwards and Jackson, 1994; Theridiidae - Rypstra, 1993; Thomisidae - Pollard, 1989). Some spiders make foraging decisions based on previous success, suggesting they use 'learnt' information to influence subsequent foraging effort (*e.g.*, Pasquet *et al.*, 1999) and some species locate webs in sites containing insect attractants (Janetos, 1986). Spiders are known to have olfactory capabilities (Foelix, 1996) and we hypothesized that the spiders found in our *Drosophila* traps may be using cues from the fermenting fruit bait in order to locate a site that is rich in dipteran prey.

The aim of this investigation was to systematically assess whether spiders were attracted to *Drosophila* bait or were simply using the traps as suitable shelters or web sites. In carrying out the investigation, we also gained information on the attractiveness of the *Drosophila* bait to hymenopteran parasitoids in New Zealand.

Methods

The investigation was carried out in six sites in the Christchurch region of Canterbury, New Zealand, between November 1998 and June 1999. The sites were chosen so as to represent a variety of different habitat types utilized by *Drosophila*. The sites were an organic orchard (Lincoln University, Canterbury), a sub-urban garden (Riccarton, Christchurch), a native forest remnant (Riccarton Bush, Christchurch), a recently planted forest restoration site (King George V Reserve, Christchurch), a conifer plantation (near Lincoln, Canterbury) and farm out-buildings (Prebbleton, Canterbury).

Plastic containers (60 mm high; 40 mm diameter) with yellow plastic lids were used as the trapping vessels. Nine holes (4mm diameter) were drilled into the lids to allow entry of animals. The traps were either baited with pieces of ripe banana ($\approx$ 20 g) or left unbaited. Thirty replicates of baited and unbaited traps were placed on the ground at each field site. The traps were placed on their sides, so that the lids were perpendicular to the ground. The traps were left in the field for 10 days and then collected and returned to the laboratory. The presence or absence of drosophilids, hymenopteran parasitoids and spiders in each trap was recorded. The distribution of each major taxa between baited and unbaited traps at each site was assessed using χ^2 tests. The heterogeneity of the distributions between sites was also assessed and, if viable, a χ^2 test using Cochrane's method was then performed on the pooled data (Zar, 1984).

Results

Four species of *Drosophila* were collected; *D. melanogaster*, *D. pseudoobscura*, *D. immigrans* and *D. neozealandica*. As expected, *Drosophila* showed a highly significant association with the banana bait (pooled data $\chi^2 = 198.4$ for 1 df; $P < 0.001$) and - with one exception - were found only in baited traps (Tables 1 and 2).

Eight different families of hymenopteran were collected (Table 1). In the first three trials, Hymenoptera were significantly more likely to be found in baited traps than in unbaited traps (Table 2). However, the Hymenoptera collected from the pine plantation showed no significant bias to either baited or unbaited traps and, due primarily to the late season, only a single specimen was collected at both the remnant forest and around the farm buildings. The distribution of Hymenoptera was, therefore, significantly heterogeneous between sites ($\chi^2 = 19.2$ for 5 df; $P < 0.01$) and the data could not legitimately be pooled for an overall analysis.

At least five families of spider (Anapidae, Linyphiidae, Lycosidae, Salticidae and Theridiidae) were found inside the traps, along with a number of unidentifiable juveniles (Table 1). Spiders were relatively rare, being present in only 30 of the 360 traps (Table 2). Although there was an overall trend towards spiders being found in baited traps this was not statistically significant within any one site or for the overall collection (Table 2; pooled data $\chi^2 = 1.8$ for 1 df; $P > 0.10$).

Discussion

The results tend to confirm the attractiveness of *Drosophila* baits to parasitoid wasps, which showed a clear preference in three of the six sites. On the last two sampling occasions too few Hymenoptera were captured to make any sound judgments. The Hymenoptera collected in the pine forest showed a spurious distribution, in that they were equally occurrent in baited traps as in unbaited. We have no immediate explanation for this result.

Table 1. Species of *Drosophila*, families of parasitoid Hymenoptera and species of spiders found in *Drosophila* traps at each of the field sites. (B - baited traps; U - unbaited traps).

	Family	Orchard		Garden		Restoration		Pines		Remanant		Farm	
		B	U	B	U	B	U	B	U	B	U	B	U
Drosophila	*D. immigrans*	✓	-	✓	-	✓	-	✓	-	✓	-	✓	✓
	D. melanogaster	✓	-	-	-	-	-	-	-	-	-	✓	-
	D. neozealandica	-	-	-	-	✓	-	-	-	✓	-	-	-
	D. pseudoobscura	✓	-	✓	-	✓	-	✓	-	✓	-	✓	-
Hymenoptera	Bethylidae	-	-	✓	-	-	-	-	-	-	-	-	-
	Braconidae	✓	-	✓	-	✓	✓	-	-	-	-	-	-
	Diapriidae	-	-	-	-	-	-	✓	✓	-	-	-	-
	Encyrtidae	✓	-	-	-	-	-	-	-	-	-	-	-
	Eulophidae	-	-	-	-	-	-	-	-	-	✓	-	-
	Figitidae	-	-	-	-	-	-	✓	-	-	-	-	-
	Ichneumonidae	-	-	-	-	-	-	✓	✓	-	-	-	-
	Pteromalidae	-	-	-	-	-	-	-	-	-	-	✓	-
Spiders													
Anapidae	*Zealanapis armata*	-	-	-	-	-	-	-	-	✓	-	-	-
Linyphiidae	*Araeoncus humilis*	-	-	-	-	-	-	-	-	✓	-	-	✓
	Diplocephalus cristatus	-	-	-	-	-	-	-	-	✓	✓	-	✓
	juveniles	✓	-	-	-	✓	-	✓	-	-	-	-	-
Lycosidae	*'Lycosa' hilaris*	✓	-	✓	-	-	-	-	-	-	-	-	-
Salticidae	juveniles	-	-	-	-	-	✓	-	-	-	-	-	-
Theridiidae	undescribed sp1	-	-	-	-	-	-	✓	✓	-	-	-	-
	undescribed sp2	✓	✓	-	-	-	-	-	-	-	-	-	-
	juveniles	✓	✓	-	-	-	✓	-	✓	-	-	✓	-
unknown	juveniles	✓	-	✓	-	✓	✓	-	-	-	-	-	-

Table 2. Number of baited and unbaited traps containing *Drosophila*, hymenopteran parasitoids and spiders at each of six sites near Christchurch, New Zealand (N = 30).

Site	Date	*Drosophila*		Parasitoids		Spiders	
		baited	unbaited	baited	unbaited	baited	unbaited
Organic orchard	30/11/98	29	0*	10	0*	5	3
Sub-urban garden	22/12/98	19	0*	11	0*	4	0
Restoration site	29/3/99	25	0*	10	2*	2	3
Pine plantation	21/4/99	10	0*	7	10	2	2
Remnant forest	20/5/99	19	0*	0	1	5	1
Farm out-buildings	1/6/99	20	1*	1	0	1	2
Total (N = 180)		122	1*	39	13	19	11

* - significant preference for baited traps identified using χ^2 test; $P<0.05$

Our casual observation that spiders were frequent visitors to *Drosophila* traps did not withstand systematic examination. We found fewer spiders than expected, and those that were collected showed no significant preference towards baited traps. However, as almost two thirds of spiders collected were caught in the baited traps, this issue may warrant further investigation. Separating spiders into different families or species may reveal more detailed patterns in preference of foraging site. Also, the use of olfactometer experiments in the laboratory may clarify whether certain species of spider are attracted to *Drosophila* resources.

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Factors of spontaneous mutations, mutability in large chromosomes and mortality from dominant lethals in *Drosophila melanogaster*.

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The ideas are widespread that changes of the natural radiation background increase the rate of mutability and are responsible for such prominent events in life on the Earth as extinction of fossil faunas (dinosaurs are mentioned most frequently), outbreaks of pest reproduction, epizooites and other phenomena believed to be associated with emergence of mutations causing resistance to pesticides and of more virulent or antibiotic-resistant microbial strains. However, 1) mutations are destructive and have nothing to do with biogenesis (Ivanov and Ivannikov, 1997), that is why the above ideas about the emergence of new forms with a heightened fitness are extremely doubtful. It is not new mutant forms that arise, it is that tolerable hereditary types preadapted to new conditions which are inherent in the given species from its very origin, but rare under the usual conditions, begin to reproduce more intensely. The species becomes adapted to various possible

conditions of its ecological niche not by means of emergence of outstanding mutant individuals with universal adaptation, but due to the enormous diversity of its tolerable hereditary types each of which is adapted in the best way to a very limited assortment of environmental conditions. 2) Extinction of faunas in the history of the Earth finds its explanation in the fact that CO_2 reserve in the atmosphere, which is replenished by mantle degasation during paroxysms of volcanic activity (geological revolutions) (Ronov, 1978; Ronov, 1980), is periodically exhausted, and this extinction may not be attributed to catastrophical radiation outbreaks, because these are extremely doubtful. In our recent work (Ivanov, 1998a) based on data known since long ago (Stern, 1960), it was demonstrated that the natural radiation background practically did not influence the spontaneous mutability, since it brings about less than 10^{-4} of all the spontaneous mutations, that is why even its repeated changes could not produce the observed changes of mutability in the populations. This and other considerations make us conclude that the mutability in nature is determined by biotic factors.

Table 1. Decomposition of the spontaneous mutation process into the main mutation types in *Drosophila melanogaster*.

Mutation type	Percent among all mutations	Percent among all DLM	Lethality of mutations	Percent among all mutations	Lethal type	Percent among all mutations	Percent among all genic mutations	Type of genic lethals	Percent among all genic lethals
CDLM	64	94			Chromosomal	64	-	-	-
GDLM	4	6	Lethals	97	Genic	33	92	GDLM	12
RLM	29	-						RLM	88
VM	3		Non-lethals	3	Non-lethals	3	8	-	-

CDLM and GDLM and sets of gametes carrying chromosomal and genic DLM, respectively. RLM and VM are sets of respective gametes

A very important result of studies of spontaneous mutagenesis in *D. melanogaster* is its division into basic mutation types (Ivanov, 1998b). It permits associating the mutation rates in separate chromosomes with the mortality of zygotes from dominant lethal mutations (DLM) in the whole genome. Therein it is assumed that constant proportions between the mutability in single chromosomes and that in the whole genome, and between rates of various mutation types, are maintained.

Under usual conditions, the fraction of mutant gametes among all gametes of *D. melanogaster* is about 3.25%. Decomposition of the spontaneous mutation process into basic mutation types in *D. melanogaster* genome is division of the set of mutant gametes into subsets that carry DLM (68%), recessive lethals (RLM) (29%), and visible mutations (VM) (3%). Therein, if VM and RLM coincide with DLM, they are recorded as DLM, and if VM coincide with RLM, they are recorded as RLM due to lethality of respective genotypes. In their turn, DLM are subdivided into chromosomal and genic ones, wherein if the genic DLM coincide with chromosomal ones, they are recorded as chromosomal (Ivanov, 1998c). All possible divisions of the set of mutant gametes are presented in Table 1. Having the decomposition of the mutation process into mutation types, the rate u_i of RLM and VM emergence in the i-th chromosome and its fraction s_i in the whole *D. melanogaster* genome, one can estimate the frequency U of DLM emergence in the whole genome, and through it the zygotes' lethality from DLM as a function $S(U)$ or $S(u_i)$.

Let, as already indicated, U be the frequency of DLM emergence in the whole genome (autosomes + X chromosome). The fraction of X chromosome in the whole (complete) genome is $s_1 = 0.19$ (Ivanov, 1998b). That is why the incomplete genome in which the X chromosome is genetically substituted by the empty Y chromosome makes up 0.81 of the complete one, and the frequency of DLM emergence in it is $0.81U$.

The probability of a female not dying from DLM is the probability of her two complete genomes turning out to be free from DLM, *i.e.* to be equal to $(1-U)^2$. The probability of her death from DLM is

$$S_f(U) = 1 - (1-U)^2 = 2U - U^2. \qquad (1)$$

The probability of a male not dying from DLM is the probability of his two genomes, the whole (complete) and the incomplete ones, turning out to be free from DLM, *i.e.* to be equal to the product $(1-U)(1-0.81U)$. The probability of his death from DLM is

$$S_m(U) = 1 - (1-U)(1-0.81U) = 1.81U - 0.81U^2. \qquad (2)$$

At the primary sex ratio of 1:1, the zygotes' death from DLM is the arithmetic mean of these quantities (1) and (2) $S(U) = 0.5[S_f(U) + S_m(U)]$, *i.e.*

$$S(U) = 1.905U - 0.905U^2. \qquad (3)$$

Now, to calculate the sought mortality, one has to estimate the value for U. Let u and u_i be the frequencies of RLM and VM in the whole genome and in the X chromosome, respectively, and s_i be the fraction of the *i*-th chromosome in the whole genome. From the proportion $u/u_i = 1/s_i$ we have: $u = u_i/s_i$, and from the proportion $U/u = 68/32$ which follows from the decomposition we have

$$U = 2.125u = 2.125u_i/s_i.$$

By means of substituting the found expression for U into equalities (1) - (3), we obtain expressions for the mortality of sexes and zygotes in general through the mutability u_i in the *i*-th chromosome:

$$\begin{aligned} S_f(u_i) &= 4.250u_i/s_i - 4.516u_i^2/s_i^2; \\ S_m(u_i) &= 3.846u_i/s_i - 3.658u_i^2/s_i^2; \qquad (4) \\ S(u_i) &= 4.048u_i/s_i - 4.087u_i^2/s_i^2. \end{aligned}$$

In particular, the expressions for zygotes' mortality from DLM through the mutability u_1 in the X chromosome and u_2 in chromosome 2 at $s_1 = 0.19$ and $s_2 = 0.36$, becomes as follows:

$$S(u_1) = 21.3u_1 - 113.2u_1^2; \qquad (5)$$

$$S(u_2) = 11.2u_2 - 31.5u_2^2. \qquad (6)$$

These formulae, unlike the approximated formulae of our previous works (Ivanov and Ivannikov,1997; Ivanov 1998a) are more precise.

From the maximal rates of spontaneous mutability known to us in large chromosomes $\hat{u}_1$ = 1.3% (Ivanov, 1998a) and $\hat{u}_2$ = 1.27% (Dubinin, 1966), by means substituting them into formulae (5) and (6), it is possible to estimate the maximal values for zygotes' mortality from DLM in populations: $S(\hat{u}_1)$ = 25.8% and $S(\hat{u}_2)$ = 13.7%. These values are rather large, from which it follows that the most important consequence of mutability increase in the population is a considerable heightening of zygotes' mortality. As it has been found (Ivanov and Ivannikov, 1997), mutability depends on the population density as an increasing function; that is why, as the species abundance increases, so does the zygotes' mortality from DLM, due to which the mutation process is one of regulators of the population numbers. This is confirmed by the fact that the basic factors of spontaneous mutagenesis have a biotic nature (paramutations, insertional mutagenesis, viruses, transduction, mutator genes, MR-factors, etc.).

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Technique Notes

Fly-FISHing: A protocol to localize single copy genes inside the nucleus in whole mount embryos.

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1. INTRODUCTION

The nucleus is the place where transcription factors, DNA- and RNA-polymerases fulfill their tasks. The chromatin is made up of an undefined number of proteins which all contribute to the way the information contained in the genome is utilized. How the nucleus is organized and how all the processes are interwoven inside the nucleus is an open and ongoing area of debate (the following books give a good overview of this field: *Chromatin: Structure & Function*, Alan Wolffe, 1998; *Chromatin Structure and Gene Expression*, Ed. Sarah C.R. Elgin, 1995; CSH Symposia on Quantitative Biology Vol. 58, 1993). Antibody stainings to proteins involved in mitosis and transcription revealed complex and dynamic patterns (Buchenau, 1993a, b, 1997). *Drosophila* offers a unique system to study changes in nuclear architecture and to reveal the dynamics of these processes. This can be done by injection of fluorescently labeled antibodies into *Drosophila* embryos or by using the GFP-technology for *in vivo* studies (Amsterdam *et al.*, 1995; Davis *et al.*, 1995; Yeh *et al.*, 1995). Alternatively fixed embryonic material can be extremely helpful for visualizing dynamics in changing structures because in each set of experiments different developmental timepoints are represented which can be sorted to reflect the developmental progression. In this technical note I describe a fluorescent *in situ* technique which can be used to localize single copy genes in three dimensions inside the nucleus. This technique was successfully used to determine the pairing kinetics of the Bithorax-complex (BX-C) during embryogenesis and to gain insights into the mechanism of transvection (Gemkow *et al.*, 1998, 1993). Localization and pairing studies for chromosomal loci on the second chromosome were also performed (Hiraoka *et al.*, 1993; Fung *et al.*, 1998 and references therein).

The protocol given here can also be used in combination with antibody stainings inside the nucleus which enabled us to co-localize the BX-C with proteins of the Polycomb-group. For this purpose a regular antibody protocol has to be performed after hybridization. An alternative protocol from Gunawardena and Rykowski (1994) can be found in: Drosophila melanogaster*: Practical Uses in Cell and Molecular Biology*; edited by Goldstein and Fyrberg (Methods in Cell Biology, Vol. 44).

2. PROTOCOL AND MATERIALS

2.1 Fixation of whole embryos

Overnight collections or embryos of any desired stage of Oregon R were dechorionated with 50% bleach for 3 min in a small basket. Embryos were thoroughly washed with 0.1 M NaCl containing 0.03% Triton X-100 followed by Millipore water. Embryos were then fixed with 4% para-formaldehyde in buffer A (60 mM KCl, 15 mM NaCl, 0.5 mM spermidine, 0.15 mM spermine, 2 mM EDTA, 0.5 mM EGTA, 15 mM PIPES, pH 7.4) and heptane (1 part buffered para-formaldehyde + 9 parts heptane) for 30 min at room temperature on a mini-shaker with eccentric rotation at 1000 rpm. After fixation the aqueous phase was taken off and embryos were devitellinized with a 1:1 mixture of heptane and methanol for 2 min by thoroughly mixing on a vortex. All embryos which sank to the bottom were washed twice with methanol and stored in 100% methanol at -20°C if not used immediately.

2.2 P1 probe preparation and labeling

We used the following P1 clones from the collection originally described by Hartl *et al.* (1994) and provided by EMBL Heidelberg: DS 03126, DS 00846 and DS 0769. These represent parts of the *Ultrabithorax*

(*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) genes, respectively.

Probes were labeled by random priming: 100-1000 ng of template were mixed with 0.1 mM dXTPs, 0.065 mM dTTP, 0.035 mM DIG and 0.6 OD_{260} units of hexanucleotide primer in a volume of 40 µl. The probes were denatured for 15 min in a boiling water bath. 10 µl of 5 times concentrated Klenow buffer (final concentration: 50 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$, 2 mM DTT) and 5 units of Klenow enzyme were added and the reaction was incubated overnight at 37°C. The reaction was stopped by boiling for 5 min. Unincorporated nucleotides were removed by gel filtration through BioRad MicroSpin 30 using 1xSSC as buffer. Labeling efficiency was checked with a spot test on nitrocellulose using an alkaline phosphatase color reaction. Incorporation was judged as adequate if 10 -100 pg of labeled probe were detectable in this test. To decrease probe length, probes were sonicated 12 times for 30 sec with 15 sec cooling intervals on ice. This procedure yielded a probe length which peaked between 200 and 300 bp.

For the hybridization 600 - 1000 ng of the labeled probe were precipitated with 10 µg of human cot1 DNA and 100 µg of yeast tRNA as carrier, dissolved in 10 µl of formamide and mixed with 10 µl of 8xSSC, 200 mM Na-PO_4 pH 7.0, 0.2% Tween 20 to reach the final concentrations used in the hybridization mixture. Denaturation was effected by boiling for 5 - 15 min, probes were then added to the embryos without prior cooling.

2.3 DNA:DNA hybridization

Fixed embryos were transferred stepwise to PBT (phosphate-buffered saline + 0.1% Tween 20) and incubated with 100-200 µg/ml RNAse A in PBT for at least 3 h at room temperature or overnight at 4°C. Embryos were transferred into the hybridization mixture via a step-gradient of 20, 50, 80 and 100% hybridization mixture (50% formamide, 4xSSC, 100 mM Na-PO_4 pH 7.0, 0.1% Tween 20), 20 min each step. All steps were done in a conical tube in a volume of 1 ml on a rotating wheel.

The embryonic DNA was denatured for 15 min at 76°C in hybridization mixture. While the embryos were still at 76°C, as much buffer as possible was taken off and the denatured probe was added. The hybridization was performed on a thermomixer at 37°C with gentle agitation for 14-17 h.

Post hybridization washes of 20 min each, were carried out at 37°C in a volume of 1 ml in the following order: 50% formamide, 2xSSC, 0.3% CHAPS; 40% formamide, 2xSSC, 0.3% CHAPS; 30% formamide (twice), 70% PBT; 20% formamide, 80% PBT; 10% formamide, 90% PBT; PBT (twice).

Embryos were thereafter washed with PBT at room temperature and stored at 4°C if not immediately used for the detection reactions.

Hybridization of the DIG-labeled probe was detected by immunofluorescence. Embryos were blocked with 3% BSA in PBT for at least 3 h at room temperature. Thereafter they were incubated with Cy3-labeled polyclonal anti-DIG Fab-fragments and monoclonal anti-lamin antibody (Risau *et al.* 1981) overnight at 4°C and washed 3×30 min with PBT. The lamin signal was detected with Cy5-labeled $F(ab)_2$-fragments goat-anti-mouse (Dianova, Jackson Labs, Hamburg, Germany). The DNA was counter stained for 5 min with 5 µM DAPI (Serva, Heidelberg, Germany). Embryos were stepwise transferred to Mowiol 4-88 (Hoechst, Frankfurt, Germany).

2.4 Antibodies

The antibodies against lamin were purified from cell culture supernatants by chromatography on a protein G Sepharose column (Pharmacia, Upsala, Sweden) before use. Cy5-labeled secondary goat IgG anti-mouse $F(ab)_2$ fragment antibodies were purchased (Dianova, Jackson Labs). Fab fragments from a sheep anti-digoxigenin antibody (Boehringer, Mannheim, Germany) were labeled with Cy3 hydroxysuccinimide (Amersham, Buckinghamshire, UK) using a protocol previously described (Buchenau *et al.*, 1993a).

2.5 Confocal laser scanning microscopy

A Zeiss 310 CLSM equipped with two internal lasers (He-Ne, 633 nm; argon ion, 488 and 514 nm) and three external lasers (He-Ne, 594 nm; He-Ne, 543 nm; argon ion, 364 nm) coupled by single-mode fiber optics was used for all measurements. Fluorophores were observed under the following conditions: Cy5, excitation at 633 nm, emission with longpass 665; Cy3, excitation at 514 nm, emission with bandpass 535 -

580 nm; DAPI, excitation at 364 nm, emission with longpass 410 nm or bandpass 450 HW40. The objective lenses used had a high numerical aperture. Primarily the Zeiss Plan Apochromat 63x, NA 1.4 was used. The voxel size was selected, so as to be three times smaller than the optical resolution of the microscope: 100x100x250 nm^3 (x,y,z).

2.6 Image processing

The image data from the CLSM were processed in SCIL IMAGE (TNO Institute of Applied Physics, TU Delft, The Netherlands), IMARIS 2.4 (Bitplane, Zürich, Switzerland), and NIH Image (National Institute of Health, Bethesda, Maryland, USA) for selection of regions of interest, smoothing, overlays, 3-D reconstructions and intensity calculations. All image processing was carried out on a Silicon Graphics Workstation.

3. CONSIDERATIONS

3.1 Which DNA probes can be used?

In principle any kind of DNA probe can be used from small vectors over lambda clones up to P1 clones. In my experience the P1 clones worked best because they gave reasonable sized signals. In an experiment one has to find a compromise between probe size, labeling quality and fluorescence signal. The P1 clones which I used in my experiments in combination with a single directly labeled antibody gave signals with a diameter between 400 nm and 1 µm and had enough fluorescent signal to allow three dimensional scans with a conventional Zeiss Laser scanning microscope. By using two or multiple layers of antibodies, signal size can be increased. An alternative for very small probes is the amplification of the signal with a peroxidase reaction using fluorescently labeled tyramids (Gemkow *et al.*, 1993 and references therein).

3.2 Which labels work best?

In my experience the incorporation of digoxigenin by random priming using Klenow fragment worked best but one can also think of using random primed PCR or nick translation to label the probes. An alternative to digoxigenin are fluorescently labeled nucleotides which are now available in a great variety having for example Cy3, Fluoresceine or Alexa dyes as labels and can be purchased from companies like Amersham or Molecular Probes. But again one has to take into account probe size and labelling intensity to match the needs for fluorescent microscopy.

3.3 Which are the critical steps in the protocol?

All steps in the procedure should be given great care, but in general the most important things are the size of the labeled probe and the denaturation temperature applied to the embryos.

For example if the probe size is too long, the probe cannot penetrate the tissue efficiently. This will probably have more of an effect than in the protocol of Tautz and Pfeifle (1989) because a Proteinase K digestion is omitted. Omitting the Proteinase K is important to be able to co-localize proteins with the hybridization signal.

The signal intensity increases by increasing the denaturation temperature for the embryonic DNA. But one has to find a compromise between an efficient denaturation and destruction of the morphology. A good value is 76 °C but this of course also depends on the target sequences and the developmental stage of the embryo. I therefore suggest trying out different temperatures and comparing the signals in the microscope.

Acknowledgments: The protocol presented was developed in the laboratory of Dr. Donna J. Arndt-Jovin.

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A *Drosophila* enhancer detector transposon marked with the *yellow* gene.

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Key words: *P*-mediated transposition; enhancer trap; *yellow* gene.

The P[lyB] enhancer trap vector (Figure 1A) was designed to be used in *P*-element-mediated mutagenesis and enhancer detection in *Drosophila melanogaster*. It contains the ß-galactosidase reporter gene and the *yellow* gene as a transformation and transposition marker. It was derived from P[lAwB] (Flybase ID = FBmc0000173), designed to allow rapid cloning and deletion analysis of genomic sequences into which it inserts, and therefore has retained the same properties.

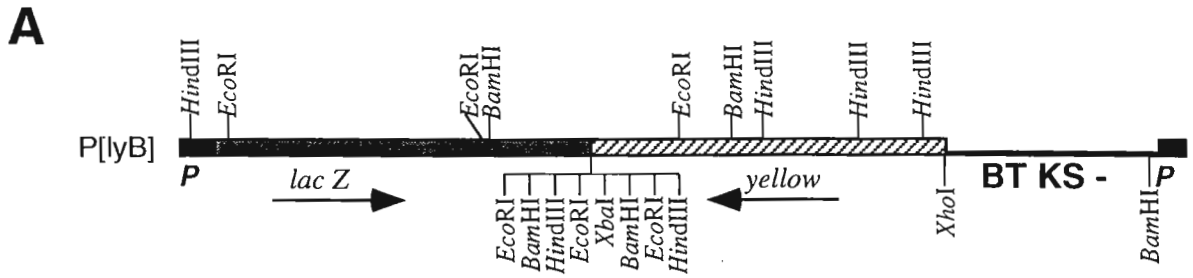
Construction of the P[lyB] vector and establishment of transgenic lines: P[lyB] was derived from P[lAwB] by replacing the *ADH* and *white* sequences from this vector with *yellow* sequences. The source of the *yellow* sequences was plasmid Dint (Geyer and Corces, 1987) that contains all DNA sequences from the *yellow* gene except the intron. The SalI fragment from Dint containing the *yellow* sequences was first subcloned into the pBluescript KS$^-$ vector, in order to place the *yellow* gene between a XhoI and a XbaI sites. Then the XhoI-XbaI fragment from this clone was ligated to XhoI- and XbaI-cut P[lAwB].

P[lyB] was injected into embryos from the JA strain (carrying both *yellow* and *white* mutations) using standard procedures described by Spradling and Rubin (1982) except that puc*hsp*Δ2-3 (Flybase ID: FBmc0002087) was co-injected as the source of transposase. Several independent transgenic lines were established. Two of them, designated J49 and J92, were characterized further by estimating the rates of transposition and excision of P[lyB]. They both carry the P[lyB] transgene on the second chromosome.

Efficient mobilization of P[lyB]: Mobilization of P[lyB] was carried out using P[ry^+Δ2-3](99B) as a stable genomic transposase source, basically using the "jumpstart" scheme of Bellen *et al.* (1989) modified as shown in Figure 1B. Virgin females from J49 or J92 transgenic line were mated to males homozygous for P[ry^+Δ2-3](99B) and carrying a *Cy* balancer second chromosome (Figure 2). In the progeny of these crosses, [Cy] males, that carry both P[lyB] and P[ry^+Δ2-3](99B), were selected and mated with virgin females from the JA stock. The occurrence of [y+;Cy] individuals in their progeny reflected transposition events to new chromosomal locations. From these experiments, estimations of transposition frequencies of P[lyB] were 4.7% when using line J49 and 3.3% when using line J92. Although these estimations are rough, because very few flies were scored in these experiments, they indicate that the rate of mobilization of P[lyB] is in the usual range (1-5%) for defective P elements mobilized by P[ry^+Δ2-3](99B) (Engels, 1989). Therefore the P[lyB] element can be used efficiently as an alternative to other enhancer trap elements in cases where the use of a yellow marker appears convenient.

Lines J49 and J92 will be available at the Umea Drosophila Stock Center and at the Bloomington Stock Center.

Acknowledgments: We are very grateful to Dr. Walter Gehring for the gift of plasmid P[lAwB] and to Nathalie Rémillieux who participated in these experiments when she was a second year student at the Université Paris XI (Orsay).



B

J49 ♀ $\frac{y\ w}{y\ w}$ $\frac{P\text{-}lyB}{P\text{-}lyB}$ $\frac{+}{+}$ x ♂ $\frac{+}{Y}$ $\frac{Cy}{Pm}$ $\frac{P\ 2\text{-}3}{P\ 2\text{-}3}$
or J92

JA ♀ $\frac{y\ w}{y\ w}$ $\frac{+}{+}$ $\frac{+}{+}$ x ♂ $\frac{y\ w}{Y}$ $\frac{P\text{-}lyB}{Cy}$ $\frac{+}{P\ 2\text{-}3}$

Line	[y;Cy]	[y+;Cy+]	[y;Cy+]	[y+;Cy]	Total	% excision	% transposition
J49	307	31	298	15	651	90.58%	4.66%
J92	1307	558	984	44	2893	63.81%	3.26%
	No mobilization		Excision	Transposition			

Figure 1: A: Sequence organization and partial restriction map of p-lyB. Thick line represents sequences from pBluescript KS-, black boxes represent P element sequences, shaded box represent LacZ sequences, striped box represents yellow sequences. Arrows indicate senses of transcription of LacZ and yellow genes. B: scheme of crosses to estimate the rate of mobilization of p-lyB by P[ry+Δ2-3](99B) and results.

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An inexpensive method for recording *Drosophila* song.

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We developed an inexpensive method for recording *Drosophila* song while observing the behaviors associated with the sounds. This apparatus was inspired by one developed by Hunt (1993) to listen to substrate vibration in leaf hoppers. We used this device to record two species of Hawaiian *Drosophila*, *D. silvestris* and *D. heteroneura*. The recording method allowed us to hear the sounds produced both by wing vibrations and by abdominal vibrations, which are relatively soft (Hoy *et al.*, 1988). The entire apparatus, exclusive of the tape recorder, was purchased and assembled for less than US$200.

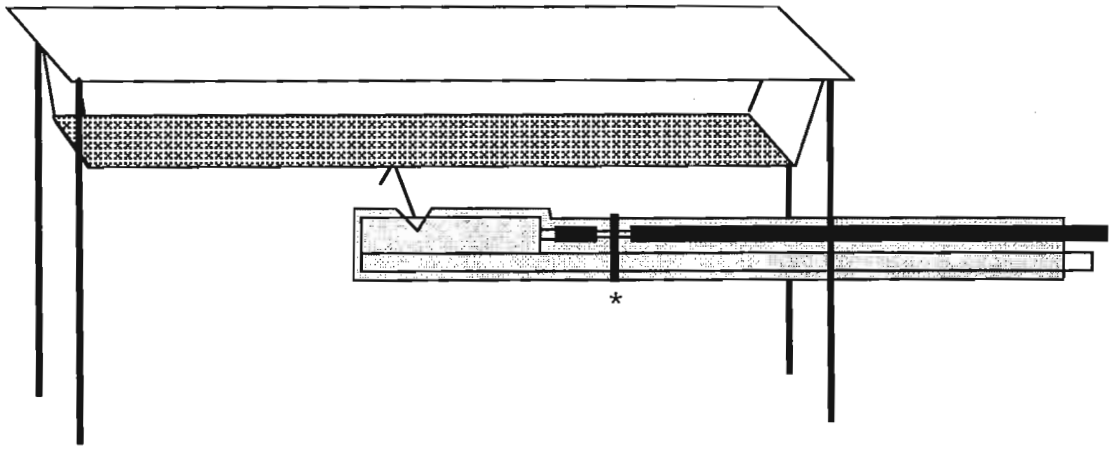

Figure 1. The recording apparatus was attached to a ring stand and placed with the insect pin in contact with the mesh floor of the chamber. Aluminum foil shielding (shown as transparent gray to show details) was placed around the recording arm. A small piece of casing was removed from the stereo cable and electrical tape was wrapped around the foil and cable at that point (*).

We used 8 oz clear, plastic delicatessen containers from which the floors were removed and replaced with nylon mesh (no seeum netting, from Recreational Equipment, Inc.) as recording chambers. Both *D. silvestris* and *D. heteroneura* spent most of their courtship time on the mesh. We used a phonograph cartridge (2.8 V output, Arista LC-4 Monaural crystal cartridges and 5 V ceramic cartridges both worked) in which the stylus had been replaced with a bent insect pin (size 000) as a microphone, which we placed in direct contact with the mesh (Figure 1). We were able to obtain cartridges with unusable needles from a local phonograph hobbyist store at minimal cost. The phonograph cartridge output was fed by stereo cable into an Optimus Model SA-155 stereo amplifier (Radio Shack). The phonograph cartridge and cable end were then mounted on a piece of dowel and the entire assembly, except the pin, was covered with aluminum foil. The foil was taped in place over a section of wire which had the rubber casing removed, thus grounding it to the cable. The foil was also grounded to the building ground wire. The foil shielded the cartridge and cable from electromagnetic interference. If, in using this apparatus, excess noise is still a problem, it may be necessary to construct a Faraday cage to reduce electrical noise further.

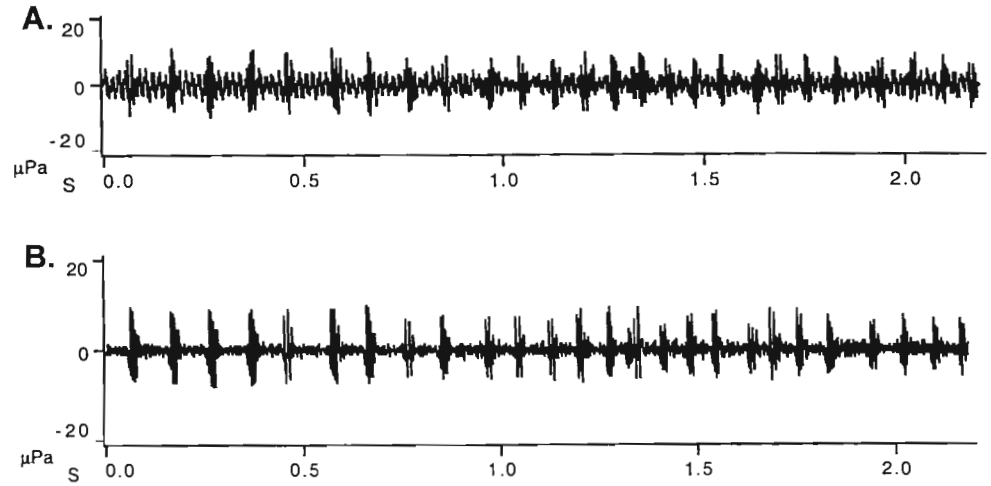

Figure 2. A shows unfiltered waveform of abdominal purring, B shows same recording after filtering.

The amplifier output was fed into an Onkyo Model TA-2600 cassette tape recorder. Recording was carried out on a vibration-isolating table in a quiet room. The table was inside a sound proof booth, but the door was kept open to allow observation of the flies' behaviors. The Hawaiian flies studied require cool temperatures, so we maintained the temperature by applying a freezer pack to the top of the chamber.

Tape recordings were exported to a desktop computer (PowerMacintosh) for analysis using Canary™ software (Cornell University). By analyzing portions of the tape with and without song, we found that we could greatly reduce background noise by filtering out signals below 100 Hz and above 600 Hz. Once filtered, we were able to analyze waveforms of the songs (Figure 2).

As a simple test of the utility of the apparatus, we used a smaller chamber with the mesh top to listen to *D. melanogaster*. The microphone apparatus was inverted and placed in contact with the mesh. Despite the smaller size of the flies, we were clearly able to hear the songs of *D. melanogaster* in a quiet room.

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A poor man's GFP desktop viewer.

