

# DROSOPHILA INFORMATION SERVICE

75

July 1994

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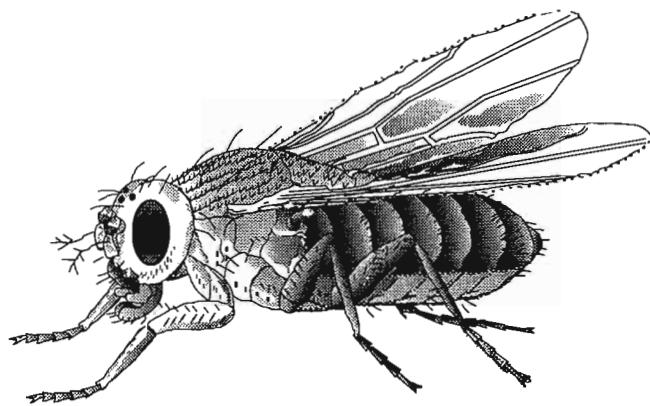
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prepared at  
Department of Zoology  
University of Oklahoma  
Norman, Oklahoma 73019



# 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 this volume on pages 207-208, DIS 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 60 years following that first issue, DIS has continued to promote open communication. In recognition of this 75th volume, a new section has been added to provide historical perspectives on *Drosophila* research. In addition to a reprint of the preface of the first volume, which summarizes the philosophy of the early researchers and includes the first directory (three of the 75 listed people were to receive Nobel Prizes for their contributions), we reprint a note by Larry Sandler on the origin of the U.S. *Drosophila* Conferences (DIS 56, 1981) and a description of the more recent conferences by Dan Lindsley.

The production of DIS 75 could not have been completed without the generous efforts of many people. Stanton Gray, Caroline Tawes, Christine LaFon, Laurel Jordan, Rick Wedel, April Sholl, Eric Weaver, and Merl Kardokus helped to prepare and check manuscripts for printing, Jean Ware and Lou Ann Lansford assisted in maintaining key records, Coral McCallister advised on artwork, and many of these individuals along with Mingull Jeung work to package and ship copies as they are requested. Special acknowledgment is due to Rick Wedel for his hard work on the cumulative subject index for volumes 1 to 71 (pages 213-301). The subject index was a challenging task since no issues were computerized prior to DIS 70. Melva Christian at the University of Oklahoma Printing Services oversaw the printing of this issue. Any errors or omissions in this volume are, however, the responsibility of the editor.

We are also grateful to the DIS Advisory Group: Michael Ashburner (Cambridge University), Daniel Hartl (Harvard University), Kathleen Matthews (Indiana University), and R.C. Woodruff (Bowling Green State University). The publication of *Drosophila* Information Service is supported in part by a grant from the National Science Foundation to R.C. Woodruff for the Mid-America *Drosophila* Stock Center, Bowling Green, Ohio. We hope that you find a lot of useful information here, and we invite you to let us know what can be done to improve DIS as an informal source of communication among *Drosophila* researchers.

James N. Thompson, jr.

Jenna J. Hellack

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## Stock Lists

**182 New Stocks of *Drosophila melanogaster***  
 Added to the  
**Mid-America Drosophila Stock Center**

at Bowling Green State University, Bowling Green, Ohio in April, 1994. See FlyBase at Indiana University (ftp ftp. bio.indiana.edu or gopher ftp.bio.indiana.edu) for the complete stock list. We can also send you a printed list or computer disk with the complete stock list. For additional information or to order stocks contact R. C. Woodruff or P. Oster at: e-mail = DMELANO@OPIE.BGSU.EDU, telephone = 419-372-2631, FAX = 419-372-2024, or write to: Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio, 43403

- 57.1 O/XYS.YL, y P[lacZ]5-45fD w P[lacZ]4-5f P[lacZ]3-52d P[lacZ]3-76a y+/C(1)RM, y pn
- 106.1 BS<sup>S</sup>Yy<sup>+</sup>/FM6/y ac sc sph<sup>4</sup>
- 111.1 BS<sup>S</sup>Yy<sup>+</sup>/y w<sup>a</sup> Ste
- 179.1 C(1)A, y/FM0, In(1)sc<sup>8</sup> In(1)dl-49 y<sup>31d</sup> w v m<sup>2</sup> f B sc<sup>8</sup>
- 209.1 C(1)A, y/T(1;Y)E1, y y<sup>+</sup> w<sup>a</sup> Ste BS
- 209.2 C(1)A, y/T(1;Y)E5, B<sup>S+</sup>
- 209.3 C(1)A, y/T(1;Y)E15, Ste<sup>+</sup>
- 209.4 C(1)A, y/T(1;Y)F12
- 209.5 C(1)A, y/T(1;Y)G8, Ste<sup>+</sup>
- 209.6 C(1)A, y/T(1;Y)L1, y y<sup>+</sup> w f B<sup>S</sup>
- 209.7 C(1)A, y/T(1;Y)N12
- 209.8 C(1)A, y/T(1;Y)N29, Ste<sup>+</sup>
- 209.9 C(1)A, y/T(1;Y)P7
- 209.91 C(1)A, y/T(1;Y)V24
- 209.93 C(1)A, y/T(1;Y)V43, Ste<sup>+</sup>
- 209.94 C(1)A, y/T(1;Y)V45
- 209.95 C(1)A, y/T(1;Y)V63, Ste<sup>+</sup>
- 209.96 C(1)A, y/T(1;Y)W19
- 469.1 C(1)DX, y w f/T(1;Y)B24, y y<sup>+</sup> B<sup>S</sup>
- 587.1 C(1)DX, y w f/T(1;Y)D5, y y<sup>+</sup> B<sup>S</sup>
- 616.1 C(1)RA, y Df(1)259/Dp(1;f)1175/XYL.YS, y Df(1)259 w
- 658.1 C(1)RM, y P[lacZ]5-45fD w P[lacZ]4-5f P[lacZ]3-52d P[lacZ]3-76a+/Y
- 701.1 C(1)TM, XYS.YLX, y<sup>31d</sup> Tu/YSX.YL, In(1)EN, y<sup>+</sup> v f B
- 741.1 Df(1)bb158, cin y w/FM7a/w<sup>+</sup>Y
- 750.1 Df(1)R46, y<sup>2</sup> v f wap<sup>3</sup>/C(1)DX, y w f/y<sup>+</sup>Ymal<sup>126</sup>
- 891.1 FM6/w uncl<sup>5</sup>/Ymal<sup>+</sup>
- 1009.1 g<sup>2</sup> mei(1)41A<sup>7</sup>/FM7a

1009.2 g<sup>2</sup> mei(1)41A29/FM7a  
 1153.03 mei(1)9RT2 f/C(1)DX, y f  
 1153.04 mei(1)9RT3 f/C(1)DX, y f  
 1153.05 mei(1)9RT4 sd/C(1)DX, y f  
 1153.06 mei(1)41A1 f/C(1)DX, y f  
 1153.2 mei(1)41InAM/C(1)DX, y f  
 1153.3 mei(1)41RT1 f/FM7  
 1153.4 mei(1)41RT2 f/FM7  
 1153.5 mei(1)41RT3 f/C(1)DX, y f  
 1153.6 mus(1)113RT3/C(1)DX, y f  
 1153.7 mus(1)114RT1 f/C(1)DX, y f  
 1179.1 rss (A2)  
 1202.1 sn<sup>3</sup> mei(1)9D1/C(1)DX, y f  
 1202.2 sn<sup>3</sup> mei(1)9D2/C(1)DX, y f  
 1202.3 sn<sup>3</sup> mei(1)9D3/C(1)DX, y f  
 1202.4 sn<sup>3</sup> mei(1)9D4/C(1)DX, y f  
 1202.5 sn<sup>3</sup> mei(1)41D7/C(1)DX, y f  
 1202.6 sn<sup>3</sup> mei(1)41D8/C(1)DX, y f  
 1202.7 sn<sup>3</sup> mei(1)41D9/C(1)DX, y f  
 1202.8 sn<sup>3</sup> mei(1)41D10/C(1)DX, y f  
 1202.9 sn<sup>3</sup> mei(1)41D11/C(1)DX, y f  
 1202.91 sn<sup>3</sup> mei(1)41D14/C(1)DX, y f  
 1202.92 sn<sup>3</sup> mus(1)109D2/C(1)DX, y f  
 1220.2 T(1;Y)J2, y y<sup>+</sup> w f B<sup>S</sup>/C(1)?  
 1220.3 T(1;Y)R12, y y<sup>+</sup> w f B<sup>S</sup>/C(1)?  
 1220.4 T(1;Y)V47/C(1)?  
 1236.01 w mei(1)41D1/C(1)DX, y f  
 1236.02 w mei(1)41D2/C(1)DX, y f  
 1236.03 w mei(1)41D3/C(1)DX, y f  
 1236.04 w mei(1)41D4/C(1)DX, y f  
 1236.05 w mei(1)41D14/C(1)DX, y f  
 1236.06 w mus(1)102D2/C(1)DX, y f  
 1236.4 w mus(1)106D1/C(1)DX, y f  
 1236.5 w mus(1)109D1/C(1)DX, y f  
 1236.6 w mus(1)115D1/C(1)DX, y f  
 1236.7 w mus(1)115D1/FM7a  
 1241.1 w<sup>1118</sup>

1290.1 y cv mus(1)111D<sup>1</sup>/C(1)DX, y f  
 1299.01 y mei(1)9<sup>a</sup>/C(1)DX, y f  
 1299.02 y mei(1)9<sup>a</sup> mei(1)41D<sup>5</sup>/FM7  
 1299.03 y mei(1)9<sup>a</sup> mei(1)41D<sup>13</sup>/C(1)DX, y f  
 1299.04 y mei(1)9<sup>a</sup> mei(1)41D<sup>14</sup>/C(1)DX, y f  
 1299.05 y mei(1)9<sup>a</sup> mus(1)101D<sup>1</sup>/C(1)DX, y f  
 1299.06 y mei(1)9<sup>a</sup> mus(1)101D<sup>2</sup>/C(1)DX, y f  
 1299.07 y mei(1)41<sup>195</sup>/C(1)DX, y f  
 1299.2 y P[lacZ]5-45fD w P[lacZ]4-5fP P[lacZ]3-52d P[lacZ]3-76a  
 1316.1 y v mei(1)41<sup>12-1007</sup>/C(1)DX, y f  
 1320.1 y w P[lacZ]3-76a  
 1390.1 Adh<sup>n5</sup> pr cn/CyO  
 1485.1 Ba<sup>DM2</sup>/CyO  
 1545.01 cn bw mus(2)202A<sup>1</sup>/CyO  
 1545.3 cn bw mus(2)205A<sup>1</sup>/CyO  
 1573.1 cn vg<sup>21-3</sup> bw  
 1574.1 cob<sup>NM2</sup>/CyO  
 1575.1 cpy<sup>SM3</sup>/CyO  
 1694.1 CyO/l(2)gl<sup>4</sup> or  
 1793.1 dif<sup>GM5</sup>/CyO  
 2080.1 ire<sup>UB6</sup>/CyO  
 2129.1 ninaC<sup>MB2</sup>/CyO  
 2142.1 pie<sup>EB3</sup>/CyO  
 2146.1 pom<sup>HM3</sup>/CyO  
 2161.1 rdgD<sup>DM5</sup>/CyO  
 2172.1 rug<sup>MM2</sup>/CyO  
 2179.1 sca<sup>OB7</sup>/CyO  
 2185.1 Sm<sup>BS</sup> (Bald)  
 2303.1 Crn in ri/TM3, Sb Ser  
 2357.1 Dr<sup>Mio</sup>/TM6  
 2371.1 emc<sup>E6</sup>  
 2454.1 In(3LR)Ubx<sup>101</sup>, Ubx<sup>101</sup>/TM9, th st Sb e l(3)DTS-4  
 2466.01 In(3R)C, Sb cd Tb ca/Dl H e<sup>S</sup> cd  
 2499.1 MKRS, P[ry<sup>+</sup>Δ2-3](99B)/TM2, P[ry<sup>+</sup>Δ2-3](99B)  
 2499.2 ms(3)sa/TM6B, Hu e Tb  
 2499.3 mus(3)302D<sup>4</sup>/TM3, Sb Ser  
 2499.4 mus(3)302D<sup>5</sup>/TM3, Sb Ser

2499.5 mus(3)302<sup>D6</sup>/TM3, Sb Ser  
 2499.6 mus(3)306<sup>D1</sup>/(TM3, Sb Ser ?)  
 2499.7 mus(3)307<sup>D1</sup>/(TM3, Sb Ser ?)  
 2499.8 mus(3)308<sup>D2</sup>/(TM3, Sb Ser ?)  
 2500.1 mus(3)312<sup>D1</sup>/TM3, Sb Ser  
 2539.1 prd FR1/SM5  
 2546.1 ri Ki pP  
 2546.2 ri Ki pP Ubx<sup>25</sup>/TM1, ri Me red  
 2546.3 ri l(3)98Da (=l(3)M3-13)/TM3, Sb  
 2456.4 ri l(3)98Db (=l(3)M3-5)/TM3, Sb  
 2546.5 ri l(3)98Dc (=l(3)M3-18)/TM3, Sb  
 2546.6 ri l(3)98Dd (=l(3)M3-3)/TM3, Sb  
 2546.7 ri l(3)98Dg (=l(3)M3-12)/TM3, Sb  
 2547.1 rn<sup>BS</sup>/TM1  
 2595.1 ru th st dic/TM3  
 2597.1 ry mus(3)305<sup>D3</sup>/TM3, Sb Ser  
 2597.2 ry mus(3)308<sup>D4</sup>/TM3, Sb Ser  
 2597.3 ry mus(3)308<sup>D5</sup>/TM3, Sb Ser  
 2597.4 ry mus(3)308<sup>D6</sup>/TM3, Sb Ser  
 2603.1 ry<sup>506</sup> P[ry<sup>+</sup> SalI](89D)  
 2640.1 st mus(3)301<sup>D1</sup>/TM3, Sb Ser  
 2640.2 st mus(3)301<sup>D2</sup>/TM3, Sb Ser  
 2640.3 st mus(3)301<sup>D3</sup>/TM3, Sb Ser  
 2640.4 st mus(3)301<sup>D4</sup>/TM3, Sb Ser  
 2640.5 st mus(3)301<sup>D5</sup>/TM3, Sb Ser  
 2640.6 st mus(3)302<sup>D1</sup>  
 2641.1 st mus(3)302<sup>D2</sup>/TM3, Sb Ser  
 2641.2 st mus(3)302<sup>D3</sup>/TM3, Sb Ser  
 2641.3 st mus(3)304<sup>D1</sup>/TM3, Sb Ser  
 2641.4 st mus(3)304<sup>D2</sup>/TM3, Sb Ser  
 2641.5 st mus(3)304<sup>D3</sup>/(TM3, Sb Ser ?)  
 2641.6 st mus(3)305<sup>D1</sup>/TM3, Sb Ser  
 2641.7 st mus(3)305<sup>D2</sup>/TM3, Sb Ser  
 2641.8 st mus(3)308<sup>D1</sup>/TM3, Sb Ser  
 2641.9 st mus(3)310<sup>D1</sup>/TM3, Sb Ser  
 2641.91 st mus(3)311<sup>D1</sup>/TM3, Sb Ser  
 2641.92 st mus(3)311<sup>D2</sup>/TM3, Sb Ser

2641.93	st mus(3)312D <sup>2</sup> /TM3, Sb Ser
2660.1	TM3, ry <sup>RK</sup> /TM6B
2661.1	TM6B, Hu e/TM8, th st Sb e l(3)DTS-4
2663.1	TMS, Sb P[ry <sup>+</sup> Δ2-3](99B)/Dr
2760.1	B <sup>S</sup> Y ; cn tra2 <sup>B</sup> bw/CyO
2760.2	B <sup>S</sup> Y/y w spl ; mle/SM1
2908.01	w ; CyO/Sco
2909.01	w <sup>1118</sup> ; nub b Sco lt stw <sup>3</sup> /CyO
2910.1	w <sup>co2</sup> v ; bw <sup>6</sup>
2920.01	y v/y <sup>+</sup> Y ; tra2/SM1
2920.2	y w ; nub <sup>7</sup> b Sco pr ca/CyO
3004.1	l(1)top1 <sup>77</sup> /FM7 ; ry <sup>506</sup>
3015.1	T(Y;3)TMS, ry <sup>506</sup> Sb P[ry <sup>+</sup> Δ2-3](99B)
3035.1	y w ; ri l(3)98De (=l(3)M1-5)/TM3, Sb
3035.2	y w ; ri l(3)98Df (=l(3)M3-7)/TM3, Sb
3036.1	y w <sup>67</sup> ; Ifm(3)3
3038.1	y <sup>2</sup> sc ; su-Hw <sup>BS</sup> /TM1
3041.1	O/C(1)RM, y v bb/X.Y, w ; net
3064.1	C(1)DX, y w f/y <sup>+</sup> YS/y <sup>2</sup> z f.YL ; spa <sup>pol</sup>
3095.1	y z/y <sup>+</sup> Y ; spa <sup>pol</sup>
3113.1	b cn ; ry <sup>8</sup> , Ifm(3)3
3122.1	bw ; c(3)GOR-12-522 bx <sup>34e</sup> /TM3
3123.1	bw ; c(3)GOR-12-570 bx <sup>34e</sup> /TM3
3193.1	CyO, S cn bw ; T(2;3)6r31, cn bw
3220.1	CyO, S cn bw ; T(2;3)8r29; cn bw (S <sup>+</sup> Cy <sup>+</sup> )
3233.1	Df(3R)sbd <sup>105</sup> , sbd <sup>105</sup> ; T(2;3)ap <sup>Xa</sup> , ap <sup>Xa</sup>
3252.1	GalN1 ; ry <sup>506</sup> (P strain)
3261.01	In(2L)Cy In(2R)Cy, Cy cn <sup>2</sup> /In(2LR)bw <sup>V1</sup> , ds <sup>33k</sup> bw <sup>V1</sup> ; emc <sup>E9</sup> /TM2, Ubx
3371.1	T(2;3)81 ; (TM3, Sb Ser ?)
3385.1	T(2;3)101 ; (TM3, Sb Ser ?)
3419.1	T(2;3)160 ; (TM3, Sb Ser ?)
3466.1	T(2;3)CyO ; TM9, th st Sb e l(3)DTS-4/Tp(3;3) <sup>M3</sup>
3466.2	T(2;3)CyO ; TM9, th st Sb e l(3)DTS-4/Pm ; H
3541.1	mus(1)112 <sup>RT2</sup> f/C(1)DX, y f ; cn bw ; ri e
3541.2	mus(1)113 <sup>RT1</sup> f/C(1)DX, y f ; cn bw ; ri e
3554.1	w ; T(2;3)ap <sup>Xa</sup> , ap <sup>Xa</sup> /SM5 ; TM6B
3565.1	y w ; T(2;3)B3, CyO, TM6B/Pin <sup>88K/+</sup>

3574.1 y ; ms(2)045/SM1 ; spa<sup>pol</sup>  
 3574.2 y ; ms(2)S3/SM1 ; spa<sup>pol</sup>  
 3582.1 Df(1)bb3a, y pn cv m f/FM7 ; TM2/Vno ; spa<sup>pol</sup>  
 3582.2 Df(1)bb74, y pn cv m f/FM7 ; TM2/Vno ; spa<sup>pol</sup>  
 3582.3 Df(1)bb452, y pn cv m f/FM7, B<sup>+</sup> ; TM2/Vno ; spa<sup>pol</sup> (B reverted)  
 3582.4 Df(1)bb456, y pn cv m f/FM7, B<sup>+</sup> ; TM2/Vno ; spa<sup>pol</sup>  
 3582.5 y w/y<sup>+</sup>Y ; ru h th st cu sr e Pr ca (ruPrica)/TM6 ; spa<sup>pol</sup>

**Stock List**  
**of**  
**The European *Drosophila* Stock Center**  
**Umeå, Sweden**

This stock list of the European *Drosophila* Stock Center at Umeå is also available on FlyBase (flybase/stocks/stock\_centers/umea/umea.txt). It is supported by a grant from the Swedish National Science Foundation. Inquiries for the Umeå Stock Center should be addressed to Asa Rasmuson, Department of Genetics, University of Umeå, S901 87 Umeå, Sweden. Telephone = (46)-90-165-275; Fax = (46)-90-167-665; e-mail = RASMUS@BIOVAX.UMDC.UMU.SE. In the following list, formatting from FlyBase has been retained, and superscripts are denoted by square brackets.

#100	0 / C(1)RM, y pn / YSX.YL, In(1)EN y w f
#10050	C(1)DX, y w f / r[89k]
#10100	C(1)DX, y w f / sc w ec
#10200	C(1)DX, y w f / sc z ec
#10600	C(1)DX, y w f / sc z w[is] wy
#10650	C(1)DX, y w f / sc z[OP6] ec
#10655	C(1)DX, y w f / sc z[OP11] ec
#10657	C(1)DX, y w f / Sh[6]
#10660	C(1)DX, y w f / sn[3] oc
#10680	C(1)DX, y w f / T(1;Y)101, yy[+]B[s]
#1070	B[S]Y / C(1)DX, y w f / y mei-9b cv ct[6] mei-218
#10700	C(1)DX, y w f / w mei-41[D5]
#10840	C(1)DX, y w f / X.Y, y w f / Dp(1;f)MM2, y[+]
#10900	C(1)DX, y w f / y rst car
#1100	B[S]Yy[+] / y w[a] f
#11000	C(1)DX, y w f / y[2] sc su(s)[2] spl
#11100	C(1)DX, y w f / y[2] sc w[a] ec cv sn[3]
#11200	C(1)DX, y w f / y[2] su(w[a]) w[a] ec
#11300	C(1)DX, y w f / y[2] su(w[a]) w[a] spl / T(1;Y)59b-2, y[59b] sc[+] su(s)[+] su(w[a])[+] sta[+]
#11410	C(1)DX, y w f / y[c4] sc[S1] B / y[59b]Y
#1150	B[S]Yy[+] / Df(1)GA-90 / FM6, l(1)69a y[31d] sc[8] dm B
#11500	C(1)DX, y w f / z w[a59k9] ec
#1160	Df(1)X-1, y ac sc / FM7b, y[31d] sc[8] w[a] lz[s] B
#11800	C(1)M3, y[2] / z[a] w[a]
#11900	C(1)RM, Basc, y[31d] sc[8] sc[S1] w[a] B / In(1)rst[3], y rst[3] car bb
#1200	Basc, sc[8] sc[S1] w[a] B
#12100	C(1)RM, y / XYL.YS(2-10T13), y[2] su(w[a]) w[a]
#1220	Basc, sc[8] sc[S1] w[a] B[+]; Pi2
#12200	C(1)RM, y / z[a69-1] spl sn[3]
#12250	C(1)RM, y f / Dp(1;f)52 / XYL.YS, Df(1)259, w
#12300	C(1)RM, y f / YSX., y cv v f

#12500 C(1)RM, y pn / FM6, y[31d] sc[8] dm B  
 #12600 C(1)RM, y v / XYL.YS, y w spl  
 #12650 C(1)RM, y v f mal[2] / Df(1)mal3, y[2] ct[6] f / Dp(1;Y)y[+]Ymal[106]  
 #12700 C(1)RM, y w / In(1)l-v231, y l(1)v231 / y[+]Y  
 #12800 C(1)RM, y v bb / X.YL(U-8e), y w / YS.YS  
 #12840 C(1)RM, y v f / Df(1)C1 / Ymal[+]  
 #12850 C(1)RM, y v f / Df(1)C4 / Ymal[+]  
 #12860 C(1)RM, y v f / Df(1)C10 / Ymal[+]  
 #1300 BB car / C(1)DX, y f / y[+]sc[8]Y  
 #13100 C(1)RM, y v f mal / In(1)dl-49 In(1)B[M1] Df(1)mal6, y sn[X2] v B[M1] mal[6] / Ymal[+]  
 #13300 C(1)RM, y v f mal[2] / Df(1)mal5, y[2] ct f mal[5] / y[+]Ymal[106]  
 #13350 C(1)TM, XYS.YLX, y[31d] Hw / YSX.YL, In(1)EN, y[+]. y v f  
 #13400 car su(f)[lts67g]  
 #13500 CIB, sc l(1)C t[2] v sl B / dor sn[3]  
 #13550 CIB, sc l(1)C t[2] v sl B / FM1, y[31d] sc[8] w[a] lz[s] B  
 #13700 CIB, sc l(1)C t[2] v sl B / R(1)2, cv v f  
 #13800 CIB, sc l(1)C t[2] v sl B / w fs(1)K10  
 #13860 de[11] / FM7a, y[31d] sc[8] w[a] v[Of] B  
 #13870 Df(1)2/9A, y mei-9 mei-41 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #13900 Df(1)16-3-22 / FM6, y[31d] sc[8] dm B  
 #13950 Df(1)16-129 / FM6, y[31d] sc[8] dm B / y[+]Ymal[106]  
 #13980 In(1)sc[SIL]sc[8R] Df(1)54, y[c4] sc[8] sc[s1] mal[65] / FM7, y[31d] sc[8] w[a] sn[x2] v[Of] g[H] B / mal[+]Y  
 #14000 Df(1)260-1 / FM4, y[31d] sc[8] dm B  
 #14040 Df(1)724, y / FM6, y[31d] sc[8] dm B  
 #14050 Df(1)A53 / FM6, y[31d] sc[8] dm B  
 #14150 Df(1)A118 / FM6, y[31d] sc[8] dm B / Ymal[+]  
 #14200 Df(1)A209 / FM7a, y[31d] sc[8] w[a] v[Of] B  
 #14300 Df(1)B263-20, B[263-20] / In(1)sc[7] In(1)AM, sc[7] car  
 #14350 Df(1)B12 / FM6, y[31d] sc[8] dm B / y[+]Ymal[106]  
 #1440 Binsn, sc[8] sc[S1] sn[X2] B / cm ct[6] su(Fl[s])  
 #1450 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)16-2-13 / mal[+]Y  
 #1455 Df(1)C74 / FM6, y[31d] sc[8] dm B  
 #1456 Df(1)C128 / FM6, y[31d] sc[8] dm B  
 #1460 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)16-3-35 / Ymal[+]  
 #14700 Df(1)C246 / FM6, y[31d] sc[8] dm B  
 #14750 Df(1)ct4b1 Dp(1;1)S93 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #14755 Df(1)ct4b1, y sn / Binsn, sc[8] sc[S1] sn[X2] B  
 #14800 Df(1)ct268-42, y ct[268-42] / FM4, y[31d] sc[8] dm B  
 #14900 Df(1)D43L1, y v f / FM6, y[31d] sc[8] dm B / y[+]Ymal[106]  
 #14930 Df(1)DA622 / FM6, y[31d] sc[8] dm B  
 #14950 Df(1)DCB1-35b / FM6, y[31d] sc[8] dm B  
 #14955 Df(1)DCB1-35c / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / y[+]Ymal[126]  
 #1500 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)16-185 / v[+]Yy[+]  
 #15000 Df(1)dm75e / FM7c, w[a] sn[X2] v[Of] g[4] B -- (Synonym: Df(1)dm[e19]=Df(1)HA44)  
 #15005 Df(1)EA113 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15100 Df(1)g-1, g[l] f B / In(1)AM  
 #15110 Df(1)G4e[L]H24i[R], M f / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15120 Df(1)GA22 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / y[+] Ymal[126]  
 #15130 Df(1)GA33 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15140 Df(1)GA37 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15145 Df(1)GA40 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15148 Df(1)GA104 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / y[+] Ymal[106]  
 #15150 Df(1)GA112 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / v[+]Yy[+]  
 #15155 Df(1)GA131 / FM6, y[31d] sc[8] dm B  
 #15200 Df(1)HA32 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15400 Df(1)HA92 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15450 Df(1)HC133 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15460 Df(1)HC194 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15500 Df(1)HC244 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15510 Df(1)HC279 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B/y[+]Ymal[106]

#15550 Df(1)HF359 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15700 Df(1)HF368 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15800 Df(1)HF396 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15805 Df(1)HM44, y w[a] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B/y[+]Ymal[+]  
 #15810 Df(1)HM430, y w[a] / FM6, y[31d] sc[8] dm B / y[+]Ymal[+]  
 #15840 Df(1)JA21 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15850 Df(1)JA22 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #15900 Df(1)JA26 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16000 Df(1)JA27 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16050 Df(1)JC4 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16080 Df(1)JC12 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16100 Df(1)JC19 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16200 Df(1)JC70 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16250 Df(1)JC77 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / y[+]Ymal[106]  
 #16280 Df(1)JF5, f car / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16400 Df(1)KA9 / FM7c, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16500 Df(1)KA10, sn[3] m / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16530 Df(1)KA14 / FM7c, y[31d] sc[8] w[a] sn[X1] v B l(1)TW-24  
 #16590 Df(1)LB6 / FM6, y[31d] sc[8] dm B / y[+]Ymal[106]  
 #16600 Df(1)LB7 / FM6, y[31d] sc[8] dm B / y[+]Ymal[106]  
 #16640 Df(1)LB23 / FM6, y[31d] sc[8] dm B/y[+]Ymal[106]  
 #16700 Df(1)M13 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #16750 Df(1)N-8 / FM7c, w[a] sn[X2] v[Of] g[4] B  
 #16800 Df(1)N264-105, N[264-105] / FM1, y[31d] sc[8] w[a] lz[s] B  
 #16900 Df(1)N12, ras v / FM6, y[31d] sc[8] dm B  
 #1700 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)17-87  
 #17000 Df(1)N19 / FM6, y[31d] sc[8] dm B  
 #17100 Df(1)N71 / FM6, y[31d] sc[8] dm B  
 #17200 Df(1)N73 / FM6, y[31d] sc[8] dm B  
 #17250 Df(1)N77 / FM6, y[31d] sc[8] dm B  
 #17300 Df(1)N105 / FM6, y[31d] sc[8] dm B  
 #17420 Df(1)Pgd35, Pgd pn / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #17450 Df(1)Q539 / FM6, y[31d] sc[8] dm B  
 #17500 Df(1)R-8A / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #17600 Df(1)R-17, y v f / FM7a, y[31d] sc[8] w[a] v[Of] B / y[+]Ymal[+]  
 #17700 Df(1)R-22, y / FM7a, y[31d] sc[8] w[a] v[Of] B  
 #17800 Df(1)R-25, y v / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #1800 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)17-137 / y[+]Ymal[+]  
 #18200 Df(1)RA2 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #18250 Df(1)RA37 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / v[+]Yy[+]  
 #18300 Df(1)RA47 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #18350 Df(1)RA67 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / y[+] Ymal[106]  
 #18400 Df(1)RC40 / FM7c, y[31d] sc[8] w[a] v[Of] g[4] B  
 #18450 Df(1)RF19 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #18470 Df(1)run1112, y f / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / y[+]Ymal[106]  
 #18500 Df(1)S39 / FM6, y[31d] sc[8] dm B  
 #18550 Df(1)S54 / FM6, y[31d] sc[8] dm B  
 #18560 Df(1)sc[V1], sc[V1] f[36a] / FM6, y[31d] sc[8] dm B  
 #18570 Df(1)sd72b / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #18600 Df(1)C128, sn[c128] / FM6, y[31d] sc[8] dm B -- (synonym Df(1)sn[c128])  
 #18650 Df(1)T2-14A / FM6, y[31d] sc[8] dm B / y[+]Ymal[106]  
 #18760 Df(1)v-L1, y[2] ec cv ct v[L1] m f / FM6, y[31d] sc[8] dm B  
 #18800 Df(1)v-L15, v[L15] / FM6, y[31d] sc[8] dm B  
 #18900 Df(1)v-N48 / Dp(1;Y)B[S-]v[+]y[+]Y / C(1)DX, y f  
 #1900 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)17-148 / Ymal[+]  
 #19100 Df(1)w, y[2] sc z w spl  
 #19150 Df(1)w62, w[62] / FM1, y[31d] sc[8] w[a] lz[s] B  
 #19200 Df(1)w67c23.2, y w[67c23.2]  
 #19300 Df(1)w67i23, y w[67i23]  
 #19350 Df(1)w67k30, w[67k30] lz ras v / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B

#19360 Df(1)w78h24, w[78h24]  
 #19400 Df(1)w258-11, y w[258-11] / In(1)dl-49, y Hw m[2] g[4]  
 #19500 Df(1)w258-42, y w[258-42] / FM4, y[31d] sc[8] dm B  
 #1955 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)17-257 / y[+]Ymal[106]  
 #1960 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)17-351  
 #1970 Binsn, sc[8] sc[S1] sn[X2] B / Df(1)17-489  
 #19800 Df(1)w-L, w[L] / In(1)dl-49, y Hw m[2] g[4]  
 #19850 Df(1)w-rJ3, y[2] w[rJ3] / In(1)dl-49, y Hw m[2] g[4]  
 #200 0 / C(1)RM, y v bb / XYL.YS(108-9), y[2] su(w[a]) w[a]  
 #20000 Df(1)y-ac[-22]  
 #20300 dnc  
 #2050 Binsn, sc[8] sc[S1] sn[X2] B / l(1)unc[16-3-212] / mal[+]Y  
 #20600 Dp(1;1)112, f  
 #2100 Binsn, sc[8] sc[S1] sn[X2] B / y w l(1)lpr-1  
 #21200 Dp(1;1)w[sp]w[a], sc z w[sp] w[a] sn  
 #21400 Dp(1;1)y w[a], y w[a] / Yw[+]Co  
 #21500 Dp(1;1)z, sc z w[is]  
 #21600 Dp(1;f)24 / In(1)sc[J1], l(1)J1 sc[J1]  
 #21700 Dp(1;f)101 / In(1)sc[8] Df(1)sc[8], sc[8] w[a]  
 #21800 Dp(1;f)107 / In(1)sc[8] Df(1)sc[8], sc[8] w[a]  
 #21900 Dp(1;f)118 / In(1)sc[8] Df(1)sc[8], sc[8] w[a]  
 #2200 Binsn, sc[8] sc[S1] sn[X2] B / y w sn[3] l(1)npr-1  
 #22000 Dp(1;f)135 / In(1)sc[8] Df(1)sc[8], sc[8] w[a]  
 #22100 Dp(1;f)R / y dor[l]  
 #22200 Dp(1;Y)Sz280, y[2] / FM6, y[2] sc[8] dm B l(1)11 / y l(1)t242  
 #22250 EA41[DC789] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #22255 eag[1]  
 #22260 eo[A7] / FM6, y[2] sc[8] dm B / y[+] Ymal[126]  
 #22270 eo[EA13] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #22280 eo[S2] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #22300 f su(f)  
 #22400 f[s] su(f)[lts67g]  
 #22550 fli[O2]  
 #22551 fliA[1]  
 #22553 fliA[3]  
 #22554 fliA[4]  
 #22555 fli[D44] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #22560 fli[DA534] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #22565 fliF[1]  
 #22567 fli[HC183] / FM6, y[31d] sc[8] dm B / y[+] Ymal[106]  
 #22570 fliI[3]  
 #22600 FM3, l(1)? y[31d] sc[8] dm B l(1)? / fs(1)384, sc v[24]  
 #22650 FM3, l(1)? y[31d] sc[8] dm B l(1)? / fs(1)508, sc v[24]  
 #22660 FM3, l(1)? y[31d] sc[8] dm B l(1)? / fs(1)1501, sc v[24]  
 #22670 FM3, l(1)? y[31d] sc[8] dm B l(1)? / y cv dec]1[(13C-73) v f  
 #22675 FM3, y[31d]sc[8]dm B / otu[11] y cv v f  
 #22676 FM3, y[31d] sc[8] dm B / otu[13] y cv v f  
 #22677 FM3, y[31d] sc[8] dm B / otu[14]  
 #22680 FM3, y[31d] sc[8] dm B / y w[a] lfl[HM46] / y[+]Ymal[106]  
 #22700 FM4, y[31d] sc[8] dm B / In(1)bb[Df], y sl[2] bb  
 #22740 FM4, y[31d] sc[8] dm B / w[cf]  
 #22760 FM6, y[31d] sc[8] dm B / Df(1)rst-vt, sc z w[+UR] rst vt  
 #22765 FM6, y[31d] sc[8] dm B / l(1)unc[JC8]  
 #22770 FM6, y[31d] sc[8] dm B / gt[X11]  
 #22800 FM6, y[31d] sc[8] dm B / In(1)N[264-84], y N[264-84]  
 #22900 FM6, y[31d] sc[8] dm B / l(1)X10  
 #2300 Binsn, sc[8] sc[S1] sn[X2] B / y w sn[3] l(1)npr-3  
 #23000 FM6, y[31d] sc[8] dm B / M(1)18C -- (synonym M(1)n)  
 #23400 FM6, y[31d] sc[8] dm B / qs  
 #23402 FM6, y[31d] sc[8] dm B / R-9-28[LB2]

#23405 FM6, y[31d] sc[8] dm B / pas[E81] -- (synonym shak-B[E81])  
 #23407 FM6, y[31d] sc[8] dm B / stn[C]  
 #23410 FM6, y[31d] sc[8] dm B / l(1)unc[586]  
 #23455 FM6, y[31d] sc[8] dm B / l(1)unc[27E2] / y[+]Ymal[106]  
 #23457 FM6, y[31d] sc[8] dm B / w fli[w2] / mal[+]Y  
 #23460 FM6, y[31d] sc[8] dm B / w l(1)unc[w5] / mal[+]Y  
 #2350 blind non-phototactic (Manning)  
 #23500 FM6, y[31d] sc[8] dm B / y ac sc pn w rb cm ct sn[3] ras[4] v m g f car  
 #23560 FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / T(1;Y)154, y y[+] B[s]  
 #23570 FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / l(1)unc[GE230]  
 #23580 FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / otu[0]  
 #23600 FM7a, y[31d] sc[8] w[a] v[Of] B / y su(Cbx) v f[36a]  
 #23700 gt w[a]  
 #23750 hdp[102] -- (synonym fli[H])  
 #23751 Hk[1]  
 #23752 Hk Hyperkinetic  
 #23755 hypoB[1]  
 #23770 hypoB[iav]  
 #23800 In(1)AM, ptg[4] / un Bx[2]  
 #23900 In(1)B[M1], v B[M1]  
 #23950 In(1)dl-49, sn[X2] v[Of] mal[2] / In(1)sc[8] Df(1)mal10, sc[8] B mal[10] / y[+]Ymal[106]  
 #2400 br w[e] ec rb t[4] / FM1, y[31d] sc[8] w[a] lz[s] B  
 #24000 In(1)dl-49, sn[X2] v mal[2] / In(1)sc[8] Df(1)y-X5, y[X5] sc[8] B  
 #24100 In(1)dl-49, sn[X2] v mal[2] / In(1)sc[8] Df(1)y-X15, y[X15] sc[8] B  
 #24200 In(1)dl-49, y Hw m[2] g[4] / N[86el]  
 #24300 In(1)rst[3], y rst[3] car  
 #2440 C(1)DX, y f / cm ct[6] sn[4] oc ptg  
 #2450 C(1)DX, y f / Df(1)64j4, y w[a] spl / w[+]Y  
 #24500 In(1)sc[4L]EN[R] Dp(1;1)B[S]TMG, y sc[4] m f B[S] / In(1)sc[7] In(1)AM, sc[7] ptg[4]  
 #24690 In(1)sc[8] In(1)dl-49, y[31d] w m[2] v f B / y cv dec1(12-403) v f  
 #24750 In(1)sc[8], sc[8] w[DZL]  
 #24755 In(1)sc[8]w[a] su(f)  
 #24757 In(1)sc[8]w[a], y[31d]  
 #24760 In(1)sc[8L]sc[S1R] In(1)S, y[S1] B / w sn[5S] bb  
 #24800 In(1)sc[9], sc[9] w[a] t f Bx  
 #2500 C(1)DX, y f / Df(1)65j26 / w[+]Y  
 #25100 In(1)sc[J1] In(1)dl-49 In(1)B[M1], l(1)J1 sc[J1] oc ptg B[M1] / Maxy b, y[c4] sc[8] sc[S1] pn w ec rb cm ct[6]  
     sn[3] ras[2] v dy g[2] f os[s] os[o] car l(1)? / l(1)J1[+]Y  
 #25200 In(1)sc[S1L]sc[8R] In(1)dl-49 Df(1)su(f)4B, y[c4] sc[8] sc[S1] w[a] v f / l(1)14-1 / y[+]Yml[126]  
 #25300 In(1)w[m4], w[m4]  
 #2550 C(1)DX, y f / Df(1)K95, y[2] cv / w[+]Y  
 #25800 In(1)y[4], y[4] w[a]  
 #25801 l(1)10Ba[4] /FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / v[+]Yy[+] (l(1)EC 230)  
 #25802 l(1)10Bb[12] f /FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B (l(1)VA 178)  
 #25803 l(1)10Bc[5] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B (l(1)VE 178)  
 #25804 l(1)10Bg / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B (l(1)v21)  
 #25805 l(1)10Bm / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B (l(1)C248)  
 #25810 l(1)C111 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #25811 l(1)DC705 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #25950 m f car[1]  
 #2600 C(1)DX, y f / Df(1)sc[8], w[a] / T(1;Y)59k9-1, y[2] sc[+] su(s)[2+] su(w[a])[+] sta[+] su(f)[+]  
 #26000 m[SL]  
 #26050 mei-9[L1]  
 #26060 N iav[+]  
 #26070 N iav[-]  
 #26100 Pgd[13] pn Zw[2]  
 #26210 Pgd[A]  
 #26220 Pgd[B]  
 #26250 Pgd[B] Fum[6] Hex-AB[2] Gpt[2] Zw[B]  
 #26400 Pgd[n39] pn Zw[2]

#26600 PgD[n39] pn Zw[27]  
 #26700 PgD[n39] pn Zw[n10]  
 #26900 PgD[n71] pn Zw[2]  
 #2700 C(1)DX, y f / Df(1)sc[8], w[a] / T(1;Y)60d12-2, y[2] sc[+] su(s)[+] su(w[a])[+] sta[+]  
 #27300 pn z  
 #27400 pn z w[is]  
 #27440 R(1)2, w[vC] / y w / y[+]Y  
 #27480 ras v dlg / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B (l(1) 10Bf and l(1)L11)  
 #27500 ras[3] m  
 #27600 ras[78f1]  
 #27700 rb cx  
 #27740 S iav[+] -- inactive mutant  
 #27741 S iav[-] -- inactive mutant  
 #27800 sc w[bl] ec  
 #27900 sc z ec  
 #2800 C(1)DX, y f / Df(1)sc[8], w[a] / T(1;Y)60d19-3, y[2] sc[+] su(s)[+] su(w[a])[+] sta[+] dor[+] hfw[+]e(dor[l])[+]  
 br[+]  
 #28200 sc z w[+UR] sn  
 #28300 sc z w[+UZ] sn  
 #28400 sc z w[+var] ec  
 #28500 sc z w[a]  
 #28600 sc z w[ch]  
 #28700 sc z w[h]  
 #28800 sc z w[is]  
 #28900 sc z w[negro] ec  
 #2900 C(1)DX, y f / Df(1)sc[8], w[a] / T(1;Y)60e17, y[2] sc[+] su(s)[+] su(w[a])[V+] sta[+]  
 #29100 sc z w[zmzrb]  
 #29150 sc z w[zmnzw]  
 #29200 sc z[59d15] w[is]  
 #29250 sn[3]  
 #29260 sn[34e]  
 #29300 sn[qr]  
 #29600 svr[poi-dish]  
 #29700 t  
 #29800 T(1;Y)59k9-2, y[2] sc[+] su(s)[2+] su(w[a])[V+] sta[V+] / y[59b] z  
 #29900 T(1;Y)60d19-4, y[2] sc[+] su(s)[2+] su(w[a])[V+] sta[2V+] / y[59b] z  
 #300 0 / C(1)RM, y v bb / XYS.YL(115-9), y[2] su(w[a]) w[a]  
 #3000 C(1)DX, y f / Df(1)sc-J4, sc[J4] / Dp(1;f)z9, z  
 #30150 v f[3N] car  
 #30170 v[24]  
 #30180 w  
 #30200 w cv sn[3]  
 #30230 w ct  
 #30250 w ct[6] f  
 #30260 w ct[6] hypoC  
 #30300 w m f  
 #30400 w mus(1)101[D1]  
 #3050 C(1)DX, y f / Df(1)svr, spl ras[2] fw / Dp(1;Y)y[2]Y[67g]  
 #30500 w mus(1)102[D1]  
 #30600 w sn[3]  
 #30700 w[56l12]  
 #30760 w[+](P)  
 #30800 w[+]Y / y w[a]  
 #30850 w[a] f su(f) / y[+]Ymal[171]  
 #30900 w[a] su(f)  
 #30950 w[a2]  
 #30960 w[a3]  
 #3100 C(1)DX, y f / Df(1)svr, svr / Dp(1;f)101, spl  
 #31000 w[a57i11]  
 #31100 w[a58l12]

#31200 w[aLue]  
 #31300 w[aMoss]  
 #31400 w[bf] f[5]  
 #31500 w[bf2]  
 #31600 w[Bwx]  
 #31700 w[ch] rb wy  
 #31900 w[ch] wy  
 #3200 C(1)DX, y f / Df(1)svr, svr spl ras[2] fw / y[2]Y[67g19.1]  
 #32000 w[co] sn[2]  
 #32050 w[col]  
 #32100 w[e]  
 #32200 w[h]  
 #32250 w[h] sn[3]  
 #32300 w[sat]  
 #32400 w[sp]  
 #32500 y w[sp2] / C(1)DX, y w f  
 #32550 w[sp55]  
 #32600 y  
 #32700 y ac w[a] ec  
 #32800 y ac w[aR60a5]  
 #32900 y ct[6] v f su(f)[lts67g]  
 #32940 y fli[sbdy]  
 #32950 y mei-9[a] mei-41[D5] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B  
 #3300 C(1)DX, y f / Df(1)v-L2, v[L2] / v[+]Yy[+]  
 #33000 y sc ec cv ct[6] v f[5] car  
 #33050 y mei-9[L1] cv / y[+]YB[S]  
 #33100 y sn[3]  
 #33200 y tuh-1  
 #33300 y v  
 #33350 y w P{w[+]-lacW}3-76a  
 #3340 C(1)DX, y f / Df(1)vt2, y vt[2] / w[+]Y  
 #33400 y w ct[6] m f  
 #33401 y w l(1)dlg-1 / Binsn, sc[8] sc[SI] sn[X2] / v[+]B[S-]Yy[+]  
 #33405 y w l(1)m2 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / v[+]B[s-]Yy[+]  
 #33406 y w l(1)m20 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / v[+]B[S-]Yy[+]  
 #33407 y w l(1)m40 / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B / v[+]B[S-]Yy[+]  
 #3345 C(1)DX, y f / Df(1)w64d, w[64d] / w[+]Y  
 #33450 y w sn[w]  
 #3350 C(1)DX, y f / Df(1)w64f1, w[64f1] / w[+]Y  
 #33600 y w[a] f / y[+]YB  
 #33650 y w[crr]  
 #33700 y w[i]  
 #33750 y w[sp3]  
 #33800 y z w[zm] sn[3]  
 #3400 C(1)DX, y f / Df(1)w258-45, y[2] w[258-45] / w[+]Y  
 #34000 y z[a]  
 #34100 y z[a1] w[ch]  
 #34200 y z[a48]  
 #34300 y[2]  
 #34400 y[2] eq / Ybb[-]  
 #3450 C(1)DX, y f / Df(1)w-rJ1, y[2] w[rJ1] spl ec sn[3] / w[+]Y  
 #34500 y[2] sc su(w[sp]) w[sp]  
 #34550 y[2] sc w[a] mei-9a / y[+]Y  
 #3460 C(1)DX, y f / Df(1)w-rJ2, w[rJ2] ec / w[+]Y  
 #34600 y[2] sc w[a] w[ch] fa  
 #3470 C(1)DX, y f / Df(1)y75a22-1.2, y[75a22-1.2] w mus(1)104[D1] / y[2]Y[67g19.1]  
 #34700 y[2] sc w[a59k1]  
 #3480 C(1)DX, y f / Df(1)z2, z[2] / w[+]Yy[+]  
 #34800 y[2] sc w[bf] spl sn[3]  
 #34900 y[2] sc w[i] w[ch]

#3500 C(1)DX, y f / Dp(1;1)3A6-3C2, y[2] ac z  
 #35000 y[2] sc w[sp]  
 #35200 y[2] su(w[a]) w[a]  
 #35300 y[2] su(w[a]) w[a] w[ch] fa  
 #35400 y[2] su(w[a]) w[a] w[is]  
 #35500 y[2] w[bf] spl sn[3]  
 #35550 y[59b] y[2] z[88h7(6)]  
 #35560 y[59b] z / y[2]Y  
 #35600 y[50k22] w[bf61d5]  
 #35700 z  
 #35800 z w[11e4]  
 #35850 z w[65a25] spl sn[3]  
 #35900 z[a1]  
 #3600 C(1)DX, y f / Dp(1;1)3C3, y[2] su(w[a]) w[a4]  
 #36000 z[var77h3]  
 #36100 Zw[A] (G-6-pd[F])  
 #36200 Zw[B] (G-6-pd[S])  
 #36300 a px or  
 #36400 ab[2] ix[2] bw sp[2] / In(2L)Cy In(2R)Cy, Cy dp[lv1] Bl cn[2] L[4] sp[2]  
 #36450 Adh[D] l(2)35Bg[OK5] pr cn / CyO, Cy dp[lv1] Adh[nB] pr cn[2] -- (synonym l(2)br26[OK5])  
 #36460 Adh[D] ck[CH52] pr cn / CyO, Cy dp[lv1] Adh[nB] pr cn[2] -- (synonym l(2)br27[CH52])  
 #36500 Adh[F]  
 #36510 Adh[n7] sna[HG31] cn (vg) / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br28[HG31])  
 #36520 Adh[n7] l(2)35Da[HG35] cn (vg) / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br35[HG35])  
 #36530 Adh[n7] lace[HG34] cn (vg), l(2)CA5[HG34] / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br36[HG34])  
 #36540 Adh[n10] l(2)35Cb[1] cn vg / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br33[HG38])  
 #36650 Adh[uf3] Su(H)[SF8] cn / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br7[SF8])  
 #36660 Adh[uf3] l(2)35Bd[SF6] rd[s] pr cn / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br9[SF6])  
 #36700 al b c sp[2]  
 #36750 al b l(2)49Ea[3] c sp[2] / SM5, al[2] Cy lt[v] cn[2] sp[2]  
 #36755 al dp eya[Ph] Sco l(2)37Cf[18] pr or[49h] / CyO, Cy dp[lv1] pr cn[2]  
 #36800 al dp b l(2)amnd[HI] / CyO, Cy dp[lv1] pr cn[2]  
 #36900 al dp b pr Bl c px sp / CyO, Cy dp[lv1] pr cn[2]  
 #3700 C(1)DX, y f / Dp(1;1)Bx[r49k], v f Bx[r49k] car  
 #37000 al dp b pr c px sp  
 #37100 al nub lt stw[2] sca[2] sp[2]  
 #37200 ap[4] / In(2LR)Rev[B], Rev[B]  
 #37300 ast ho ed dp cl  
 #3800 C(1)DX, y f / Dp(1;1)sc[V1] Df(1)mal3, y[2] y[+] sc[+] sc[V1] ct[6] f mal[3] / y[+]Ymal[+]  
 #38111 Asx[XF23] / CyO, Cy dp[lv1] pr cn[2]  
 #38180 b Adh[n1]  
 #38300 b Adh[n2] l(2)35Bc[AR6] pr cn / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br13[AR6])  
 #38400 b Adh[n4] l(2)35Bf[SF18] / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br2[SF18])  
 #38450 b Adh[S] pr cn  
 #38500 b el rd[s] pr cn  
 #38600 b el[2] Adh[F] / In(2L)t In(2R)Cy, Cy Roi pr cn  
 #38660 b el[8i1] cn[1] / CyO, Cy dp[lv1] pr cn[2]  
 #38700 b wb[SF20] Adn[n4], l(2)CA4 / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br1[SF20])  
 #38800 b wb[SF25] Adh[n2] pr cn / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br1[SF25])  
 #38900 b l(2)35Bc[AR1] pr / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br4[AR1])  
 #38950 b Su(H)[S5] pr / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br7[S5])  
 #3900 C(1)DX, y f / Dp(1;1)w[+R], y[2] z  
 #39000 b l(2)34Fc[SF9] Adh[n4] / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br8[SF9])  
 #39100 b l(2)35Be[AR3] pr / In(2L)Cy In(2R)Cy, al[2] Cy pr Bl cn[2] vg c sp[2] -- (synonym l(2)br10[AR3])  
 #39200 b l(2)35Aa[SF12] Adh[n4] / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br12[SF12])  
 #39300 b l(2)br14[SF23] Adh[n4] / CyO, Cy dp[lv1] pr cn[2]  
 #39400 b l(2)34Fd[CR5] Adh[n2] pr cn[2], l(2)CA3 / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br15[CR5])  
 #39500 b l(2)34Dg[SF10] Adh[n4] / In(2L)Cy In(2R)Cy, al[2] Cy pr Bl cn[2] vg c sp[2] -- (synonym l(2)br17[SF10])  
 #39600 b l(2)34Db[SF29] Adh[n2] pr cn / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br20[SF29])  
 #39650 b l(2)34Ea[SF15] Adh[n4] / CyO, Cy dp[lv1] pr cn[2] -- (synonym l(2)br24[SF15])