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The Green Fluorescent Protein (GFP) is more and more widely used as a powerful tool for biologists, and especially drosophilists. One application that is unique to *Drosophila* is the construction of green balancers (Reichhart and Ferrandon, 1998). The advantages of using GFP expression as a reporter or as a dominant marker for balancer are clear; however, not everyone has an epifluorescence microscope in the laboratory, and even those who have one may feel inconvenienced to have to turn on the microscope every time one wants to observe a sample. Here is the description of the "GFP viewer", a Do-It-Yourself benchtop device designed for the observation of S65T modified GFP (Heim *et al.*, 1995). The GFP viewer can be used as a complement of an epifluorescence microscope or as a low-price/low-bulk alternative for the observation of S65T-GFP specimens. It allows observation and rapid sorting of GFP expressing bacterial clones, P{Gal4}/UAS-GFP or GFP balancer larvae, and can be used in combination with a standard binocular microscope. It can be turned on and off at will, when one needs to check for GFP

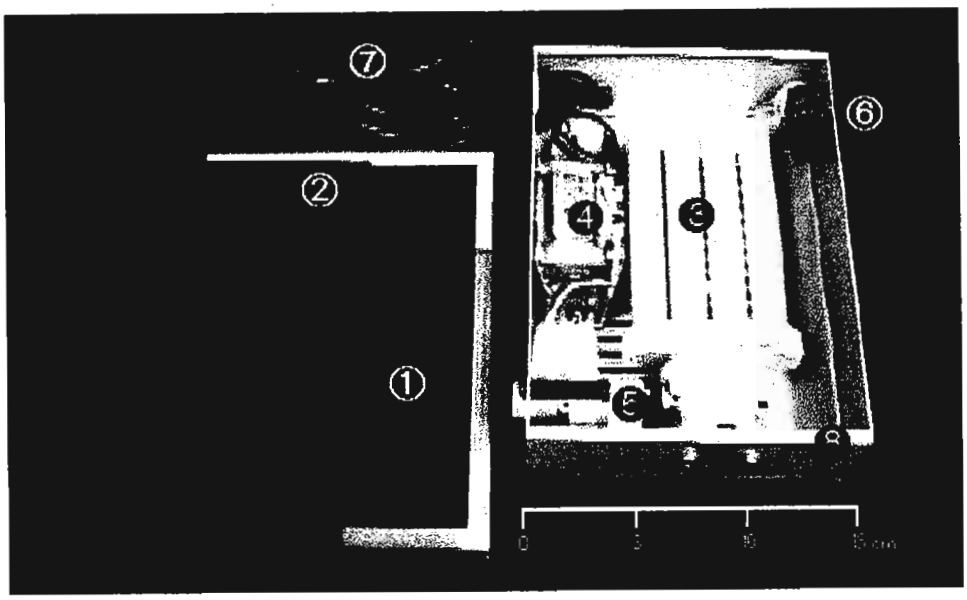

Figure 1. The GFP viewer.

expression of EGFP or S65T-GFP transgenic samples. It can be built easily, one needs just some soldering ability and common tools (screwdriver, drill); Its design is quite simple and illustrated by Figure 1:

The elements needed for building the GFP viewer are:

– A set of polycarbonate blue and yellow color filters such as those used by the lighting industry such as roscolux/supergel filters. Two Glass or Plexiglas 200 × 145 mm rectangular plaques (2) are used to fix two or three layers of blue filter on the box top. Putting the yellow filter (two layers) into a stationery plastic holder (1) allows for handling it over the observed samples. The blue filter is chosen to cut wavelength longer than 500 nm, whereas the yellow filter eliminates wavelength shorter than 500 nm.

– A compact fluorescent lamp (3) with socket. It should emit around 480 nm. A Blue compact fluorescent lamp should be preferred to the Daylight type.

– A ballast (4) for the neon tube and a starter lamp (5) with socket; interrupter (6), fuse, cable and plug (7).

– A plastic, wood or metal box (8), preferably reflective or white inside. Putting a rectangular mirror under the fluorescent lamp gives a good increase in luminosity.

Figure 2. Observation of GFP expressing larvae: At left: 3rd instar larvae. upper: control, lower: Bloomington stock 4559 (FM7i-pAct-GFP). At right: P{Gal4} enhancer/UAS-GFP 3rd instar larva: GFP Expression is visible in the imaginal discs and salivary glands.

The two major elements, crucial for efficiency, the fluorescent lamp emission spectrum and the filter set transmission spectrums, have to be chosen carefully so as to fit the excitation/emission spectra of the S65T-GFP (Heim *et al.*, 1995). When leaving the viewer on for long times, temperature buildup could occur, especially if using a daylight lamp. Ventilation has to be assured by drilling holes in the box or using metallic meshes, to allow air flow.

Pictures (Figure 2) were taken using the GFP viewer and a Sony DKC5000 CCD (contrast and luminosity have been altered to accommodate grayscale images, originals visible online).

Using the GFP viewer, it is possible to observe the expression pattern and easily discriminate P{Gal4} enhancer trap/ UAS-GFP larvae due to the high expression of GFP. Sorting larvae that carry actin 5c-GFP balancers (Reichhart and Ferrandon, 1998) requires more attention as the pattern and level of expression may be lower in those larvae.

Inquiries for more technical details are also welcome by E-mail at peyre@c.metro-u.ac.jp or by mail at the laboratory. More figures and color pictures are available online at: http://www.sci.metro-u.ac.jp/~akira/gfpview.html. For people interested in buying an already-made version of the GFP viewer, a company will start producing it soon. We will be happy to send its name and address upon request.

Acknowledgment: J-B Peyre is supported by a scholarship from the Rotary Club - Yoneyama Foundation.

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New type of CyO and TM3 green balancers.

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For differentiation between mutant homo- or heterozygotes during different stages of development, recessive visible mutations can be used (Lindsley and Zimm, 1992). More recently, transgenes expressing dominant markers were introduced into balancer chromosomes, allowing identification of balancer hetero- or homozygotes throughout development. Reichhart and Ferrandon (1998) used the 5C actin gene promotor driving GFP expression to construct "green balancers". In order to avoid the strong maternal contribution of GFP seen in crosses using these balancers, we constructed a new set of CyO and TM3 green balancers. With the help of P{w[+m]hsp70:GAL4} and P{w[+m]UAS:GFP} elements which had been adjusted to heat shock independent expression after a series of remobilization crosses, balancers were established which allow easy differentiation between mutant homo- and heterozygotes during early embryogenesis, throughout all larval stages, in pupae and in adults. Finally, a series T(Y;2)CyOGFP, T(Y;3)TM3GFP and T(2;3)CyOGFP-TM3GFP translocations have been isolated. The T(2;3)CyOGFP-TM3GFP translocations combine the staining pattern of both CyOGFP and TM3GFP and are especially useful for genetic analysis.

The P{w[+m]UAS:GFP} element expresses the natural form of GFP (Yeh *et al.*, 1995), allowing GFP detection under normal UV microscope with DAPI filter. In our experiments, we use a Zeiss UV dissecting microscope. For analysis of embryos (without dechorionation) and early first instar larvae a regular fluorescence microscope can be used.

The green balancers were constructed by the following steps:

(1) Jump of P{w[+m]UAS:GFP} onto CyO and TM3, Ser: an X chromosomal P{w[+m]UAS:GFP} insert was remobilized in males of the constitution P{w[+m]UAS:GFP}/Y; CyO/+; P{(ry[+])2-3} Sb/+ and P{w[+m]UAS:GFP}/Y; P{(ry[+])2-3} Sb/TM3, Ser males, respectively. P{ry[+])2-3} is described in Robertson *et al.* (1988). Individual F_1 w[+m] CyO or w[+m] TM3 males were tested for linkage of w[+m] with the dominant Cy and Ser marker mutations, respectively. From the set of about 10 to 15 CyO, P{w[+m]UAS:GFP} and TM3, P{w[+m]UAS:GFP} chromosomes, representative lines expressing a homogenous yellowish eye color were selected for further work.

(2) Jump of P{w[+m]hsp70:GAL4} onto CyO, P{w[+m]UAS:GFP} and TM3, P{w[+m]UAS:GFP}: a phenotypically nearly red eye second chromosomal P{w[+m]hsp70:GAL4} and third chromosomal P{w[+m]hsp70:GAL4} line was obtained from J. Urban and G. Technau (University of Mainz, Germany). Males of the genotypic constitution P{(ry[+])2-3}/Y; CyO P{w[+m]UAS:GFP}/ P{w[+m]hsp70:GAL4} and P{(ry[+])2-3}/Y; TM3, y[+] Ser P{w[+m]UAS:GFP}/ P{w[+m]hsp70:GAL4} were crossed to w/w females. In the F_1 generation, Cy or Ser males expressing a wild type red eye color were collected. Linkage of the red eye color phenotype with Cy or Ser dominant markers were tested by a backcross to w/w females. Balancers were selected which gave strong green fluorescence after heat shock treatment. In order to select for heat shock-independent expression of green fluorescence, further remobilizations were performed.

(3) For remobilization of P{w[+m]hsp70:GAL4 on CyO, P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP} and TM3, Ser P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP} balancer-bearing females were crossed to TM3, ry^{RK} Sb e P[(ry^{+})2-3]/Df(3R)C4 (Reuter *et al.*, 1993) or Sb P[(ry+)2-3]/TM6 males, respectively. Offspring males w/Y; CyO, P{w[+m]hsp70:GAL4} P{w[+m}UAS:GFP}/+; TM3, ry^{RK} Sb e P[(ry^{+})2-3]/+ and w/Y; TM3, Ser P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP}/Sb P[(ry^{+})2-3] were crossed to w/w females. Flies were allowed to lay eggs on small petri dishes, which were inspected for freshly hatched first instar larvae with strong GFP fluorescence. Individual larvae were collected and grown to adults. From those CyO, P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP}/Sco and TM3, Ser P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP}/Sb stocks were constructed. Finally, chromosomes were selected which expressed heat shock-independent green fluorescence beginning in early embryogenesis and continuously throughout the rest of development.

(4) We also isolated a series of CyGFP-TM3GFP, Y-CyOGFP, Y-TM3GFP and Y-CyOGFP-TM3GFP translocations after irradiation of w/Y; CyOGFP/+; TM3GFP/+ males with 4000R of X-rays. The

irradiated males were crossed to w/w females and F_1 w/Y; CyOGFP/+; TM3GFP/+ and male offspring tested for linkage between Cy and Ser or Cy and Ser and the Y chromosome, respectively, after backcrossing to w/w females. From 4717 males tested, 160 translocations were identified (142 T(2;3)CyOGFP-TM3GFP, 4 T(Y;2)CyGFP; 7 (T(Y;3)TM3GFP and 7 T(Y;2;3)CyGFP-TM3GFP). From these, representative translocations were selected, inspected for GFP expression and strains constructed.

Expression Pattern of GFP: Strongest GFP expression is seen in T(2;3)CyOGFP-TM3GFP. None of the balancers show any maternal contribution of GFP, and mutant homo- and heterozygotes can be distinguished from the stock in ca. 10-12 hour old embryos (without dechorionation). The most pronounced fluorescence is visible in salivary glands and the midgut region. In addition to the strong fluorescence of salivary glands and midgut, a more homogenous background fluorescence is also found (*e.g.*, imaginal discs, brain, fat body and gut) throughout larval stages of development. Heat shock treatment strongly enhances GFP expression, but even at 18°C the T(2;3)CyOGFP-TM3GFP translocation allows unambigous differentiation of balancer-bearing animals. We have successfully used these balancers in determining the lethal phase of recessive lethal PEV modifier mutations.

Selected pictures showing GFP expression in embryos and larvae can be viewed at http://www.biologie.uni-halle.de/Genetics/Drosophila/GreenBalancers/index.html.

The following stocks were sent to Umea and Bloomington stock centers:
CyOGFP: w[1]; CyO, P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP}/Sco[1]
TM3GFP: w[1]; TM3, y[+] ri[1] p[p] bx[34e] e[s] Ser[1] P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP}/Sb[1]
T(2;3)CyOGFP-TM3GFP: w[1]; Sco[1]; T(2;3)CyO, P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP}; TM3, y[+] ri[1] p[p] bx[34e] e[s] Ser[1] P{w[+m]hsp70:GAL4} P{w[+m]UAS:GFP}/Sb[1].
Other translocation lines are available upon request.

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A weakly biased *Drosophila* trap.

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Traps always present some bias. However, we noticed that most traps used in Brazil to collect *Drosophila* are strongly biased against flies of the *tripunctata* species group (one of the two most abundant in South American Forests). To try to minimize this problem, we tested 32 different models of traps obtained by successive changes of 7 basic types, starting out with the trap described by Tidon and Sene (1988). Twenty eight experiments in the laboratory and in the field were carried out. We chose the trap that performed best on the following criteria: total number of flies caught for the same volume of bait; smallest bias in relation to the species frequency in the environment (known in laboratory experiments); retention of collected animals; protection of flies from dying by gaseous accumulation or by sticking to the bait; and being less cumbersome.

In the last experiment, we compared, in the field, the trap against the traditional method of collecting *Drosophila* – collection over banana baits. Nine pairs of trap and bait were set up at least 20 meters from each other. In each set the distance between the bait and the trap was about 4 to 8 meters. No significant differences were detected for the sex ratio per class (Figure 1a). For the species composition, we found similar results for the two methods (Figure 1b), although there were still differences in the class frequencies. Yet, the similarity is better than we expected, and differences are almost negligible when compared to the differences among pairs (not shown).

The trap is made with three transparent 2 litre soft-drink bottles and a piece of panty hose (Figure 2c). In part 3, four windows measuring 0.5 cm × 2 cm are opened. Parts 1, 2 and 3 are assembled and fixed. Two pieces of soft paper (Figure 2b) are introduced with a wire to fill the spaces where the flies could die (Figure 2f). Dark parts of the figures are painted with black ink. The cap can receive a piece of filter paper to remove

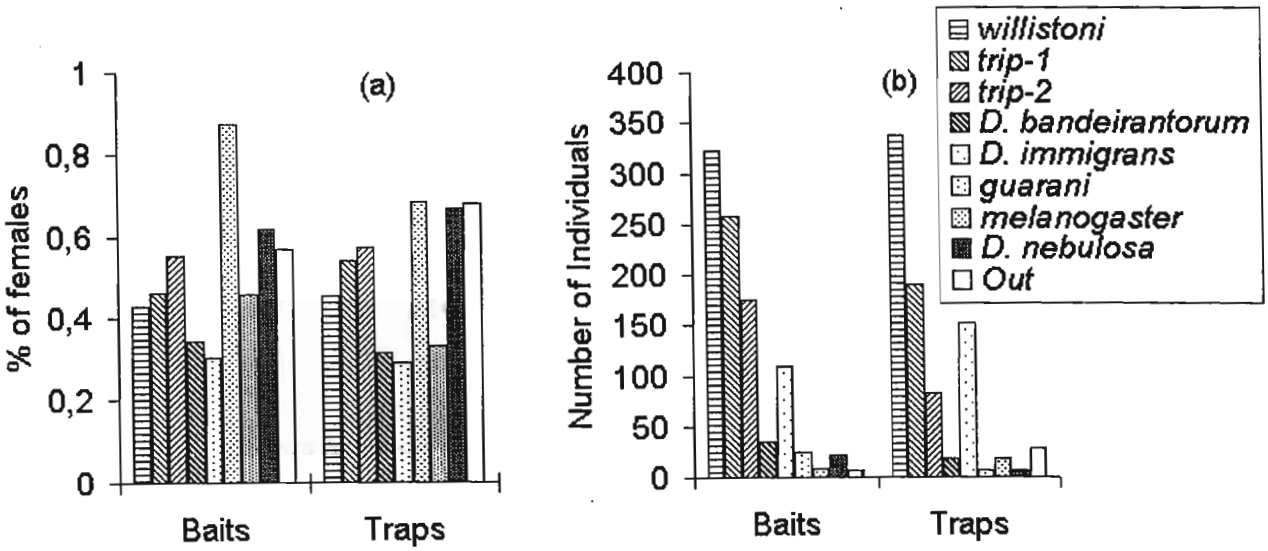

Figure 1. (a). Relative frequency of females per class for nine sets with trap and bait; (b). Total number of flies by class. *willistoni* = *willistoni* group-*D. nebulosa; Trip* I = *tripunctata* group; Trip2 = *tripunctata* group-*D. bandeirantorum; melanogaster, guarani* and *repleta* are species group.

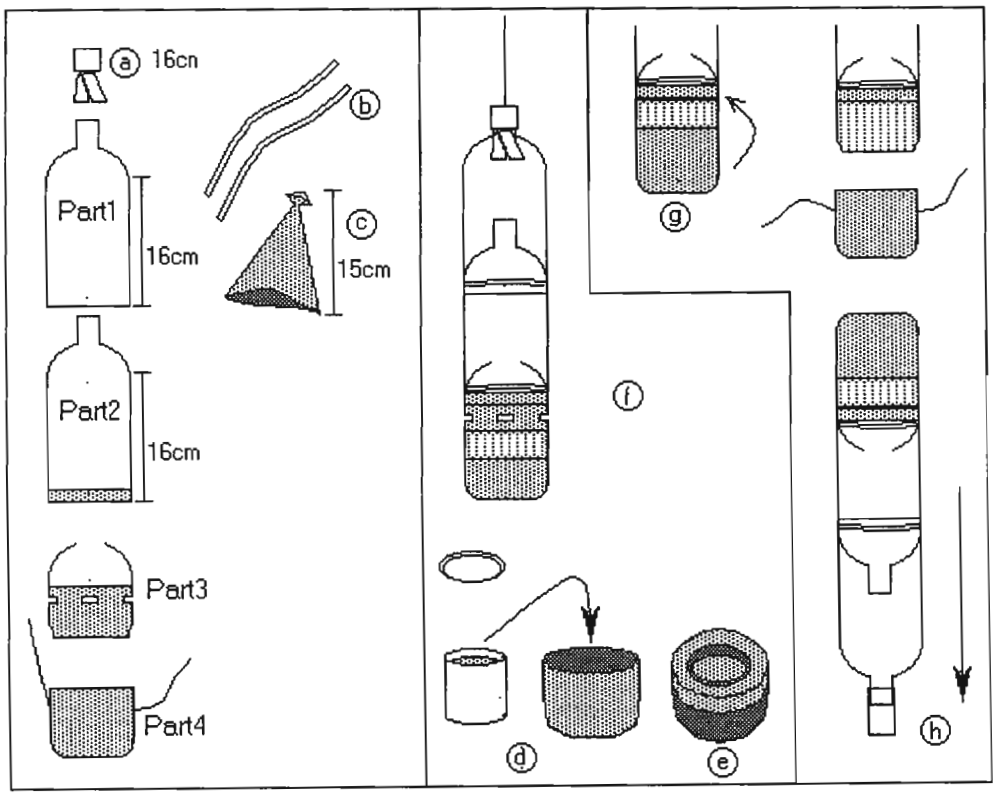

Figure 2. Making and using the trap. Explanations in the text.

humidity (Figure 2a). To set up the trap in the field, bait is put inside part 4 (directly or inside a vial as showed in Figure 2d). The piece of panty hose is set up and pressed in such a way that it gets attached to the bait (this contact is very important for the efficiency of the trap) (Figure 2e). Then part 4 is adjusted to part 3 and both are attached. The trap can then be hung on the vegetation (Figure 2f). To collect the trapped flies, the panty hose is pulled up closing the entrances (Figure 2g). Part 4 is separated and the bait removed. To transfer the flies, the cap is removed, and the trap, turned upside down, is knocked vigorously in a vial (Figure 2h).

Closing the entrances with the panty hose can be useful allowing the transport of the trap in the field preventing flies from outside the study area to get into the trap. By now, we have collected more than a thousand samples with this model of trap. It has proven to be resistant to rain and practical to use in the field.

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Using the MARCM system to positively mark mosaic clones in *Drosophila*.

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Introduction to the MARCM system: Mosaic analysis is a powerful tool to analyze gene functions in many biological processes. In *Drosophila*, the introduction of highly efficient FLP/FRT systems (Golic and Lindquist, 1989) and effective ways of marking clones (Xu and Rubin, 1993) allowed for both functional analysis of candidate genes and identification of new genes by mosaic screening. In the widely used marking system (Xu and Rubin, 1993), the marker transgene is placed distal to the FRT site in trans to the mutant of interest, such that homozygous mutant cells are the only cells that are not labeled. In many cases, it is desirable to visualize the mutant cells but not heterozygous parents or homozygous wild type siblings. The MARCM (for *M*osaic *A*nalysis with a *R*epressible *C*ell *M*arker) system we developed (Lee and Luo, 1999) serves this purpose.

An organism subjected to MARCM analysis must contain at least six transgenes: two transgenes for two homologous FRT sites, one for the FLP recombinase, one for a UAS-marker, one for a GAL4 driver, and lastly one for the *tubP-GAL80* transgene. The *tubP-GAL80* must be placed distal to the FRT site in trans to the mutant gene of interest. In heterozygous cells, GAL80 inhibits GAL4 induced UAS-marker expression. Only in homozygous mutant cells, in which the *tubP-GAL80* transgene is not present, will the marker be expressed (Lee and Luo, 1999).

Much interest has been generated in using the MARCM system since the publication of our original paper. In this Technique Note, we describe some of the recent additions to the MARCM system and discuss different parameters that affect the use of the system.

Completion of the MARCM chromosome arms: Since the publication of the original paper (Lee and Luo, 1999), we have been able to generate *tubP-GAL80* transgene insertions on 2L and 3L, using D2-3 mediated P-element transposition (Robertson *et al.*, 1988). Thus a complete MARCM set is available for all major chromosome arms. New *tubP-GAL80* insertions were initially tested for their ability to suppress the phenotype created by *Drac1L89* expression in the eye using the UAS-GAL4 system (Brand and Perrimon, 1993; Lee and Luo, 1999). We also confirmed the ability of these *tubP-GAL80* transgenes to suppress GAL4 induced marker expression in larval brains and discs. We found and discarded many *tubP-GAL80* insertions that only partially suppressed GAL4 induced gene expression in at least one of the above assays, presumably due to positional effects that reduced the expression of *tubP-GAL80* in certain cells.

All *tubP-GAL80* insertions have been recombined with the most commonly used FRTs. These recombinant chromosomes, as well as other related strains, are available at the Bloomington Stock Center under the stock number 5128-5148 (for X, 2R, 3R), and 5190-5192 (for 2L, 3L). There is also a web page for strains containing the *tubP-GAL80* insertions (http://flystocks.bio.indiana.edu/gal80.htm).

FLP enhancer trapping: One of our original goals of establishing the MARCM system is to enable consistent labeling of identifiable neurons in different organisms. The best way to conduct tissue-specific mosaic analysis is to restrict FLP activity spatially. Unfortunately, one cannot use the combination of *UAS-*

FLP (Duffy *et al.*, 1998) and tissue specific GAL4 lines in the MARCM system because the expression of GAL80 inhibits the expression of *UAS-FLP* in the first place to prevent mitotic recombination. To achieve tissue-specific expression of FLP, we conducted an enhancer trap screen. We inserted the open reading frame of the yeast FLP recombinase (Golic and Lindquist, 1989) into an enhancer trap vector (Giniger *et al.*, 1993). Using third instar larval brain as the assay system, we screened through 750 independent insertions on the autosomes for FLP under the control of specific enhancer elements. We used two assays to test FLP activity (Figure 1). While almost 50% of the FLP enhancer trap lines gave patterns in the brain using the flip-out assay (Figure 1A) (Struhl and Basler, 1993; Pignoni and Zipursky, 1997), very few lines gave reproducible patterns using the trans-FRT assay (Figure 1B). For those few lines that yielded patterns in the CNS in the trans-FRT assay, each animal had a different subset of labeled neurons, suggesting that we trapped several pan-CNS enhancers, and each neuroblast lineage has a small frequency of productive mitotic recombination. These experiments suggest that either the frequency of FLP/FRT mediated mitotic recombination is much lower than that of intra-chromosomal flip-out, or that most of the lines that score positive for flip-out assay but negative for trans-FRT assay start their expression in post-mitotic neurons, or both.

The low frequency of FLP-induced recombination was also confirmed by another experiment, in which we tested the efficiency of *GMR-FLP* using the trans-FRT assay. *GMR-FLP* is expressed posterior to

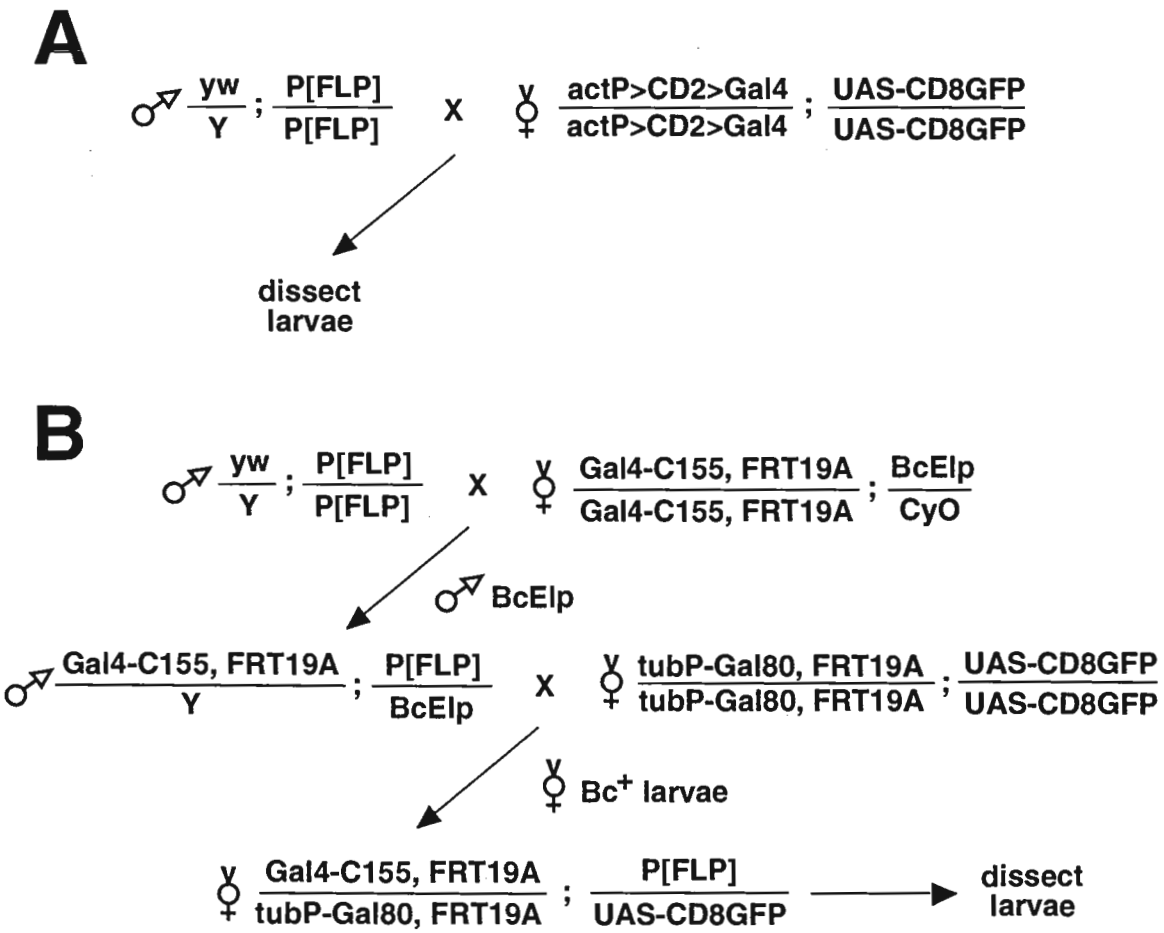

Figure 1. Genetic schemes to detect FLP enhancer trap activity. (a) Flip-out assay: the two FRT sites (>) are on the same chromosome. (b) Trans-FRT assay: the two FRT sites are on two homologous chromosomes. The scheme only illustrates the cases in which FLP is located on the second chromosome.

the morphogenetic furrow, and is thus capable of generating clones in R1, R6 and R7 photoreceptor cells (Pignoni *et al.*, 1997). In theory, there are potentially 3 × 800, or 2,400 mitosis that are targets of FLP-mediated mitotic recombination that gives rise to photoreceptor neurons. In each mitosis there is a 25-50% chance (Lee and Luo, 1999) that recombination would occur in a way such that one of the two daughter cells would lose *tubP-GAL80*, and a further 50% chance that the GAL80-negative daughter cell would be a photoreceptor neuron. Thus the estimated GAL80 deprived photoreceptor neuron is 300-600 if every *GMR-FLP* expressing precursor would undergo mitotic recombination. However, we observed an average of 30 neurons that were labeled in each eye when the MARCM clones were examined in the mid-pupal stage. This gave us an indication that even when FLP is under the control of a strong synthetic promoter like GMR, the frequency of generating mitotic recombination is between 5-10%.

We found that *hs-FLP* is the most efficient way to generate mitotic recombination at a reasonable frequency for MARCM analysis. Although *hs-FLP* lacks spatial control, one can sometimes choose heat shock window to favor the generation of clones in desired cell type, as only cells under going active proliferation are likely to be targets for mitotic recombination (Lee and Luo, 1999). For instance, we were able to generate mushroom body-specific clones by performing heat shock at the time when MB neuroblasts are preferentially active in proliferation (Lee and Luo, 1999).

Choice of GAL4 lines: In order to mark mosaic clones efficiently, strongest GAL4 drivers are always the best choice. This is especially true when one wants to visualize single-cell clones. There is no extra cell division to dilute the GAL80 protein inherited from the parental heterozygous cells. Consider the example of generating two-cell clones in MB lineage. We previously reported that the frequency of generating two-cell clones is much lower compared with Nb clones in the MB lineage when we used the *GAL4-C155* as a driver, whereas the theoretical prediction should be 1:1 (Lee and Luo, 1999). Subsequently we found that if we examined male progeny, in which *GAL4-C155* expression is at a higher level due to X-chromosome dosage compensation, the frequency of two-cell clones was higher. Recently, we have used *GAL4-201Y*, a MB GAL4 driver located on chromosome 2R, to visualize MB clones in combination with *FRT (2R-G13)*. We found the predicted 1:1 ratio of MB neuroblast clones to two-cell clones. In addition, all of the two-cell clones were much more strongly labeled (Lee *et al.*, 1999). This intense labeling was presumably due to the strong expression of *GAL4-201Y* in MB neurons as well as the homozygosity of the *GAL4-201Y* transgene in mitotic clones.

Ideally, the GAL4 driver should be ubiquitous, such that all mutant cells devoid of *tubP-GAL80* can be visualized. This was the reason we developed the *tubP-GAL4* transgene (Lee and Luo, 1999). However, we found that a high level of ubiquitous GAL4 expression is toxic to the organism based on the following lines of evidence. First, the frequency of generating *tubP-GAL4* transgenic lines was 5-10 fold lower than other transgenes of comparable size. Second, it was very difficult to generate new insertions of *tubP-GAL4* using D2-3 transposase-induced P-element transposition. Third, of the all the insertions we obtained, none of them was homozygous viable. The homozygous lethality can be rescued by the presence of one copy of the *tubP-GAL80* transgene, thus identifying the high level of GAL4 expression as the cause of the lethality.

For certain applications, it may not be necessary to have GAL4 expressed in all GAL80-negative cells. In fact, sometimes it is advantageous to visualize only a subset of cells that have lost *tubP-GAL80*. For instance, selectively visualizing MB neurons using MB GAL4 lines in the MARCM system helped us to decipher the development of axon projection in wild-type animals (Lee *et al.*, 1999). If FLP is under the control of a specific promoter, there may only be a limited numbers of cells that can undergo mitotic recombination. The GAL80-negative and GAL4-negative "background" cells, when mutant, may not influence the development of cells of interest (GAL80-negative, GAL4-positive) due to, for instance, physical distance. In those cases using cell type-specific GAL4 lines to visualize only the cells of interest may facilitate mosaic analysis.

UAS-Marker: The membrane-targeted mCD8-GFP (Lee and Luo, 1999) has proven to be a good marker. We have generated insertions on X, 2L, 2R, and third chromosomes, which are all available at the Bloomington Stock Center.

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An efficient mutagenesis screen to generate duplications of polytene section 8 on the X chromosome of *Drosophila melanogaster*.

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SUMMARY

Duplications of the X chromosome are invaluable tools for conducting detailed genetic analysis of X chromosome genes. Appropriate duplications are essential for defining complementation groups within a given interval. Although 77% of the euchromatic X chromosome is covered by duplications, several regions remain devoid of these tools. Interval 8B is one such region, which prompted us to design a method to create a duplication that was specific to this segment. We initiated a genetic screen to isolate duplications that covered the 8B region. Our strategy utilized an X-Y translocation and an attached X-Y chromosome to generate viable pieces of the X chromosome, connected to a complete Y chromosome. Our results demonstrate an efficient method for generating duplications of the X chromosome and describe five new duplications that span region 8B. Two of these duplications bridge the gap between previously described duplications, while the other three cover only a portion of these segments. These new duplications provide important tools that will permit a systematic genetic analysis of the 8B interval to be conducted.

1. INTRODUCTION

Screening for lethal mutations on the X chromosome requires that the region of interest be covered by a duplication that resides elsewhere within the genome. These duplicated segments enable lethal mutations to be carried in hemizygous males that possess only one X chromosome. By carrying mutations in males, complementation tests can then be performed to facilitate the characterization of alleles within a given interval. Unfortunately, regions that lack appropriate duplications cannot be genetically dissected, because complementation analysis is not possible. Thus, the availability of appropriate duplicated segments is essential for conducting detailed genetic analysis of X chromosome genes. Studies have shown that approximately 77% of the euchromatic X chromosome is covered by duplications (Hilliker *et al.*, 1980; Eberl *et al.*, 1992).

One region which is devoid of available duplications is the 8B interval. Our interest in this region initiated with the observation that a cDNA sequence that we had identified, termed *dlim1*, mapped to this segment. The results of which will be presented elsewhere (Lilly *et al.*, 1999, submitted). To our knowledge all pre-existing duplications that spanned this segment had been lost and were unavailable for our use. As a consequence, to carry out a genetic analysis of the region we were compelled to generate a duplication that covered the 8B interval. We designed a screen to create a X chromosome duplication on a complete Y. Y-linked X chromosome duplications are particularly useful because they segregate from the X chromosome in males and facilitate complementation analysis.

The strategy that we used was based on the method of Brosseau *et al.*, (1960), in which they described the generation of Y chromosomes bearing specific sections of the X chromosome. The design of their screen was dependent upon the fulfillment of two requirements. First, that the translocations used had breakpoints near the region of interest. Secondly, that the irradiated translocations retained small enough fragments to

prevent hyperploidy. Using this as a framework, our screen took advantage of a pre-existing X-Y translocation with breakpoints near 8B. By inducing a recombination event between our translocation and an attached X-Y chromosome, we provided an intact Y chromosome. The complete Y chromosome supplied the neccessary elements for proper segregation and fertility. Using the recombined version of the translocation chromosome, we then induced breaks by γ rays and screened for viable males. Viable males with potential 8B duplications were first screened for their ability to complement *oc[1]* in 8A, and subsequently with neighboring alleles.

From our screen we generated five duplications that complemented *oc[1]* and extended into region 8B. Our results describe an efficient method for generating viable pieces of the X chromosome attached to a complete Y chromosome. The strategy we used allowed us to screen very few flies to recover several individual chromosomes with the region of interest. The duplications that were generated cover segments spanning 8B, a region which had previously lacked available duplications. These new duplications provide additional useful tools for the genetic analysis of genes that reside within this interval. These tools will allow for a systematic complementation analysis to be achieved, and facilitate the characterization of genes within region 8B.

2. MATERIALS AND METHODS

(i) Fly stocks

All fly strains were maintained on standard cornmeal, molasses, yeast, and agar medium. Detailed description of most stocks can be found in Lindsley and Zimm (1992) and/or in Flybase. Stocks used in this study were, *y[1] btd[1]/FM7c; y[1] lz[89d18-15] f[1]/Dp(1;Y)lz; ct[n] oc[1]/FM1;* and *lz[K];* obtained from Bloomington stock center. Stocks, *FM4, w[1] B[+]/T(1;Y)156, y[1] B[S]; 0/C(1)M4, y[2]/XYL-YS, y[2] su[wa] wa; C(1)RM, y/XYL-YS, y[2] su[wa] wa; 0/C(1)RM, y[1] v[1] bb[**]/C(1;Y)129-16, y[2] y[+] su(w[a]) w[a];* and *w[1] otd[1]/FM7c* were obtained from Mid-American stock center. *C(1)DX, y[1] w[1] f[1]; Dp(1;2)FN107/bw[D]* was provided by Robert Finkelstein. The *Nrg* allele, *In(1)RA35/FM7c*, was obtained from Corey Goodman. New chromosomes generated in this study are diagrammed in Figures 1 and 3.

(ii) Mutagenesis screen

For the duplication screen, 2100 males of genotype, *XYL-YS, y[+] T(1;Y)156, y[1] B[S]* carrying the newly recombined translocation, diagrammed in Figure 1, were subjected to 3500 rad of gamma irradiation (Gammacell-1000). The irradiated males were allowed to recover for eight hours, and then mated *en masse* to *y[1] w[1]* females for 72 hours, after which the males were discarded. Females were allowed to lay eggs for another 96 hours, 7 days in total. Individual F1 viable male progeny from this cross, that possessed at least one of the Y chromosome markers, *B[S]* and *y[+]*, were crossed to three females of genotype, *ct[n] oc[1]/FM1*. F2 male progeny from this cross carrying the *ct[n] oc[1]* chromosome and a putative duplication on the Y, were screened for the presence of ocelli, indicating the duplication contained X chromosome material that rescued the locus. The F2 males carrying duplications that rescued the *oc* phenotype were established as balanced stocks. Duplications were maintained using the compound chromosome *C(1)DX, y[1] w[1] f[1]* in females, and an embryonic lethal allele, *otd[1]* in males.

(iii) Complementation tests

Complementation tests to map the breakpoints of the duplications were conducted similar to those for the *oc[1]* allele in Figure 2. Males carrying the newly generated duplications *Dp(1;Y)* and *otd[1]* were crossed to females carrying the allele of interest, and an X chromosome balancer. The ability of the duplications to complement lethal alleles was determined by the presence of male progeny that carried markers for the duplication, *y[+] B[S]*, and for the allele of interest. For the viable *lz[K]* allele, all males were screened for the rescue of the rough eye lozenge phenotype. For each cross greater than 100 progeny were scored.

3. RESULTS

(i) Generation of a recombined X-Y translocation

The goal of the duplication screen was to generate a piece of the X chromosome that contained region 8B attached to a complete Y chromosome. An entire Y chromosome was an essential requirement to provide all the fertility genes and the Y centromere for proper segregation. We utilized the availability of a previously described X-Y translocation stock that had breakpoints near region 8B. This provided an advantage to creating a viable piece of the X chromosome with breakpoints near the region 8B. We chose X-Y translocation *T(1;Y)156* (Lindsley and Zimm, 1992) that has the long arm of the Y chromosome attached to the X chromosome portion 7D-20 to the centromere, while the short arm of the Y chromosome is attached to X chromosome portion 1A-7C.

The starting translocation is shown in Figure 1A. Since our interest was in the 8B region we sought to place the portion of the X chromosome 7D-20 containing 8B on to a complete Y chromosome. To do this we recombined the sequences of the translocation *T(1;Y)156* with that of an attached X-Y chromosome, *XYL-YS 129-16, y[2] y[+] su(w[a]) w[a]* (Figure 1B). By doing so, we generated a translocation that contains the original short arm of the Y chromosome attached to X chromosome 1-7C, and a newly recombined chromosome. The recombined chromosome contained the original long arm of the Y chromosome attached to X chromosome 7D-20, but was now joined to a complete Y chromosome from the attached X-Y chromosome (Figure 1E). The newly recombined translocation was designated, *XYL-YS, y[+] T(1;Y)156, y[1] B[S]*. Each recombined translocation was established as an individual stock using a compound chromosome *C(1)M4, y[2]*. Because the two pieces of the translocation segregated independently, the stock consisted of females with the compound chromosome *C(1)M4* and *Y[S]* with X chromosome 1-7C. The males of this stock contained both portions of the X-Y translocation (Figure 1E). All other combinations of these three chromosomes resulted in non-viable progeny which permitted the stock to be balanced. The females of this stock, containing the compound chromosome and a part of the translocation required additional care to maintain. Presumably the combination of the translocation segment 1-7C, and the compound chromosome had an adverse affect on their viability. With the generation of the recombined translocation we had the primary tool that we needed for the production of duplications that covered our region of interest, 8B.

Table 1. Results of the duplication screen.

Number of irradiated males	2100	
Number of males recovered	1608	
y^1w^1	1113	
$y^1w^1B^S$	329	
y^+w^1	56	} 495
$y^+w^1B^S$	110	
Number of males crossed to oc^1	495	
Number of fertile males	153	
Number that rescued oc^1	5	

Recovered males were categorized based on *y* and *B* markers. Only males with a y^+ and/or B^S marker were tested to complement oc^1.

Table 2. Complementation tests.

	Alleles				
Duplications	*nrg*	*oc*	*dlim-1*	*lz*	*btd*
Dp(1;Y)578	—	+	+	—	—
Dp(1;Y)619	—	+	+	—	—
Dp(1;Y)850	—	+	+	—	—
Dp(1;Y)867	—	+	+	+	—
Dp(1;Y)921	—	+	+	+	—

Complementation tests with the new duplications. (—) duplication fails to complement allele. (+) duplication complements the allele.

(ii) Mutagenesis screen for X chromosome duplications

Our mutagenesis screen was designed to induce double strand breaks in the newly recombined chromosome, that would generate viable pieces of the translocation in males that possessed an additional X chromosome. By using a translocation that had breakpoints near region 8B, we essentially predetermined the distal breakpoint. Therefore, our screen was simplified by having only to generate a proximal breakpoint that

was small enough to be compatible with viability. Since the two pieces of the translocation segregated independently from one another, it allowed us to screen by markers for viable males that carried the portion of the translocation that contained region 8B. Males that retained too much of the translocation would not survive as a consequence of hyperploidy (Patterson *et al.*, 1937). Thus, only males that had lost a considerable part of the X chromosome material would be viable. This provided us with a powerful and efficient screen by eliminating all males that retained unuseable translocations.

The design of the screen is diagrammed in Figure 2. Males carrying the translocation were subjected to γ irradiation, allowed to recover and crossed to *y[1] w[1]* females. By discarding the irradiated males after 72 hours we insured that the resulting progeny would be derived from irradiated sperm. As expected, the majority of the progeny recovered were males. Table 1 summarizes the results of the mutagenesis screen. 2100 irradiated males produced 1608 viable males, possessing four different phenotypes. Only 15 females were recovered, and were probably a result of low frequency non-disjunction from the parents. The four phenotypic groups of the males recovered (Table 1), reflected the probable outcomes resulting from chromosome breaks due to the γ rays. As shown in Figure 2 the dominant markers, *y[+]* and *B[S]* were transmitted from the Y portion of the recombinant translocation. Males that lacked both of these markers made up 69% of the viable progeny. The absense of these markers in viable males suggested that these flies had recieved translocations that had lost significant portions of the chromosome, or lacked it altogether. The three other phenotypes, making up 31% of the progeny, contained one or both of the dominant markers, *y[+]* and *B[S]*. However as a consequence of hyperploidy, these males must have lost most the X chromosome to be viable. Surprisingly, no males were recovered that retained the *w[+]* marker of the smaller translocation. The reason for this is unclear; however, its proximity to the tip of the X may have reduced the number events which would allow for its transmission.

The males that displayed one or more of the translocation markers with which the 8B region segregated with, *y[+]* and *B[S]*, shown in Figure 2C were crossed to *oc[1]* females. 495 males were crossed to *oc[1]* females, of which only 153 were fertile and produced progeny. From the 153 crosses, five individual lines rescued the *oc[1]* phenotype and were presumed to contain a duplication of the 8A1 region of the X chromosome. Four of these retained the *y[+]* and *B[S]* markers, and were designated, *Dp(1;Y)578, Dp(1;Y)619, Dp(1;Y)867,* and *Dp(1;Y)921, w*hile *Dp(1;Y)850* retained only the *y[+]* marker and lacked *B[S]*. Males with duplications that rescued *oc[1]* were maintained as stocks over a lethal *oc* allele, *otd[1]* in males and a compound X chromosome, *C(1)DX* in females. To verify the regions that these duplications covered, the five duplications were mapped by complementation tests using surrounding alleles outlined in Table 2.

(iii) Complementation tests of duplications

In generating duplicated segments that complemented the *oc[1]* allele, we had predicted that the neighboring region, 8B would be included in one or more of these new duplications. To determine if the 8B region was covered, and to define the breakpoints of these duplications, we carried out complementation tests using alleles that surrounded the *oc* locus at region 8A. Because of the lack of duplications that span 8B-C, few alleles exist that have been cytologically mapped to this region. For purposes of clarity we used only well characterized alleles whose cytological locations had been verified by molecular means for our complementation analysis. Table 2 outlines the results of the complementation tests. The most distal and most proximal alleles that failed to complement these duplications are shown from left to right, respectively. Figure 3 illustrates the mapping data of the five new duplications. Shown are two previously described duplications that flank the 8B region. *Dp(1;2)FN107*, covers the X chromosome region 7A8-8A5 (Craymer and Roy, 1980), and *Dp(1;Y)lz* spans region 8D(7-9)-9A(4-5) (Santamaria and Randsholt, 1995).

The new duplications span the gap that was previously not covered by any available duplications. All five duplications have a distal breakpoint that lies between *Nrg* at 7F1, and *oc* located at 8A1. This is not surprising, considering the original translocation had a breakpoint that was reported to be near 7D (Lindsley and Zimm, 1992). The duplications overlap *Dp(1;2)FN107* and fill the gap that had existed within this interval. The proximal breakpoints of these five duplications vary to a much greater degree. Two duplications *Dp(1;Y)867* and *Dp(1;Y)921* complement *lz,* and thus overlap with the previously described duplication *Dp(1;Y)lz*. Neither of these two duplications complement the *btd* allele, indicating that their breakpoints lie between the interval 8D(8-9) and 9A1. The three other duplications, *Dp(1;Y)578, Dp(1;Y)619,* and

Dp(1;Y)850, failed to complement the *lz* allele. The most proximal allele tested, for which these duplications complement is a newly described gene, *dlim1*, (Lilly *et al.*, 1999, submitted) which has been mapped to region 8B(1-2) by cytology and deficiency analysis. Thus, the proximal breakpoint of these duplications lies somewhere between 8B(1-2) and the *lz* allele at 8D(8-9). Using a host of other alleles scattered along the X chromosome, these duplications failed to complement any of them. This suggests that the duplications are made up of a contiguous stretch of the X chromosome within the 8A-B region and lack any other X chromosome material. In addition, each new duplication was analyzed by cytology using the *dlim1* cDNA, as a marker. Because the Y chromosome does not polytenize it was difficult to map the duplicated segments by cytology. The analysis of the squashes supported our complementation data in that we were able to see pieces of the X chromosome that hybridized with our probe (not shown).

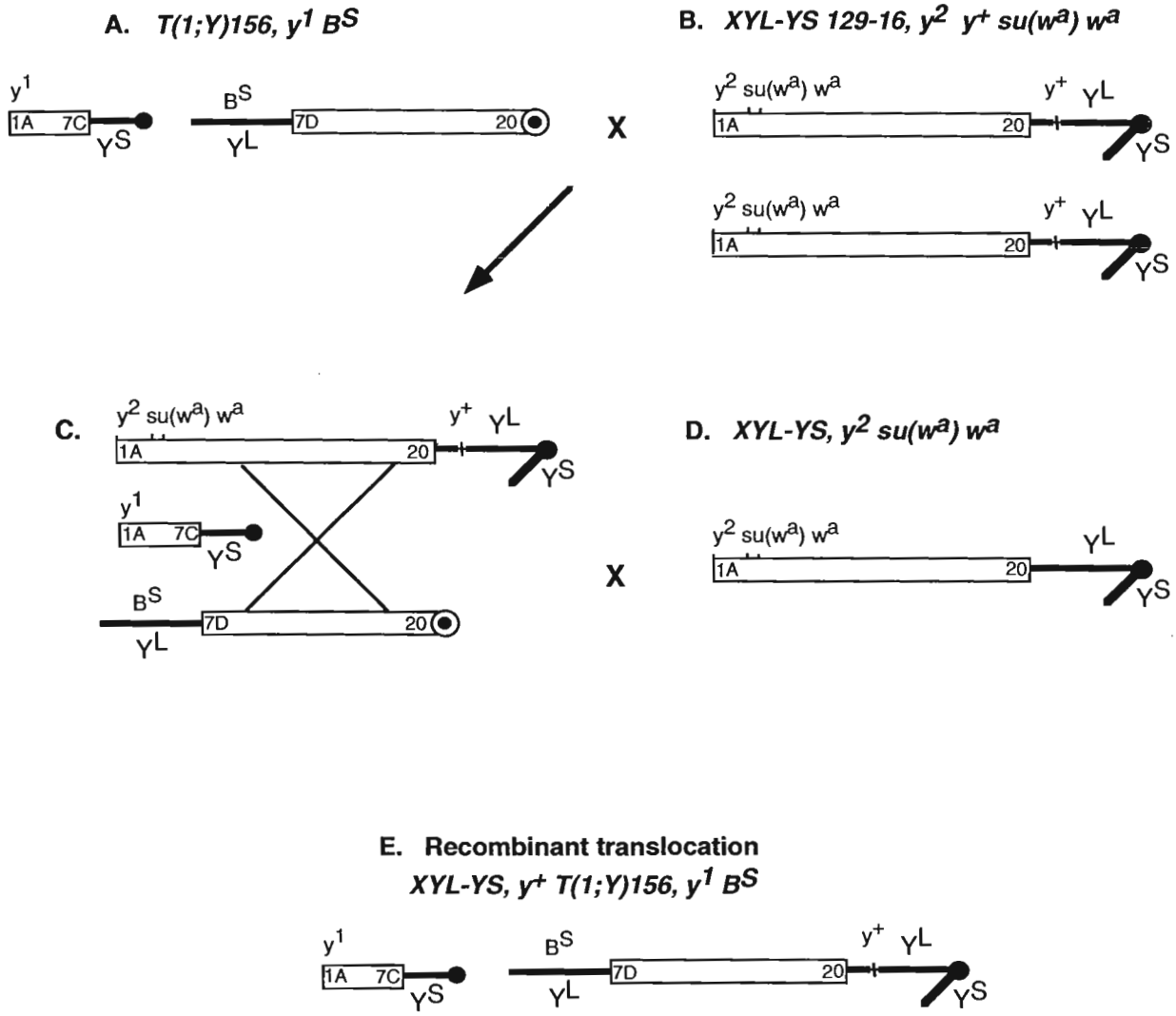

Figure 1. Generation of a recombined translocation chromosome. Open boxes represent X chromosome material and thick black lines represent Y chromosomes. (A) Males carrying translocation *T(1;Y)156, y[1] B[S]* were crossed to females (B), *XYL-YS 129-16, y[2] y[+] su(w[a]) w[a],* having two attached X-Y chromosomes, to generate females, with an attached X-Y and the translocation (C). These females, from which the recombination event was designed to occur, were crossed to males (D), *XYL-YS, y[2] su(w[a]) w[a],* an attached X-Y chromosome. Recombination events were identified by the presence of *y[+]* and *B[S]* markers segregating together in males (E).

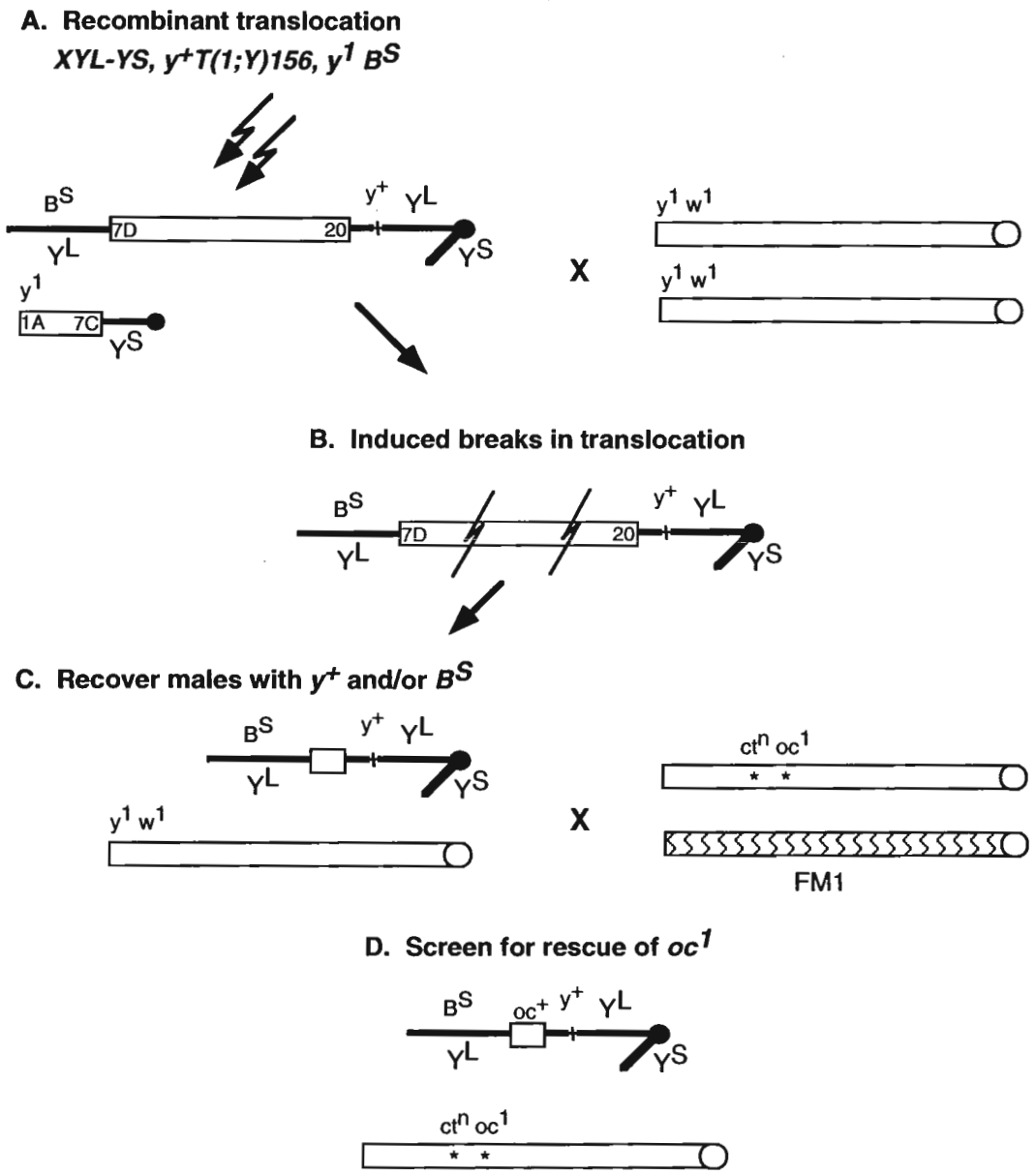

Figure 2. Mutagenesis screen for X chromosome duplications. Open boxes represent X chromosomes and thick black lines represent Y chromosomes. (A) Males carrying the recombined translocation chromosome were subjected to γ irradiation and crossed to *y[1] w[1]* females. (B) Chromosomal breaks produced viable male progeny that retained one or both markers from the recombined translocation chromosome. These males (C), shown with both the *y[+]* and *B[S]* markers, were crossed to *ct[n] oc[1]/FM1* females. The progeny from this cross were screened for rescue of the *oc* phenotype (D). The diagrammed duplicated segment (C), represents just one example of the potential viable chromosomes. Many other possibilities exist due to the random breaks induced by the γ rays. Only males with a *y[+]* and/or *B[S]* markers were crossed to *ct[n] oc[1]* females.

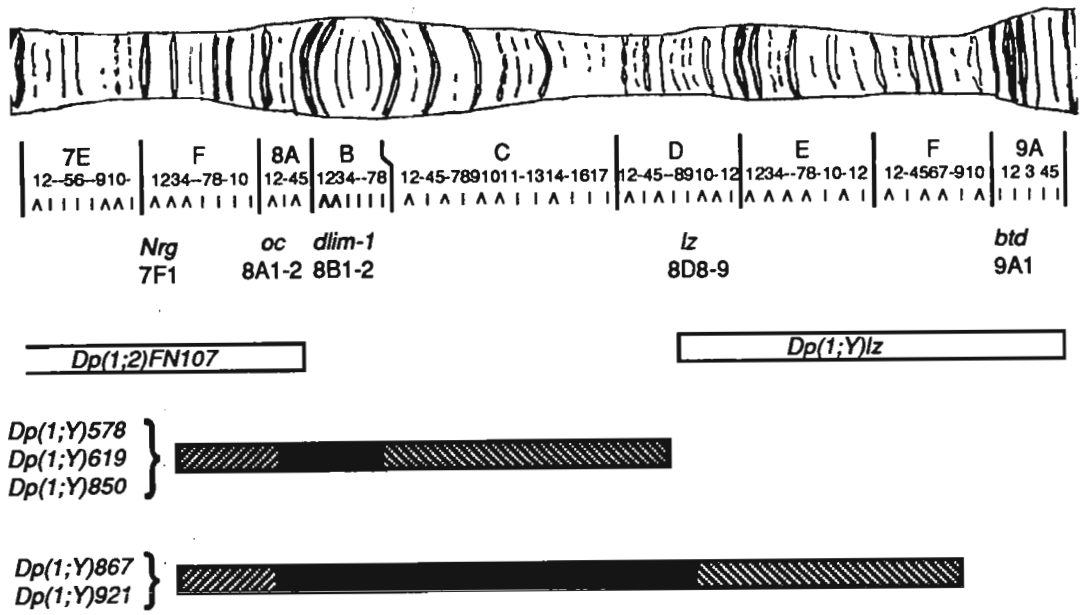

Figure 3. Cytogenetic map of the interval covered by the duplications. The breakpoints of the five new duplications were defined by complementation tests with alleles surrounding *oc*. The alleles used are shown with their map position along the chromosome. Two preexisting duplications are shown as open boxes. The distal duplication, *Dp(1;2)FN107* has breakpoints, 7A8-8A5, and covers *oc*. The proximal duplication, *Dp(1;Y)lz* has breakpoints, 8D(7-9)-9A(8-9), and covers *lz* and *btd*. The new duplications are represented as filled boxes. All have a distal breakpoint between *Nrg*, 7F1 and *oc*, 8A1. Three of these duplications have proximal breakpoints between *dlim1*, 8B(1-2) and *lz*, 8D(8-9). The two remaining duplications break proximally between *lz* and *btd* (9A1). The strips within the boxes represent the region of uncertainty in the breakpoints.

4. DISCUSSION

To facilitate the analysis of a newly discovered gene found in the 8B region of the X chromosome, we undertook a genetic screen to create duplications that covered interval 8B. This region has lacked sufficient characterization because of the absence of duplications that span it. Duplications of the X chromosome enable one to test by complementation the allelelism of mutations within a given region. Thus, without appropriate duplicated segments, comprehensive genetic screens can not be performed. To generate new duplications we utilized an efficient mutagenesis scheme, that was based on that described by Brousseau *et al.*, (1961). The screen design took advantage of a pre-existing X-Y translocations with breakpoints near region 8B, and the lethality associated with hyperploidy of the *Drosophila* X chromosome. Through a recombination event we attached an intact Y chromosome to the translocation which was a neccessary element for generating usuable duplications.

From this screen we generated five new Y-linked duplications that covered the 8B interval. Of these duplications, two span a gap of the 8B region that covers an uncharacterized region. The other three duplications break within this interval and cover smaller portions of the 8A-C region. Our method allowed us to generate duplications of the 8B region with great efficiency and specificity. By taking advantage of the lethality caused by hyperploidy and the translocation breakpoints near 8B, our screening process was highly selective. This scheme could be modified and used for other selected regions of the X chromosome. The duplications that were generated and described here provide us with valuable tools for dissecting out the

genetics of the X chromosome. This study should greatly facilitate further genetic and molecular analysis of loci mapping within this region.

Acknowledgments: The authors thank the Bloomington and Mid-America stock centers for providing fly stocks. We wish to acknowledge Robert Finkelstein and Corey Goodman for providing alleles used in this study. BL was supported by a National Institutes of Health postdoctoral fellowship. This work was supported by grants from the National Institutes of Health and the National Science Foundation to JB.

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Mutation Notes — *Drosophila melanogaster*

Saturation mutagenesis of region 82F.

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In an attempt to isolate mutations in the 82F gene responsible for the late puff there, an X ray and an ENU mutagenesis were run, starting with an isogenic *mwh red e* chromosome and testing against *Df(3R)3-4* (82F3-4;82F10-11, my cytology, does remove the puff) for lethals and visibles in the F_2. Out of 5756 fertile F_1 tests of X-rayed chromosomes (78% of males were fertile, 97% of females) there were 21 lethals = 3.6×10^{-3} for ca 9 bands; out of 3130 fertile F_1 tests of ENU-treated chromosomes (92% of males fertile, 95% of females) there were 14 lethals and one semi-lethal = 4.8×10^{-3}. These were then sorted into three regions by complementation testing against *Df(3R)6-7* (82E3;82F3-7, not my cytology, does remove the puff) and *Df(3R)110* (82C4;82F3-7, not my cytology, does not remove the puff). All mutations within a region were crossed to each other and also to all mutations in the adjacent region(s); 11 compementation groups resulted, with only one (*l(3)82Fh*) being at all complicated, and one deficiency was recovered: *Df(3R)ME15,* 81F3-6;82F5-7. All X-ray induced mutations had their cytology checked; if no aberration is indicated below, then the 82F region had no obvious cytological defect.

In group 1 (lethal over all four deficiencies):

l(3)82Fa: X ray alleles 1 and 2, ENU alleles 3 and 4. Larval-pupal lethals with inclusions; mitotic index low.

l(3)82Fb: ENU alleles 1 and 2. Both alleles are only semi-lethal; escapers have small heads and broad abdomens.

In group 2 (lethal over *Df(3R)6-7, Df(3R)ME15,* and *Df(3R)3-4;* viable over *Df(3R)110):*

l(3)82Fc: X ray alleles 1, 2, and 3, ENU allele 4. *In(3R)82Fc*^{1}, het;82F3-11; *In(3R)82Fc*^{2}, het;82F3-7. Pre-larval lethals.

l(3)82Fd: X ray alleles 1, 2, 4, 5, 6, 7, 8; ENU allele 3. *Tp(3;Y)82Fd*^{1}, 82F3-11;98F8-14; *T(2;3)82Fd*^{1}, 42E3-7;82F5-7; *Tp(3;3)82Fd*^{7}, 82F5-7;92D1+;92F3-5, new order 61A1-82F5|92D2-92F5|82F5-92D1|92F5-100. All alleles are eclosion lethals over deficiencies; flies assisted from their pupal cases are alive, and weaker combinations give significant levels of escapers who had been wing-stuck. Alleles 2 and 3 have brown eyes over deficiencies and allele 1 has variegated brown eyes (since the parent chromosome carries *red,* this phene has not been assayed in the heteroallelic combinations); the rest have wild-type eyes. Allele 3 is homozygous viable and fertile; all other alleles are stronger, with roughly the order (strongest) 1, 2; 4, 7; 8; 5, 6 (weakest) based on relative viabilities over deficiencies and each other, although slightly different orders result from different comparisons, suggesting that the gene may be somewhat complex -- as indeed is already suggested by the variation in eye color effects. Allele 4 does not puff the 82F puff, suggesting that *l(3)82Fd* is the puff gene itself.

l(3)82Fe: X ray allele 1, ENU allele 2. *In(3)82Fe*^{1}, het;82F3-7. Pre-larval lethals.

In group 3 (lethal over *Df(3R)3-4* only; viable over *Df(3R)110, Df(3R)6-7* and *Df(3R)ME15):*

l(3)82Ff: one allele induced with ENU, early pupal lethal

l(3)82Fg: one allele induced by X rays, mid-pupal lethal

l(3)82Fh: alleles 1, 2, and 5 induced with X rays; alleles 3, 4, 6, 7, and 8 induced with ENU. *T(2;3)82Fh*^{2}, 57F3-11;82F7-11. Alleles 1 and 2 appear to be amorphic (from stages of death of hypomorphs over them) and are pre-larval lethals; the rest of the alleles are increasingly hypomorphic, with alleles 6, 7, and 8 complementing each other in all combinations. Lethal phases of hypomorphic

combinations range from pre-larval lethality through hanging up as wandering third instars to late pupal lethality.

l(3)82Fi: alleles 1 and 2 induced with X rays, allele 3 with ENU. *T(2;3)82Fi*^{2}, 57A10-B1;82F10-83A1. Pre-larval lethal.

l(3)82Fj: one allele induced with X rays, pre-pupal lethal. *Ab(het;3R)82Fj*^{1}, het;83A1+.

l(3)82Fk: one allele induced with ENU, leaky late pupal/eclosion lethal.

Other mutations recovered from X rays:

Df(3L)ru-22, 61F8;62A3-5. Detected because the *Df(3R)3-4* chromosome used carried *ru*^{1} although this wasn't indicated on its label.

In(3LR)Sai^{1}, 69D2-6;84E12-F3. Dominant outheld wings, recessive lethal allele of the *mirr* complementation group = *mirr*^{Sai1}.

Sai^{2}, dominant outheld wings; no cytological defect, maps genetically to 3-37.9 relative to *h* and *th.* Recessive lethal allele of the *mirr* complementation group = *mirr*^{Sai2}.

Sai^{1}, *Sai*^{2}, *D*^{1}, *D*^{3}, and *mirr*$^{DH-1}$ (homozygous viable hypomorphic *mirr* allele) fail to complement each other; *Sai*^{1} is the strongest allele, then *D*^{1} = *Sai*^{2}, then *D*^{3}. *D*^{3}/*mirr*$^{DH-1}$ is nearly completely viable, though with mild head defects and missing bristles.

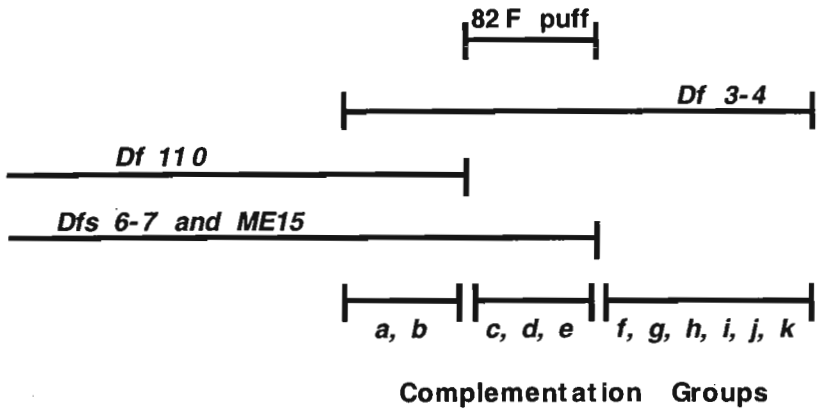

Figure 1.

Other mutations recovered from ENU: saw several, kept only a scarlet (= *st*^{33}), again detected because the *Df(3R)3-4* chromosome carried a *st* allele that wasn't indicated on its label.

Thirty-five mutations across 11 complementation groups = 3.2 hits per gene on average; although the distribution of numbers of hits per gene observed is very far off that expected from the Poisson distribution, that distribution predicts that the number of lethally- or visibly-mutable genes missed is 0.5.

New lethal mutations in the 97B1-10 to 97D13 region of the *Drosophila melanogaster* 3rd chromosome.

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In F2 EMS screens for mutations in the *dPC2* gene, we recovered sixteen lethal mutations and one visible mutation over *Df(3R)Tl-X* and *Df(3R)ro80b*. Together, the deficiencies cover the 97B1-10 to 97D13 region and overlap in the 97D1-2 region (Anderson *et al.*, 1985; Knibb *et al.*, 1993). Nine lethal mutations and the visible mutation fail to complement both deficiencies and thus map to the 97D1-2 region that includes the *dPC2* gene. These mutations are described elsewhere (D.T., A.R.K., and M.B., manuscript in preparation). Three (*dt6, dt12, dt14*) of the remaining 7 mutations recovered in our screens fail to complement *Df(3R)Tl-X* but complement *Df(3R)ro80b* and therefore are located beween 97B1-10 and 97D1 (Figure 1). The *dt6, dt12,* and *dt14* mutations fail to complement one another and also fail to complement *l(3)673*, a previously identified lethal in the region (K. Anderson, unpublished). These mutations have recently been shown to be allelic to

scribble (Bilder and Perrimon, personal communication). Four mutations recovered in our screens fail to

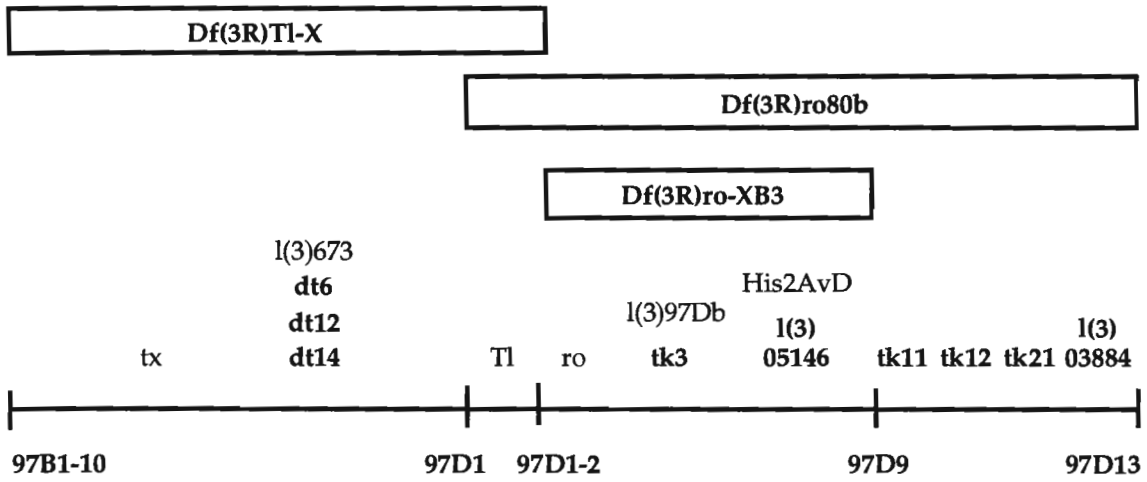

Figure 1. New lethal mutations in the 97B1-10 to 97D13 interval. The seven lethal EMS-induced mutations reported here and two lethal P element insertions are shown in bold type. Three previously identified lethal mutations (K. Anderson, unpublished; van Daal and Elgin, 1992; Knibb *et al.*, 1993) are shown in light type. Three other mutations (*taxi*, *tx*; *Toll*, *Tl*; and *rough*, *ro*) are also shown in light type for reference. The extents of *Df(3R)Tl-X*, *Df(3R)ro80b* and *Df(3R)ro-XB3* are shown by open bars at the top. Cytological positions determined from deficiency endpoints are indicated below the vertical hatch marks.

complement *Df(3R)ro80b* but complement *Df(3R)Tl-X* and therefore are located between 97D1-2 and 97D13 (Figure 1). One of these (*tk3*) fails to complement *Df(3R)ro-XB3*, a deficiency removing 97D2-9 (Knibb *et al.*, 1993). The *tk3* mutation fails to complement *l(3)97Db* (Knibb *et al.*, 1993). The other three mutations (*tk11, tk12, tk21*) complement *Df(3R)ro-XB3* and therefore are located between 97D9 and 97D13. The *tk11*, *tk12* and *tk21* mutations complement one another, defining three separate genes in this region (Figure 1).

Four lethal P-element insertions have been mapped within or near the 97B1-10 to 97D13 region by *in situ* hybridization [*l(3)neo59*, 97C/D (Cooley *et al.*, 1988), *l(3)03077*, 97C1-2; *l(3)05146*, 97D3-6; *l(3)03884*, 97D6-9 (Spradling *et al.*, 1995)]. The *l(3)03884* mutation complements *Df(3R)Tl-X* and *Df(3R)ro-XB3* but fails to complement *Df(3R)ro80b* and therefore maps to the 97D9 to 97D13 region (Figure 1). The *l(3)03884* mutation complements *tk11*, *tk12* and *tk21*, indicating that it defines a separate gene in this region. The *l(3)05146* mutation complements *Df(3R)Tl-X* but fails to complement *Df(3R)ro80b* and *Df(3R)ro-XB3* and therefore maps to the 97D1-2 to 97D9 region (Figure 1). The *l(3)05146* mutation fails to complement *His2AvD*, a lethal mutation that maps to this region (van Daal and Elgin, 1992). The other two P element insertion mutations complement *Df(3R)Tl-X* and *Df(3R)ro80b* and therefore map outside the 97B1-10 to 97D13 region defined by *Df(3R)Tl-X* and *Df(3R)ro80b* or carry lethal mutations unlinked to the P element insertion mapped by in situ hybridization.

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Mutation Notes — Other Species

cinnabar, cn: A spontaneous mutation in *Drosophila ararama.*

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Drosophila ararama Pavan and Cunha, 1947, belongs to *annulimana* group which is endemic to the Neotropical region. According to Vilela and Bächli (1990), 15 species, mostly cryptic, have been ascribed to the *Drosophila annulimana* group and they are distinguished only by the male terminalia.

On January 16th 1997 an unfertilized female of the *annulimana* group was collected at *Serra do Cipó*, state of Minas Gerais, Brazil (19°15' S, 43°30' W). Having a suspicion that this specimen could belong to the species *D. ararama*, Dr. C.R. Vilela mated the wild-caught female with a male from an isofemale line (F19F3) from Belém (state of Pará), previously identified by him as *D. ararama* on the basis of the analysis of the F_1 generation males. The F19F3 strain, which was established in 1988, was subsequently lost. The mating was successful, the first generation of imagines was obtained in March 1997, and the second generation one month later. The new strain was then called I61F5. Several males (87) and females (80) showing bright red eyes emerged among a larger amount of wild flies.

The eye color of wild *D. ararama* was described by Pavan and Cunha (1947) as blackish-red, although it looks more like wine.

Virgin females and males both with bright red eyes were crossed and a mutant strain was established (I61F5M1). A wild stock (I61F5M2) was isolated again from several wild-eyed flies of the F_2 generation from the original isofemale line (I61F5).