#39700 b lt l(2)? cn mi sp / In(2R)bw[VDe1], b bw[VDe1]  
 #39750 b pr cn eve[3.77] sca / CyO, Cy dp[lv1] pr cn[2]  
 #39752 b pr cn wx[wxt] Kr[9] If / SM1, al[2] Cy cn[2] sp[2]  
 #39753 b pr cn wx[wxt] Kr[17] If / SM1, al[2] Cy cn[2] sp[2] -- (synonym Kr[IV])  
 #39754 b pr cn wx[wxt] Kr[21] If / SM1, al[2] Cy cn[2] sp[2] -- (synonym Kr[V])  
 #39755 b pr cn wx[wxt] Kr18 If / SM1, al[2] Cy cn[2] sp[2] -- (synonym Kr[VI])  
 #39756 b pr cn wx[wxt] Kr[22] If / SM1, al[2] Cy cn[2] sp[2] -- (synonym Kr[VII])  
 #39757 b pr cn wx[wxt] Kr[23] If / SM1, al[2] Cy cn[2] sp[2] -- (synonym Kr[VIII])  
 #39758 b pr cn wx[wxt] Kr[24] If / SM1, al[2] Cy cn[2] sp[2] -- (synonym Kr[IX])  
 #39759 b pr cn wx[wxt] Kr[25] If / SM1, al[2] Cy cn[2] sp[2] -- (synonym Kr[X])  
 #39760 b pr cn wx[wxt] Kr[26] If / SM1, al[2] Cy cn[2] sp[2] -- (synonym Kr[XI])  
 #39761 b pr fs(2)PC4-U[133(IV)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39762 b pr grh[300(II)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39764 b pr l(2)PC4-A[138(I)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39766 b pr l(2)PC4-B[110(I)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39768 b pr l(2)PC4-D[202(II)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39770 b pr l(2)PC4-E[119(III)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39771 b pr l(2)PC4-F[198(III)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39773 b pr l(2)PC4-G[223(III)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39774 b pr l(2)PC4-H[236(III)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39775 b pr l(2)PC4-K[363(III)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39776 b pr l(2)PC4-M[420(III)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39777 b pr l(2)PC4-P[41(IV)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39779 b pr l(2)PC4-Q[267(IV)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39782 b pr l(2)PC4-T[459(IV)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39783 b pr Pcl[216(I)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #39785 b pr Pcl[E90] / CyO, Cy dp[lv1] pr cn[2]  
 #39786 b pr thr[313(I)] cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2]  
 #400 0 / C(1)RM, y v f / YSX.YL, In(1)dl-49, y v f car  
 #4000 C(1)DX, y f / Dp(1;1)w[+R61e19], z  
 #40240 b sna[19] pr cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2] -- (synonym sna[4.26])  
 #40250 b sna[20] pr cn wx[wxt] bw / CyO, Cy dp[lv1] pr cn[2] -- (synonym sna[18.19])  
 #40400 b Tft / CyO, Cy dp[lv1] pr cn[2] l(2)513[DTS]  
 #40460 bib[ID05] cn bw sp / CyO, Cy dp[lv1] pr cn[2]  
 #40500 B1 L[2] / SM5, al[2] Cy lt[v] cn[2] sp[2]  
 #40600 bur fs(2)E1 / SMS5, al[2] Cy lt[v] cn[2] sp[2]  
 #40630 bw  
 #40650 bw[D]  
 #40700 C(2)EN, b pr  
 #40800 C(2)EN, bw sp  
 #40900 C(2)EN, cn bw  
 #40950 C(2)RM, b ; C(2R)RM, cn  
 #4100 C(1)DX, y f / Dp(1;1)w[a], w[a]  
 #41000 C(2L)RM, b / C(2R)RM, vg  
 #41200 C(2L)RM(P3), j[63] / C(2R)RM(P4), px  
 #41300 C(2L)RM(P4), dp / C(2R)RM(P4), px  
 #41400 chl l(2)bw bw[2b] mr[2] / SM5, al[2] Cy lt[v] cn[2] sp[2]  
 #41500 cn  
 #41505 cn Asx[IIF51] bw sp / CyO, Cy dp[lv1] pr cn[2]  
 #41508 cn bw  
 #41510 cn bw Kr / SM1, al[2] Cy cn[2] sp[2]  
 #41520 cn bw sp Kr[2] / SM1, al[2] Cy cn[2] sp[2]  
 #41550 cn en Hx  
 #41560 cn mam[IB99] bw sp / CyO, Cy dp[lv1] pr cn[2]  
 #41580 CyO, Cy dp[lv1] pr cn[2] / Ddc[43] -- (synonym;Ddc[pl])  
 #41590 CyO, Cy dp[lv1] pr cn[2] / Ddc[ts1] fs(2)TW[1] pr sp l(2)248 absent, E(fs)[-]  
 #41600 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)64j, L[2]  
 #41605 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A48, b cn bw  
 #41606 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A63, b pr cn  
 #41607 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A72, b cn bw

#41608 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A220, b cn bw  
 #41609 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A245, b cn bw  
 #41611 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A260, b cn bw  
 #41612 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A263, b cn bw  
 #41613 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A266, b cn bw  
 #41614 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A267, b cn bw  
 #41617 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)A446, b cn bw Tp(2)446  
 #41620 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)fn1  
 #41621 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)fn5, pr cn  
 #41622 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)fn7, pr cn  
 #41623 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)fn30 pr cn  
 #41624 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)Sco7 -- (synonym Df(2L)Sco[rv7])  
 #41625 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / In(2LR)A379 Df(2L)A379, b cn bw  
 #41626 CyO, Cy dp[lv1] Adh[nB] pr cn[2] / l(2)34Eb[CH61] Adh[D] pr cn -- (synonym l(2)br31[CH61])  
 #41627 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TE35Bc(R)GW8, pr cn sp -- (synonym Df(2L)TE36(R)GW8)  
 #41628 CyO, Cy dp[lv1] b[81f1] pr cn[2] / Df(2L)b82a1, b[82a1] Adh[uf3] pr cn  
 #41629 CyO, Cy dp[lv1] b[81f1] pr cn[2] / Df(2L)b82a2, b[82a2] Adh[uf3] pr cn  
 #41630 CyO, Cy dp[lv1] pr cn[2] / cn Psc[IIN48] bw sp  
 #41631 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW2, Tft l(2)74i -- (synonym Df(2L)2)  
 #41635 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW3, l(2)74i -- (synonym Df(2L)3)  
 #41640 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW9, Tft cn -- (synonym Df(2L)9)  
 #41660 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW84 -- (synonym Df(2L)84)  
 #41670 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW119, cn bw -- (synonym Df(2L)119)  
 #41680 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW130, cn bw -- (synonym Df(2L)130)  
 #41700 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW137, M(2)? cn bw  
 #41750 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW150, cn bw -- (synonym Df(2L)150)  
 #41760 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW158, cn bw -- (synonym Df(2L)158)  
 #41800 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TW161, M(2)? cn bw -- (synonym Df(2L)161)  
 #41850 CyO, Cy dp[lv1] pr cn[2] / Df(2L)A16, cn bw  
 #41860 CyO, Cy dp[lv1] pr cn[2] / Df(2L)A20, cn bw  
 #41900 CyO, Cy dp[lv1] pr cn[2] / Df(2L)b75, b[75]  
 #4200 C(1)DX, y f / Dp(1;1)w[a], z w[a] w[a4]  
 #42000 CyO, Cy dp[lv1] pr cn[2] / Df(2L)b80c1, b[80c1] Sco  
 #42050 CyO, Cy dp[lv1] pr cn[2] / Df(2L)C75RL In(2L)75c[L]C158.1[R]  
 #42100 CyO, Cy dp[lv1] pr cn[2] / Df(2L)c11, cl[1] Adh[nC1] cn bw  
 #42200 CyO, Cy dp[lv1] pr cn[2] / Df(2L)c12, cl[2] Adh[nC1] cn bw  
 #42300 CyO, Cy dp[lv1] pr cn[2] / Df(2L)c17, cl[7] Adh[nC1] cn bw  
 #42350 CyO, Cy dp[lv1] pr cn[2] / Df(2L)E55, rdo hk pr  
 #42380 CyO, Cy dp[lv1] pr cn[2] / Df(2L)fn3, pr cn  
 #42385 CyO, Cy dp[lv1] pr cn[2] / Df(2L)H20, b pr cn sca  
 #42386 CyO, Cy dp[lv1] pr cn[2] / Df(2L)hk-UC1 -- (synonym Df(2L)hk[326])  
 #42449 CyO, Cy dp[lv1] pr cn[2] / Df(2L)noc10, b noc[10] cn bw  
 #42450 CyO, Cy dp[lv1] pr cn[2] / Df(2L)noc11, b noc[11] cn bw  
 #42451 CyO,Cy dp[lv1] pr cn[2] / Df(2L)OD15  
 #42453 CyO, Cy dp[lv1] pr cn[2] / Df(2L)osp18, osp[18] pr cn  
 #42455 CyO, Cy dp[lv1] pr cn[2] / Df(2L)osp29, osp[29] Adh[uf3] pr cn  
 #42457 CyO, Cy dp[lv1] pr cn[2] / Df(2L)osp38, osp[38] pr cn  
 #42460 CyO, Cy dp[lv1] pr cn[2] / Df(2L)osp144, osp[144] Adh[uf3] pr cn  
 #42486 CyO,Cy dp[lv1] pr cn[2] / hk[C1] or[49h] (hk[C1] EMS-Gamma induced)  
 #42500 CyO, Cy dp[lv1] pr cn[2] / Df(2L)pr76, Sco pr[76]  
 #42510 CyO, Cy dp[lv1] pr cn[2] / Df(2L)prd1.7, b pr cn sca  
 #42550 CyO, Cy dp[lv1] pr cn[2] / Df(2L)Sco10  
 #42555 CyO, Cy dp[lv1] pr cn[2] / Df(2L)Sco14, b  
 #42670 CyO, Cy dp[lv1] pr cn[2] / Df(2L)Sco25  
 #42680 CyO, Cy dp[lv1] pr cn[2] / Df(2L)T317, b pr cn sca  
 #42681 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TE35BC-GW4, b pr pk cn -- (synonym Df(2L)TE36GW4)  
 #42682 CyO, Cy dp[lv1] pr cn[2] / Df(2L)TE35BC-GW3, b pr pk cn sp -- (synonym Df(2L)TE36(R)GW3)  
 #42683 CyO,Cy dp[lv1] pr cn[2] / Df(2L)TE42-B7  
 #42684 CyO,Cy dp[lv1] pr cn[2] / Df(2L)VA17, Sco pr  
 #42684A CyO,Cy dp[lv1] pr cn[2] / Df(2L)VA19, Sco rdo pr

#42685 CyO, Cy dp[lv1] pr cn[2] / Df(2R)en-A, en[-A]  
 #42687 CyO, Cy dp[lv1] pr cn[2] / Df(2R)eve1.27, cn  
 #42688 CyO, Cy dp[lv1] pr cn[2] / Df(2R)gsb-ES1, b pr  
 #42689 CyO, Cy dp[lv1] pr cn[2] / Df(2R)Pc4  
 #42690 CyO, Cy dp[lv1] pr cn[2] / Df(2R)pk78k, pk[78k]  
 #42695 CyO, Cy dp[lv1] pr cn[2] / Df(2R)pk78s In(2R)pk[78s]  
 #42745 CyO, Cy dp[lv1] pr cn[2] / Dox-A2[2] pr or[49h]  
 #42755 CyO, Cy dp[lv1] pr cn[2] / Ddc[n27] l(2)37Cf[18] pr or[49h]  
 #42760 CyO, Cy dp[lv1] pr cn[2] / dp odd[5-36] b pr cn bw sp -- (synonym odd[IIIID36])  
 #42765 CyO, Cy dp[lv1] pr cn[2] / Dox-A2[mfs1] Bl  
 #42800 CyO, Cy dp[lv1] pr cn[2] / Dp(2;2)GYS, Df(2L)b80e3, b[80e3] pr bw  
 #42810 CyO, Cy dp[lv1] pr cn[2], Dp(2;2)n(2)m[+] / Df(2L)TW50, cn  
 #42820 CyO, Cy dp[lv1] pr cn[2] / el[3] Adh[uf3] cn  
 #42830 CyO, Cy dp[lv1] pr cn[2] / el[82f1]  
 #42845 CyO, Cy dp[lv1] pr cn[2] / Flg, b pr cn sca  
 #42846 CyO, Cy dp[lv1] pr cn[2] / fs(2) TW1[3]  
 #42850 CyO, Cy dp[lv1] pr cn[2] / In(2L)osp[22], osp[22] Adh[uf3] rd[s] pr cn  
 #42855 CyO, Cy dp[lv1] pr cn[2] / In(2L)Sco[R+2]  
 #42856 CyO, Cy dp[lv1] pr cn[2] / In(2L)Sco[R4] Df(2L)ScoR4  
 #42857 CyO, Cy dp[lv1] pr cn[2] / In(2L)Sco[R+8]  
 #42858 CyO, Cy dp[lv1] pr cn[2] / In(2L)Sco[R+17], b pr  
 #42859 CyO, Cy dp[lv1] pr cn[2] / In(2L)TW47, l(2)74i  
 #42859A CyO, Cy dp[lv1] pr cn[2] / In(2LR)bw[VI], ds[33k] bw[VI] l(2)18[DTS]  
 #42860 CyO, Cy dp[lv1] pr cn[2] / In(2LR)el[6], b el[6] cn bw  
 #42870 CyO, Cy dp[lv1] pr cn[2] / In(2LR)noc[4], b noc[4] cn bw  
 #42880 CyO, Cy dp[lv1] pr cn[2] / In(2LR)Sco[R+1]  
 #42890 CyO, Cy dp[lv1] pr cn[2] / In(2LR)Sco[R+9]  
 #42891 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bb[9] or [49h]  
 #42892 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bc[fs2] pr  
 #42893 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bd[4] or [49h]  
 #42894 CyO, Cy dp[lv1] pr cn[2] / l(2)37Be / or 49[h]  
 #42895 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bg / or 49[h]  
 #42896 CyO, Cy dp[lv1] pr cn[2] / l(2)37Cg[1] pr or[49h]  
 #42900 CyO, Cy dp[lv1] pr cn[2] / l(2)91[DTS]  
 #42901 CyO, Cy dp[lv1] pr cn[2] / l(2)7431 pr  
 #42940 CyO, Cy dp[lv1] pr cn[2] / l(2)35Ba[AR10] Adh[n11] pr -- (synonym l(2)br22[AR10])  
 #42943 CyO, Cy dp[lv1] pr cn[2] / l(2)37Ba[fs1]  
 #42945 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bc[10] (BL106)  
 #42946 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bc[fs1] pr  
 #42947 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bc[fs3] cn bw  
 #42948 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bc[fs1] cn bw  
 #42949 CyO, Cy dp[lv1] pr cn[2] / l(2)37Bc[fs3] cn bw  
 #42950 CyO, Cy dp[lv1] pr cn[2] / l(2)br30[HG15] Adh[n1] rd[s] pr cn, l(2)CA8[HG15]  
 #42960 CyO, Cy dp[lv1] pr cn[2] / l(2)amd[H60] cn bw (amd[9])  
 #42965 CyO, Cy dp[lv1] pr cn[2] / l(2)amd[H89] (amd[6])  
 #43000 CyO, Cy dp[lv1] pr cn[2] / M(2)38b[G]  
 #43050 CyO, Cy dp[lv1] pr cn[2] / MR-102 bw[V77k10(6)]  
 #43060 CyO, Cy dp[lv1] pr cn[2] / MR-T007  
 #43063 CyO, Cy dp[lv1] pr cn[2] / odd[3] b pr cn sca -- (synonym odd[1.36])  
 #43065 CyO, Cy dp[lv1] pr cn[2] / Pcl[13] -- (synonym Pcl[X21])  
 #43066 CyO, Cy dp[lv1] pr cn[2] / Pcl[15] -- (synonym Pcl[XM3])  
 #43067 CyO, Cy dp[lv1] pr cn[2] / Pcl[XM82]  
 #43068 CyO, Cy dp[lv1] pr cn[2] / prd[4], b pr cn sca -- (synonym prd[2.45])  
 #43069 CyO, Cy dp[lv1] pr cn[2] / prd[7] -- (synonym prd[32.12])  
 #43070 CyO, Cy dp[lv1] pr cn[2] / P%, cn bw sp  
 #43070A CyO, Cy dp[lv1] pr cn[2] / Su(z)2[1]  
 #43071 CyO, Cy dp[lv1] pr cn[2] / Su(z)2[IR]  
 #43072 CyO, Cy dp[lv1] pr cn[2] / scw[4-18] b pr -- (synonym scw[005-18])  
 #43073 CyO, Cy dp[lv1] pr cn[2] / scw[1-14] b pr -- (synonym scw[CC13-14])  
 #43074 CyO, Cy dp[lv1] pr cn[2] / scw[3-16] b pr -- (synonym scw[N5-16])

#43075 CyO, Cy dp[lv1] pr cn[2] / scw[4-4] b pr -- (synonym scw[SS12-4])  
#43076 CyO, Cy dp[lv1] pr cn[2] / rdo hk l(2)37Cf[1] pr or[49h]  
#43080 CyO, Cy dp[lv1] pr cn[2] / wg[l-X2] b pr -- (synonym wg[CX2])  
#43085 CyO, Cy dp[lv1] pr cn[2] / wg[l-X3] b pr -- (synonym wg[CX3])  
#43090 CyO, Cy dp[lv1] pr cn[2] / wg[l-X4] b pr -- (synonym wg[CX4])  
#43095 CyO, Cy dp[lv1] pr cn[2], Dp(2;2)M(2)m[+] / Df(2L)TW2, Tft l(2)74i  
#43095A CyO, Cy dp[lv1] pr cn[2], Dp(2;2)M(2)m[+] / Df(2L)TW203, M pr cn  
#43096 CyO, Cy dp[lv1] pr cn[2], Dp(2;2)M(2)m[+] / Df(2L)M(2)m[S6]  
#43096A CyO, Cy dp[lv1] pr cn[2], Dp(2;2)M(2)m[+] / Df(2L)VA18, m pr  
#43098 CyO, Cy Roi cn[P] bw / Pi ;Pi2  
#43100 da abo / In(2L)Cy In(2R)Cy, Cy cn[2]  
#43200 Df(2L)75c / In(2L)Cy In(2R)Cy, al[2] Cy pr Bl cn[2] vg c sp[2]  
#43600 Df(2L)A178, b rd[s] pr cn / In(2L)t In(2R)Cy, Cy Roi b[77.1x] bw[45a] sp[2] or[45a]  
#43700 Df(2L)A217, b cn bw / In(2LR)Gla, Gla l(2)34De[SF16] l(2)br3[TA2] -- (synonym l(2)br16[SF16])  
#4400 C(1)DX, y f / Dp[2](1;1)3C3, z w[a] w[a4]  
#4450 C(1)DX, y f / eo[47-2], In(1)sc[8], y[31d]w[a]47-2 / y[+]Ymal[+]  
#44900 Df(2L)nNXF1, b Adh[nNXF1] / In(2L)t In(2R)Cy, Cy Roi pr cn -- (synonym Df(2L)Adh1)  
#4500 C(1)DX, y f / f B os[o] car  
#45000 Df(2L)nNXF2, b Adh[nNXF2] / In(2L)t In(2R)Cy, Cy Roi pr cn -- (synonym Df(2L)Adh2)  
#45110 Df(2L)ast1 / SM1, al[2] Cy cn[2] sp[2]  
#45120 Df(2L)ast2 / SM1, al[2] Cy cn[2] sp[2]  
#45130 Df(2L)ast3 / SM1, al[2] Cy cn[2] sp[2]  
#45140 Df(2L)ast4 / SM1, al[2] Cy cn[2] sp[2]  
#45150 Df(2L)ast5 / SM1, al[2] Cy cn[2] sp[2]  
#45160 Df(2L)ast6 / SM1, al[2] Cy cn[2] sp[2]  
#45170 Df(2L)b87e25 / In(2L)NS  
#45200 Df(2L)C' / SM1, al[2] Cy cn[2] sp[2]  
#45210 Df(2L)cl-h1, cl[h1] / In(2L)Cy[L]t[R] In(2R)Cy, Cy Roi cn sp  
#45220 Df(2L)cl-h2, cl[h2] / In(2L)Cy[L]t[R] In(2R)Cy, Cy Roi cn sp  
#45225 Df(2L)cl-h3, cl[h3] / In(2L)Cy[L]t[R] In(2R)Cy, Cy Roi cn sp  
#45230 Df(2L)cl-h4, cl[h4] / In(2L)Cy[L]t[R] In(2R)Cy, Cy Roi cn sp  
#45231 Df(2L)dp79b, dp[DA] cn / In(2LR)bw[VI], b  
#45232 Df(2L)dp-h24, dp[h24] / In(2L)Cy[L]t[R] In(2R)Cy, Cy Roi cn sp  
#45233 Df(2L)dp-h25, dp[h25] / In(2L)Cy[L]t[R] In(2R)Cy, Cy Roi cn sp  
#45240 Df(2L)edSz-1, al b / SM5, al[2] Cy lt[v] sn[2] sp[2]  
#45250 Df(2L)el-77, Coi el[77] / In(2LR)Gla, Gla  
#45270 Df(2L)esc10, b pr / CyO, Cy dp [lv1] pr cn[2]  
#45300 Df(2L)Gdh-A, dp L / SM1, al[2] Cy cn[2] sp[2]  
#45350 Df(2L)J136-H52 / SM5, al[2] ds[33k] Cy lt[v] cn[2] sp[2]  
#45400 Df(2L)J-der-2 / SM1, al[2] Cy cn[2] sp[2]  
#45450 Df(2L)J-der-27 / SM1, al[2] Cy cn[2] sp[2]  
#45455 Df(2L)l(2)gl-net3 / Cy  
#45460 Df(2L)l(2)gl-U314 / Cy  
#45470A Df(2L)net14 / Cy  
#45470B Df(2L)net18 / Cy  
#45471 Df(2L)net62 / Cy  
#45472 Df(2L)net l(2)gl[78;30]  
#45475 Df(2L)net-PM22 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45476 Df(2L)net-PM29A / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45480 Df(2L)net-PM47C /SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485 Df(2L)net-PM86A / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485A Df(2L)PMA / SM1, al[2] Cy cn[2] sp[2]a  
#45485B Df(2L)PMC / SM6a, al[2] Cy dp[lv1] cn[2] sp2]  
#45485C Df(2L)PMD / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485D Df(2L)PMF / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485E Df(2L)PMG / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485F Df(2L)PM1 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485G Df(2L)PM4 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485H Df(2L)PM11 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485K Df(2L)PM44 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]

#45485L Df(2L)PM45 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485M Df(2L)PM51 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485N Df(2L)PM73 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485O Df(2L)PM82 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485P Df(2L)PM85 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45485R Df(2L)PM91 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45487 Df(2L)Pr1 / CyO, Cy dp[lv1] pr cn[2]  
#45490 Df(2L)r10, cn / In(2LR)O, Cy dp[lv1] pr cn[2]  
#45500 Df(2L)S2, S / In(2L)Cy In(2R)Cy, E(S) Cy cn[2]  
#45600 Df(2L)S3, S / SM1, al[2] Cy cn[2] sp[2]  
#45650 Df(2L)TE75, w[+] / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#45670 Df(2L)TW161, M pr cn bw / CyO, Cy dp[lv1] pr cn[2]  
#45700 Df(2L)wg-CX3, b pr / CyO, Cy dp[lv1] pr cn[2]  
#45800 Df(2L)spdX4, In(2L)spdX4, al dp / CyO, Cy dp[lv1] pr cn[2]  
#45850 Df(2L)TW84 / CyO, Cy dp[lv1] pr cn[2]  
#45900 Df(2R)42, en / SM1, al[2] Cy cn[2] sp[2]  
#45910 Df(2R)44CE, al dp b pr / CyO, Cy dp[lv1] pr cn[2]  
#45940 Df(2R)AA21, In(2R)AA21, c px sp / SMI, al[2] Cy cn[2] sp[2]  
#45950 Df(2R)bwS46 / Cy ?  
#45960 Df(2R)cn9 / Cy Roi  
#4600 C(1)DX, y f / f B[i]B[i]  
#46000 Df(2R)Kr-AP1, b pr Bl c If[R] / SM1, al[2] Cy lt[v] cn[2] sp[2]  
#46010 Df(2R)Kr-SB1, b pr Bl c If[R] / SM1, al[2] Cy lt[v] cn[2] sp[2]  
#46050 Df(2R)cn88b, cn / SM5, al[2] Cy lt[v] cn[2] sp[2]  
#46090 Df(2R)Kr10, b pr Bl e If[R] / SMI, al[2] Cy cn[2] sp[2]  
#46100 Df(3R)M-c33a, M(2)c[33a] / In(2LR)bw[V32g], bw[32g]  
#46150 Df(2R)PuD17, cu bw sp / SM1, al[2] Cy cn[2] sp[2]  
#46300 Df(2R)M-S8, M(2)S8 / SM1, al[2] Cy cn[2] sp[2] -- (synonym Df(2R)M-S2[8])  
#46400 Df(2R)M-S10, M(2)S10 / SM1, al[2] Cy cn[2] sp[2] -- (synonym Df(2R)M-S2[10])  
#46500 Df(2R)Px2, bw sp Px[2] / SM1, al[2] Cy cn[2] sp[2]  
#46550 Df(2R)Px4, dp b Px[4] / In(2LR)Gla, Gla  
#46600 Df(2R)r10a, lt rl[10a] cn / In(2LR)bw[V1], al[4] ds[33k] lt bw[V1]  
#46650 Df(2R)r10b, lt rl[10b] cn / In(2L)Cy In(2R)Cy, al[2] Cy lt[3] cn[2] (L[4]) sp[2]  
#46670 Df(2R)trix / Cy?  
#46750 Df(2R)vg135 / CyO, S dp cn bw  
#46800 Df(2R)vg-B, vg[B] / SM5, al[2] Cy lt[v] cn[2] sp[2]  
#4700 C(1)DX, y f / fu  
#47050 Df(2R)vg-D, vg[D] / SM5, al[2] Cy lt[v] cn[2] sp[2]  
#47070 Df(2R)vg-W, vg[W] / In(2L)t In(2R)Cy, Roi cn[2] bw or sp[2]  
#47150 dp wg[CP1+RC6] cn bw / SM5, al[2] ds[33k] Cy lt[v] cn[2] sp[2]  
#47160 dp wg[CP1+RC7] cn bw / SM5, al[2] ds[33k] Cy lt[v] cn[2] sp[2]  
#47200 Dp(2;2)S, net S ast S ast[4] dp cl / In(2L)Cy In(2R)Cy, E(S) Cy cn[2]  
#47300 ds S G b pr / In(2L)Cy In(2R)Cy, al[2] Cy lt[3] cn[2] L[4] sp[2]  
#47350 ds[rv] ft dp[v2] l(2)M b pr / SM5, al[2] ds[33k] Cy lt[v] cn[2] sp[2]  
#47400 ds[W] / In(2L)Cy[L]t[R], Su(S) b pr  
#47500 ex ds S[X] ast[X] / SM1, al[2] Cy cn[2] sp[2]  
#47550 eya  
#47560 eya[ph]  
#47600 fs(2)B Alu lt / SM5, al[2] Cy lt[v] cn[2] sp[2]  
#47650 Got-2[6] Gpdh[2] cMdh[6] Adh[2] Dip-A[2] Hex-C[2] Amy[2,3] / SM1, al[2] Got-2[4] Cy Gpdh[4] cMdh[4]Adh[4] cn[2] Dip-A[4] Hex-C[4] Amy[1] sp[2]  
#47700 hy a px sp / SM1, al[2] Cy cn[2] sp[2]  
#47900 In(2L)Cy, Cy / In(2LR)bw[V1], ds[33k] bw[V1]  
#4800 C(1)DX, y f / gt[13z] / w[+]Y  
#48000 In(2L)Cy In(2R)Cy, al[2] Cy lt[3] cn[2] L[4] sp[2] / S Sp Bl bw[D]  
#48100 In(2L)Cy In(2R)Cy, al[2] Cy pr Bl cn[2] L[4] sp[2] / rk[5] ta cn bw  
#48200 In(2L)Cy In(2R)Cy, al[2] Cy pr Bl cn[2] vg c sp[2] / In(2L)dp[1], dp Adh[nC1] cn bw  
#48300 In(2L)Cy In(2R)Cy, al[2] Cy pr Bl cn[2] vg c sp[2] / In(2L)dp[2], dp Adh[nC1] cn bw  
#484000 In(2L)Cy In(2R)Cy, Cy cn[2] / M(2)40c[2] -- (synonym M(2)40c[G])  
#48500 In(2L)Cy In(2R)Cy, Cy cn[2] / M(2)60E[2] -- (synonym M(2)c[G])

#48600 In(2L)Cy In(2R)Cy, Cy cn[2] / M(2)25A[2] -- (synonym M(2)z[G1])  
 #48800 In(2L)Cy In(2R)Cy, Cy cn[2] / MR[h12] Bl  
 #48900 In(2L)Cy In(2R)Cy, Cy cn[2] / MR[h12] bw[v76j61]  
 #4900 C(1)DX, y f / In(1)AB  
 #49000 In(2L)Cy In(2R)Cy, Cy cn[2] L[4] sp[2] / ms(2)2 cn mr  
 #49150 In(2L)Cy In(2R)Cy, Cy pr / Su(H)  
 #49200 In(2L)Cy In(2R)Cy, E(S) Cy cn[2] / S  
 #49300 In(2L)Cy In(2R)Cy, S[2] Cy pr Bl cn[2] L[4] bw sp[2] / In(2L)NS In(2R)NS, px sp  
 #49400 In(2L)Cy In(2R)Cy, S[2] E(S) Cy dp[lv2] cn[2] / lm  
 #49500 In(2L)Cy In(2R)Cy bw[V34kR], Cy bw[V34k] / M(2)30A[3] -- (synonym M(2)e[S])  
 #49600 In(2L)Cy In(2R)NS, Cy / nw[2]  
 #49700 In(2L)t In(2R)Cy, Roi cn[2] bw sp or / In(2R)vg[U], vg[U]  
 #49850 In(2L)wg[P] / In(2LR)Gla, Gla  
 #49900 In(2LR)102, ds[W] sp[2] / SM1, al[2] Cy cn[2] sp[2]  
 #500 0 / C(1)RM, z sn / YSX.YL, In(1)EN y w f  
 #50000 In(2LR)bw[V1], ds[33k] bw[V1] / px bw mr sp  
 #50010 In(2LR)Gla, Bc / In(2R)bw[VDe2]  
 #50100 In(2LR)Gla, Gla / In(2R)bw[VDe2L]Cy[R], bw[VDe2]  
 #50101 In(2LR)Gla, Gla / wg[CP1+RC3]  
 #50110 In(2LR)Gla, Gla l(2)34De[2] / In(2LR)O+Df(2R)cnS5, Cy dp[lv1] pr cn[-] -- (synonym br16[SF16])  
 #50115 Kr[L14] / SM1, al[2] Cy cn[2] sp[2] -- (synonym Df(2R)Kr14)  
 #50200 l(2)2[DTS] / SM1, al[2] Cy cn[2] sp[2]  
 #50250 l(2)crc cn / R(2)Bl  
 #50300 l(2)gl a px or / SM5, al[2] Cy lt[v] cn[2] sp[2]  
 #50350 l(2)M6 / Cy  
 #50351 l(2)M11 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
 #50360 l(2)PM13 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
 #50361 l(2)PM59 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
 #50370 l(2)R2 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
 #50371 l(2)R23 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
 #50374 l(2)r213 / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
 #50700 M(2)58F[1] / SM1, al[2] Cy cn[2] sp[2] -- (synonym M(2)l[2])  
 #50800 M(2)41A[1] / SM5, al[2] Cy lt[v] cn[2] sp[2] -- (synonym M(2)S2[3])  
 #50900 M(2)41A[2] / SM5, al[2] Cy lt[v] cn[2] sp[2] -- (synonym M(2)S2[9])  
 #5100 C(1)DX, y f / In(1)sc[4L]sc[8R], y sc[4] sc[8] / y[+]Y  
 #51000 M(2)53 / SM5, al[2] Cy lt[v] cn[2] sp[2] -- (synonym M(2)S7)  
 #51100 M(2)30A[3] / SM5, al[2] Cy lt[v] cn[2] sp[2] -- (synonym M(2)S11)  
 #51200 M(2)25A[1]es / SM5, al[2] Cy lt[v] cn[2] sp[2] -- (synonym M(2)z)  
 #51400 MR-102  
 #51660 pys  
 #51700 shr bw[2b] abb sp / SM5, al[2] Cy lt[v] cn[2] sp[2]  
 #51900 SM5, al[2] Cy lt[v] cn[2] sp[2] / Sp Bl L[rm] Bc Pu[2] Pin  
 #5200 C(1)DX, y f / In(1)sc[4L]sc[8R] In(1)S, y sc[4] / y[+]Y  
 #52100 vg  
 #52200 vg bw  
 #52600 abl[1] kar red e / TM6B, e Tb ca  
 #52700 abx[1] pbx[1] e[11] / TM1, Me ri sbd[1]  
 #52900 Acph-1[B]  
 #5300 C(1)DX, y f / In(1)sc[7] In(1)B[M1], sc[7] w[43b] B[M1]  
 #53000 Acph-1[C]  
 #53100 Acph-1[n13]  
 #53250 Antp[73b] / TM3, y[+] ri sep Sb bx[34e] e[s]  
 #53255 Antp[73b + R21] / TM3, y[+] ri sep Sb bx[34e] e[s]  
 #53260 Antp[D42] / TM3, y[+] ri sep Sb bx[34e] e[s]  
 #53270 Antp[D43] / TM3, y[+] ri sep Sb bx[34e] e[s]  
 #53300 bx[34e] Mc / Tp(3)bx[107], bx bxd[107] sr e[s]  
 #53600 C(3)x, M(3)x e[x] / ru h th st p[p] H e[s] ro  
 #5370 C(1)DX, y f / In(1)sc[8] Df(1)48-2, w[a] / y[+]Y mal  
 #53700 C(3L)RM, ri / C(3R)RM, sr  
 #53800 C(3L)RM, se h[2] rs[2] / C(3R)RM, sbd gl e[s]

#53900 C(3L)RM(P3), ri / C(3R)RM(P3), sr  
 #5400 C(1)DX, y f / In(1)sc[S1L]sc[4R] In(1)S, y sc[4] sc[S1] / y[+]sc[8]Y  
 #54100 ca K-pn  
 #54200 Cbx Ubx gl[3] / TM1, Me ri sbd[I]  
 #54300 cp in ri p[p]  
 #54400 cu kar ry[8]  
 #54500 cu kar Sb / Df(3R)kar-D1, In(3R)AFA, e  
 #54600 cu kar Sb / Df(3R)kar-D3, In(3R)AFA, e  
 #54620 CyO, Cy dp[lv1] pr cn[2] / rdo Ddc[101]  
 #54640 Df(3L)29A6, ri p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #54643 Df(3L)81K19 / TM6B, e Tb ca  
 #54645 Df(3L)ACI, roe p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #54650 Df(3L)BK9 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #54652 Df(3L)Cat / TM6, Hn[P] ss[P88] bx[34e] Vbx[P15] e  
 #54655 Df(3L)fzD21 / TM6B, e Tb ca  
 #54660 Df(3L)fzGF3b / TM6B, e Tb ca  
 #54670 Df(3L)GN24 / TM8, l(3)4[DTS] th st Sb e  
 #54670A Df(3L)h-i22, Ki roe p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #54671 Df(3L)HR119 / TM6B, e Tb ca  
 #54671A Df(3L)HR232 / TM6B, e Tb ca  
 #54672 Df(3L)HR370 / Dp(3;3)T33[L]f19R  
 #54680 Df(3L)in61, in[61jl] / TM1, Me ri p[p] sbd[I]  
 #54683 Df(3L)Ly, mwh / TM1, Me re jv sbd[lv1]  
 #54685 Df(3L)M21, ri p[p] / Dp(3;3)T33[L]f19[R]  
 #54690 Df(3L)Pc, cp in ri Pc p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #54691 Df(3L)Pc-MK / TM3, y[+] ri[p] sep Sb bx[34e] e[s] Ser  
 #54700 Df(3L)Pc Df(3R)bxd100, cp in ri Pc p[p] bxd[100] / TM1, Me ri sbd[I]  
 #54720 Df(3L)pbl-X / TM6B, e Tb ca, h D[3] Hu e (w floating)  
 #54740 Df(3L)rdgC, th st in ri p[p] / TM6C, Tb Sb cu e[s]  
 #54750 Df(3L)ri79c / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #54760 Df(3L)R / TM6C, Tb Sb  
 #54780 Df(3L)st7 / TM3, y[+] ri[p] sep Sb bx[34e] e[s] Ser  
 #54790 Df(3L)st-f13, Ki roe p[p] / TM6B, e Tb ca  
 #54800 Df(3L)st4, st[4] gl[2] e[4] / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e  
 #54900 Df(3L)st6, st[6] gl[2] e[4] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #5500 C(1)DX, y f / In(1)sdx, sdx  
 #55100 Df(3L)th102, th[ss102] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #55320 Df(3L)vin2 / TM3, y[+] ri[p] sep Sb bx[34e] e[s] Ser  
 #55330 Df(3L)VW3 / TM3, y[+] ri[p] sep Sb bx[34e] e[s] Ser  
 #55340 Df(3L)W4, ru h e ca / TM6B, e Tb ca (w[+R4])  
 #55350 Df(3L)W10, ru h sbd[2] / TM6B, e Tb ca (synonym w[+R10])  
 #55400 Df(3R)84B3, ri / TM1, Me ri sbd[I]  
 #55401 Df(3R)awd-KRB, ca / TM3, y[+] ri[p] sep Sb bx[34e] e[s] Ser  
 #55403 Df(3R)C4, p[?] / Dp(3;3)P5, Sb  
 #55404 Df(3R)crbS87-4, st e / TM3, Ser  
 #55405 Df(3R)crbS87-5, st e / TM3, Ser  
 #55406 Df(3R)ChaM7 / TM6B, Tb  
 #55410 Df(3R)89E1-2 / TM1, Me ri sbd[I]  
 #55430 Df(3R)e-RI / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #55440 Df(2R)E229, th st / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym Df(3R)229)  
 #55450 Df(3R)Antp17 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym Df(3R)Antp[Ns+R17])  
 #55460 Df(3R)bxd100, bxd[100] / TM1, Me ri sbd[I]  
 #55465 Df(3R)by10, red e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #55470 Df(3R)C1 Abd-B[M1] e[11] ro ca / TM1, Me ri sbd[I]  
 #55500 Df(3R)cu, cu Sb / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e -- (synonym Df(3R)cu[40])  
 #55600 Df(3R)dsx5, bx sr e[s] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym Df(3R)dsx[D+R5])  
 #55800 Df(3R)E-079 / MKRS, ry[2] Sb  
 #55860 Df(3R)E307 / TM3, y[+] ri[p] sep Sb bx[34e] e[s] Ser  
 #55870 Df(3R)e-N19 / TM2, Ubx[130] e[s]  
 #560 l(1)A112[GF314] / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B

#5600 C(1)DX, y f / y l(1)zw1[b22] / w[+]Y  
 #56000 Df(3R)kar37, kar[37] e / l(3)B[DTS] Sb e  
 #56200 Df(3R)kar3l, kar[3l] / MKRS, ka r ry[2] Sb  
 #56300 Df(3R)karD2 In(3R)AFA, kar[D2] e / cu kar Sb  
 #56330 Df(3R)karH10 / TM2, Ubx[130] e[s]  
 #56350 Df(3R)kar-LG27 In(3R)G27 / MKRS, kar ry[2] Sb  
 #56500 Df(3R)kar-Sz-8, kar[Sz8] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #56600 Df(3R)kar-Sz-11, kar[Sz-11] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #56700 Df(3R)kar-Sz-12, kar[Sz-12] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #56800 Df(3R)kar-Sz-16, kar[Sz-16] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #56900 Df(3R)kar-Sz-21, kar[Sz-21] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #5700 C(1)DX, y f / z l(1)zw1[d13] sgg[4] spl sn[3] / w[+]Y -- (synonym l(1)zw3[d13])  
 #57100 Df(3R)kar-Sz-29, kar[Sz-29] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #57200 Df(3R)kar-Sz-31, kar[Sz-31] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #57300 Df(3R)kar-Sz-33, kar[Sz-33] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #57400 Df(3R)kar-Sz-37, kar[Sz-37] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #57500 Df(3R)kar1w, kar[1w] e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #57550 Df(3R)kar3J, ru h th st kar[3J] sr e[s] ca / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #57600 Df(3R)kar3L, kar[3L] / MRS, ry[2] Sb  
 #57800 Df(3R)karH5, kar[H5] / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e  
 #57940 Df(3R)l26c, kar[2] / MKRS, kar ry[2] Sb  
 #57950 Df(3R)IC4a / MRS, ry[2] Sb  
 #57952 Df(3R)M-Kx1 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #57954 Df(3R)pXT103, ru st e ca / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #57955 Df(3R)P9 / Dp(3;3)P5  
 #5800 C(1)DX, y f / sgg[1] / w[+]Y (synonym l(1)zw3[b12])  
 #58000 Df(3R)P-16 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #58100 Df(3R)P-21 / MKRS, kar ry[2] Sb  
 #58150 Df(3R)P47 / Dp(3;3)MRS, ry[2] Sb  
 #58200 Df(3R)P-58 / MRS, ry[2] Sb  
 #58250 Df(3R)red1 / TM1, Me ri red sbd[1]  
 #58290 Df(3R)red21, red[21] / TM1, Me ri red sbd[1]  
 #58300 Df(3R)red3l, red[3l] / MKRS, kar ry[2] Sb  
 #58350 Df(3R)red-P93, l(3)tr red[P93] Sb / In(3L)P In(3R)P18, Me Ubx e[4]  
 #58355 Df(3R)ry, ry / In(3LR)Ubx[130], Ubx[130] e[s]  
 #58400 Df(3R)ry36, ry[36] / MKRS, kar ry[2] Sb  
 #58550 Df(3R)ry81, ry[81] / MKRS, kar ry[2] Sb  
 #58590 Df(3R)ry614, ry[614] / MKRS, kar ry[2] Sb  
 #58700 Df(3R)ry615, ry[615] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #58751 Df(3R)ry619, ry[619] / MKRS, kar ry[2] Sb  
 #58755 Df(3R)sbd26, mwh sbd[26] e / TM2, Ubx[130] e[s]  
 #58760 Df(3R)sbd45, mwh sbd[45] e / TM2, Ubx[130] e[s]  
 #58850 Df(3R)Scr, Scr p[p] e[s] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #58880 Df(3R)TI-P, e ca / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #58890 Df(3R)Tp110, Dp(3;3) Fdf[rvXI], ri p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] ec Ser  
 #58900 Df(3R)T-01, ri cu sr e[s] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #59100 Df(3R)T-05, (ri) cu sr e[s] / MKRS, kar ry[2] Sb  
 #59200 Df(3R)T-10, ri cu sr e[s] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser (Df(3R)TE10)  
 #59300 Df(3R)T-25, (ri) cu sr e[s] / MKRS, kar ry[2] Sb  
 #59400 Df(3R)T-29, (ri) cu sr e[s] / MKRS, kar ry[2] Sb (Df(3R)TE29)  
 #59600 Df(3R)T-41, ri cu sr e[s] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser (Df(3R)TE41)  
 #59700 Df(3R)T-43, ri cu sr e[s] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser (Df(3R)TE43)  
 #59750 Df(3R)T-45 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser (Df(3R)TE45)  
 #59800 Df(3R)T-47, (ri) cu sr e[s] / MRS, ry[2] Sb (Df(3R)TE47)  
 #59900 Df(3R)T-55, (ri) cu sr e[s] / MRS, ry[2] Sb (Df(3R)TE55)  
 #59950 Df(3R)T-61, (ri) cu sr e[s] / MRS, ry[2] Sb (Df(3R)TE61)  
 #59970 Df(3R)XTAI, th st ri roe p / Dp(3;3)Su M(3)w13, st e  
 #600 amn[PS801]  
 #6000 C(1)DX, y f / l(1)zw6[e13] / w[+]Y  
 #60000 DI H e[s] cd / In(3R)P, spr

#60100 Di[14] / In(3R)P, Cyd  
 #60150 Dp(3;3)P146 Df(3R)P115 M(3)67C[4] e[11] / TM1, Me ri sbd[l] -- (synonym M(3)i[55])  
 #60180 Dp(3;3)S2, ru[g] jv Ly red cv-c sbd[2] / In(3LR)HR33[L] bxd[106R]  
 #60200 Dp(3;3)VV7, gl[2] e[4] / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e  
 #60250 Dr[ME4] / TM6B, Tb  
 #60260 Dr[ME8] / TM6B, Tb  
 #60300 dsx[60] / TM1, Me ri sbd[l]  
 #60350 e[4] wo ro  
 #60400 e[11]  
 #60500 e[s] ca[nd] / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e  
 #60600 eg rn[3] p[p] bx sr e[s] ca / In(3LR)Ubx[130], ri Ubx[130] e[s] ca  
 #60700 Est-6[4] Est-C[6] Odh[2] Lap-D[4] AcpH-1[2] Tpi[6] / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e Est-6[6] Est-C[4] Odh[4] Lap-D[2] AcpH-1[4] Tpi[4]  
 #60900 Est-6[F] Lap-A[O]  
 #61000 Est-6[F] Lap-A[F]  
 #61200 Est-6[S] Est-C[S]  
 #61250 Est-6[S] Lap-A[O]  
 #61300 st-6[S] Lap-A[F]  
 #61350 Est-C[VF]  
 #61360 flr[3] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser Ser  
 #61500 h Pc[2] sr e[s] / TM1, Me ri sbd[l]  
 #61600 h th st cp in ri p[p] ss[a] bx[3] sr e[s] / TM1, Me ri sbd[l]  
 #61700 th st cp in ri Pc[2] sr[61j2] / TM2, ri Ubx[130] e[s] ca  
 #61750 hb[FF8] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s]  
 #61800 H[2] / Tp(3)Vno, Vno  
 #61900 Idh[6] To[2] Pgm[6] Ald[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser Idh[4] To[4] Pgm[4] Ald[4]  
 #62000 In(3L)D, D[3] / In(3L)P  
 #62100 In(3L)Fd / TM1, Me ri sbd[l]  
 #62200 In(3L)fz[3], fz[3] gl[2] e[4] / TM1, Me ri sbd[l]  
 #62400 In(3L)P, Me / M(3)69E[5] -- (synonym M(3)h[y])  
 #62500 In(3L)VV11, gl[2] e[4] / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e  
 #62600 In(3L)VV14, p[p] gl[2] e[4]  
 #62700 In(3LR)CxD, D / W Sb  
 #62900 In(3LR)DcxF, ru h D / th st cp Pc[2]  
 #6300 C(1)DX, y f / l(1)zw11[b18] / w[+]Y  
 #63100 In(3LR)Ubx[101], Ubx[101] / Sb  
 #63200 In(3LR)Ubx[130], ri p[p] Ubx[130] e[s] / th st cp in ri Antp[Scx] p[p] sr[R947]  
 #63400 In(3LR)Ubx[130], Ubx[130] / M(3)67C[5] -- (synonym M(3)i[G1])  
 #63500 In(3LR)Ubx[130], Ubx[130] / M(3)67C[6] -- (synonym M(3)i[G2])  
 #63700 In(3R)Antp[B], Antp[B] / TM1, Me ri sbd[l]  
 #63800 In(3R)C, cd / Sb H  
 #63900 In(3R)C, e l(3)e / M(3)w[124] -- (synonym M(3)95A[2])  
 #64000 In(3R)C, l(3)a / k[D] e[s] Bd[G]  
 #64100 In(3R)C, l(3)a / M(3)96C[2] -- (synonym M(3)be[36e])  
 #64300 In(3R)Dl[B], st Dl[B] / In(3R)P, st l(3)W ca  
 #64400 In(3R)Hu, Hu Sb[Spi] / Payne, l(3)PL l(3)PR  
 #64500 jv Hn[r] h  
 #64600 kar[2]  
 #64700 Ki  
 #64750 l(3)1.13 / TM6, Tb  
 #64760 l(3)1.17 / TM6B, Tb  
 #64770 l(3)2.1 / TM6B, Tb  
 #64790 l(3)2.3 / TM6B, Tb  
 #64900 l(3)3[DTS] / TM2, Ubx[130] e[s]  
 #650 B  
 #6500 C(1)DX, y f / l(1)zw12[k9] / w[+]Y  
 #65000 l(3)4[DTS] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #65100 l(3)5[DTS] pb p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #65200 l(3)4g(SzC) / MKRS, kar ry[2] Sb  
 #65300 l(3)7[DTS] st p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser

#65350 l(3)155 / TM6B, Tb  
 #65400 l(3)SG36 red e / TM1, Me ri sbd[l] -- (synonym l(3)11m254)  
 #65500 l(3)12m137 red e / TM3, e Sb Ser  
 #65600 l(3)87A-02, e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #65700 l(3)ac e[s] M(3)95A[1] / LVM, pe l(3)L VML l(3)L VMR -- (synonym M(3)w)  
 #65800 l(3)86Fe[1] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck5[e88])  
 #65900 l(3)86Ft[1] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck6[e145])  
 #6600 C(1)DX, y f / lz[3]  
 #66000 l(3)87Aa[3] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck8[e296])  
 #66100 l(3)87Ab[6] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck9[e260])  
 #66300 l(3)87Ac[3] / MKRS, kar ry[2] Sb -- (synonym l(3)ck10[e170])  
 #66400 l(3)87Ae[1] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck12[e77])  
 #66500 l(3)87Ae[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck12[e177])  
 #66600 l(3)87Ae[3] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck12[e294])  
 #66700 l(3)87Ba[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck13[e72])  
 #66800 l(3)87Ba[3] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck13[e152])  
 #66900 l(3)87Ba[5] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck13[e248])  
 #6700 C(1)DX, y f / lz[34]  
 #67000 l(3)87Bc[1] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck15[e61])  
 #67100 l(3)87Bd[1] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] -- (synonym l(3)ck16[e22])  
 #67200 l(3)87Bd[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck16[e300])  
 #67300 l(3)87Be[4] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck17[e180])  
 #67400 l(3)87Be[7] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck17[e280])  
 #6750 C(1)DX, y f / para[ts]  
 #67500 l(3)87Bf[3]p / TM3, y[+] ri p[p] se Sb bx[34e] e[s] Ser -- (synonym l(3)ck18[e312])  
 #67600 l(3)87Bg[3] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck19[e211])  
 #6765 C(1)DX, y f / R(1)2, y ct[6] f  
 #6770 C(1)DX, y f / R(1)2, y v / y[+]Y  
 #67700 l(3)87Bh[1] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck20[e214])  
 #67800 l(3)87Bh[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck20[e214])  
 #67900 l(3)ck21[e310] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #6800 C(1)DX, y f / sc z w[+UZ] f  
 #68000 l(3)87Bi[2] / MKRS, kar ry[2] Sb -- (synonym l(3)ck21[hs48])  
 #68100 l(3)87Bj[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck22[e293])  
 #68200 l(3)87Bj[5] / MKRS, kar ry[2] Sb -- (synonym l(3)ck22[nf30])  
 #68300 l(3)87Bk[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] -- (synonym l(3)ck23[e115])  
 #68400 l(3)87Bk[3] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck23[e158])  
 #6850 C(1)DX, y f / sn  
 #68500 l(3)87Bk[4] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck23[e162])  
 #68600 l(3)87Bl[1] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck24[e33])  
 #68700 l(3)87Bm[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] -- (Ser lost, synonym l(3)ck25[e99])  
 #68800 l(3)87Bm[1] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)ck25[e11])  
 #68900 l(3)e20 e / TM1, Me ri sbd[l]  
 #6900 C(1)DX, y f / sn[36a]  
 #69000 l(3)ecd[1] st ca  
 #69100 l(3)87Ca[14] / MKRS, kar ry[2] Sb -- (synonym l(3)m107(SzA))  
 #69200 l(3)87Cd[16] / MRS, ry[2] Sb -- (synonym l(3)m114(SzD))  
 #69300 l(3)87Ca[5], e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)SzA[5])  
 #6950 C(1)DX, y f / su(s)[2] v  
 #69500 l(3)87Ca[7], e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)SzA[7])  
 #69600 l(3)87Cb[5], e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym l(3)SzB[5])  
 #69750 l(3)tr Ubx / TM1, Me ri sbd[l]  
 #6980 C(1)DX, y f / w sn[5]  
 #69800 LVM, pe l(3)L VML l(3)L VMR / Ly Sb  
 #69900 M(3)40l30 / Payne, Dfd l(3)PL l(3)PR ca  
 #700 B car bb / C(1)DX, y f  
 #7000 C(1)DX, y f / w[+]Y / y[2] l(1)zw7[e3] spl sn[3]  
 #70100 M(3)67C[7] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym M(3)i[G3])  
 #70200 mei-1  
 #70250 TM6B, Hu e Tb ca / MKRS, kar ry[2] Sb