Table 1. Parental crosses and number of wild and *cinnabar* males and females obtained in the F_1 and F_2 generation.

cross	F_1			F_2				
female X male	wild		total	wild		*cinnabar*		total
	male	female		male	female	male	female	
cinnabar x wild	304	275	579	2,276	2,016	600	556	5,448*
wild x *cinnabar*	298	311	609	2,651	2,234	817	742	6,444

* The deviation of the ratio 3:1 of wild to *cinnabar* was significant at 5% level (chi-square test)

Virgin bright-red-eyed females were mated with wild males (10 pair matings) and virgin wild-eyed females were crossed with bright-red-eyed males (13 pair matings). All the F_1 flies (Table 1) were dark-wine-eyed, leading to the hypothesis that the mutation should be recessive. Furthermore, as the reciprocal crosses cited above yielded only wild-eyed males and females, the gene should be autosomal.

In the crosses of wild females with bright-red-eyed males, the ratio between wine and bright-red eyed F_2 flies (Table 1) was statistically different from 3:1 (Chi-square = 41.54). This may be a consequence of the excess of wild males. Additional experiments are being done to try to figure out the cause of this deviation. In the reciprocal crosses, nearly 3:1 ratio between dark-wine and light-red-eyed flies was found in F_2. This led me to conclude that bright-red eye color in *Drosophila ararama* is a recessive monogenic autosomal mutation. As far I know, this is the first mutant strain to be isolated in any species belonging to the *annulimana* group of *Drosophila*. This mutation is being called *cinnabar* as I am assuming its homology with a similar phenotype produced by an autosomal and recessive mutation known to occur in *Drosophila melanogaster.*

Acknowledgments: I thank Dr. C. R. Vilela for the species identification, for his help in the fieldwork, for suggestions during the development of this work and for the critical reading of the manuscript. I am indebted to Roselia and Oswaldo Machado for providing hospitality and a wonderful working environment at *Fazenda Monjolos Pousada* (*Serra do Cipó*, state of Minas Gerais).

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References: Pavan, C., and A.B. da Cunha 1947, Bolm. Fac. Filos. Ciênc. Letr. Univ. S. Paulo (86), Biologia Geral 7: 20-66; Vilela, C.R., and G. Bächli 1990, Mitt. Scweiz. Ent. Ges. 63 (Suppl.): 1-332.

A spontaneous double mutant in *Drosophila bipectinata*.

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Drosophila bipectinata is a member of the *bipectinata* complex of *ananassae* subgroup of *melanogaster* species group. It is distributed in South East Asia including India. In laboratory stocks of this species, spontaneous mutations such as brown eye, sepia eye, and cut wings have already been described (Hegde and Krishna, 1995; Singh *et al.*, 1995; Banerjee and Singh, 1996). In the present study, we report a spontaneous autosomal double recessive mutation in this species.

We detected several males and females with purple eyes and spread wings double mutant characters in one of our laboratory stocks which was established from a naturally inseminated isofemale line collected from Mysore, Karnataka in 1994. These mutant flies were aspirated out and maintained in separate vials containing food. The crosses between purple eyes and spread wing males and females yielded purple eyes and spread wings, indicating that the stock is pure for both purple eyes and spread wings.

Table 1. Normal and reciprocal crosses between wild and double mutant (purple eyes and spread wings) in *Drosophila bipectinata*.

Class	Number observed	Number expected	χ 2	Number observed	Number expected	χ 2
Wild	459	450	0.18	339	342	0.03
Purple	156	150	0.24	120	114	0.316
Spread	148	150	0.02	116	114	0.035
Purple and Spread	51	50	0.02	36	38	0.10

p value = insignificant at 0.05 level.

The pattern of inheritance of this mutant was studied by crossing mutant males with wild type females. Reciprocal crosses were also made using wild males and virgin mutant females. F_1 progeny consisted of only wild type flies. This shows that the mutant phenotype is recessive. Reciprocal crosses also yielded the same results. Therefore, purple eyes and spread wing mutants are autosomal recessive mutations. The F_1 inbreeding gave both wild and mutant flies in a 9:3:3:1 ratio (Table 1). This shows that the two genes, purple eyes and spread wings, assort independently. Test cross results (Table 2) confirm the difactorial inheritance of the purple eyes and spread wings. This is the first report of a spontaneous double mutation in *D. bipectinata*.

Table 2. The cross between F_1 female and double mutant (purple eyes and spread wings) males in *Drosophila bipectinata*.

Class	Number observed	Number expected	χ 2
Wild	140	133	0.37
Purple	122	133	0.90
Spread	138	133	0.18
Purple and Spread	129	133	0.12

p value = insignificant at 0.05 level.

Acknowledgments: The authors are grateful to the Chairman, Department of Studies in Zoology, University of Mysore, for providing facilities.

References: Hegde, S.N., and M.S. Krishna 1995, Dros. Inf. Serv. 76: 80; Singh, B.N., S. Sisodia, and R. Banerjee 1995, Dros. Inf. Serv. 76: 83; Banerjee, R., and B.N. Singh 1996, Dros. Inf. Serv. 77: 147.

Spontaneous mutant phenotypes found in urban *D. paulistorum* and *D. willistoni* populations.

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The lack of stable, available mutant strains of the species of the *Drosophila willlistoni* subgroup is one of the difficulties to study the genetics of these Neotropical flies, despite their challenging evolutionary characteristics. We are trying to obtain and to maintain all the phenotypic variants of these species that appear in our cultures and collections, since our group is currently performing field studies both in urban and in wild places in the Southernmost State of Brazil (Rio Grande do Sul). Along several years of work, we observed that in urban places, it is relatively common to see the appearance of phenotype alterations in several of the species captured, including the report of an extremely mutant strain of *D. simulans* (Loreto *et al.*, 1998). Here we report some of the variants detected in the sibling *D. willistoni* and *D. paulistorum*, both found in our collections and subject of several studies in our laboratory.

The *D. paulistorum* and *D. willistoni* mutants.

Some *Drosophila paulistorum* and *D. willistoni* strains obtained in periodic collections in the urban area of Porto Alegre city (30°02' S; 51°14' W) show phenotype characteristics resembling those of mutants described in other species of the genus *Drosophila*. In certain cases it was possible to determine their types of inheritance. In others, however, it was only possible to describe and to characterize phenotypically each mutation, because they were apparently sterile.

D. willistoni.

1. Antenna-to-leg transformation and ectopic eyes. After 22 generations of a single strain culture established in our laboratory, a *D. willistoni* male emerged with the phenotype presented in Figure 1a and b. Its phenotype includes: a small leg in the place of antenna, with large portions of eye ommatidea tissue. This male was mated with virgin females of the same strain, and all the offspring produced presented the wild type phenotype. No other individuals with the same phenotype were observed in the subsequent generations. This bizzarre phenotype called our attention because recently Loreto *et al.* (1998) described a similar mutant in a hypermutable strain of *D. simulans,* also captured in the same Porto Alegre city. In that case, the mutant presented incomplete penetrance and its expression is strongly influenced by the temperature of the culture chamber, apparently being 29°C the more effective one. Until now we have not performed shift down and shift up temperature assays to verify if the mutant here described in *D. willistoni* is an analogous mutant with that spontaneously found in *D. simulans*.

2. One-winged flies. Figures 1c and d show a female of *D. willistoni* of the other strain. This female was mated with wild males of the same strain, but no offspring was produced, although no anatomic abnormalities were detected in the reproductive organs of this fly. In *D. subobscura,* one-winged flies can arise in the *Va/Ba* strain, and in the descendents of the cross between the mutant strain with wild individuals. The mutant genes *Va* and *Ba* present very variable expressivity, being lethal in homozygous condition. It was also observed that the absent wing is always replaced by a thoracic structure of variable size, very similar to that described in Figures 1c and d. (Sperlich *et al.,* 1977; Mestres and Busquets, 1991; Orengo and Mestres, 1993; Orengo *et al.*, 1997).

3. Haltere to wing transformation. A single, sterile female of *D. willistoni* presented the transformation of one haltere to one wing in the third thoracic segment. No other alteration besides the infertility was observed (Figure 1e). It is similar to mutants of the homoeotic *Ultrabithorax* (*Ubx*) gene, which normally affects both the metathoracic (T3) and the first abdominal segments (A1); the haltere primordia in T3 expands to the size of wing primordia (White and Wilcox, 1985).

Figure 1. Mutant spontaneous phenotypes in *Drosophila willistoni*: a, b) homeotic leg to antenna transformation with ectopic eyes in the transformed leg; c, d) one-winged fly; e) wing to haltere transformation. In *D. paulistorum*: f) *white* eyes; g) *Bar* eyes; h) *lozenge*-like eye mutant.

D. paulistorum.

1. The *white* mutant (Figure 1f). In the offspring of two laboratory populations of *D. paulistorum* we observed six males with the characteristic *white* phenotype, inherited as a X-linked recessive allele (see Lindsley and Zimm, 1992).

2. The *Bar* mutant. Figure 1g shows one female descendent of the F1 of the mass cross between three mutant males and seven wild females found in a population of *D. paulistorum*. This mutant strain presented extremely variable phenotypic expression, being the fly in Figure 1h representative of the more extreme expression of the gene. As it occurrs in *D. melanogaster*, this gene is X-linked in *D. paulistorum*. This phenotype disappeared from the strain after a bottleneck induced by temperature accidental elevation of the culture chamber. The lack of the mutant phenotype may be also a consequence of a reversion of the phenotype to the wild one, or both phenomena. The first known *Bar* mutation was isolated by Tice as a single male in 1914. Homozygous or hemizygous *Bar* flies have narrow eyes in which the facet number has been reduced from the wild-type number. The mutations are all associated with chromosomal rearrangements as *tandem* duplications or inversions and translocations sharing a common breakpoint within the 16A1-2 region of the X chromosome (Tsubota *et al.*, 1989). Zeleny (1919, 1921) reported the instability of the mutation and its revertion to the wild type at the frequency of 1 in 1000 to 2000. Sturtevant (1925) suggested that this mutation was restricted to females and associated with the recombination, unequal crossing-over being the phenomenon responsible for *Bar* instability.

3. The *lozenge*-like mutant (Figure 1h). Seven eye-mutant males emerged in a same strain after six generations of rearing in laboratory. Both phenotype and genetic pattern are similar to those described in *D. melanogaster* (see Lindsley and Zimm, 1992) and in the same hypermutable strain of *D. simulans* already mentioned (Loreto *et al.*, 1998). The sterility of the homozygous female is a consequence of the detected absence of spermathecae.

4. The *yellow* mutant. Six yellow-pigmented body males were found in a recently established strain at the fourth generation (photos not shown). The gene is X-linked, similar to the same gene reported for other species of the genus (see Lindsley and Zimm, 1992). This strain is stable, being easily kept in our laboratory.

Acknowledgments: The authors thank CNPq, FAPERGS, FINEP and PROPESQ-UFRGS for fellowships and grants.

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Recovery and mapping of an *Antennapedia* mutation in *Drosophila simulans.*

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Hox genes are critical players in determining the basic body patterns of all animals and have attracted much attention from both developmental and evolutionary biologists (Carrol, 1995). While performing an X-ray mutagenesis screen (~ 4000 rad) for X chromosome deletions in *Drosophila simulans*, we recovered an apparent *Hox* gene mutation — a dominant *Antennapedia*-like allele (*Antp*). Most mutations in *D. simulans* are homologous to known mutations in *Drosophila melanogaster*. For recessive mutations, homology is easily established by complementation tests in species hybrids. However, because the *D. simulans Antp*-like mutation is dominant and likely homozygous lethal (*i.e.*, we were unable to construct homozygous lines), complementation tests were not possible. We thus attempted to infer homology by mapping *Antp* using visible markers available within *D. simulans*.

We crossed virgin *Antp* females to a multiply-marked *D. simulans* stock carrying the recessive visible mutations *forked* (*f*: 1-56), *net* (*nt*: 2-0), *plum* (*pm*: 2-100), *scarlet* (*st*: 3-49), *ebony* (*e*: 3-63). (Third chromosome map positions from Jones and Orr, 1998). F1 *Antp* males were then backcrossed to virgin *f; nt pm; st e* females. Their male and female progeny were scored for *Antp* and each of the five markers. The results showed that *Antp* is not on the X chromosome as both male and female progeny showed the mutant phenotype. We further found that while 17.4% (n = 218) of *nt pm* progeny also showed *Antp*, no *st e* progeny (n = 168) showed *Antp*. Therefore, like *D. melanogaster*, *Antp* in *D. simulans* is on the third chromosome.

Table 1. Backcross progeny from F1 *Antp* females x *f, nt pm; st e* males.

Genotype	Progeny
st + e	750
st Antp e	1
st Antp +	63
st + +	93
+ + e	149
+ Antp e	8
+ Antp +	355
+ + +	514

We determined *Antp*'s map position on the *D. simulans* third by backcrossing F1 *Antp* females to *f; nt pm; st e* males. Nearly 2000 progeny were then scored for the presence of *Antp* and the two third chromosome markers, *st* and *e* (Table 1). It should be noted that gene orders in *D. melanogaster* and *D. simulans* are essentially the same with the exception of loci included in a known paracentric inversion on the right arm of the third chromosome (Ashburner, 1989). In fact we found that the order of the three genes in *D. simulans* (*st e Antp*) differs from that in *D. melanogaster* (*st Ant e*) indicating that *Antp*, along with *e*, is included in the 3R inversion of *D. simulans*. Distances between markers are presented in Table 2. These map distances place *Antp* at 3-78.7. After accounting for the inversion difference between species, the map distances between the three loci in *D. simulans* are 27% higher than those in *D. melanogaster*. This is consistent with the known average 30% genome-wide increase in recombination rate in *D. simulans* relative to *D. melanogaster* (Ashburner, 1989).

Table 2. Map distances (cM).

Intervals	*D. simulans*	*D. melanogaster* †
st e	16.2	26.7
e Antp	31.9	23.2
st Antp	48.1*	3.5

**st Antp* distance is the sum of the smaller distances.

*† Data are from Lindsley and Zimm (1992)

These findings indicate that the *Antp* mutation reported here is likely a lesion at the *D. simulans* homologue of the *Antennapedia* locus in *D. melanogaster*.

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References: Ashburner, M., 1989, *Drosophila: A Laboratory Handbook.* Cold Spring Harbor Laboratory Press; Carrol, S.B., 1996, Nature 376: 479-485; Jones, C.D., and H.A. Orr 1998, Dros. Inf. Serv. 81: 137-138; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York.

Recurrence of *yellow* mutation in *Drosophila subobscura*.

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Two males of *yellow* phenotype were detected in a homokaryotypic stock (O_{3+4+22}/O_{3+4+22}) of *D. subobscura*. These males were crossed with virgin females of the *yellow* stock. All offspring individuals were *yellow*, confirming that the original males presented the *yellow* mutation. This mutation has appeared many times in our laboratory stocks of *D. subobscura* (Mestres, 1996; Solé, 1997). In all cases the *yellow* mutation has arisen in the *cherry curled* strain or in stocks obtained by means of genetic crosses with this strain. This suggests that the *yellow* mutation originates in the *ch cu* strain, and that some transposable genetic element could be involved.

References: Mestres, F., 1996, Dros. Inf. Serv. 77: 148; Solé, E., 1997, Dros. Inf. Serv. 80: 105.

Teaching Notes

In this section, we reprint a selected number of articles from previous issues of Drosophila Information Service that might be of interest for laboratory course development. Some are from many years ago. Thus, while the specific stocks they mention may not still be available in exactly the same conformation, these notes might at least offer ideas that can be applied to currently available strains. All tables and some figures have been redrawn to make them consistent with current Dros. Inf. Serv. formatting.

We would like to expand this section in future issues, and we welcome your submissions on teaching exercises, crosses, problems sets, and other material that can be used in teaching genetics using *Drosophila* as the experimental organism. In addition to these reprinted notes, one new teaching article, by Ron Woodruff and Jim Thompson, is included at the end of the Teaching Notes section.

Additional uses of the "C-scan" stock.

Roberts, P.A. Oregon State University, Corvallis, Oregon. [reprinted from DIS 48: 159, 1972].

Students desirous of learning cytogenetic techniques using *Drosophila* stocks may find this exercise instructive without being cookbookish. Wild-type males are exposed to at least 4000 R of X-rays and mated *en masse* to homozygous "C-scan" (*sc f*; *al b sp*; *ve st ca*) females. F1 females are individually mated to several C-scan males in vials or 1/4 pint bottles and the F2 progeny are scored for crossover suppressors on X, 2L, 2R, 3L, and 3R. The details of techniques for inducing, scoring, detecting, balancing and examining the recovered rearrangements cytologically are described in Roberts (Genetics, 1970, 65: 429-448). At least 1 in 4 F1 female offspring of males receiving 4000 R should carry a crossover suppressor effective enough to be detected using this stock if care is taken to transfer females and keep down bacterial growth in the less fertile cultures which are often semisterile owing to chromosomal rearrangement. Usually the suspected rearrangements are balanced then examined cytologically because this not only preserves the rearrangement but makes it possible to pick out larvae heterozygous for the aberration. If it is desirable to shorten the procedure, half the offspring of F2 males carrying wild type alleles in the arm or arms in which crossing over is reduced should show the rearrangement in salivary gland chromosomes. This procedure not only

demonstrates radiation mutagenesis, but provides the careful student with an opportunity for the discovery of a novel crossover suppressor since each rearrangement is unique. A wide variety of rearrangements including pericentric and paracentric inversions, reciprocal and insertional translocations, (and occasionally, a duplication or transposition) has been recovered using this procedure (see the above reference).

We have also used the C-scan stock in more routine experiments with beginning students in genetics. Students are given mated F1 females each of which is heterozygous for C-scan and a stock rearrangement and are asked to determine from the pattern of crossover suppression and (or) pseudolinkage, what sort of rearrangement is present.

A demonstration of compensation for an inherited biochemical defect in *D. melanogaster*.

Potter, J.H. University of Maryland, College Park, Maryland. [reprinted from DIS 47: 134, 1971].

A simple demonstration of compensation for an inherited biochemical defect can be carried out by beginning students using *D. melanogaster*. In essence, students supply kynurenine to larvae of vermilion mutants which cannot convert tryptophan to kynurenine, one of the steps in the synthesis of ommochrome pigments. Since students frequently do not distinguish vermilion from wild type flies, they use the white-eyed, double mutant, vermilion brown. Vermilion brown larvae fed kynurenine develop brown eyes. To emphasize the specificity of the block, students also feed kynurenine to the double mutant, cinnabar brown. Cinnabar brown mutants develop white eyes whether or not they receive kynurenine.

Experimental procedure: Students set up two cultures each of vermilion brown and cinnabar brown mutants in 80 × 25 mm shell vials containing 5 ml of Carolina Instant *Drosophila* Medium. As soon as larvae appear, the parents are removed and the medium in one vial of each genotype is injected with 0.2 ml of a kynurenine-antibiotic solution. The medium in the other two vials is injected with 0.2 ml of plain antibiotic solution. The injections are made with a 2 1/2 ml syringe without a needle inserted in a hole made in the medium with an applicator stick. Injections are repeated every two days until pupae appear. The adults are scored in the usual way. The kynurenine treated, vermilion brown, flies are mated after scoring and their progeny scored for eye color to demonstrate that the genotype has not been changed by the kynurenine treatment.

The kynurenine antibiotic solution is similar to that used by Parsons and Green (1959) for culturing eye discs: 0.05% streptomycin, 0.033% penicillin and 1.00% D.L. kynurenine, which can be obtained from Sigma Chemical Company, St. Louis, Missouri.

References: Parsons, P.A., and M.M. Green 1959, Proc. Nat. Acad. Sci., USA 45: 993.

Demonstration of intra- and inter-chromosomal effects of inversions on crossing over.

Moree, Ray, and Donald T. Grahn. Washington State University, Pullman, Washington. [reprinted from DIS 44: 135, 1969].

The following experiment must be in use in many teaching laboratories, yet I do not recall any mention of it during conversation. It may therefore be worth a note since it adds an interesting contrast to the types of experiments traditionally in use. Using the stocks *y w*, In(1)*y*,In(1)*w* (see DIS 35:7), *Cy*/*Pm*;*D*/*Sb*, and any wild type stock, F1 females of the following four types are produced: (1) *y w*/++;+/+;+/+, (2) In(1)*y*,In(1)*w*/++;+/+;+/+, (3) *y w*/++;*Cy*/+;*D*/+, and (4) In(1)*y*,In(1)*w*/++; *Cy*/+;*D*/+. These females are then crossed to *y w* males. As carried out by the class the crosses have given, respectively, the following percentages of crossing over between *y* and *w*: 1.5, 0.3, 8.1, and 2.4. Some students often fail to identify *D* in selecting F1 females, so the maximum enhancing effect is probably greater than that obtained. Results are clear cut and can be appreciated without resort to a statistical test. The experiments are easily performed and yet introduce an aspect of genetics quite novel to beginning students. That no satisfactory explanation exists for the increase in crossing over is disappointing to some students but intriguing to others.

A "*Drosophila* Kit" for the genetics teaching lab.

Waddle, F. Fayetteville State University, Fayetteville, North Carolina. [reprinted from DIS 70: 260-261, 1991].

I have developed a "*Drosophila* Kit" which consists of a 3 × 6 × 15 inch plastic tray containing the following items:

1. Instant *Drosophila* medium in 220 ml container.
2. Tegosepted cotton (0.1%) in 4 1/2 oz. container.
3. Tegosepted water (0.1%) in squeeze bottle.
4. Yeast in small plastic vials (2).
5. Foam plugs (for 20mm test tube) in large container.
6. Scoopula (small stainless steel scoop).
7. Microspatula.
8. Labels.
9. Test tube brush.
10. Etherizer.
11. Ether bottle (with medicine dropper held in attached test tube).
12. Sorting plate.
13. Sorting brush (fine pointed artist's brush).
14. Lens paper.
15. Dissecting needles (2).
16. Forceps, very fine pointed.
17. Glass slides (in container, not loose).
18. #1 Cover slips.
19. Siliconed lens paper (for making squashes, not for lenses).
20. Razor blade.

Each student is given a kit. Each kit is numbered. The containers and equipment within the kit are numbered the same as the kit. This discourages students from "borrowing" from one another. Ideally, the student should have a cabinet with lock in which to keep the kit and a stereomicroscope.

To go with the kit, the student has ready access (either directly or on request) to:

1. Test tubes, 20 × 150mm.
2. Test tube racks.
3. Salivary gland stains in dropper bottles.
4. Refills for fly food, plugs, etc.

The 20 × 150mm test tubes require less food than bottles or vials and are excellent for single pair matings. Moreover, breakage is considerably less than for glass vials. Carolina Biological Supply Company sells an inexpensive 50 tube rack that works well. Tegosepted water is used to prepare food in the tubes and the tubes are slanted. The tegosepted cotton is for use at the bottom of the slant. Fisher's etherizer is far superior to Carolina Biological's, provided the cotton in the etherizer is replaced with a larger piece and tightly packed. To minimize the ether hazard, we use small, corked (not rubber), ether bottles and put the dropper for it in a small test tube rubber banded to the bottle.

With the student thus equipped, the faculty work load can be reduced while the student learns the basics of *Drosophila* research from making food, collecting virgins and making crosses to washing the glassware afterwards. Since batch matings are avoided, fewer virgins are required for equivalent result while even the unintentional use of a nonvirgin in a particular vial can provide a learning experience without disastrous result.

Some stocks for the teaching lab.

Waddle, F. Fayetteville State University, Fayetteville, North Carolina USNA. [reprinted from DIS 70: 262, 1991].

I use the following "unknowns" in the teaching lab: w^a (sc^6), w^e, ras^2, g^F, *car*, *lt* (*stw*), or^{45a} (sp^2), *se* (*h*), p^p (*ri*), *red*. The following stocks are used for tests with the unknowns: Ore-R, *b*, *e*, *cv f*, CyO/*Sp bw*; In(3LR)DcxF, *D/Sb e*.

The eye color mutations, including the garnet allele, are all easily distinguishable from wild type. Sex linked mutations were chosen such that any competent mapping test with *cv* and *f* can distinguish one locus from the others. Genes in parentheses are "teacher markers". They are mostly rank 2 or 3 mutations not obvious to the student but which allow the instructor to distinguish among stocks of similar eye color.

Each student is given one sex linked and one autosomal unknown plus the stocks to test them with. No two students have the same combination of unknowns. Depending on the autosomal unknown, either *b* or *e* is given, without identification, so that the student can do dihybrid crosses. The CyO/*Sp bw*; In(3LR)DcxF, *D/Sb e* stock is excellent for determining which chromosome an autosomal unknown is on. If the student chooses to make chromosome squashes of Ore-R heterozygotes, he/she has the opportunity to observe that one of the bands near the tip of 2R is heterozygous for a deletion.

Transplanting *Drosophila* tissues.

Ursprung, H. [reprinted from DIS 37: 146, 1963].

In teaching transplantation of *Drosophila* imaginal discs (see *Biology of* Drosophila, ed., M. Demerec, New York, Wiley, 1950, pp. 350 ff, for a description of the method) one of the most frequent difficulties encountered is that the tissues get stuck in the micropipette. Siliconizing the glass capillary may overcome this problem, but a still simpler procedure is to suck a piece of larval fat body, that usually floats in the dissecting drop, into the pipette several times. This procedure coats the interior of the pipette with a thin film and effectively prevents clogging.

Class demonstration of allelic complementation at the *ma-1* locus and maternal effect of the $ma\text{-}1^+$ gene.

Goldschmidt, Elizabeth. [reprinted from DIS 39: 138, 1964].

In *D. melanogaster* intra-locus complementation can be observed at the morphological level in heterozygotes for different pseudo-alleles at any one of a series of loci, such as *fa-spl* or *m-dy*. Complementation at the *ma-1* locus which has been demonstrated at the enzymatic level (Glassman, 1960) may be preferable for class work. Heterozygous females of the constitution $ma\text{-}1/ma\text{-}1^{bz}$ synthesize considerable quantities of the enzyme xanthine dehydrogenase (XDH) which is virtually absent in either homozygous mutant. Although this effect is amenable to investigation with advanced enzymological and immunological techniques, its main features may be illustrated by one-dimensional chromatography of mutant flies, following Hadorn and Mitchell (1951).

One of the immediate products of XDH activity is isoxanthopterine (IX). Neat separation of IX from bodies of female flies may be obtained by chromatography of Malphighian tubules. the Malphighian tubules of 8 females are applied to each start point of a sheet of filter paper. After development in (2:1) propanol:5% ammonia solution in a darkened room, the IX spot with its characteristic violet fluorescence will be seen under a U.V. lamp (360 mμ), in chromatograms of Malphighian tubules derived from normal females and of $ma\text{-}1/ma\text{-}1^{bz}$ heterozygotes, but not in those of either homozygous mutant type. Partial complementation of the amount of red pigment (drosopterine), the synthesis of which is also correlated with the XDH level, can be demonstrated by chromatography of single female heads of the same four genotypes.

The maternal effect of *ma-1*$^{+}$ females on the XDH content of their mutant offspring may be shown by chromatography of testes of mutant males. The testes of two males applied to each start point yield large spots of IX. The maternally affected males should be obtained from the following crosses:

1) *y ma-1*$^{+}$:= × *ma-1*

2) *y ma-1*$^{+}$:= ; *ry/ry* × *ma-1*;+/+

The latter cross demonstrates that *ry/ry* females although devoid of XDH can exert a maternal effect on the production of the enzyme in their *ma-1* sons.

For technical details and further references see: Glassman, E., 1960, Science 131: 1810; Glassman, E., and J. McLean 1962, Proc. Nat. Acad. Sci. 48: 1712; Hadorn, E., and H.K. Mitchell 1951, Proc. Nat Acad. Sci. 37: 650.

Simple demonstration of modified ratios using *b* and *e*.

Moree, Ray. [reprinted from DIS 36: 132, 1962].

Laboratory experiments relating to modified ratios and genic interaction can be made both simple and surprising by using stocks of *b* and *e*. That the F1 is wild type is surprising to many. The F2 is classified by most students into wild type and "dark", in 9:7 ratio if the sample size is large enough. But some students detect what they consider different degrees of darkness, so the possibilities of getting a 9:3:4 or a 9:6:1 ratio are pointed out: it can also be indicated that if a simple chemical test were available it might even be possible to recognize a 9:3:3:1 ratio. That the ratio of wild to dark may be about 1:1 in F2 progeny and 1:3 in testcross progeny from the cross F1 × *b/b;e/e* is usually somewhat surprising, too. The results emphasize the way in which inferences as to interaction, epistasis, etc., depend upon the possibilities of discriminating among the individual progeny of a cross.

An inexpensive, simple etherizer for classroom use.

Bennett, Jack. [reprinted from DIS 33: 178, 1959].

A simplification of an etherizer reported by Lloyd L. Arnold (Amer. Biology Teacher 19: 248-251) has proved very useful in the student and research laboratory. It consists of a polyethylene bottle of the type used to dispense catsup, mustard, or salad dressing, which has a long (3 cm) pointed spout with a small (approx. 1 mm) hole in the end. The bottle is loosely packed with cotton, 5 cc of diethyl ether is added (sufficient for several hours' use), and the cap and spout are replaced. In use, the vial or bottle containing flies is gently inverted and the spout is carefully inserted past the cotton plug; ether vapor is expelled into the vial or bottle by pressing the sides of the etherizer. CAUTION: Remove spout before releasing sides of the etherizer or the flies may be drawn into the etherizer. When the flies succumb they fall on the cotton plug, which can then be removed and the flies shaken off for examination. The polyethylene bottles cost about 25 cents (and are often thrown away by restaurants and housewives), and are virtually unbreakable. They require much less ether than most other types, and are adaptable to almost any type of container, including the polyethylene population cages.

Use of the w^{vc} chromosome in class laboratories.

Hinton, Claude W. [reprinted from DIS 32: 173, 1958].

Several phenomena rarely encountered in undergraduate genetics laboratories can be easily demonstrated in simple crosses involving the unstable w^{vc} ring chromosome (Genetics 40: 951-961), for example, w^{vc} *f* / *y w* females by *y v f* / Y males. As a consequence of elimination of the w^{vc} chromosome, gynandromorphs are frequently encountered among the progeny; those gynandromorphs mosaic in the head may be used to illustrate gene hormones. Exceptional males are also abundant among the offspring; and, although many of these are the result of w^{vc} loss, others are due to primary nondisjunction. This phenomenon

is also responsible for exceptional (non-forked) females occurring in the progeny. Position-effect variegation is manifested by the eye pigment of the parental females but not in the F1 w^{vc} females; the effect of the Y chromosome in suppressing position-effect variegation is demonstrated by the exceptional females' phenotype. To insure maintenance of instability, multiple lines of the stock must be carried and selected frequently.

Genetics of behavior.

Rizki, M.T.M. [reprinted from DIS 31: 183, 1957].

We have been doing some experiments on the response of normal and mutant strains of *D. melanogaster* to the smell of food, in order to demonstrate the possible influence of inheritance on the behavior pattern of flies. A simple apparatus constructed from a cardboard box with a transparent cover is sufficient to carry out these experiments (see drawing). Air saturated with the desired mixture is blown through rubber tubing into the box. Flies are generally starved overnight and conditioned in the plastic-

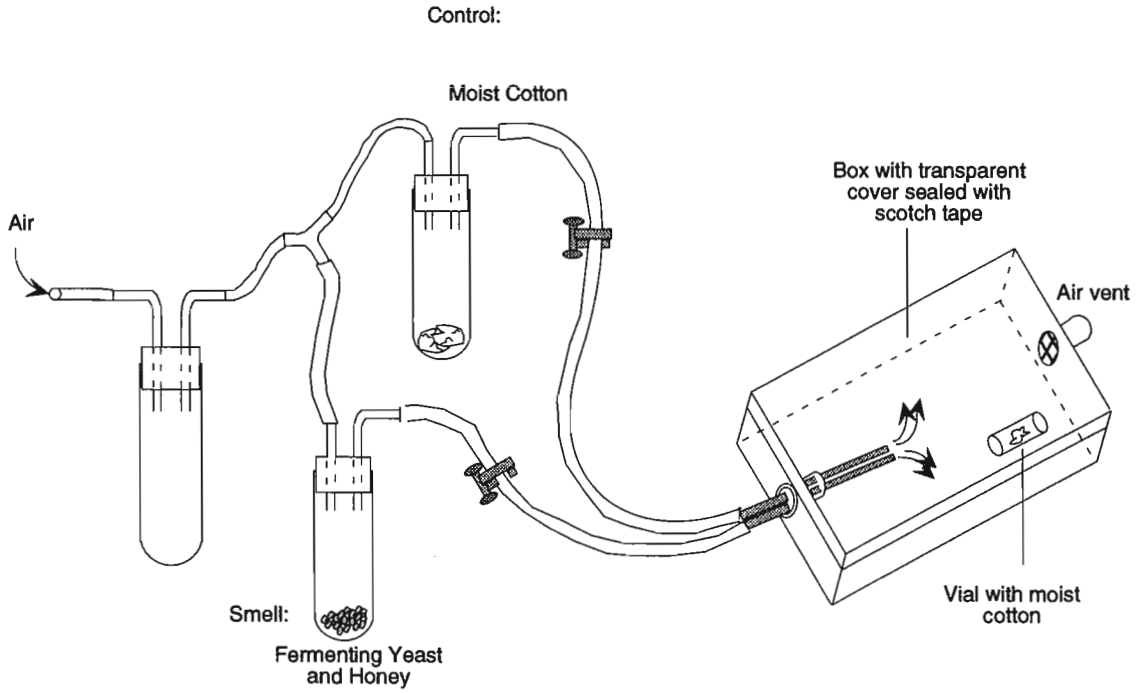

Figure 1.

covered box, which contains a small vial of moist cotton. The flies will respond to the control of moist air if water is not available during the conditioning period. Desiccation is also avoided. When air saturated with the odor of yeast and honey is blown into the box, the following components of behavior of Oregon-R flies can be observed in an orderly sequence: (a) fluttering of wings, (b) shaking of abdomen, (c) looping or circling, (d) walking straight to the orifice of the tube which is the origin of the odor. This experiment can be modified by introducing other variables, such as different kinds of smells and different mutants. Students have found these experiments interesting and instructive, particularly those who are interested in psychology and behavior.

A two-factor sex-linked cross involving gene interaction.

Hexter, W.M. [reprinted from DIS 31: 182-183, 1957].

The mutants g^{53d} and w^a, both sex-linked recessives 42.9 map units apart, are phenotypically indistinguishable. The phenotype of these mutants is orange, varying somewhat with age. The class is given as parents, females of one mutant and males of the other, and the cross is designated simply as "orange 1 × orange 2". F1 females are wild type and F1 males are orange. F1 are interbred to raise an F2. The F2 females are expected to be wild type and orange in equal frequencies, and the F2 males theoretically should be approximately 60 percent orange (parental types) and 40 percent crossovers, half of which are wild type and have double mutant (w^a g^{53d}). Actual class data were: females, 1530 wild type, 1402 orange; males, 690 wild type, 1420 orange, 454 white. Deviations from equality were due primarily to differential viability of the various genotypes. The student is confronted with the following facts: a wild-type F1 female; a mutant F1 male; a new phenotype (white) in the F2 but confined to one sex. From this information the student should conclude that orange 1 and orange 2 are not alleles and are recessive; that one of them is sex-linked; and that white is probably due to the combined action of orange 1 and orange 2. The student then usually assumes a second gene that is autosomal. This assumption will not account for the data. The second gene is then assumed also to be sex-linked, and the conditions of the problem are satisfied if linkage is assumed to be about 45 percent. In addition to the unexpected phenotypes and the challenging yet not too complicated analysis, this experiment has the advantage of simple and rapid classification.

Monohybrid for sophisticated students.

Burdick, A.B. [reprinted from DIS 29: 181, 1955].

One of our *Bar* stocks has two lethals on the first chromosome with *Bar*. These lethals, for reasons unknown, stay in the stock in high frequency and provide an interesting experiment in our advanced laboratory course. At the beginning of the semester we propose a sex-linked monohybrid involving *Bar* as the first experiment. Although this is quite beneath many of the students, the results challenge all of them for interpretation. We ask them to make five single pair matings of *B/B* female × + male. With the two lethals in the stock, these matings may be of several types:

1) l^1 *B/B* female × + male	gives	2 wide Bar females: 1 Bar male
2) *B/B* l^2 female × + male	gives	2 wide Bar females: 1 Bar male
3) l^1 *B* l^2 / *B* female × + male	gives	about 3 wide Bar females: 1 Bar male
4) *B/B* female × + male	gives	1 wide Bar female: 1 Bar male

We also get some matings that appear to involve l^1 *B/B* l^2 females which indicates that one of these "lethals" must, occasionally, go through the male.

The data of any given student usually contains one or more bottles with aberrant sex ratios. They find heterogeneity with Chi-square and, if they have only 2:1 and 1:1 ratios, usually come up with a lethal in their interpretation. But, when 3:1 (and sometimes 10:1) ratios appear, they seem to lose confidence in lethals especially when they consider how a female could get to be l^1 *B/B* l^2 to give an approximately 10:1 ratio. I feel they learn quite a bit about critical handling of data from this experiment. It evokes considerable discussion and frequently leads to interesting interpretations.

Xasta in class experiments.

Burdick, A.B. [reprinted from DIS 29: 181, 1955].

Good, clear, uncomplicated translocation segregation data is difficult to obtain in *Drosophila*. Most translocations are not well marked and multichromosomal recessive testers frequently conflict in expression

Table 1.

	Males	Females
Xa	34	53
(Xa)m	44	44
m, cn, e	29	41
cn, e	35	44

with translocation markers. One combination that has worked well for us is T(2;3)*Xa* with *m;cn;e*. We cross *m;cn;e* females × T(2;3)*Xa*/*Ubx*^{130} males and backcross: *m*/+; *cn-e*/T(2;3)*Xa* females × *m;cn;e* males.

Typical backcross data are those of Mr. V.M. Sahni, last year (Table 1). We never obtain any recombination between *cn* and *e* which is unfortunately due to the fact that *Xa* also carries In(2R)*Cy*, which covers *cn*, and In(3R)P with a break near *e*. This prevents location of the break-points of *Xa* from the data and leads everyone to conclude that the breaks must be very close to both *cn* and *e*. I think the recessive tester stock could be improved by making it *B;cl;ss*^{a} which should give some recombination between *cl*, *ss*^{a}, and *Xa* to allow genetic estimation of the break-points.

Phenocopy and species hybrid in class work.

Goldschmidt, Elisabeth. [reprinted from DIS 29: 182, 1955].

The following simple experiments are familiar to geneticists from the literature, but it is not generally realized how easily their success in class work can be ensured.

a) "Yellow" phenocopy on silver nitrate medium. Medium containing 0.1%, 0.05%, and 0.025% $AgNO_3$, respectively, is prepared by stirring appropriate volumes of a 5% $AgNO_3$ solution into standard food mixture cooled to 60°C directly after boiling. Since stocks vary in their sensitivity to the salt, the concentration which produces a high percentage of phenocopies (50-80%) without being too toxic to the flies is determined each year by a test series before starting the course. At least 10 pairs of parents should bc introduced into each half-pint bottle. Students test different isogenic strains (or different *Drosophila* species) on the same medium or one stock on a series of concentrations. Light-colored flies are transferred to normal medium to demonstrate nonheritability of the effect in their offspring.

b) Hybrid *D. melanogaster* × *D. simulans*. Virgin males and females, aged for different periods, are shaken without etherizing into "creamers", according to the method described by Uphoff (Genetics 34: 314-327). Thirty to fifty percent of students' crosses prepared in this way are fertile. Hybrid larvae are utilized for salivary preparations to demonstrate the inversion constituting the main cytological difference between the parent species. Adult female hybrids are tested for sterility. Change of dominance relations in the hybrid can be demonstrated by utilizing *melanogaster* females carrying dominant genes. Thus, when employing *Cy L/Pm* females, *Cy* is found to be dominant, while *L* and *Pm* are recessive in the hybrids.

Trihybrid with duplicate independent factors.

Burdick, A.B. [reprinted from DIS 28: 173, 1954].

We have made up a stock in which *v* (1-33.0), *cn* (2-57.5), and *st* (3-44.0) are all homozygous and which has eye color indistinguishable from either *v*, *cn*, or *st*, that is, a right vermilion color. We refer to this stock as "Bright" and use it in the student laboratory as an unknown mutant type.

If a virgin Bright (*i.e.*, *v;cn;st*) female is mated with a wild-type male, the F1 males are Bright and the F1 females are wild type. This leads to the tentative conclusion that Bright is a sex-linked recessive. However, when an F2 is produced from F1 × F1 mating, it is in a ratio of about 2.5 Bright to 1 wild type; or when an F1 female is testcrossed to a Bright male parent, the ratio is 7 Bright to 1 wild type. The Bright trait, which in F1 looked like a sex-linked recessive, now looks somewhat like a dominant.

On the basis of the above data the student may conclude either (1) three duplicate independent genes, one sex-linked, or (2) two duplicate sex-linked genes with about 26% recombination. Test matings of F2 Bright types with Bright parents are interesting if time permits. They yield 1:1, 3:1, and 7:1 ratios and indicate that conclusion (1) is correct.

Effect of environment on segregation results.

Burdick, A.B. [reprinted from DIS 28: 173, 1954].

We have a stock with *vg* (2-67.0) and *c* (2-75.5) both homozygous. The flies appear *vg* at room temperature, but if they are reared at 80°-85°F the *vg* wings become almost normal and the stock appears *c*. If the stock is outcrossed to wild type, and F1 females testcrossed to *vg c*, the following testcross proportion results (Table 1).

Table 1.

Phenotype	At 70° F	At 80°F
vg	50	4
+	46	46
c	4	50

Results may vary depending on the temperature used and the ability of the student to detect an "almost normal" *vg* wing. However, the experiment provides an impressive illustration of gene-environment interaction and, used late in the semester, moderates a student's faith in genetic ratios as such.

Phenotypically identical but genotypically unique *Drosophila* "unknown" stocks for genetics laboratory courses.

MacIntyre, R. Cornell University, Ithaca, New York. [reprinted from DIS 51: 158, 1974].

At Cornell, all biological sciences majors are required to take a course in general genetics which includes a mandatory 2 hour laboratory component. As part of the laboratory, each student is required to genetically analyze an unknown strain of *D. melanogaster* and to write a detailed report on his analysis. This is a six-seven week project and, accordingly, contributes substantially to his grade in the course. Unknowns have generally 3 - 6 mutant genes and are crossed to the following marker stocks: (1) wild type, to identify the genes and their individual phenotypic effects and to localize them to the X or to autosomes; (2) *Cy/Pm;Sb/Ubx* to place autosomal genes on the 2nd, 3rd, or 4th chromosome; (3) *BL L/Cy;Ly Sb* or *Gl Sb/LVM;f B*, to map genes on the 2nd, 3rd, and X chromosomes, respectively. Most students work very hard on this project, and it is not surprising that the "fruit fly experiment" has a widespread, rather notorious reputation. I have always felt, however, that the hard work put in by the students was justified by and, in fact, enhanced the tutorial value of the genetic analysis.

Recently, it came to my attention at a wedding reception in a fraternity house, that there were extensive collections of old *Drosophila* reports at several places on and around the campus. The inebriated fraternity member bragged to me how valuable these collections were to the genetics "students" seeking short cuts to high grades. Usually by the F1 generation there would be enough information to enable the short-cutter to find an already complete analysis of his unknown in one of these collections. Because of the obvious unfairness of this practice to the serious, hard working student and because of the gamesmanship involved, I decided to construct a set of "unknowns" which would force all students to complete the analysis. After examining several combinations, I found one which involves no difficult phenotypic interactions with the marker stocks. This is a combination of orange eyes, black bodies and incomplete wing veins. In the stocks I've constructed so far, orange eyes are due to the interaction of *car;st*, *car;kar*, *pn;st*, *pn;kar*, *v car* or *pn v*. Body color is due to *b* or *e* and wing veins to *shv* or *ve*. Thus, I have 24 genotypically unique but phenotypically identical or at least very similar stocks (*b* and *e* as well as *shv* and *ve* show different phenotypes to the trained eye). I plan to multiply this number by incorporating *s* and *rb* or *ca* as additional body color and brown eye mutants.

A temperature-sensitive yellow eye color.

Erickson, J. Western Washington University, Bellingham, Washington, USA. [reprinted from DIS 59: 146, 1983].

I've found that the yellow eye color trait which I reported previously (DIS 51: 22, 1974) shows an interesting change with temperature.

The trait, $w^{se\text{-}y}$, originated spontaneously in my sepia stock, and I use it in this way, that is, $w^{se\text{-}y}$;*se*. The eyes are a clear lemon-yellow color at 25°C. At 18°, the eye color of the flies of this stock is indistinguishable from *w*.

I have found that sepia-yellow works well to show the effect of temperature on phenotype. Students simply make up cultures from stock and incubate them at the two temperatures, or at several temperatures. One may also use the trait, of course, for temperature-shift experiments, so that students may observe what stage of development is sensitive to temperature, in the development of pigment in this case.

Demonstration of the heat shock response by means of ADH activity in a transformed line of *Drosophila melanogaster*.

Morrison, W.J. Shippensburg University, Shippensburg, Pennsylvania. [reprinted from DIS 66: 178, 1987].

For the past three years my undergraduate genetics students have conducted a simple experiment that demonstrates both the heat shock response and an enzyme deficiency. The experiment employs a construct in which the coding region of the alcohol dehydrogenase gene (*Adh*) has been joined to the promotor region of the hs70 heat shock protein gene (Bonner *et al.*, 1984). This heat shock-sensitive *Adh* gene has been introduced into the genome of a strain homozygous for a null *Adh* allele by germline transformation at 61C on chromosome 3. When such flies, identified as Adh^{hs61c}, are maintained at 25°C and are heat shocked by exposure to 37°C for one hour followed by a recovery period of 24 hr, they synthesize ADH in nearly all tissues (Bonner *et al.*, 1984). Heat shocked Adh^{hs61c} flies are compared in ADH activity to wild type, null strain, and non-heat shocked Adh^{hs61c} flies by a simple, direct test. The basis of the test is that flies possessing ADH activity convert pentynol to a toxic compound that causes paralysis and eventual death.

Five to ten adult flies of each strain/treatment category are placed in empty shell vials (25 mm × 95 mm) plugged with cotton. In a fume hood, two drops of 5% (v/v) pentynol (1-pentyne-3-ol; Pfalz & Bauer, Inc., 172 East Aurora St., Waterbury, CT 06708) are absorbed into a square (2 cm × 2 cm) of thick filter paper (Schleicher & Scheull, grade 470), which is inserted into a shell vial containing the flies to be tested. The vial then is immediately sealed with Parafilm, and the student closely observes the condition of the flies while noting the elapsed time. The wild type and the heat shocked Adh^{hs61c} flies begin to exhibit paralysis after about five minutes, whereas neither the null mutant nor the non-heat shocked Adh^{hs61c} flies show any early response to the pentynol. (However, after several minutes, all flies succumb to this treatment.)

The experiment may be done conveniently and effectively as a lecture demonstration by inserting the pentynol-moistened paper squares into vials containing flies whose silhouettes are projected onto a screen with an overhead projector. This experiment sparks the interest of students by vividly indicating a heretofore unseen difference in the flies. Furthermore, it involves the students with a project of recombinant DNA technology.

References: Bonner, J.J., *et al.*, 1984, Cell 37: 979-991; O'Donnell, J. 1975, Genetics 79: 73-83.

Incidence of *Drosophila melanogaster* flies with melanotic tumors for demonstrating conditionality, penetrance and variable expression.

Perez-Chiesa, Y. University of Puerto Rico, Rio Piedras PR, USA. [reprinted from DIS 60: 228, 1984].

A sex-linked, temperature-sensitive melanotic tumor mutation in *Drosophila melanogaster*, $tu(1)Sz^{ts}$ (Rizki and Rizki, 1980) is excellent for demonstrating conditionality, penetrance and variable expression with changes in temperature. It also allows for learning the chi-square contingency test and for discussing dosage compensation in *Drosophila*, as well as other aspects of insect physiology. As reported by Rizki and Rizki (1980) $tu(1)Sz^{ts}$ larvae develop melanotic tumors at 26°C, whereas 18°C inhibits tumor formation. However,

penetrance may vary in melanotic tumor strains depending also on genetic background, crowding conditions and food media used (Sparrow, 1978).

Experimental Procedure: Students are given two stocks of *D. melanogaster*: wild type, non-tumor forming strain, and *tu*(1)*Sz*^{ts}. They set up two cultures of each stock and place them in incubators: one culture of each at 18°C, the others at 26°C. Three days later the parents are removed and their progeny is allowed to continue development at the same temperature at which they started. After eclosion students classify the flies in terms of sex and mutant phenotype: presence of melanotic tumors. The tumors are usually found in the abdomen and less frequently elsewhere. The students are asked to determine whether there are significant differences in the incidence of flies with tumors between the sexes and between the stocks used at each temperature. We have done the experiment at 22°C vs 29°C; there will be tumor formation at 22°C, but the incidence of flies with tumors is still significantly different from that of flies grown at 29°C. Cultures can be coded to avoid bias.

References: Rizki, T.M., and R.M. Rizki 1980, Wilhelm Roux's Archives 189: 197-206; Sparrow, J.C., 1978, In: *The Genetics and Biology of* Drosophila (Ashburner, M., and T.R.F. Wright, eds.), volume 2b: 277-313. Academic Press, London.

A laboratory simulation of natural selection in *Drosophila*.

Marengo, N.P. C.W. Post College of Long Island University, Greenvale, New York. [reprinted from Dros. Inf. Serv. 52: 185, 1977].

In an undergraduate course in genetics, a laboratory exercise was devised to illustrate how, in a single generation of *D. melanogaster*, a significant shift in phenotype proportions in a population could be achieved by introducing a selective environmental hazard. This would be a structure or condition in the environment limiting the viability and reproductive capacity of one of the phenotypes and therefore favoring the viability and reproductive capacity of the other.

The success of the experiment was of such a significant degree, that its communication to the educational and scientific communities appears warranted.

Large numbers of wild type (+) and vestigial (*vg*) flies were cultured separately in conventional milk bottle cultures of tomato-paste agar (Lewis, 1942). After emerging and aging for several days they were issued to students. Some students received five pairs of each phenotype and some received ten. Both population samples yielded similar results.

The students worked in twos, with one responsible for the "control" culture, and the other the "experimental".

The cultures were identical except that the experimental culture had a 20 mm × 60 mm piece of flypaper (Dars bug-all flycatcher, Dars-Met-All Industries, Inc., Long Island City, New York 11101) pierced twice by a swabstick and held suspended over the food mass by having the top of the swabstick pushed through the foam plug of the bottle (Figure 1).

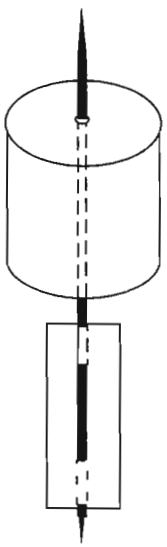

Figure 1. Sketch of the selective hazard consisting of a piece of flypaper attached by a swabstick inserted through bottle plug.

Each class met twice per week and the experiment was completed in three weeks. The first period was taken up with issuing five or ten pairs of each phenotype to each student. These were issued etherized and were placed in small plugged test tubes until active, at which time they were transferred to the breeding bottles. One period of the second week was used to check the fertility of the cultures and remove the surviving parental phenotypes originally introduced.

At room temperature, the cultures were ready for counting two

Table 1.

Control		Experimental	
+	vg	+	vg
1561	1361	702	2038

weeks after the start. Two counts were made of each culture, and each student's totals were entered on a master tally-sheet. The figures were then consolidated and compared. A total of thirty student-cultures provided the data. The consolidated results are shown in Table 1.

The striking difference in the proportion of phenotypes in the control and experimental cultures indicates that the introduction of a selective hazard can in one generation change significantly the genetic constitution of a population.

References: Lewis, M.T., 1942, Science 96(No. 2490): 282.

A search for pleiotropic effects of a mutant gene: An exercise in ecological genetics.

Huber, I. Fairleigh Dickinson University, Madison, New Jersey. [reprinted from Dros. Inf. Serv. 58: 181-182, 1982].

In studying a population, ecologists often make the simplifying assumption that all members of a population are genetically identical. A large body of recent studies in population genetics, especially with electrophoretic techniques, indicates that this assumption is unrealistic. Ecological genetics is a field that has developed at the boundary of ecology and population genetics and is concerned with the effects of genes (such as those for eye color or body color in *Drosophila*) on biologically important parameters, such as longevity and fertility, as well as aspects of physiology and behavior which may affect viability.

From the viewpoint of the organismic geneticist, there is a major phenotypic effect by which the presence of a gene is identified and a multiplicity of minor effects on many aspects of the anatomy, physiology and behavior of an animal. Collectively, these effects are referred to as pleiotropic. As an example, the sickle-cell gene in homozygotes, in addition to causing sickling of erythrocytes, has an effect on virtually all organ systems (Neel and Schull, 1954). Both phenomena, namely the existence of pleiotropic effects of a mutant gene and the fact that these effects may be important in population dynamics, can be demonstrated with *Drosophila melanogaster*.

Materials and Methods: Three groups of *D. melanogaster* will be tested: wild-type, a recessive mutant, and their F_1 hybrid. Students are asked to select a trait unrelated to the major phenotypic effect and test samples of all three groups. Examples of adult traits which have been studied with interesting results are: dry weight, wet weight, longevity (for faster results, deprive flies of food but not water), longevity at elevated temperatures, resistance to standard dose of insecticide, number of eggs laid/female/day, O_2 consumption/gram body weight/hour. Other traits which could be investigated include behavioral characteristics such as walking speed, frequencies of grooming movements and components of courtship and rates of maze-learning ability.

Means for each group will be determined and (depending on the amount of statistical knowledge of the class) statistical tests run. For weights of flies, it is best to group them. For example, weigh four groups of 25 flies of each genotype, expressing the results as mean weight/25 flies.

Interpretation: Several kinds of outcomes are possible for this exercise. All of the mutants studied are recessive.

1. All three genotypes have the same mean. In this case, the mutant has no pleiotropic effect on the trait measured. This is entirely possible, though not as interesting, as the other kinds of results.
2. The heterozygote has a mean not significantly different from the wild-type. Since the mutant is completely recessive, the pleiotropic trait parallels the expression of the major phenotypic effect. This is the extreme of the range of values mentioned in #3 (below).
3. The heterozygote mean is closer to wild-type than to the mutant.
4. The heterozygote has a mean intermediate between that of the wild-type and the mutant. Therefore, the trait shows incomplete dominance in contrast to the recessive inheritance pattern of the mutant.
5. The heterozygote is closer to the mutant than to the wild-type.
6. The heterozygote mean is identical with the mutant mean. This is the extreme of the range of values mentioned in #5 (above).

7. The heterozygote mean is more extreme than either homozygotes, a phenomenon called overdominance.

Whether a gene is regarded as dominant, overdominant, incompletely dominant or recessive depends entirely on the criterion by which an individual is classified and is thus relative. The criterion may be the major phenotypic effect, a pleiotropic effect (or amounts of protein products of the genes). For example, by appropriate choice of criteria, the sickle-cell gene can be shown to resemble most of the dominance relationships listed above (see Mange and Mange 1980: 189-193 for a useful discussion).

References: Mange, A.P., and E.J. Mange 1980, Genetics: Human aspects, Saunders, Philadelphia; Neel, J.V., and W.J. Schull 1954, *Human Heredity*, Univ. Chicago Press, Chicago.

SDS-PAGE technique for demonstrating sex linked genes.

Ramesh, S.R., and W.-E. Kalisch. Ruhr-Universit≅t Bochum, FR Germany. [reprinted from Dros. Inf. Serv. 67, 1988].

Classical experiments to demonstrate the inheritance of X-chromosomal genes involve reciprocal crosses to compare heterozygous F1-female and hemizygous F1-male flies phenotypically, the latter ones getting their sex linked genes exclusively from their mothers. Here, we describe a simple technique involving the use of a 13.7% SDS-PAGE (Sodium Dodecyl Sulfate – PolyAcrylamide Gel Electrophoresis) to demonstrate electrophoretically the sex linkage of several genes coding for major glue protein fractions in the larvae of *D. n. nasuta* and *D. n. albomicans*. Interspecific crosses of these two *Drosophila* species belonging to the *nasuta* subgroup of the *immigrans* group are used for the following reasons: (1) The *nasuta* subgroup members show species-specific glue protein patterns. The major glue protein fractions are very prominent; therefore, one pair of salivary glands (from a single individual) is sufficient for obtaining the electrophoretic pattern (Ramesh and Kalisch, 1987). (2) The major glue protein fractions in *D. n. nasuta* and *D. n. albomicans* show different kd-values, as a result of which the patterns of protein fractions can easily be analyzed in the heterozygous F1-females (Ramesh and Kalisch, 1988).

We crossed the species *D. nasuta albomicans* (stock number: 15112-1751.0, obtained from the National Drosophila Species Resource Center, Bowling Green, Ohio, USA) and the *D. nasuta nasuta* [stock Mysore I from our lab (Ramesh and Kalisch, 1988), but the 15112-1781.0 stock from Bowling Green can also by used]. Standard cornmeal medium was used; cultures and crosses were maintained at 22±1°C.

The advantages of this experiment for teaching purposes are the little amount of tissue required (*i.e.*, only one larva is needed) and the clear identification of the prominent glue protein fractions, by which, an unequivocal interpretation of the results is possible even by beginners.

Preparation of samples: Samples from third-instar larval salivary glands from parental and F1-hybrid cultures are prepared. Well-grown larvae are washed, separated according to the sex, and dissected in a 0.03 M phosphate buffer (pH 6.8) with 0.04 M KCl, 0.011 M NaCl, 0.003 M $CaCl_2$, and 0.021 M $MgCl_2$ (Ashburner, 1970), at 20±1°C. The glands are transferred for 20 min. into a 1.5 ml microfuge tube filled with cold 10% TCA. The tissue is washed with the help of a Pasteur pipette (each time for 20 min.) in 95% ethanol, a mixture of methanol and chloroform (1:1); finally it is dried at 37°C (which requires about 15 min.). To the dried tissue, 30µl of sample buffer [0.0625 M Tris-HCl (pH 6.8), 1% SDS, 1% β-mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue] is added and the lid of the microfuge tube is tightly closed. After 2-3 hr., it is heated in boiling water for 10 min., cooled to room temperature and then centrifuged for 3 min. at 1,000 rpm. These extracts can be stored at -20°C for 8-10 days.

Table 1.

Solutions	Lower gel	Upper gel
Lower gel buffer	7.5 ml	—
Upper gel buffer	—	4.0 ml
Acrylamide solution	18.0 ml	4.0 ml
Dist. water	4.5 ml	7.76 ml
1% Ammonium persulfate	200.0 µl	160.0 µl
20% SDS	225.0 µl	240.0 µl
TEMED	20.0 µl	16.0 µl

Solutions required for preparation of the gels: (1) *lower gel buffer*: 18.15 g Tris dissolved and made up to 100 ml with dist. water, pH adjusted to 8.8 with conc.

HCl; **(2)** *Upper gel buffer*: 6.0 g Tris dissolved and made up to 100 ml with dist. water, pH adjusted to 6.8 with conc. HCl; **(3)** *Acrylamide solution*: 22 g acrylamide and 0.8 g bis acrylamide dissolved and made up to 100 ml with dist. water; **(4)** *Ammonium persulfate*: 1% solution (should be prepared fresh); **(5)** *Sodium dodecyl sulfate*: 20% solution.

Stacking gel and separating gels are prepared by mixing different proportions of solutions as shown in Table 1. The amount of gel solution mixtures are enough for casting 15 x 10 cm gels of 1 mm thickness. For details with regard to gel casting, see Ostermann (1984). It is preferable to cast the gels in cold. The prepared gel may be used immediately or may be stored at 8-10°C for 24-48 hr.

Electrophoresis: 15µl of the samples prepared is injected into each slot of the gel, which are then carefully layered with running (tray) buffer [0.05 M Tris, 0.384 M glycine buffer (pH 8.3) containing 0.1% SDS]. The electrophoresis is performed with 165 volts (35 mA max.) for 3 ½ hr at 20±1°C. The gel is treated overnight with a prestaining solution [50% TCA and isopropanol (1:1)]. 0.175% Coomassie brilliant blue R-250 (in 50% methanol and 10% acetic acid) is used for staining the gel for 2 hr., and a different mixture (25% methanol and 7.5% acetic acid) is used for destaining.

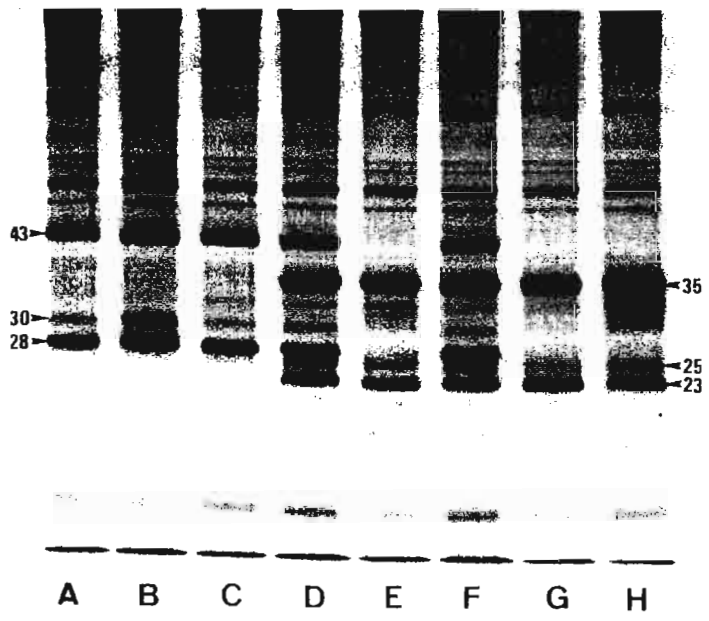

Figure 1. X-chromosomal linkage of major glue protein fractions in *D. n. nasuta* and *D. n. albomicans*. SDS-PAGE patterns of protein extracts from salivary glands of third-instar larvae. **(A)** male and **(B)** female of *D. n. nasuta*. **(C)** F1-male and **(D)** F1-female from a *D. n. nasuta* mother and a *D. n. albomicans* father. **(E)** F1-male and **(F)** F1-female from the reciprocal cross. **(G)** male and **(H)** female of *D. n. albomicans*. Note that in (C) as well as in (E) the F1-male shows the pattern of its mother, whereas in (D) as well as in (F) the F1-female shows a heterozygous pattern of both parents. Each protein extract is from one pair of salivary glands by the use of an individual larva. Differences in protein staining are based on gland size as well as species- and stage-specific development. Arrow-labeled bands indicate major glue protein fractions in both species which are coded by X-chromosomal genes. The approximate kd-values are given according to Ramesh and Kalisch (1988). Coomassie-blue staining.

Figure 1 shows the SDS-PAGE patterns of protein fractions from the salivary glands. The prominent bands between 43-23 kd belong among others to the glue proteins (Ramesh and Kalisch, 1987). It is evident from Figure 1, that the three labeled glue protein fractions in both species are produced by genes located in the X-chromosome, since the F1-hybrid males (C and E) show the phenotype of the P-females used for the cross; while the F1-females (D and F), irrespective of the direction of the cross, are heterozygous for the maternal and paternal patterns. Chromosomal linkage of the remaining major and minor glue protein fractions will be published elsewhere (Ramesh and Kalisch, in prep.).

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References: Ashburner, M., 1970, Chromosoma 31: 356-376; Osterman, L.A., 1984, In: *Methods of Protein and Nucleic Acid Research*, Springer-Verlag, Berlin and N.Y., p21-25; Ramesh, S.R., and W.-E. Kalisch 1987, Dros. Inf. Serv. 66: 117; Ramesh, S.R., and W.-E. Kalisch 1989, Genetica (in press).

Useful population cage experiments for demonstrating directional and balancing selection.

Sperlich, D. University of Tηbingen, F.R. Germany. [reprinted from Dros. Inf. Serv. 59: 147-148, 1983].

In a basic course in population genetics we are using successfully for years a simple cage experiment to demonstrate the effect of natural selection in populations carrying a recessive lethal. Depending on the population system the lethal is either going to be balanced or eliminated (Sperlich and Karlich, 1970). Strains used for the experiment are: a wild-type strain of *D. melanogaster* (*D. pseudoobscura* can be also used; see Sved and Ayala, 1970) and a L Cy/Pm strain with good expression of the markers (occasional selection of the strain for good manifestation of Cy is recommended). Using the ordinary marker strain technique pairs of lines are established (see Figure 1) carrying the same wild chromosome II in homozygous condition ($+^A/+^A$) or in combination with L Cy (L Cy/$+^A$). At least ten such pairs of lines (A/A – L Cy/A, B/B – L Cy/B ..., J/J – L Cy/J) must be available for the experiment. Lines with homozygotes A/A being lethal (about 30 percent!) must be discarded. "Monochromosomal" populations are then started each by founder flies from one pair of the lines only; *e.g.*, line C/C and line L Cy/C. Wild flies are taken from the C/C line and L Cy- phenotypes from the L Cy/C line (there are wild-type flies in this line too which must be either discarded or counted as "wild"-type C/C). The ratio between C/C and L Cy /C genotypes in the founder population is chosen 2:1. "Polychromosomal" populations are founded in the same way but wild-type flies are now taken equally from all different pairs of lines (A/A, B/B, ... , J/J) and L Cy flies from the corresponding L Cy-line (L Cy/A, L Cy/B, ... , L Cy/J). The ratio wild: L Cy is again 2:1. Any population cage system can be used to keep the populations for three to four months. The temperature should be 25°C which ensures good manifestation of Cy and a generation time of about 15 days.

Egg samples should be taken every second generation (this means monthly) by inserting 4-6 fresh vials into the cage for 24 hours. The flies hatching from these samples are then counted. The relative frequency of the L Cy chromosomes in the cage populations is easily calculated. Three to four samples are usually enough to demonstrate that L Cy is almost completely eliminated in this short period from the gene pool of the polychromosomal populations but appears balanced in the monochromosomal populations (Sperlich and Karlich, 1970; Sved, 1971). Starting with a L Cy frequency of q = 0.167 (= 1/6) it becomes nearly zero in the polychromosomal and around q = 0.25 to almost q = 0.50 in the monochromosomal populations.

The population system is very simple since L Cy/ L Cy phenotypes are completely lethal. Putting the relative fitness of the heterozygotes (*e.g.*, L Cy/A) first, only the fitness of the wild-type "homozygotes" (*e.g.*, A/A in mono. and A/B ... D/D in poly.)

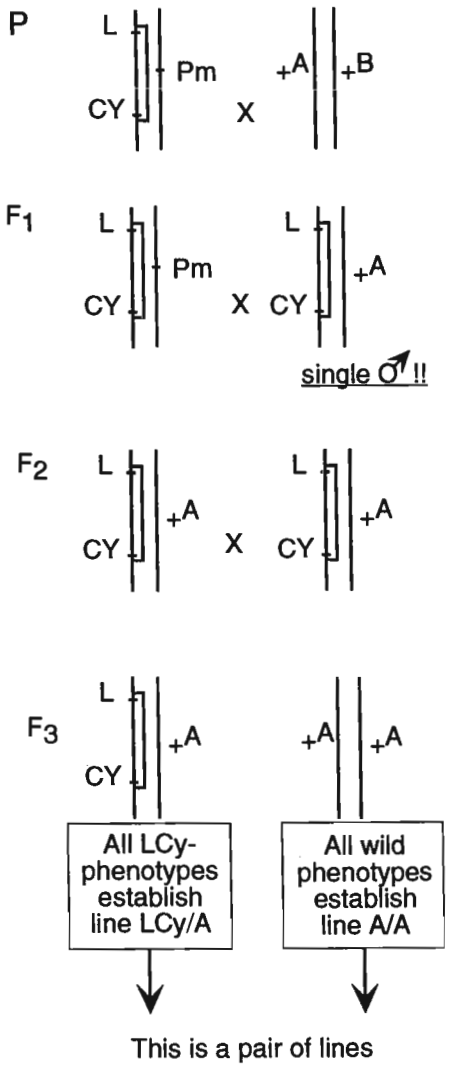

Figure 1. Crossing procedure for the construction of "monchromosomal" and "polychromosomal" populations.

Table 1.

	A/A	LCy / A	LCy / LCy	
Fitness	W	1	0	population fitness = $\overline{W}$
Frequency	p^2	$2pq$	q^2	$= W \bullet p^2 + 2pq$
	$q_1 = \frac{p_0 q_0}{W p_0^2 + 2 p_0 q_0} = \frac{q_0}{W p_0 + 2 q_0}$			($q_0 = .1667$; $W = 1.4, 1.2, 1.0, 0.8, 0.6, 0.4, 0.2, 0.0$)

remains unknown. Starting with q = 0.167, the following fitness distribution calculations can be easily made with any pocket calculator by the students: By iteration of this formula and by using different W values students can easily gain basic understanding for selection processes maximizing population fitness. Additional discussions arise automatically about genetic load, balancing selection and computer simulations of population systems (Sperlich *et al.*, 1982).

References: Sperlich, D., and A. Karlik 1970, Genetica 41:265-304; Sved, J., 1971, Genet. Res. 18: 97-105; Sved, J., and F.J. Ayala 1970, Genetics 66: 97-113; Sperlich, D., A. Karlik, and P. Pfriem 1981, BiolZbl. 101: 395-411.

Salivary preparations from *D. pseudoobscura.*

Bryant, S.H. Western Illinois University. [reprinted from Dros. Inf. Serv. 55: 169, 1980].

I have found that the use of *D. pseudoobscura* rather than *D. melanogaster* is much easier for salivary preparations. The larvae are much larger, and so are the salivary glands. Students also have a much easier time extracting the glands from *D. pseudoobscura* than they do from *D. melanogaster*. While not as many interesting chromosome aberrations are available in *D. pseudoobscura*, one excellent balanced lethal stock is available which has a very nice single inversion loop in chromosome 2. This stock is ①/Ba: Delta/Bare^{Inv}.

New white-eyed *Drosophila* "unknown" stocks for genetics laboratory courses.

Pye, Q. Cornell University, Ithaca, New York. [reprinted from Dros. Inf. Serv. 55, 171, 1980].

In his teaching note MacIntyre (DIS 51:158) discussed the utility of some phenotypically identical (orange eye, dark body, and incomplete wing veins) but genotypically unique *Drosophila* "unknown" stocks for genetics laboratory courses. I have constructed a set of "unknown" stocks that are comparable to his, except that they all have white eyes. White eyes are due to three different situations, (1) the interaction of *bw*, *bw*^{D}, or *w*^{Bwx} with *cn*, *st* or *v*, (2) the epistatic interaction of *w* with *ca*, *bw*, *st*, *bw*^{D} or *v*, or (3) the allele *w*. Another difference between the orange and white-eyed stocks is that some of the white unknowns contain two wing vein mutations (e.g. *shf*^{2}; *ve* or *ve ri*) that interact to produce the wing phenotype.

A method for transferring etherized flies into a container of active flies.

Wright, C.P. Western Carolina University, Cullowhee, North Carolina. [reprinted from Dros. Inf. Serv. 55: 172, 1980].

In working with fruit flies, it is sometimes necessary to transfer etherized flies into a container of flies which are awake and active. It is usually best to make such a transfer without etherizing or disturbing the active flies in the container. One method which is useful in this situation involves the use of a Pasteur pipette. An etherized fly can be gently brushed or sucked head-first into the small end of a Pasteur pipette. The Pasteur pipette containing the etherized fly can then be carefully inserted along the side of the stopper or cover

of the container of active flies. This can be done in such a way that the active flies in the container do not escape and are not disturbed. After the end of the Pasteur pipette which contains the etherized fly has entered the chamber of the fly container, gentle air pressure can be applied with the pipette bulb or by mouth. The etherized fly will be forced out of the pipette into the fly container. The etherized fly should be deposited on a dry surface such as the side of the container and allowed to remain on the dry surface until it wakes up and becomes active.

I have found this to be a useful technique in the situation of introductory genetics labs where beginning genetics students sometimes have difficulty in handling flies. If students try to etherize all the active flies in a container in order to introduce a few etherized flies of another genotype, sometimes all the flies will be killed as a result of accidental over-etherization. This can cause problems, especially in the situation where the active flies are virgin females which might be difficult to replace if they are killed. The use of the method of transfer decreases the amount of ether to which the flies are exposed, and thus increases their chance for survival.

A teaching exercise combining Mendelian genetics and gene therapy concepts in *Drosophila.*

Woodruff, R.C.[1], and J.N. Thompson, jr.[2] [1]Department of Biological Sciences, Bowling Green State University, Bowling Green, OH, and [2]Department of Zoology, University of Oklahoma, Norman, OK. [reprinted from Dros. Inf. Serv. 80: 107-108, 1997].

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

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

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

Females					Males				
$\frac{w^{1118}\ P[w^+]038}{w^{1118}\ P[w^+]038}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	X	$\frac{+}{Y}$	$\frac{CyO}{Sp}$	$\frac{ry^{506}\ Sb\ P[ry^+\ \Delta2\text{-}3](99B)}{TM6,\ Ubx}$	$\frac{+}{+}$	

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

Females					Males			
$\frac{w}{w}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	X	$\frac{+}{Y}$	$\frac{CyO}{+}$	$\frac{Sb}{+}$	$\frac{+}{+}$

Record Number of F1 Flies with Mosaic Eye Spots

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

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

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

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

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

The F1 females will have red eyes, because they are w^{1118}/+ (w^{1118} is a recessive mutation), whereas, one half of the F1 males will have eyes with red and white mosaic spots. These mosaic eyes are caused by white spots in which the P[w^+] element has excised during fly development, yielding w^{1118} cells on a background of cells that are red, *i.e.*, w^{1118} P[w^+]. Note that for cells to be white, the P[w^+] element has to excise and then not insert into a new chromosomal position, or the P[w^+] element has to lose part of the white DNA during a transposition event; such imprecise P-element excisions do occur. The size of the white spots will be larger the earlier the P[w^+] excisions occur during eye development.

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

In introducing this exercise to students, one could review Mendelian genetics, *Drosophila* cytogenetics, sex linkage, and gene symbolism. In addition, gene therapy could be reviewed, including how

gene replacement was first performed in *Drosophila* (Spradling and Rubin, 1982; Rubin and Spradling, 1982). This could then lead to a general discussion of transposable DNA elements, how elements such as Alu and *mariner* have been observed to cause gene and chromosomal mutations in humans (Cooper and Krawczak, 1993), and the current status of gene therapy in humans.

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

An alternative to ether.