#70300 mwh e  
#70300A mwh jv  
#70301 mwh jv red sbd[2] e[11] l(3)M52 ro ca / TM1, Me ri red sbd[l]  
#70302 mwh jv red sbd[2] e[11] l(3)M61 ro ca / TM1, Me ri sbd[l]  
#70303 mwh jv red sbd[2] e[11] l(3)M63 ro ca / TM1, Me ri sbd[l]  
#70304 mwh jv red sbd[2] e[11] l(3)M72 ro ca / TM1, Me ri sbd[l]  
#70305 mwh jv red sbd[2] e[11] l(3)M212 ro ca / TM1, Me ri sbd[l]  
#70308 mwh jv red sbd[2] e[11] l(3)M255 ro ca / TM1, Me ri sbd[l]  
#70309 mwh jv red sbd[2] e[11] l(3)M258 ro ca / TM1, Me ri sbd[l]  
#70310 mwh jv red sbd[2] e[11] l(3)M263 ro ca / TM1, Me ri sbd[l]  
#70311 mwh jv red sbd[2] e[11] l(3)M268 ro ca / TM1, Me ri sbd[l]  
#70313 mwh jv red sbd[2] e[11] l(3)M279 ro ca / TM1, Me ri sbd[l]  
#70316 mwh jv red sbd[2] e[11] l(3)M291 ro ca / TM1, Me ri sbd[l]  
#70318 mwh jv red sbd[2] e[11] l(3)M1610 ro ca / TM1, Me ri sbd[l]  
#70319 mwh jv red sbd[2] e[11] l(3)M2915 ro ca / TM1, Me ri sbd[l]  
#70320 mwh jv red sbd[2] e[11] l(3)M2918 ro ca / TM1, Me ri sbd[l]  
#70330 mwh jv red sbd[2] e[11] l(3)ME15 ro ca / TM1, Me ri sbd[l]  
#70331 mwh jv red sbd[2] e[11] l(3)ME21 ro ca / TM1, Me ri sbd[l]  
#70332 mwh jv red sbd[2] e[11] l(3)ME212 ro ca / TM1, Me ri sbd[l]  
#70350 Pc[11] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym Pc[XL5])  
#70360 ru h th st Pc[12] Msc sr e[s] ca / TM3, Sb Ser -- (synonym Pc[XMI])  
#70400 Pr Dr / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
#70440 mvh jv st red sbd[2] e[11] l(3)11b[M184] ro ca / TM1, Me ri sbd[l]  
#70441 mvh jv st red sbd[2] e[11] l(3)11b[M216] ro ca / TM1, Me ri sbd[l]  
#70442 mvh jv st red sbd[2] e[11] l(3)11b[M239] ro ca / TM1, Me ri sbd[l]  
#70443 mvh jv st red sbd[2] e[11] l(3)11b[M251] ro ca / TM1, Me ri sbd[l]  
#70444 mvh jv st red sbd[2] e[11] l(3)11b[M273] ro ca / TM1, Me ri sbd[l]  
#70445 mvh jv st red sbd[2] e[11] l(3)11b[M283] ro ca / TM1, Me ri sbd[l]  
#70446 mvh jv st red sbd[2] e[11] l(3)11b[M2913] ro ca / TM1, Me ri sbd[l]  
#70447 mvh jv st red sbd[2] e[11] l(3)11b[M2919] ro ca / TM1, Me ri sbd[l]  
#70447A mwh jv st red sbd[2] e[11] ro ca abd-A[M1] / TM1, Me ri sbd[l]  
#70447B mwh jv st red sbd[2] e[11] ro ca abd-A[M1] Abd-B[M8] / TM1, Me ri sbd[l]  
#70447D mwh jv st red sbd[2] e[11] ro ca abd-A[M3] / TM1, Me ri sbd[l]  
#70447E mwh jv st red sbd[2] e[11] ro ca Abd-B[M1] / TM6, Hu e Tb ca  
#70447F mwh jv st red sbd[2] e[11] ro ca Abd-B[M5] / TM1, Me ri sbd[l]  
#70447G mwh jv st red sbd[2] e[11] ro ca Ubx[9.22] Abd-B[M1] / TM6B, Hu e Tb ca  
#70447H mwh jv st red sbd[2] e[11] ro ca Ubx[9.22] Abd-B[M5] / TM1, Me ri sbd[l]  
#70448 mwh jv st red sbd[2] Ubx[9.22] Df(3R)C1 e / TM1, Me ri sbd[l]  
#70448A mwh jv st red sbd[2] e[11] ro ca Ubx[M4]  
#70448B mwh jv st red sbd[2] e[11] ro ca Ubx[MX6] abd-A[M1] / TM1, Me ri sbd[l]  
#70448D mwh jv st red sbd[2] e[11] ro ca Ubx[MX17] / TM1, Me ri red sbd[l]  
#70449 Odh[NCI] / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e  
#70500 ri ss  
#70600 rn[3] p[p] bx sr e[s] / TM1, Me ri p[p] sbd[l]  
#70700 ru Aph[O] e ca Acpn-1[n13]  
#70750 ru h cp in ri p[p] Scm[4] sr e[s] ca / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym Scm[XF24])  
#70760 ru h th st ri Scr[XF9] p[p] cu sr e[s] ca / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
#70800 ru h st p[p] ss e[s]  
#70900 ru h th st cu sr e[s] ca / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
#7100 C(1)DX, y f / w[+]Y / z l(1)zw9[k18] spl sn[3]  
#71000 ru h th st cu sr e[s] Pr ca / TM6B, Hu e Tb ca  
#71050 ru h th st hb[IIU03] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
#71055 ru h th st Pc[12] Msc sr e[s] ca / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser -- (synonym Pc[XM1])  
#71060 ru kl h[IL79K] / TM3, y[+] ru kl ri p[p] sep Sb bx[34e] e[s] Ser  
#71065 ru kl opa[IIP32] / TM3, y[+] ru kl ri p[p] sep Sb bx[34e] e[s] Ser  
#71070 (ru) st kni[FC13] e (ca) / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
#71080 ru th st cp in ri kl ftz[E193] p[p] / TM3, y[+] ru kl ri p[p] sep Sb bx[34e] e[s] Ser  
#71250 ry[506]  
#71300 ry cd  
#71310 Scr[XF24] Antp[73b+R29] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser

#71330 Scr[XH21] Ki / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #71350 shm[E320-5], th st cp in ri p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #71400 se  
 #71450 se e[11]  
 #71500 ss[a]  
 #71600 st  
 #71700 st Antp[Ns] cu  
 #71800 st c(3)G ca / TM1, Me ri sbd[]]  
 #71840 st e E(spl)[8D06] / TM3, y[+] ri p[p] Sb bx[34e] e[s]  
 #71850 st hb[FB92] e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #71860 st kni[1-19] e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #71865 st kni[17-3] e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #71900 st p e  
 #7200 C(1)DX, y f / w[a4]  
 #72000 st ry e  
 #72050 st Scr[XH44] e / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #72100 st ss e[11]  
 #72200 su(ve) ru ve h th  
 #72250 (th st cp in ) ri Antp[Scx] p[p] Ubx / TM1, Me ri sbd[l]  
 #72255 th st cp in ri p[p] hb[349] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #72260 th st cp in ri kni[301] p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #72265 th st cp in ri kni[357] p[p] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #72300 th st cp in ri p[p] ss bxd sr e[s] / TM1, Me ri p[p] sbd[l]  
 #72350 th st hth[5E04] cu sr e[s] ca / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #72400 th st pb p[p] cu kar su(Hw)[2] jvl ss bx sr gl / TM6, (Hn[P]) ss[P88] bx[34e] Ubx[P15] e  
 #72500 TM1, Me ri sbd[l] / TM3, y[+] ri p[p] sep Sb bx[34e] e Ser  
 #72515 TM1, Me ri sbd[l] p[p] / ru h th st Ubx[XM1] Msc sr e[s] ca  
 #72520 TM1, Me ri sbd[l] p[p] / st Ubx[X31] e  
 #72530 TM6B, e Tb ca / “ -letal; ”2[  
 #72560 Ubx[Mx11] abd-A[M1] Abd-B[M8] / Sb[1] Dp(3;3)P5  
 #72570 Ubx[MX16] abd-A[M1] Abd-B[M9] / Sb[1] Dp(3;3)P5  
 #72575 We[E12] / TM6B, Tb  
 #72580 We[E15] / TM6B, Tb  
 #72585 We[X1] / TM6B, Tb  
 #72590 We[X2] / TM6B, Tb  
 #72600 ar / ey[D]  
 #72700 bt ey[R] sv[n]  
 #72800 C(4)RM, ci ey[R].gvl sv[n]  
 #72900 C(4)RM(P1), ci ey[R].gvl sv[n]  
 #7300 C(1)DX, y f / w[a59k13]  
 #73000 C(4)RM(P2), ci ey[R].gvl sv[n]  
 #73100 ci ey[R]  
 #73300 ci gvl ey[R] sv[n]  
 #73500 ci[D] / l(4)5  
 #73600 ci[D] / l(4)14  
 #73900 ci[W]  
 #7400 C(1)DX, y f / w[ch] rb wy  
 #74000 Df(4)G / ci[D]  
 #74050 slow A spa  
 #74100 spa[pol]  
 #74200 sv[n]  
 #7450 C(1)DX, y f / XYL.YS, y v f car / Dp(1;f)MMRing, y[+]  
 #74700 b ; T(Y;2)B  
 #74800 C(1)DX, y f car ; bw[D] ; T(1;2)sn[+72d]  
 #7500 C(1)DX, y f / y w spl sn[3] / y[2]Y[61l]  
 #75000 C(1)DX, y f / In(1)y[3PL]sc[S1R] In(1)S, y[-] ac[-] sc[-] ; In(2L)Cy In(2R)Cy, Cy cn[2] ; Dp(1;2)sc[19], sc[19]  
 #75200 C(1)DX, y f / Dp(1;Y)1E, y / y sc ; cn bw  
 #75250 C(1)DX, y f / Dp(1;Y)2E / v car l(1)64 ; cn bw  
 #75300 C(1)DX, y w f / Df(1)A113 ; Dp(1;2)w[+64b]  
 #75400 C(1)DX, y w f / Df(1)KA7 ; T(1;2)v[65b]

#75500 C(1)DX, y w f / Df(1)N69h9, N[69h9] rb ; Dp(1;2)w[+51b7]  
#75600 C(1)DX, y w f / Df(1)N71h24.5, N[71h24.5] ; Dp(1;2)w[+51b7]  
#75700 C(1)DX, y w f / Df(1)v-L3, v[L3]; Dp(1;2)v[+63i]  
#75800 C(1)DX, y w f / Df(1)v-L15, y v[L15] ; T(1;2)v[+75d]  
#75900 C(1)DX, y w f / Df(1)w67c23.3, w[67c23.3] ; Dp(1;2)w[+51b7]  
#7600 C(1)DX, y f / y[2] sc w[i] ec  
#76100 C(1)DX, y w f ; T(1;2)rb[+71g], ct[6] v  
#76200 C(1)DX, y w f ; T(1;2)v[65b], lz[50e] v[65b]  
#76300 C(1)DX, y w f ; T(1;2)w[64d], w[64d] cm ct[6] sn[3]  
#7640 C(1)DX, y w f / cm ct[6] sn[3]  
#7643 C(1)DX, y w f / cm Df(1)ct4b1 / ct[+] Y  
#7645 C(1)DX, y w f / ct[n] oc  
#76450 C(1)DX, z sn[3] / Df(1)w, y[2] sc w spl ; SM5, al[2] Cy lt[v] cn[2] sp[2] / TE.2w[+78c28]  
#7647 C(1)DX, y w f / Df(1)64c18, g sd / w[+]Y  
#76600 C(1)RM, y / YSX.YL, In(1)EN, y ; SM1, al[2] Cy cn[2] sp[2] ; T(Y;2)A80, y[+] B[S]  
#76700 C(1)RM, y / YSX.YL, In(1)EN, y ; SM1, al[2] Cy cn[2] sp[2] ; T(Y;2)B190, y[+] -- Obs 77200  
#76800 C(1)RM, y / YSX.YL, In(1)EN, y ; SM1, al[2] Cy cn[2] sp[2] ; T(Y;2)B224, y[+] B[S]  
#76905 C(1)RM, y / YSX.YL, In(1)EN, y ; SM1, al[2] Cy cn[2] sp[2] ; T(Y;2)H52, y[+] B[S]  
#76910 C(1)RM, y / YSX.YL, In(1)EN, y ; SM1, al[2] Cy cn[2] sp[2] ; T(Y;2)H116, y[+] B[S]  
#76915 C(1)RM, y / YSX.YL, In(1)EN, y ; SM1, al[2] Cy cn[2] sp[2] ; T(Y;2)L52, y[+]  
#76920 C(1)RM, y / YSX.YL, In(1)EN, y ; SM1, al[2] Cy cn[2] sp[2] ; T(Y;2)L110, y[+] B[S]  
#76950 C(1)RM, y / YSX.YL, In(1)EN, y ; In(2LR)Cy, Cy pr cn sp ; T(Y;2)R31, y[+]  
#77000 C(1)RM, y / YSX.YL, In(1)EN, y ; In(2L)Cy In(2R)Cy, Cy cn[2] ; T(Y;2)A62, y[+] B[S]  
#77200 C(1)RM, y / YSX.YL, In(1)EN, y ; In(2L)Cy In(2R)Cy, Cy cn[2] ; T(Y;2)B224, y[+] B[S]  
#77600 C(1)RM, y / YSX.YL, In(1)EN, y ; In(2L)Cy In(2R)Cy, Cy cn[2] ; T(Y;2)P51, y[+] B[S]  
#77700 C(1)RM, y / YSX.YL, In(1)EN, y ; In(2L)Cy In(2R)Cy, Cy cn[2] ; T(Y;2)P58, y[+] B[S]  
#77900 cl[2] ; T(Y;2)E  
#7800 C(1)DX, y w f / Df(1)R-7 / y[+]Ymal[+]  
#78000 CIB, sc l(1)C t[2] v sl B ; T(1;2)Bld, Bld  
#78100 cn[3] ; T(Y;2)C  
#78110 CyO, Cy dp[lv1] pr cn[2] / odd[5] dp prd[9] cn eve[ID] ; T(Y;2)eve[2.37], b pr cn sca  
#78120 CyO, Cy dp[lv1] pr cn[2] / odd[5] dp prd[9] cn eve[ID] ; T(Y;2)odd[2.31], b pr cn sca  
#78125 CyO, Cy dp[lv1] pr cn[2] / odd[5] dp prd[9] cn eve[ID] ; T(Y;2)odd[4.13], b pr cn sca  
#78127 CyO, Cy dp[lv1] pr cn[2] / odd[5] dp prd[9] cn eve[ID] ; T(Y;2)odd[4.25], b pr cn sca  
#78130 CyO, Cy dp[lv1] pr cn[2] ; T(1;2)odd[1.10], b pr cn sca  
#78135 CyO, Cy dp[lv1] pr cn[2] ; T(1;2)r[+75c]  
#78140 CyO, Cy dp[lv1] pr cn[2] ; T(Y;2)Kr[AP2], b pr cn wxt (or) If[R]  
#78150 Df(1)R-29, y / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B ; T(1;2)R-29  
#78160 Df(1)R-38, y / FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B ; T(1;2)R-38  
#78200 Df(1)w, y[2] sc z w ; SM5, al[2] Cy lt[v] cn[2] sp[2] / TE.2(w[+78el])  
#78300 Df(1)w, y[2] sc z w spl ; SM5, al[2] Cy lt[v] cn[2] sp[2] / TE.2(w[+78h24])  
#78400 Df(1)w, y[2] sc z w spl ; SM5, al[2] Cy lt[v] cn[2] sp[2] / TE.2(w[+80f7])  
#78500 Df(2L)Mdh-A ; T(1;2)Bld, Bld ; T(1;2)OR64  
#78550 Df(2L)sc19-8 / Cy Roi ; Tp(2;1)B19, y ed dp[o2] cl  
#78600 Df(2L)Sco-R+23, b pr Dp(2;1)Sco[R+23], el[+] rd[+] / In(2LR)O, dp[lv1] Cy pr cn[2]  
#78605 Df(2R)en30 / SM5, al[2] Cy C+[v] cn[2] sp[2] ; B[S]Y  
#78610 Df(2R)G100-L141, y[+] / SM1, al[2] Cy cn[2] sp[2] ; C(1;Y)Y[S]XY[L], In(1)EN, y / C(1)RM, y  
#78650 Dp(2;Y)A161, B[S] / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#78660 Dp(2;Y)L124, B[S] / SM6a, al[2] Cy dp[lv1] cn[2] sp[2]  
#78700 FM6, y[31d] sc[8] dm B ; T(1;2)N[264-10], N[264-10]  
#78800 g[3] ; bw  
#78900 In(1)AM ; T(1;2)f[257-15], f[257-15]  
#7900 C(1)DX, y w f / Df(1)R-9 / Ymal[+]  
#79050 In(1)w[m4], w[m4] ; E(var)5 / In(2L)Cy In(2R)Cy, Cy cn[2]  
#79100 In(1)w[m4], y w[m4] ; E(var)7  
#79150 In(1)w[m4], w[m4] ; E(var)8 / In(2L)Cy, Cy  
#79200 In(2L)Cy, al[2] Cy cn[2] lt[3] sp[2] / M(2)e ; T(1;2)B[bd], B[bd]  
#79300 In(2L)Cy In(2R)Cy, al[2] Cy cn[2] sp[2] ; T(1;2)lt, eq lt[m]  
#79400 In(2L)Cy In(2R)Cy, al[2] Cy pr Bl cn[2] vg c sp[2] ; T(1;2)cl[1], cl Adh[nC1] cn bw  
#79500 lt ; T(Y;2)A

#79550 sc z w[is]; CyO, Cy dp[lv1] pr cn[2] / Su(z)2[5]  
 #79600 sc z w[is]; CyO, Cy dp[lv1] pr cn[2] / Su(z)3[1.y]  
 #800 B[S]Y / C(1)DX, y f / Df(1)K-5, y w f  
 #80100 sc z w[is] ; In(2L)Cy In(2R)Cy, Cy cn[2] / Su(z)5  
 #80200 sc z w[is] ; In(2L)Cy In(2R)Cy, Cy cn[2] / Su(z)6  
 #80300 sc z w[is] ; In(2L)Cy In(2R)Cy, Cy cn[2] / Su(z)7  
 #80400 su(s)[2]; bw  
 #80500 T(Y;2)CyO, Cy dp[lv1] pr cn[2] l(2)513[DTS]  
 #80600 v ; bw  
 #80700 v ; cn  
 #80750 w ; b Df(2L)TE36(R)GW3 pr pk cn sp / CyO, Cy dp[lv1] pr cn[2]  
 #80751 w ; b Df(2L)TE36(R)GW7 pr pk cn sp / CyO, Cy dp[lv1] Adh[nB] pr cn[2]  
 #80753 (y) w ; b Df(2L)TE36(R)GW24 pr pk cn sp / CyO, Cy dp[lv1] pr cn[2]  
 #80754 (y) w ; b Df(2L)TE36(R)GW28 pr pk cn sp / CyO, Cy dp[lv1] pr cn[2]  
 #80755 (y) w ; b Df(2L)TE36(R)GW29 pr pk cn sp / CyO, Cy dp[lv1] pr cn[2]  
 #80756 (y) w ; b Df(2L)TE36(R)GW31 pr pk cn sp / CyO, Cy dp[lv1] pr cn[2]  
 #80757 (y) w ; b Df(2L)TE36(R)GW34 pr pk cn sp / CyO, Cy dp[lv1] pr cn[2]  
 #80760 w; CyO, Cy dp[lv1] pr cn[2] /Df(2L)3OC  
 #80770 w ; l(2)C7 / Cy  
 #80771 w ; l(2)M5 / Cy  
 #80772 w ; l(2)M12 / Cy  
 #80773 w ; l(2)PM6-16 / Cy  
 #80800 w ; shv b bw  
 #80840 w / y[+]Y ; CyO, Cy dp[lv1] Adh[nB] pr cn[2] / Df(2L)TE146(Z)GW7, al dp b pr l(2)pwn cn  
 #80841 (w) / y[+]Y ; CyO, Cy dp[lv1] pr cn[2] / Df(2L)TE146(Z)GW1, al dp b pr l(2)pwn cn  
 #80842 (w) / y[+]Y ; CyO, Cy dp[lv1] pr cn[2] / Df(2L)TE146(Z)GW4, al dp b pr l(2)pwn cn  
 #80843 (w) / y[+]Y ; CyO, Cy dp[lv1] pr cn[2] / Df(2L)TE146(Z)GW6, al dp b pr l(2)pwn cn  
 #80850 w[a] fa[g] ; CyO, Cy dp[lv1] pr cn[2] / Df(2R)Jp1  
 #80860 w[a] fa[g] ; CyO, Cy dp[lv1] pr cn[2] / Df(2R)Jp8, w[+]  
 #80900 w[ch] ; In(2L)Cy In(2R)Cy, Cy cn[2] / Su(w[ch])  
 #8100 C(1)DX, y w f / Df(1)R-21 / y[+]Ymal[+]  
 #81000 w[ch] wy ; cn  
 #81050 y ; C(2L)SH1 / F(2R)VH2, bw  
 #81100 y ; C(2R)RM, cn / F(2L)dp  
 #81150 y ; CyO, Cy dp[lv1] pr cn[2] ; Dp(1;2)sc[8]  
 #81200 y ; CyO, Cy dp[lv1] pr cn[2] / Su(z)2[4]  
 #81305 y ; dp wg[CP1] cn bw / SM1, al[2] Cy cn[2] sp[2]  
 #81306 y / + ; SM1, al[2] Cy cn[2] sp[2] / wg[CP1+RC1]  
 #81307 y / + ; SM1, al[2] Cy cn[2] sp[2] / wg[CX1] b pr  
 #81310 y ac sc pn ; Df(2L)sc19-1, sc[19-1] / In(2L)Cy[L]t[R] In(2R)Cy, Cy Roi cn sp ; Dp(2;1)B19, ed dp[o2] cl  
 #81320 y ac sc pn ; Df(2L)sc19-3, sc[19-3] / In(2L)Cy[L]t[R] In(2R)Cy, Cy Roi cn sp ; Dp(2;1)B19, ed dp[o2] cl  
 #81350 y ac sc pn ; Df(2L)sc19-6, sc[19-6] / In(2L)Cy[L]t[R] In(2R), Cy Roi cn sp ; Dp(2;1)B19, ed dp[o2] cl  
 #81400 y[2] w[a] ; cn  
 #81410 z w[11E4]; Su(z)3 / CyO, Cy dp [lv1] pr cn[2]  
 #81550 Antp[XMI] Msc / TM3, y[+ri] p[p] sep Sb bx[34e] e[s] Ser ; T(Y;3)MAS, ru st e ca  
 #81600 B[S]Y / w[a] ; st tra / TM2, Ubx[130] e[s] (w[a]-temperature sensitive)  
 #81700 B[s]Y ; mu-2 st  
 #81730 B[S]Yy[31d] ; mus(3)312 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #81800 C(1)DX, y f / Df(1)w67d6, y w[67d6] sn[3] ; Dp(1;3)w[vco], w[vco]  
 #81900 C(1)DX, y f / Df(1)w258-48, y sc[5] w[258-48] spl ; Dp(1;3)w[vco], w[vco]  
 #8200 C(1)DX, y w f / Df(1)R-36 l(1)Q464 / y[+]Ymal[+]  
 #82100 C(1)DX, y f ; T(1;3)ras[v], ras[v]  
 #82200 C(1)DX, y w f / Df(1)ct-J4, ct[J4] f ; Dp(1;3)sn[13a] ; Ki  
 #8250 C(1)DX, y w f / Df(1)R-44, y v / Ymal[+]  
 #82500 C(1)DX, y w f / Df(1)w258-45-64, y[2] w[258-45-64] sn[3] ; Dp(1;3)w[+67k27]  
 #82550 C(1)DX, y w f / Sh[6] ; e  
 #82600 C(1)M3, y[2] / YSX.YL, In(1)EN, y ; TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e ; T(Y;3)A78, y[+] B[S]  
 #82800 C(1)M3, y[2] / y z[a] w[Bwx] ; sbd[2] ss bx[34e] / TM1, Me ri sbd[l]  
 #82810 C(1)M3, y[2] bb / YSX.YL, In(1)EN, y ; TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e ; T(Y;3)A14, y[+] B[S]  
 #82830 C(1)M3, y[2] bb / YSX.YL, In(1)EN, y ; TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e ; T(Y;3)R153, y[+] B[S+]

#82900 C(1)RM, y / In(1)sc[29], sc[29] w[a] ; se h  
 #8300 C(1)DX, y w f / Df(1)rst2, rst[2]  
 #83100 CIB, sc l(1)C t[2] v sl B ; T(1;3)w[vco], w[vco] v f  
 #83200 Df(1)w, y[2] sc w spl ; TE.3(w[+])  
 #83200A Df(3R)2-2 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser ; Dp;(3;1)w[1118] / ? -- ( synonym Tp(3;1)2-2 )  
 #83201 Df(3R)B81, P{ry[+]rp[49]}F2-80Ae / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser ; Dp(3;1)67A  
 #83210 Df(3R)L127 / TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e ; Dp(3;1)B152  
 #83215 Df(3R)P115, e[11] / TM1, Me ri sbd[l] ; Dp(3;1)P115=Tp(3;1)P115  
 #83220 Df(3R)ry506-85c / MKRS, kar ry[2] Sb ; Dp(3;Y)ry[506-85C]  
 #83300 Dp(3;1)P115 ; Df(3R)Scb Df(3R)P9 / TM2, Ubx[130] e[s]  
 #83350 Df(3R)XS, Dp(3R)XS, asp ats p[p] / TM6B; e Tb ca ; y / y[+]Y  
 #83400 FM3, l(1)? y[31d] sc[8] dm B l(1)? ; T(1;3)sta, sta ss[a]  
 #83500 FM6, y[31d] sc[8] dm B ; T(1;3)f[+71b]  
 #83600 FM6, y[31d] sc[8] dm B ; T(1;3)sc[260-15], sc[260-15]  
 #83650 FM7, y[31d] sc[8] w[a] sn[X2] v[Of] g[4] B ; T(1;3)v[+74c]  
 #83750 In(1)dl-49, SxI[fl] v[Of] g[4] ; Dp(1;3)sn[13a1]  
 #83900 In(1)sc[8] Df(1)sc[8], sc[8] w[a] ; Dp(1;3)sc[J4], y[+]? sc[J4]  
 #8400 C(1)DX, y w f / Df(1)w, y[2] sc z w spl mei-9b cv  
 #84080 In(3L)Fd ; Dp(1;3)B[S]  
 #84100 In(3LR)Ubx[130], ri Ubx[130] e[s] ; Dp(3;1)in[61j2], in[61j2]  
 #84200 sc z w[is] ; E(z) / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #84300 sc z w[is] ; Su(z)4 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #84360 Scr[XF9], ru hth st cp in ri p[p] cu sr e[s] ca / TM3, y[+] ri sep Sb bx[34e] e[s] ; T(Y;3)MA9, ru st e ca  
 #84380 T(3;Y)Abd-B[S10] / Sb Dp(3;3)P5  
 #84400 T(1;3)C195  
 #84500 T(1;3)w[R+19], st / TM3, y[+](?) ri p[p] sep Sb bx[34e] Ser  
 #84600 T(Y;3)P80  
 #84700 T(Y;3)P102  
 #84730 TM1, Me ri sbd[l] / TM3, y[+] ri sep Sb bx[34e] e[s] ; T(Y;3)Ubx[79C]  
 #84740 TM1, Me ri sbd[l] / TM3, y[+] ri sep Sb bx[34e] e[s] ; T(Y;3)Ubx[79f-2]  
 #84800 tuh-1 ; tuh-3  
 #84900 tuh-1 ; tuh-3[I127]  
 #84920 v ; Df(3L)vi3, vi[3] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #84930 v ; Df(3L)vi4, vi[4] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #84940 v ; Df(3L)vi5, vi[5] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #84950 v ; Df(3L)vi6, vi[6] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #84960 v ; Df(3L)vi7, vi[7] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #8500 C(1)DX, y w f / Df(1)w64c18, w[64c18] g / w[+]Y  
 #85010 w; Df(3L)GN50 / TM8, l(3)4DTS th st Sb e  
 #85020 w ; H2AvD[810] / TM6B, Tb  
 #85030 w ; l(3)835 / TM3, y[+] ri p[p] sep bx[34e] e Sb  
 #85050 w ; ry[506] (P ry[+] delta 2-3)  
 #85100 w ; se  
 #85200 w ; ve st e  
 #85300 w ; ve e ca  
 #85350 w sn[3] ; e[11]  
 #85400 w v ; ve e  
 #85500 w v ; ri e  
 #85550 w[1118] ; Df(3R)3450 / TM6B, Hu e  
 #85600 w[a] ct[6] ; mei-1  
 #85700 w[c] ; Est-6[F] Est-C e ro  
 #85740 y ; mwh jv Df(3R)C1 / TM1, Me ri sbd[l]  
 #85745 y ; mwh jv Pc[3] abd-A[M1] Abd-B[M8] e[11] ro ca / TM6B, Hu e Tb ca  
 #85748 y w spl ; Df(3L)66C-G28 / TM3, y[+] ri p[p] sep bx[34e] e[s]  
 #85750 z ; E(z) / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser  
 #85800 0 / C(1)RM, y w / YSX.YL, In(1)EN y w f ; TE.4(w[+])  
 #85900 B[S]Y / C(1)DX, y f / y Hw w ; C(4)RM, spa[pol]  
 #8600 C(1)DX, y w f / Df(1)w78h24, sc z w[78h24] sn  
 #86050 C(1)DX, y f / R(1)5, In(1)w[m4], w[m4] ; Dp(1;4)w[m258-18], y w[m258-18]  
 #86100 C(1)DX, y f ; T(1;4)B[S], B[S]

#86200 C(1)DX, y f ; T(1;4)sc[8], sc[8] w[a] B  
 #86300 C(1)DX, y f ; T(1;4)w[m5], w[m5]  
 #86400 C(1)DX, y f / y mei-218 / y[+]Y ; spa[pol]  
 #86550 Df(1)r-D1, v f / C(1)DX, y w f ; Dp(1:4)r[+] / +  
 #86560 Df(1)r-D17, y[31d] sc[8] dm B / FM6, y[31d] sc[8] dm B ; Dp(1:4)r[+] / +  
 #86600 ey[D] ; T(1;4)w[m5], w[m5]  
 #86900 FM1, y[31d] sc[8] w[a] Iz[s] B ; T(1;4)w[258-21]  
 #8700 C(1)DX, y w f / Df(1)y74k24.1, y[74k24.1] / y[2]Y  
 #87000 In(1)dl-49, y Hw m[2] g[4] ; T(1:4)w[m258-18], y w[m258-18]  
 #87200 y ; ci[D] spa[pol] / l(4)10  
 #87300 y ; ci[D] spa[pol] / l(4)16  
 #87400 y ; l(4)8 / spa[Cat]  
 #87500 y ; l(4)35 / spa[Cat]  
 #87700 b cn bw ; ri  
 #87740 b Su(er)[+] bw ; st er  
 #87750 bw[D] ; Su-283 ; T(2;3)ap[Xa], ap[Xa]  
 #87860 bw ; st p[p]  
 #87900 bw ; ve st  
 #8800 C(1)DX, y w f / Df(1)z-lx12, y ac sc z[lx12] / Y.w[+]Co  
 #88000 bw[75] ; st  
 #88100 cn bw ; e[11]  
 #88150 C(2)EN, c bw ; C(3L)RM / C(3R)RM  
 #88155 Cbx Ubx gl[3] ; T(2;3)ap[Xa], ap[Xa]  
 #88156 Cy / bw[V1] ; H / Sb -- (synonym Pm)  
 #88157 Cy / bw[V1] ; Sb / Ser -- (synonym Pm)  
 #88160 CyO, Cy dp[lv1] pr cn[2] / Df(2L)osp141, osp[141] pr cn ; T(2;3)CA7 T(2;3)CA8  
 #88170 CyO, Cy dp[lv1] pr cn[2] / In(2L)C158.1  
 #88180 CyO, Cy dp[lv1] pr cn[2] / In(2L)Sco[R+11]  
 #88190 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)eve[1.18], b pr cn sca  
 #88195 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)eve[5.5], b pr cn sca  
 #88200 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)ho[5], ho[5] Adh[nC1] cn bw  
 #88250 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)H16, ho[2] l(2)br[37GE1]  
 #88260 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)odd[3.29], b pr cn sca  
 #88300 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)pk[78t], pk[78t]  
 #88320 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)prd[2.27.3], b pr cn sca  
 #88340 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)Sco[R+7]  
 #88345 CyO, Cy dp[lv1] pr cn[2] ; T(2;3)sna[2.40], b pr cn sca  
 #88350 CyO, Cy dp[lv1] pr cn[2] / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser; T(2;3)P10  
 #88370 Df(3R)2-2 / TM3, y[+] ri p[p] sep Sb bx[34e] e[s] Ser ; Dp(3;1)2-2, w[1118] / ?  
 #88400 Df(2R)bw5, bw[5] sp[2] ; T(2;3)ap[Xa], ap[Xa]  
 #88430 Df(3R)by62, T(2;3)by[62], red e / TM1, Me ri sbd[l]  
 #88440 Df(3R)p712, T(2;3)p[712], red e / TM3, y[+] ri sep Sv bx[34e] e[s]  
 #88445 Df(3R)p819, T(2;3), Tp(3;3), In(3LR), red e / TM3, y[+] ri sep Sv bx[34e] e[s]  
 #88450 Df(3R)P14, sr gl ; T(2;3)ap[Xa], ap[Xa]  
 #8850 C(1)DX, y w f / Dp[2](1;1)3A6-3C2, y ac z  
 #88500 e[s] ca[nd] ; T(2;3)ca[v], e[s] ca[v]  
 #88600 In(2L)Cy In(2R)Cy, al[2] Cy lt[3] cn[2] L[4] sp[2] ; T(2;3)bw[VDe4], bw[VDe4]  
 #88700 In(2L)Cy In(2R)Cy, al[2] Cy lt[3] cn[2] L[4] sp[2] ; T(2;3)rn In(2R)M, al rn  
 #88800 In(2L)Cy In(2R)Cy, al[2] Cy pr Bl cn[2] vg c sp[2] ; T(2;3)dp[5], dp Adh[nC1] cn bw  
 #89000 In(2L)Cy In(2R)Cy, Cy cn[2] ; T(2;3)Hm, Hm  
 #89050 In(2L)Cy [L]t[R] In(2R)Cy, Cy Roi cn ; T(2;3)dp[h27]  
 #89100 In(2LR)bw[V1], dp b / SM6a, Cy dp[lv1] pr ; TM6B, h D[3] Hu e / In(3R)Mo, Sb sr  
 #89300 In(3R)Mo, sr ; T(2;3)ap[Xa], ap[Xa] ca  
 #89400 M(3)86D ; T(2;3)Me, Me sbd[l] -- (synonym M(3)S31)  
 #89500 M(3)S32 ; T(2;3)Me, Me sbd[l]  
 #89600 M(3)76A ; T(2;3)Me, Me sbd[l] -- (synonym M(3)S34)  
 #89700 M(3)S36 ; T(2;3)Me, Me sbd[l]  
 #89800 Payne, Dfd l(3)PL l(3)PR ca ; T(2;3)101, ru h e[4] ro ca  
 #89900 Payne l(3)PL l(3)PR ; T(2;3)rn, rn Sb

#89950 SM1, al[2] Cy cn[2] sp[2] Pgk[4] Pgi[4] / Pgk[2] Pgi[2] ; TM6, Hn[P] ss[P88] bx[34e] Ubx[P15] e Men[4] Xdh(ry)[4] Aldox[4] mMdh[4] / Men[6] Xdh(ry)[2] Aldox[8] mMdh[2]

#900 B[S]Y / C(1)DX, y f / In(1)sc[4L] sc[8R], y sc[4] sc[8] cv v B

#9000 C(1)DX, y w f / Dp(1;1)w[+R], y[2] z

#90000 shv cn bw ; e

#90200 SM1, al[2] Cy cn[2] sp[2] ; TM2, Ubx[130] e[s] ; T(2;3)Sb[V] In(3LR)P35 In(3R)Mo, Sb[V]

#90250 SM1, al[2] Cy cn[2] sp[2] ; T(2;3)DII, Pc[3] (e)

#90500 ss bxd k e[s] ; T(2;3)ap[Xa], ap[Xa]

#90540 Su(er) tu-bw ; st su(tu-bw) er

#90550 su(Hw)[2] sbd ; T(2;3)ap[Xa], ap[Xa]

#90600 T(2;3)spy, spy

#90650 TM3, y[+] ri p[p] sep Sb bx[34e] e[s] ; T(2;3)osp[3], Adh[uf3] pr cn

#90670 TM3, y[+] ri sep Sb bx[34e] e[s] ; T(2;3)Scr[XF5]

#90700 Tp(3)bx[100], ri bxd[100] ; T(2;3)Me, Me sbd[l]

#90950 wg[F] ; st e

#91000 CyO, Cy dp[lv1] pr cn[2] / Df(2L)b81a1, b[81a1] Adh[uf3] cn ; +4 / spa[pol]

#91100 Df(4)M62f ; T(2;4)ey[D] ey[D] Alp[eD]

#91200 In(2L)Cy In(2R)Cy, Cy pr cn[2] ; ey[2] ; T(2;4)a

#91300 In(2L)Cy In(2R)Cy, Cy pr cn[2] ; ey[2] ; T(2;4)b

#91350 In(2L)Cy In(2R)Cy, Cy pr cn[2] ; T(2;4)d

#91400 In(3L)P, Me ; T(3;4)f

#91500 In(3L)P, Me ca ; T(3;4)A2

#91600 In(3L)P, Me ca ; T(3;4)A13, ve ca

#91800 In(3LR)Ubx[130], Ubx[130] e[s] ; T(3;4)e

#91900 Payne, Dfd l(3)PL l(3)PR ca ; T(3;4)c

#92100 C(1)DX, y f / X.YS, y / YL.sc[S1] ; cn bw ; +3 / e

#92200 sc z w[is] ; In(2L)Cy In(2R)Cy, Cy cn[2] ; In(3LR)Ubx[130], Ubx[130] ; T(2;3)ap[Xa], ap[Xa]

#92300 w ; b ; ve ca

#92440 y[2] sc w[a] w[ch] fa ; In(2L)Cy In(2R)Cy, Cy ; In(3LR)Ubx[130], Ubx[130] ; T(2;3)ap[Xa], ap[Xa]

#92445 In(1)w[m4], w[m4] ; In(2L)Cy, Cy ; T(2;4)Su(var)2, Su(var)2

#92450 Df(2L)A400, b cn bw / CyO, dp[lv1] Cy Adh[nB] pr cn[2] ; T(2;3;4)CA5

#92500 y ; net ; sbd[2] ; spa[pol]

#92600 Pod-R

#92700 sc z w[is] ; net ; sbd[2] ; spa[pol]

#9300 C(1)DX, y w f / Dp(1;1)w[sp], sc z w[sp] ec

#9500 C(1)DX, y w f / Dp(1;1)z[59d15], sc z[59d15]

#9600 C(1)DX, y w f / eo[S3M] / y[+]Ymal[106]

#9700 C(1)DX, y w f / FM7a, y[31d] sc[8] w[a] v[Of] B

#9800 C(1)DX, y w f / gt w[is]

#9900 C(1)DX, y w f / r[9]

#9950 C(1)DX, y w f / r[39k]

#S10 Drosophila novamexicana

#S11 Drosophila pseudoobscura

#S12 Drosophila simulans

#S12a Drosophila simulans -- Lankenau Yale

#S12b Drosophila simulans -- Barcelona

#S12c Drosophila simulans; st e

#S12e Drosophila simulans -- St Antico

#S12f Drosophila subobscura

#S13a Drosophila teissieri

#S13b Drosophila transversa

#S14 Drosophila virilis

#S14a Drosophila virilis -- Taura

#S14d Drosophila virilis -- w; b; gp2; cd; pe

#S14e Drosophila virilis -- b; gp; cd; pe

#S15a Drosophila yakuba -- Ivory Coast

#S1a Drosophila ananassae

#S2 Drosophila busckii

#S3a Drosophila funebris

#S4 Drosophila hydei -- Degeberga

#S4a	Drosophila hydei -- Finland
#S4b	Drosophila hydei
#S5	Drosophila immigrans
#S6a	Drosophila littoralis -- Uppland
#S7	Drosophila lummei
#S7a	Drosophila lummei -- Kuopio
#S7b	Drosophila lummei -- Vaajasalo
#S7c	Drosophila lummei -- white
#S8	Drosophila mauritiana
#S9	Drosophila nasuta
#S9a	Drosophila miranda
#W10	Algeria -- (Algeria)
#W1030	Wien -- (Austria; Wien, 1966)
#W1050	Zabcice -- (Czechoslovakia; Brno, 1977)
#W110	Birsk -- (USSR; 1974)
#W120	Boa Esperance -- (Brazil; Minas Gerais)
#W125	Bygdeå -- (Sweden; 1988)
#W130	Canton-S -- (USA; Ohio, Canton)
#W135	Cardwell -- (Australia; Queensland)
#W140	Champetieres -- (France; virusfree 78e)
#W141a	Chateau Tahbilq -- (Australia 1989)
#W141b	Chateau Tahbilq -- (Australia 1989)
#W141c	Chateau Tahbilq -- (Australia 1989)
#W141d	Chateau Tahbilq -- (Australia 1989)
#W141e	Chateau Tahbilq -- (Australia 1989)
#W145	Chichijima -- (Japan; 1979)
#W150	Chieti-v -- (Italy)
#W155	Chongju -- (Korea)
#W157	Coffs Harbour -- (Australia; New South Wales)
#W170	Crkwenica -- (Czechoslovakia)
#W180	Curitiba -- (Brazil; Curitiba)
#W20	Alma-Ata -- (USSR; Kazakstan, 1978)
#W200	Fairfield-2 -- (Australia; Melbourn 1980)
#W240	Fairfield-11 -- (Australia; Melbourn 1980)
#W250	Fairfield-12 -- (Australia; Melbourn 1980)
#W260	Fairfield-15 -- (Australia; Melbourn 1980)
#W270	Fairfield-16 -- (Australia; Melbourn 1980)
#W290	Fairfield-18 -- (Australia; Melbourn 1980)
#W30	Amherst-3 -- (USA)
#W300	Fairfield-19 -- (Australia; Melbourn 1980)
#W310	Falsterbo -- (Sweden)
#W320	Florida -- (USA)
#W370	Gelendzhik -79 -- (USSR; 1979)
#W40	Anapa-78 (USSR; 1978)
#W420	Gruta (Argentina)
#W430	Gurzuf USSR; 1961)
#W460	Hikone (Japan)
#W470	Hikone-R (Japan; resistant to BHC, DDT, parathione, nicotine)
#W482	Hämeenlinna (Finland 1972)
#W5	Akayu (Japan; 1976)
#W50	Anapa-79 (USSR; 1979)
#W501	Israel (Israel, 1954)
#W520	Karsnäs (Sweden; Karsnäs)
#W530	Karsnäs-R (Sweden; Karsnäs, Hg-resistant)
#W560	Krasnodar (USSR; 1974)
#W590	Kurdamir (USSR; Azerbaijan, 1977)
#W60	Ashtarak (USSR; Armenia, 1977)
#W600	Lausanne-S (USA; Wisconsin; Lausanne, 1938)
#W608	Luminy
#W611	Medvastö -29 (Finland; 1992)

#W612 Medvastö -21 (Finland; 1992)  
#W613 Medvastö -22 (Finland; 1992)  
#W614 Medvastö -23 (Finland; 1992)  
#W615 Medvastö -24 (Finland; 1992)  
#W616 Medvastö-25 (Finland; 1992)  
#W617 Medvastö-28 (Finland; 1992)  
#W618 Medvastö -29 (Finland; 1992)  
#W620 Moltrasio (Italy)  
#W630 Moravsky Pisek -- (Czechoslovakia; South Moravia, 1972)  
#W640 Naantali -- (Finland; Naantali, 1973)  
#W644 Nokia -- (Finland 1971)  
#W660 Oregon-K -- (USA; Oregon)  
#W670 Oregon-R -- (USA; Oregon, 1925)  
#W70 Aspra -- (Italy)  
#W72 Avondale-2 -- (Tasmania)  
#W729 Qiryat-Anavin -- (Israel; Jerusalem 1953)  
#W731 Qiryat-Anavim 81  
#W732 Qiryat-Anavim 83  
#W740 Salvador -- (Brazil; Bahia)  
#W750 Samarkand -- (USSR; Uzbek Republic; Samarkand, 1936)  
#W760 San Miguel -- (Argentina; Buenos Aires)  
#W80 Bacup  
#W800 Siniy Gay -- (USSR; 1979)  
#W820 Slankamen -- (Belgrad; Yugoslavia 1982.)  
#W830 Stäket -- (Sweden)  
#W840 Strängnäs-74 -- (Sweden; Strängnäs, 1974, isofemale)  
#W860 Strämsvreten 8 -- (Sweden; Åkersberga, 1969)  
#W890 Suchumi  
#W90 Berlin -- (Germany; Berlin)  
#W900 Swedish-B -- (Sweden; Stockholm, 1923)  
#W910 Tashkent (USSR; Uzbekistan, 1977)  
#W930 Turku (Finland; Turku, 1966)  
#W950 Uman-66 (USSR; 1966)  
#W970 Uman-79 (USSR; 1979)  
#W975 Umeå-15 (Sweden; 1992)  
#W976 Umeå-16 (Sweden; 1992)  
#W977 Umeå-17 (Sweden; 1992)  
#W978 Umeå-18 (Sweden; 1992)  
#W979 Umeå-19 (Sweden; 1992)  
#W990 Valdemarsvik-79 (Sweden, Valdemarsvik, 1979, isofemale)

## Mutation Notes - *Drosophila melanogaster*

### Report of Regina Chorsky and John Belote.

Department of Biology, Syracuse University, Syracuse, NY 13244.

*genitalia missing (gem)*: An autosomal recessive mutant that affects development of the genital disc derivatives

During the course of an X-ray mutagenesis screen designed to isolate mutants in the 73AD region (Belote, *et al.*, 1990), a deficiency of the *hairy* locus was discovered as a chromosome that failed to complement the *h<sup>2</sup>* allele present on the *Df(3L)st-81k17* tester chromosome. During the initial characterization of this *hairy* deficiency, called *Df(3L)h<sup>i22</sup>*, it was noticed that when this chromosome was made heterozygous with the *Df(3L)tra* chromosome, a genitalia-defective phenotype was produced (*e.g.* all or parts of the genital disc derivatives were missing). The mutant present on the *Df(3L)tra* chromosome responsible for this phenotype was mapped by meiotic recombination to a position very near the *h* locus. This new mutant, named *genitalia missing (gem)*, is assumed to lie within the region deleted in the *Df(3L)h<sup>i22</sup>* deficiency (*i.e.*, 66D10-14). The phenotypes of *gem/gem* and *gem/Df(3L)h<sup>i22</sup>* flies are similar, suggesting that the *gem* allele is an amorph or a strong hypomorph. Penetrance of this mutant is high, but the expressivity is variable: some mutant flies lack external genitalia, while others are missing only parts of the genital disc derivatives. Both males and females are affected, although the phenotype in males is more conspicuous. In addition to the genitalia defects, mutant flies also show abnormal wing veins (LV is incomplete). Both of these phenotypes map to the same position and are presumably due to the same genetic lesion.

Acknowledgments: The *Df(3L)h<sup>i22</sup>* deficiency was isolated in a screen carried out in collaboration with Dr. M. McKeown in the laboratory of Dr. B. Baker, UCSD. The *Df(3L)st-81k17* chromosome was provided by Dr. M.M. Green. This work was supported by a grant from the NIH.

Reference: Belote, J.M., F.M. Hoffmann, M. McKeown, R. Chorsky, and B.S. Baker 1990, Genetics 125: 783-793.

### Report of Zhimulev, I.F. Institute of Cytology and Genetics, Novosibirsk, 630090, Russia.

*Drosophila melanogaster* linkage data.

<i>In(3R)P23</i>	gamma-rays (4.0 kR) (Solovjova) <i>In(3R)9OD;100C1-2</i>
<i>In(1)AB</i>	(received from R. Woodruff). <i>In(1)9E7-8;13E1-2</i> . Homozygous viable.
<i>In(1)AM</i>	(received from B. Rasmuson). <i>In(1)8D6-12;16EF</i> . Homozygous viable.
<i>In(1)123</i>	(received from D. Nash). <i>In(1)9E1-1;20</i> . Homozygous lethal.
<i>In(1)BM2</i>	(received from B. Rasmuson). <i>In(1)16A1-6;20</i> . Homozygous viable.
<i>T(1;Y)B105</i>	(received from J. Merriam). X-chromosome break is located in the middle part of the band 10A1-2.
<i>flw</i>	Mutation was found to be within <i>Df(1)HC133</i> , <i>Df(1)vL15</i> , and <i>Df(1)sbrK1</i> but not within <i>Df(1)ras-v17Cc8</i> , <i>Df(1)vP5</i> , <i>Df(1)vL11</i> , <i>i.e.</i> , this mutation localized in 9B9-9C4 interval.
<i>ny</i>	Cytologically located in 10B3-10B17 interval: within <i>Df(1)RA37</i> , <i>Df(1)N71</i> , <i>Df(1)KA7</i> , not within <i>Df(1)vL3</i> and <i>Df(1)vL1</i> . Both expressivity and penetrance are stronger at 18°C; more than 90% of flies have notches on both margins of wings at 18°C, and less than 40% at 25°C and 30°C (only in inner margins of wings).

### Report of Chatterjee, R.N.

Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta-700 019, India.  
Allele of *intersex (ix)*.

The *intersex (ix)* locus is one known sex determining mutant gene in *D. melanogaster*. It was discovered by Morgan *et al.* (1943). The phenotypes of mutations of *ix/ix* and *ix<sup>2</sup>/ix<sup>2</sup>* flies were also recorded by Lindsley and Grell (1968) and Lindsley and Zimm (1985). The mutant *ix* is maintained as *B<sup>s</sup>Y, pr cn ix/SM5*. Using the same stock, provided by Dr. Rolf Nöthiger, Zoologisches Institut der Universität Zurich, Switzerland, I noted that, in some *B<sup>s</sup> Y, ix/ix* males, the

teeth of the sex combs were frequently arranged in more than one row (Figure 1). Some lines of homozygous  $ix^4/ix^4$  males were recovered and crossed with a balancer, SM5, for the stock. This mutant also caused certain alteration of caetotaxal pattern of the basitarsus of the foreleg in males. Homozygosity for the new mutant,  $ix^4$ , also caused diplo X individuals to develop as intersexes; however, phenotypes of external genitalia of  $ix^4$  intersexes are quite variable. This mutant is allelic to  $ix$ .

References: Lindsley, D.L. and E.H. Grell 1968, Carnegie Inst. Wash. Publ. 627; Lindsley, D.L. and G. Zimm 1985, Dros. Inf. Serv. 62; Morgan, T.H., H. Redfield and L.V. Morgan 1943, Carnegie Inst. Wash. Yearbook 42: 171-174.



Figure 1. Example of a sex comb of  $ix^4/ix^4$  male. Note that the teeth of the sex comb (arrow) arranged in more than one row.

**Report of Scheel, D.W., and W.W. Doane.** Department of Zoology, Arizona State University, Tempe, AZ 85287-1501.  
*P*-Tagged narrow mutant in *Drosophila melanogaster*.

In *Drosophila melanogaster*, narrow (*nw*) mutants have wings with somewhat pointed tips that are narrower and often longer than wild-type. Known *nw* mutations, whether dominant or recessive, cause reduced fertility. The *nw* locus is at 2-79.3 on the genetic map of chromosome 2R (Doane and Dumapias, 1987). We are studying a *CyO*-balanced mutant strain of flies that contains a defective *P* element in chromosome 2 and expresses a recessive "narrow-like" wing phenotype. Mutant males are completely sterile, while mutant females display reduced fertility.

The above mutant strain was derived from a stock in the PZ series maintained in the laboratory of T. Yamazaki (Kyusu University, Japan). The original strain was part of a collection of stocks with *P*-tagged second and third chromosomes (provided by H. Shibata) that had been isolated following mobilization of the defective *PZ* element in the X-chromosome of strain *p123.1*. After detecting the "narrow-like" wing phenotype in homozygotes of one of these PZ stocks, we replaced chromosomes 1 and 3, and made chromosome 2 isogenic. The derived strain is being analyzed on the premise that its mutant phenotype is due to insertion of a *PZ* transposon into the *nw* gene. We therefore refer to this strain as *CyO/nw<sup>PZ[ry+]</sup>; ry<sup>506</sup> e*.

The *CyO* balancer chromosome in our derived "narrow-like" strain carries the wild-type *nw<sup>+</sup>* allele. It is homozygous for the *ry<sup>506</sup>* mutant allele, but expresses the *ry<sup>+</sup>* gene carried in the *PZ* construct. Ebony (*e*) body color insures presence of a *ry<sup>506</sup>/ry<sup>506</sup>* background, since both markers are recessive and linked. (See Lindsley and Zimm, 1992, for explanations of genetic symbols). Homozygous *nw<sup>PZ[ry+]</sup>; ry<sup>506</sup> e* females are routinely crossed to *CyO/nw<sup>P[ry+]</sup>; ry<sup>506</sup> e/ry<sup>506</sup> e* males in order to maximize the number of mutant homozygotes among their progeny. At room temperature, only 26.6% of these progeny are homozygous for *nw<sup>P[ry+]</sup>; ry<sup>506</sup> e*, instead of the predicted 50%. This reflects the reduced viability of the mutants.

The wing phenotype of the putative *nw<sup>P[ry+]</sup>* mutants is compared to that of wild-type in Figure 1, C and A, respectively. In a test for allelism, a "narrow-like" wing phenotype was expressed by heterozygous *nw<sup>2</sup>/nw<sup>P[ry+]</sup>* flies (Figure 1, B). The most convincing wing defect appeared at their tips, which were slightly pointed in these heterozygotes when compared to the more rounded wild-type. Homozygotes of known recessive *nw* alleles (Lindsley and Zimm, 1992) often overlap wild-type in the range of their expression at 25°C, but can usually be distinguished by their pointed wing tips. Results from our test for allelism are consistent with the new mutation being an allele of the *nw* gene, since it did not fully complement *nw<sup>2</sup>* in the *trans* configuration.

The site of *PZ* insertion in the second chromosome carrying *nw<sup>P[ry+]</sup>* was located by *in situ* hybridization of a biotinylated probe to polytene chromosomes. A biotin-labelled probe containing *P* sequences (Carnegie 20) was made by nick translation (BRL Bio-Nick Labeling System) and hybridized to spreads of late third instar larval salivary gland chromosomes according to standard techniques (Ashburner, 1989), as modified by Johng Lim (University of Wisconsin-

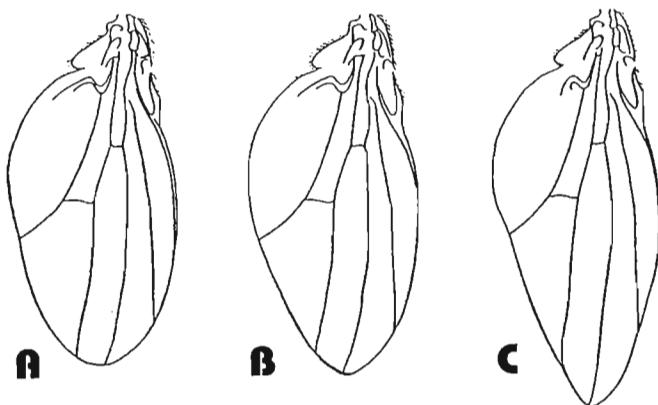


Figure 1. Wing phenotypes are compared among three genotypes: homozygous wild-type,  $nw^+/nw^+$  (A); heterozygous  $nw^{PZ[ry+]}/nw^2$  from the test for allelism (B); and homozygous  $nw^{PZ[ry+]}/nw^{PZ[ry+]}$  (C).