Hedgley, E.J., and M.J. Lamb. Birkbeck College, University of London, England. [reprinted from Dros. Inf. Serv. 50: 203, 1973].

Although diethyl ether is traditionally used for anaesthetizing *Drosophila* in genetics experiments, it is highly inflammable and subject to possible abuse. It is therefore a serious potential hazard in teaching laboratories. Chloroform is a possible alternative anaesthetic but it has both acute and chronic toxic effects. We have found that methylene chloride (dichloromethane) is a cheap and adequate noninflammable substitute for ether. Methylene chloride is ten times less toxic than chloroform and, unlike the latter, it appears not to generate chronic ill effects, although of course it is still necessary to ensure that, as with ether or chloroform, the laboratory in which it is used is adequately ventilated.

For anaesthetic purposes one may use methylene chloride in exactly the same way as ether. The length of time that the flies need to be left in the vapor and the time taken for recovery are similar to those for ether. However, the behaviour of the flies while anaesthetized is rather different. Initially the wings of the flies may be held vertically above the body, *i.e.*, they may appear to be "over-etherised", but this effect is temporary. The flies also tend to twitch in a way which may be slightly disconcerting to *Drosophila* workers who are used to handling etherised flies. Although this twitching might possibly detract from the use of methylene chloride for some experiments, *e.g.*, those involving bristle counting, we have in general found no difficulty in training students to use it for work involving mutants.

New Teaching Notes

A one-generation assay for induced genetic damage.

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Usually it is difficult to demonstrate the induction of genetic damage in a higher organism in a teaching environment. There are several reasons for this. Chemical mutagens are potentially hazardous, especially when used by students with little laboratory experience. Furthermore, many assays for germ-cell mutations are multi-generation crosses (for example, screens for recessive sex-linked lethals in *Drosophila melanogaster*), and one-generation assays like those for visible mutations on the X chromosome are inappropriate because the inexperience of students in identifying new visible phenotypic changes can yield many misclassifications. Finally, germ-cell mutation rates are low, so extensive data are needed to evaluate them. This creates a large work load in media preparation and physical processing of crosses. Hence, we have tested a new DNA repair-defective assay in *D. melanogaster* (Negishi *et al.*, 1991) using UV and X-ray treatments. This one generation assay is shown to be a safe and efficient method to demonstrate induced genetic damage. Description of the mutations is given in Lindsley and Zimm (1992).

In this assay, the eggs and first instar larvae of the following cross are treated.

C(1)DX, *y w f* / Y females (repair efficient) × *sc*^{1} *z*^{1} *w*$^{+(TE)}$ *mei-9*^{a} *mei-41*^{D5} / Y males (defective in DNA excision repair and postreplication repair)

↓

Score for the number of F1 matroclinous females (yellow body color, white eyes, and forked bristles) and F1 patroclinous males (yellow eyes). A treatment with a mutagenic agent will reduce the male to female ratio as compared to this ratio in untreated F1 eggs and larvae.

In the X-ray experiment, F1 eggs and larvae up to four days old were given 500R of irradiation or were untreated (control). In the UV experiment, F1 eggs and first-instar larvae up to three days old were exposed for 10 minutes to a UV transilluminator. The *Drosophila* food carrying the eggs and larvae in open vials was held directly against the UV transilluminator.

Table 1. X-ray treatment.

	C(1)DX, *y w f* Females	sc^{1} z^{1} w$^{+(TE)}$ mei-9^{a} mei-41^{D5} Males
Control		
Vial 1	21	179
2	89	179
3	136	130
4	110	166
Total	256	654
	% male = 654/910 = 71.87%[a]	
500 R		
Vial 1	99	170
2	106	191
3	16	92
4	117	109
Total	338	562
	% male = 562/900 = 62.44%[a]	

[a] $P < 0.0001$

Table 2. U-V treatment.

	C(1)DX, *y w f* Females	sc^{1} z^{1} w$^{+(TE)}$ mei-9^{a} mei-41^{D5} Males
Control		
Vial A	32	32
B	20	33
C	31	56
D	19	39
E	34	51
F	30	50
G	20	50
H	15	31
I	37	49
J	34	33
K	24	42
L	28	52
Total	324	518
	% male = 518/842 = 61.52%[b]	
UV- 10 min		
Vial A	31	15
B	29	36
C	39	18
D	13	25
E	12	15
F	16	13
G	19	31
H	12	8
I	8	9
J	13	6
K	7	4
L	7	7
Total	206	187
	% male = 187/393 = 47.58%[b]	

[b] $P < 0.01$

The results are shown in Tables 1 and 2. They show that X-rays and UV cause a significant increase in somatic cell genetic damage that leads to the death of flies that are defective in DNA repair. This quite logical result, in turn, supports the use of this assay as a way to demonstrate induced genetic damage in a classroom exercise. The common availability of a UV source makes this a treatment of choice when considering safety concerns in working with students who have limited laboratory experience.

Acknowledgments: We thank Diane Jackson for her work on the X-ray experiment.

References: Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, NY; Negishi, T., T. Shiotani, K. Fujikawa, and H. Hayatsu 1991, Mut. Res. 252: 119-128.

Hardware Prototype and Remote Operations Testing in Preparation for Multiple Generation *Drosophila* Experiments on the International Space Station.

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FOREWORD

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For copies of project documents cited in this paper, please address your request to:

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Note: All Geneve and Taipei web pages and images referenced in this paper have been relocated and can now be found at: http:// spaceprojects.arc.nasa.gov/space_projects/flylab/flyhome.html

Acronym Table

CDS – Communications and Data Systems
CSA – Canadian Space Agency
FCU – Fly Collection Unit
HTML – Hypertext markup language
MG – Multiple generation test group

MGSU – Multiple generation separation unit
NASA – National Aeronatics and Space Administration
PI – Principal Investigator
RH – Relative humidity
SB – Stock bottle test group
SHARP –Summer High School Apprenticeship Research Program
SSBRP – Space Station Biological Research Project
STELLAR – Science Training for Enhancing Leadership and Learning through Accomplishments in Research
UOF – User Operations Facility
VD – Food volume and depth test group
WWW- World Wide Web

INTRODUCTION

Life sciences research during the Space Station era will allow for much longer experiment duration than was ever possible on the Space Shuttle. The capability of having a full time laboratory in space will allow biologists to study the effects of long duration exposure to the space environment on biological systems. In order to conduct long duration experiments with insects in space, fruit flies in particular, a suitable method must be devised to separate successive generations of flies for planned 90 day Space Station increments.

NASA plans to provide a habitat that will allow for separation of successive generations on orbit. This capability is required by the international insect science community so that multiple generation experiments can be conducted on long duration Space Station increments (Givens and Wade, 1996). Several methods of separating generations have been examined by the Space Station Biological Research Project and other groups (Pence *et al.*, 1994; Fish *et al.*, 1995; Pence, 1996a). Based on test results, a natural selection method was eliminated because it relies on the insects' attraction to light or some other locomotive behavior. This method is dependent upon the behavior, which may not be reliable and may not work with all strains of a species. A specific example of this problem could occur with a gene expressed in the larval stage of development known as the foraging (*for*) gene in *D. melanogaster*. There are two types of alleles for this gene: rover (a dominant allele) and sitter (a recessive allele). This locus is also acted upon by other modifier genes and the environment. The *for* gene is responsible for the larvae either migrating through medium or staying localized in one area of the medium (Sokolowski and deBelle, 1990). Therefore, a technique that relies on larval motility would not be desirable. If a larvae separation technique was utilized to allow larvae to migrate through food from one container to another container, the technique may select for or against groups of genes that are linked to the same chromosome as the *for* gene. Similar situations could occur with other techniques that rely on light attraction, smell attraction or some other behavioral response.

Two previous tests were completed using a mechanical method multiple generation separation unit (MGSU) prototype. Separation of generations in the first test failed due to a behavioral preference of the females to deposit eggs into the old food cylinder rather than into the new food cylinder that was being provided. The second test used food with a furrowed surface and was successful in stimulating the females to lay eggs onto the new food in the outgoing cylinder. The second test succeeded in separating generations, but demonstrated that overcrowding during development was due to overpopulation and a finite amount of food. The study found that access to the new food must be controlled to maintain a stable population (Pence, 1996a).

The objectives of the third test were to better define the method of separating multiple generations of fruit flies by developing a food slide cover mechanism or other suitable method that limits access to food during larval development and egg deposition, and helps to control overpopulation and mixing of generations. Other factors also necessary in conducting multiple generation experiments in space include evaluating remote video monitoring to observe development and determine separation time of generations, and evaluating techniques, collection prototypes devices, and operations which would simulate the process astronauts would use to collect adult flies and return them to Earth.

MATERIALS AND METHODS

MGSU Prototype

The MGSU prototype is based on a sequential specimen container configuration joined by a food filled cylinder system (Figure 1). The MGSUs are composed of two parts: a specimen container top and a medium cylinder base. The specimen container top is composed of five 48 mm × 33 mm × 50 mm (79 cc) Lexan sheet cut containers arranged in a "staggered" configuration. The medium cylinder base is constructed from solid Lexan and holds five medium cylinders in parallel. Each cylinder contains a nutrient medium volume of 10 cc (20 mm depth) and an exposed surface area of 3.65 cm^2. Six MGSUs and one spare were used in the test. Each specimen container, except the last, provides space for two food cylinders. One cylinder is the incoming cylinder that brings in egg laden food and provides the same food for the developing larvae. The second cylinder is the outgoing cylinder. It is also filled with food and is used as a receptacle to collect eggs deposited by the newly developed and matured females. When adults become mature, the old food is covered and the new food is exposed. Once the females deposit their eggs, the second cylinder is moved to a new container.

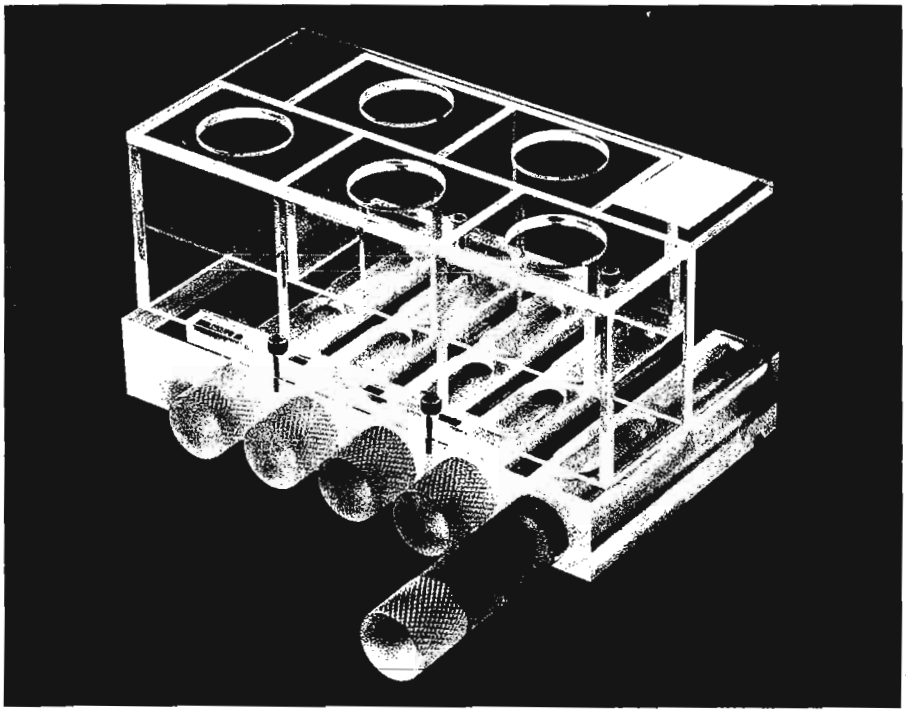

Figure 1. Multiple Generation Separation Unit (MGSU) prototype.

World Wide Web Data Collection System Development and Testing

To simulate the Insect Habitat space data collection operations and to simulate data collection by the principal investigator (PI), a web-based environmental and video data collection system was developed (Sun *et al.*, 1996). For the multiple generation separation unit (MGSU) prototype testing, the plan was to simulate data collection on orbit and use the images to monitor fly development and to make operational decisions regarding movement of food cylinders. The system is comprised of computer hardware, as well as software components, and several laboratory instruments.

The hypertext markup language (HTML) user interface for the development of the web site pages was designed using the WebMagic application (Silicon Graphics, Inc., Mountain View, CA) and BBEdit for Macintosh (Bare Bones Software, Bedford, MA). A Java applet to view real-time environmental data was compiled using Java Development Kit for SGI Irix (Sun Microsystems Inc., Santa Clara, CA). The web site was maintained on a SGI Indy workstation (Taipei: 100 MHz MIPS R4000 processor, 64 MB RAM running Irix version 5.3) in the insect laboratory with video data routed to the main computer collection system loaded on the SGI workstation Geneve in the User Operations Facility (UOF) at Ames Research Center. The data collection system on Geneve is a searchable database (Oracle Inc., Redwood City, CA). Video data was

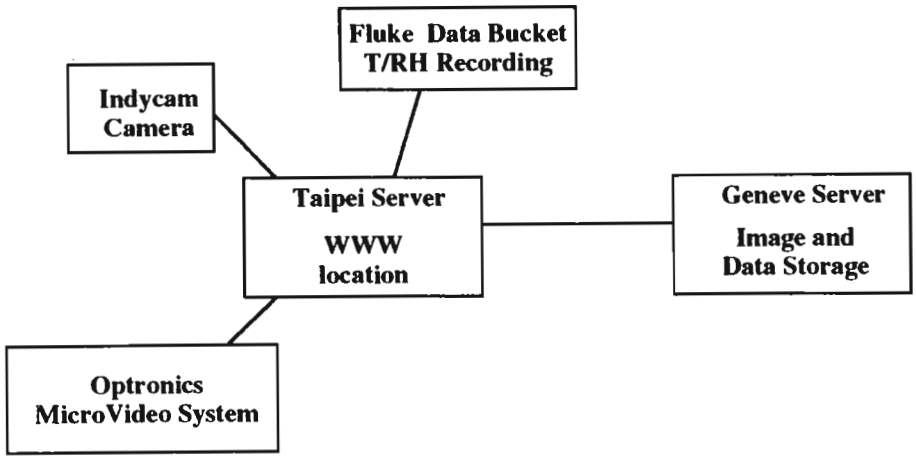

Figure 2. WWW system schematic.

collected by a SGI Indycam (Model # CMNB006C, Silicon Graphics, Inc., Mountain View, CA) and the environmental data (temperature/ humidity) was collected by Optronics dual probes (model # HT46R, Rotronic Instrument Corp., Huntington, NY) and stored via a Fluke Hydra Data Bucket (Model 2635A and 2620A-100 Input Module, John Fluke Mfg. Co., Inc., Everett, WA) to a PCMCIA card (IC Memory Card, Prod # 927512 256 KB Seiko Epson Corporation, Torrance, CA) and converted on Microsoft Excel. Later testing data was routed to Geneve and embedded with the collected images by a Common Gateway Interface shell script (Appendix I). The video and environmental data system was tested and images were collected during the MGSU food isolation mechanism test (Figure 2).

During functional testing, the video camera was positioned over the multi-generation slide cover test unit and images were collected at 20 minute intervals. The collection interval was remotely adjustable through a password-protected section of the web site. Prior to experiment use, the camera system was examined for proper lighting, options for camera positioning, necessary frame rate and resolution of images. The web site was tested for ease of use and accessibility of data and images.

Magnified Video Imaging

In addition to the Indycam video imaging system, a video microscope camera system (VI-470 CCD Video Camera System and VI-470 Camera Head, Optronics Engineering, Goleta, CA) (PVM-1351Q Video Display, Sony Electronics, Park Ridge, NJ) (Haines, 1994) was connected to the World Wide Web data collection system to test the feasibility of using magnified video on *Drosophila melanogaster* to visualize the Drosophila. A Unix shell script (Appendix II) was added to the site to simultaneously capture video from the Indycam and video micro-camera to support the multi-generation test and magnified video images collected. Images were collected over a five day period of a metamorphosing pupa in a 15 mm × 30 mm Petri dish positioned in front of the 10x objective and stored on the Taipei server. Movies were created using SGI IRIS Movie Maker 2.1 then converted to mpeg using mv2mpg shareware created by Andreas Paul (1996) http://wwwzenger.informatik.tu-muenchen.de/persons/paula.html.

Adult Fruit Fly Anesthetization and Collection Unit Prototype (FCU) Testing and Collection Operations Development

To better understand the equipment required and the amount of time necessary to collect flies, a simple prototype system was constructed to anesthetize and remove adults from the MGSU (Figures 3 and 4). The system consisted of two components: an anesthetic section and a vacuum section. The anesthetic section consisted of a small hand-held bicycle tire inflator device (Superflate, Innovations in Cycling Inc., AZ) that dispensed compressed CO_2 from a gas cylinder (CO_2 Powerlet -Model 2311, Copperhead Co., East Bloomfield, NY) via a gas flow regulator (Nupro Co., Willoughby, OH). The vacuum section was comprised

of a commercial mini-vacuum (Model # 440, MicroComputer Accessories Inc., Ingelwood, CA) and an insect aspirator (Bioquip Products, Gardena, CA). Adult flies were collected in snap cap 1.5 ml microcentrifuge tubes (Applied Scientific, South San Francisco, CA) that were attached to the modified aspirator. A small "finger" brush was also constructed from a small paint brush to aid in collecting flies stuck in crevices and corners of the test units.

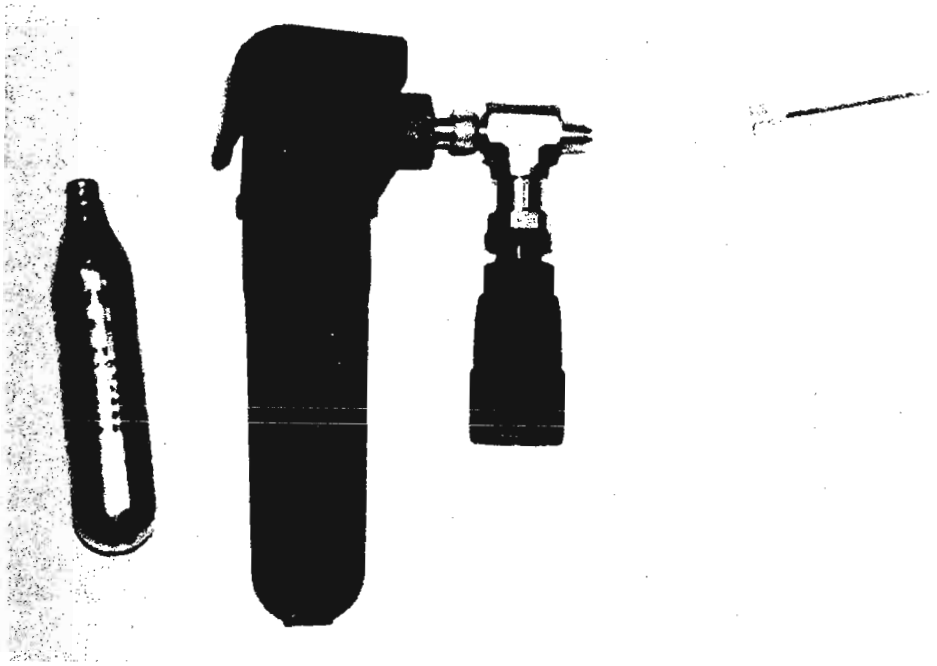

Figure 3. Anesthetization section of the FCU with CO_2 cylinder at left.

Figure 4. FCU prototype hardware. Test vacuums (upper left, center, and upper right) were used during prototype testing. The unit on the right was selected for the multiple generation test. The anesthetization unit (lower left) and 1.5 ml collection vials (lower right) are shown.

Several vacuums were tested prior to the final system selection for tne FCU system manufacture and functional testing (Model # MS-4, Metropolitan Co., Suffern, NY). Operation of the system was practiced prior to the multi-generation test and during the food tests.

During the integrated multi-generation test, two "crew members" conducted the "on-orbit" adult collection while a third observer recorded the times for each operation. Operations were split into functional categories. The shared tasks for all groups were sterilization of the hood, readying the units, gassing the flies, removing the flies, replacing the fly collection vial, and sterilizing the lab work bench after each collection. Tasks included moving cylinders, sterilizing the new chamber covers, and replacing the CO_2 cylinders. This data was collected to give an estimate of how much crew time will be necessary to perform an adult collection during a multigeneration experiment and gauge the level of difficulty it may take to complete a specimen collection task in orbit. No attempts were made to accelerate tasks during collection procedures and procedures were not practiced between collections. Operations were performed by the same personnel at each collection interval.

Food Isolation Mechanism Test

Due to their small size, fruit fly larvae are capable of squeezing through very tight spaces to gain access to food. This behavior was observed during medium volume tests when larvae squeezed between a container wall and a food retaining wall of less than 0.5 mm. The food isolation test was conducted to verify a possible method of eliminating access of the developing larvae and adults to new food.

To conduct the test, one of the prototypes was fitted with five square Lexan sheets (dimensions: 2.3 mm × 3.3 mm × 0.3 mm and cover rail dimensions: 4.7 mm × 0.3 mm × 0.5 mm) in each of the five sequential generation specimen chambers. The assessment criterion was set that if even one larva breached the slide cover, the current slide cover would be considered unsatisfactory for the complete five generation test. The prototype was equipped with the food isolation covers to limit access to the food and a second prototype was equipped only with food cylinders that were rotated 180° to limit access to the food. Four groups of one hundred adults (Oregon R wild type, P: 60 females/40 males) were each housed in the two separate specimen containers in the two multiple generation prototype units. Flies were raised and housed in a temperature and lighting controlled incubator (L:D 12:12, 24.9 ± 0.4 SD °C, 52.7 ± 11.3 SD % RH). RH was not controlled. The adults were allowed to mate and lay eggs for twenty-four hours with the intention that the female adults would lay enough eggs to create an overcrowded environment. The adults were removed with the collection prototype and the embryos were allowed to develop through the adult stage (fourteen days). The developing larvae were observed and video images were recorded with the World Wide Web video collection system to observe possible larval breach of the food isolation cover.

Integrated Five Generation Test

The multiple generation separation unit five generation test included biological testing of the MGSU and FCU prototypes, use of the web video data collection system, and performance of crew operations.

The MGSUs were biologically tested for information about population dynamics and development over five successive generations by collecting data on adult number, adult female group mass, and total development time for each generation. Adults were counted and sexed at the end of each generation and the females were dried and weighed *en masse*. Flies that were stuck in the food during transfers or collection, or pupae that were uneclosed at time of collection, were counted in the group population totals, but were not included in the female mass measurements. Development was scored by the appearance of a development stage in each test container at lights on each morning and in the late afternoon. Each measured parameter was used to assess possible stress conditions of overcrowding (Ohba, 1961). Statistical analysis was completed using unpaired student t test between and within the test groups. The prototype was also assessed for its ability to maintain a stable population by controlling food exposure through all five generations. The minimum criterion was one mating pair in each prototype to perpetuate generations.

Two other groups were maintained during the five generation test: a food volume and depth (VD) group and a standard *Drosophila* stock bottle group (SB). The VD control group consisted of six acrylic containers (TAP Plastics, Mountain View, CA) with a 40 mm × 40 mm × 72 mm outer dimension and a 100 cc volume 20 mm depth medium (10 cc), and 10 initial adults (6 female: 4 male). The SB group consisted of six standard laboratory polypropylene *Drosophila* containers (Applied Scientific, South San Francisco, CA), 103 mm height, 55 mm width at the bottom, tapering to 35 mm at the lid, with a total volume of 170 cc, 20 mm depth medium (45 cc) and 10 initial adults (6 female: 4 male). The *Drosophila* stock bottle container (SB) was assumed as the control container for housing flies. The multi-generation prototype is based on a system of moving food with newly laid fly eggs on the food surface. The control containers are based on an adult moving scheme allowing adults to emerge, then moving the new adults to new food containers. Due to the differences between the multigeneration prototype and the "controls," the plan was that the two groups would be coupled to one another chronologically to account for differences from generation to generation when adults emerge, when they are exposed to the new food, and when the food cover and cylinder is moved.

Similar container covers were used for both test units and controls. The container openings were covered with a small sheet of acrylic fitted with a small foam filled slit to allow air flow and retain container food moisture. Sterile technique was used throughout the experiment. For the parental (P) generation of the MGSU and the control groups, flies were removed with normal collection techniques and tools, and not the FCU.

All food was made prior to the start of the 5 generation test. For the multi-generation cylinders, food was cooked, placed into cylinders while melted, wrapped in aluminum foil, taped together by generation of use, placed into an autoclave bag, then autoclaved. Stock bottles were filled with melted cooked food, plugged, autoclaved, then sealed with parafilm after cooling. Volume and depth control containers were ethanol sterilized, filled with sterile food, then parafilmed after cooling. All food was stored at 5°C until use.

Mature, axenic (microbe-free) adult fruit flies (5-10 days old) were utilized (Oregon R wild type strain, Mid-America Stock Center, Bowling Green, OH) and the experiment was conducted in an incubator at 24.9 ± 0.5°C, 38.5 ± 10.7% uncontrolled relative humidity, and an environmental lighting regime of light:dark 12:12 (Pence and Hult, 1997). Flies were reared and tested on a nutrient medium of cornmeal (Sigma C-6304) 135 g/L, Grandma's unsulfured molasses 100 ml/L, and *Candida utililis* yeast (Sigma YCU) 40 g/L, agar (Sigma A-9915) 20 g/L, p-hydroxy benzoate methyl ester (Sigma H3647, 10% in 95% EtOH, 1.5 ml/L) 15 ml/L and propionic acid (8 ml/L) in distilled H_2O.

Mating groups (6 females: 4 males 10-12 days adult age) were placed into the six test units and removed 24 hours later. Two groups of controls were also simultaneously started; the food depth/volume (VD) control and a *Drosophila* stock bottle (SB) baseline control. The video camera was set on unit #5 and image collection was set at a 20 minute interval.

RESULTS AND DISCUSSION

World Wide Web Data Collection System Development and Testing

The insect web data collection system was developed jointly between the User Operations Facility Computer Data System group and the insect science lead in an iterative process. The core functions of the web site consisted of a database query form (Figure 5) and a selectable functions page (Figure 7). The information that simulated "on orbit" data retrieval was the habitat monitor page (Figure 6) which listed the "habitat" camera image, data sample time, temperature, relative humidity, the MGSU #, chamber #, and notes of the image.

The selectable functions page allows the PI to select several options of data visualization, image frequency and data collection, "snapshot" imaging, instantaneous image and data collection, and graphic data visualization. The automatic image and data collection function allows the PI to select how often images are automatically collected and conceivably could control other habitat functions, such as temperature and lighting cycle. The collection frequency could be programmed from minutes to years. For the MGSU test, the image collection frequency was set to collect images automatically at 20 minute intervals. Increasing or decreasing

frequency is possible with the current system; however, there is a limit to data storage space. The snapshot image and data collection allows the PI to collect image and data instantaneously. Once the habitat monitor page is retrieved, the PI can add notes about the image, then store the image in the database. With the instantaneous data option, the habitat monitor page is "streamed" approximately every 10 seconds, without storing the data to the database. The graphic database option allows the PI to view the environmental data over a period of time.

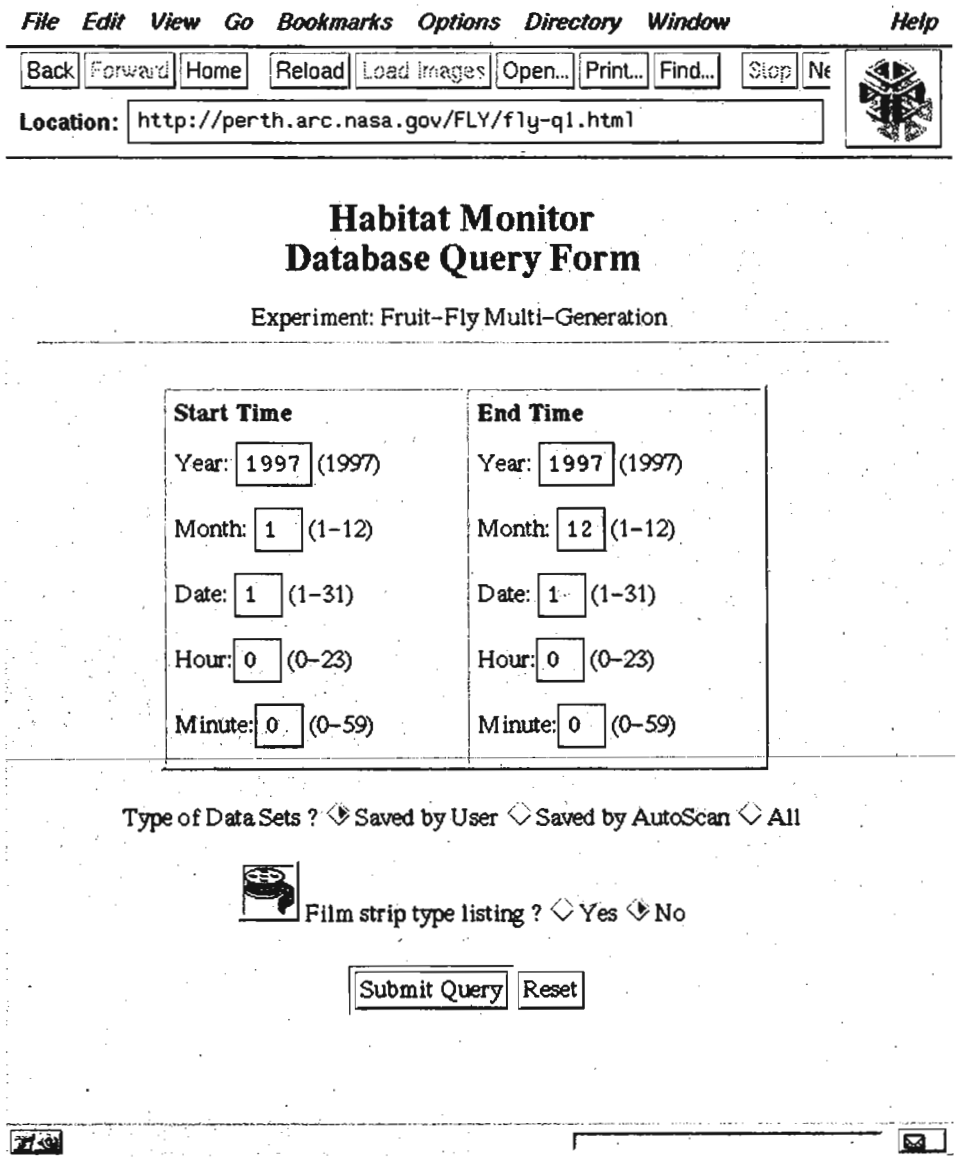

Figure 5. Database Query Form.

The database query form is contracted to allow the PI to retrieve all stored habitat monitor pages. The page is set up to allow the PI to select the start and end time of data to retrieve by year, month, day, hour and minute and gives options to collect autosaved or snapshot image data and the option to view the images as a film strip. (All habitat monitor pages for the food isolation mechanism test and the MGSU test are available through the database query form page on the Space Projects server. Images from the food isolation mechanism test are retrievable from (test dates: 9/17 - 10/1, 1996) and images from the MGSU test are

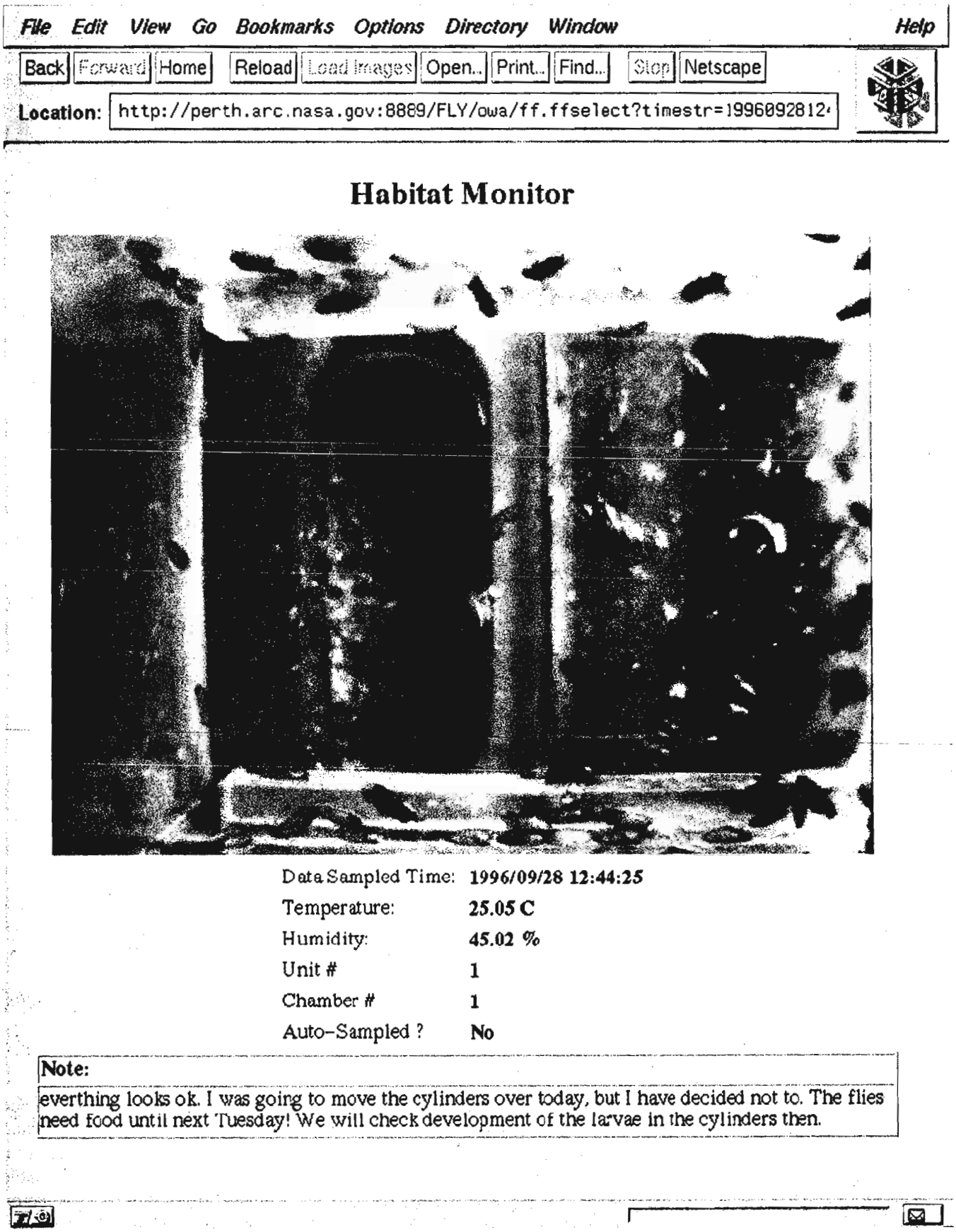

Figure 6. Habitat Monitor Page.

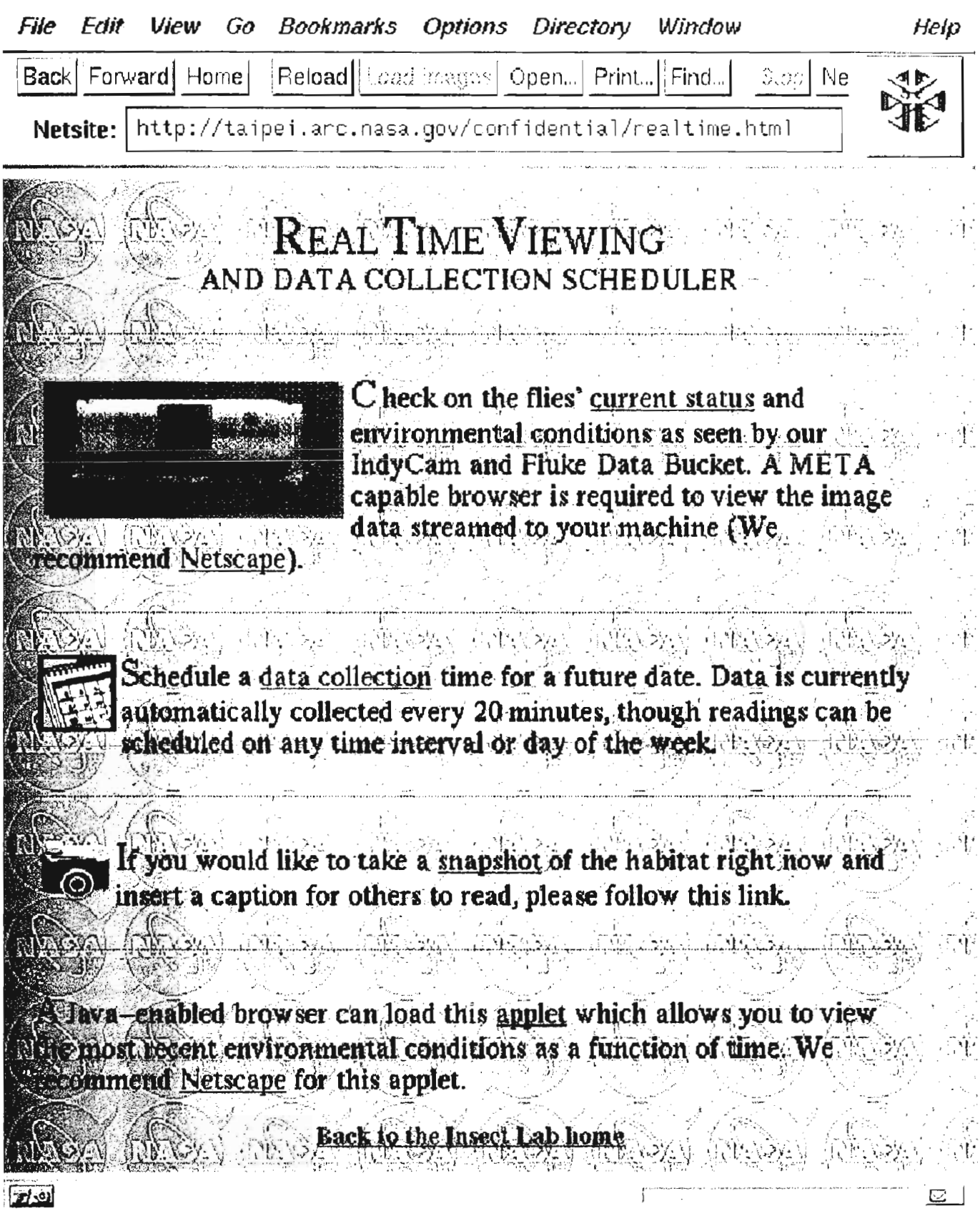

Figure 7. Selectable Functions Page.

retrievable from (test dates: 12/11, 1996 - 2/7, 1997).) A time-lapse video was made from the food isolation mechanism test collected images and is available on the Space Projects server (larvae.mpg).

World Wide Web System Performance

The data retrieval worked very well, but typing in the dates on the database query form was less than optimal and the numbers were reset to the lowest number in each group each time the page was reloaded. It may be better to set up the page as a mouse clickable set of numbers. During set up of the system, a conflict was found between the web data collection system and the laboratory temperature/humidity collection system. The Fluke Data Bucket was not designed to send data to more than one output and therefore could not send data to the PCMCIA card and send data to the web system simultaneously or even sequentially. The temperature and humidity data was critical for documentation of the incubator environment during the MGSU test. Due to the experimental nature of the web system, and as a safety precaution, the temperature/humidity data was not sent to the web data collection system. The realtime data transmittal to the web site was achieved successfully after completion of the multi-generation test. There were some reliability problems during the test including the loss of the main server one day, although collected images could be retrieved at a later date, and a data file problem at the start of the new year, in which all images were lost for approximately a 24 hour period, during a critical development period of the test. There was also a time stamp problem one day during the test, which caused no problem to the image collection, but did cause some confusion in temporal review of the images. Reliability is extremely important and the new system needs to be fully tested prior to system use in flight operations.

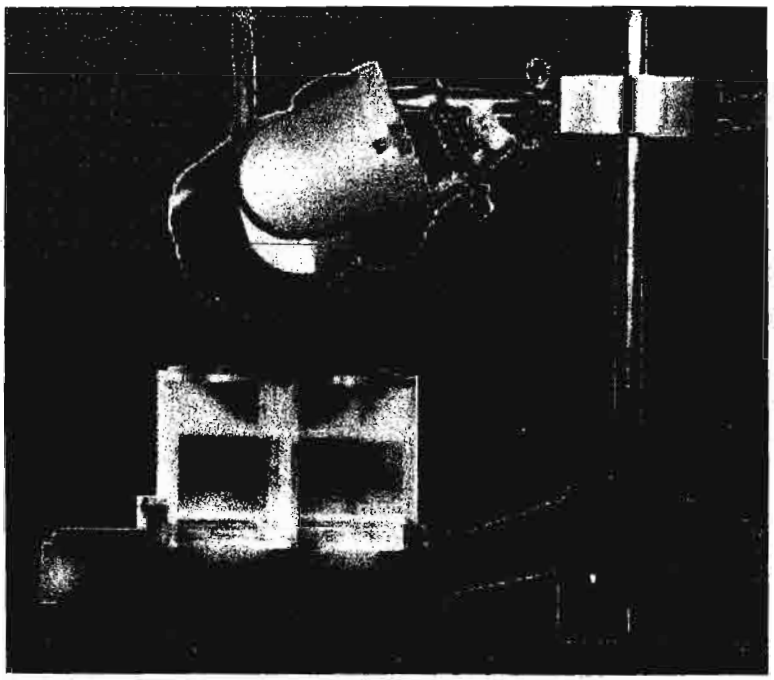

Figure 8. Indycam camera and MGSU configuration.

The Indycam camera images were of satisfactory quality, and lighting and positioning were adequate to observe fruit fly development. There were some difficulties with focusing the camera, maintaining color balance, and line dropouts of the images. The camera was focused by screwing the lens in and out of the camera, but it was difficult to get the exact focus needed to view developing larvae in the food. Autofocusing would have been desirable. For a space based system, serious consideration will have to be given to image quality. For the tests, the camera was positioned over the test units and a small foam filled acrylic plastic sheet (6 cm × 3 cm) was used to allow viewing of the test chamber and to allow passive air flow through the chamber (Figure 8).

An acrylic sheet was used on all the test units to allow image collection of the other units as needed. To maintain the proper image color, the camera was white balanced using camera software to return the camera to proper color after spectral drifting as needed. Drifting was usually caused by camera exposure to laboratory fluorescent lighting when the incubator door was opened, but the camera also exhibited an inherent drift. Another problem that occurred with the Indycam system was line

dropout of some of the images which resulted in reduced image quality. The reason for the dropout was unknown, but was probably caused by the supporting camera software.

Aside from the functional and feasibility testing of the web system, educational information was added to the web site by the addition of other html pages. Information added to the site includes past insect flight history and literature references, STELLAR (Science Training for Enhancing Leadership and Learning through Accomplishments in Research) and SHARP (Summer High School Apprenticeship Research Program) laboratory interns information, and project documentation. Links were made to the Canadian Space Agency (CSA); an international Insect Habitat development partner, NASA sites, and other fruit fly web pages.

The web site has also been extremely helpful in providing information to the CSA and international insect science advisors. This page has also been linked to the SSBRP home page and to Summer 1996 STELLAR teacher Judy Jones' classroom web site, to aid in Mrs. Jones' presentation to students of lessons prepared at NASA ARC.

Magnified Video Imaging

The magnified video imaging system was not used on the MGSU test units due to the small focal plane of the camera and close focusing range of the lens, the size of the MGSU containers, and the space needed for the Indycam. Images were collected of a stationary metamorphosing pupa in a 15 mm × 30 mm Petri dish. Time-lapse movies of the developing pupa were created and are available on the Space Projects server (pupdev.mpg, pupdev2.mpg). Magnified imaging of stationary specimens is probably feasible on orbit, and may be required for some developmental studies. 10× magnified imaging was adequate for visualization of a single fruit fly pupa. For mobile targets, however, a target tracking and focusing system would be necessary.

Adult Fruit Fly Anesthetization and Collection Prototype (FCU) and Collection Operations Development

Prototype Trials

The CO_2 gas system anesthetized groups of adult flies in stock bottles within 3-5 seconds and then flies were given a 30 second dose to maintain the anesthetic effect for collection. The flies stayed anesthetized for approximately 2-3 minutes after gassing. The total collection time for a single chamber was approximately 2 minutes, which was faster than expected.

Fly Collection During the Food Isolation Mechanism Test

On the second day of the food isolation mechanism test, fly collecting procedures were practiced to test the FCU. The FCU worked well, except for repeated activation of a motor shutoff switch. The switch was activated automatically when the motor overheated, due to continual high suction pressure created when collecting the flies in multiple containers. During the test, the operator had to wait for the motor to cool before the motor could start again. After the test, a carburetor was added to the vacuum line so that vacuum pressure could be controlled manually. This increased vacuuming time and the motor would still occasionally shut off. A more durable motor is needed for this task.

The average collection time of flies during the functional test was approximately six and one half minutes per multiple generation unit for the parental generation (Table 1) and two and one half minutes for the F1 generation (Table 2). Vacuum motor overheating created the delay between the P and F1 times. In the P generation only 1 gas cylinder was used, but during the F1 generation 1 cylinder changeout occurred. It required 2 minutes for cylinder changeout.

Table 1. Parental (P) generation FCU collection data.

Test Unit/chamber	gas time	tube change	vacuum/overheat?	total time
Unit 1/chamber 1	1.5 min.	1 min.	4 min./Y	6.5 min.
Unit 1/chamber 2	2 min.	1 min.	4 min./Y	7 min.
gas cyl. change	0 min.	N/A	N/A	0 min.
Unit 2/chamber 1	2 min.	1 min.	3 min./Y	6 min.
Unit 2/chamber 2	2 min.	0.75 min.	3.5 min./Y	6.25 min.
Average time	1.9 min.	0.9 min.	3.6 min.	6.4 min.
Total time				25.8 min.

Table 2. 1st Familial (F1) generation FCU collection data.

Test Unit/chamber	gas time	tube change	vacuum/overheat?	total time
Unit 1/chamber 1	1 min.	1 min.	1 min./N	3 min.
Unit 1/chamber 2	0.5 min.	0.75 min.	0.75 min./N	2 min.
gas cyl. change	2 min.	N/A	N/A	2 min.
Unit 2/chamber 1	0.5 min.	1 min.	0.75 min./N	2.25 min.
Unit 2/chamber 2	0.8 min.	1 min.	0.8 min./N	2.6 min.
Average time	0.7 min.	0.9 min.	0.8 min.	2.5 min.
Total time				11.9 min.

When using the fly collection prototype, fly collection was easier in the MGSU lacking the food isolation mechanism, although there were no large differences in the collection times. The food isolation cover rails created crevices for the adults to hide in, which made it difficult to remove the flies. After the test, a small "finger" brush was constructed to aid in collecting flies that were stuck in crevices and corners of the test units. An advantage of using the compressed CO_2 gas in the non-slide modified units was that you could use the gas stream to blow all of the adult flies into a corner, which made it extremely easy to vacuum them and you would not need the small brush.

Also during the test, there were some problems with flies becoming stuck in the vacuum tubing when the flies were vacuumed. To overcome this problem, clear tubing was added to the vacuum line to make viewing of the flies easier as they were vacuumed. The clear tubing was later used during the five generation integrated test.

Biological Testing of the Food Isolation Mechanism

The food slide cover mechanism failed to stop the developing larvae from entering the new food cylinder. As the overcrowded larvae population developed and exhausted the food supply in the first cylinder, the larvae began searching for another source of food. Many larvae were able to crawl underneath the cover and access the food (Figure 9). The time-lapse video also shows that the larvae were able to physically move the slide to the side when locomoting between the slide and the chamber wall. Individual images of the larvae breaching the slide are available at the Space Projects web site (test date 09/23 -9/24/96).

With the failure of the slide cover, it was decided to rotate the food cylinders by 180° to limit access to the food and continue the five generation test without a redesign of a slide cover system. Another problem that occurred during the later portion of the test was food drying in the exposed cylinders in both test units. This was due to the small food volume, large chamber volume and air exchange within the incubator. During the test, test chambers were capped with a foam plug. For the five generation test, the foam plug was replaced by

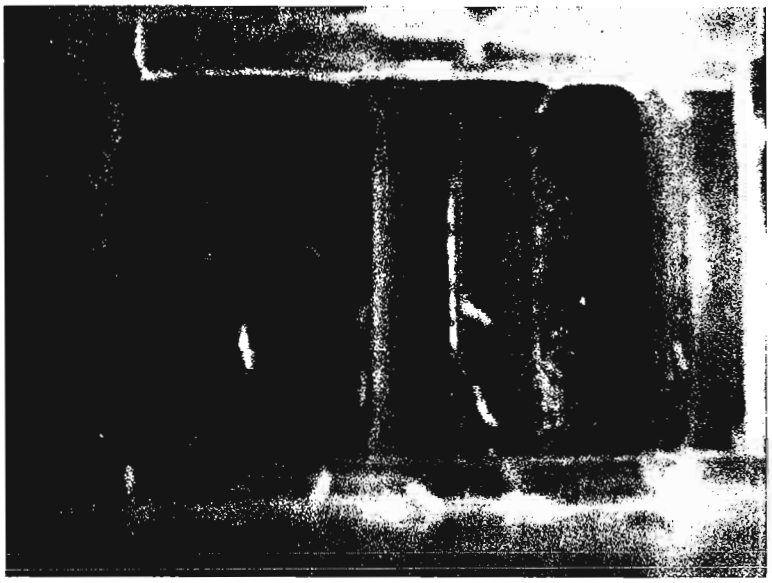

Figure 9. Overhead view of a food isolation cover slide within an MGSU chamber. 3rd instar larvae can be seen crawling beneath the slide on the left.

a clear acrylic sheet perforated with small foam-filled holes to maintain air exchange and to allow video imaging.

During the testing of the food isolation mechanism, problems persisted with bacterial growth on the fly food (Pence, 1996c). At this time a microbially sterile population protocol was developed based on a technique described by Michael Ashburner (Ashburner, 1989) and a Test/Operation Procedure was created (Pence and Hult, 1997) and used for the five generation integrated test.

A rectangular food container could provide sufficient food for a larger population whereas the cylinder does not provide an adequate quantity of food to sustain a large population of developing adults. Further engineering design and manufacture will be required to implement a larvae-proof seal.

Five Generation Integrated Test

Detailed generation to generation test results are described in Appendix III.

Assessment of Development: Population, Female Mass, and Development Time

Population

The population was adequately maintained in four of the six MGSUs during the test and it appears that the exposure time to the food (Figure 11) was adequate to help maintain the population. It is unclear if the decrease in exposure time at the F2/F3 transition made any difference to the next generation, since the vast majority of eggs were probably laid immediately after lights off. Flies were killed and their offspring not transferred to the next generation, due to entrapment in the food during anesthetization to transfer flies to new SB and VD containers, or when collecting the adults. A few flies were also killed in moving cylinders in the MGSU and several flies also became stuck in the MGSU food as well. In the F3 generation there was death of flies in the VD units due to the inadequate warming of food and the resultant accumulation of fluid in the bottom of the chambers. (Table 3). The most significant loss of flies occurred in the SB F2 and F4 and the VD F1, F2, and F3. In the SB F2, most flies were uneclosed pupae. The high number of uneclosed may be due to overcrowding, but the mass and population data do not support overcrowding (Figure 12). The only statistically significant difference between group populations occurred between the SB and VD groups ($P < 0.005$) in the F3 generation (Table 4) and the difference in mass of these groups was also statistically significant (Table 6). No conclusion can be drawn, however, since no other groups exhibited change in population with a correlation to a difference in mass. When comparing groups from generation to generation (Table 5), there was a significant increase in the SB from F1 to F2 ($P< 0.05$). The VD had a significant increase from F1 to F2 ($P < 0.0001$) a significant decrease from F3 to F4 ($P < 0.001$), and another significant increase from F4 to F5 ($P < 0.01$). The VD group population seemed to be the most unstable population, but

there were no significant differences in groups from generation to generation except in the F3 between the VD and SB. The MGSU appeared to be the most stable population from generation to generation, with no statistically significant differences. This may be due to the fact that the MGSUs were used to determine length of food exposure and timing of generation interval.

There was no particular evidence of stressed development, although there were uneclosed pupae in the F2 and F4 SB groups at the time of exposing the generations to new chambers.

Table 3. Uncollected flies % of total population. Dead and uneclosed.

Unit	F1	F2	F3	F4	F5
MG 1	0	2	0	0	0
MG 2	0	16/15u	0	0	0
MG 3	4	1/2u	0	0	0
MG 4	0	0	0	0	10u
MG 5	0	0	0	0	0
MG 6	0	0	-	-	-
avg. %	3%	8%	0%	0%	2%
SB 1	3	2/5u	0	21/11u	0
SB 2	1	3/14u	0	6/6u	0
SB 3	4	3/49u	0	1/5u	0
SB 4	4	1/38u	0	13/4u	0
SB 5	5	1/10u	0	4/7u	0
SB 6	1	1/7u	0	2/5u	0
avg. %	9%	20%	0%	22%	0%
VD1	2	1/13u	12	1	0
VD2	2	2/24 u	8	2/1u	0
VD3	0	4/16u	17	2u	0
VD4	0	18	3	18	0
VD5	0	3/18u	5	8/1u	0
VD6	1	41	0	2	0
avg. %	2%	21%	15%	13%	0%

u = uneclosed pupae

Table 4. Population unpaired student t test analysis between test groups (P values).

	F1	F2	F3	F4	F5
MG vs. VD	ns	ns	ns	ns	ns
MG vs. SB	ns	ns	ns	ns	ns
VD vs. SB	ns	ns	< .005	ns	ns

Table 5. Population unpaired student t test analysis within test groups (P values).

	F1 to F2	F2 to F3	F3 to F4	F4 to F5
MG	ns	ns	ns	ns
SB	< 0.05	ns	ns	ns
VD	< 0.0001	ns	< 0.001	< 0.01

Female Mass

It was hard to define the standard control mass in the test groups, although the SB group had consistently higher mean mass than the VD or MGSU groups in all of the generations, which could indicate that the SB group had the healthiest flies. The mean mass of the SB however varied from generation to generation, peaking at the F2 and decreased continually afterwards. In the F1, the MGSU and VD groups were both significantly less than the SB group ($P < 0.05$ and < 0.01, respectively), although the population (Figure 10) between the three groups was not different, which could indicate stressed development. In the F2 generation, the mass of the VD ($P < 0.0001$) was significantly less than the SB. The large standard error in the MGSU was due to weighing the two extremely small populations in the MGSUs, #3 and #6 which skewed the data. The removal of these two populations probably would have made the MGSU significantly less than the SB control. In the F3 generation, there was no significant difference between the MGSU and SB groups. There was a significant difference between the MGSU and VD ($P < 0.0001$) and the SB and VD ($P < 0.0001$). The VD group mean was at an all time population high with a mean of 120 adults, which could indicate stressed development with a decreased mean mass. The VD did display some delayed development, but not different than the MGSU group (Figure 13). In the F4 generation, there were no significant differences in female mass. This was also the generation when the mean population was at its lowest for the VD and MGSU groups, although, as stated before, the mean populations were not significantly different than the other generations (Figure 10). In the F5 generation, all three group's female masses were significantly different with the VD and SB groups being the most significantly different ($P < 0.0001$), followed by the VD and MG groups ($P < 0.002$) and MG and SB groups ($P < 0.01$).

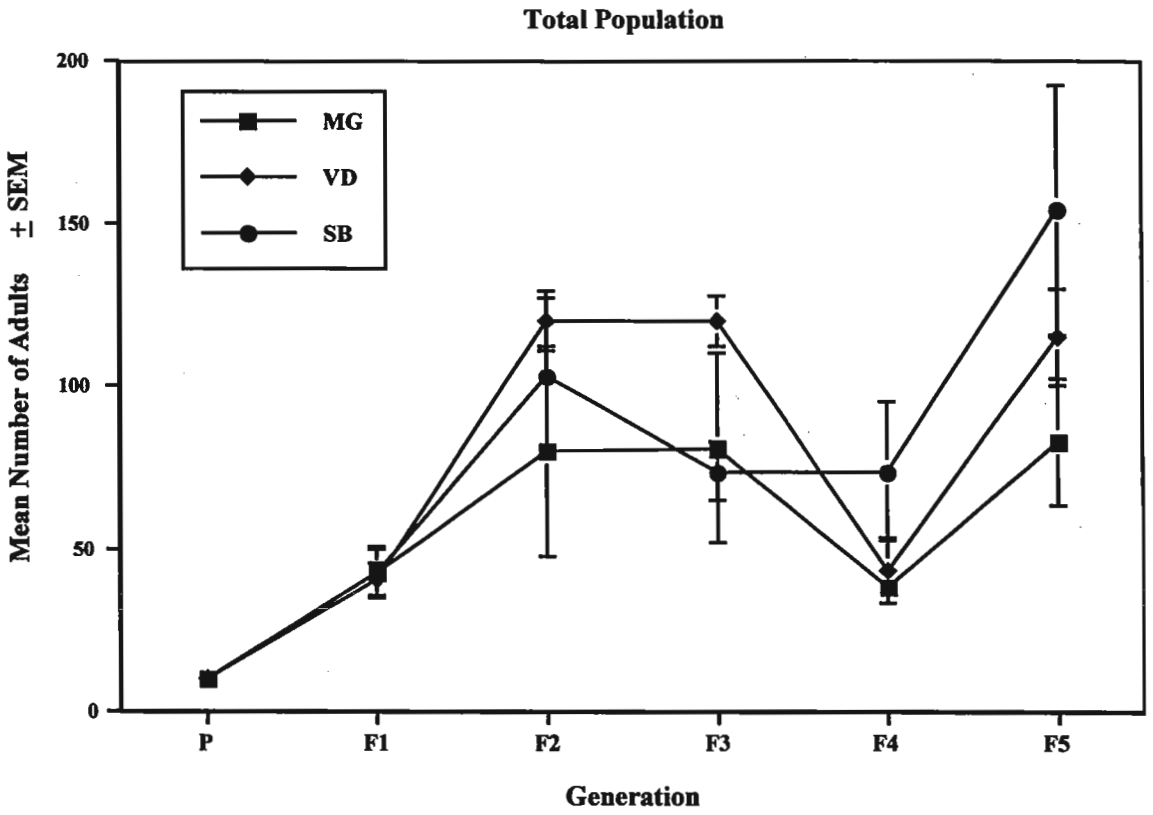

Figure 10. Total Population.

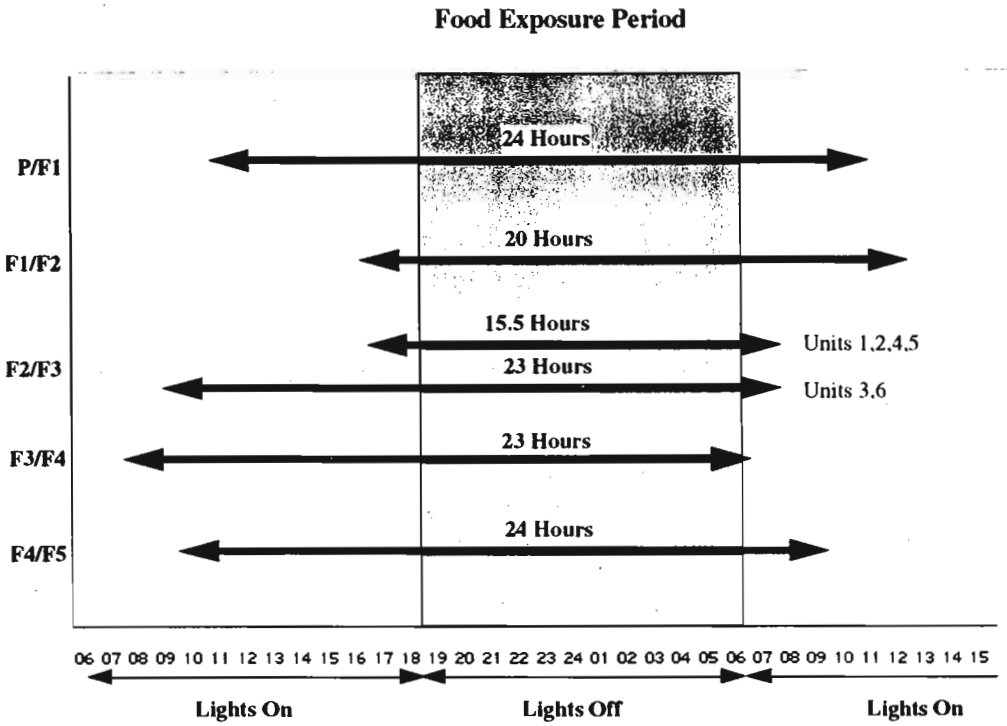

Figure 11. Food exposure periods.

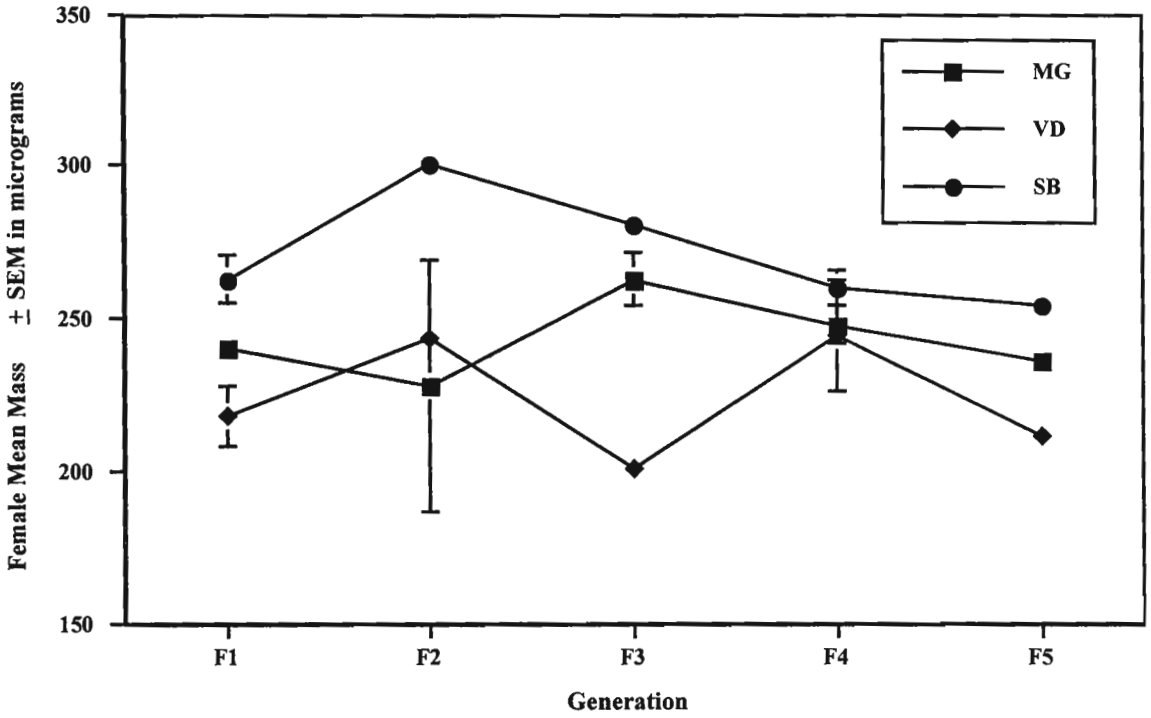

Figure 12. Female Mass.

Table 6. Female mass unpaired student t test analysis between test groups (P values).

	F1	F2	F3	F4	F5
MG vs. VD	ns	ns	< 0.0001	ns	< 0.002
MG vs. SB	< 0.05	ns	ns	ns	< 0.01
VD vs. SB	< 0.01	< 0.0001	< 0.0001	ns	< 0.0001

Development Time

In the F1 generation, it appeared that the majority of the SB and VD controls were 12-18 hours in advance of the MGSUs at the time of adult emergence. In the F2 generation, there appeared to be a 12-24 hour delay in development of the MGSUs as compared to both control groups, although some uneclosed pupae were still present in the SB controls when adults were collected. In the F3 generation, it appeared that, for the first time in the start of the experiment, all of the test groups were developing at approximately the same rate. In the F4 generation, the SB controls were slower in development, but it did not appear to be caused by an exceptionally large population. In the F5 generation, the VD group appeared to develop at the fastest rate.

There was a definite observed difference in development timing between groups, which was somewhat unexpected, unless there was profound delayed development due to overcrowding, but this does not seem to be the case. The VD and MGSUs were expected to develop at the same rate due to the same food volume and depth dimensions. It is also interesting how the fastest developing group varied from generation to generation. Overall, the F2 and F3 groups developed slower than the other three generations and the adults were collected one day later in the F2 and F3 generations. There is no apparent reason why these generations were slower or faster, and the decision to collect adults was based upon overall readiness of the generations.

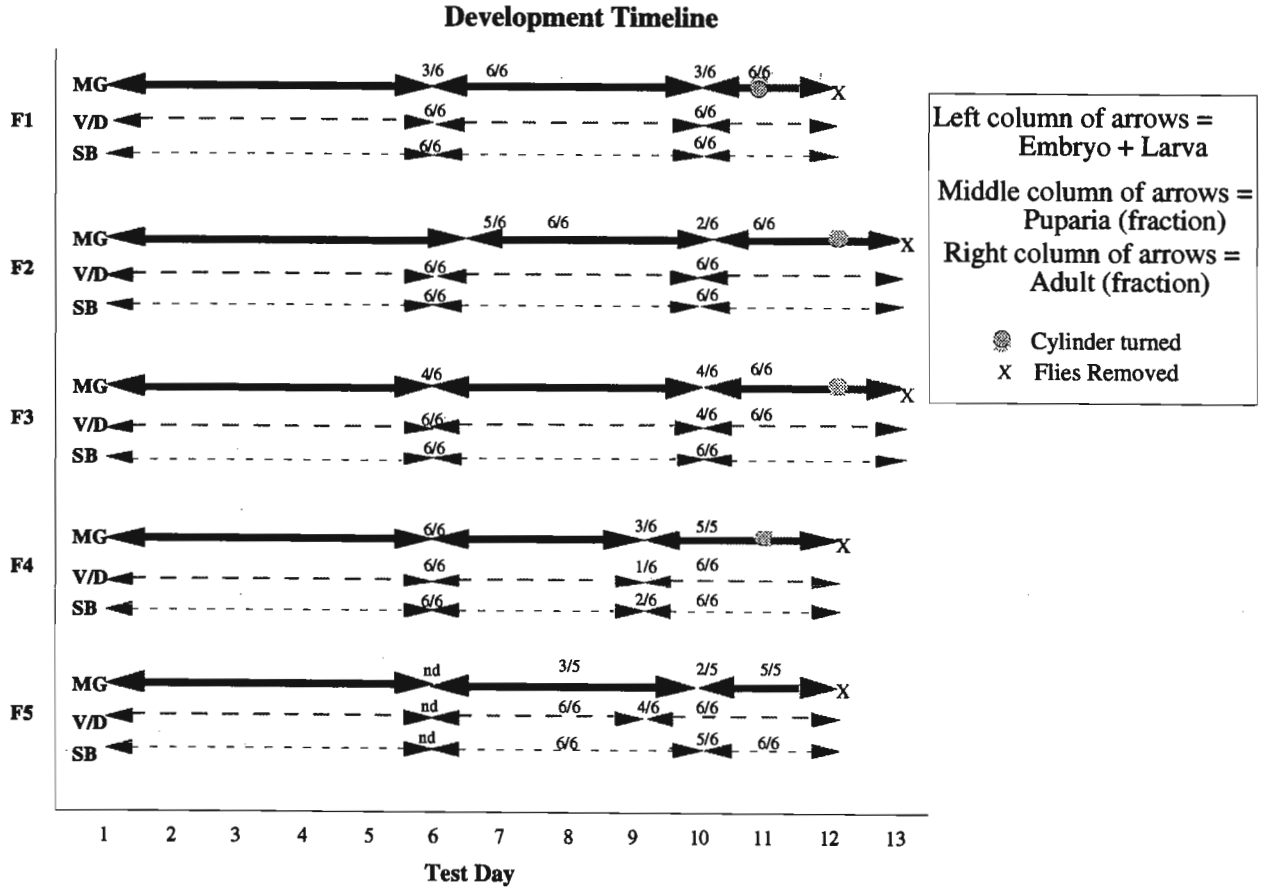

Figure 13. Development Timeline.

Crew Operations

Figure 14 shows the total time it took to perform the operations necessary to collect the flies from all test chambers for each generation. MGSU flies were collected with the FCU prototype and the operations also included the time necessary to move new food cylinders to the next chamber. The operation time for the MGSUs in the F1-F3 took significantly longer, due to the problems in moving the cylinders in two of the units, and due to the FCU motor overheating. Time to perform the operations significantly decreased when one malfunctioning unit was replaced and the other was discontinued (Figure 14). At the worst case, collection from six MGSUs, took approximately 1.5 hours to complete. The total operation time may have decreased due to familiarity of performing the operations after multiple collections. This can be seen in the VD and SB groups over the five generations of collecting the flies with the fly collection tools. Figure 15 shows the amount of time it took to perform each task of the operation. The extra operations of moving cylinders, replacing cylinders, removing flies, and sterilizing covers, took longer than control groups due to the vacuum unit problems, and replacing collection vials took longer due to reattachment of the aspirator to the vacuum.

A significant amount of time was necessary during operations in sterilization of equipment and facilities for all groups. On average, it appears that it took twice as long to perform operations with the MGSUs than with the two other groups.

It may be difficult to perform multiple generation experiments with replicate groups, but options exist to automate some parts of the operations or to collect only the last generation of adults. Video observation was essential in monitoring developmental timing in order to perform generation separation operations. Flexible

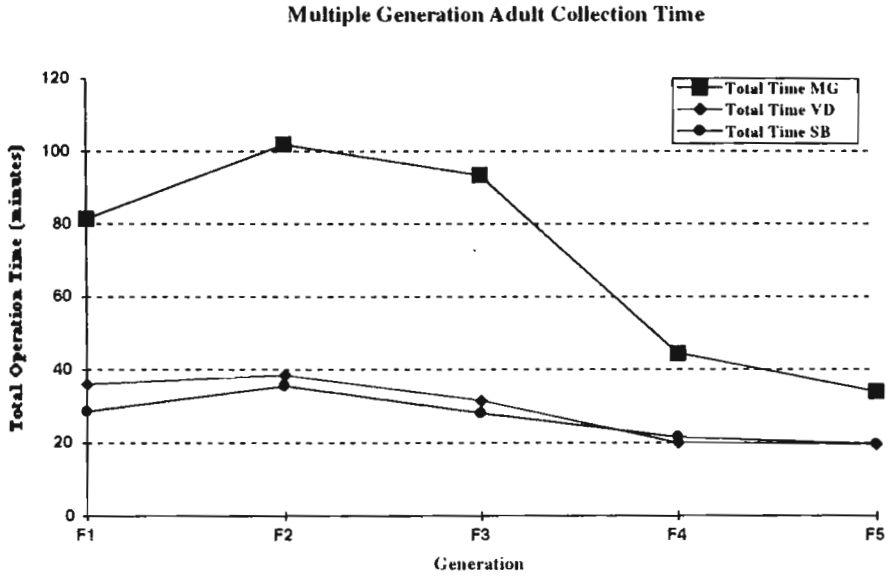

Figure 14. Operation Duration. Notice that the MGSU generations F4 and F5 are reduced. This is due to removal and or replacement of malfunctioning units.

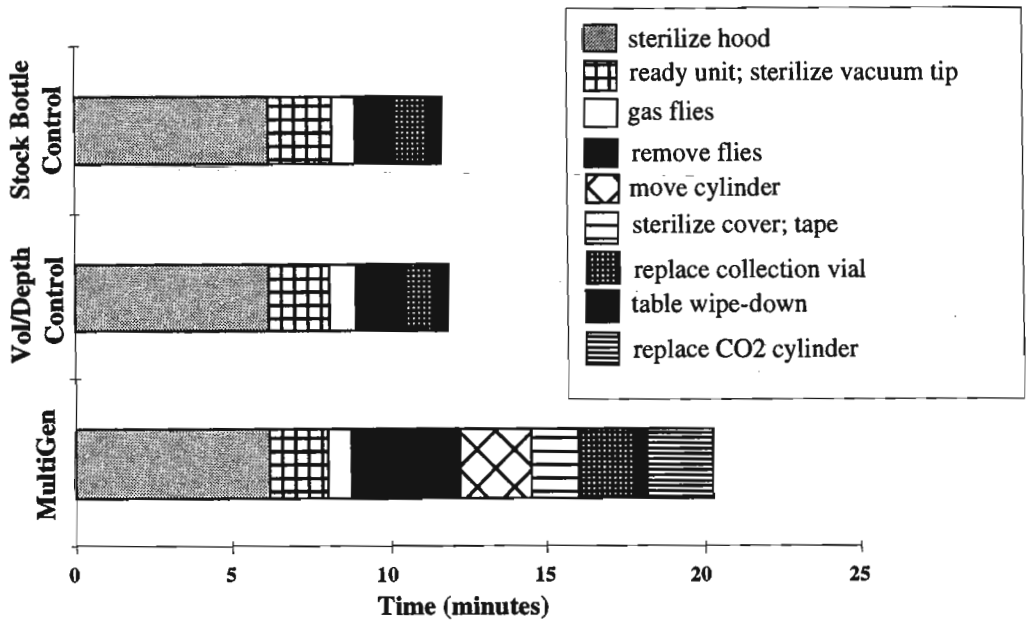

Figure 15. Mean adult removal procedure times.

scheduling with the crew may be difficult and moderately intensive training will be necessary to perform the operations.

Flexibility in crew scheduling will be necessary, but operations can assuredly be performed during a normal crew shift. It is recommended that two crew members will be necessary to perform the tasks. Crew member 1 performs the activities of collecting flies and crew member 2 monitors procedures, operates the

collection vacuum, and tracks collected specimens. Since the adult collections are a fairly infrequent event, approximately every 2 weeks, it is feasible for the crew to perform the operations, although significant training would be necessary, as well as continued refresher training while on orbit.