F.E. Dumapias 1987, Dros. Inf. Serv. 66:49; Lindsley, D.L. and G.G. Zimm 1992, *The Genome of Drosophila melanogaster*. Academic Press, NY; Thompson, D.B., L.G. Treat-Clemons, and W.W. Doane 1992, J. Exp. Zool. 262:122-134.

**Report of Zusman, S., T. Steet, S. Lam, and E. Mendelsohn.** Department of Biology, University of Rochester, Rochester, NY.

New lethal mutations obtained on a *white*, *FRT* chromosome.

X-linked lethal mutations have been induced on a *white*, *FRT*<sup>18A</sup>-containing chromosome (Xu and Rubin, 1993) by mutagenizing male flies with EMS. We screened 2052 *w*, *FRT* chromosomes and established 742 (36%) independent X-linked lethal containing-lines. Of these, 32 mutations were determined to be in the polytene region 11DEF-12B7, since hemizygous lethal-containing males carrying *Dp(1,f)y<sup>+</sup>*, *w y<sup>+</sup>* were viable. Although the precise endpoints of *Dp(1,f)y<sup>+</sup>*, *w y<sup>+</sup>* are not known, the left breakpoint of this duplication is proximal to the left breakpoint of *Df(1)N12* at polytene bands 11D1-2 (Lindsley and Zimm, 1992) and the right breakpoint is distal to *garnet* at polytene bands 12B6-7 (Lindsley and Zimm, 1992). As a result of complementation tests with previously identified alleles we determined that 3 mutations are in the *l(1)Eb* locus and all 32 mutations complement the loci, *l(1)Ec* and *l(1)Ed*.

Mitotic recombination was induced at the *FRT*<sup>18A</sup> sequences in *w*, *lethal FRT*<sup>18A</sup>/*FRT*<sup>18A</sup>; *FLP*<sup>38</sup>/*FLP*<sup>38</sup> flies by inducing expression of a *FLP* recombinase-producing transposon, 24-36 hours after fertilization, as described in Chou and Perrimon (1992) and Xu and Rubin (1993). The resulting flies were examined for the presence of *white* eye clones and obvious morphological abnormalities. Eye clones were induced in approximately 71% of control *w*, *FRT*<sup>18A</sup>/*FRT*<sup>18A</sup> flies. 228/742 mutations (31%) yielded no homozygous *white* clones and are most likely cell lethal in the eye. We also found that 2 mutations produce abnormally small eyes, 3 mutations produce notched wings, 2 mutations produce scalloped wings, 18 mutations produce wing blisters and 3 mutations produce random wing abnormalities. Of the 18 mutations that produce wing blisters, 4 are new alleles of *inflated* and 2 are new alleles of *myospheroid*. The effect of homozygous mutant clones on eye development is presently being sought.

References: Xu, T. and G. Rubin 1993, Development 117:1223-1237; Lindsley, D.L. and G.G. Zimm 1992, *The Genome of Drosophila melanogaster*, Academic Press Inc; Chou, T. and N. Perrimon 1992, Genetics 131:643-653.

Eau Claire; personal communication). A single site of hybridization was observed. This was in 54E of the polytene map for chromosome 2R, again consistent with the new mutation being a *P*-induced *nw* allele. Thus, the cytological location of the *nw* gene appears to be in region 54E. Assuming that *nw* has been molecularly tagged, sequences flanking the *PZ* insert can be recovered and used as probes to clone and sequence the *nw<sup>+</sup>* allele. This work is in progress. Cloning the *nw* gene will permit analysis of its role in wing development and gamete formation. It also will provide a starting point for a short "chromosomal walk" to the nearby *mapP* gene, a *trans*-acting regulatory gene that controls expression of alpha-amylase structural genes in the posterior midgut of flies at the level of *Amy* RNA abundance (Thompson *et al.*, 1992).

References: Ashburner, M. 1989, *Drosophila: A Laboratory Manual*. Cold Spring Harbor Lab. Press; Doane, W.W. and A.G. Clark 1984, Dros. Inf. Serv. 60:234; Doane, W.W. and

## Mutation Notes - Other Species

**Report of Coyne, J.A.,<sup>1</sup> and P. Sniegowski.<sup>2</sup>** <sup>1</sup> Department of Ecology and Evolution, The University of Chicago, 1101 E. 57th St., Chicago, IL 60637, and <sup>2</sup>The Center for Microbial Ecology, PSS, Michigan State University, East Lansing, MI 48824. A third-chromosome balancer in *Drosophila simulans*.

We report here a third-chromosome balancer stock of *D. simulans* that contains previously undescribed paracentric inversions. We obtained a stock of *D. simulans* heterozygous for *Ubx<sup>m</sup>/K<sup>s</sup>* (*K<sup>s</sup>* is a mutation producing spread wings) from T.K. Watanabe, who originally obtained it from E.H. Grell. The spread-wing mutant was not visible in the stock, but all flies showed the *Ultrabithorax* phenotype. As *Ubx<sup>m</sup>* is homozygous lethal, this observation implied the presence of an inversion in this strain. We crossed *Ubx<sup>m</sup>/K<sup>s</sup>* females to males heterozygous for the third-chromosome dominant mutation *Delta*. The *Ubx<sup>m</sup>/Dl* F<sub>1</sub> females were backcrossed to wild-type *D. simulans* males in an attempt to produce, through recombination, a single chromosome containing both *Ubx<sup>m</sup>* and *Dl*. As these two genes are more than 7 map units apart in *D. melanogaster* (*Ubx<sup>m</sup>* at 3-58.8 and *Dl* at 3-66.2), we expected a substantial number of recombinants. We observed none, however, among 527 offspring. This again suggested an inversion on the *Ubx<sup>m</sup>*-carrying chromosome. The F<sub>1</sub> *Ubx<sup>m</sup>/Dl* flies were interbred, producing a strain in which all flies showed both mutations for more than ten generations. These observations further suggested the presence of an inversion, probably of the *Ubx<sup>m</sup>*-containing chromosome. We made a number of salivary-gland squashes of larvae from the *Dl/+* x *Ubx<sup>m</sup>/+* cross, and observed that about half the flies contained a moderately-sized paracentric inversion on the right arm of the third chromosome, an inversion that itself harbored a very small included inversion (see Figure 1). We call this inversion *In(3R)Ubx*.

The normal 3R of *D. simulans* also differs from that of *D. melanogaster* by a paracentric inversion of 9 numbered divisions: using the *D. melanogaster* system, the chromosome order of the normal *D. simulans* 3R is 61-84F/93F-84F/93F-100 (Ashburner, 1989). Again using the *melanogaster* numbering, the breakpoints of the inversion on the *D. simulans* *Ubx<sup>m</sup>*-containing chromosome are 81F1 and 89E1, with the small included inversion having



Figure 1. Salivary-gland chromosome squash of *D. simulans* heterozygous for *In(3R)Ubx*.

breakpoints at 84B1 and 84E1. Relative to *D. melanogaster*, then, the *D. simulans* *Ubx*<sup>m</sup> chromosome has the sequence: 61-81F1/89E1-93F/84F-84E1/84B1-84E1/84B1-81F1/89E1-84F/93F-100. (The underlined region is the region inverted relative to the normal *D. simulans* 3R).

*Delta* is cytologically located in region 92A2 in *D. melanogaster*, and *Ubx* at 89E1 (Lindsley and Zimm, 1992). *Ubx* is, therefore, located at one inversion breakpoint, and *Dl* is within the inverted region. It is, therefore, not surprising that no recombinants were observed.

Although the region balanced by this chromosome is rather small, the stock is useful because it allows one to keep two dominant alleles in *trans* condition without selection.

References: Ashburner, M. 1989, *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory Press; Lindsley, D.L. and G.G. Zimm 1992, *The Genome of Drosophila melanogaster*. Academic Press, Inc., San Diego.

## Guide to Contributors

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## Technique Notes

**Strecker, T., S. McGhee, S. Shih, and D. Ham.** Pomona College, Claremont, CA. A modified protocol for permeabilization and culture of living *Drosophila* embryos.

organic solvent for permeabilizing the vitelline membrane in older (12-13 hr) *Drosophila* embryos (Lynch *et al.*, 1988). The application of hexane at this late stage of embryogenesis did not affect embryonic viability, but its effect on the development of early embryos was not addressed in this study. Furthermore, permeabilization was measured as a function of observed plasmolysis when treated embryos were exposed to a 1M sucrose solution, but the degree and uniformity of permeabilization in these embryos was also not assessed. Here we report a modified protocol for permeabilization and culture of living embryos within the first hours of embryogenesis using hexane as a permeabilizing agent. With this protocol we found hexane to be more effective at permeabilizing the vitelline membrane than octane and have reported a comparison of the effects of these solvents on uniformity of permeabilization and embryonic viability during the first 7 hrs of embryogenesis (Strecker *et al.*, 1994).

The culture medium, MBIM, used in these experiments is a modification of the original basic incubation medium (BIM) which was described by Limbourg and Zalokar (1973) as:

MgCl <sub>2</sub> · 6H <sub>2</sub> O	0.22 g
MgSO <sub>4</sub> · 7H <sub>2</sub> O*	0.297 g
NaH <sub>2</sub> PO <sub>4</sub> *	0.042 g
Glutamic Acid*	1.21 g
Glycine*	0.605 g
Malic Acid*	0.066 g
Sodium Acetate	0.0027 g
Glucose*	0.22 g
dd H <sub>2</sub> O	86 ml.

Reagents marked with an asterick (\*) are tissue culture reagent grade (Sigma). This solution was neutralized with a mixture of equal amounts of 5% NaOH and 5% KOH to pH 6.8, after which 0.099 g of CaCl<sub>2</sub> was added, and the final volume of the solution was adjusted to 100 ml with the addition of sterile, distilled water. Then, the buffer was sterilized through a 0.2 micron filter (Nalgene).

In preparation for culturing living *Drosophila* embryos, siliconized deep well slides were irradiated with a UV light in a tissue culture hood for approximately 20 minutes. Embryos were collected for 1 hr at 25°C in 100 ml plastic beakers over yeasted grape juice/agar plates (Nusslein-Volhard, 1977), and then incubated at 25°C until they reached a desired stage during early development. Following the incubation period, eggs were dechorionated by flooding the plate's surface with 2.6% sodium hypochloride solution (50% Clorox bleach; Hill, 1945). While dechorionated embryos were drying, 25 microliters of MBIM was added to an irradiated, siliconized deep well slide followed by 500 microliters of organic solvent. Between 20-30 embryos were transferred to the slide well. Using a drawn pasteur pipette, three quarters of the organic phase was removed and the embryos were aligned end to end, in a ring, by gentle tapping. After the complete evaporation of the organic phase, 75 microliters of MBIM was added to the well to cover the embryos. The slide was incubated at 25°C and 55-60% relative humidity in a petri dish lined with a moist Kim-Wipe for 15 minutes.

After incubation, 56S halocarbon oil (Halocarbon Products Corp.) was slowly added to the side of the well, displacing most of the MBIM to the opposite side. Using a microcapillary tube connected to an adjustable vacuum line, the MBIM was completely removed from the well, taking extra care to suction away medium surrounding each embryo. After MBIM was removed, the slide was incubated at 25°C and 55-60% relative humidity in a petri dish lined with a moist Kim-Wipe for 24 hrs. Although we describe only 20-30 embryos treated in one slide well, we routinely used three, triple walled slides at a time, so a total of 240-360 embryos were used in a given experiment. This procedure can be repeated four to six times a day resulting in over one thousand treated embryos.

We observed hexane to be a more effective permeabilizing agent of early embryos than octane, with more embryos exhibiting dark and uniform staining with vital dyes following a brief exposure to this organic solvent (Strecker *et al.*, 1994). Although hexane is found to permeabilize the vitelline membrane of the *Drosophila* egg more effectively than

The organic solvents, octane and heptane, have been used routinely to permeabilize the hydrophobic vitelline membrane which surrounds the *Drosophila* embryo (Limbourg and Zalokar, 1973; Arking and Parente, 1980; Mitchison and Sedat, 1983). Recently, it has been reported that hexane is a more effective

octane, it does decrease the viability of embryos exposed between 0 and 3 hrs of age (Strecker *et al.*, 1994). Embryos between 3 and 7 hrs old exhibited equally high viability following hexane exposure as those embryos treated with octane. Molecules with a molecular mass greater than or equal to 984 daltons were not absorbed into the embryo following treatment with either hexane or octane. Given the importance of the *Drosophila* embryo as a model system for early development, this procedure should enable investigators to more successfully introduce small molecules uniformly throughout living *Drosophila* embryos in culture.

References: Arking, R. and A. Parente 1980, J. Exp. Zool. 212:183-184; Hill, D.L. 1945, Dros. Inf. Serv. 19:62; Limbourg, B. and M. Zalokar 1973, Dev. Biol. 35:382-387; Lynch, D.V., S. P. Myers, S.P. Leibo, R.J. MacIntyre, and P.L. Steponkus 1988, Dros. Inf. Serv. 67:89-90; Mitchison, T.J. and J. Sedat 1983, Dev. Biol. 99:261-254; Nusslein-Volhard 1977, Dros. Inf. Serv. 52:166; Strecker, T.R., S. McGhee, S. Shih, and D. Ham 1994, Biotech. Histochem. 69:25-30.

**Lin, James C., and A. Villavaso.** Northwestern State University, Natchitoches, Louisiana, U.S.A. A stepper motor driven microinjecting device.

micrometer gauge with fingers is quite unreliable, it often balloons up the body or without delivering enough material to the insect.

To use a stepper motor with precise stepping control has been found to be a very satisfactory improvement over hand injection. The stepper motor and the stepping control unit are available commercially as a complete kit or sold by different companies as separate items\*. They are not popular enough to be found in most of the electronic stores in the United States. If you can buy a kit including both a motor and a controller, follow the instructions which come with the kit to assemble the unit, and follow the steps at the end of this paper to couple the unit with the injector syringe. If not, the following schematic diagram (Figure 1) and description will help you to assemble your own controller board and decipher the wires coming from the motor in case the colors of the wires are not the same as the ones in the schematic diagram.

The stepper motor you buy must have to be a 12 V, four-phase (four-coil) unipolar motor (A bipolar motor requires two separate voltage supplies, the SAA 1042 chip can only drive a unipolar stepper), and it doesn't require more than 1 ampere. If the coil current is not given, calculate it by dividing the stepper motor voltage (in volts) by the coil resistance (in ohms).

A four-phase (four-coil) stepper motor may have 5 to 8 wires that connect to the coils. See Figure 2 below.

If the motor has 8 wires, all the coil ends (terminals 1-8 above) are available externally. A 6-wire motor has two pairs of coils internally connected, for example, terminals 2-3 internally connect coils A and B together and terminals 6-7 internally connect coils C and D together. If the motor has 5 wires, one wire is the common point for all four coils, for example, terminals 2-3-6-7 connected together, with one wire for each coil remaining.

The following discussion and the schematic diagram provided in Figure 1 use a 6-wire motor configuration. The colors of the wires from the motor do not follow a standard code; therefore, it is necessary to identify the proper coil terminals. For a 36-ohm, 6-wire motor, three wires should emerge from each half of the stator. An ohmmeter should read 72 ohms across two of the wires (terminals 1-4 and 5-8), and 36 ohms between the other two pairs (terminals 1-2 & 3-4 and 5-6 & 7-8). Once the wires are identified, the stepping sequence of the coils for shaft rotation can then be determined. First use a marker pen to put a dot on one side of the shaft to make movement easier to see. Next, connect the two common wires (terminals 2-3-6-7) to the positive (+) terminal of the power source. Arbitrarily select one of the remaining four wires to be phase coil "A." Touch this wire momentarily to the power source ground (-) terminal to establish the shaft's position. Now, select one of the two unconnected wires from the other stator half, touch it momentarily to ground, and observe the direction of rotation of the shaft. If the shaft turns clockwise, this is coil C. If the shaft doesn't turn clockwise, again touch the coil A wire momentarily to ground and then touch the other unconnected wire of the other stator half momentarily to ground. This should cause the motor to turn clockwise. This wire is coil "C." The other unconnected wire from the coil A stator half is coil "B" and the other unconnected wire from the coil C stator half is coil "D." The sequence of coils A-C-B-D being touched to the ground terminal should result in the continuous movement of the shaft in a clockwise direction. Repeat the pattern of touching the ground wire individually with each of the four wires several more times to verify the sequence. The connections between each of the coils (phases) and each of the four motor controller pins in the SAA 1042 can be made by the schematic in Figure 1.

The switch S1 on the control board in Figure 3 allows the change of a full step ( $7.5^\circ$ ) to half step ( $3.75^\circ$ ) to

Microinjection is a commonly used method in assaying mutagenicity and toxicity of chemical reagents in insects. One of the difficulties involved in the application of this technique is to deliver a repeatable amount of material to each specimen. Turning the

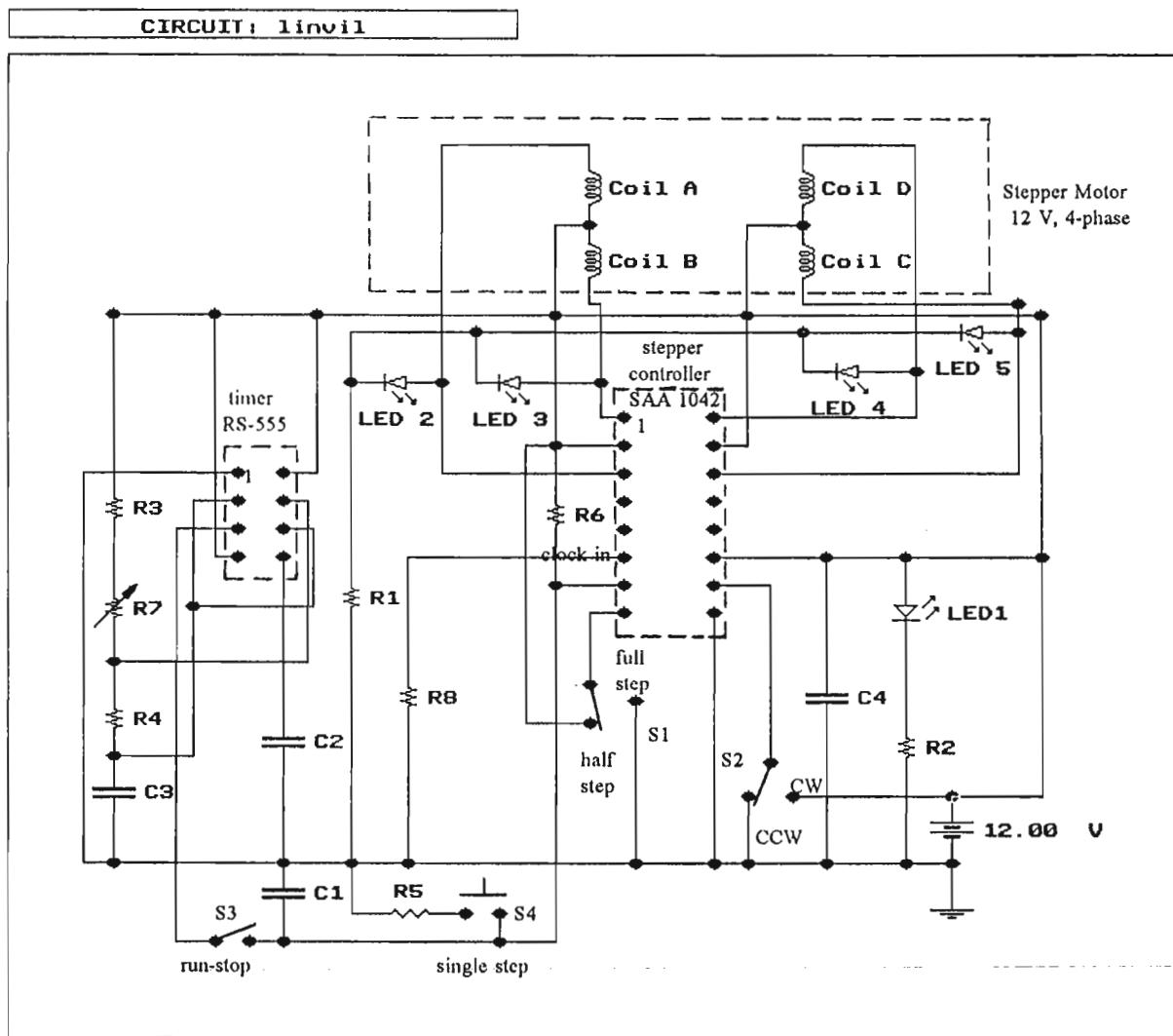


Figure 1. Schematic diagrams of the stepper motor and the stepper motor controller board.

decrease the amount of material delivered in each stepping. The S2 switch changes the direction of movement from clockwise to counter clockwise, so the syringe plunger can be pulled back to refill the syringe with injection solution through the glass needle. Switch S3 speeds up the movement of the micrometer forward or backward. Switch S4 is a momentary switch which makes the motor step once when it is triggered. I use it when I do my injection. When the needle is outside the insect body, 1 microliter of insect saline is delivered for 5 triggerings of the momentary switch. The pressure inside the body of the insect is high, it may not deliver as much. But the amounts delivered to different insects are quite uniform.

To adapt the stepper motor to the syringe micrometer control is simple. The coupler is made of

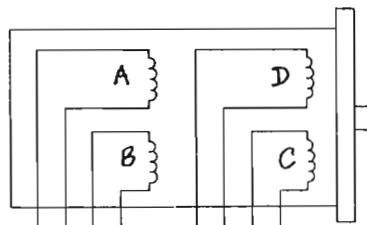


Figure 2. The coils and wires of the stepper motor.

Figure 3 (at the right). The stepper motor and the stepper motor controller.

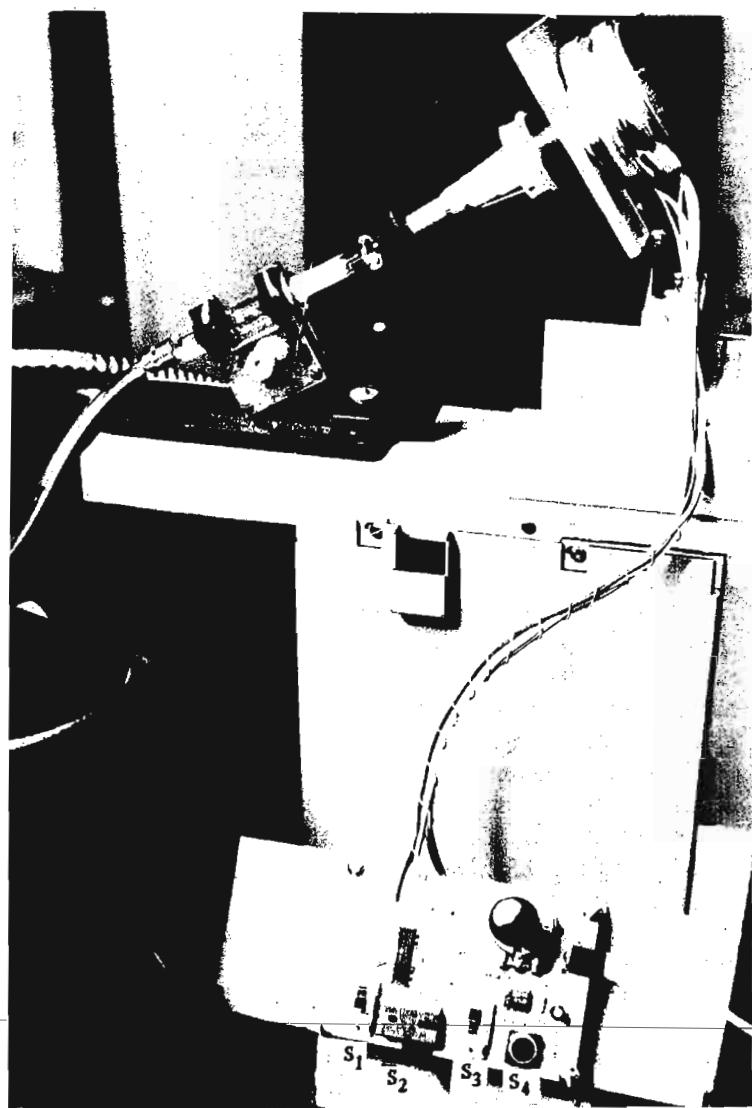


Figure 4. Coupler assembly. A, stepper motor; B, a 1 cm slice cut from a rubber stopper with a central hole which can fit the motor shaft. Two thin nails are pushed through on the sides of this piece. C, a 0.5 cm slice cut from a rubber stopper with a central hole which fits to the top of the micrometer, and two side holes for the two needles to slide through. D, micrometer.

one No. 6 rubber stopper and two thin 4.5 mm long nails (or thick dissecting pins). The stopper is sliced into a 1 cm thick large end piece and a 0.5 cm thick middle piece. Discard the small end piece. Drive the nails through the 1 cm thick piece (A) as shown in Figure 4. The two small holes bored on the 0.5 cm piece (B) should be large enough to allow free sliding of the two nails when the motor shaft turns. The central holes of the two pieces can be bored according to the diameters of the motor shaft and the micrometer head. The motor is attached to a thin wooden board with a central hole. The unit is, in turn, fastened to a wrap-around hinge (about 3.5 cm in length) which is mounted on the top of a wooden pillar (3 x 2 x 4 cm). A large wooden base is used to glue the pillar on. The size of which depends on the height of the syringe micrometer. The one made for my unit has dimensions of 10 x 10 x 5 cm.

Microinjection is an effective technique in genetic studies, but I was often frustrated in finding a way to stop the leak of injecting solution at the junction between the glass needle and the needle holder. After trying many different things I finally found that the best way is to use a piece of silicon rubber. Cut a very small piece of cured silicon rubber, poke a hole through it with a pin, and pass the end of the glass needle through the needle holder cap and the silicon block before going through the collar and the holder core. When the cap is tightened, I seldom find leaks any more.

References: Iovine, John, 1992, Build an astrophoto platform, *Astronomy*, Nov. 1992, pp 60-63; Mendelson Electronic Co., Inc., 1992, Stepper motor drive II assembly notes. MECI, Dayton, Ohio.

\* The complete kit of stepper motor and motor controller parts is available at the Mendelson Electronics Co., Inc., 340 East First Street, Dayton, Ohio, USA 45402. The stepper motor can be obtained from The Images Company North, P.O. Box 14072, Staten Island, N.Y., USA 10314-0024.

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fermenting fruit have been eliminated. Many of them use agar to make the medium firm, but in our days, the cost of the agar is high. This is, in some countries, an unsolved problem which makes the use of fruit flies for research difficult. In this note, we report the carrageenans as a practical substitute of the agar in the culture medium. These types of gums will reduce the cost of media by about 80%.

The carrageenans are sulfated polymers made up of galactose units. The common backbone of carrageenans consists of a main chain of D-galactose residues linked alternately  $\alpha$ -(1-3) and 8-(1-4). The differences between the types (Iota, Kappa, Lambda, Mu, Nu) are due to the number and position of the sulfate groups. The botanical sources are red seaweeds (Rhodophyceae) which have a broad geographical distribution (France, Argentina, Chile, Peru, the North Atlantic coastal regions, Philippines, Indonesia, Brazil and Senegal) of the genera *Gigartina*, *Chondrus*, *Iridaea*, *Euchemia* and *Hypnea*.

The species used for the industrial extraction and the fraction obtained from them are *Euchemia cottoni* (K), *E. spinosum* (I), *Gigartina acicularis* ( ) and *Chondrus crispus*, *G. stellata*, *Iridaea* sp (K + I + ). The carrageenans have many uses in the food industry as a gelling agent and as a thickener in dairy desserts, chocolate milks, meat preserves and pet foods as well as in other industries (e.g., cosmetics and toothpaste) or in *in vitro* meristem culture. Due to the differences in the capacity of gelling among different fractions, a mixture containing a refined extract of red seaweeds of the Kappa type (gelling agent, standardized with sugar) and an extract of the Lambda type (thickener, standardized with dextrin) in the proportion of 3:7 (v/v), was employed. In this way a resistant and flexible gel suitable for *Drosophila* cultures could be obtained. In our laboratory we used the following recipe to prepare the media: 1250 ml water, 18 ml complex of gums, 85 ml sugar, 220 ml corn meal, 55 g dry beer yeast, 0.4 g nipagin, 4 ml propionic acid; 870 ml of the water was added to mix the gums with the sugar and 380 ml to mix the corn meal and the dry beer yeast. The first mixture was heated until boiling and the nipagin was added, the second mixture was added to the first, boiled for a few minutes before being removed from the fire, the propionic acid was added and well mixed. Subsequently the medium was filled into dry bottles with no need to sterilize them.

We have been culturing pure strains of *Drosophila melanogaster* in this medium for more than a year, and heretofore no differences have been observed compared to cultures with agar media, neither in size of population, fertility of flies or viability of eggs oviposited in the media, nor in changes of phenotypes or spontaneous mutations. In fact, no mites were present since the carrageenans were incorporated into the culture medium.

In conclusion, the carrageenans have several advantages which make them suitable substitutes of agar in the culture media of *Drosophila melanogaster*. Moreover, there are companies that produce and supply them, in Europe and America, without any problem.

References: Demerec, M., and B.P. Kaufman 1961, *Drosophila Guide*, 7th ed., Carnegie Institution of Washington Publication, Washington D.C.; King, R.C., 1975, *Drosophila melanogaster*: an introduction. In: R.C. King (ed.) *Handbook of Genetics*, Vol. 3, Plenum Press, New York, pp. 625-652; Würgler, F.E., F.H. Sobels, and E. Vogel 1984, *Drosophila* as an assay system for detecting genetic changes. In: Kilbey, B.J., M. Legator, W. Nichols and C. Ramel (eds.) *Handbook of Mutagenicity Test Procedures*, 2nd. ed., Elsevier Science Publishers. Amsterdam, pp. 555-601.

**Allison, Greg and Holly Hilton.** Rutgers University, Piscataway, N.J. A combination of techniques for the control of *Drosophila* mites.

here. In order to prevent future infestation, we experimented with our mites using stocks of *D. virilis*.

Attempts at reducing mite populations by transferring to fresh food every other day were unsuccessful, as the mites and/or mite eggs remained on the individual flies. To prevent the transfer of mites, the flies were anesthetized with triethylamine (Carolina Biological Supply Co.'s FlyNap), and the flies were tapped on to a piece of dark-colored construction paper. The mites remained active despite the anesthetic and a large majority crawled off their hosts onto

*Drosophila* can be raised on a variety of fermenting plants, like grapes or ripe banana with yeast added. Indeed, these were the first media used but have implied serious troubles such as excess of moisture, molding and lower firmness. However, there exist numerous recipes on how to get *Drosophila* to flourish like those described by Demerec and Kaufmann (1961), King (1975) and Würgler *et al.* (1984), in which the problems with

fermenting fruit have been eliminated. Many of them use agar to make the medium firm, but in our days, the cost of the agar is high. This is, in some countries, an unsolved problem which makes the use of fruit flies for research difficult. In this note, we report the carrageenans as a practical substitute of the agar in the culture medium. These types of gums will reduce the cost of media by about 80%.

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Our laboratory experienced an outbreak of anoetid mites (*Histiostoma laboratorium*) on numerous lines of *Drosophila virilis* and *D. melanogaster*. We were successful in eradicating the mites while saving our fly lines by using a combination of techniques described

Table 1. Survival time up to twenty minutes on construction paper (left column) or anti-mite paper (right column). The two groups were significantly different at the  $p < 0.0001$  level.

Control	Anti-Mite
20:00	3:12
20:00	3:30
20:00	3:41
20:00	4:54
20:00	2:07
20:00	2:07
20:00	3:15
20:00	2:45
20:00	2:11
20:00	3:05

Table 2. Survival time up to twenty minutes after transfer from: construction paper to construction paper (left column), one minute on mite paper to construction paper (middle column) and thirty seconds on mite paper to construction paper (right column). Planned contrasts showed significant differences ( $p < 0.0001$ ) between the control versus both treatments and control versus each treatment individually. The thirty and sixty second trials were also significantly different ( $p < 0.0056$ ).

Control	60 sec	30 sec
20:00	3:25	20:00
20:00	3:49	12:39
20:00	20:00	3:40
20:00	4:01	10:59
20:00	3:59	8:22
20:00	5:30	4:52
20:00	3:18	20:00
20:00	4:17	11:36
20:00	7:19	12:59
20:00	4:01	11:36

from the starting point. In a separate control group, ten mites were transferred in the same manner to a piece of construction paper. All mites survived this treatment, living a full 20 min (see Table 1). The two groups were significantly different in an ANOVA at the  $p < 0.0001$  level.

To assess whether mites could survive migrating to closely spaced vials despite a short exposure to anti-mite shelf paper, a second set of trials were conducted. Mites were exposed to the anti-mite shelf paper for 30 or 60 sec, after which the mites had no choice but to crawl onto construction paper. The mites were timed from the moment they touched the shelf paper until they were no longer able to move on the construction paper. Nine out of ten mites did not survive a full minute on the anti-mite shelf paper, although two mites out of ten survived the 30 sec exposure. A separate control group of ten mites was placed on a piece of construction paper, and the mites were then forced to crawl on to an identical sheet. This entire group survived the full twenty minutes (see Table 2). A single factor ANOVA with planned comparisons was used to analyze the effects of exposure of mite paper on mite survivorship. Contrasts between the control group and both timed short exposure to mite paper was significantly different at  $p < 0.0001$ . Individual contrasts between the control group and each of the short times were both significant at the  $p < 0.0001$  level. The 30 sec and 60 sec exposure were also different at the  $p < 0.0059$  level.

It appears that fresh shelf paper can be an effective deterrent against mite infection. The vials should not be too closely spaced, however, and a extra measure of mite paper should be placed around the outside of the racks. No mite traveled further than 15 cm in one minute, an exposure which was enough to quickly kill most mites.

References: Ashburner, M. 1989, *Drosophila, A Laboratory Handbook*. Cold Spring Harbor Laboratory Press.

the construction paper. (This is observable with a dissecting scope.) Loose mites were crushed with the blunt end of a paint brush and the flies were then gently brushed onto a second piece of construction paper. The first piece of construction paper was disposed of in a dish of soapy water. At this stage, each individual fly was examined for mites. If any mite or mite egg was seen on a fly, the mite was brushed off and killed. Dead flies were also removed as they may have had hidden mite eggs on them. The clean flies were transferred to new media vials, which were plugged with dense balls of cotton. Cotton is a denser material than styrofoam plugs, and is known to prevent mites from reinfesting vials (Ashburner, 1989). Vials were placed in vial racks that had been sterilized and were lined with fresh anti-mite shelf paper. All laboratory surfaces and the incubators had been cleaned with soap and disinfectant.

*Effects of Temperature.* The effects of temperature on mite survival and development were assessed by placing vials containing adult mites and vials containing mite eggs in each of three different temperatures for 24 hrs: 4°C (refrigerator), -20°C (freezer), and 25°C (incubator). Vials placed in the latter temperature served as a control group.

In the control group, adult mites survived and new eggs were deposited. Eggs also developed into adults in the incubator. In the refrigerator, adult mites survived and eggs developed although they took longer to develop. At the freezing temperature, adults died and eggs did not develop.

Our results suggest that refrigeration of media vials will not prevent infestation of anoetid mites. In order for freezing temperatures to act as an effective deterrent, vials must be frozen for at least twenty-four hours prior to use.

*Effects of "Pest Control" Paper.* The effectiveness of Carolina's Anti-Mite shelf paper in preventing the migration of mites to new vials was assessed. In the first trial, ten mites were placed on a piece of shelf paper treated with methylcarbamate (2,2-dimethyl-1,3-benzodioxyl-4-ol). Each mite was timed from the moment it touched the anti-mite paper until it was no longer able to move. No mite survived longer than 5 minutes or walked further than 20 cm

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use the P element in *D. melanogaster* as a model system to determine the feasibility of using transposable genetic elements to drive genes into natural populations of insects. A full account of the results of these experiments will be presented elsewhere. Here we report only details of the methods used for *in situ* hybridization.

The technique we used employs DIG nucleic acid labeling. Detection kits were supplied by Boehringer Mannheim. Labeled DNA probes were generated with Taq DNA polymerase by PCR incorporation of a nucleotide analog, digoxigenin-11-dUTP. DIG-labeled DNA was detected, after hybridization to the target site, by enzyme-linked immunoassay using an antibody conjugate and an enzyme-catalyzed color reaction.

#### DIG-dUTP-labeled probe preparation using the Polymerase Chain Reaction (PCR).

Amplification of a DNA probe larger than one kilobase was performed by incorporation of digoxigenin-11-dUTP using PCR. The probe used for amplification and labeling was plasmid p $\pi$ 25.1, containing a 2.9 kb complete P element isolated from *D. melanogaster* (O'Hare and Rubin, 1983). PCR was carried out using the entire plasmid in a DNA Thermal Cycler (Perkin-Elmer Cetus) according to the following protocol: 5 ul Cetus 10x PCR buffer; 3 ul of 25 mM MgCl<sub>2</sub>; 4 ul of 10x concentrated dNTP labeling mixture (1 mM/1 of dATP, 1 mM/1 of dCTP, 1 mM/1 of dGTP, 0.65 mM/1 of dTTP and 0.35 mM/1 of DIG-dUTP); primer: #829 (terminal inverted repeats of P element), 5 ul (132 ng); 1 ul template p $\pi$ 25.1 plasmid DNA (including 2.9 kb P element insert) (100 ng/ul); 0.5 ul AmpliTaq DNA polymerase; sterile deionized water (31.5 ul) was added to the mixture to achieve a final volume of 50 ul; Mix and overlay with one drop of mineral oil (Sigma Co.) in 0.5 ml Eppendorf reaction tubes.

The amplification was performed for 30 cycles with 1-minute denaturation at 95°C and 1-minute annealing at 50°C, followed by a 3-minute extension at 72°C. The temperature was then dropped to 4°C for 15 minutes to overnight. The PCR product was removed from under the oil and placed in new tubes. The results of PCR were confirmed by electrophoresis using 5 ul on 1% agarose minigel. Then the product is cleaned using a Quick Precipitation Method\* and stored at -20°C.

#### \* Quick Precipitation Method

1. Begin with 45 ul PCR solution.
2. Add 1/2 volume 7.5 M NH<sub>4</sub>OAc (22.5 ul).
3. Add 1 volume cold 100% EtOH (67.5 ul).
4. Vortex and let sit for 5 minutes at room temperature.
5. Spin 5 minutes in a microcentrifuge (12,000 rpm).
6. Decant off EtOH and add 1 volume 70% EtOH (67.5 ul).
7. Spin for 1 minute (12,000 rpm).
8. Decant 70% EtOH.
9. Speed vacuum to dry.
10. Resuspend in 40 ul TE buffer (10 mM Tris-HCl, pH 8.0/1 mM EDTA).
11. Leave for 30 minutes at room temperature and spin briefly, then store at -20°C.

#### Denaturation of labeled probe DNA

1. Add reagents to a sterile microfuge tube (on ice) in the following order: 10 ul of 20x SSC; 25 ul of deionized formamide; 8 ul of 50% dextran; 2 ul of deionized sterile water; 5 ul of DNA probe in TE buffer. Mix and spin briefly. The 50 ul of final volume is enough for hybridization of 8 slides.
2. Denature the probe at 95°C for 5 minutes, and chill on ice quickly for at least 5 minutes before use.
3. Either apply the probe to a slide for hybridization, or store at -20°C for future use.

We have modified a technique for *in situ* hybridization to polytene chromosomes, using DIG nucleic acid labeling, in order to determine the number and chromosomal locations of P element constructs in a series of experiments using *Drosophila melanogaster*. These experiments were designed to investigate the population dynamics of P element constructs carrying the *ry*<sup>+</sup> gene, following genetic transformation by microinjection (see research report by Kim *et al.*, this issue). The overall purpose of the experiments was to

use the P element in *D. melanogaster* as a model system to determine the feasibility of using transposable genetic elements to drive genes into natural populations of insects. A full account of the results of these experiments will be presented elsewhere. Here we report only details of the methods used for *in situ* hybridization.

**Preparation of salivary gland polytene chromosomes**

1. Select climbing third instar larvae for squashing. (Larvae should be grown at 18°C with a few drops of 5% yeast suspension. Female larvae are preferable for examining the X chromosome).
2. Wash the larvae in 0.7% NaCl prior to dissection.
3. Dissect the salivary glands in a drop of 45% glacial acetic acid on a silicone-treated slide.
4. Transfer the glands to a small drop (6-10 ul) of fixative\* on a clean silicone-treated 18 x 18 mm coverslip. Fix the glands for about 3 to 4 minutes.

\* Fixative is composed of lactic acid (86%), water, and glacial acetic acid in a ratio of 1:2:3, respectively.

5. Pick up the coverslip containing the fixed glands with a clean, nonsiliconized slide coated with Denhardt's solution\*. Invert so that the coverslip is facing upwards. To make the squash, the thumb should be firmly pressed on the coverslip above the glands under paper (Whatman #1).

**\* Preparation of slides coated with Denhardt's solution**

- a. Incubate slides in SSC-Denhardt's solution\*\* at 65°C for 2.5 hours.
- b. Dip in distilled water for a few seconds.
- c. Fix the slides in ethanol-acetic acid (3:1) at room temperature for 20 minutes.
- d. Air dry.
- e. Store at 4°C until needed.

**\*\*SSC-Denhardt's solution**

In order to make 800 ml of solution, enough for 2-3 dishes of 20 slides each, combine the following:

120 ml of 20x SSC  
 16 ml of 1% (w/v) PVP 360 (polyvinylpyrrolidone)  
 16 ml of 1% ficoll (MW: 400,000)  
 16 ml of 1% nuclease-free BSA (Sigma A-7906)  
 Add water to the mixture to achieve 800 ml.

6. Keep the slides with the squashes between two plates of glass and press them with a weight (about 500 g) at room temperature over night (18-20 hours).
7. Place the slides on dry ice (block shape) for about 30 minutes.
8. Pry off each coverslip individually from the dry ice using a razor blade.
9. Place the slides, without coverslips, into a jar containing 70% ethanol for about 10 minutes at room temperature. Transfer the slides from 70% ethanol to a new jar containing 95% ethanol and store the slides about 10 minutes at room temperature.
10. Air dry the slides, completely. After that, slides can be stored for a few months before hybridization.

**Pretreatment of polytene chromosomes for hybridization**

1. Place the slides in 2x SSC and incubate at 65°C for 30 minutes.
2. Dehydrate the slides twice by first placing in 70% ethanol and then in 95% ethanol, each for 5 minutes at room temperature. After incubation, air dry completely.
3. Denature the chromosomes in freshly prepared 0.07 N NaOH for 2 minutes.
4. Wash the slides in 2x SSC three times, for 5 minutes each, and dehydrate the slides with ethanol by incubating as same direction in step 2.
5. Air dry completely. These slides can now be used for hybridization.

**Hybridization**

1. Apply 6 ul of denatured probe to each slide.
2. Cover with a coverslip (24 x 24 mm).
3. Keep the slides in a humidity chamber and hybridize for about 20 hours at 37°C.

**Immunological detection**

1. Remove the coverslip and place the slides in 3x SSC-50% formamide twice for 10 minutes each at 37°C.
2. Wash the slides twice for 5 minutes each in 2x SSC at room temperature.
3. Wash the slides twice for 5 minutes each in 1x SSC at room temperature.
4. Wash the slides twice for 3 minutes each in Buffer 1\* at room temperature.

**\* Buffer 1:**

150 mM NaCl  
100 mM Tris-HCl, pH 7.5

5. Incubate the slides in Buffer 2\* for 30 minutes at room temperature.

**\* Buffer 2:**

0.25 g Blocking reagent (Genius Kit)  
50 ml Buffer 1

Buffer 2 does not dissolve very rapidly, so prepare the solution 1 hour in advance and dissolve at 50-70°C with gentle mixing.

6. Wash the slides in Buffer 1 for 5 minutes.

7. Place the slides horizontally in a light protected box and add 600 ul of diluted antibody conjugate\* onto each slide and incubate at room temperature for 60 minutes.

**\* Diluted antibody-conjugate (1:5,000)**

1 ul antibody-conjugate (Genius kit)  
5 ml Buffer 1

8. Wash the slides twice, for 15 minutes each, in Buffer 1.

9. Wash the slides twice, for 15 minutes each, in Buffer 3\*.

**\* Buffer 3:**

100 mM Tris-HCl (pH 9.5)  
100 mM NaCl  
50 mM MgCl<sub>2</sub>

10. Apply 120 ul of color solution\* on each slide and cover the solution with a 24 x 50 mm coverslip.

**\* Color solution:**

4.5 ul NBT solution (Genius Kit)  
3.5 ul X-phosphate solution (Genius Kit)  
1.0 ml Buffer 3

11. Incubate at room temperature for 15 - 30 minutes in a light-protected box.

12. Check the condition of the color reaction with a phase contrast microscope.

13. If the hybridized band is clear (deep blue color), remove the coverslip and fix the reaction for about 10 minutes with Buffer 4\*.

**\* Buffer 4:**

10 mM Tris-HCl (pH 8.0)  
1 mM EDTA

14. The chromosomes with hybridized band are photographed with a drop of Buffer 4 under the coverslip. The slides were dried in air and stored in the dark at 4°C.

**Acknowledgments:** This work was partially supported by a grant to Dr. M.G. Kidwell from WHO, and a grant from the Korean Science and Engineering Foundation (KOSEF) to Dr. W. Kim. We thank Drs. M.F. Wojciechowski

and A. Dickerman for help and advice in the plasmid preparation and PCR, respectively. We are also indebted to Dr. J. Clark for supplying the P element primers.

Reference: O'Hare, K. and G.M. Rubin 1983, Cell 35:25-35.

**Isono, K., A. Harada, K. Ozaki, \*G. Lazarova and Y. Tsukahara.** Graduate School of Information Sciences, Tohoku University, Sendai 980-77, Japan. \*Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria. Computer-controlled power supply system for field-inversion gel electrophoresis.

compared with ordinary electrophoresis systems.

Field-inversion electrophoresis is a method developed by Carle *et al.*, where large DNA samples can be electrophoretically separated by simple forward and backward movement in the agarose gel through alternating DC polarity (Carle *et al.*, 1986). Since the system only requires temporal control, we have converted an 'ordinary' electrophoresis unit to the field-inversion type by adding to the DC power supply system a simple device controlled by a personal computer.

#### Field Inversion Gel Electrophoresis

Status	Run	Stop	Wait
	Forward	Backward	
Clock	Present Time / 13:50:48	Time Passed / 01:50:48 ( 10%)	
	Initial Time ~ Final Time	Time Remaining	
Ramp Set	[A]12:00:00 [B]06:00:00	16:09:12 ( 90%)	
	Initial Cycle Final Cycle [Present Cycle]		
	[C] + 10 sec [E] + 60 sec [ + 15 sec ]		
	[D] - 2 sec [F] - 5 sec [ - 2 sec ]		
Command	[X]Start [Y]Stop [Z]Exit		

Figure 1 (above). A printout of the computer screen monitoring the conditions of field-inversion gel electrophoresis. Key strokes A-F change the parameter values for run time and for switching cycles. X,Y and Z are for starting and stopping the program.

Figure 2 (at the right). Separation of chromosomal and YAC DNAs by customized field-inversion gel electrophoresis. Electrophoresis was carried out on 0.8% agarose gel in 0.5x TBE buffer for 12 hours at an alternating forward and backward DC of 150 V (5 V/cm). The alternating cycle was changed from initial values of 10 and 5 seconds (forward and backward migration, respectively) to those of 60 and 5 seconds. Left: bacteriophage T4 DNA (166Kb) + Lambda DNA (49 Kb); middle: DY1 (YAC containing 120 Kb *Drosophila* genomic DNA); right: DY4 (YAC containing 100 Kb *Drosophila* genomic DNA). Yeast chromosome DNAs I (225 Kb), VI (295 Kb), III (375 Kb) are marked by small arrows. YAC DNAs are marked by white arrows. Larger chromosomal DNAs are not resolved under these conditions.

The only hardware required is a computer board mounted relay for switching main power and the polarity of the DC output. A simple program written in C language controls the switch and enables custom settings of various parameters such as running period and pulse duration for 'forward' and 'backward' modes. The pulse-time can also be programmed to create an interval ramp during electrophoresis to improve size separation of DNAs.

Most *Drosophila* genomic fragments cloned in yeast artificial chromosomes (YACs) are 100-300 Kb with a mean of around 170 Kb (Garza *et al.*, 1989) and can be separated from the yeast chromosomes only by pulse-field electrophoresis. Pulse-field systems usually entail special apparatus to control the electric field both spatially and temporally, including an elaborate chamber and power supply. These systems are very expensive

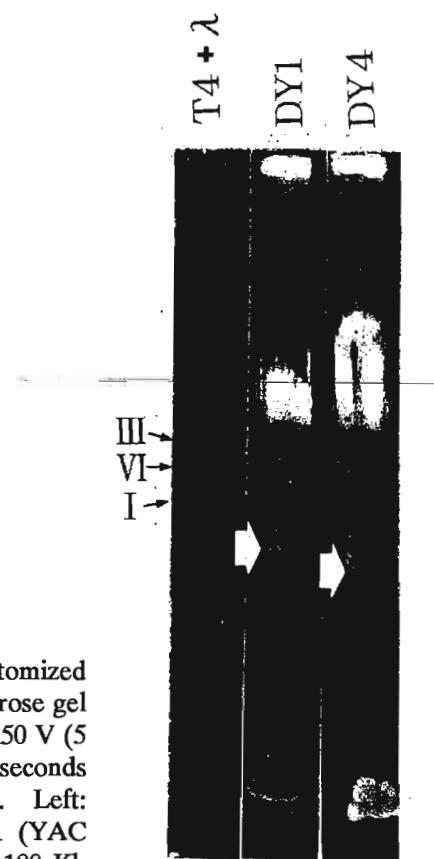


Figure 1 is a screen dump showing the various parameters during electrophoresis. A typical result with this system using two *Drosophila* YAC clones is shown in Figure 2. Without any modifications to the gel electrophoresis system, we obtained separation of chromosomal DNAs of up to 500-600 Kb in size. The resolution is, therefore, more than enough to isolate 100-300 Kb YAC DNAs. For larger DNAs, however, it is recommended that a smaller amount of samples be used to obtain better resolution than with other types of pulse-field system.

The program and the switching board were designed for a NEC computer (PC9801). But with minor modifications they should perform well with IBM and compatibles. The program and source code are available on request.

Acknowledgment: The YAC clones were gifts from Dr. D. Hartl's lab at Washington University. This work was supported by grant-in-aid for Scientific Research (C) 03680216 to one of us (K.I.).

References: Carle, G.F., M. Frank, M.V. Olson 1986, Science 232:65-68; Garza, D., J.W. Ajioka, D.T. Burke, and D.L. Hartl 1989, Science 246:641-646.

## Special Issues

DIS 73: *Drosophila* Genes/Chromosomes

DIS 74: Bibliography of *Drosophila*, 1982-1993

published by the FlyBase Consortium

DIS 73 is a compendium of four tables. Three, compiled by the FlyBase Consortium, are major revisions of their DIS 69 counterparts describing *D. melanogaster* genes. The fourth is a detailed description of the polytene maps of *D. melanogaster*.

GENETIC LOCI lists genes sorted alphabetically by abbreviation and listing gene name, map position, gene product information and literature citations.

GENE FUNCTION lists loci assorted by the function of the gene's product.

GENE and ALLELE SYNONYMS lists synonyms alphabetically together with their corresponding valid genetic symbols.

SALIVARY GLAND CHROMOSOME MAPS by T.I. Heino, A.O. Saura and V. Sorsa consist of light and electron micrographs of the polytene chromosomes, and histograms of chromatin and DNA per band.

DIS 74, also compiled by the FlyBase Consortium, is an exhaustive reference list of *Drosophila* from 1982 through 1993 inclusive, presented in standard bibliographic order.

Each volume is about 675 pages. Each costs \$29.00 plus shipping. Domestic UPS (U.S.A. Only) is \$4.50 per copy; Canada and Mexico is \$7.00 per copy; other foreign surface shipping is \$6.00 per copy. Foreign air parcel post costs per copy are: Canada and Mexico \$8.00; Central and South America \$13.00; Europe \$22.00; other foreign destinations \$30.00.

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## Research Notes

**Inoue, Y., Y. Igarashi and T. Watanabe.** Osaka University of Foreign Studies, Japan. Inversion polymorphism of Japanese *Drosophila melanogaster* populations in 1970's and 1980's.

of which are found once for all (Mettler *et al.*, 1977). But in fact the border between *Recurrent* and *Unique Endemics* is not clear. More and more often, *Unique Endemics* are recorded as a number of surveys are carried out, and a few out of them are found to show the temporal polymorphism by the large scale of survey for one population. In this note we classified the polymorphic type of inversions to the following three: *Common Cosmopolitans*, *Rare Cosmopolitans* and *Endemics* (= just not cosmopolitan). A total of 17 populations (Figure 1) distributing in middle and western Japan and the South-east islands were surveyed in the 1970's (Table 1) and compared with 10 geographically corresponding populations which were surveyed in the 1980's (actually from 1979 to 1992) (Table 2). The locality number approximately corresponds in Table 1, Table 2, and Figure 1. Inversions were identified through direct observation of single  $F_1$  larva chromosomes from each isofemale line which was established immediately after collection. Frequency of each inversion was calculated per each chromosome arm. For all 27 populations the correlation was calculated among total inversion frequencies of each chromosome arm, being proved to be positive and all statistically significant; right and left arms of the second chromosomes ( $\gamma = 0.954$ ,  $p < 0.01$ ), right and left arms of the third chromosomes ( $\gamma = 0.885$ ,  $p < 0.01$ ), and the second and third chromosomes ( $\gamma = 0.961$ ,  $p < 0.01$ ). Therefore the average frequency of polymorphic inversions per major autosome arm was used to compare the degree of polymorphisms among populations. It is suggested the inversions of all chromosome arms respond to selective pressure in a same way. Adaptive differences among chromosomes of various gene arrangements might not be due to the genic components included in each inversion, but due to the inverted structure itself.