One of the most critical aspects of conducting multiple generation experiments is maintaining a stable population. To control the population the containers need continual monitoring. The key parameters to monitor were early development duration, eclosion timing of adults, adequate mating duration, adequate egg laying duration, and total population from generation to generation. The health of the population was the key issue in maintaining the generations. Overall during the five generation integrated test, all six units were monitored and alterations were made in the planned schedule to maintain a steady population in all six units. Populations varied between the units, and in order to maintain a steady population, operations in some units had to be altered on a unit by unit basis. For instance, due to mechanical difficulties in two of the units, the population decreased in these two units and the exposure time of females to the food for egg laying had to be extended. Equipment must also be mechanically sound to prevent failure. Delays in development resulted in the necessity of delaying cylinder advance until all units were ready to advance. All groups were held in the old containers until most adults were mature before the adults were exposed to the new food for each generation. This was done so the newly laid eggs were of synchronized age. It will be a challenge to maintain a steady population over multiple generations and to maintain multiple populations.

Table 7. CO_2 cylinder used.

Generation	cylinders used
1	2
2	2
3	3
4	2
5	2

FCU Prototype

Overall the prototype developed for the test worked extremely well, and has good potential for future applications, when development of an adult collection device is necessary on orbit. One of the problems that persisted in the MGSU test was vacuum motor shutoff. More work will have to be done to correct this problem, as engine shutoff persisted even with removal of the circuit breaker during the multiple generation test. This problem can easily be fixed with testing of other commercial motors that are not part of a vacuum designed to clean computer keyboards.

Some other additions that may be beneficial to a FCU device and crew operation would be to supply pre-sterilized vacuum tips that can be changed after each use, if sterile operations are required. A transparent vacuum hose would be a requirement to be able to view flies in the tube to make sure they are all collected. Another possible change to the current prototype design would be to use the vacuum tube as the collection tube instead of using a microcentrifuge tube. The tubing would have to be changed after each use, but it may make design of the FCU easier and may give other options in preserving the specimens for return to Earth. A flexible tube may give more options than a flip-cap microcentrifuge tube. Something that may be required to make collecting flies easier is to add a brush to the end of the vacuum tip instead of a separate brush that has to be utilized by the crew.

The bigger challenge is to make a crew and fruit fly "friendly" device that is compatible with chamber design. Another challenge is to make the system operational in space. It is unknown how fly behavior and response to the anesthetic in space will impact the ability to collect specimens.

When collecting flies it is still unknown how the fly behavior in space and reaction to the anesthetic will be altered as compared to the ground. For example, when collecting flies with the FCU, it was very easy to use the CO_2 gas flow to push the anesthetized flies into a corner and then to quickly vacuum them into a vial, but in reduced gravity, it is unknown if the flies will adhere to the walls or float within the chamber. Other designs may need to be considered to accommodate this issue. Air flow through the chamber may be a way to gather flies for collection.

Stowage of supplies will also be a consideration when performing multiple generation experiments. It is recommended that "kits" will have to be made to store the necessary supplies for collecting adult fruit flies and for processing of tissues as needed. Kits that may be necessary include: a FCU box, a gas cylinder kit and

vacuum tubing kit, a vial/specimen storage kit for each generation, a facilities and equipment sterilization kit with sterile towels and sterile gloves, and possibly a tool kit for special tools needed to collect flies, operate the FCU, or to gain access to specimen chambers. For instance, in collecting flies during the five generations, a total of 11 CO_2 cylinders were used (Table 7), which would require stowage space.

SUMMARY/CONCLUSION

- A World Wide Web site was created in conjunction with the SSBRP Communications and Data System group for the purpose of simulating on-orbit observation of fruit fly multiple generation experiments. The system was designed to collect and archive video images and environmental data and was used to monitor development in the five generation experiment to aid in decision making for performing experimental operations. During the five generation test, the system worked very well to provide simulated remote observation capability of the experiment. There were a few reliability problems during the test with data storage, but overall the system exceeded the original expectations. More user friendly options for retrieval of data and the need for camera coverage of more test units is necessary. The 10× magnified video system attached to the web site also performed well. At the higher magnification, a World Wide Web capable system should be developed for cameras to track and focus on individual specimens for successful remote observations.

  After the development of the site for the five generation test, the function of the site expanded to provide information about the SSBRP insect laboratory, the studies conducted in the lab, and as a database for past insect space research information. The site was also expanded to provide information about SHARP and STELLAR interns. The Summer '96 STELLAR teacher used the site to educate her grade school students about the work she performed at NASA Ames Research Center. The site was also recently enhanced to provide server access to Canadian Space Agency personnel for document review and distribution.

- A prototype adult fruit fly collection unit (FCU) was developed with modified commercial off-the-shelf products to collect flies during the five generation test. The prototype was designed to anesthetize fruit flies with gas from a portable CO_2 cylinder regulated by a small flow valve, then to collect anesthetized flies into 1.5 ml tubes using a small vacuum driven aspirator. The prototype system performed extremely well and "crew" operation times were collected during the five generation test. There were a few problems with the commercial vacuum motor performance, although the system was functional during testing. The concept is feasible for specimen collection on orbit by crew members, although the weightless environment may have an impact on fly behavior to anesthetic and crew operation techniques will have to be refined. Design of specimen chambers and interfaces with a FCU will also need to be considered. More design work and testing will be necessary to create a unit for flight.

- The food cover mechanism added to a Multiple Generation Separation Unit (MGSU) prototype that was designed to eliminate developing larvae from gaining access to food failed to work, but the backup method worked very well. Previous testing had shown that a mechanism was needed to keep larvae from accessing food designated for a future generation of fly eggs in a specimen chamber with two food cylinders. Due to their small size, fruit fly larvae were capable of squeezing through a very small gap between the cover and the unit, to gain access to the food. The food isolation test was conducted to verify a possible method of limiting access of the developing larvae and adults to new food on the previously developed MGSUs. During the five generation test, a backup method of rotating food cylinders was used to eliminate larval access to the food with complete success. If a food presentation design solution other than a cylinder is used, the seal/slide system will have to be created.

- A five generation test utilizing the MGSU and two control groups was successfully completed. The five generation units worked very well to separate generations and to maintain populations. There were some mechanical difficulties with two of the units due to sterilization of the units. This resulted in the loss of one of the six populations of fruit flies by the 3rd generation.

Fly populations were adequately maintained in the MGSUs with no clear evidence of delayed development due to overcrowding. Development in the MGSUs was 12-24 hours slower than controls in the F1 and F2 generations, but this was not due to a large population. It appears that limiting access to food appeared to be successful in limiting the population growth. There were no significant differences between populations of the MGSU or controls, from generation to generation, except between the volume/depth control and stock bottle control in the F3 generation. The MGSUs had the most stable mean population from generation to generation, while the VD control appeared to have the most unstable population. Flies were killed during the experiment when they became stuck in the food during anesthetization for transfer or collection, or during movement of food cylinders in the MGSUs, which could have had an impact on future generations. The fly mass data showed that the SB controls consistently maintained a higher mean female mass from generation to generation which may indicate a healthier population when compared to the other two groups.

The fly collection unit worked very well in the five generation test and crew operations were performed with two "crew members." One operator handled the FCU and MGSUs and the second operator helped to operate the vacuum, load collection vials, and unload and label the collected flies. The maximum amount time to perform adult collections and move cylinders in six MGSU on Earth was approximately 1.5 hours in an 11-12 day period, which also includes the time it took to move cylinders in mechanically defective units. Collection of adult flies is probably possible on orbit, but further development of the unit and supporting supplies would be necessary.

The World Wide Web system worked well to aid in decision making regarding the development of flies in the MGSUs and direct visual observation helped to corroborate the web images. Images of all of the test group's development times would have significantly aided the decision to expose food, move cylinders and collect flies. In a space-based multiple generation system, video data will be indispensable in monitoring the development of individual test chambers to assess the need to perform experiment operations. Time critical operations will need to be quickly viewed and assessed by the PI to plan crew or automated system operations and video will probably be the most important data to collect.

During the five generation test, making decisions to maintain the six populations of flies was a somewhat complex task to perform due to the variation between populations and differences in development between the test groups. Similar problems may be encountered on orbit and when maintaining control populations on the ground.

Acknowledgments: I would like to thank Charles Sun, May Windrem, and Lou Picinich for design and development of the World Wide Web site data collection system. I would also like to thank Kjell Hult for significant development of the web page, web video script development, and for help in development of the multiple generation test operations. I would also like to thank Mayur Trivedi and Sam Black for assisting in the muliple generation test. I would also like to thank Mayur Trivedi, Gabrielle Meeker and Melissa Kirven-Brooks for review of the final report.

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and L. Picinich 1996, Application of World Wide Web (W3) Technologies in Payload Operations. Proceedings of the Fourth International Symposium on Space Mission Operations and Ground Data Systems (SpaceOps 96). Article # SO96.2.033. Munich, Germany, http://www.esoc.esa.de/external/mso/SpaceOps/2_33/2_33.htm.

APPENDIX I

Script to collect timed video data from the micro video system and download to the SGI Indy workstation.

```
#!/bin/sh
# A simple shell script to take video snapshot from SGI Indy video system.
# Uses input from S-VHS CCD camera
# Reduces image size 50% and saves as .rgb for movie compilation
# original source: unknown
# modified by Charles Sun 4/22/96
# modified by Kjell Hult 12/20/96
# set TIME as variable to alphabetize pictures in order taken.
TIME=`date '+%Y%m%d%H%M'`
cd /usr/people/guest/public_html/pupdev
# Save .rgb image to pupdev/ directory and use TIME variable as name
# Redirect vidtomem output to /dev/null/usr/sbin/vidtomem -v 1 -f $TIME 2>/dev/null
# Scale new image file down 50% and remove -00000 suffix
/usr/local/bin/convert -geometry 50%x50% $TIME*.rgb $TIME.rgb
# Copy large image to other directory for web access and convert to .jpg
cp /usr/people/guest/public_html/pupdev/$TIME-00000.rgb \
/usr/people/guest/public_html/pupdev/current/current.rgb
/usr/sbin/imgcopy
/usr/people/guest/public_html/pupdev/current/current.rgb \
/usr/people/guest/public_html/pupdev/current/current.jpg 2>/dev/null
# cat "current.jpg"
# Delete old (large) image files
rm -f $TIME-0*rm -f /usr/people/guest/public_html/pupdev/current/current.rgb
```

APPENDIX II

Script to collect temperature and humidity data from the Fluke Data Bucket and download it to an SGI workstation.

```
#!/bin/sh
# A simple shell script to replace fly-a1.html for easier access to
# other executables via shell
# Charles Sun 4/22/96
# HTTP handshake w/ client
echo Content-type: text/html
echo
# define vars
name=$REMOTE_HOST
date=`date`
# read temp and humidity sensors from ch5 & ch3
temphigh="29"
templow="15"
```

```
#temp="25.05"
temp=`/usr/people/csun/fly/flydaq 5 `
#hum="55.02"
hum=`/usr/people/csun/fly/flydaq 3 `
echo "<HTML><META HTTP-EQUIV=\"Refresh\" CONTENT=\""
echo $QUERY_STRING
echo "\">"
echo "<HEAD><TITLE>Habitat Snapshot</TITLE></HEAD>"
if test $temp -gt $temphigh
then echo "<BODY BGCOLOR=\"red\">"fi;
if test $temp -lt $templow
then  echo "<BODY BGCOLOR=\"red\">"fi;
echo "<CENTER>"
echo "<H2>Habitat Monitor</H2><HR>"
echo "<IMG SRC=\"http://taipei.arc.nasa.gov/cgi-bin/capture.cgi\">"
echo "<TABLE><TR><TD>Data Sampled Time: <TD><STRONG>"
echo $date
echo "</STRONG><TR><TD>Temperature: <TD><STRONG>"
echo $temp
echo " C</STRONG><TR><TD>Humidity: <TD><STRONG>"
echo $hum
echo " %</STRONG></TABLE>"
echo "</CENTER></BODY></HTML>"
echo
```

APPENDIX III

Five Generation Integrated Test Notes

The following sections address the performance of each of the 5 generations and the parental generation in the MGSU and controls. Performance is divided into the four categories of Biology, MGSU prototypes, FCU Prototypes and Operations, and the web system.

Parent (P) Mating and Egg Deposition

Biology- Flies were added to the multiple generation separation unit, SB and VD controls, and exposed to the new food on the morning of Day 0. The flies were exposed to the new food for 24 hours. After removal of the flies on the morning of Day 1, an adequate number of eggs (20-50) were seen in all 6 units as well as the controls.

MGSU Prototypes- Most MGSUs functioned well, although there was some trouble in loading the starting food cylinders into two of multiple generation units and a small hammer had to be used to load the cylinders. It appears that the cylinder ports were slightly distorted when the bottom portion of the units were autoclaved.

FCU Prototypes and Operations- Adult flies were added on test day 0 and removed on test day 1 with the normal fly collection tools and CO_2 system. There were no anomalies in the test start operations, other than cylinder loading described in the prototype section.

WWW- The system worked well and baseline images of the units and controls were taken during the beginning of the experiment. The Indycam camera was set to collect images at 20 minute intervals on MGSU #5. There was a problem with the image date stamp command on day 0 that was fixed by CDS personnel.

First Generation (F1) Development

Biology- On the morning of day 6, pupariation had occurred in 50% of the MGSUs, and 100% of the SB controls. Pupariation had occurred in 100% of the VD controls, but all of the pupae were seen in the food. In all groups, most pupae formed close to the food and at most, crawled to only the bottom 3rd of the container walls to pupate. Some of the SB controls also had pupae in the food. On the morning of day 7, most larvae had pupated in MGSUs, although some 3rd instar larvae were still seen in the MGSUs and the SB controls. On the afternoon of test day 9, wing pads and eyes were clearly present in all of the SB controls and most of the VD controls. Wing pads were visible in most of the MGSU pupae. On the morning of day 10, the MGSUs had no adults. All the VD and SB controls had adults, with each still having some pupae uneclosed. Late on day 10, MGSUs only had adults in 50% of the containers. VD and SB controls all had adults with some pupae still uneclosed. On the morning of day 11, adults had appeared in all the MGSUs, although there were still some uneclosed pupae. The VD and SB controls had all adults eclosed, although one container of the SB controls had 2 uneclosed pupae. Overall it appeared that the majority of the SB and VD controls were 12-18 hours in developmental advance of the MGSUs.
MGSU Prototypes- By the completion of the generation, there was moderate drying of the food in the units and controls due to low RH in the incubator.
FCU Prototypes and Operations- Nothing to report.
WWW- Images were collected of all MGSUs and control groups during the F1, in addition to the continual 20 minute interval image collection of MGSU # 5.

F1 Finish and F2 Start

Biology- Adults were exposed to the new food on the afternoon of day 11 and the egg-laden cylinders were moved to the F2 chambers on the afternoon of day 12. Adults from all groups were only exposed to the food after the slowest groups, which were in the MGSUs, had time to reach maturity. More than 100 eggs were visible in each test unit and controls. During the transfer process, some flies became stuck in the food and were not transferred (Table 3). Some eggs were also lost when moving the cylinder.
MGSU Prototypes- Most units operated well when new cylinders were inserted at day nine, exposure of new food and closing of old food on day 11, and transfer of food to the F2 chambers on day 12. It was still very difficult to move the cylinders without the aid of a small hammer and vise. Four adults were mashed in the process of turning the cylinder, and some of the egg-laden food was splattered onto the wall of the F1 chamber when the cylinder was moved on day 12. Some flies were also stuck in the food of the control groups and not moved to the next generation containers (Table 3).
FCU Prototype and Operations- The timing of operations was dependent upon the development time of the adults in the MGSUs and the control groups. The needed information was gathered by video observation and by direct visual observation of the groups. These scheduling issues persisted as the experiment progressed and the fly population fluctuated from generation to generation. The sequence of decisions that required continual monitoring of development to determine when to perform the operations were:

1. When to add new food cylinders: The cylinders were not added until after the entire population of 3rd instar larvae had pupated (if possible). For the F1 generation, new food cylinders were added on day 9.

2. When the cylinders are rotated and for how long: The best time to rotate cylinders (expose new food and close old food) is after all of the adults are eclosed and a reasonable amount of time has passed for the adults to sexually mature. Cylinders were rotated on day 11 in the F1 generation. The number of adults that emerge, and their ability to lay eggs, are the most important factors in deciding how long to expose the new food. Another factor that was not considered was that it will probably not be feasible to expose or move food in the dark cycle of the photoperiod, unless the system is automated. The females lay the most eggs immediately after onset of the dark cycle, which makes it difficult to monitor the exposure period.

3. When to vacuum flies and move food cylinders to the next chamber: For this experiment , the decision was made to remove the adults prior to moving the cylinders to eliminate the risk of adults moving into the next generation chamber and mating with their offspring. Turning of the cylinder prior to vacuuming also made collection easier. Until an effective method of preventing adults from moving to the next chamber is

devised, this was the safest method. Adult collection with the gas and vacuum units and cylinder movement was generally successful. Flies became stuck several times when the vacuum tip was dipped in ethanol and not given adequate time to evaporate.
WWW- Ideally, remote observation of all test units and controls would be helpful to gauge development rate in all groups. By using the images collected during the F1, it was possible to make reasonable estimates of when to perform the specific operations.

Second Generation (F2) Development

Biology- On the morning of F2 day 1 (test day 12) eggs were visible in all groups. It was difficult to see any eggs in one of the MGSU because of the disturbed medium. On the afternoon of test day 17 (F2 day 6), pupae began to appear in the VD and SB controls. Pupae were not yet visible in the MGSUs. On the afternoon of day 7, pupae were present in most of the MGSUs. On day 9, wing pads were present in the VD and SB controls. Wing pads were not present in the MGSUs until the afternoon. Late on day 10, adults were present in both the VD and SB controls. Adults were present in only 2 of the 6 MGSUs. On the morning of day 11, flies were present in all units, except one MGSU, although it had a large number of pupae ready to eclose (215 total final adults), which may have caused a delay in development. Overall, there appeared to be a 12-24 hour delay in development of the MGSUs as compared to both control groups, although some uneclosed pupae were present in the SB controls.
WWW- The system continued to work well, however 20 minute interval collected images were lost on January 1st (test day 21, F2 day 10) due to the lack of a directory to accept images with a 1997 date. Images could still be accessed from the web page to see fly activity, but they could not be stored. The error was not corrected until January 2nd.

F2 Finish and F3 Start

Biology- Adults were exposed to the new food on the afternoon of day 22, F2 day 11 and on the morning of day 12. Flies were collected and the egg-laden food cylinders were moved to the F3 chambers on test day 24/F2 day 13. There were some differences noticed in the population sizes of two of the six test units. Food exposure times were split between the two low populated units to allow the other four units with smaller populations to have more time to lay eggs. The well developed units were exposed for approximately 14 hours to limit any overpopulation, while the two lower population units were exposed for approximately 23 hours. There were still only five adults visible in one unit at the time of egg laying. On day 13 at the time of cylinder rotation there were still some uneclosed pupae seen in all the units and some flies became stuck in the food during collection (Table 3). On the morning of day 13, there appeared to be plenty of eggs in most of the units except.
MGSU Prototypes- There were major difficulties in loading food cylinders and rotating food cylinders in two MGSUs. At the start of the F3 generation, the decision was made to replace one MGSU with the backup unit. There were no problems with the backup unit when the egg laden food cylinder was transferred to it.
FCU and Operations- New food cylinders were inserted on the morning of F2 test day 11. During adult collection with the FCU, there were some difficulties with the vacuum motor due to overheating as described previously. This delayed the collection times.
WWW- The remote video collection system continued to provide the needed information on the developing flies.

Third Generation (F3) Development

Biology- On the morning of F3 day 1, test day 24, there appeared to be plenty of eggs in four of the six units. On the afternoon of test day 4, one unit still had no signs of larval development, and the food was still unworked. On the morning of day 6, pupae were present in four of the six MGSUs and controls. It is believed that the mechanical striking of the cylinders when moving them to the F3 chambers, severely affected the developing eggs and caused extremely low or non-existent populations. On the morning of day 7, there were

some pupae in MGSU #3. On the late afternoon of day 9, wing pads and eyes were visible on MG units and controls, although no adults had appeared. There were still very active 3rd instar larvae in MGSU #2. Adults began to appear on the morning of day 10. Adults were present in all SB controls, 66% of the VD controls and 66% of the MGSUs. On the morning of day 11, almost all adults had eclosed in all groups, although many of the adults looked very recently eclosed. It appeared that for the first time since the start of the experiment, that all of the test groups were developing at the same rate.
MGSU Prototypes- Nothing to report.
FCU and Operations- Nothing to report.
WWW- The remote video collection system continued to provide the needed information on the developing flies.

F3 Finish and F4 Start

Biology- The flies in one MGSU appeared to have made a good comeback after a low population in the F2 generation. One unit had no flies. The populations in the four other units were variable. During presentation of the new food to the VD controls, some of the adults became stuck in condensed food fluids that were created during warming (Table 3). The reduced exposure time may have helped lower the SB population.
MGSU Prototypes- There were no hardware problems with the prototypes at this time.
FCU and Operations- Due to the large number of flies in most test and control containers, it was planned to expose the flies to the food only during the light cycle and collect flies late in the day. The flies however, laid very few eggs during the day and adult collection was delayed until the next morning. Therefore, the flies were exposed to the new food for 23 hours. It was not planned to conduct fly collection during lights out, due to the difficulty in performing operations under red filtered light. It is also unlikely that crew members could perform these operations. Adults were collected on the morning of day 13 with no new anomalies. As with the last collection, the motor shut off, after intermittently overheating.
WWW- The remote video collection system continued to perform well.

Fourth Generation (F4) Development

Biology- Eggs were visible on the food surfaces of the MGSUs and controls. On the evening of day 5, no pupae were present in any of the groups. On the morning of day 6, pupae were present in all of the containers of all of the groups, except one MGSU. One container of the VD control had all of the pupae in the food. On the morning of day 8, wing pads and eyes were visible in some of the containers of all of the groups.

By the afternoon of day 9, adults were visible in 50% of the MGSU containers, 17% of the VD containers, and 33% of the SB containers. On the morning of day 10, adults were present in all containers, although a lot of the adults in the containers appear to be recently eclosed and callow. The SB control group still had a large number of unclosed pupae and some 3rd instar larvae were still visible. In the F4 generation, the MGSUs and VDs appeared to be developing a slightly faster than the SB controls. The SB controls were slower in development, but it did not appear to be caused by an exceptionally large population.
MGSU Prototypes- Nothing to report.
FCU and Operations- Nothing to report.
WWW- The remote video collection system continued to provide the needed information on the developing flies to determine how the flies were developing in the MGSUs.

F4 Finish and F5 Start

Biology- Nothing to report.
MGSU Prototypes- One MGSU was discontinued from testing due to the absence of any new flies in the unit. All other units performed well.
FCU and Operations- There were no new FCU problems.
WWW- Nothing to report.

Fifth Generation (F5) Development and F5 Collection

Biology- On the morning of F5 day 1, test day 47, eggs were visible in all the containers of all groups. On the morning of day 5, 3rd instar larvae in the VD control were crawling on the walls and preparing to start pupariation, although no pupae were seen yet. Other groups only had larvae crawling in the food. No information was collected on days 6 or 7. On the morning of day 8, pupae were present in 60% of the MGSUs, and 100% of the VD and SB groups. 3rd instar larvae were still present in the MGSU and the SB groups. On the afternoon of day 9, 67% of the VD group had adults, while no adults were present in either the MGSU or SB groups, although wing pads and eyes were visible in the pupae. On the morning of day 10, adults were present in 87% of the SB group and 40% of the MGSU group. On the morning of day 11, adults were in 100% of the SB and MGSU groups, although uneclosed pupae were still present in some of the individual containers. On adult collection at day 12, there were still 10 uneclosed pupae in one MGSU.

MGSU Prototypes- Nothing to report.

FCU and Operations- Adult flies were collected on the morning of day 12 with no problems. The FCU worked flawlessly at this time.

WWW- Images were not stored for the entire day of test day 53, for an unknown reason.

Conference Programs

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

40th Annual *Drosophila* Research Conference
24 to 28 March 1999, Bellevue, Washington, U.S.A.

The 40th Annual *Drosophila* Research Conference was held at the Double Tree Hotel, Bellevue, Washington, and the 1999 Program Chairs were Barbara Wakimoto (University of Washington) and Susan Parkhurst (Fred Hutchinson Cancer Research Center). The conference was sponsored by the Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814-3998. Large numbers of platform talks and posters were presented and summarized in a 384 page Program and Abstracts Volume.

Plenary Session Lectures:

Anderson, Kathryn (Department of Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, NY). Rel signaling pathways in development and immunity.

Botas, Juan (Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX). LIM genes in limb development.

Carlson, John (Department of Biology, Yale University, New Haven, CT). A large family of candidate odor receptors and a gene that regulates them.

Denell, Rob (Division of Biology, Kansas State University, Manhattan, KS). Beetle developmental genetics: it takes more than one insect to study evolution.

Heberlein, Ulrike (Department of Anatomy, UCSF, San Francisco, CA). Flies on drugs: genetic approaches to acute and chronic responses to alcohol and psychostimulants.

Hogness, David (Department of Biochemistry, Stanford, CA). From colony hybridization to DNA microarrays.

Kellum, Rebecca (Department of Biology, University of Kentucky, Lexington, KY). Coupling transcription to replication: new insight into an old question.

Lasko, Paul (Department of Biology, McGill University, Montreal, Canada). Translational control of gene expression in the germ line: who needs transcription factors?

Minden, Jon (Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA). Life, death, and pattern repair in the embryo.

Perrimon, Norbert (Genetics Department, Harvard Medical School, Boston, MA). Heparan sulfate proeoglycans: what they are and what they do?

Ruohola-Baker, Hannele (Department of Biochemistry, University of Washington, Seattle, WA). Axis formation: networking in the eggchamber.

Sandler, Iris. Mendel's forgotten law: the neglect continues!

Workshops:

Behavior.
McNabb, Susan (Department of Zoology, University of Washington), and Kim Finley (Department of Molecular Biology and Virology, Salk Institute, La Jolla, CA)

Cytoskeletal Filament Interactions.
Foe, Victoria, and Garrett Odell (Department of Zoology, University of Washington)

Ecdysone Workshop.
Riddiford, Lynn, and James Truman (Department of Zoology, University of Washington)

Evolution of Insect Development.
Brown, Susan J. (Division of Biology, Kansas State University), Mary Anne Pultz (Department of Biology, Western Washington University), and Reinhard Schröder (Institut für Biologie, Abt. Genetik der Tiere, Tübingen, Germany)

Genetic Responses to Environmental Stimuli.
Johnson, Eric (Department of Biochemistry, Stanford University)

Growth Control.
Gallant, Peter, and Laura Johnston (Fred Hutchinson Cancer Research Center, Seattle)

RNA.
Mount, Stephen M. (Cell Biology and Molecular Genetics, University of Maryland)

Technical Advances.
Ish-Horowicz, David (Developmental Genetics Laboratory, Imperial Cancer Research Fund, London)

Fourth International Conference on *Drosophila* Heterochromatin

Arthur J. Hilliker (University of Guelph, Ontario, Canada) reports that The Fourth International Conference on *Drosophila* Heterochromatin was held June 20-24, 1999 at Alfred State College in Alfred, New York The speakers and their titles are provided below. The next conference will be held in the spring/summer of 2001 in Cortona, Italy, and the organizers are Maurizio Gatti and Sergio Pimpinelli.

Gunter Reuter (Martin Luther University, Germany). "The control of heterochromatin-dependent gene silencing by Su(var)3-9 and its interacting proteins."

Joel Eissenberg (Saint Louis University Medical School). "Versatility of conviction: HP1 is both a silencer and activator of transcription."

Sergio Pimpinelli (Universita di Roma, Italy). "The involvement of Pc-G and trx-G proteins in heterochromatin organisation."

Vladimir Gvozdev (Russian Academy of Sciences). "Evolution of paralogous stellate and Su(Ste) repeats and their ability to silence a reporter gene."

FangLin Sun (Washington University). "Chromosome organisation, chromatin structure and gene silencing in heterochromatin."

John Tomkiel (Wayne State University). "Fourth chromosome-induced position effect variegation of the rDNA."

Lori Wallrath (University of Iowa). "Lessons learned from heterochromatic heat shock transgenes."

Vett Lloyd (Dalhousie University, Canada). "Genomic imprinting and position effect variegation in *Drosophila*."
Kent Golic (University of Utah). "Y chromosome imprinting in *Drosophila*."
Bruce McKee (University of Tennessee). "Meiotic chromosome segregation: cis and trans acting factors."
Lenny Robbins (Universita di Siena, Italy). "Heterochromatic elements involved in disjuction and meiotic drive."
Erwin Schmidt (Johannes Gutenberg University, Germany). "Stimulation of gene expression by tandem repeats in transgenic lines."
Peter Harte (Case Western Reserve University). "Polycomb group proteins required for repeat-induced gene silencing."
Steven Henikoff (Fred Hutchinson Cancer Research Center). "Satellites, cenromeres and histone H3-like proteins."
Kumar Hari (The Salk Institute). "Molecular analysis of DNA and proteins required from chromosome inheritance."
Mary Lou Pardue (M.I.T.). "*Drosophila* telomeres: Two transposable elements with important roles on the chromosome."
James Mason (NIEHS). "Genetic consequences of telomeric interactions."
Patrick Maxwell (Syracuse University). "The TART family of telomeric retrotransposons."
Maurizio Gatti (Universita di Roma, Italy). "The genetic and molecular basis for telomeric fusions in *Drosophila*."
Caroline Conte (INSERM, France). "Insertions of the retroelement idefix modulate the enhancer effect of the retroelement ZAM at the white locus."
Giacomo Cavalli (Institut de Genetique Humaine, Montpelier, France). "PcG and trx-G proteins and the regulation of cellular memory."
Arthur Hilliker (University of Guelph, Canada). "Intercalary heterochromatin." (Group discussion).
Robert Glaser (Wadsworth Center, New York State Department of Health). "Alteration of DNA structure during polyploid development reveals replication barriers within heterochromatin."
Alexander Reugels (University of Koln, Germany). "Structural and evolutionary aspects of Y chromosomal mega-genes in *Drosophila* sp."
Manfred Steinemann (Technische Universitat Darmstadt, Germany). "*Drosophila miranda*: A model system for Y chromosome evolution."
Phing Zhang (University of Connecticut, Storrs). "Fertility factors and novel genetic elements of the heterochromatic Y chromosome and their involvement in *Drosophila* spermatogenesis."
Allan Lohe (Australian National University). "Functional and nonfunctional ribosomal RNA genes in heterochromatin of sibling species of *Drosophila melanogaster*."
Barry Honda (Simon Fraser University, Canada). "Genetic and molecular studies of heterochromatic loci in *Drosophila*."

Evolution '99

The joint meetings of the Society for the Study of Evolution, the American Society of Naturalists, and the Society of Systematic Biologists met at the University of Wisconsin, Madison, WI, U.S.A., on 22 to 26 June 1999. The local organizer was Don Waller (University of Wisconsin). The editors thank Mr. Robert Klitzman (Department of Zoology, University of Oklahoma) for extracting from the Program information on talks and posters that involve research with *Drosophila*. Listings are organized according to general topic, with some minor modifications from the published program.

Analysis of Life Histories

Chien, S. A. (Brown University, Providence, RI), N. K. Priest, and M. Tatar. Negligible senescence during reproductive diapause in *Drosophila melanogaster*.

Stearns, S. C. (University of Basel, Seattle, WA). The experimental evolution of life history traits in *Drosophila*.

Artificial Selection

Chippindale, A. K. (Department of Biology, University of California, Santa Cruz, CA), J. R. Gibson, and W. R. Rice. Gender and the genetics fitness in *Drosophila*.

Teotonio, H. (University of California – Irvine, Irvine, CA) and M. R. Rose. Reversibility in the experimental evolution of *Drosophila melanogaster*.

Biogeography/Geographic Variation: Insects

Gilchrist, G. W. (Department of Biology, Clarkson University, Potsdam, NY), R. B. Huey, D. Berrigan, and M. Carlson. Male and female body size clines in new World and Old World *Drosophila subobscura*.

Matzkin, L. M. (SUNY at Stony Brook, Stony Brook, NY), B. Verrelli, and W. F. Eanes. Life history variations in wild populations of *Drosophila melanogaster* along a latitudinal cline.

Verrelli, B. C. (Department of Ecology and Evolution, SUNY at Stony Brook, Stony Brook, NY) and W. F. Eanes. Clines revisited: examining geographic variation in *Drosophila melanogaster* metabolic enzymes at the nucleotide level.

Developmental Evolutionary Biology

Marcus, J. M. (Department of Zoology, Duke University, Durham, NC) and C. P. Klingenberg. Morphometric analysis of a *Drosophila* mutant with a wing phenotype of variable expressivity.

Roberts, S. P. (Department of Organismal Biol. and Anatomy, University of Chicago, Chicago, IL) and M. E. Feder. Developmental consequences of natural hyperthermia and heat-shock proteins in *Drosophila melanogaster*.

True, J. R. (University of Wisconsin, Madison, WI) and S. B. Carroll. *Drosophila* melanin synthesis genes as candidates in pattern evolution.

Ecological Genetics

Jones, C. D. (University of Rochester, Rochester, NY). The genetics of *Drosophila sechellia's* adaptation to its host.

Hybridization and Hybrid Zones

Alipaz, J. (University of Chicago, Chicago, IL), T. Karr, and C. Wu. The nature of racial differentiation in *Drosophila melanogaster*.

Rand, D. M. (Brown University, Providence, RI) and D. M. Weinreich. Microsatellite markers reveal selection against Zimbabwe genome in an experimental hybrid zone of *Drosophila melanogaster*.

Mating/Breeding Systems

Gronlund, C. J. (University of Chicago, Chicago, IL), M. P. DeAngelis, and J. A. Coyne. Mate grasping in *Drosophila pegasa*.

Mechanisms of Post-Reproductive Isolation

Presgraves, D. (Biology Department, University of Rochester, Rochester, NY). A genetic test for the mechanism of *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila*.

Molecular Evolution

Bachtrog, D. (Institute Fuer Tierzuent/Genetik, Vienna, Austria) and C. Schloetterer. Microsatellite mutation rates differ between dinucleotide repeat motifs - evidence from *Drosophila melanogaster*.

Bergman, C. M. (University of Chicago, Chicago, IL), M. Ludwig, and M. Kreitman. Binding site evolution in *Drosophila*.

Bettencourt, B. R. (Department of OBA, University of Chicago, Chicago IL), J. A. Maresca, and M. E. Feder. Natural variation and response to selection at *Drosophila* hsp70 loci.

Clark, A. G. (Penn State University, University Park, PA), B. Todd, and D. J. Begun. Polymorphism and divergence in *Drosophila* accessory gland proteins.

Duvernell, D. D. (Department of Ecology and Evolution, SUNY at Stony Brook, Stony Brook, NY) and W. F. Eanes. Contrasting rates of molecular evolution for three hexokinase genes in *Drosophila melanogaster* and *D. simulans*.

Yi, S. (University of Chicago, Chicago, IL) and B. Charlesworth. Molecular evolution of genes on the neo-sex chromosomes of *Drosophila miranda*.

Molecular Systematics: Arthropods

Kliman, R. M. (Rutgers University, Piscataway, NJ) and J. Hey. Addressing the "gene tree/species tree" problem in the *Drosophila simulans* complex.

Population Genetics

Andolfatto, P. (University of Chicago, Chicago, IL) and M. Przeworski. An excess of linkage disequilibrium in the *Drosophila* genome.

Bauer, V. L. (Cornell University, Ithaca, NY), J. Fay, and C. F. Aquadro. Nucleotide variability at the Notch locus in *Drosophila melanogaster*: inferences from a local reduction in variability.

Dermitzakis, E. T. (Penn Sate University, University Park, PA), J. P. Masly, and A. G. Clark. Sex ratio distortion in *Drosophila simulans*/*D. sechellia* introgression lines: simple or complex trait?

Fay, J. C. (University of Chicago, Chicago, IL) and C.-I. Wu. Molecular signature of positive selection in regions of low recombination in *Drosophila*.

James, A. C. (Chicago Field Museum, Chicago, IL) and J. W. O. Ballard. Population dynamics of *Wolbachia*infestation in *Drosophila* species.

Klingenberg, C. P. (Department of Zoology, Duke University, Durham, NC) and S. D. Zaklan. Developmental integration of fluctuation asymmetry in fly wings.

Silva, J. C. (University of Arizona, Tucson, AZ), and M. G. Kidwell. Population genetics of *D. willistoni*, a widespread New World Drosophilid.

Ting, C.-T. (University of Chicago, Chicago, IL), S. C. Tsaur, and C.-I. Wu. Population genetics of the Odysseus locus in *Drosophila simulans* clade.

Quantitative Genetics

Fry, J. D. (Department of Biology, Utah State University, Logan, UT). The rate of mildly deleterious mutation in *Drosophila*: new estimates, a reanalysis of the old studies, and the effects of genotype-environment interactions of the estimates.

Montooth, K. L. (Penn State University, University Park, PA), J. H. Marden, and A. G. Clark. Metabolism as a complex trait in *Drosophila*.

Pletcher, S. D. (Max Planck Institute/Demograph. Res, Rostock, Germany) and C. J. Geyer. Quantitative genetics of function-valued traits: estimating mutational variance for age-specific mortality rates in *Drosophila*.

Tanikawa, A. Y. (Cornell University, Ithaca, NY), H. P. Yang, W. A. Van Voorhies, J. C. Silva, and A. S. Kondrashov. Mutational evolvability: the impact of EMS on several fitness-related traits in *Drosophila melanogaster*.

Sexual Selection

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