In Yamagata (Locality No. 1 in Figure 1) 1972 and 1977, four *Common Cosmopolitans* and one *Rare Cosmopolitan* were found.  $In(2L)t$  frequencies were high (19.4% and 18.8%), and  $In(3L)P$  was very low (0% and 0.9%) in both years.  $In(3R)C$  was also stably found with frequencies of 6.3% and 3.4%. The average

The naturally occurring inversions of *D. melanogaster* have been categorized to four classes, considering their geographical distribution and frequencies; *Common Cosmopolitans*, *Rare Cosmopolitans*, *Recurrent Endemics* which are frequent in only the restricted regions, and *Unique Endemics* each

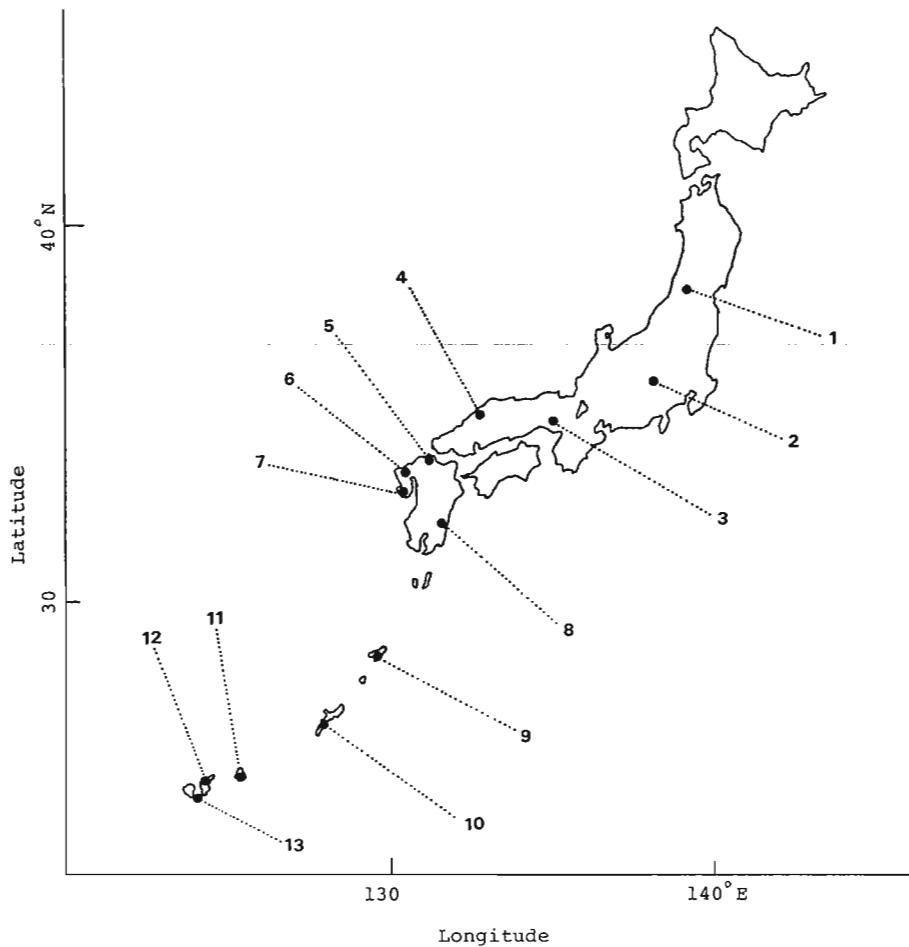


Figure 1. Thirteen Japanese natural populations of *D. melanogaster* examined for inversion frequency in the present study.

Table 1. The polymorphic inversion frequencies per major autosome arm in Japanese natural populations in nineteen seventies.

Localities\$	Year	No. of Chromosomes analyzed	Common Cosmopolitans				Rare Cosmopolitans			Endemics		Arm # Average
			2Lt	2RNS	3LP	3RP	3LM	3RC	3RMo	2LA	92C;96A	
1. Yamagata	1972	144	.194	.063	0	.097	0	.063	0	0	0	.068
	1977**	430	.188	.115	.009	.107	0	.034	0	0	0	.091
2. Yamanashi	1970*	200	.155	.140	.025	.165	0	.170	.030	0	0	.171
3. Hyogo	1974*	406	.197	.133	.022	.121	.005	.165	.027	0	0	.168
4. Shimane	1970	40	0	0	0	0	0	0	0	0	0	0
5. Fukuoka	1977**	350	.106	.049	.037	.052	0	0	0	0	0	.049
6. Saga	1970	226	.008	.004	0	0	0	0	0	.027	0	.010
7. Nagasaki	1970	462	.004	.004	.002	0	0	0	0	.022	0	.008
8. Miyazaki	1973	798	.015	.011	.004	.019	0	.001	.003	.016	0	.017
9. Amami (Naze) (Koniya)	1973	134	.254	.333	.370	.341	0	.123	.072	0	0	.373
10. Okinawa (Nago)	1973	332	.051	.012	.024	.075	.003	0	.060	0	0	.056
	(Naha)	62	.468	.355	.355	.548	.032	.048	.032	0	0	.460
11. Miyako	1973	500	.010	.008	.004	.008	0	0	.002	.008	.006	.006
12. Ishigaki	1971	58	.707	.397	.466	.828	0	0	0	0	0	.600
	1973	878	.614	.353	.386	.746	.001	.024	.004	0	.005	.533
13. Taketomi	1971	92	.609	.315	.315	.696	0	.022	0	0	0	.489

\$ Locality No. corresponds to Fig. 1. # Average frequencies of inversions per major autosome arm. \* Data from Inoue et al. 1984.

\*\* Data from Yamazaki et al. 1984.

Table 2. Inversion frequencies in nineteen eighties.

Localities\$	Year	No. of Chromosomes analyzed	Common Cosmopolitans				Rare Cosmopolitans				Endemics		Arm # Average
			2Lt	2RNS	3LP	3RP	3LM	3RC	3RMo	3RK	2LW	3LY	
1. Yamagata	1982	200	.180	.075	.010	.110	0	.025	.020	0	0	0	.105
2. Yamanashi	1990	200	.140	.050	.020	.195	0	.020	.005	0	.010	0	.110
3. Osaka	1992	240	.175	.096	.054	.096	.004	.067	.046	0	0	0	.134
4. Shimane	1982	70	.029	0	0	0	0	0	0	0	0	0	.007
5.													
6. } Kitakyushu	1982	96	.021	0	0	.021	0	0	0	0	0	.010	.013
7.													
8. Minamikyushu	1982	132	0	0	0	0	0	0	0	0	0	0	0
9. Amami (Ogachi)	1982	260	.089	.081	.092	.085	0	.050	.008	0	.004	.004	.103
10. Okinawa (Nago)	1982	256	.642	.353	.358	.599	.016	.043	.035	.004	0	0	.511
12. Ishigaki	1979*	200	.555	.380	.455	.825	.025	.005	.010	0	0	0	.564
13. Iriomote	1982	62	.532	.323	.500	.855	0	.065	0	0	0	0	.569

\$ Locality No. corresponds to Table 1 and Fig. 1. # Average frequencies of inversions per major autosome arm. \* Data from Inoue et al. 1984.

values were 6.8% and 9.1%, respectively. In 1982, the overall polymorphic phase was not changed, although one *Rare Cosmopolitan*, *In(3L)M*, was added with a frequency of 2%. In Yamanashi (No. 2) 1970, 2Lt, 2RNS, 3RP and 3RC showed more than 15%, and 3LP and 3RMo were found with a frequency of 2-3%. The average was 17.1%, which was the highest of all mainland populations. In 1990, although 3RP increased a little, 2RNS and 3RC were much decreased (from 14% to 5% and from 17% to 2%, respectively). The average was 11%, which was almost the same as Yamagata 1982. Hyogo (No. 3) 1974 was compared with Osaka 1992, because they were the neighbouring districts. The Hyogo 1974 population had 2Lt, 2RNS, 3RP and 3RC with individual frequencies of more than 10%. The average was 16.8%, to be as high as Yamanashi 1970. In the Osaka 1992 population the decrease of 2Lt, 2RNS, 3RP and 3RC were recognized, and it resulted in an average decrease of 13.4%. Three kinds of *Rare Cosmopolitans* (3LM, 3RC and 3RMo) were sampled in both 1974 and 1992 in this region. In Shimane (No. 4) 1970 no inversion was observed, although the sample was small, 40 genomes. This population showed an almost monomorphic phase also in 1982. In the Kyushu region (Fukuoka: No. 5, Saga: No. 6, Nagasaki: No. 7 and Miyazaki: No. 8), the frequencies of all inversions were only a few percent except the Fukuoka 2Lt case (10.6%) in the 1970's. The averages were all low: 4.9%, 1%, 0.8%, and 1.7%, respectively. In both Kitakyushu and Minamikyusyu of 1982, some small samples were pooled, respectively. Kitakyusyu hardly kept inversions, the average being 1.3%, and Minamikyusyu did not keep inversions at all. In western Japan all the regions proved to be inversion-free or almost inversion-free in the 1970's were confirmed in the 1980's. In Amami-oshima (No. 9), three sampling sites were a little separated, and the results varied from one another. In 1973 four *Common Cosmopolitans* showed high frequencies with the range from 25.4% to 37%, and 3RC was 12.3%. The average was very high at 37.3%, which was more than twice that of the mainland populations. But in the sample of 1974, these inversions' frequencies were much decreased to show the average of 5.6%, which was in the level of the mainland populations. Then in 1983 the frequency level of each inversion was between the 1973's and 1974's samples. In Okinawa (No. 10) two populations were sampled in 1973, and they showed the similar results. Four *Common Cosmopolitans*

were in much higher frequencies than Amami 1973, the average being 52% and 46%, respectively. The sample of 1982 also showed the same result of many *Common Cosmopolitans*, the average being 51.1%. Three *Rare Cosmopolitans* (3LM, 3RC and 3RMo) were found in all three Okinawa samples, and especially 3RK was found in 1983 with a frequency of 0.4%. The Ishigaki-jima (No. 12) samples of 1971, 1973 and also 1979 showed the same polymorphisms as Okinawa, the frequencies of four *Common Cosmopolitans* being very high, from 35.3% to 82.8%. The averages were 60% (1991), 53.3% (1973) and 56.4% (1974), and were rather more than Okinawa's averages. Taketomi (No. 13) and Iriomote are small islands near Ishigaki-jima. The samples of Taketomi 1971 and Iriomote 1982 showed the same patterns as the Okinawa and Ishigaki populations, the averages being 48.9% and 56.9%, respectively. There was no difference due to time-gap.

Thus, the South-west Islands' populations have many more inversions than the mainland populations. Furthermore the sample of Okinawa, Ishigaki, Taketomi and Iriomote display the common phenomenon specific to the South-west Islands, that is, the frequencies of all four *Common Cosmopolitans* were similarly very high in comparison with those of the mainland populations. The 3LP frequencies were always lower than the other three *Common Cosmopolitans* in the mainlands. Amami-oshima (No. 9) exists geographically between Minamikyusyu (almost no inversion region) and Okinawa (high inversion frequency region), and it might be related to the fact that the frequencies of Amami samples varied year by year. But the Amami populations also display the characteristic of the South-west Islands mentioned above, that is, 3LP is not the least inversion among *Common Cosmopolitans* in all samples of 1973, 1974 and 1983, regardless of their average differences. There is one more thing to be noted. Although Miyako-jima (No. 11) is a small island between Okinawa and Ishigaki-jima both of which are the regions of high inversion frequency, its average was extremely low (0.6%) in 1973. Sampling scale was not small, but almost no inversion was observed in Miyako-jima. This fact might be related to the habitat differences. In Okinawa and Ishigaki flies are sampled in the pine-apple yards and factories with large populations of flies, and Miyako-jima does not have such appropriate places for flies propagation. As a whole, the phases of inversion polymorphisms were not changed in the mainlands and South-west Islands, respectively, during the 1970's and 1980's. For *Endemic* inversions, 2LA was found in several populations and 3R(92C;96A) was polymorphic in only the Miyako population in the 1970's. But in the 1980's, in place of these two inversions, 2LW and 3LY were newly observed in several localities.

References: Inoue, Y., T. Watanabe and T.K. Watanabe 1984, Evolution 38: 753-765; Mettler *et al.* 1977, Genetics 87: 169-176.

**Benado, M.<sup>1,2</sup> and M. Budnik<sup>2</sup>.** <sup>1</sup>Departamento de Estudios Ambientales, Universidad Simon Bolivar, P.O. Box 89000, Caracas, Venezuela and <sup>2</sup>Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Santiago, Chile. Adult competition between *Drosophila subobscura* and *D. simulans* and between *D. subobscura* and *D. pavani*.

serial transfer techniques that include the effect of adult interactions upon population sizes. We report on serial-transfer competition experiments between *D. subobscura* and *D. simulans*, and between *D. subobscura* and *D. pavani*, all commonly found in Chile. The null hypothesis is that the species' population sizes are the same when competing homo- and hetero-specifically.

#### MATERIAL AND METHODS

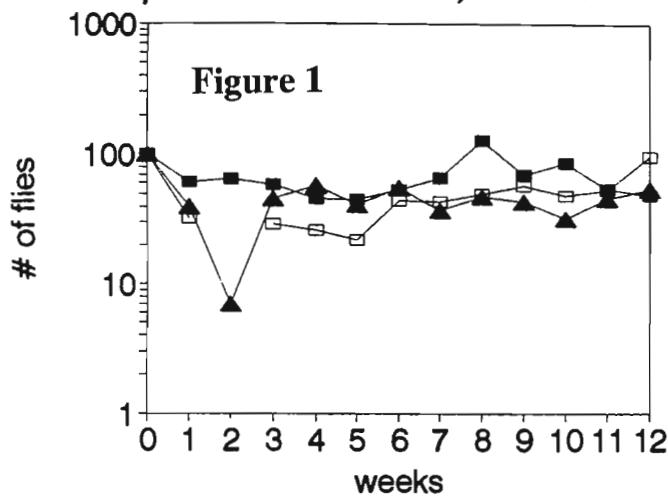
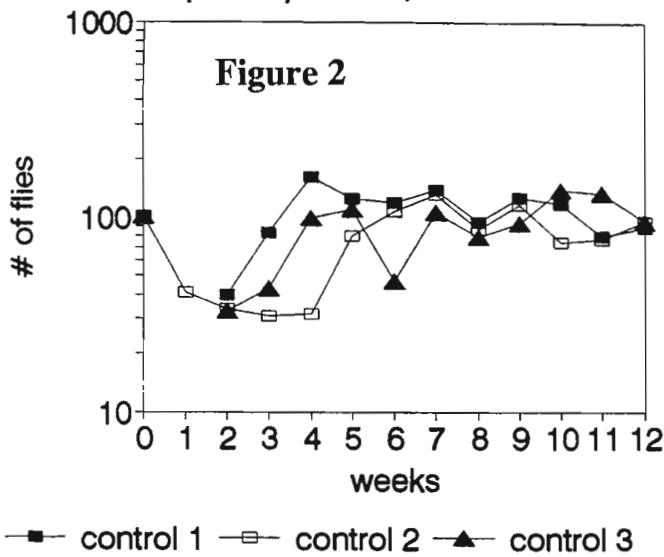
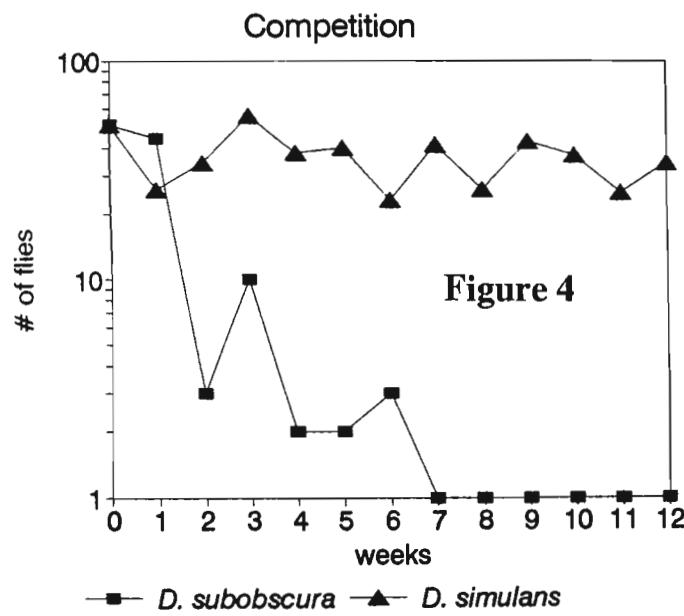
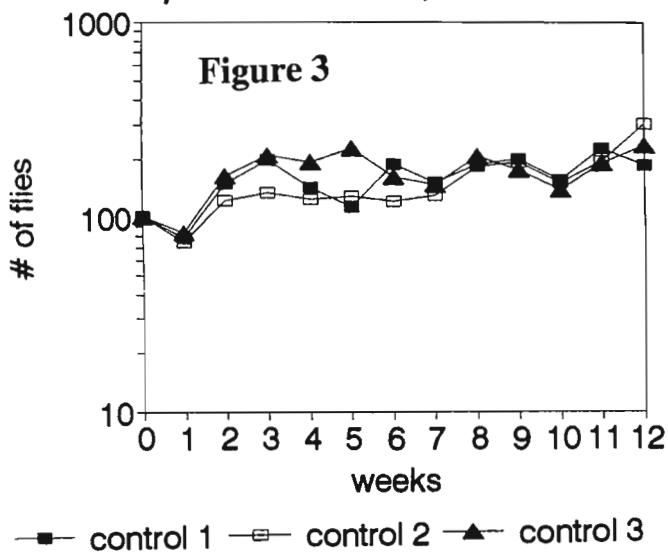
All the experiments were carried out in 9.5 cm L x 2.5 cm D vials with 10 cc food, at 19°C, and were started on March 24, 1992, with 100 individuals/vial. The food formula was the same used in previous experiments with larvae (see References). All the strains came from La Florida, in the outskirts of Santiago (Benado *et al.*, 1993).

We used a serial-transfer protocol with one week transfer time. We run three controls for each of the species (monospecific experiments), and three competition experiments for each pair of species, started with adults in the proportions 2:8, 5:5, and 8:2, respectively (Table 1). The flies were transferred, but not counted, until the first adults began emerging in the series. The experiments were terminated the 12th week for the controls and when no further flies emerged for any of the two species in the competition series; this happened by the 12th week.

#### RESULTS AND DISCUSSION

The outcomes of competition are indicated in Table 1, and some population trajectories are depicted in Figures 1,

*Drosophila subobscura* is a palearctic species that invaded Chile about 15 years ago (Budnik *et al.*, 1982). Since then, it has become a permanent member of the Chilean fauna (Benado *et al.*, 1993). Our understanding of this colonization process has been increased by studying in the laboratory larval competitive interactions between this species and other resident *Drosophila* (Budnik *et al.*, 1974, 1982, 1983; Brncic *et al.*, 1982, 1987). The experiments that we report here make use of

*Drosophila subobscura*, controls*Drosophila pavani*, controls*Drosophila simulans*, controls

## Competition

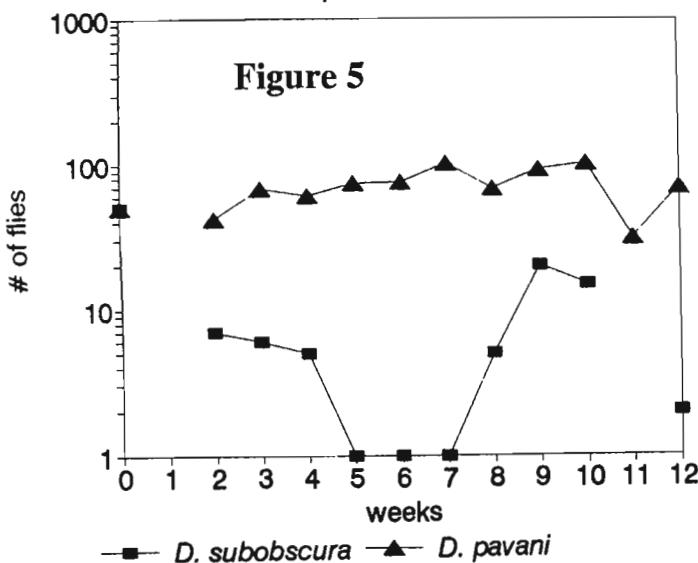


Table 1.- Experimental design and results of competition.

Species	Replicates	Competitor*	Species Fixed
SIM	1, 2, 3	—	—
PAV	1, 2, 3	—	—
SUB	1, 2, 3	—	—
SUB	—	(0.20) SIM	SIM
SUB	—	(0.50) SIM	SIM
SUB	—	(0.80) SIM	SIM
SUB	—	(0.20) PAV	PAV
SUB	—	(0.50) PAV	PAV
SUB	—	(0.80) PAV	PAV

SIM: *D. simulans*; SUB: *D. subobscura*; PAV: *D. pavani*.

\*: ( ) competitor's initial frequency.

2, and 3 (controls), and in 4 and 5 (competition). *D. simulans* outcompeted *D. subobscura*, and *D. pavani* outcompeted *D. subobscura*.

The results indicate that *D. subobscura* is a bad competitor when adult interactions are taken into consideration. Furthermore, they agree with data that show that the species is also a bad competitor at larval stages (Budnik *et al.*, 1982, 1983; Brncic *et al.*, 1987). Thus, it seems unlikely that competitive displacements of resident *Drosophila* by *D. subobscura* is a mechanism involved in the successful colonization of Chile by the species.

Acknowledgments: Funded by FONDECYT, 193078.

References: Benado, M. and D. Brncic 1993, in press, Z. zool. Syst Evolut-forsch. An eight year phenological study of a local drosophilid community in Central Chile; Brncic, D. and M. Budnik 1982, Arch. Biol. Med. Exp. 13: 481-490; Brncic, D. and M. Budnik 1987, Genetica Iberica 39: 249-267; Budnik, M. and D. Brncic 1974, Ecology 55: 657-661; Budnik, M. and D. Brncic 1982, Actas V Congres. Latinoam. Genetica 177-188; Budnik, M. and D. Brncic 1983, Oecologia 58: 137-140.

**Benado, M.<sup>1,2</sup> E. Klein,<sup>1</sup> and D. Brncic<sup>2</sup>**

<sup>1</sup>Departamento de Estudios Ambientales, Universidad Simon Bolivar, P.O. Box 89000, Caracas, Venezuela, and

<sup>2</sup>Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Santiago, Chile. Eight-year stability of abundance series of *Drosophila subobscura*.

there are long-term trends in the abundance series of the species. We interpret null or positive trends as the species being a successful colonizer.

#### MATERIAL AND METHODS

The flies were collected monthly in La Florida, in the outskirts of Santiago, Central Chile, from August, 1984, to December, 1991 (see Benado *et al.*, 1993 for details).

The collections can be interpreted as autocorrelated time-series (Benado *et al.*, 1993). Thus, after log-transforming, we have used Durbin's two-step iterative procedure (Johnston, 1972) to extract from the series linear trends, that are essentially regressions adjusted for the autocorrelations in the data.

#### RESULTS AND DISCUSSION

Abundances and trends are given in Figure 1. The trend was not significantly different from zero. We conclude that the abundance series was stable.

On the basis of yearly spring collections made in 1956-1986, Brncic *et al.* (1987) pointed out that the drosophilid community in La Florida changed rather drastically by 1978, when *D. subobscura* was first detected in Chile. The species became the most abundant one there, as well as in many other localities along Chile. Our data do not go that far back, and hence inferences about the time of *D. subobscura*'s introduction can not be made. However, we have showed that from 1984 to 1991 the species shows a stable abundance series. We interpret the result as *D. subobscura* having settled permanently in Central Chile.

References: Benado, M. and D. Brncic 1993, in press, Z. zool. Syst. Evolutforsch. An eight year phenological study of a local drosophilid community in Central Chile; Brncic, D. and M. Budnik 1987, Genetica

#### INTRODUCTION

*Drosophila subobscura* is a palearctic species that was detected in Chile about 15 years ago (Budnik *et al.*, 1982). Since then, it has successfully colonized the country, as suggested by its widespread distribution and common occurrence (Benado *et al.*, 1993; Brncic *et al.*, 1987; Budnik *et al.*, 1982). However, long-term tendencies in population numbers have not been analyzed quantitatively. In this report, we test whether

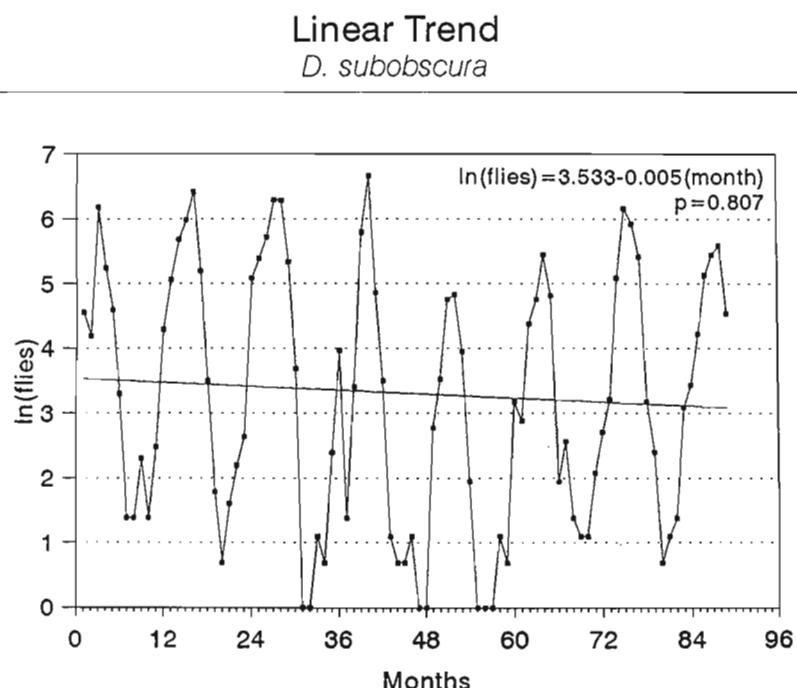


Figure 1. FLIES = # of individuals + 1

Iberica 39: 249-267; Budnik, M. and D. Brncic 1982, Actas V Congr. Latinoam. Genet. 177-188; Johnston, J. 1972, *Econometric Methods*, 2nd ed., McGraw-Hill, New York.

**Johnson, Norman.** Department of Ecology and Evolution, 1101 E. 57th St., Chicago, IL 60637. Age effects on mating propensities in *Drosophila neotestacea*.

*Drosophila neotestacea* (then classified as *D. testacea*, see Grimaldi *et al.*, 1992, for the reclassification of this species), which were parasitized by *Howardula* nematodes, had a reduced mating success in comparison with unparasitized males under laboratory conditions. In contrast, James and Jaenike (1992) in a field experiment detected no significant difference in the prevalence of parasitism among *D. neotestacea* males observed copulating and randomly caught males. James and Jaenike (1992), however, were able to detect a significant size difference between copulating and randomly caught males.

Table 1. Proportion of pairs of *D. neotestacea* mating within 40 minutes.

TRIAL 1 (July 88)		% Mated	Total
Age (in days) male	female		
4	4	44.6	83
4	6	63.1	84
6	4	63.5	85
6	6	79.5	83

The first (4/4) is significantly different from the next two (4/6, 6/4) ( $\chi^2 = 5.759$ ,  $p < 0.02$ ;  $\chi^2 = 6.077$ ,  $p < 0.01$ ) which are significantly different from the last one (6/6) ( $\chi^2 = 5.504$  and 5.241;  $p < 0.02$  for both).

TRIAL 2 (Sept. 88)		% Mated	Total
Age (in days) male	female		
4	4	46.7	75
4	6	64.9	77
6	4	64.9	77
6	6	67.9	78

The 4/4 series is significantly different from the other three ( $\chi^2 = 5.143$ ,  $p < 0.02$ ;  $\chi^2 = 7.075$ ,  $p < 0.01$ ).

6/4 treatments did not differ, but these both differed significantly from the 6/6 treatment. Assuming a null model, the proportion of completed matings in the 4/4 treatment (A) should be equal to BC/D, where B is the proportion of matings in the 6/4 treatment, C is the proportion in the 4/6 treatment, and D is the proportion in the 6/6 treatment. To test for deviations from this null model (that is, interactions between the male and female component), I performed a 2 x 2 contingency test for independence using the proportion of successful matings in each of the treatments multiplied by the lowest sample size in each of the cells. This test is similar to that used by Davis *et al.* (1994). There was little evidence of an interaction in either trial; the  $\chi^2$  values were 0.187 for the first trial and 0.907 for the second.

Although the proportions mating in 40 minutes are identical for the 4/6 and 6/4 pairs, the proportions mating in 10 minutes are significantly different: in the first trial 33 of 84 in the series with 4 day old males and 6 day old females mated within 10 minutes whereas only 20 of 85 did so in the series with 6 day old males and 4 day old females ( $\chi^2 = 5.86$ ,  $p = 0.01$ ).

These results demonstrate that small differences in the age of *Drosophila neotestacea* have substantial effects on the mating propensities of both sexes. These large effects need to be heeded in experimental studies as they could manifest as systematic errors.

Acknowledgment: I thank John Jaenike for support during the course of this experiment.

References: Davis, A.W., E.G. Noonburg, and C.-I. Wu 1994, Evidence for complex genic interactions between conspecific chromosomes underlying female sterility in the *Drosophila simulans* clade. *Genetics* (submitted); Grimaldi,

The behavior, and in particular, the mating behavior, of individuals is affected by a myriad of different factors. Thus under different conditions, the results of an experiment may be substantially altered. For instance, Jaenike (1988) found that males of *Drosophila*

The differences between the results under laboratory conditions and those under natural conditions may, in part, be due to differences in receptivity of laboratory and wild-caught females. One of the factors which could influence receptivity is age. Here I show that relatively small differences in age have a large effect on the mating propensities of both males and females of *D. neotestacea* under laboratory conditions.

#### METHODS AND MATERIALS

Virgin males and females (from lab stocks of recently wild-caught *D. neotestacea*) were collected and placed in individual one-dram vials containing media consisting of one gram of instant Carolina *Drosophila* media, 1.5 ml of water, and a small slice of mushroom (*Agarius* sp.). The males and females were allowed to age for 4 or 6 days. Males and females were placed into the mating board, a device which allows ten pairs of flies to be observed simultaneously. The pairs were observed until copulation or for a period of 40 minutes, whichever came first, and the time to copulation was recorded.

#### RESULTS

In both trials, increasing the age of either the male or the female increased the mating propensity. Table 1 shows the proportion of pairs that mated within 40 minutes for each of the different treatments. In the first trial, there was a significant difference between the sets with 4 day old males and 4 day old females (4/4) and the sets with 6 day old females and 4 day old males (4/6) and 6 day old males and 4 day old females (6/4). The 4/6 and

D., A.C. James, and J. Jaenike 1992, Ann. Entomol. Soc. Am. 85: 671-685; Jaenike, J. 1988, Amer. Natur. 131: 774-780; James, A.C. and J. Jaenike 1992, Anim. Beh. 44: 168-170.

**Krebs, R.A., J.M. Krebs, and V. Loeschcke.**

Department of Ecology and Genetics, University of Aarhus, Denmark. Pupal density affects rate of emergence of *D. buzzatii* adults.

also must be controlled, as increased numbers of pupae may contribute to mortality independently of other stresses. Density dependent mortality is well known for larval *Drosophila* (Barker and Podger, 1970), and specifically for *D. buzzatii* (Krebs and Barker, 1991), due primarily to resource limitation. However, density effects on mortality are unexpected in pupae, because no resource other than space is required, and to our knowledge, density effects on pupae have not previously been reported.

For seven *D. buzzatii* populations (Table 1, six described in Krebs and Loeschke, 1994, plus one mass population from Cordoba, Spain, which was kindly provided by Dr. Jean David), flies were placed in 200 ml flasks per locality that contained instant *Drosophila* medium (Carolina Biological Supply). In each flask, ca. 20 pairs of adults oviposited for two days and were then discarded. Eight days later, 2 x 5 cm pieces of filter paper were folded lengthwise and placed within the flasks to provide an attractive surface for pupation. These papers were removed from each flask 24 hours later, after which the lowest cm, which was embedded in medium, was removed. The remainder of each filter was transferred to a vial that contained agar medium. After another 24 hours, these vials were placed at 41.5°C, incubator temperature, for 100 min. These pupae had first been conditioned to high temperatures 6 hours previously by placing in a 38°C incubator for 1 hr. The proportion of adults emerging in each vial was scored, and these proportions were arcsine-square root transformed for statistical analysis, but actual values have been presented below.

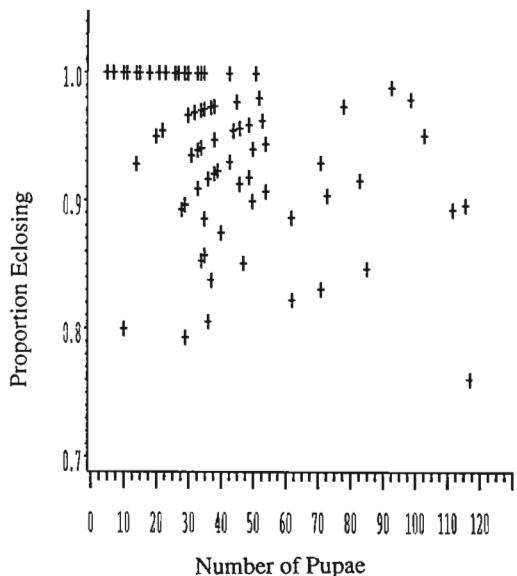


Figure 1. The proportion of adults that eclosed plotted against the number of pupae sharing the same 2 x 4 cm piece of filter paper.

Numbers of pupae were drawn more or less at random as filter papers from bottles (Figure 1), and number of pupae explained 15% of the variation in survival in a regression model for transformed data ( $p < 0.001$ ),

Proportion eclosing =  $1.46 - 0.0024$  (s.e. = 0.00062) Pupae,  
or for untransformed data,

Proportion eclosing =  $0.97 - 0.0008$  (s.e. = 0.00026) Pupae.

The transformation corrected for unequal variances at proportions close to one. For this transformation, 1.57 is

For comparisons of resistance to various forms of stress, groups of flies often are pooled to measure the proportion of survivors (e.g., Hoffmann and Parsons, 1991; Loeschke *et al.*, 1994). Most studies of stress have examined effects on adults, where numbers easily may be controlled. Here we report that pupal density

also must be controlled, as increased numbers of pupae may contribute to mortality independently of other stresses. Density dependent mortality is well known for larval *Drosophila* (Barker and Podger, 1970), and specifically for *D. buzzatii* (Krebs and Barker, 1991), due primarily to resource limitation. However, density effects on mortality are unexpected in pupae, because no resource other than space is required, and to our knowledge, density effects on pupae have not previously been reported.

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Table 1. The proportion of pupae eclosing after exposure to thermal stress, 41.5°C for 100 min (N = number of vials of pupae for each population).

Locality	N	Proportion Eclosing
Tenerife, Canary Islands	11	0.957 ± 0.015
Cordoba, Spain	12	0.937 ± 0.019
Dixalea, Qld., Australia	12	0.910 ± 0.023
Bulla, Vic., Australia	10	0.965 ± 0.015
Oxford Downs, Qld., Australia	12	0.940 ± 0.016
Metz, NSW, Australia	11	0.898 ± 0.018
El Chañar, Argentina	11	0.942 ± 0.012

From the exposed *D. buzzatii* pupae, adults emerged from 93.4%, which was significantly below 96%, a value recorded for a lesser temperature (39°C for 6 hr). Initial analysis suggested that this overall small reduction in pupal survival was specific to one or two populations (Table 1), with differences among localities near to significance ( $p = 0.07$ ). However, subsequent analysis indicated that differences among vials primarily were due to the number of pupae on the filter papers. Including the number of pupae as a covariate in the ANOVA eliminated the effect of locality (now not significant), and a significant effect on emergence rate was found for pupal density ( $p < 0.05$ ).

equivalent to 100% survival, with the intercept, 1.46, corresponding to a survival of 99%. Mortality of pupae at the higher densities reported would be predicted to increase by 4 - 5%, due only to the greater number of pupae crowded on the filter paper.

From observations during long term research on *D. buzzatii*, we note that larvae often pupate in clumps at pupation sites, at least within bottles and vials, even where open space is available. On a hard medium, pupation occurs on or near the surface, but larvae leave the medium when it is soft. Out of the medium, larvae appear preferentially to pupate beside, or if possible, under existing pupae, a behavior which we speculate is an avoidance defense against parasitic wasps. Such activity can dislodge older pupae and cause them to drown in soft medium. The results reported here were for pupae on damp filter paper that was transferred to vials containing 2% agar, eliminating any effects of drowning. Therefore, density effects must have been due solely to packing, which either smothered or damaged some pupae, or prevented them from being able to eclose.

Acknowledgment: This work was supported by the Danish Natural Science Research Council (No. 11-9639-1 and 11-9719-1).

References: Barker, J.S.F. and R.N. Podger 1970, Ecology 51:170-189; Krebs, R.A. and J.S.F. Barker 1991, Aust. J. Zool. 39:579-593; Krebs, R.A. and V. Loeschke 1994, Response to environmental change: genetic variation and fitness in *Drosophila buzzatii* following temperature stress, in *Conservation Genetics* (eds. V. Loeschke, J. Tomiuk and S. K. Jain), Birkhäuser, Basel (in press); Loeschke, V., R.A. Krebs, and J.S.F. Barker 1994, Genetic variation for resistance and acclimation to high temperature stress in *Drosophila buzzatii*, Biol. J. Linn. Soc. (London) (in press); Hoffmann, A.A. and Parsons, P.A. 1991, *Evolutionary Genetics and Environmental Stress*, Oxford Science Publications, Oxford.

**Dai, Zhuohua.** Department of Biology, Peking University, Beijing 100871, P.R. China. The metaphase karyotype and structure of salivary gland chromosomes of *Drosophila immigrans*.

Wasserman, 1957; Calvez, 1953; Lin, *et al.*, 1974; Wakahama, *et al.*, 1983) has been described for *D. immigrans*. The results from China in this study with the type II,  $2n = 8$ , 1V,1J,2R, there are three pairs of autosomes, two of which are rods (3rd and 4th chromosome) and the other one is a V-shaped (2nd chromosome), a pair of sex chromosomes of which the X is small J-shaped with an acrocentric and the Y is also J-shaped (Figure 1).

In the female larval salivary gland nuclei, there are as expected five long arms and a short one. The system of band coding according to Bridges (1935) was used. Each of the two telocentric chromosomes numbered III and IV are divided into 20 numbered divisions. However, chromosome I is as the X chromosome in which the long arm of acrocentric chromosome IR is divided into 20 numbered divisions while the short arm IL is divided into only 5 divisions. In addition, in one pair of metacentric chromosomes numbered II, each of the two arms is divided into 20 divisions. Of course, division numbering is continuous from chromosome IL through the 4th chromosome. In order to make detailed localization possible, a conspicuously staining band was used for each subdivision. Each of these divisions is subdivided again into five segments designated A,B,C,D and E. The total number of salivary chromosome subdivisions was 525 (Figure 2).

In this experiment, the metaphase configuration and the structure of the polytene chromosomes of *D.*

Cytogenetical studies were carried out on the metaphase karyotype and salivary gland chromosomes derived from *D. immigrans* (Sturtevant) which was collected from Chengde, China.

Previously, two karyotypes which were 1V,3R, type I (Metz and Moses, 1923; Word, 1949; Clayton and Wharton, 1943) and 1V,1J,2R, type II (Emmens, 1937; previously, two karyotypes which were 1V,3R, type I (Metz and Moses, 1923; Word, 1949; Clayton and Wharton, 1943) and 1V,1J,2R, type II (Emmens, 1937;



Figure 1. Photographs of the metaphase karyotype of *D. immigrans* (a. female, b. male).



Figure 2. Maps of the salivary gland chromosomes of *Drosophila immigrans* Sturtevant (Chengde, China).

*immigrans* have been studied and compared with earlier descriptions. It has been showed to be quite different in the following:

1. The X and Y chromosomes are not R-shaped, but J-shaped with acrocentric. Thus, my observation generally agrees with the descriptions of Emmens (1937) and Wharton (1943). They referred one of the autosomes to an acrocentric chromosome.

2. As has been indicated above, I get a result the salivary gland chromosomes have five long arms and one large "dot"; one of the five long arms is the right arm of the x chromosome. Furthermore, the number of elements of the shortest one is not the 4th chromosome, but the left arm of the X chromosome in the maps of salivary gland chromosomes of *D. immigrans*.

I used the modified Air-dry methods (Dai *et al.*, 1986). It is possible to identify one chromosome from the other morphologically similar chromosomes. The metaphase karyotype and the structure of polytene chromosomes of *D. immigrans* (Sturtevant) (Chengde, China) have a lot of differences to the Emmens' result. It is largely due to the entity of the interspecific variations and differences of the methodology.

References: Clayton, F.E. and M.R. Wheeler 1975, *Handbook of Genetics* (ed., R.C. King) 3: 471-478; Patterson, J.T. and W.S. Stone 1952, *Evolution in the genus Drosophila*. Macmillan, New York, 152-153; Calvez, J.L. 1953, Chromosoma. Bd. 6 S: 170-174; Lin, F.J. *et al.* 1974, Genetica 45:133-144; Wakahama, K.I. *et al.* 1983, Jpn. J. Genet. 57: 315-326; Dai Zhuohua *et al.* 1986, Acta Genetica Sinica 13(4): 285-294.

**Singh, B.N. and M.B. Pandey.** Department of Zoology, Banaras Hindu University, Varanasi, India. Pattern of mating between wild strains of *Drosophila biarmipes*.

wing patch have greater mating success than those without wing patch which provides evidence for the role of visual stimuli in mating behavior of *D. biarmipes* (Singh and Chatterjee, 1987). The males without a patch lack the visual element of courtship display, and they have to court the females for a longer time to stimulate the females beyond the acceptance threshold and achieve copulation (Chatterjee and Singh, 1991). A purple eye colour mutation which was detected in a wild laboratory stock of *D. biarmipes* has been found to affect the mating propensity of both sexes (Singh and Pandey, 1993). Mutant males are less successful in mating than wild males, but mutant females are more receptive than wild females.

The phenomenon of sexual isolation has been extensively studied in the genus *Drosophila* and has been found to be widespread (Spieth and Ringo, 1983; Chatterjee and Singh, 1989). The results of numerous investigations have revealed that complete sexual isolation occurs in most cases. However, incomplete sexual isolation has also been observed between species in different species groups. But isolation is maintained because of preferential mating between males and females of the same species. Incipient sexual isolation among different geographic strains of the same species has also been reported in a number of species (for references see Singh and Chatterjee, 1985) which originates as a side effect of genetic divergence. During the present study, three wild strains of *D. biarmipes* were tested for intraspecific sexual isolation and the results are reported in this note.

To test intraspecific sexual isolation, the following strains of *D. biarmipes* were used:

1. Ng - established from a female collected from Nagpur, Maharashtra in 1990.
2. Rc - raised from a female collected from Raichur, Karnataka in 1991.
3. My - obtained from Mysore stock centre in 1993 (origin Mysore).

All the stocks were maintained for several generations in the lab before they were tested for sexual isolation. To test sexual isolation, the multiple-choice technique was used and mating was directly observed in an Elens-Wattiaux mating chamber. All the experiments were carried out at approximately 24° under normal laboratory light conditions from 7:00 to 11:00 am. Virgin females and males (with patch) were collected from each strain and were aged for 7 days in small batches. In all the experiments, 15 flies of each sex were used, i.e. 15 females of each of two stocks were introduced into the mating chamber with 15 males of each of the two strains and were observed for 60 minutes. When a pair commenced mating, it was aspirated out, and the types of individuals mated were recorded. To identify the females and males of different strains in each cross, the flies of one stock were marked with a small spot of marking pen on the scutellum. Before making the cross, the effect of marking was tested, and no effect of marking was found either on the performance of flies or outcome of sexual isolation tests. In each cross, 5 trials were run. To measure the degree of sexual isolation among different strains, the isolation estimate was calculated using the formula of Merrell (1950): Isolation Estimate = (number of heterogamic matings)/(number of homogamic matings).

*Drosophila biarmipes* was described by Malloch in 1924 from Coimbatore, India. Males of *D. biarmipes* possess a dark apical black patch on their wings. However, males without black wing patch have also been found in laboratory stocks of *D. biarmipes*. Males with

Table 1. Results of multiple-choice experiments involving three wild strains of *Drosophila biarmipes* (data based on 5 replicates in each cross)

Strains	Numbers of homogamic and heterogamic matings				Isolation Estimate	$\chi^2$
	Homogamic	Heterogamic	Homogamic	Heterogamic		
Nagpur x Mysore	Ng ♀ x Ng ♂ 19	Ng ♀ x My ♂ 32	My ♀ x My ♂ 24	My ♀ x Ng ♂ 20	1.21	0.86
Nagpur x Raichur	Ng ♀ x Ng ♂ 19	Ng ♀ x Rc ♂ 31	Rc ♀ x Rc ♂ 19	Rc ♀ x Ng ♂ 30	1.60	5.34*
Mysore x Raichur	My ♀ x My ♂ 22	My ♀ x Rc ♂ 25	Rc ♀ x Rc ♂ 22	Rc ♀ x My ♂ 31	1.27	1.44

\*Significant, df = 1, p < 0.025

Isolation estimate ranges from zero to infinity. If isolation estimate is one, there is no sexual isolation between the strains tested. If it is zero, then isolation is complete. Since the strains of the same species were tested,  $\chi^2$  values were calculated to measure the difference between the homogamic and heterogamic matings under the assumption

of random mating. Any significant deviation from randomness would indicate non-random mating.

The results of multiple-choice experiments involving three wild strains of *D. biarmipes* are given in Table 1. In all the three crosses, isolation estimate is above one which is due to more heterogamic matings than homogamic ones. In one cross, i.e. Nagpur x Raichur, the difference between homogamic and heterogamic matings is significant ( $p < 0.025$ ). These results suggest that the strains of *D. biarmipes* tested during the present study do not show preference for homogamic matings and thus there is no evidence for sexual isolation within *D. biarmipes*.

Acknowledgment: The financial support from CSIR, New Delhi, in the form of SRF to MBP is thankfully acknowledged.

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**Das, A., S.K. Singh, and B.B. Parida.** Genetics Laboratory, Department of Zoology, Utkal University, Bhubaneswar 751 004, India. Intra- and interpopulational variation in starvation tolerance in Indian natural populations of *Drosophila ananassae*.

Hoffmann and Parsons, 1989; Da Lage *et al.*, 1990). The physiological significance of starvation tolerance has been widely discussed in *D. melanogaster* (David *et al.*, 1975; Da Lage *et al.*, 1989) and the artificially selected lines were also compared (Service, 1987; Service *et al.*, 1985). Interpopulational variation of starvation tolerance has also been detected in *D. melanogaster* (Da Lage *et al.*, 1990).

India, being a tropical country, harbours several species of *Drosophila* including the two cosmopolitan species, *D. melanogaster* and *D. ananassae*. Recently, intrapopulation variation in starvation and desiccation tolerance in *D. melanogaster* has been presented in an Indian natural population (Das *et al.*, 1993 a,b). However, no such type of studies have been conducted in *D. ananassae*, so far.

In the present study, five population samples of *D. ananassae* were collected and isofemale lines were constructed. The F1 male and female individuals of each isofemale line were subjected to starvation stress at 25°C as described in Das *et al.* (1993 a,b). The results are presented in Table 1. In this table the mean survival time in different isofemale lines of the five natural populations are shown. Also, the differentiation between the male and female individuals for the tolerance was measured, and the values for t, correlation coefficient (r), and analysis of variance (F) are shown in this table. It is evident from this table that both intra- and interpopulational variation and sexual differentiation occur with respect to survival duration under starvation conditions in Indian *D. ananassae*. On the whole, the females seem to be more tolerant than their male counterparts to the stress.

The present findings on the intra- and interpopulational variation in tolerance to starvation in *D. ananassae* is very interesting, because this phenomenon has been investigated in this species for the first time. Apart from a tropical and humid environment, where not many food resources are available in all the seasons in India, the survivability of *D. ananassae* under starvation stress in a natural environment is really a cause of concern. As we have used the natural population samples, the observed results must reflect the situation that prevails in nature. Thus, it is expected from the

In recent years significance of abiotic environmental stress to the evolutionary biology of *Drosophila* species is getting attention, and research in this field has shown that tolerance to different stresses in *Drosophila melanogaster* could be mediated by the basic physiological process, particularly a lower resting metabolic rate (David *et al.*, 1983; Parsons, 1983, 1987;

results obtained that the tolerance to starvation stress is having a genetic basis in Indian *D. ananassae*. Our results are supported by the conclusions drawn by the observation in natural populations of *D. melanogaster* from various parts of the world including India, that the greater difference in starvation tolerance sometimes observed between geographic populations has a genetic basis (Da Lage *et al.*, 1990; Das *et al.*, 1993 a,b). Although it has been identified in *D. melanogaster* that in the presence of water (starvation condition) males survive longer than females, the difference was not always significant. The difference, however, is more pronounced when the individuals were kept at higher temperature (Da Lage *et al.*, 1989). The present results provide some evidence in support of sexual differentiation for the tolerance, although the experiments were carried out at 25°C. Thus, a more detailed study is necessary in order to elucidate the nature of tolerance to starvation in natural populations of Indian *D. melanogaster*.

**Acknowledgments:** The financial support in the form of a Research Associateship to A. Das is thankfully acknowledged.

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Table 1. Mean survival time of male and female *Drosophila ananassae* in different isofemale lines of five natural population samples of India exposed to starvation condition.

Population	Isofemale line	Male		Female	
		No. of individuals analysed	Mean survival time (in hrs.) ± SE	No. of individuals analysed	Mean survival time (in hrs.) ± SE
Cuttack	1	10	68.0 ± 1.19	10	8.8 ± 4.49
	2	10	45.3 ± 1.20	10	58.2 ± 0.71
	3	10	88.7 ± 3.37	10	70.0 ± 1.95
	4	10	41.4 ± 1.61	10	56.3 ± 0.51
	5	10	47.7 ± 0.94	10	65.0 ± 1.43
	6	10	60.3 ± 0.37	10	16.3 ± 3.70
	7	10	51.1 ± 0.59	10	64.6 ± 0.86
	8	10	43.6 ± 1.38	10	50.8 ± 0.06
	9	10	58.0 ± 0.13	10	70.3 ± 1.99
	10	10	62.9 ± 0.65	10	53.9 ± 0.26
For ♀ vs ♂ : t = 0.65, d.f. = 18, P > 0.20 insignificant, r = -0.18, d.f. = 8, P > 0.10 insignificant, F = 0.41, P > 0.20 insignificant.					
Bhubaneswar	1	10	58.4 ± 0.49	10	77.6 ± 0.85
	2	10	47.6 ± 0.64	10	77.6 ± 0.85
	3	10	48.6 ± 0.53	10	65.9 ± 0.37
	4	10	52.2 ± 0.15	10	62.4 ± 0.74
	5	10	50.4 ± 0.34	10	65.6 ± 0.84
	6	10	59.3 ± 0.59	10	75.7 ± 0.65
	7	10	56.6 ± 0.30	10	68.9 ± 0.09
	8	10	56.6 ± 0.30	10	53.9 ± 0.16
	9	10	54.7 ± 0.10	10	75.8 ± 0.06
	10	10	52.6 ± 0.11	10	76.2 ± 0.70
For ♂ vs ♀ : t = 4.08, d.f. = 18, P < 0.001 significant, r = +0.06, d.f. = 8, P > .10 insignificant, F = 16.6, P < 0.001 significant.					
Ratnagiri	1	10	74.4 ± 0.82	10	69.6 ± 0.43
	2	10	59.7 ± 0.72	10	56.6 ± 1.78
	3	10	73.8 ± 0.75	10	68.5 ± 0.53
	4	10	65.9 ± 0.07	10	79.1 ± 0.66
	5	10	63.9 ± 0.94	10	72.1 ± 0.15
	6	10	56.4 ± 1.07	10	75.0 ± 0.14
	7	10	69.4 ± 0.29	10	82.7 ± 0.95
	8	10	64.2 ± 0.25	10	71.9 ± 0.17
	9	10	73.2 ± 0.69	10	81.5 ± 0.83
	10	10	65.2 ± 0.14	10	79.9 ± 0.66
For ♂ vs ♀ : t = 2.07, d.f. = 18, P > 0.05 insignificant, r = +0.03, d.f. = 8, P > 0.10 insignificant, F = 4.28, P > 0.05 insignificant.					
Balasore	1	10	55.1 ± 0.30	10	68.1 ± 0.15
	2	10	56.7 ± 0.13	10	66.6 ± 0.00
	3	10	63.3 ± 0.55	10	67.6 ± 0.10
	4	10	60.4 ± 0.25	10	63.1 ± 0.36
	5	10	63.6 ± 0.46	10	71.7 ± 0.53
	6	10	57.5 ± 0.05	10	64.8 ± 0.18
	7	10	55.9 ± 0.22	10	64.0 ± 0.27
	8	10	60.3 ± 0.24	10	65.5 ± 0.11
	9	10	58.2 ± 0.02	10	68.0 ± 0.14
	10	10	59.7 ± 0.07	10	67.3 ± 0.07
For ♂ vs ♀ : t = 4.67, d.f. = 18, P < 0.001 significant, r = +0.12, d.f. = 8, P > 0.10 insignificant, F = 21.77, P < 0.001 significant.					
Howrah	1	10	46.5 ± 0.49	10	80.7 ± 0.28
	2	10	53.2 ± 0.21	10	73.7 ± 0.45
	3	10	48.6 ± 0.27	10	65.7 ± 1.29
	4	10	48.1 ± 1.06	10	76.8 ± 0.12
	5	10	54.1 ± 0.30	10	82.1 ± 0.43
	6	10	47.4 ± 0.40	10	74.2 ± 0.40
	7	10	54.9 ± 0.39	10	75.3 ± 0.28
	8	10	49.7 ± 0.39	10	81.5 ± 0.36
	9	10	57.3 ± 0.64	10	85.1 ± 0.74
	10	10	53.1 ± 0.20	10	85.5 ± 0.79
For ♂ vs ♀ : t = 10.03, d.f. = 18, P < 0.001 significant, r = +0.60, d.f. = 8, P > 0.05 insignificant, F = 100.42, P < 0.001 significant.					

**Singh, B.K. and S. Dash.** Department of Zoology, Kumaun University, Naini Tal, India. Record of further new species of Drosophilidae from Uttarakhand region, India.

The Drosophilid fauna of most countries are now well established in view of the great significance of Drosophilidae in genetic and evolutionary studies. The Indian subcontinent, however, still remains an exception. Although recent surveys have yielded considerable data on Indian Drosophilid species (Singh and Gupta, 1977, 1981; Gupta and Singh, 1979; Dwivedi and Gupta, 1979, 1980; Kumar and Gupta, 1988; Gupta and Kumar, 1986; Singh

and Bhatt, 1988; Singh and Negi, 1989, 1992) many parts of the Indian subcontinent still await exploration. The present paper deals with the results of surveying studies undertaken in Uttarakhand region of Uttar Pradesh, previously unexplored.

The Uttarakhand region includes Kumaun and Garhwal regions which are the hilly areas of the state of Uttar Pradesh. The Kumaun region includes three border districts, viz. Nainital, Almora and Pithoragarh, whereas the Garhwal region includes five border districts, viz. Dehra Dun, Chamoli, Tehri Garhwal, Pauri Garhwal and Uttarkashi. The Kumaun region, a wild hilly area, is located at an elevation of 6,000 ft from the sea level on the north-east periphery of the state of Uttar Pradesh. The Garhwal region is located at an elevation of 5,000 ft from the sea level and is characterized by evergreen forest. In continuation with our study of the Drosophilid fauna of these regions, further collections were made from different geographical regions which have yielded sixteen (16) new species of Drosophilidae belonging to different genera and subgenera. Table 1 shows the name of the species, genus, subgenus and collection locality which were recorded as new species during the present investigations.

Based on our present and past surveying results, it can be predicted that the Uttarakhand region is very rich in Drosophilid fauna particularly the species of the subgenus *Drosophila* of the genus *Drosophila*. It is believed that further surveying studies of this region will yield interesting results in the future.

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**Vandana and R. Parkash.** Biosciences Department, M.D. University, Rohtak, India. Patterns of electrophoretic variation in *D. takahashii* and *D. lutescens*.

of electrophoretic variability for eight gene-enzyme systems in laboratory strains of these two sibling *Drosophila* species. Isofemale lines of *D. takahashii* and *D. lutescens* were characterised after Bock (1980). About 12-14 homogenates of single individuals of both species were loaded in each horizontal starch gel slab (15 x 10 x 1 cm) and run electrophoretically at 250 V and 30 mA at 4°C for 4 hrs, and the gel slices were stained for different gene enzyme systems (Shaw *et al.*, 1970). The polymorphic zones of ODH, ACPH, APH-3, MDH-1 and AO are represented by segregating single-band variants (fast or slow) and triple-banded patterns. On the contrary, segregating two-banded patterns (conformational isozymes) for ADH and alpha-GPDH represent allelic isozymes. The banding patterns of heterozygous individuals depict subunit structure of allozymes, and accordingly monomeric proteins (two-banded heterozygotes) include esterases; dimeric proteins (triple-banded heterozygotes) include ODH, ACPH, APH, MDH and AO. The monomorphic zones include EST-1 to EST-6, APH-1 and -2 and MDH-2, and the electrophoretic mobility values of all such zones are identical in both the sibling species. The identical electrophoretic phenotypes occur at most of the polymorphic zones except ACPH, APH-3 and AO.

Table 1. Showing the name of species, genus, subgenus and collection locality.

Genus, Subgenus, species	Collection Locality
Genus- <i>Drosophila</i>	
Subgenus- <i>Sophophora</i>	
1. <i>D. neobaimaii</i> sp.n.	Bageshwar, Pithoragarh
2. <i>D. neohaoyama</i> sp. n.	Bageshwar, Pithoragarh
3. <i>D. saraswati</i> sp. n.	Bageshwar, Pithoragarh
Subgenus- <i>Drosophila</i>	
4. <i>D. bageshwarensis</i> sp. n.	Bageshwar, Pithoragarh
5. <i>D. serrata</i> sp. n.	Bageshwar, Pithoragarh
6. <i>D. paramarginata</i> sp. n.	Bageshwar, Pithoragarh
7. <i>D. hexaspina</i> sp. n.	Okhalkanda, Nainital
8. <i>D. surangensis</i> sp. n.	Surang, Nainital
9. <i>D. paharpaniensis</i> sp. n.	Paharpani, Nainital
10. <i>D. khansuensis</i> sp. n.	Khansu, Nainital
11. <i>D. elongata</i> sp. n.	Khansu, Nainital
Subgenus- <i>Scaptodrosophila</i>	
12. <i>D. hirsuta</i> sp. n.	Okhalkanda, Nainital
Genus- <i>Leucopenga</i>	
13. <i>L. angulata</i> sp. n.	Okhalkanda, Nainital
14. <i>L. okhalkandensis</i> sp. n.	Okhalkanda, Nainital
15. <i>L. clubiata</i> sp. n.	Okhalkanda, Nainital
Genus- <i>Scaptomyza</i>	
16. <i>S. quadruangulata</i> sp. n.	Okhalkanda, Nainital

Table 1. Data on distribution of allelic frequencies, heterozygosities, Wright's fixation index ( $f$ ) and G-values for log-likelihood  $\chi^2$  test for fit to Hardy-Weinberg expectations at 8 loci in *Drosophila takahashii* and *D. lutescens*.

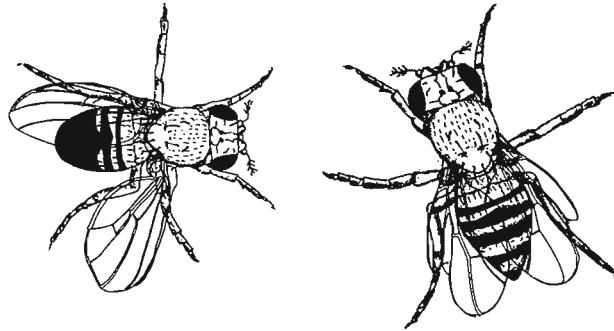
Locus	<i>D. takahashii</i>			<i>D. lutescens</i>		
	N	F	S	H <sub>o</sub> /H <sub>e</sub>	1	G-values (d.f.)
ADH	114	0.16	0.84	28 / .27	-0.03	2.85
ODH	104	0.71	0.29	.42 / .41	-0.02	0.93*
MDH-1	134	0.75	0.25	.45 / .37	-0.19	5.03*
$\alpha$ -GPDH	164	0.91	0.09	.11 / .16	-0.12	9.46*
EST-7	130	0.29	0.71	.40 / .41	0.02	0.17
AO	130	1	2	3	4	
APH	132	0.29	0.60	0.11	—	
ACPH	180	0.07	0.82	0.33	0.62	

N, sample size; fast (F) and slow (S) represent diallelic loci while 1, 2, 3 and 4 refer to allelic variants at tetra-allelic loci. H<sub>o</sub> and H<sub>e</sub>, observed and expected heterozygosities. The +ve and -ve values of Wright's fixation index ( $f$  = 1 - obs. het / exp. het.) indicate deficiency of heterozygotes and excess of heterozygotes, respectively. Log-likelihood  $\chi^2$  test (G-test) was used to obtain statistical significance of the difference between obs. and exp. genotypes. \* G-values significant at 5% level.

The data on allelic frequencies, the observed and expected heterozygosities, Wright's fixation index and the G-values for log-likelihood  $\chi^2$  test for fit to Hardy-Weinberg expectations at eight polymorphic loci in sibling species (*D. takahashii* and *D. lutescens*) have been represented in Table 1. The two loci (ACPH and AO) are highly polymorphic (high allelic content) in the two sibling species, while the other loci are mostly diallelic (Table 1). The patterns of genetic indices, i.e. the number and frequency of alleles and the amount of heterozygosity, are almost identical at four loci (ADH, ODH, alpha-GPDH and EST-7). However, the two sibling species have revealed differential patterns of genetic indices at another four loci (MDH-1, AO, APH-3 and ACPH). The range of heterozygosities observed at various polymorphic loci correlates well with the number of alleles and allelic frequencies in both species. Significant deviations from Hardy-Weinberg expectations have been observed at AO, APH-3, alpha-GPDH and ODH in *D. takahashii* and at ACPH, AO and MDH-1 in *D. lutescens*. The data on Wright's fixation index ( $f$ ) in *D. lutescens* indicate deficiency of heterozygotes at the MDH locus and excess of heterozygotes at the AO locus.

The sibling species pair of *D. takahashii* and *D. lutescens* are indistinguishable morphologically and are known to differ in their geographical distribution, i.e. *D. takahashii* occurs in South-east Asia as well as from India and Japan while *D. lutescens* is endemic to Korea and Japan. The present observations reveal some genetic differentiation at 50% of the loci examined in the two sibling species. The results on allelic frequencies and heterozygosities at polymorphic loci in *D. lutescens* concur with the data reported earlier (Fukatami, 1977). Comparison of the genetic structures of *D. takahashii* and *D. lutescens* reveal that ADH, ODH, alpha-GPDH and EST-7 constitute non-differentiating loci since both the electrophoretic phenotypes as well as allelic frequencies are similar for these loci. However, MDH-1, AO and APH-3 constitute species discriminating loci because, except for a few shared alleles, the allelic frequency patterns are differential in the two sibling species. The present observations do not reveal a diagnostic locus with distinct mobilities and/or non-sharing of major alleles in the two sibling species.

References: Bock, I.R. 1980, Syst. Entomol. 5: 341; Shaw, C.R. and R. Prasad 1970, Biochem. Genet. 4: 297; Fukatami, A. 1977, Jpn. J. Genet. 52: 341.



**Parkash, R. and Vandana.** Biosciences Department, M.D. University, Rohtak, India. Allozyme variation among three *Drosophila* species of *montium* subgroup.

species group comprises 115 species under 13 species subgroups which include the largest *montium* species subgroup

Gel electrophoretic technique has been widely applied to natural populations of cosmopolitan as well as other *Drosophila* species of temperate regions, but there is lack of such information on the drosophilids occurring in the oriental region. The *Drosophila melanogaster*

(50%), 4 major subgroups (25%) and 8 minor subgroups (25%) (Bock, 1980). A single study has been made so far to examine the extent of genic variation in laboratory strains of 6 species of the *montium* subgroup (Triantaphyllidis *et al.*, 1978) Thus, the information on most of the species of the *montium* subgroup is still lacking. The present paper reports the comparative patterns of electrophoretic variability for 8 gene-enzyme systems in 3 species of the *montium* subgroup (Figure 1).

Table 1. Data on the distribution of genotypes, allelic frequencies, heterozygosities (obs. / exp.), Wright's fixation index (f) and effective number alleles ( $n_e$ ) and G-values for log-likelihood  $\chi^2$  test for fit to Hardy-Weinberg expectations at esterase loci in three species of *montium* species subgroup (A = *D. jambulina*, B = *D. punjabensis*, C = *D. kikkawai*).

Locus	Species	Obs/exp. genotypes			Sample size(N)	Allele frequency		Het.		f	$n_e$	G-values
		FF	SS	FS		F	S	Obs./exp.	f			
EST-1	A	39 / 28.81	42 / 31.21	39 / 59.98	120	.49	.51	.32 / .50	.36	2.0	14.99*	
EST-2	A	45 / 41.39	30 / 25.56	57 / 65.05	132	.56	.44	.43 / .51	.15	1.97	2.08 n.s.	
	B	6 / 7.53	48 / 49.76	42 / 38.71	96	.28	.72	.43 / .40	-.07	1.67	0.67 n.s.	
EST-3	A	30 / 29.84	41 / 41.11	69 / 70.05	141	.46	.54	.49 / .50	.02	2.0	0.04 n.s.	
	B	15 / 7.13	72 / 64.12	27 / 42.75	114	.25	.75	.24 / .37	.35	1.6	14.19*	
EST-4	A	42 / 26.24	52 / 36.16	30 / 61.60	124	.46	.54	.24 / .50	.52	2.0	39.13*	
EST-5	A	26 / 18.83	46 / 38.99	40 / 54.18	112	.41	.59	.36 / .48	.25	1.92	7.71*	
	C	11 / 9.26	40 / 38.15	34 / 37.59	85	.33	.67	.40 / .44	.09	1.78	0.75 n.s.	
EST-7	A	36 / 30	36 / 30	48 / 60	120	.50	.50	.40 / .50	.20	2.0	4.85*	
	B	9 / 10.06	36 / 37.90	42 / 39.04	87	.34	.66	.48 / .45	-.06	1.82	0.43 n.s.	
EST-8	B	30 / 31.26	12 / 12.77	42 / 39.97	84	.61	.39	.50 / .48	-.04	1.91	0.20 n.s.	

*D. jambulina* is monomorphic for EST-6 and EST-8 loci; *D. punjabensis* is monomorphic for EST-1, EST-4, EST-5 and EST-6 loci, while *D. kikkawai* is monomorphic at all the esterase loci except EST-5. \* significant at 5 % level; n.s. = non significant.

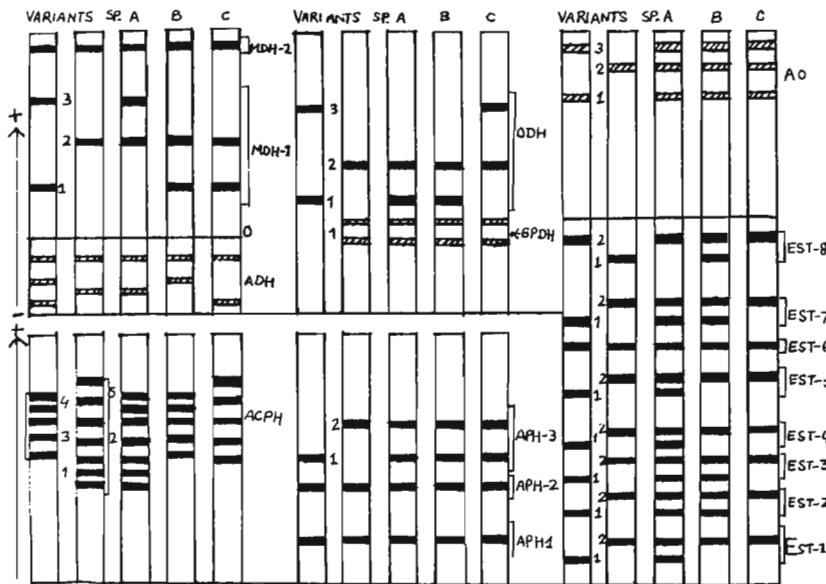


Figure 1. Schematic comparative representation of allelic isozymes (allozymes) of eight gene-enzyme systems in three *Drosophila* species of the *montium* species subgroup. Each gene-enzyme system has been shown in five bar-type diagrams. Allelic variants (electromorphs) for each enzyme are shown in first two bars while species specific allozymes of *D. jambulina*, *D. punjabensis* and *D. kikkawai* are shown in bars 3, 4 and 5, respectively. The bands have been drawn according to their electrophoretic mobilities. The banding patterns of cathodal ADH and anodal alpha-GPDH have been superimposed. O refers to the origin for sample homogenates.

Isofemale lines of *D. jambulina*, *D. punjabensis* and *D. kikkawai* were characterised after Parshad and Paika (1964), Bock and Wheeler (1972), and Bock (1980). About 12-15 homogenates of species specific single individuals were loaded in each horizontal starch gel slab (15 x 10 x 1 cm) and run electrophoretically at 250 V and 30 mA at 4°C for 4 hrs, and the gel slices were stained for different gene-enzyme systems (Smith, 1976; Harris and Hopkinson, 1976).

The single polymorphic ACPH zone revealed either three-banded or five-banded ACPH patterns, and the mobility patterns are species specific. The progeny of isofemale lines depicting particular triple-banded patterns did not reveal segregation of the ACPH bands in any of these 3 species. Genetic crosses involving 2 distinct types of triple-banded ACPH phenotypes resulted in five-banded ACPH patterns in the F1 individuals. Genetic crosses among five-banded individuals resulted in offspring having 2 distinct types of triple-banded patterns and five-banded patterns in accordance with Mendelian segregation ratio of 1:2:1. Thus, the genetic data indicated that the observed complex ACPH patterns in each of the 3 species is controlled by a duplicate locus, and three-banded and five-banded phenotypes represent homozygous and heterozygous ones, respectively.

The data on allelic frequencies, observed and expected heterozygosity, Wright's inbreeding coefficients and G-values for log-likelihood  $\chi^2$  test for fit to Hardy-Weinberg expectations at various polymorphic loci in *D. jambulina*,

*D. punjabensis* and *D. kikkawai* have been represented in Table 1. The patterns of allelic frequencies are almost identical at three loci (ADH, alpha-GPDH and AO) while the three species seem to be genetically different at other loci (MDH-1, APH-3, ACPH and EST loci) due to differential distribution of allelic frequency patterns (Table 1).

The range of heterozygosities observed at various polymorphic loci correlate well with the number of alleles and allelic frequencies in three species. Significant deviation from Hardy-Weinberg expectations have been observed at ACPH, AO, MDH-1, APH-3, EST-1, 4, 5, 7 in *D. jambulina*; at ACPH, AO, EST-3 in *D. punjabensis* and at AO and APH-3 loci in *D. kikkawai*. The high values of Wright's coefficient at EST-1 and -4 loci in *D. jambulina* and ACPH, EST-3 and EST-8 in *D. punjabensis*, and at ACPH and APH-3 loci in *D. kikkawai* indicate deficiency (positive f) or excess (negative f values) of heterozygotes at such loci.

The earlier electrophoretic analysis of 5 species (*D. auraria*, *D. biauraria*, *D. triauraria*, *D. serrata* and *D. kikkawai*) of the *montium* subgroup revealed species interrelationship based on genic variation patterns for 5 enzyme systems (EST-6, EST-C, ACPH, alpha-GPDH and LAP). Thus, Triantaphyllidis *et al.* (1978) reported a maximum of 66.6% genetic similarity between *D. serrata* and *D. kikkawai* while a greater degree of genetic differentiation was observed between *D. auraria*, *D. biauraria* and *D. triauraria*. In the present studies also, the data on interrelationships between 3 species of the *montium* subgroup (*D. jambulina*, *D. punjabensis* and *D. kikkawai*) are based on present similarity of allozymes among species pairs. The data revealed maximum genetic/allozymic similarity (86.67%) between *D. jambulina* and *D. punjabensis* which constitute the known sibling species pair. However, *D. kikkawai* is also closely related to the other 2 species of the *montium* group. The present studies point out the need to survey genetic polymorphism in many other species of the *montium* subgroup which occur in India and thereby to establish their overall phylogenetic relationships.

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**Shamina and R. Parkash.** M.D. University, Rohtak India. Ethanol and acetic acid tolerance in three drosophilids.

Ethanol is the end product of fermentation, and ethanol vapors provide a normal energy source in *D. melanogaster* (Parsons, 1983). Ethanol tolerance analysis revealed significant divergence in *D. melanogaster* and *D. simulans* and thus provided a mechanism for niche separation for these sibling species (Parsons, 1977; Parsons and Spence, 1981). Most studies on ethanol tolerance had been made on *D. melanogaster* and *D. simulans* populations from Europe and Africa (David *et al.*, 1986) and Australia (Parsons, 1979, 1980; McKenzie and Parsons, 1972), but the information on several drosophilids from temperate as well as tropical parts of the world are still lacking. The objective of the present studies is to analyse acetic acid and ethanol utilisation as well as *Adh* polymorphism of adult as well as larval forms in *D. melanogaster*, *D. ananassae* and *D. busckii*.

Mass bred populations of *D. melanogaster*, *D. ananassae* and *D. busckii* from Rohtak (northern populations; 28°.54' N) were used for ethanol and acetic acid utilisation as well as ADH polymorphism. Homogenates of single individuals were subjected to electrophoresis at 250 V and 25 mA at 4°C for 4 hours. The gel slices were stained for ADH gene-enzyme system by standard staining procedures (Harris and Hopkinson, 1976). Ethanol and acetic acid tolerance patterns were assessed following the procedure of Starmer *et al.* (1977) and David and Van Herrewege (1983).

The distribution of ADH genotypes, allelic frequencies, observed and expected heterozygosity and log-likelihood chi-square test for fit to Hardy-Weinberg expectations at the *Adh* locus in *D. melanogaster*, *D. ananassae* and *D. busckii* are shown in Table 1. All the three species showed deviations from Hardy-Weinberg equilibrium at the *Adh* locus. The larval ethanol tolerance threshold response revealed significant variation between three different *Drosophila* species, i.e. *D. busckii* (3.2%); *D. ananassae* (4.2%) and *D. melanogaster* (10%). Thus, *D. melanogaster* larval forms revealed much higher ethanol tolerance than the two other *Drosophila* species (Table 2). The increased longevity data revealed lesser effect in *D. busckii* (84.5 hrs) as compared with *D. ananassae* (165 hrs) and *D. melanogaster* (300 hrs). The adult ethanol threshold values were found to be lower in *D. busckii* (2.3%) and *D. ananassae* (3.4%) but higher for *D. melanogaster* (13.0%). The LC<sub>50</sub> values were found as 2.0% in *D. busckii*, 3.5% in *D. ananassae* and 10.8% in *D. melanogaster*. Interestingly, the larval and adult stages of all the three *Drosophila* species could utilise acetic acid in a parallel way to that of ethanol. The increased longevity data revealed parallel but lesser effect of acetic acid utilisation, i.e. 66 hrs in *D. busckii*, 110 hrs in *D. ananassae* and 216 hrs for *D. melanogaster*. The data on species specific acetic acid threshold

Table 1. Alcohol dehydrogenase (*Adh*) genotypes, allelic frequencies, heterozygosities (Obs./exp.) and G-values for log-likelihood chi-square test for Hardy-Weinberg expectations in three *Drosophila* species from Rohtak.

Species	Adh genotypes			Sample size	Adh frequency		Het.	
	FF	SS	FS		F	S	Obs./exp.	G-values
<i>D. busckii</i>	4	92	4	100	.06	.94	.04 / .11	18.39*
<i>D. ananassae</i>	44	16	28	88	.66	.34	.32 / .45	7.38*
<i>D. melanogaster</i>	62	13	28	103	.74	.26	.27 / .38	8.54*

\* significant at 5 % level.

Table 2. Comparison of ethanol as well as acetic acid tolerance indices (increase in longevity LT<sub>50</sub> hrs; LT<sub>50</sub> maximum/LT<sub>50</sub> control; adult and larval threshold concentrations and LC<sub>50</sub>) in *Drosophila* species from Rohtak.

Species	Increase in longevity*		Threshold concentration		LC <sub>50</sub> values **
	LT <sub>50</sub> (hrs.)	LT <sub>50</sub> max./LT <sub>50</sub> control	Larval	Adult	
<b>A) Ethanol</b>					
<i>D. busckii</i>	84.5	1.76	3.2	2.3	2.0
<i>D. ananassae</i>	165.0	2.39	4.2	3.4	3.5
<i>D. melanogaster</i>	300.0	3.12	10.1	13.0	10.8
<b>B) Acetic Acid</b>					
<i>D. busckii</i>	66.0	1.38	3.0	3.1	3.0
<i>D. ananassae</i>	110.0	1.60	3.9	4.6	4.0
<i>D. melanogaster</i>	216.0	2.25	9.0	12.6	9.0

\* Increase in longevity at 1 percent in *D. busckii* and *D. ananassae* as well as at 6 percent in *D. melanogaster*. \*\* LC<sub>50</sub> was measured in *D. busckii* (on 2nd day); in *D. ananassae* (on 4th day) and in *D. melanogaster* (after 6th day).

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University, Rohtak, India. Microspatial differentiation  
of ethanol tolerance in *D. melanogaster* from India.

Higher ethanol tolerance together with higher *Adh*<sup>F</sup> allele frequency in winery populations (Briscoe *et al.*, 1975; Hickey and McLean, 1980). Other studies, however, showed that increased ethanol tolerance is not invariably associated with a higher *Adh*<sup>F</sup> allele frequency in winery populations of *D. melanogaster* from Australia and California (McKenzie and McKechnie, 1978; Marks *et al.*, 1980). Thus, the present study was undertaken to analyse *Adh* frequencies, ethanol utilization as well as ethanol tolerance in Rohtak sugar mill and fruit stall populations of *D. melanogaster* which are 6 Km apart. The population samples were analysed electrophoretically for ADH polymorphism, for ethanol utilisation and tolerance levels. Homogenates of single individuals from isofemale lines were subjected to starch gel electrophoresis at 250 V and 25 mA at 4°C for 4 hours, and the gel slices were stained for ADH (Harris and Hopkinson, 1976). The genetic indices were calculated by following standard statistical formulae (Ferguson, 1980). The ethanol utilisation (at 1 to 16 percent) and tolerance (8 to 32 percent) were studied by exposing *Drosophila melanogaster* adults to ethanol and water vapors following the procedure of Starmer *et al.* (1977; Van Herrewege and David, 1978, 1980; David and Van Herrewege, 1983).

The data on genotypes, allelic frequencies, heterozygosity and statistical fit to Hardy-Weinberg equilibrium at the *Adh* locus in *D. melanogaster* populations collected from local sugar mill campus and fruit stalls located 6 Km apart are given in Table 1. The fruit stall populations (with lower ethanol concentration) revealed *Adh*<sup>F</sup> frequency as 0.74 and higher heterozygosity (0.27) and showed significant deviation from the Hardy-Weinberg expectations. On the contrary, the three samples of sugar mill population (indoor with very high concentration of ethanol due to fermentation of molasses etc.) revealed consistently significant increase in *Adh*<sup>F</sup>, i.e. about 0.90 and lower heterozygosity (about 0.19) and the samples were found to be in Hardy-Weinberg equilibrium. Thus, the extent of *Adh* polymorphism did

values, LC<sub>50</sub> values, mortality and longevity responses further supported that acetic acid was utilised as a resource in the three *Drosophila* species (Table 2).

These species seem to have adaptively partitioned their ecological niches in terms of concentration of alcoholic resources available in man-made indoor fermenting and outdoor fermenting resources. Since the fermenting food sources as well as the biotic (yeast and other fermenting organisms) and abiotic factors (temperature, humidity) and so forth markedly differ in the Indian sub-continent, the observed alcoholic utilisation profiles of the three cosmopolitan and domestic species such as *D. melanogaster*, *D. ananassae* and *D. busckii* reflected species specific characteristics in alcoholic metabolism.

References: David, J.R. and J. Van Herrewege 1983, Comp. Biochem. Physiol. 74A: 283-288; David, J.R., H. Mercot, P. Capy, S.F.

*D. melanogaster* populations living in wine cellars (ethanol rich) and in the surroundings (with low ethanol concentration) revealed micro-differentiation in the alcohol tolerance as well as in *Adh*<sup>F</sup> frequency.

Comparative studies of natural populations have reported

significantly vary between two local contrasting habitats with varying ethanol concentrations. The patterns of ethanol utilization in *D. melanogaster* from sugar mill revealed significant increased life duration of 432 hours ( $LT_{50}$  hrs) or almost 18 days at 6% ethanol (Table 3). On the contrary the fruit stall populations showed maximum survival only as 212 hours ( $LT_{50}$  hrs) at 4% ethanol. The adult ethanol threshold concentrations were found to be 10% and 15% in fruit stall and sugar mill populations, respectively. The  $LC_{50}$  data revealed values similar to those of ethanol threshold values, i.e. 9% in fruit stall and 13.8% in sugar mill populations (Table 2).

In order to know the level of ethanol tolerance, the populations were exposed to higher concentrations (18 to 32%) of ethanol supplemented with sucrose (so as to check starvation). The maximum tolerated hours by fruit stall populations of *D. melanogaster* were found to be 430 hours while sugar mill population revealed a significantly higher value (648 hours). The ethanol threshold concentrations were found as 19% for sugar mill population and 10% for fruit stall populations. The mortality data at four days of ethanol treatment also revealed significant differences in  $LC_{50}$  values, i.e. 14.7 for fruit stall population and 22.5 for sugar mill population.

Table 1. Distribution of observed genotypes, allelic frequencies, heterozygosities and G-values for log-likelihood  $\chi^2$  test for fit to Hardy-Weinberg expectations at *Adh* locus in *D. melanogaster* populations sampled from fruit stalls (a) and from co-operative sugar-mill, Rohtak (b)

Population	Observed Adh genotypes			Sample size	Adh allelic frequency		Het.	
	FF	SS	FS		F	S	Obs./exp.	G-values
a) Fruit stall population	62	13	28	103	.74	.26	.27/.38	8.54*
b) Sugar-mill population	110	0	25	135	.90	.10	.19/.18	2.72

\* significant at 5 percent level.

Table 2. Comparison of ethanol utilisation and ethanol tolerance indices in *D. melanogaster* populations sampled from fruit stalls and from co-operative sugar-mill, Rohtak.

Populations	Ethanol utilisation				Ethanol tolerance			
	LD <sub>50</sub> hrs at 6%	Ethanol threshold conc. (%)	LT <sub>50</sub> max. / LT <sub>50</sub> control	LC <sub>50</sub> (%)	LD <sub>50</sub> hrs at 10%	Ethanol threshold conc. (%)	LT <sub>50</sub> max. / LT <sub>50</sub> control	LC <sub>50</sub> (%)
From fruit stalls	170	10.0	2.15	9.0	430	10.0	1.04	14.7
From sugar-mill	432	15.0	6.0	13.8	648	19.0	2.04	22.5

The present observations showed higher levels of  $Adh^F$  frequency (90%) in the population samples collected from indoor fermentation vats in the sugar mill as compared with  $Adh^F$  frequency (74%) in the fruit stall population located at a distance of 6 km. The analysis of both ethanol utilization and ethanol tolerance of adult flies from sugar mill and fruit stall areas revealed significant divergence in both these traits, i.e. the sugar mill population was found to be characterised as a sub-divided population (metapopulations) with distinctly higher potential of ethanol utilization as well as tolerance for a wide range of alcohol concentrations. Thus, the present results concur with the arguments of Hickey and Mclean (1980) that populations located 3 or more km apart reveal significant differences in both  $Adh^F$  frequency as well as ethanol tolerance. The present data on ethanol utilization as well as ethanol tolerance through independent experiments lend further support to the occurrence of microspatial differentiation due to selection pressure imposed by ethanol rich environments over a short range distance of about 6 km.

References: Briscoe, D.A., A. Robertson and J.M. Malpica 1975, Nature 255: 148-149; David, J.R. and J. Van Herrewege 1983, Comp. Biochem. Physiol. 74A: 283-288; Ferguson, A. 1980, *Biochemical Systematics and Evolution*. Wiley, New York; Hickey, D.A. and M.D. Mclean 1980, Genet. Res. 36: 11-15; McKenzie, J.A. and S.W. McKechnie 1978, Nature 272: 75-76; Marks, R.W., J.G. Brittnacker, J.F. McDonald, T. Prout, and F.J. Ayala 1980, Oecologia 47: 141-144; Harris, H. and D.A. Hopkinson 1976, *Handbook of Enzyme Electrophoresis in Human Genetics*, North Holland, Amsterdam; Starmer, W.T., W.B. Heed and E.S. Rockwood-Sluss 1977, Proc. Natl. Acad. Sci. U.S.A. 74: 387-391; Van Herrewege, J. and J.R. David 1978, Experientia 34: 163-164; Van Herrewege, J. and J.R. David 1980, Heredity 44: 229-235.

**Parkash, R. and Vandana.** Biosciences Department, M. D. University, Rohtak, India. Enzyme polymorphism in *D. melanogaster*.

Several investigations have been made on the electrophoretic analysis of the pair of cosmopolitan sibling species, *D. melanogaster* and *D. simulans*, from various temperate regions of the world (Girard *et al.*, 1977; Singh *et al.*, 1982). On the contrary, there are no reports on the electrophoretic analysis of genic variations occurring in *D. melanogaster* populations of the Indian

reports on the electrophoretic analysis of genic variations occurring in *D. melanogaster* populations of the Indian

sub-continent. The present report describes the patterns of genic variations for seven gene-enzyme systems in wild populations of *D. melanogaster*.

Data on distribution of enzyme genotypes, allelic frequencies, observed and expected heterozygosity, Wright's coefficient (*f*) and log-likelihood  $\chi^2$  test for fit to Hardy-Weinberg expectations at the seven loci are given in Table 1. The range of heterozygosity observed at the various loci correlate well with the incidence of number as well as allelic frequency distribution patterns. The positive and negative values of Wright's index (*f*) indicate deficiency and excess of heterozygotes, respectively. Data in Table 1 reveal that the population deviates from Hardy-Weinberg equilibrium at the EST-6, ODH and AO loci. Electrophoretic analysis at the MDH locus in *D. melanogaster* has revealed the occurrence of one most common allele (0.93) and one rare allele (0.07), while each of the other six loci are represented by two frequent alleles.

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

Locus	Obs./exp. Phenotypes			Sample size	Adh frequency		Het. Obs./exp.	$n_e$	<i>f'</i>	G-values
	SS	FF	FS		S	F				
APH-3	8 / 11.36	38 / 41.31	50 / 43.33	96	.34	.66	.52 / .45	1.82	-.15	2.36 n.s.
EST-6	98 / 95.4	5 / 2.13	23 / 28.5	126	.87	.13	.18 / .23	1.3	.19	4.0*
ADH	8 / 7.30	60 / 59.14	40 / 41.56	108	.26	.74	.37 / .38	1.62	.03	0.14 n.s.
ODH	14 / 6.48	93 / 85.44	32 / 47.08	139	.216	.784	.23 / .34	1.51	.32	12.62*
AO	7 / 2.49	98 / 93.93	22 / 30.58	127	.14	.86	.17 / .24	1.31	.28	8.29*
$\alpha$ -GPDH	10 / 8.12	75 / 73.13	45 / 48.75	130	.25	.75	.34 / .37	1.57	.08	0.83 n.s.
MDH-1	96 / 96.87	0 / 0.55	16 / 14.58	112	.93	.07	.14 / .13	1.14	-.07	1.24 n.s.

\* significant at 5 % level; n.s. = non significant.

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

Population*	EST-6		APH-3		ADH		$\alpha$ -GPDH		ODH		AO		MDH	
	F	S	F	S	F	S	F	S	F	S	F	S	F	S
1. Canada (Hamilton) (43.3° N)	.54	.46	1.0	—	.76	.24	.81	.19	.93	.07	.89	.11	—	1.0
2. U.S.A. (Texas) (25.8° N)	.64	.36	.98	.02	.14	.86	.68	.32	.62	.38	.50	**	—	1.0
3. West Africa (6.3° N)	.63	.37	.98	.02	.03	.97	1.0	—	.87	.13	**	.57	.14	.86
4. Vietnam (11° N)	.77	.23	.94	.06	1.0	—	.77	.23	1.0	—	.77	.23	—	1.0
5. India (28.94° N)	.44	.56	.66	.34	.83	.17	.90	.10	.80	.20	.79	.21	.08	.92

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

A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95, and accordingly all the seven loci analysed in *D. melanogaster* are polymorphic. The present observations on the genetic basis and subunit structure of allozymic variants at seven loci in *D. melanogaster* concur with those reported earlier (Cabrera et al., 1982). The incidence of the frequent allele at most of the polymorphic loci is also in agreement with other studies, but the allelic frequency patterns are not in agreement with those of the populations of temperate regions (Table 2). Such differences in allelic frequency patterns at some loci (ADH and alpha-GPDH) could be argued on the basis of the earlier reported evidences of the action of temperature as an agent of natural selection (Oakeshott et al., 1982; Alahiotis et al., 1977). The observed low level of genic polymorphism at the MDH locus concurs with the functional constraint hypothesis that the glucose metabolising enzymes which are involved in crucial metabolising pathways are least variant as compared to non-glucose-metabolising enzymes. Present studies need to be extended to several ecogeographical populations of *D. melanogaster*.

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Progress report on invasion of *Drosophila subobscura* in a California locality.

On July 2, 1993, Th. Dobzhansky and others collected there from 1940

*Drosophila subobscura* Collin recently invaded many North and South American localities, becoming the most abundant *obscura* group species in some areas (Beckenbach and Prevosti, 1986; Ayala et al., 1989). I report here my collection from the Robert Louis Stevenson State Park area of Mt. St. Helena, California, on July 2, 1993. Th. Dobzhansky and others collected there from 1940

through the 1970's (Anderson *et al.*, 1975), and there was no report of *subobscura*'s presence.

Table 1 shows the domination of *D. subobscura* relative to the other *obscura* group species. Collections of isofemale lines of *D. pseudoobscura* or *D. persimilis* from this site are now much more difficult since the females cannot be easily distinguished from *D. subobscura* or *D. azteca* females. Single male *D. subobscura* were also captured in my collections at Mather (July 4, 1993, N-326) and Auburn, CA (July 5, 1993, N-10), showing the apparent easternmost border of the invasion.

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**Koryakov, D.E., O.V. Demakova, M.L. Balasov, S.A. Demakov, I.V. Makunin, and I.F. Zhimulev.** Institute of Cytology and Genetics of the Russian Academy of Sciences, Novosibirsk, 630090, Russia. Chromosome rearrangements in four *Drosophila melanogaster* populations from the Altai region.

Data about inversions found in four natural Altai populations (Russia) are presented. The flies were caught in sites with relatively different environments, above all in regard to antropogenic pollution. All populations were screened in September. The inversions were analyzed after individual crosses of  $F_1$  males from isofemale lines with virgin females of the *Batum-L* strain, homozygous for standard-sequenced chromosomes.

Table 1. Total number of inversions and their distribution among chromosomes\*

Altai populations	No. of examined haploid sets	No. of inversions	No. of inversions in chromosomes				
			1	2L	2R	3L	3R
Pospelikha	367	23 ( 6.3)	5 (1.3)	3 (0.8)	0	3 (0.8)	12 (3.3)
Gorno-Altaisk	331	19 ( 5.7)	1 (0.3)	5 (1.5)	1 (0.3)	1 (0.3)	11 (3.3)
Zmeinogorsk	175	13 ( 7.4)	0	1 (0.6)	5 (2.9)	1 (0.6)	6 (3.4)
Biisk	149	21 (14.1)	0	9 (6.0)	1 (0.7)	1 (0.7)	10 (6.7)

\* In parentheses are the frequency percentages

Table 2. Distribution of breakpoints and inversion frequencies (%)

Chromosome arm	Rearrangement	Break-points	Rearrangement frequency in populations			
			Pospelikha	Gorno-Altaisk	Zmeinogorsk	Biisk
1	In (1) UC	6F-13B	0	0.3	0	0
1	In (1) AB	10B-13F	1.3	0	0	0
2L	In (2L) t	22E-33F	0.8	1.5	0.6	5.3
2L	*In (2L) Alt-1	24A-30B	0	0	0	0.7
2R	*In (2R) Alt-2	44B-51F	0	0.3	0	0
2R	In (2R) NS	52A-56F	0	0	2.9	0
2R	*In (2R) Alt-3	53B-56F	0	0	0	0.7
3L	In (3L) P	63C-72E	0.8	0.3	0.6	0
3L	In (3L) M	66B-71A	0	0	0	0.7
3R	*In (3R) Alt-4	83A-88E	0	0	0	0.7
3R	*In (3R) Alt-5	85C-87E	0	0.3	0	0.7
3R	In (3R) St-F	86D-88C	0	0	0	0.7
3R	In (3R) St-c	87A-100F	0	0.3	0	0
3R	In (3R) PS	87E-92E	0	0.6	0	0.7
3R	*In (3R) Alt-6	87E-94E	0	0	0	0.7
3R	*In (3R) Alt-7	90E-99A	0	0	0	0.7
3R	In (3R) PI	90F-96A	0	0	1.1	0
3R	In (3R) C	92D-100F	1.6	0.9	2.3	1.2
3R	In (3R) Mo	93D-98F	1.6	0.6	0	1.7
3R	*In (3R) Alt-8	97F-99A	0	0.3	0	0.7

Asterisk marks new endemics

The inversions found and their frequencies are shown in Tables 1 and 2. The total number of detected inversions was 20, all of them being paracentric. Twelve of the 20 inversions we detected were described earlier in natural populations (Lindsley and Zimm, 1992). Among 7 known cosmopolitan type inversions (Mettler *et al.*, 1977), 4 were found in populations studied (*In(2L)t*, *In(3L)P*, *In(3R)C*, *In(3R)Mo*). Another inversion (*In(3R)PS*) was described as an endemic in a Korean population (Lindsley and Zimm, 1992). Eight inversions which are not listed in the catalog of Lindsley and Zimm (1992) are thought to be new endemics.

The distribution of breaks was

non-random: more than half of the inversions occurred in 3R-chromosome. The significantly higher total inversion frequency and spectrum was observed in the Biisk population. Approximately 50% of the inversions found in Biisk proved to be new endemics.

References: Lindsley, D.L. and G.G. Zimm 1992, *The Genome of Drosophila melanogaster*. Academic Press, Inc.; Mettler, L.E., R.A. Voelker, and T. Mukai 1977, Genetics 87: 169-176.

**Kokoza, E.B. and I.F. Zhimulev.** Institute of Cytology and Genetics, Novosibirsk, Russia. Ectopic stretches labelled by DNA clones from the 10A1-2 band of *D. melanogaster* X-chromosome.

tein in regions of ectopic contacts, proposed by Kaufmann and Iddles (1963), implies either relationship of nucleic acids and proteins or similar composition of proteins or nucleic acids. Data of *in situ* hybridization of DNA probes on polytene chromosomes demonstrate the presence of homologous DNA sequences either in both conjugating chromosome regions and ectopic stretch between them or only in one chromosome region and ectopic stretch.

Five models of ectopic conjugation formation were suggested: i) Late replication in cluster of repeats characteristic to intercalary heterochromatin (IHC) results in underreplication and chromosome breaks with formation of "cohesive" ends which lead to formation of ectopic contacts (Zhimulev *et al.*, 1982; Scouras and Kastritsis, 1988). ii) Proposed repetitive elements of several types constitute IHC regions, and individual regions of IHC have some of them in common; ectopic contact could be formed by homologous DNA sequences (Bolshakov *et al.*, 1985). But if DNA probe contains a non-common repetitive element, the label would be found only in one region and the ectopic stretch. Two other models are based on replication fork migration after some disturbances in the replication process. iii) Ectopic pairing could occur due to limited homology of single strand DNA sequences from one region with another replicating region (Ashburner, 1980), so DNA probe from one region of ectopic contact would not mark second ectopic region. iv) Another way of stretch forming involves proteins with high affinity to any single DNA strand, which bind them together, and ectopic links would arise despite absence of sequence homology (Laird *et al.*, 1987). v) According to the fifth model ectopic stretches are formed at the expense of homology of proteins in sites of IHC (Zhimulev, 1993).

Among cloned DNA sequences from a walk of the 9F12-10A7 region of *D. melanogaster* (Kozlova *et al.*, 1993), there are two variants of X-chromosome specific repetitive element - K1 and K2, for which localization is precisely determined (Kokoza *et al.*, 1993). Using DNA clones from the 10A1-2 region containing both repeated and unique DNA sequences for *in situ* hybridization, we have found two types of ectopic contacts revealed with biotinylated probes: between two labelled sites (*i.e.*, two sites of repeat localization) and between labelled and nonlabelled sites (*i.e.*, only one of the sites contained repeated sequences) (see Table, Figure 1). Ectopic stretches were always labelled.

Clone K206.1 comprising no repetitive sequences dispersed in genome demonstrates ectopic stretch between the labelled 10A1-2 band and the unlabelled 10B1-2 band (Figure 1i).

There are three examples of partial labelling of second ectopic region: 87D (Figure 1e) and 7B (Figure 1j,l) which could be explained by overlapping of strongly labelled ectopic stretch on chromosome body.

So, these data give more new examples of both types of labelling: i) labelling of both contacting bands and stretch between them, ii) labelling of only one band and stretch with unlabelled band.

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The hypotheses of ectopic conjugation phenomenon are based on ideas of either homology of conjugating chromosome regions (Bridges, 1935, 1936; Prokofyeva-Belgovskaya, 1941) or nonspecific chromomere interaction, terminal or heterochromatic (Bauer, 1936). The existence of homologous nucleoprotein in regions of ectopic contacts, proposed by Kaufmann and Iddles (1963), implies either relationship of nucleic acids and proteins or similar composition of proteins or nucleic acids. Data of *in situ* hybridization of DNA probes on polytene chromosomes demonstrate the presence of homologous DNA sequences either in both conjugating chromosome regions and ectopic stretch between them or only in one chromosome region and ectopic stretch.

Table 1.

Clone	Repeat	Regions involved in ectopic contacts	Reference on figure 1
1. Contact between two labelled regions			
M 325	K1	10A1-2-9F12 10A1-2-10B1-2	a
3.18.8	K1	9F10-12-9DE	b
3 B	K1	10A1-2-9A	c
K 191	K2	10A1-2-9E1-3 10A1-2-7DE	f g
K 172	K1	10A1-2-8A	k
2. Contact between labelled and nonlabelled regions			
3.18.8	K1	8A-5D	b
3 B	K1	10A1-2-21A	c
5 F	K1	10A3-5-23D	d
K 172	K1	10A1-2-87D	e
K 191	K2	10A1-2-26C	g
K 192	K2	10A1-2-11A	h
K 206.1	none	10A1-2-10B	i
M 193	K2	10A1-2-7B	j
K 175	K1	10A1-2-7B	j



Figure 1 (opposite page). Labelling of ectopic contact stretches upon *in situ* hybridization of biotinylated DNA probes from the 10A1-2 band of *D. melanogaster* X-chromosome on polytene chromosomes of wild type strains Canton S (CS), Oregon R (OR), Novosibirsk (Nov) and *D. simulans* (sim): (a) clone M325 on CS chromosomes; (b) clone 3.18.8 on CS; beside conjugation between 9F10-12 and 9DE there is another ectopic contact: between region 8A and 5D, only first of them has K1 repeat presented in the probe; (c) clone 3B on CS; (d) clone 5F on CS; (e) clone K172 on CS; (f) clone K191 on OR; (g) clone K191 on CS; ectopic stretch from the band 10A1-2 ties up with two overlapping regions from different chromosomes -X and 2L, so it is not clear which region is participating in ectopies -7DE having repeated sequence homologous to the probe or 26C without that repeat; (h) clone K192 on CS; (i) clone K206.1 on CS; (j) clone M193 on sim; (k) clone K172 on sim; sites of K1 repeat are not seen because of some interspecific divergence of repeated element sequence and low specific labelling of the probe (compare with signals on Canton S chromosomes with the same probe K172 and higher specific labelling on Figure 1e); (l) clone K175 on Nov.

Thick arrows mark the band where the probe originates (or possesses sequences, homologous to the probe, as on Figure 1b), thin arrows - labelled ectopic stretches.

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**Kozhemikina, T.A. and D.P. Furman.** Institute of Cytology and Genetics SO RAN, Novosibirsk-90, Russia. Copy number and chromosomal distribution of P and Hobo transposable elements in an Altai population of *Drosophila melanogaster*.

1971). Three preparations were analyzed for each of the transposable elements.

**P element.** The number of P element hybridization sites on the polytene chromosomes for the Biysk population is presented in Table 1. The average number was 28.4, which is higher than that for other Soviet populations. In the rundown submitted by Ronsseray and associates (1989) on 12 populations, the number ranges from 7.7 to 19.0 and just for one population it hits 27.5, which is close to what we have got. The average number of sites occupied by P elements per genome in two Azerbaijani populations was set at 23 and 25 (Pasyukova and Gvozdev, 1986).

Chromosome I bears fewer P elements than any other chromosome: 4.7 of them are in arm 2L, 5.0 in arm 2R. At the same time, the X chromosome contains 5.9 copies, 3L has 6.7 and 3R has 6.1.

As a rule, wild populations are noted for a reduced TE copy number and a strong variety of TE localizations in the X chromosome, compared with autosomes (Leibovitch, 1990). However, no such

In this work we present data on the polymorphism of the genomic distribution of P and hobo transposable elements in an Altai population of *D. melanogaster* (the city of Biysk), that had not been studied before. The localization of P elements was determined in 13 and hobo in 12 isofemale lines by *in situ* hybridization on salivary gland polytene chromosomes (Pardue and Gall, 1971).

Table 1. Distribution of the P and hobo hybridization sites in the genome of *Drosophila melanogaster* from the Altai wild population.

Mobile element	Chromosomes					genome
	X	2L	2R	3L	3R	
P	5.9 ± 0.2	5.0 ± 0.2	4.7 ± 0.2	6.7 ± 0.1	6.1 ± 0.2	28.4 ± 0.4
hobo	2.7 ± 0.1	5.1 ± 0.2	5.4 ± 0.1	4.6 ± 0.1	4.9 ± 0.2	22.7 ± 0.3

Table 2. Hotspots of P and hobo localized on polytene chromosomes of wild *Drosophila melanogaster* population of Biysk. Sites of intercalar heterochromatin are typed in bold.

Mobile element	Chromosomes				
	X	2L	2R	3L	3R
P	2C	<b>25A</b>	47EF	<b>67DE</b>	85B
	3C	26A	50CD	<b>70C</b>	<b>87F</b>
	4D	<b>35C</b>	<b>56F</b>	<b>71C</b>	<b>89DE</b>
	7B	<b>36CD</b>	—	<b>75C</b>	90B
	14B	—	—	77AB	<b>98C</b>
hobo	—	—	—	—	100C
	4D	<b>25A</b>	47F-48A	<b>67E</b>	<b>83D</b>
	7B	26A	49F	<b>70C</b>	<b>89E</b>
	—	<b>30A</b>	50C	<b>79E</b>	98E
	—	<b>35CD</b>	<b>56F</b>	—	—
	—	<b>36CD</b>	<b>57A</b>	—	—

situation has been observed for P elements. Analogous data were earlier reported by a range of authors (Yamaguchi *et al.*, 1987; Ronsseray *et al.*, 1989).

**Hobo element.** The average number of hobo localization sites in the genome was 22.7 (Table 1). This is a lower number than those reported by other authors. Streck *et al.* (1986) sets the average number of hobo per H-line genome at 50. For some highly inbred lines, the magnitude of 28.53 was obtained (Biemont *et al.*, 1988).

The pattern of distribution of hobo elements throughout the chromosomes is unlike that of P elements. The average hobo copy number in the X chromosome is 2.7. At the same time, chromosome I is noted for an increased number of hybridization sites of this transposon (5.1 in 2L and 5.4 in 2R), which is somewhat higher than that of chromosome III (4.6 on 3L and 4.9 on 3R).

**Hotspots.** It is possible to clearly distinguish chromosomal regions where P and hobo elements are found in at least 50% of individuals (the so-called "hotspots"). The hotspots are listed in Table 2. Twenty three such sites have been revealed for P and 18 for hobo. From Table 2 one can also acquaint oneself with the cases when hotspots coincide with the regions of intercalary heterochromatin determined with the data by Zhimulev and associates (1982). The percentage of such coincidences is 57% for P and 67% for hobo, *i.e.*, a considerable share of hotspots of localization of these transposable elements concentrates in the regions of intercalary heterochromatin. It is apparently associated with structural differences of eu- and heterochromatin regions: intercalary heterochromatin is known to have a more condensed structure than euchromatic chromosome regions. Chromatin decondensation sets up conditions for TEs to move. The structure of intercalary heterochromatin in contrast impedes transpositions and sets up conditions for mobile elements to accumulate in these regions.

**P and hobo co-occurring.** We have analyzed the cases when P and hobo occupied the same sites, the results gathered in Table 3. The highest percentage of coincidence was shown by 2L and X chromosomes (40% and 33%, respectively). With other chromosomes, this characteristic was about the same: 21% for 2R, 17% for 3L, and 24% for 3R.

The results of an analysis of the sites shared at a time by P and hobo in "hotspots" are presented in Table 3. The percentage of coincidence here is higher than that in the total list of the sites (41% and 26%, respectively). There are clear-cut chromosomal differences. 2L demonstrates the highest percentage of coincidence, 80%. Then go 2R with 60%, X chromosome with 40%, and 3L with 33%. The lowest index is shown by 3R, just 13%.

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**D.P. Furman.** Institute of Cytology and Genetics, SO RAN, Novosibirsk-90, Russia. Properties of the *scute* mutation of *Drosophila melanogaster*, induced in the P-M system of hybrid dysgenesis.

mutants (Lindsley and Grill, 1968). Some of the mutants arose spontaneously, some under radiation or chemical mutagenesis. We induced the *scute* mutations in P-M hybrid dysgenesis system. For this purpose females of the *Cy/Pm;D/Sb* laboratory stock (Lindsley and Grill, 1968) were crossed with males of *C(1)y w f<sup>2</sup>*, kindly provided by Dr. Engels (Engels, 1979). The mating scheme is presented in Figure 1. Mutant males were selected for the loss of bristles and tested by a cross with *Df(1)sc<sup>V1</sup>f<sup>26a</sup>/FM6, y<sup>31d8</sup> sc dm B* females. Deficiency of *Df(1)sc<sup>V1</sup>* = *Df(1)L48-C3*

Table 3. The number of hybridization sites and hotspots of localization of P and hobo in the Biysk population of *Drosophila*.

Mobile element	Chromosomes					genome
	X	2L	2R	3L	3R	
sites, total	21	20	29	24	33	127
coinciding sites, Qty	7	8	6	4	8	33
coincidence, %	33	40	21	17	24	26
hotspots, total	5	5	5	6	8	29
coinciding hotspots, Qty	2	4	3	2	1	12
coincidence, %	40	80	60	33	13	41

The *scute* gene (1-0.0) in *Drosophila melanogaster* is in fact a series of step alleles (Dubinin, 1933). Phenotypically these mutations show in that flies lose various series of the large bristles (macrochaetes). Loss of macrochaetes in mutant stocks occurs at a probability level (penetrance). Collections number over 20 *scute*

Table 1. Bristle loss frequencies in the *scute* mutants induced in the P-M system of hybrid dysgenesis (class I alleles)

Bristles	Bristle loss frequency, %											
	<i>sc<sup>38P</sup></i>		<i>sc<sup>37P</sup></i>		<i>sc<sup>53P</sup></i>		<i>sc<sup>34P</sup></i>		<i>sc<sup>80P</sup></i>		<i>sc<sup>226P</sup></i>	
	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>
oc <sup>1-2</sup>	8.5	32.0	2.5	1.5	—	—	—	—	—	—	—	—
pv <sup>1-2</sup>	24.0	80.5	3.5	1.5	1.5	—	57.0	60.5	51.0	74.5	37.5	50.0
or <sup>1</sup>	31.5	80.5	9.0	6.0	6.5	3.0	—	—	27.0	29.0	14.5	15.0
or <sup>2</sup>	87.0	78.0	72.0	54.5	61.5	10.0	—	—	2.5	19.0	—	—
or <sup>3</sup>	22.5	35.0	3.0	2.0	3.0	—	—	—	—	—	—	—
v <sup>1</sup>	5.0	54.0	2.5	—	1.5	—	—	—	—	—	—	—
v <sup>2</sup>	3.0	—	5.5	—	0.5	0.5	—	—	—	—	—	—
dc <sup>1-2</sup>	11.0	—	7.5	4.5	—	—	—	—	—	—	—	—
dc <sup>3-4</sup>	53.5	—	33.0	11.5	3.5	—	—	—	—	—	—	—
sc <sup>1-2</sup>	46.0	36.5	48.5	20.5	3.0	—	—	—	—	1.0	—	—
sc <sup>3-4</sup>	87.5	71.0	76.0	35.0	30.0	—	—	—	—	4.5	—	—
h <sup>1</sup>	39.0	76.0	6.0	4.5	4.5	2.5	60.5	54.5	—	—	—	—
h <sup>2</sup>	16.0	26.5	3.0	2.0	3.5	—	58.5	56.0	—	—	—	—
ps	4.0	1.5	—	1.0	—	—	—	—	—	—	—	—
n <sup>1</sup>	19.5	56.5	7.0	3.0	1.5	—	—	—	—	—	—	—
n <sup>2</sup>	18.5	0.5	7.5	1.5	16.0	—	—	—	—	—	—	—
sa <sup>1-2</sup>	8.5	0.8	5.2	1.2	—	—	—	—	—	—	—	—
pa <sup>1-2</sup>	12.8	15.0	12.5	2.2	—	—	—	—	—	—	—	—

Data as gathered on just generated mutants

Table 2. Bristle loss frequencies in the *scute* mutants induced in the P-M system of hybrid dysgenesis (class II alleles)

mutants	loss frequency, %			
	h <sup>1</sup>		h <sup>2</sup>	
	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>
sc <sup>55P</sup>	29.0	8.5	34.5	11.5
sc <sup>59P</sup>	32.0	19.5	35.5	21.5
sc <sup>96P</sup>	39.5	46.5	62.0	68.0
sc <sup>219P</sup>	54.5	41.0	62.0	49.0
sc <sup>232P</sup>	52.5	46.0	53.5	53.5
sc <sup>63P</sup>	66.0	71.5	49.5	56.0
sc <sup>146P</sup>	72.5	64.0	77.0	63.5
sc <sup>126P</sup>	75.5	65.5	78.0	71.0
sc <sup>133P</sup>	90.0	78.5	89.0	84.0
sc <sup>147P</sup>	88.0	85.5	88.0	83.5

Data as gathered on just generated mutants.

Table 3. Bristle loss at different temperatures in class I mutants

Bristles	<i>sc<sup>38P</sup></i>						<i>sc<sup>34P</sup></i>					
	18°		25°		30°		18°		25°		30°	
	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>	♀♀	♂♂ <sup>i</sup>
oc <sup>1-2</sup>	17.0	20.0	3.0	15.0	11.0	44.5	—	0.5	—	5.5	10.5	10.5
pv <sup>1-2</sup>	17.0	78.0	25.0	65.0	36.5	75.5	1.5	3.0	86.0	86.0	31.0	45.0
or <sup>1</sup>	21.0	32.0	36.0	49.0	41.0	70.0	—	—	—	—	—	—
or <sup>2</sup>	21.0	32.0	36.0	49.0	41.0	70.0	—	—	—	—	—	—
or <sup>3</sup>	27.0	69.0	17.0	52.0	19.0	52.5	—	—	—	—	—	—
v <sup>1</sup>	10.0	82.0	16.0	55.0	29.5	74.0	—	—	—	—	—	—
v <sup>2</sup>	5.0	27.0	8.0	12.0	10.0	29.5	—	—	—	—	—	—
dc <sup>1-2</sup>	20.0	—	12.0	1.0	5.5	11.0	—	—	—	—	—	—
dc <sup>3-4</sup>	75.0	3.0	48.0	1.0	25.5	3.0	—	—	—	—	—	—
sc <sup>1-2</sup>	60.0	65.0	36.0	49.0	36.0	76.0	—	—	—	—	—	—
sc <sup>3-4</sup>	92.0	77.0	84.0	69.0	74.5	75.5	—	—	—	—	—	—
h <sup>1</sup>	19.0	62.0	16.0	41.0	27.0	50.0	62.5	64.0	83.0	76.0	89.5	49.5
h <sup>2</sup>	22.0	7.0	11.0	5.0	13.5	20.5	58.5	63.0	83.5	75.0	87.0	48.0
ps	10.0	5.0	5.0	24.0	25.0	61.5	—	—	—	—	—	—
n <sup>1</sup>	21.0	78.0	27.0	62.0	37.0	73.0	—	—	—	—	—	—
n <sup>2</sup>	67.0	6.0	51.0	8.0	23.5	11.5	—	—	—	—	—	—
sa <sup>1</sup>	5.0	72.0	4.0	52.0	9.5	52.0	—	—	—	—	—	—
sa <sup>2</sup>	14.0	9.0	18.0	4.0	9.0	10.5	—	—	—	—	—	—
pa <sup>1</sup>	18.0	37.0	18.0	35.0	8.5	33.5	—	—	—	—	—	—
pa <sup>2</sup>	—	51.0	1.0	46.0	16.5	70.0	—	—	—	—	—	—

overlaps region 1B1/2-1B4/5, where the *achaete-scute* complex is localized (Lindsley and Grill, 1968). Penetrance of the mutations in homozygotes and compounds was assessed with a sample of 100 males and 100 females of each genotype. As a train of interest, we took bristle loss percentage. Out of 17,347 X-chromosomes passed through the dysgenic cross, 18 were identified as being mutant.

The phenotypes of 16 mutants at a breeding temperature of 22°C are presented in Tables 1 and 2. Compared with most of the known *scute* alleles, these mutants have more bristles, and penetrance reaches 100%.

Qualitatively, i.e., as regards lacking bristle variety, the mutants can be classified into two groups: one holding six mutants (*sc<sup>34P</sup>-sc<sup>226P</sup>*) and one holding ten (*sc<sup>55P</sup>-sc<sup>147P</sup>*). In group one, the most impressive carrier of the mutant phenotype is *sc<sup>38P</sup>* with missing 17 out of 20 pairs of bristles from head, thorax and scutellum (Table 1). Group two mutants lose only one pair of bristles, namely humerals (*h<sup>1-2</sup>*), yet the penetrance is different, ranging between ~10% (*sc<sup>55P</sup>* males) and ~90% (*sc<sup>133P</sup>* females).

Now compare the resulting mutations to the known, "classical", noted for the following properties: hypomorphism (phenotypically, females are closer to the wild type than males are); temperature dependence of penetrance; invariance of qualitative differences of alleles under temperature variations; local dominance of the norm (presence of bristles) over the mutation (lack of bristles) in the compounds, i.e., the compound loses only those bristles that are lacking in both its component homozygotes (Dubinin, 1933; Child G. 1935; Furman D.P. et al., 1979).

Different phenotypic manifestations of the mutation between males and females is observed in most of the induced stocks. Females often have more bristles lost and higher penetrance than do males, i.e., two doses of the gene produce a more

Table 4. Bristle loss in the compounds of ♀♀  $sc^{38P}$  × ♂♂  $sc^n$  crosses

Bristles	Bristle loss frequency, %									
	$sc^{37P}$		$sc^{53P}$		$sc^{34P}$		$sc^{133P}$		$sc^{147P}$	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
oc <sup>1-2</sup>	9.5	21.0	9.0	17.0	—	1.0	1.0	1.0	—	24.5
pv <sup>1-2</sup>	8.0	36.0	10.0	28.5	—	5.5	—	0.5	—	45.5
or <sup>1</sup>	15.0	43.0	25.0	38.5	—	7.0	—	2.0	—	42.5
or <sup>2</sup>	82.0	77.5	87.0	57.0	—	33.0	—	35.0	—	58.0
or <sup>3</sup>	12.5	34.0	16.0	26.5	—	4.5	—	2.0	—	21.0
v <sup>1</sup>	6.0	50.0	4.5	31.0	—	3.0	—	0.5	—	30.5
v <sup>2</sup>	6.5	0.5	16.0	2.5	—	—	—	0.5	—	—
dc <sup>1-2</sup>	16.0	0.5	20.5	—	—	1.0	4.0	—	—	—
dc <sup>3-4</sup>	54.5	1.0	52.0	—	—	2.5	2.5	—	—	—
sc <sup>1-2</sup>	59.5	22.0	71.0	28.0	—	5.0	—	4.5	—	28.0
sc <sup>3-4</sup>	92.0	52.0	92.0	41.0	—	12.0	—	10.5	—	50.5
h <sup>1</sup>	7.0	50.5	16.5	27.0	2.5	2.5	11.5	10.5	26.5	51.5
h <sup>2</sup>	11.5	6.0	29.5	4.0	1.5	1.0	12.0	8.0	28.0	19.0
ps	1.0	—	—	0.5	—	—	—	—	—	—
n <sup>1</sup>	13.5	53.0	20.0	30.0	—	1.5	—	—	—	42.5
n <sup>2</sup>	31.0	—	44.0	1.5	—	—	—	1.5	—	—
sa <sup>1</sup>	0.5	—	—	—	—	—	—	—	—	—
sa <sup>2</sup>	19.5	1.5	20.5	0.5	—	—	—	—	—	—
pa <sup>1</sup>	28.0	31.5	38.5	21.5	—	1.0	—	1.0	—	29.0
pa <sup>2</sup>	1.0	3.5	—	—	—	—	—	—	—	2.0

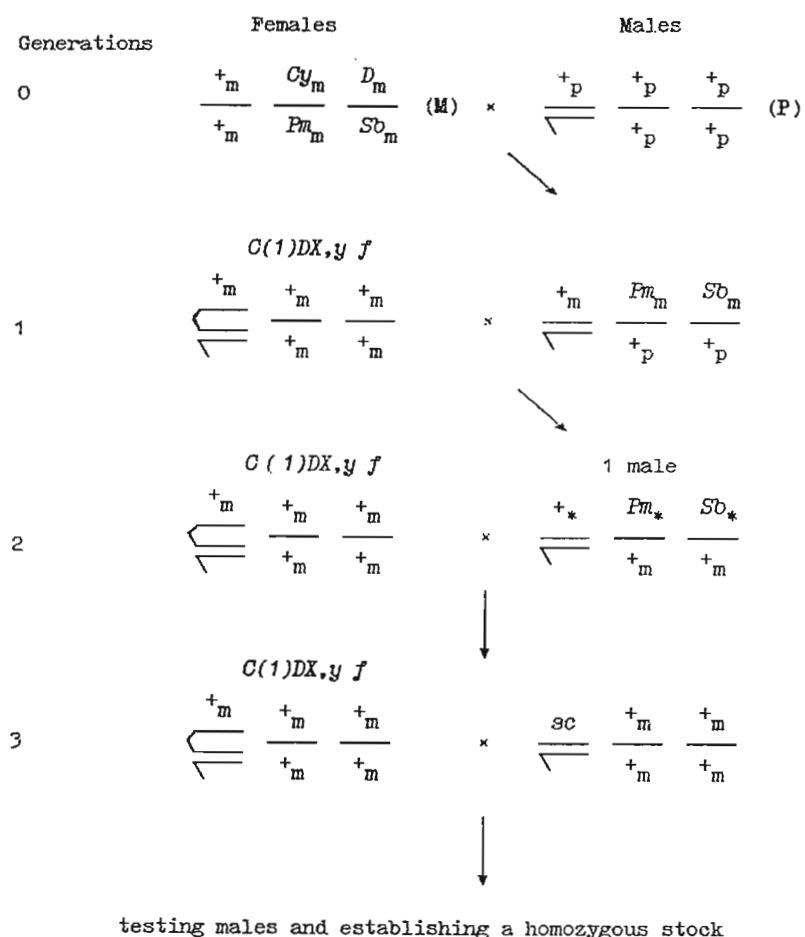


Figure 1. Mating scheme for the induction of scute mutations. The subscripts m and p indicate wild-type chromosomes of M and P strain origin, respectively. Asterisk subscripts denote M-derived chromosomes, passed through the P-M system of hybrid dysgenesis.

"anomalous" phenotype (Tables 1 and 2).

**Temperature treatment.** To make the background uniform within the stocks, these were first isogenized with respect to autosomes using the  $Cy/Pm;D/Sb$  balancer stock (Lindsley and Grill, 1968). The phenotypic characteristics of "dysgenic" mutations have been read at  $18^\circ$ ,  $25^\circ$  and  $30^\circ\text{C}$ , the results shown in Table 3. Penetrance remarkably varies as temperature changes. At that, a mutant's bristles may not produce the same response to temperature change, their topographic nearness not necessarily ensuring similar responses (e.g.,  $oc^{1-2}$  and  $pv^{1-2}$  in  $sc^{38P}$ ). Namesake bristles of different mutants differ in response ( $pv^{1-2}$  in  $sc^{38P}$  and  $sc^{34P}$ ), nor do otherwise even those of the males and females within one stock ( $sc^{1-2}$  in  $sc^{38P}$  etc.).

Nevertheless, no temperature change has deprived a mutant of its qualitative properties or has made it go over to another class. The only exception was  $sc^{34P}$  at  $18^\circ\text{C}$  (Table 3).

**Compounds.** The induced mutants were crossed pair-to-pair in all direct and reciprocal combinations possible. The typical properties of the compounds of group one alleles and the intergroup compounds are illustrated in Table 4. This table presents the results of the cross between  $sc^{38P}$  females and the males of some mutant stocks of groups one and two (see Tables 1 and 2). As is seen, interallelic relationships do not only reduce to domination of the norm over the mutation with respect to separate bristles. There are exceptions to this rule, that is bristle loss is dominating or a total recovery of the normal phenotype takes place, as in the

*sc<sup>38P</sup>/sc<sup>34P</sup>* compound.

Group two alleles show their regular behavior in crosses *inter se*: the compounds only lose h<sup>1-2</sup> bristles (data not shown).

**Penetrance depends on genetic background.** Comparison of progeny males derived from crosses among mutant stocks of group two and especially with group one mutants provides evidence that penetrance depends on the genetic background both in direct and reciprocal combinations of alleles. Quite illustrative in this respect are the phenotypes of the sons coming from a cross of *sc<sup>38P</sup>* females to *sc<sup>133P</sup>* and *sc<sup>147P</sup>* males, the two stocks noted for close penetrance (Table 2). Heterozygous for autosomes, the sons inherited their X chromosome from *sc<sup>38P</sup>*. Phenotypically, these males differ considerably from one another and from *sc<sup>38P</sup>* (Table 4). Since the mutation in the *scute* gene remains the same, the differences should apparently be related to the genetic background. Different genetic backgrounds - different penetrance in the mutants of group two. Penetrance appears to be under polygenic control.

Thus, the comparison of the qualitative and quantitative properties of the "classical" and the newly emerging *scute* alleles allows us to see a hard line between the two. Indeed, none of the induced alleles has an exact match among those present in the collections.

The stability of qualitative phenotypic differences among the mutants under varying temperature may provide evidence, by analogy with the results obtained earlier for the "classical" *scute* alleles (Furman, *et al.*, 1979), that these differences are conditioned by structural alterations in the locus. Quantitative differences among the alleles as regards penetrance may be associated with changes in the polygenic loci and/or an upset of their balance.

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**Jamiruddin, S.K., A. Das, and B.B. Parida.** Genetics laboratory, Department of Zoology, Utkal University, Bhubaneswar, 751 004, India. Chromosomal polymorphism in some Indian Populations of *Drosophila ananassae*.

Chromosomal inversions have been conducted by many workers around the world (Kikkawa, 1938; Dobzhansky and Dreyfus, 1943; Shirai and Moriwaki, 1952; Freire-Maia, 1955, 1961; Jha, 1976; Sreerama Reddy and Krishnamurthy, 1972; Singh, 1974, 1984, 1989). Three paracentric inversions (alpha in 2L, delta in 3L and eta in 3R) have been found to be widespread and present in almost all natural populations of *D. ananassae* so far screened. The frequency of different gene arrangements due to these three inversions and the level of inversion heterozygosity vary in different natural populations of this species.

Table 1. Frequency (in percent) of three commonly occurring inversions and correlation coefficient (*r*) with the latitude and longitude in five natural populations of *D. ananassae* presently studied.

Population	State	Latitude (°N)	Longitude (°E)	Total no. chromosomes examined	Inversion Frequency (in percent)			Mean no. of heterozygous inversions per individual
					AL(2L)	DE (3L)	ET (3R)	
Bhubaneswar	Orissa	20.1	85.5	104	8.00	18.00	28.00	1.03
Cuttack	Orissa	20.2	85.5	118	3.00	25.00	10.00	0.54
Ratnagiri	Orissa	20.4	86.2	142	18.00	37.00	20.00	1.29
Balasore	Orissa	21.2	86.5	66	12.00	39.00	20.00	1.18
Howrah	West Bengal	22.4	88.7	62	13.00	16.00	26.00	1.10
Correlation Coefficient with Latitude				+ 0.11	- 0.14	+ 0.26		
Probability				> 0.10	> 0.10	> 0.10		
Correlation Coefficient with Longitude				+ 0.37	+ 0.47	+ 0.30		
Probability				> 0.10	> 0.10	> 0.10		

India, being a tropical country, harbours *D. ananassae*, and several natural population samples of this species have been screened for chromosome inversion polymorphism. However, the east-coastal part of India has not been analysed for this phenomenon, thus, we became interested to obtain a general idea

on inversion polymorphism in *D. ananassae* from these localities. For this, five population samples were collected and isofemale lines were constructed. The F1 larvae from isofemale lines were analysed cytologically for inversions, and, for frequency determination, the karyotype of one F1 larva was considered. The chromosomal analysis revealed the presence of all the three cosmopolitan inversions in all populations. The frequencies of different karyotypes were obtained independently for each inversion and subjected to Hardy-Weinberg expectation. Except in two instances (eta in Ratnagiri and Bhubaneswar), deviation from the expectation could not be detected (data not shown). The frequency of all the three inversions and the mean number of heterozygous inversions per individual was calculated and shown in Table 1. While the frequency of all the three inversions varies among populations, the delta inversion was most frequent in the populations of *Drosophila ananassae*. The mean number of heterozygous inversions per individual also varies among populations and ranges from 0.54 to 1.29. In order to see the influence of latitude and longitude on the frequency distribution of these inversions, the correlation coefficient was calculated independently and shown in Table 1. In all cases the  $r$  was found to be statistically insignificant but, the relation is positive in some cases while negative in others. Different intra- and interchromosomal associations for these three inversion karyotypes were obtained and, in instances, found to be random.

The results on the whole are in agreement with the previous suggestion that: (1) the three inversions are cosmopolitan in distribution in the polytene chromosome of *Drosophila ananassae*; (2) genetic differentiation does exist among populations of different geographical areas; in our study a direct correlation between inversion frequency and latitude and longitude was measured. Though not significant in any of the comparisons, which may be due to a narrow range, some relationship between the variables could be observed; (3) the lack of evidence for intra- and interchromosomal interactions, although in some natural populations of *Drosophila ananassae* from India linkage disequilibrium between two inversions could be detected (Singh, 1988).

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**Parkash, R. and Vandana.** M.D. University, Rohtak, India. Allozyme variation in *D. jambulina* and *D. punjabensis*.

the electrophoretic mobilities of all such zones are identical in both the sibling species. Out of eight esterase zones of activity, most of the zones are represented by segregating single band variants and two-banded patterns while the EST-6 zone is monomorphic. The single polymorphic ACPH zone revealed either three-banded or five-banded ACPH patterns, and the mobility patterns are species specific. The progeny of isofemale lines depicting particular triple-banded patterns did not reveal segregation of the ACPH bands in any of these two species. Genetic crosses involving two distinct types of triple-banded ACPH phenotypes resulted in five-banded ACPH patterns in the F1 individuals.

The data on allelic frequencies, observed and expected heterozygosity, Wright's inbreeding coefficients, and G-values for likelihood  $\chi^2$  test for fit to Hardy-Weinberg expectations at twelve polymorphic loci in sibling species (*D. jambulina* and *D. punjabensis*) have been represented in Table 1. The patterns of allelic frequencies are almost identical at four loci (ADH, ODH, alpha-GPDH and AO) while the two sibling species seem to be genetically different at several loci (MDH-1, APH-3, ACPH and EST loci) due to differential distribution of allelic frequency patterns. Significant deviations from Hardy-Weinberg expectations have been observed at ACPH, AO, MDH-1, APH-3, EST-1, -4, -5, -7 in *D. jambulina* and at ACPH, AO, EST-3 in *D. punjabensis*. The high values of Wright's Inbreeding coefficient at EST-1 and -4 loci in *D. jambulina* and at ACPH, EST-3 and EST-8 in *D. punjabensis* may be due to sampling errors. Genetically determined allozyme (allelic isozyme) polymorphism at enzyme loci have been found useful in distinguishing sibling species, other closely related sub-species and incipient species of mosquitoes (Munstermann, 1980; Mathews *et al.*, 1983). The present observations reveal genetic differentiation at 60% of the loci examined in these two sibling species. The comparison of genetic structure of *D. jambulina* and *D. punjabensis* has revealed that ADH, ODH, alpha-GPDH and AO constitute non-differentiating loci, since both the electrophoretic

The present paper reports the patterns of electrophoretic variability in eight gene-enzyme systems in the natural population samples of *D. jambulina* and *D. punjabensis*. The monomorphic zones include alpha-GPDH, ADH, APH-1 and -2 and MDH-2; and

Table 1. Data on distribution of allelic frequencies, heterozygosities ( $H_o$  and  $H_e$ ), Wright's inbreeding coefficient ( $f$ ) and G-values for log likelihood  $\chi^2$  test for fit to Hardy-Weinberg expectations at twelve loci in *D. jambulina* and *D. punjabensis*.

Enzyme/ Locus	<i>D. jambulina</i>							<i>D. punjabiensis</i>						
	N	F	M	S	Ho/He	f	G-values	N	F	M	S	Ho/He	f	G-values
ACPH	168	.08	.66	.26	.36 / .50	.28	42.08*	142	.73	.27	—	.22 / .40	.45	23.95*
AO	112	.16	.44	.40	.44 / .62	.29	56.00*	95	.16	.47	.37	.53 / .62	.14	88.85*
MDH-1	128	.78	.22	—	.43 / .35	.23	15.90*	120	—	.42	.58	.45 / .49	.08	0.75
	F	S						F	S					
ODH	92	.30	.70	.43 / .42	.02	0.09	105	.36	.64	.52 / .46	.13	.13	.214	
APH-3	136	.67	.33	.52 / .44	.17	4.20°	81	.39	.61	.38 / .48	.19	.19	3.85	
EST-1	120	.49	.51	.32 / .50	.36	15.00*	110	1.0	—	—	—	—	—	
EST-2	132	.56	.44	.43 / .50	.14	2.08	96	.28	.72	.43 / .42	.02	.02	0.67	
EST-3	141	.46	.54	.49 / .50	.02	0.04	114	.25	.75	.23 / .38	.40	.40	14.15*	
EST-4	124	.46	.54	.24 / .50	.52	39.13*	100	1.0	—	—	—	—	—	
EST-5	112	.41	.59	.36 / .48	.25	7.71*	90	1.0	—	—	—	—	—	
EST-7	120	.50	.50	.40 / .50	.20	4.85*	87	.34	.66	.46 / .48	.05	.05	0.43	
EST-8	100	1.0	—	—	—	—	84	.61	.39	.50 / .47	.37	.37	0.20	

*N* = number of individuals analysed; *F*, *M* and *S* refer to fast, medium and slow allelic electromorphs. \* significant at 5%; *Ho* and *He* refer to observed and expected heterozygosity. The values of average number of alleles (*A*), proportion of polymorphic loci (*P*), mean heterozygosity (*H*) are 1.72, 0.55 and 0.23 in *D. iambulina*; 1.63, 0.50, and 0.22 in *D. punjabensis*, respectively.

phenotypes as well as allelic frequencies are similar for these loci. However, MDH-1, ACPH, APH-3, and most of the EST loci constitute species discriminating loci because, except for a few shared alleles, the allelic frequency patterns are differential in the two sibling species. The present observations have not revealed a diagnostic locus, with distinct mobilities and/or non sharing of major alleles in the two sibling species.

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

Colonising species populations of *D. melanogaster* offer excellent material for microevolutionary studies (Endler, 1977; Knibb, 1982). Most of the allozymic studies in *D. melanogaster* were made on U.S., European, Australian and Afrotropical populations

while Indian populations remained unexplored. However, chromosome polymorphism and inversion clines have been reported recently in Indian populations of *D. melanogaster* (Singh and Das, 1992). In the present study, we analysed allozymic variation at six polymorphic loci in Indian populations of *D. melanogaster*. The data on allozymic frequencies in Indian populations revealed significant genetic divergence and thus favoured the Selectionist's hypothesis.

The population samples of *D. melanogaster* were bait-trapped from eight latitudinally varying sites (Cochin, 10°N, to Dalhousie, 33°N; Figure 1). The homogenates of single individuals were analysed electrophoretically in 12% starch gel at 250 V and 30 mA at 4°C for four hours. The gel slices were stained for seven gene-enzyme systems (Harris and Hopkinson, 1976; Richardson *et al.*, 1986). The enzymes included: esterases (EST); acid phosphatases (ACPH); alcohol dehydrogenases (ADH); octanol dehydrogenases (ODH); aldehyde oxidases (AO); malate dehydrogenases (MDH) and alpha-glycerophosphate dehydrogenases (alpha-GPDH). The genetic interpretation of electrophoretic data and calculation of genetic indices were followed from standard sources (Workman and Niswander, 1970; Zar, 1984; Weir, 1990).

Table 1. Distribution of gene frequencies at seven loci among eight Indian natural populations of *Drosophila melanogaster*



Figure 1. Map of India sub-continent showing the collection sites for wild caught individuals of *Drosophila melanogaster*. The sites include (1) Cochin,  $9^{\circ}58'N$ ; (2) Tirumala,  $13^{\circ}40'N$ ; (3) Hyderabad,  $17^{\circ}20'N$ ; (4) Bhopal,  $23^{\circ}16'N$ ; (5) Phuntsholing,  $26^{\circ}84'N$ ; (6) Rohtak,  $28^{\circ}54'N$ ; (7) Dehradun,  $30^{\circ}19'N$ ; (8) Dalhousie,  $30^{\circ}0'N$ .

The data on the distribution of allelic frequencies at six polymorphic loci in eight Indian populations of *D. melanogaster* are given in Table 1. The *Acph* locus revealed monomorphism while *Adh*, *Odh*, *Ao*, *Mdh* and *alpha-Gpdh* loci revealed two common alleles in all the Indian populations of *D. melanogaster*. The allelic frequency data revealed significant changes among distantly located Indian populations of *D. melanogaster*. The changes in the allelic frequencies patterns for the most frequent allele in all the eight natural populations of *D. melanogaster* from latitudinally varying sites are shown in Figure 2. Allele frequencies of *Est-6<sup>S</sup>*, *Adh<sup>F</sup>*, *alpha-Gpdh<sup>S</sup>*, *Odh<sup>F</sup>*, *Mdh<sup>S</sup>* and *Ao<sup>S</sup>* were found to be significantly and positively correlated with latitude. The data on the  $F_{ST}$  values at the various polymorphic loci in

Figure 2. Patterns of changes in allelic frequencies, simple correlation coefficient ( $r$ ) and Wright Fixation ( $F_{ST}$ ) values, at six polymorphic loci in latitudinally varying eight natural populations of *D. melanogaster* from India sub-continent.

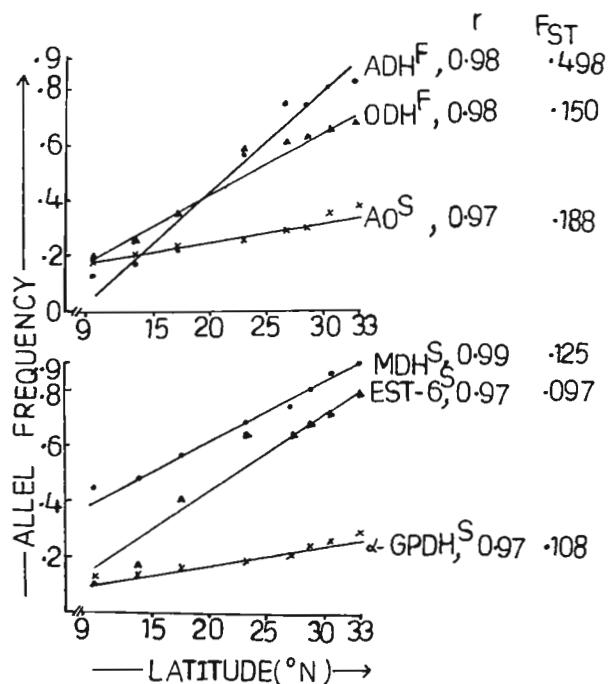


Table 2. Comparison of allelic frequencies (*Acph<sup>F</sup>*, *Adh<sup>F</sup>*, *Odh<sup>F</sup>*, *Est-6<sup>S</sup>*, *alpha-Gpdh<sup>S</sup>* and *Mdh<sup>S</sup>*) in geographical populations of *D. melanogaster* from temperate and tropical regions.

Populations*	N	Latitude	<i>Acph<sup>F</sup></i>	<i>Adh<sup>F</sup></i>	<i>Odh<sup>F</sup></i>	<i>Est-6<sup>S</sup></i>	<i>alpha-Gpdh<sup>S</sup></i>	<i>Mdh<sup>S</sup></i>
1. France	16	$45^{\circ}, 0' - 52^{\circ}, 0' N$	.99	.96	—	.76	.43	—
2. Greece	5	$35^{\circ}, 10' - 41^{\circ}, 10' N$	.99	.90	—	.61	.32	.999
3. Japan	26	$32^{\circ}, 0' - 42^{\circ}, 0' N$	.99	.69	.93	.82	.24	.997
4. Canada	1	$43^{\circ}, 3' N$	—	.76	.93	.46	.19	1.0
5. Massachusetts	1	$42^{\circ}, 04' N$	1.0	.48	.91	.51	.17	1.0
6. Tunisia	1	$35^{\circ}, 0' N$	—	.95	.99	.75	.55	—
7. Australia	1	$33^{\circ}, 4' S$	.98	.78	1.0	.62	.21	1.0
8. Egypt	1	$31^{\circ}, 25' S$	—	.76	.96	.83	.57	—
9. Taiwan	1	$25^{\circ}, 0' N$	.98	.95	.95	.81	.12	1.0
10. China	6	$20^{\circ}, 02' - 34^{\circ}, 17' N$	1.0	.75	1.0	.83	.46	—
11. India	8	$9^{\circ}, 58' - 33^{\circ}, 0' N$	1.0	.52	.49	.51	.19	.68
12. Vietnam	1	$11^{\circ}, 0' N$	1.0	1.0	1.0	.77	.23	1.0
13. Seychelles	1	$5^{\circ}, 0' S$	—	.85	1.0	.74	.49	—

\* Populations include: 1-7 = temperate region; 8-13 = tropical region. N = number of populations

all the geographical populations of *D. melanogaster* are shown in Figure 2. The observed  $F_{ST}$  values indicated significantly higher genetic differentiation at *Adh* and *Est-6* loci; moderate at *Odh* and *Mdh* loci; and lower genetic differentiation was observed at *Ao* and *alpha-Gpdh* loci. Latitudinal correlation coefficients between the frequencies of the most

common allele of the polymorphic loci are given in Figure 2.

The present observations on clinal variation concurred with other reports on *D. melanogaster* populations from different continents (Nagylaki, 1975; Endler, 1986). The allelic frequencies at six polymorphic loci from twelve continental populations were compared with those of Indian populations of *D. melanogaster* and it was found that allelic frequencies of *Adh<sup>F</sup>*, *Est-δ<sup>S</sup>* and *alpha-Gpdh<sup>S</sup>* loci revealed significant genetic differentiation among different continental populations (Table 2). However, *Acph* and *Mdh* loci revealed either one allele with more than 95% frequency or monomorphism at these loci in different geographical populations. The Indian populations of *D. melanogaster* revealed monomorphism at *Acph* locus, but *Odh* and *Mdh* loci were represented by diallelic variation (Table 2).

The data on the extent of genic differentiation in *D. melanogaster* populations supported the contention that the ecologically and climatically variable habitats were the predictors of the observed high levels of the genic diversity in this colonising species. Thus, the observed levels of genetic polymorphism in *D. melanogaster* populations were being adaptively maintained by natural selection mechanisms.

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**Parkash, R. and Shamina.** M.D. University, Rohtak, India. Patterns of ethanol and acetic acid tolerance in two *Drosophila* species populations.

metabolites are generally found in natural habitats of the *Drosophila* species. The alcohol dehydrogenase of *D. melanogaster* converts a wide range of alcohols to aldehydes, and more than 90 percent of the external alcohols are metabolised in a pathway initiated by ADH (Geer *et al.*, 1989). Natural populations of *D. melanogaster* were found to be polymorphic at the *Adh* locus and generally contained two common electrophoretic alleles: "slow" - *Adh<sup>S</sup>* and "fast" - *Adh<sup>F</sup>*. Ethanol tolerance in *D. melanogaster* was found to be *Adh* genotype dependent, *i.e.* *Adh<sup>F</sup>* homozygotes revealed higher ethanol tolerance (David, 1988). The objective of the present studies is to report the acetic acid and ethanol utilisation as well as *Adh* polymorphism for assessment of these two metabolites as resources and stresses for adult as well as larval forms of northern and southern populations of *D. melanogaster* and *D. ananassae* from India.

Mass bred populations of *D. melanogaster* and *D. ananassae* from Rohtak (northern populations; 28°.54' N) and from Madras (southern populations; 13°.04' N) were used for ethanol and acetic acid utilisation as well as ADH polymorphism. Homogenates of single individuals were subjected to electrophoresis at 250 V and 25 mA at 4°C for 4 hrs. The gel slices were stained for ADH gene-enzyme system by standard staining procedures (Harris and Hopkinson, 1976). Ethanol and acetic acid tolerance patterns were assessed following the procedures of Starmer *et al.* (1977) and David and Van Herreweghe (1983).

*Drosophila* species exploit a wide array of fermenting and decaying fruits and vegetables, other organic materials and man-made alcoholic environments. Ethanol is converted into acetic acid via acetaldehyde, and thus concentrations of these two

Table 1. Comparison of LT<sub>50</sub> (hrs); LT<sub>50</sub> maximum / LT<sub>50</sub> control values as well as adult threshold concentration for ethanol and acetic acid tolerance in northern versus southern populations of two *Drosophila* species.

Species	Populations* / Adh <sup>F</sup> allelic freq.	LT <sub>50</sub> (hrs)**		LT <sub>50</sub> max. / LT <sub>50</sub> control		Adult threshold values	
		Ethanol	Acetic Acid	Ethanol	Acetic Acid	Ethanol	Acetic Acid
<i>D. melanogaster</i>	North / 0.74	300	216	3.12	2.25	13.0	12.6
	South / 0.13	156	160	1.20	1.23	10.0	8.5
<i>D. ananassae</i>	North / 0.66	165	110	2.39	1.60	3.4	4.6
	South / 0.47	150	100	1.67	1.30	2.4	2.1

\* North and South populations represent Rohtak (28°. 54' N) and Madras (13°. 04' N), respectively.

\*\*Data on LT<sub>50</sub> (hrs) were made at 1 percent and 6 percent in *D. ananassae* and *D. melanogaster*, respectively.

The data on *Adh* allelic frequencies in northern and southern populations of *D. melanogaster* as well as *D. ananassae* revealed genetic divergence (Table 1). The frequency of *Adh<sup>F</sup>* was found to increase with latitude, *i.e.* 3% for

*D. melanogaster* and 1.5% for *D. ananassae*. The southern populations of Madras and northern populations (Rohtak) of *D. melanogaster* revealed 156 hours and 300 hrs as maximum longevity periods with ethanol; and 160 hrs and 216 hrs in the case of acetic acid. The LT<sub>50</sub> maximum/LT<sub>50</sub> control data of southern and northern populations of *D. melanogaster* were 1.20 and 3.12 for ethanol treatment and 1.23 and 2.25 for acetic acid treatment, respectively. The adult ethanol and acetic acid threshold values were found to be 10 and 8.5% for the southern population and 13.0 and 12.6% for the northern population of *D. melanogaster* (Table 1).

The *D. ananassae* southern population (Madras) and northern population (Rohtak) revealed 150 hrs and 160 hrs as maximum longevity periods with ethanol; and 110 and 100 hrs with acetic acid. The LT<sub>50</sub> maximum/LT<sub>50</sub> control data of southern and northern populations of *D. ananassae* were 1.67 and 2.39 for ethanol treatment and 1.30 and 1.60 for acetic acid treatment, respectively. The adult threshold values were 3.4% and 2.4% for ethanol and 4.6% and 2.1% with acetic acid in northern and southern populations of *D. ananassae*.

The northern and southern populations of India revealed significant genetic divergence in their potential to utilise ethanol as well as acetic acid, i.e. the increased longevity data (LT<sub>50</sub> hrs), adult threshold values, LC<sub>50</sub> values for ethanol and acetic acid were found to be significantly higher in northern populations of *D. melanogaster* and *D. ananassae* as compared with the southern populations. The northern populations of *D. ananassae* revealed significantly higher threshold values for acetic acid utilisation as compared with ethanol. However, lesser differences were observed in the southern populations of *D. ananassae*. The lower threshold values of ethanol (2.4%) and acetic acid (2.1%) utilisation in southern populations of *D. ananassae* seem to be correlated with the lower levels of alcohols in diverse types of the fermented sweet fruits in the tropical parts of the Indian sub-continent.

The observed genetic differentiation of ethanol tolerance in north and south Indian populations of *D. melanogaster* concur with other continental populations from Africa and Australia (Parsons, 1980; David *et al.*, 1986; David, 1988). Thus, present results on the comparative profiles of alcoholic utilisation in the abundantly available *D. melanogaster* and *D. ananassae* reflect the species specific adaptive characteristics in the tropical habitats.

References: David, J.R. 1988, in *Population Genetics and Evolution*, Springer Verlag, Berlin, pp. 63-172; David, J.R. and J. Van Herrewege 1983, Comp. Biochem. Physiol. 74A: 283-288; David, J.R., H. Mercot, P. Capy, S.F. McEvey and J. Van Herrewege 1986, Genet. Sel. Evol. 18: 405-416; Geer, B.W., P.W.H. Heinstra, A.M. Kapoun, and A. Van Der Zel 1989, in *Ecological and Evolutionary Genetics of Drosophila* (eds., Barker, J.S.F. *et al.*), pp 231-252, Plenum; Harris, H. and D.A. Hopkinson 1976, *Handbook of Enzyme Electrophoresis in Human Genetics*, North-Holland, Amsterdam, pp 300; Parsons, P.A. 1980, Experientia 36: 70-71; Starmer, W.T., W.B. Heed and E.S. Rockwood-Sluss 1977, Proc. Natl. Acad. Sci. USA 74: 387-391.

## Announcement

### Announcement from Imogene Schneider.

Department of Entomology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

The following cell lines have been available by sending requests to the me for more than 20 years:

*Drosophila melanogaster*, lines 1, 2, and 3

*Drosophila immigrans*

*Drosophila virilis*

However, I expect to retire in early 1995 and suggest that anyone who anticipates using any of the lines, with the exception of *D. melanogaster*, line 2, request them before the end of 1994. *D. mel* (2), the most frequently requested line, has been placed in the ATCC under acquisition number CRL-1963 and should be available anytime in the future.

**Demakova, O.V., D.E. Koryakov, M.L. Balasov, S.A. Demakov, and I.F. Zhimulev.** Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, 630090, Russia. Variation in frequency of gamma-irradiation induced chromosome aberrations in *Drosophila melanogaster* in successive generations.

1976). Some of the irradiated males were used for cytological analysis part of the males of every new generation were used for individual crossing with *Oregon-R* females.

Among 308 F1 haploid chromosome sets, 40 carried various types of aberrations. Except for two deletions, all rearrangements observed were translocations and inversions. The distribution of breaks among both chromosome arms and chromosome regions seemed to be non-random. There was a significant excess of rearrangements involving X- and 3R-chromosomes. Among 82 breaks, 26 were localized in the distal part of 3R-chromosome (sections 90-100) (Table 1 and 2). The reasons for such a high sensitivity of these regions to inducing rearrangements after irradiation are not clear yet; nevertheless, the same results were obtained by Hessler (1958). The frequency of aberrations decreased during subsequent generations, being 13.0% for F1 and 2.1% for F4 (Table 2, Figure 1). Since F4, the total frequency of aberrations has been constant (for F10 it was 2.0%).

Frequencies of autosomal aberrations are shown in Table 3 and Figure 1. These two types of aberrations were presented in F1 in approximately equal frequencies, but the following fate of their carriers was different. The frequency of translocations decreased twice per generation becoming zero by F4. Some types of translocations are known to result in male sterility when heterozygous (review: Ashburner, 1989); 20% of individual crosses of F1 males showed male sterility. The fertility of F2 males turned out to be practically normal, which seemed to

Gamma-Ray induced chromosome aberrations were obtained to study their changes in frequency and spectrum in successive generations (Table 1). *Oregon-R* 2-4-day-old males were irradiated (1.3 kR) and then mated to females of the same stock. The brooding technique was used for taking into analysis mainly chromosomes of irradiated spermatids and late spermatocytes (review: Sankaranarayanan and Sobels,

Table 1. The list of the  $\gamma$ -ray induced chromosomal rearrangements in F1 - F4, F10 generations.

<u>1st generation</u>					
Translocations		Inversions		Deletions	
1.	T (1; 2L)	12E-34C	1.	In (1)	4A-14D
2.	T (1; 2R)	5E-41A	2.	In (1)	4D-12E
3.	T (1; 2R)	6F-47C *	3.	In (1)	10F-17E
4.	T (1; 2R)	7C-53F	4.	In (2R)	51D-56C
5.	T (1; 3L)	3C-67F	5.	In (2R)	41A-51F
6.	T (1; 3L)	3D-67A	6.	In (3L)	64C-67D
7.	T (1; 3L)	11A-73C	7.	In (3R)	82C-84D
8.	T (1; 3R)	1F-99F	8.	In (3R)	85D-94E**
9.	T (1; 3R)	16D-96D	9.	In (3R)	84B-92D
10.	T (1; 3R)	16D-98B	10.	In (3R)	90B-97D
11.	T (1; 3R)	20-96F	11.	In (3R)	92A-94C*
12.	T (1; 2; 3)	10A-37B	12.	In (3R)	92B-95A
		40F-99A	13.	In (3R)	92C-87C
13.	T (2L; 2R)	21F-57C	14.	In (3R)	93F-98C
14.	T (2L; 2R)	34A-59E	15.	In (3R)	96B-98A **
15.	T (2L; 3L)	24F-64D*	16.	In (3R)	96C-98D
16.	T (2L; 3R)	25B-92C			
17.	T (2L; 3R)	27A-98C			
18.	T (2L; 3R)	39A-91A			
19.	T (2R; 3R)	41A-98C			
20.	T (2R; 3R)	52A-82C			
21.	T (2R; 4)	60C-101F			
22.	T (3L; 4)	67D-101F			
<u>2nd generation</u>					
1.	T (2L; 2R)	23B-53C *	1.	In (2L)	33B-36E
2.	T (2R; 3R)	41A-94B	2.	In (2R)	41F-52A
3.	T (3L; 3R)	65B-99B	3.	In (2R)	42A-53C *
4.	T (3R; 4)	96A-101F	4.	In (2R)	50C-54A
			5.	In (3L)	67D-89E
			6.	In (3L)	65F-75F
			7.	In (3L)	67E-75C
			8.	In (3R)	86D-87D
			9.	In (3R)	87E-88F
			10.	In (3R)	99B-100F
<u>3rd generation</u>					
1.	T (2R; 3R)	49E-85D **	1.	In (1)	7B-11A
2.	T (3L; 3R)	65B-99B (3 times)	2.	In (1)	7C-9B
			3.	In (1)	7C-13A
			4.	In (1)	7D-12A *
			5.	In (2L)	39E-39D
			6.	In (3L)	64F-71C *
			7.	In (3R)	86B-98E **
			8.	In (3R)	87E-88F
			9.	In (3R)	95F-97B
<u>4th generation</u>					
1.			1.	In (1)	10E-15E
2.			2.	In (3L)	72A-76A
3.			3.	In (3R)	84B-85C
4.			4.	In (3R)	87E-88F *
5.			5.	In (3R)	96B-99E *
<u>10th generation</u>					
1.			1.	In (2R)	53E-56E
2.			2.	In (3L)	62B-64C
3.			3.	In (3L)	70C-74A
4.			4.	In (3R)	85D-87B
5.			5.	In (3R)	87E-88F
					2 times

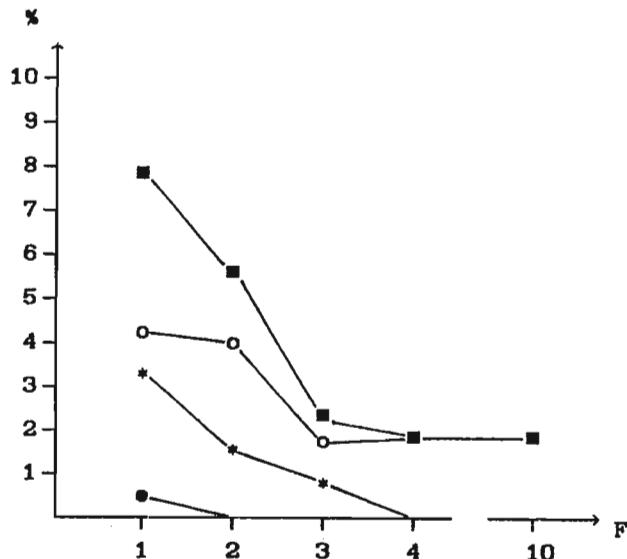


Figure 1. Frequencies of the rearrangements in F1-F4 and F10 generations. Frequencies of total rearrangements, inversions, translocations and deletions are marked with filled squares, open circles, asterisks and filled circles, respectively.

Table 2. The rearrangements frequencies in F1-F4, F10 generations.

Generations	No. of haploid sets examined	No. of rearrangements*	No. of rearrangements break points				
			1	2L	2R	3L	4
1	308	40 (13.0 ± 1.9)	20	9	12	7	32
2	258	14 ( 5.6 ± 1.4)	0	3	8	6	10
3	287	13 ( 4.8 ± 1.3)	8	2	1	5	10
4	282	6 ( 2.1 ± 0.9)	2	0	0	4	6
10	300	6 ( 2.0 ± 0.8)	0	0	2	4	6

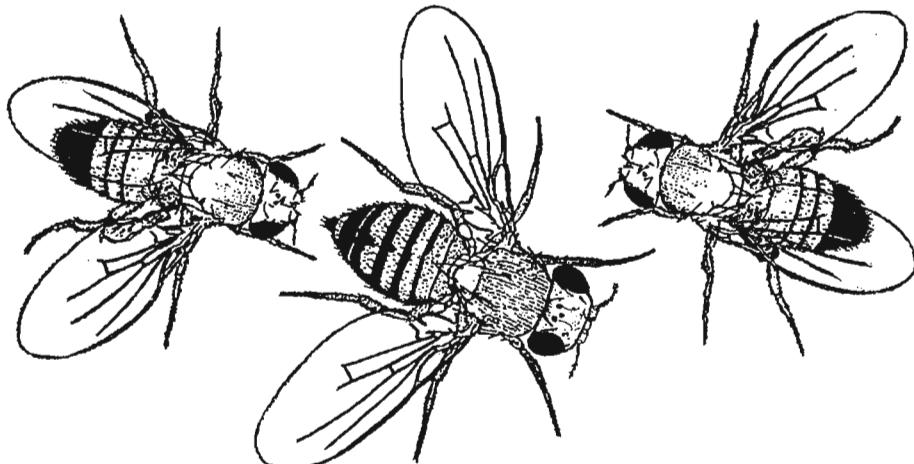
\* The frequency percentages are in parentheses.

Table 3. The frequency of different types of autosomal rearrangements in F1-F4, F10 generations.

Generations	No. of rearrangements (%)	No. of		
		inversions	translocations	deletions
1	24 (7.8)	13 (4.2)	10 (3.2)	1 (0.3)
2	14 (5.4)	10 (3.9)	4 (1.6)	0
3	7 (2.4)	5 (1.7)	2 (0.7)	0
4	5 (1.8)	5 (1.8)	0	0
10	6 (2.0)	6 (2.0)	0	0

The frequency percentages are in parentheses.

Spring Harbor Laboratory Press: p. 439; Hessler, A.Y. 1958, Genetics 43: 395-403; Sankaranarayanan, K. and F.H. Sobels 1976, in: *The Genetics and Biology of Drosophila* (Ashburner, M. and E. Novitski, eds.) Academic Press Inc., 1c: 1090-1254.



correlate much with translocation frequency decreasing, although translocations are not considered to be the only reason for F1 male sterility.

As for inversions, the curve for their frequency decreasing during F1-F4 generations demonstrates the only significant change in F2. Figure 1 reflects behaviour of paracentric inversions in a laboratory population, since only one pericentric inversion was observed in experiments. The analysis of F10 showed the absence of inversion frequency changes. Apparently, the contribution of paracentric inversions to "genetic load" of laboratory populations was not very significant for selective pressure against their carriers.

References: Ashburner, M. 1989, *Drosophila. A Laboratory Handbook*, Cold

**van 't Land, Jan, Albert Kamping and Wilke van Delden.** Department of Population Genetics, University of Groningen, P.O. Box 14, 9750 AA Haren, the Netherlands. Differences in some fitness parameters among two geographically distinct populations of *Drosophila melanogaster*.

1977; and see Parsons, 1983, and Lemeunier *et al.*, 1986, for reviews). In this study we compared 50 iso-female lines from Panama (code: Pan, see van 't Land *et al.*, 1993, for more details) and 32 iso-female lines derived from a population from the Netherlands (code: D83, described by Oudman *et al.*, 1991) for development time, viability, wing length and thorax length. Females of each line were allowed to lay eggs during 4 hours on dishes with fresh standard medium. Eggs were transferred to vials containing 8 ml fresh standard medium, 75 eggs per vial. For each line, 3 replicate vials were incubated at 2 temperatures: 25°C and 29°C.

Development time was measured for males and females by checking each vial, every 4 hours (29°C) or every 6 hours (25°C) for adult flies. Mean development time was calculated for each vial by taking the weighted average of the mid-interval times of the individual flies.

Table 1. Mean values (s.e. between parentheses) for some fitness characters of flies from different geographic origins.

		Panama			The Netherlands			N	
		25°C	29°C	Overall	N	25°C	29°C		
Thorax length (mm * 100)	♂ <sup>†</sup>	87.49 (0.18)	84.21 (0.21)	85.88 (0.14)	1395*	88.30 (0.19)	84.71 (0.22)	86.26 (0.16)	1112*
Wing length (mm * 100)	♂ <sup>†</sup>	123.05 (0.21)	114.23 (0.21)	118.70 (0.19)	1395*	130.22 (0.23)	120.78 (0.28)	124.85 (0.23)	1112*
Develop. time (hrs.)	♀	214.09 (0.77)	192.30 (0.83)	203.35 (0.86)	286 <sup>‡</sup>	218.42 (0.84)	204.64 (1.17)	211.53 (0.87)	192 <sup>‡</sup>
	♂ <sup>†</sup>	221.24 (0.83)	200.12 (0.94)	210.87 (0.88)	287 <sup>‡</sup>	223.63 (0.79)	209.42 (1.11)	216.53 (0.85)	192 <sup>‡</sup>
Egg-Pupae Viability (%)	♀ + ♂ <sup>†</sup>	54.25 (1.64)	61.80 (1.69)	57.95 (1.20)	292 <sup>‡</sup>	59.44 (1.79)	65.07 (1.62)	62.26 (1.22)	192 <sup>‡</sup>
Egg-Adult Viability (%)	♀ + ♂ <sup>†</sup>	49.13 (1.57)	54.27 (1.59)	51.65 (1.13)	292 <sup>‡</sup>	55.78 (1.83)	51.97 (1.90)	53.88 (1.32)	192 <sup>‡</sup>

\* Number of individual measured flies; <sup>‡</sup> Number of vials

The number of pupae and the number of eclosed adult flies divided by the amount of eggs ( $N = 75$ ) resulted in egg-pupae viability and egg-adult viability, respectively. Viabilities were angular transformed before statistical analysis.

Wing length (anterior crossvein to wingtip) and thorax length of 5 males per vial were measured with a Reflex stereo microscope ( $d = 0.02$  mm). Statistics have been performed on ln-transformed data.

Table 1 gives a summary of the observed data. At both temperatures flies from Panama developed significantly faster than Dutch flies (25°C, males:  $p = 0.049$ ; females:  $p < 0.001$ ; 29°C, males:  $p < 0.001$ ; females:  $p < 0.001$ ), and although both populations developed faster at 29°C (Pan/D83 males/females:  $p < 0.001$ ), Pan flies reacted more to the higher temperature (by developing even faster, ANOVA,  $F(\text{popul} \times \text{temp}) = 32.54$ ,  $p < 0.001$ ).

We observed no overall significant difference in thorax length between the populations ( $t = 1.81$ ,  $p = 0.07$ ). Only at 25°C thoraxes of Pan males were smaller than the thoraxes of the D83 males ( $t = 3.16$ ,  $p = 0.002$ ). However, wings of the Pan males were significantly shorter at both temperatures and overall (latter:  $t = 20.38$ ,  $p < 0.001$ ) compared to the wings of the D83 males. Both populations had shorter wings and shorter thoraxes at higher temperatures.

Overall egg-adult viabilities were not significantly different between Pan and D83 (ANOVA,  $F(\text{popul-effect}) = 2.34$ ,  $p = 0.127$ ). However, the populations reacted differently on the two temperatures (ANOVA,  $F(\text{popul} \times \text{temp}) = 6.32$ ,  $p = 0.012$ ): Pan flies showed a higher egg-adult survival at 29°C compared to 25°C ( $t = -2.56$ ,  $p = 0.011$ ), but there was no significant difference between temperatures for D83 flies ( $p = 0.17$ ). As egg-pupae survival did not show such an opposite reaction to higher temperature (at both temperatures, D83 survived slightly [but not significantly] better than Pan), and both survived significantly better at 29°C (Pan:  $p < 0.001$ ; D83:  $p = 0.017$ ), it can be concluded that the D83 population has a higher pupal mortality at 29°C.

Geographic variation for life-history and morphological characters among populations of *D. melanogaster* has been recognized for some time, although research has been carried out mainly on African, North-American and Eurasian populations. In general, fitness traits such as wing length, thorax length, body weight, development time and viability are found to be larger among temperate populations (David *et al.*, 1977;

and see Parsons, 1983, and Lemeunier *et al.*, 1986, for reviews). In this study we compared 50 iso-female lines from Panama (code: Pan, see van 't Land *et al.*, 1993, for more details) and 32 iso-female lines derived from a population from the Netherlands (code: D83, described by Oudman *et al.*, 1991) for development time, viability, wing length and thorax length. Females of each line were allowed to lay eggs during 4 hours on dishes with fresh standard medium. Eggs were transferred to vials containing 8 ml fresh standard medium, 75 eggs per vial. For each line, 3 replicate vials were incubated at 2 temperatures: 25°C and 29°C.

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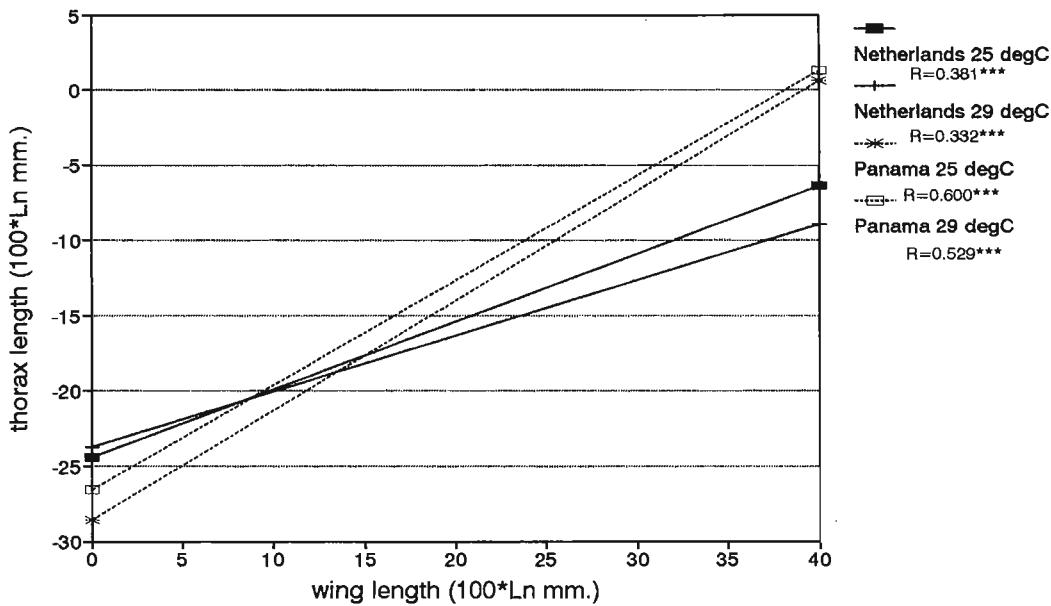


Figure 1. Correlation between wing length and thorax length of males from a Panamanian and a Dutch population, raised at two different temperatures: 25°C and 29°C. Due to the large amount of datapoints, only regression lines are shown. \*\*\* p < 0.001

Another interesting result is the difference between the two populations in correlation between wing length and thorax length (Figure 1), (ANCOVA,  $F(\text{popul}) = 94.92$ ,  $p < 0.0001$ ). This difference is independent of temperature, and confirms the results presented in Table 1: Pan flies have shorter wings but similar thorax lengths. The correlations themselves are rather low (see r-values in legend of Figure 1), but are significant for all four population/temperature groups.

It can be concluded that flies from Panama are, as expected, more adapted to a higher temperature because of lower pupal mortality and shorter development time than the Dutch population. The two morphological traits (i.e., wing length and thorax length) are supposed to be only slightly genetically correlated (David *et al.*, 1977) and indeed we find low (but significant!) correlations. The populations differ only for wing length: Pan flies have shorter wings, in accordance with Bergman's rule which states that morphological characters increase in size with increasing latitude. Stalker (1980) observed a larger wing-load index for flies adapted to high air-temperature, which means that they have similar body weights but smaller and more rapidly beating wings. As wing beat frequency was not measured in this study, this hypothesis could not be tested.

References: David, J.R., C. Bocquet and M. de Scheemaeker-Louis 1977, Genet. Res. 30:247-255; van 't Land, J., W. van Delden and A. Kamping 1993, Dros. Inf. Serv. 72:102-104; Lemeunier, F., J.R. David, L. Tsacas and M. Ashburner 1986, In: *The Genetics and Biology of Drosophila*, (eds. M. Ashburner, H.L. Carson and J.N. Thompson jr.) vol. 3e:147-256, Academic Press, London; Oudman, L., W. van Delden and A. Kamping 1991, Heredity 67:103-115; Parsons, P.A., 1983, *The Evolutionary Biology of Colonizing Species*, Cambridge Univ. Press, London and New York; Stalker, H.D., 1980, Genetics 95:211-223.

**Catchpole, R.D.J.** Drosophila Population Biology Unit, University of Leeds, Leeds, LS2 9JT, U.K. Wing length is not the best predictor of body size.

The use of wing length as an indicator of body size has generally been widely accepted for drosophilids (Sokoloff, 1965). In most circumstances the assumption that length is the best predictor of mass is often made without any quantitative support (e.g., Monclus and Prevosti, 1970).

One of the few studies to examine the relationship was undertaken by Sokoloff (1965) who found that there was in fact a significant correlation between wing length and body mass for *Drosophila persimilis* and *D. pseudoobscura*. Although the author also examined both wing width and thorax length no direct comparisons were made between the various measures. In the following note such a comparison will be made for *D. melanogaster* and *D.*

*virilis*.

Twenty adult male and female flies were randomly selected from large populations of approximately five hundred individuals. After collection the samples were frozen and then dried until a constant weight was achieved. During measurement all individuals were kept in a sealed container with a desiccant until weighing had occurred. Once mass had been determined the following morphometric measurements were made using an ocular micrometer: scutellum length, femur length, wing length, wing width and wing area (length\*width). The exact wing dimensions which were taken are indicated in Figure 1.

Table 1.

<i>D. melanogaster</i> females			
Variable	Statistic	Sample size	Significance level
Kendall			
scutellum length	0.182	20	0.031
wing length	0.374	20	0.024
<u>wing width</u>	0.466	20	0.006
Spearman			
femur length	0.444	20	0.050
wing length	0.475	20	0.035
<u>wing width</u>	0.648	20	0.002
wing area	0.464	20	0.040

Table 2.

<i>D. melanogaster</i> males			
Variable	Statistic	Sample size	Significance level
Kendall			
femur length	0.517	20	0.002
scutellum length	0.404	20	0.023
wing length	0.442	20	0.008
wing area	0.426	20	0.010
Spearman			
<u>femur length</u>	0.677	20	0.001
scutellum length	0.520	20	0.019
wing width	0.578	20	0.008
wing area	0.558	20	0.011

Table 3.

<i>D. virilis</i> males			
Variable	Statistic	Sample size	Significance level
Kendall			
femur length	0.337	19	0.049
scutellum length	0.511	19	0.004
wing length	0.577	19	0.001
wing width	0.596	19	0.001
<u>wing area</u>	0.625	19	< 0.001
Spearman			
femur length	0.665	19	0.002
wing length	0.741	19	< 0.001
wing width	0.737	19	< 0.001
wing area	0.796	19	< 0.001

Table 4.

<i>D. virilis</i> females			
Variable	Statistic	Sample size	Significance level
Pearson			
wing length	0.526	20	0.017
wing width	0.524	20	0.018
<u>wing area</u>	0.558	20	0.010

N.B. the most significant variables are underlined

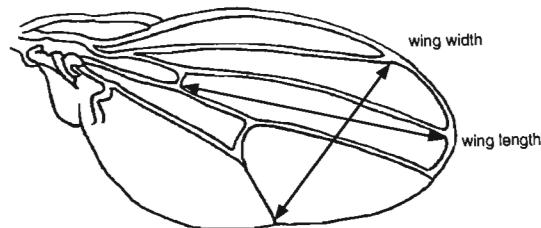


Figure 1. Wing dimension measurements (redrawn from Robertson and Reeve, 1952).

It was necessary to analyse the data using non-parametric methods as most of the variables were not normally distributed and could not be transformed effectively. Simple correlation matrices were computed for both males and females of each species using Kendall's and Spearman's rank correlation coefficients. All significant results are given in Tables 1-3. No significant results were obtained for *D. virilis* females. In consequence, Pearson's correlation coefficient was also calculated; the significant values given in Table 4 are valid as these particular variables were normally distributed.

As can be seen from Tables 3 and 4, the best predictor of mass for both male and female *D. virilis* is wing area. All wing variables in this case show little differentiation in terms of significance. In contrast the mass of *D. melanogaster* males is most significantly predicted by femur length, while for females, wing width is the most significant variable. Interestingly, wing length is the next most significant variable for *D. melanogaster* males. Such a width/length difference may reflect a greater wing loading in females which is clearly not present in *D. virilis*. This is not surprising as body size is not as dimorphic in *D. virilis* as it is in *D. melanogaster*.

In general terms, the results would appear to suggest that it may not be acceptable to assume that wing length is always the best indicator of body size; not only between species but also between sexes. It is conceivable that in some cases its use could be unacceptable, not only as the best predictor, but as any sort of predictor.

References: Monclus M. and A. Prevosti 1970, Evolution 25:214-217; Robertson F.W. and E. Reeve 1952, Journal of Genetics 50:416; Sokoloff A., 1965, Evolution 20:49-71.

**Shorrocks, B., C. Dytham and K. Dooher.** Drosophila Population Biology Unit, Department of Pure and Applied Biology, University of Leeds, Leeds, LS2 9JT, UK. Three-species competition between domestic species of *Drosophila*.

under conditions which approximate those experienced in a 'wild' situation, such as a fruit market (Atkinson and Shorrocks, 1977; Rosewell, 1989; McNamee, 1994) or vineyard (Capy, *et al.*, 1987). Grapes were chosen as food since they represent discrete patches of resource which do not dry out readily and can support quite large numbers of *Drosophila*. They provide a system where the dynamics of competition between species on a divided and ephemeral resource can be studied (Shorrocks, Atkinson and Charlesworth, 1979; Atkinson and Shorrocks, 1981).

Four microcosms were set up, each with 100 individuals of three species: *D. immigrans*, *D. hydei* and *D. virilis*. In Yorkshire, England, these three species are found in breweries (Newbury, *et al.*, 1984) and *D. immigrans* and *D. hydei* are found in fruit markets (Atkinson and Shorrocks, 1977; Rosewell, 1989; McNamee, 1994). The results are shown in Figure 1. Only the proportions for *D. hydei* and *D. virilis* are shown, those for *D. immigrans* can be obtained by subtraction from one. Multi-species populations that are dominated by *D. immigrans* will approach zero. Populations that reach the diagonal line have lost *D. immigrans*. Those dominated by either of the other two species will approach 1.

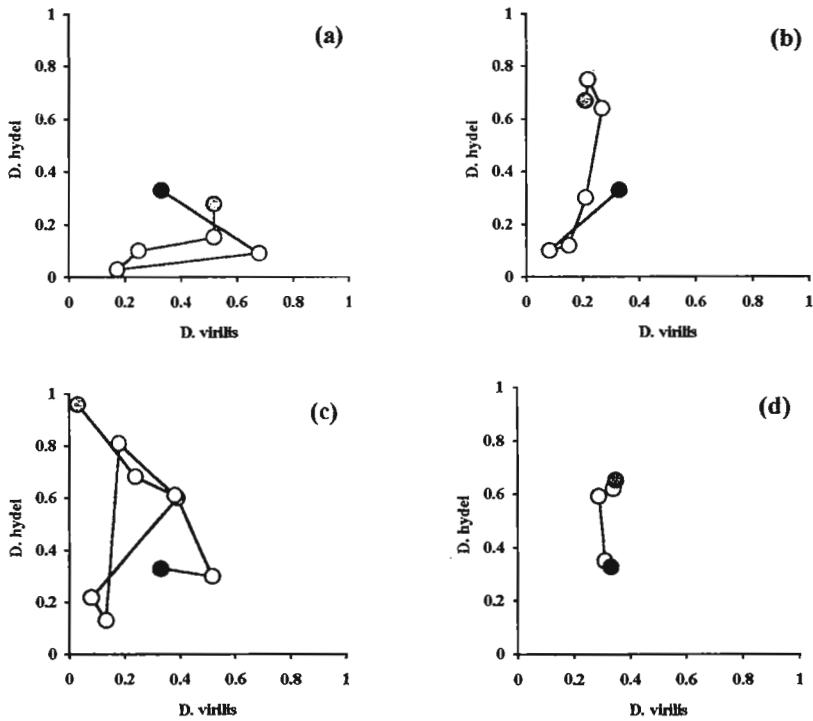


Figure 1. Frequencies of *D. hydei* and *D. virilis* (and by subtraction *D. immigrans*) in four, three-species competition experiments, conducted in microcosms. The black circle marks the start of the trajectory, the grey circle marks the end. The diagonal line represents the upper limit of possible frequencies.

Although these are large systems containing over 1000 resource patches and maintaining populations of several thousand flies, population trajectories wander extensively. Three populations initially moved towards zero origin of the graphs (dominance by *D. immigrans*). This species has the fastest developmental time and therefore the emergence of the early generations dominates overall frequencies. Both *D. hydei* and *D. virilis* have a slower response time, but gradually emerge as the more successful competitors. Three population trajectories ended in the region dominated by *D. hydei*. These preliminary experiments emphasize the need for population studies in large systems that effectively mimic natural habitats.

References: Atkinson and Shorrocks 1977, Oecologia 29:223-232; Atkinson and Shorrocks 1981, J. Anim. Ecol. 50:461-471; Capy *et al.* 1987, Acta Oecologia 8:435-471; Dytham, *et al.* 1992, Dros. Inf. Serv. 71:253; McNamee 1994,

PhD Thesis, University of Leeds; Newbury *et al.* 1984, Biol. J. Linn. Soc.; Rosewell 1989, in *Living in a Patchy Environment* (B.Shorrocks and I. Swingland, eds.) 63-74, OUP; Shorrocks, Atkinson and Charlesworth 1979, J. Anim. Ecol. 48:899-908.

**Shorrocks, B. and J. Miller.** Drosophila Population Biology Unit, Department of Pure and Applied Biology, University of Leeds, LS2 9JT, UK. A comparison of species abundance in ecologically "marginal" and ecologically "central" populations of *Drosophila*.

northwest of Leeds, in North Yorkshire, were both woodlands (Highfolds Scar: SE 895672 and Malham Fen: SE 882669) were between 380 m and 400 m above sea level. Malham Tarn, in the Pennine Hills, has more severe weather than Leeds, with longer winters and generally lower temperatures (Manley, 1956). In each geographical area one woodland was dominated by larch (*Larix decidua*) (LF and MF) and one was mixed deciduous (AD and HS). The survey was carried out between June 1977 and September 1978, and used "bottle-traps" with four different types of bait (Basden, 1954; Shorrocks, 1975). The four baits were: fermenting banana, fermenting apple, rotting tomato and fermenting malt. The three fruit baits were prepared by cutting fresh fruit into 1 cm cubes and placing them in a sealed container one week before use. The malt bait was made using the recipe of Lakovaara (1969), on the day before traps were placed in the wood. At each wood, traps were placed at two sites, and at each site, four traps (the four baits) were placed at ground level and four traps (the four baits) at 4-5 m in the tree canopy. Traps were left out for two weeks and then returned to the laboratory where all captures were removed. The results of the trapping are shown in Table 1.

Baited traps of the type used here are a convenient way of continuously sampling *Drosophila* from an area. The technique provides a relative estimate of population size but is sensitive to the activity of the flies and the type of bait used. The use of four baits rather than one helps to negate the second problem while activity (on a trapping scale of 14 days) is largely influenced by local temperature. Although on a yearly basis Malham is colder on average than Leeds, during the summer and autumn period when most of the flies were caught temperatures were similar. It is unlikely therefore that the greater number of flies in the two Leeds woodlands is due to a temperature/activity difference between the two geographical areas. There are also species differences that probably reflect real differences in abundance between the ecological "margins" and "centres". *Drosophila subobscura* ( $F_{3,132} = 9.41$ ), *D. obscura* ( $F_{3,132} = 11.55$ ) and *D. subsilvestris* ( $F_{3,132} = 4.08$ ) all show significantly higher numbers at the Leeds woods, while *D. tristis* shows the opposite effect, which is not however significant ( $F_{3,132} = 1.06$ ). All ANOVA's were carried out on log transformed numbers. All the first three species show the same rank order of woods, which is AD > LF > HS > MF.

References: Basden, 1954, Trans. R. Soc. Edin. 62:603-654; Lakovaara, 1969, Dros. Inf. Serv. :128; Manly, 1956, Annual Report of the Council for the Promotion of field Studies 56:43-56; Shorrocks, 1975, J. Anim. Ecol. 44:851-864.

Two areas were examined in this survey, and in each area flies were trapped in two woodlands. The ecologically "central" area was 10 miles north of Leeds, in West Yorkshire, where both woodlands (Adel Dam: SE 274412 and Lofthouse Farm: SE 332432) were between 100 m and 130 m above sea level. The ecologically "marginal" area was Malham Tarn, 43 miles

Table 1. Numbers of *Drosophila* species collected in woodlands, at baited traps, in two ecologically 'central' and two ecologically 'marginal' areas of northern England

Species	sex	Adel Dam	Lofthouse Farm	Highfolds Scar	Malham Fen	Total
<i>D. subobscura</i>	female	944	802	220	376	2342
	male	458	395	146	205	1204
<i>D. obscura</i>	female	511	419	71	45	1046
	male	367	241	3	23	668
<i>D. tristis</i>	female	23	18	51	25	117
	male	11	9	41	24	86
<i>D. subsilvestris</i>	female	32	28	10	5	75
	male	33	10	7	6	56
<i>D. ambigua</i>	female	3	3	9	0	15
	male	0	0	7	0	7
<i>D. phalerata</i>	female	9	15	4	4	32
	male	0	10	3	3	16
<i>D. deflexa</i>	female	3	0	1	0	4
	male	1	9	0	3	13
<i>D. confusa</i>	female	2	5	6	5	18
	male	0	6	4	5	15
<i>D. funebris</i>	female	0	3	0	0	3
	male	1	3	0	0	4
<i>D. melanogaster</i>	female	18	1	0	3	22
	male	15	5	1	2	23
<i>D. cameraria</i>	female	2	1	0	0	3
	male	1	0	0	0	1
<i>D. immigrans</i>	female	1	0	0	0	1
	male	0	0	0	1	1
Total		2435	1983	618	735	5771

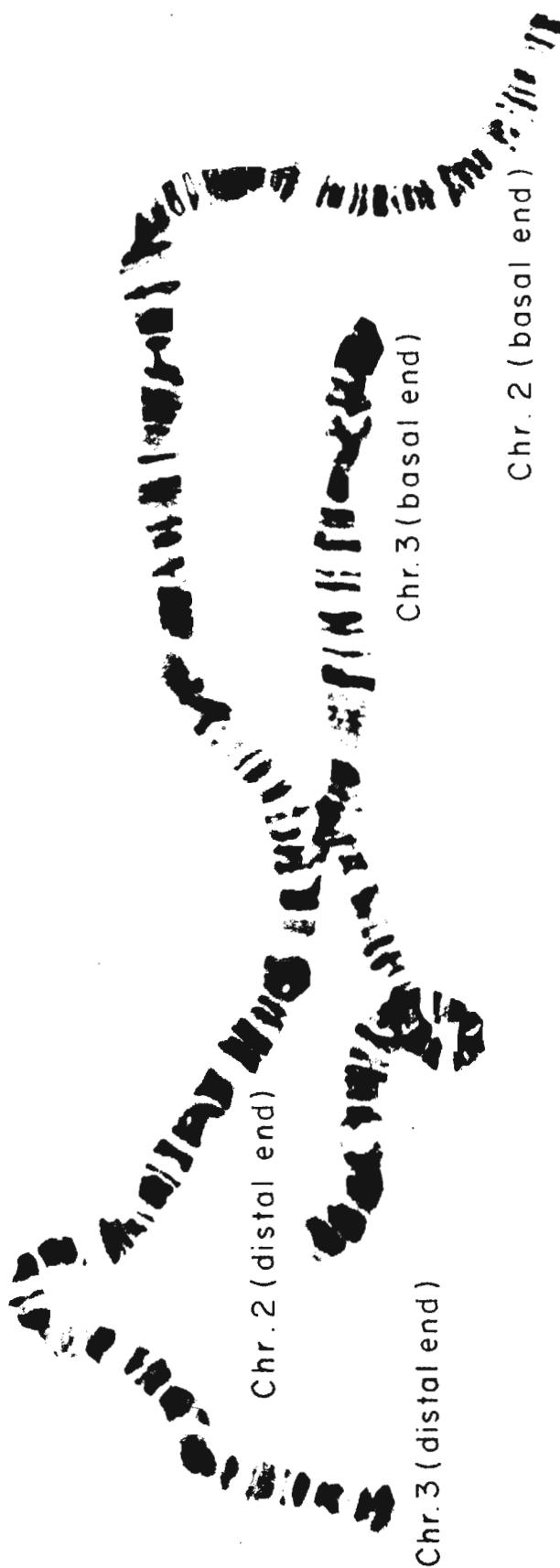
**Brncic, D. and G. Manríquez** Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Independencia 1027, Correo (7), Santiago - Chile. Natural occurrence of reciprocal translocations in *Drosophila pavani*.

The *mesophragnatica* group of species of the genus *Drosophila* comprises a cluster of Neotropical species that form a phyletic unit (Brncic, 1957). Eight of the species: *D. mesophragnatica*, *D. pavani*, *D. gaucha*, *D. gasici*, *D. altiplanica*, *D. viracochi*, *D. orkui* and *D. brncici* have been deeply studied regarding the chromosomal structure of their populations (Brncic, 1973). Brncic *et al.* (1957, 1971) established the phylogenetic relationships of six of the species based mainly on chromosomal comparisons. As a main conclusion that can be drawn from the work done is that, with the exception of small changes in the heterochromatin content, all the observed chromosomal differences could be attributed to paracentric inversions. In this respect the *mesophragnatica* group follows the same tendency observed in other phyletic groups of the genus. Paracentric inversions have been the most common changes observed when related species have been compared, although changes related with the heterochromatin, Robertsonian fusion/fission changes and translocations have been occasionally observed. For instance in *D. pavani* whose populations have been profusely analysed with regard to their chromosomal polymorphisms in the last 40 years), some reciprocal translocations very seldom appeared in the offspring of females collected in nature. The translocation heterozygote between chromosomes 2 and 3 (see photomicrograph) has been observed in the offspring of one female collected over rotten parts of the columnar cactus *E. chilensis* in the locality of Til-Til in Central Chile.

This finding is of importance because it indicates that, although there are no indications of translocations in the phylogeny of the group, changes at this level are possible.

Acknowledgments: Partially supported by FONDECYT grant No. 1930769.

References: Brncic, D. 1957, Las especies Chilenas de Drosophilidae, Mongr. Biol. U. de Chile, Stgo.; Brncic, D. 1973, J. Heredity 64: 175-180; Brncic, D. and S. Koref-Santibáñez 1957, Evolution 11: 300-310; Brncic, D., P.S. Nair and M.R. Wheeler 1971, Studies in Genetics VI. Univ. Texas Publ. 7103: 1-16.



**Bingley, M. and B. Shorrocks.** Drosophila Population Biology Unit, Department of Pure and Applied Biology, University of Leeds, Leeds, LS2 9JT, UK. A survey of fungal-breeding *Drosophila* using *Agaricus bisporus* as "bait".

and Jaenike, 1984; Jaenike and James, 1991; Kearney, 1979; Rocha Pite and Brandao Ribeiro, 1985; Shorrocks and Charlesworth, 1980, 1982; Shorrocks and Wood, 1973). Although surveys involving these natural substrates are essential, they do present problems since they effectively involve a collection programme using a variety of baits (the fungi) at different times and places. This makes comparative studies and examination of other environmental factors difficult. It is also difficult to carry out manipulative experiments (Hairston, 1989), in the field and the laboratory, using naturally occurring fungi, since the required types are not always available in sufficient numbers. Recently, we have successfully carried out manipulative experiments in the laboratory (Shorrocks and Bingley, 1994) using the domestic mushroom (*Agaricus bisporus* forma *albida* J.Lge). The survey reported here uses this same mushroom to "collect" wild flies, partly to support the "naturalness" of this successful laboratory system and partly to explore its potential as a more standard field bait for ovipositing females.

The 12 month survey was carried out in Adel Dam, a small ( $0.075 \text{ km}^2$ ) nature reserve situated 8 km north of Leeds (OS map reference SE 274413) (Shorrocks, 1975). Two sampling sites were selected from within the woodland. Whole mushrooms (*A. bisporus*) were exposed on plastic seed trays divided into 12 compartments. Each compartment contained an upright pin, onto which the stipe of a single mushroom was impaled. Surrounding the pin, and the stipe, was a layer of damp sand. This damp sand not only provided support for the upright mushroom, along with the pin, but also provided a source of water, allowing the mushroom to expand, mature and rot once in the field. At the end of each week, the trays were brought back to an outdoor insectary, with all the mushrooms covered with a plastic pot that allowed air to enter but not insects. Mushrooms were checked for emerging flies every day for two months. Over the winter period, when pupae of some species may have been diapausing, trays were kept for six months. The survey was started on the 31<sup>st</sup> May 1989 and ended on the 13<sup>th</sup> June 1990.

In total, 39,970 individuals were reared and identified in the survey. The total numbers of *Drosophila* collected are shown in Table 1. These emergence data from experimental sites show many similarities to data obtained from natural sites. In the survey of Shorrocks and Charlesworth (1980), five *Drosophila* species were reared from fungi (126 species) collected in the field. These were the four species collected here, plus *D. transversa* Fallen. However, this latter species was not very common in their collections (1.65% of all *Drosophila*) and was not reared from Adel Dam. In both surveys, *D. phalerata* Meigen and *D. camheraria* Haliday are the numerically dominant species emerging, 99% in the present collections and 94% in those from natural fungi, from Adel Dam (Shorrocks and Charlesworth, 1980).

Like most drosophilids, spatial variation in numbers (between different breeding sites) shows intense aggregation for fungal-breeding species (Jaenike and James, 1991; Rosewell *et al.*, 1990). This over-dispersion is summarised, in Table 1, using the exponent *k* of the negative binomial distribution. The values in Table 1 correspond very well with those from natural breeding sites given by Rosewell *et al.* (1990), with most values much less than *k* = 0.5.

For drosophilids, including the fungal breeding species (Jaenike and James, 1991; Rosewell *et al.*, 1990), the degree of aggregation is density-dependent and conveniently summarised using the power law relationship  $s^2 = am^b$ , where *m* and *s*<sup>2</sup> are the mean and variance over patches, and *a* and *b* are parameters estimated from the linear regression of the logarithm of variance on the logarithm of the mean (Taylor, 1961). The values of *a* and *b*, and therefore the subsequent power law relationships, obtained from these experimental collections are very similar to those obtained by rearing flies from natural fungi (Jaenike and James, 1991; Rosewell *et al.*, 1990).

For *Drosophila* species, therefore, we can conclude that these experimental breeding sites mimic natural fungal sites very well. This is the case for both general species composition and spatial variation in numbers. Such an experimental system will therefore prove invaluable for long-term comparative studies on both species diversity and population dynamics.

References: Burla and Bachli 1968, Vierteljahrsschrift der Naturforschenden Gesellschaft in Zurich 113:311-336; Driessen *et al.*, 1990, Nether. J. Zool. 40:409-427; Grimaldi and Jaenike 1984, Ecology 65:1113-1120; Hairston 1989,

The fungal breeding *Drosophila* offer particularly exciting possibilities for evolutionary and ecological research since their breeding sites are small, discrete and easily delimited, and there have been a number of surveys that looked at the distribution and abundance of these species by collecting naturally occurring fungi (Burla and Bachli, 1968; Driessen *et al.*, 1990; Grimaldi

Table 1. Numbers of fungal-breeding *Drosophila* reared from *Agaricus bisporus* placed at Adel Dam, Yorkshire, England. *a* and *b* are parameters in the Power Law relationship between variance and mean. *k* is the exponent in the negative binomial distribution.

species	Numbers	<i>b</i> ± SE	<i>a</i>	<i>k</i>
<i>D. camheraria</i>	6177	1.25 ± 0.05	11.87	0.089
<i>D. confusa</i>	12			
<i>D. phalerata</i>	2450	1.38 ± 0.07	8.12	0.077
<i>D. subobscura</i>	42	1.50 ± 0.05	10.34	0.009

*Ecological Experiments: Purpose, Design, and Execution*, Cambridge University Press; Jaenike and James 1991, J. Anim. Ecol. 60:913-928; Kearney 1979, PhD thesis, University of Leeds; Rocha Pité and Brandao Ribeiro 1985, Boletim da Sociedade Portuguesa de Entomologia (Suplemento 1, Actas II Congresso Ibérico de Entomologia) 1:189-199; Rosewell *et al.*, 1990, J. Anim. Ecol. 59:977-1001; Shorrocks 1975, J. Anim. Ecol. 44:851-864; Shorrocks and Bingley 1994, J. Anim. Ecol., in press; Shorrocks and Charlesworth 1980, Ecol. Ent. 5:61-78; Shorrocks and Charlesworth 1982, Biol. J. Linn. Soc. 17:307-318; Shorrocks and Wood 1973, J. Nat. Hist. 7:61-78.

**Danielson, P.B. and J.C. Fogleman.** University of Denver, Denver, CO, USA. Inhibition of cytochrome P450 enzymes by eye pigments in *D. mojavensis*.

For many years the focus for many years has been on the metabolism of xenobiotics, especially insecticides (Agosin, 1985; Hodgson, 1985) and a number of genotoxic compounds (Hallstrom *et al.*, 1983). In lepidopterous larvae, the bulk of P450 enzymatic activity has been localized to the midgut, fat body and Malpighian tubes, and, in orthoptera, the gut and Malpighian tubes show the greatest enzyme activity. Because of their small size, however, the P450 activity levels in different tissues of dipterans have been more difficult to investigate.

Early work on *Musca domestica* seemed to suggest that the P450 activity was greatest in the gut and abdominal tissues. What is not clear is whether the greater activity associated with isolated abdomens is due to the *in vivo* P450 activity of the tissues being assayed or the elimination of some endogenous inhibitor. The abundant eye pigment, xanthommatin, is a known endogenous inhibitor of P450 activity in a number of insect species (Wilkinson and Brattsten, 1972) including *Drosophila* (Ryall and Howells, 1974). As a potent electron acceptor that is readily reduced by NADPH cytochrome P450 reductase, it has been suggested that the pigment may act to divert electrons away from cytochrome P450s thereby lowering the apparent P450 content and *in vitro* activity levels.

Table 1. Total cytochrome P450 content (mean  $\pm$  SD) expressed as nmoles P450/mg microsomal protein. Results of one-way ANOVA tests are also given.

Treatment	N	Decapitated	N	Intact	% Decrease	F
None	6	0.33 $\pm$ 0.05	3	0.09 $\pm$ 0.01	72.6	63.5
Saguaro	3	0.33 $\pm$ 0.01	3	0.12 $\pm$ 0.02	60.6	216.6
Senita	4	0.30 $\pm$ 0.03	2	0.15 $\pm$ 0.01	49.0	42.9
PB	3	0.42 $\pm$ 0.03	3	0.22 $\pm$ 0.04	48.5	48.0

one-minute period and then separating heads and appendages (legs and wings) from bodies (thoraces + abdomens) by rapid vibration on a 0.8 mm<sup>2</sup> wire mesh screen. The efficiency of separation of heads from bodies routinely approached 100%. Microsomes were prepared by standard methods (Waters *et al.*, 1983) and were stored in 100mM sodium phosphate buffer containing 40% glycerol at -70°C until used. Total P450 content was determined spectrophotometrically by the method reported in Frank and Fogleman (1992).

Total cytochrome P450 content was determined for both decapitated and intact *D. mojavensis* adults that were either uninduced or treated with cactus isoquinoline alkaloids (from saguaro or senita cactus tissue) or the known P450 inducer, phenobarbital. These alkaloids have been shown to induce both P450 content and *in vitro* activity in larvae of several species of desert *Drosophila* (Frank and Fogleman, 1992). Results are summarized in Table 1. Regardless of whether the flies were uninduced or treated with cactus alkaloids or phenobarbital, there was a significant depression in the measured values of total P450 content when microsomal suspensions prepared from intact adult flies were used in the assay relative to that when isolated bodies were the source of the microsomes. The degree of depression ranged from a low of 48.5% in phenobarbital treated *D. mojavensis* to a high of 72.6% in the uninduced organisms. In spite of the depressed levels of detectable P450 in microsomal suspensions prepared from whole flies, the increase in total P450 content induced by exposure to phenobarbital relative to the uninduced flies was evident for both whole and decapitated flies. The impact of eye pigments on *in vitro* metabolism assays appeared to be even more deleterious in that there was a complete suppression of alkaloid metabolizing P450 activity in microsomal suspensions prepared from intact flies (data not shown). On the basis of the above findings, it is apparent that there is a very significant reduction in the *in vitro* activity and detectability by spectrophotometric methods of P450 enzymes when microsomal fractions are prepared from whole organisms. This appears to be due to the presence of endogenous inhibitors, most likely eye pigments associated with the heads of the intact flies. We cannot overemphasize the importance of eliminating the effect of these endogenous inhibitors either through physical separation of heads prior to

Insects have emerged as an increasingly important group of organisms through which to investigate the multiplicity, substrate specificity and regulation of cytochrome P450 enzymes. While P450s are responsible for the metabolism of a number of endogenous com-

Here, we report a comparison of assayed P450 content using microsomal suspensions prepared from whole versus decapitated adult *D. mojavensis*. This species feeds and breeds in necrotic cactus tissue in the Sonoran Desert of Mexico (see Fogleman and Heed, 1989). Decapitated flies were prepared by freezing 2-5 day post-eclosion adults in liquid nitrogen, repeatedly and vigorously vortexing the frozen organisms over a

homogenization or by dialysis afterwards.

Acknowledgments: This research was supported by NSF Grant BSR-9111430 to J.C.F.

References: Agosin, M. 1985, in: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 12 (G.A. Kerkut and L.I. Gilbert, eds.) Pergamon Press, Oxford; Fogleman, J.C. and W.B. Heed 1989, in: *Special Biotic Relationships in the Arid Southwest* (J.O. Schmidt, ed.), University of New Mexico Press, Albuquerque; Frank, M.R. and J.C. Fogleman 1992, PNAS 89:11998-12002; Hallstrom, I., A. Blanck, and S. Atuma 1983, Chem. Biol. Interact. 46:39-54; Hodgson, E. 1985, in: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 11 (G.A. Kerkut and L.I. Gilbert, eds.) Pergamon Press, Oxford; Ryall, R.L. and A.J. Howells 1974, Insect Biochem. 4:47-61; Waters, L.C., C.E. Nix and J.L. Epler 1983, Chem. Biol. Interact. 46:55-66; Wilkinson, C.F. and Brattsten, L.B. 1972, Drug Metab. Rev. 1:153-228.

**Bruins, B.G. and G.E.W. Thörig.** University of Utrecht, Department of Evolutionary Genetics, Padualaan 8, 3518 CH Utrecht, The Netherlands. Compounds that interfere with the biosynthesis of porphyrins affect light induced mortality.

oxygen radicals are important for the reduction in survival under illumination (Bruins *et al.*, 1991b; Massie *et al.*, 1993).

Porphyrins and their derivatives are involved in many biocatalysts (e.g., cytochromes, vitamin B12, and prosthetic groups of enzymes). Porphyrins do have photodynamic properties. Exposure to light can result in damage and can be lethal (Docampo and Moreno, 1984). This suggests that alterations in the biosynthesis of porphyrins may affect light sensitivity. However, almost nothing is known about the metabolism of porphyrins in *Drosophila*. Therefore we have investigated the effect of DDC and hemin on survival of *Drosophila melanogaster*. Both components affect the synthesis of porphyrins in mammals. DDC (= diethyl 1,4 dihydro 2,4,6, trimethylpyridine 3,5 dicarboxylate) is an inducer of 5-aminolevulinic acid synthetase, the rate controlling enzyme of the heme pathway (Granick, 1966). Hemin is a repressor of the synthesis of 5-aminolevulinic acid synthetase (Granick, 1966).

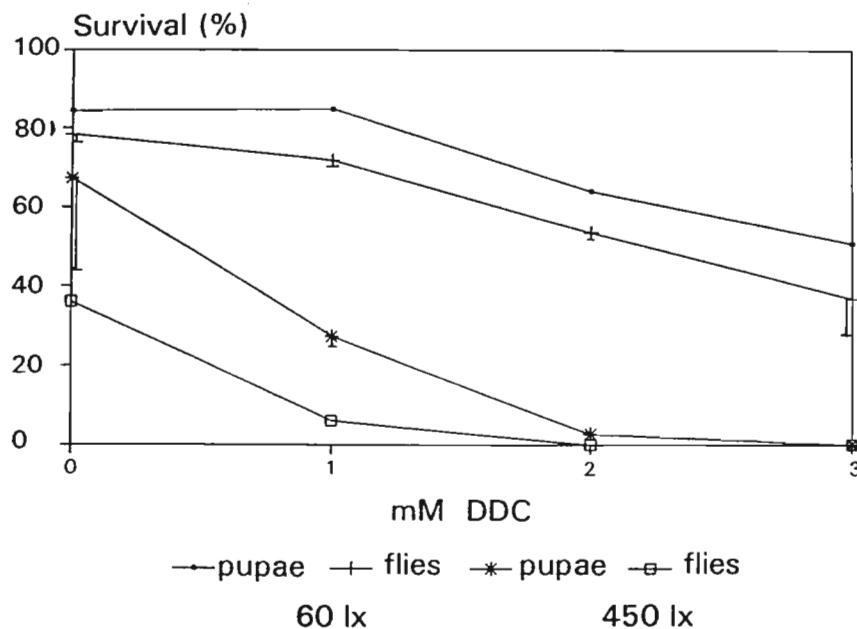


Figure 1. Effect of DDC on survival on 24G (24 g yeast/l and propionic acid) food media under different light intensities. n = 3, n gives number of vials tested at each condition. Interaction between illumination and DDC is significant ( $F = 7.0$  with  $p < 0.005$ ).

Visual light of low intensity reduces survival and retards developmental time of *Drosophila melanogaster* (Bruins *et al.*, 1991a). The noxious effect of light is diet-dependent. Important components which affect survival under illumination are pyridoxine, riboflavin (both are dietary essentials for *Drosophila*) and vitamin C (Bruins *et al.*, 1991b). It has been suggested that

We found that light induced mortality is enhanced on food media supplemented with DDC (Figure 1). The effect of hemin is reported in Figure 2. Under high illumination, survival on 16G (16 g yeast/l) food media is reduced. Survival improved on media supplemented with hemin. Improvement was better according as the hemin concentration increased. Under low illumination hemin scarcely affected survival.

The effect of DDC and hemin on survival suggests that porphyrins are involved in light induced mortality. However, we need more investigations to elucidate the relation between porphyrins and mortality. Nevertheless addition of hemin to the food medium prevents mortality by exposure to illumination, whereas DDC enhances the noxious effect of light.

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

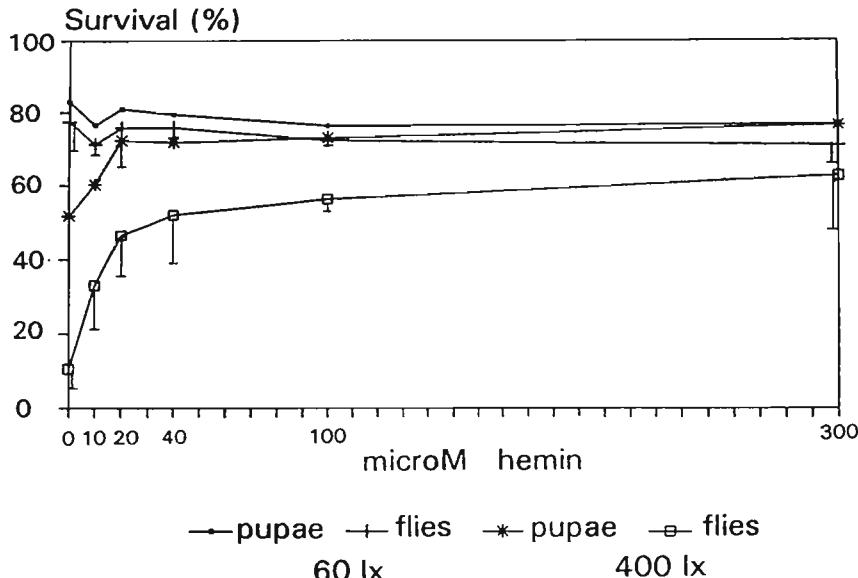


Figure 2. Effect of hemin on survival on 16G under different light intensities.  $n = 4$ ,  $n$  gives number of vials tested at each condition. Interaction between illumination and hemin is significant ( $F = 12.8$  with  $p < 0.001$ ).

Bruins, B.G., W. Scharloo and G.E.W. Thörig 1991b, Insect Biochemistry 21:541-544; Massie H.R., V.R. Aiello and T.R. Williams 1993, Mechanisms of Ageing and Development 68:175-182; Docampo R. and S.N.J. Moreno 1984, In: *Free Radicals in Biology*. Vol. VI. (W.A. Poyov, ed.). Academic Press, London; Granick, S. 1966, JBC 241:1359-1375.

**Bowen, Laura, and J.H. Williamson.** Department of Biology, Davidson College, Davidson, NC, USA. Adriamycin does not inhibit malic enzyme, 6-PGD or isocitrate dehydrogenase from *Drosophila*.

IDH. Since the major cardiac IDH associated with infarction is the NADP-dependent form of this enzyme, we decided to test adriamycin for its effects on several NADP-dependent enzymes using our *Drosophila* model system. In addition, we tested the drug's effects on mitochondrial IDH, an NAD-dependent form of the enzyme.

NADP-malic enzyme, NADP-6-phosphogluconate dehydrogenase and NADP-IDH from the Canton-S strain of *D. melanogaster* were purified 200-400 fold, using affinity chromatographic techniques (Williamson *et al.*, 1980). NAD-IDH from the same strain was purified approximately 100-fold (Fox, 1971). Enzyme activities were monitored in 1 ml reaction mixtures at 30°C by following the reduction of co-enzyme for three minutes (Geer *et al.*, 1979; Williamson *et al.*, 1980).

At concentrations ranging from 2.5 uM to 100 uM, adriamycin had no inhibitory effects on any of the *Drosophila* NADP-enzymes, even when incubations were extended to 60 minutes at 30°C. These observations suggest that adriamycin may have differential effects on activities of *Drosophila* and mammalian NADP-dependent enzymes.

References: Fox, D.J., 1971, Biochem. Genet. 5:69-80; Geer, B.W., *et al.*, 1980, Comp. Biochem. Physiol. 65B:25-34; Krochko, D. and J.H. Williamson 1981, Comp. Biochem. Physiol. 66B:51-57.

**Bruins, B.G. and G.E.W. Thörig.** University of Utrecht, Department of Evolutionary Genetics, Padualaan 8, 3518 CH Utrecht, The Netherlands. Pupation site of *Drosophila melanogaster* affected by illumination, propionic and acetic acids.

Adriamycin is an anthracycline antibiotic used against acute leukemia and many solid neoplasms, although its efficacy is limited due to its cardiotoxicity. This side effect might be a consequence of inhibition of cardiac isocitrate dehydrogenase; Yasumi *et al.*, (1980) described inhibitory effects of adriamycin on rat heart

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(1991). The results are reported in Figure 1. Under high illumination less larvae of both strains pupated on the glass wall, and more larvae pupated on the surface of the food medium (Figure 1). This effect is dependent on the yeast concentration in the food. On high yeast food media (16G) more larvae pupated on the glass wall than on low yeast media.

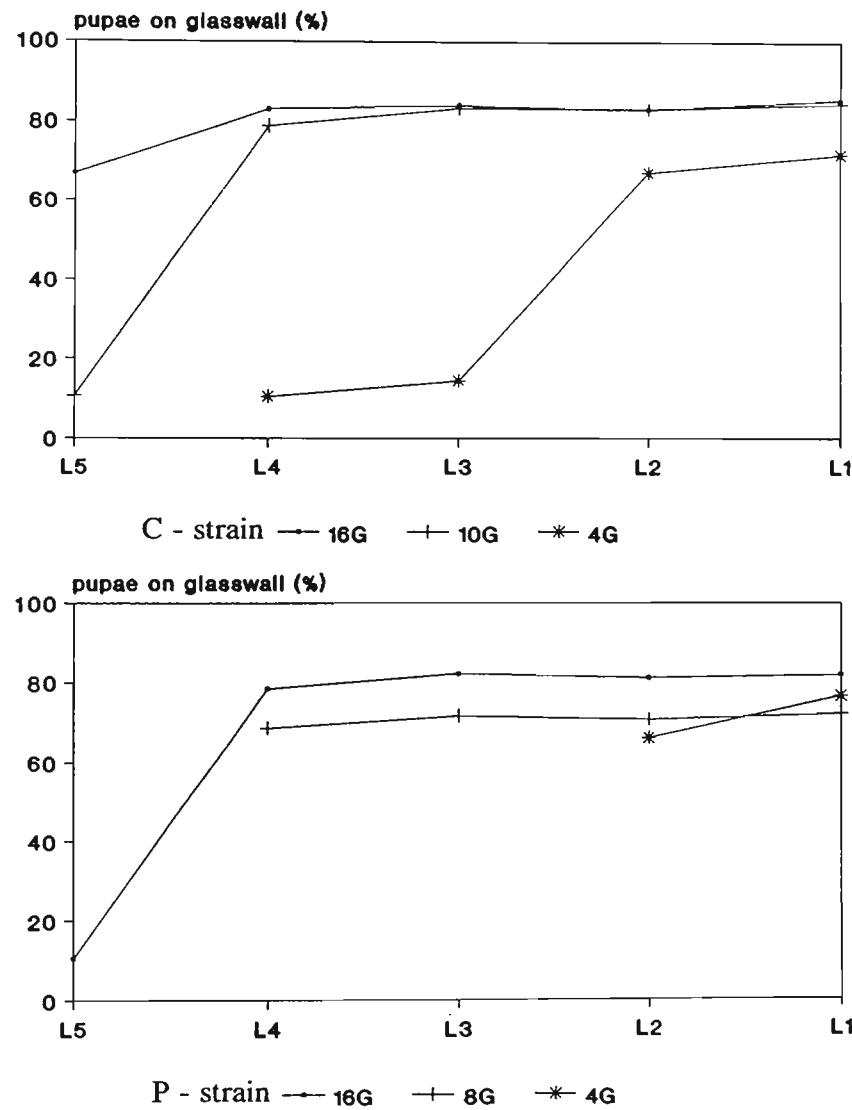


Figure 1. Effect of light intensity on pupation site in two (C and P) strains of *Drosophila melanogaster*. L5 to L1 encompasses a range of light intensities, by which L5 refers to the highest intensity and L1 to the lowest (Bruins *et al.*, 1991).

Under high illumination, 89.5% of the larvae of the P strain and 33.1% of the larvae of the C strain pupated on the surface of the 16G food medium. Under low illumination the differences in pupation site between the C and P strain were insignificant.

In another experiment, we measured pupation height in cm above the surface of the food-medium in 35 ml vials under different light intensities. The results are reported in Figure 2. Pupation sites are affected by acetic and propionic acids. Under high and low illumination, pupation sites on 16G media supplemented with acetic acid are higher than on the unsupplemented 16G diet (Figures 2a and 2b).

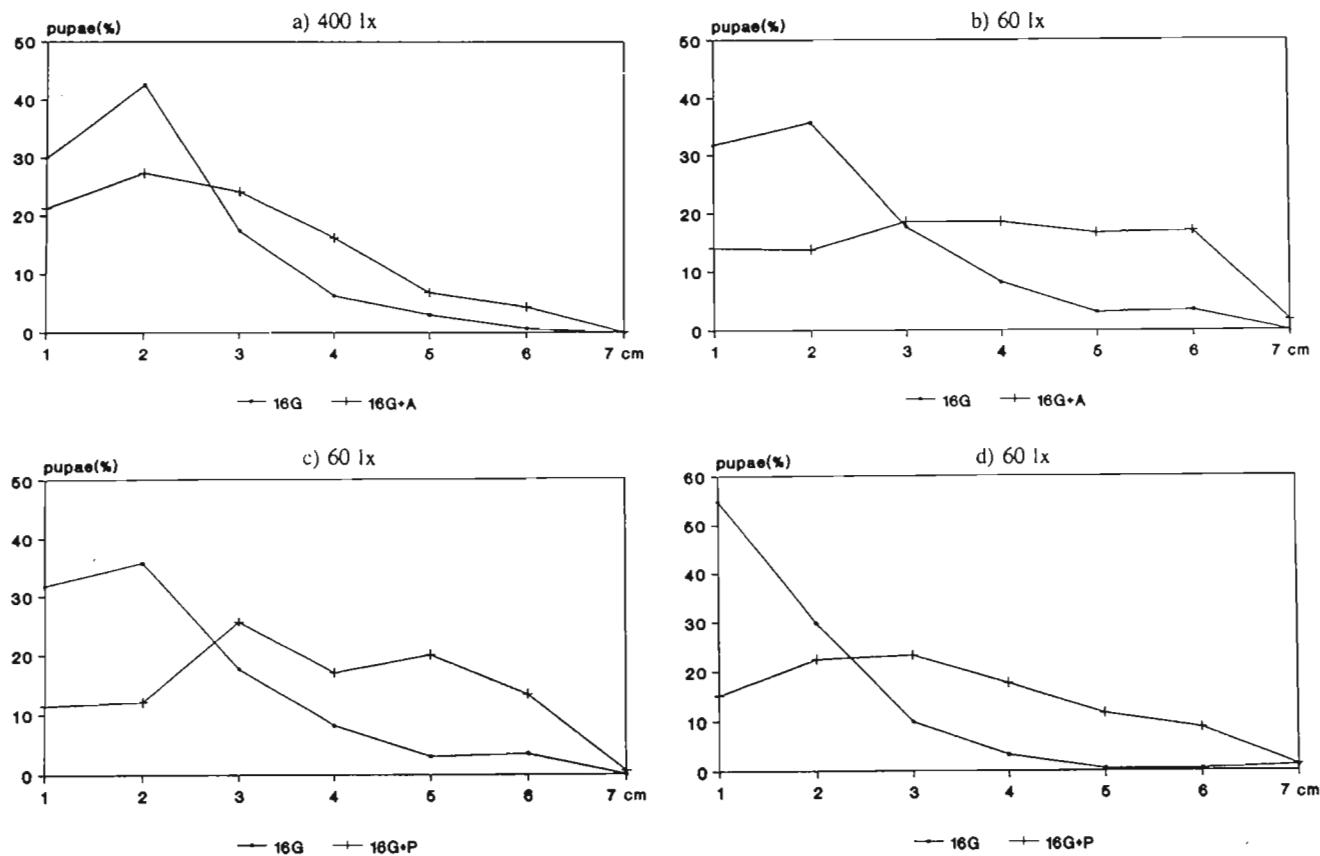


Figure 2. Effect of light intensity, acetic and propionic acids on pupation height in *Drosophila melanogaster*. 1 to 7 cm refers to the height above the surface of the food medium. 16G = 16 g yeast/l; A = 13 ml acetic acid/l; P = 13 ml propionic acid/l. Light sources were Philips 58W/33 fluorescent tubes (in a, b and c, with n = 4, except in 16G + P then n = 3) and 20W halogen lamps (in d, with n = 3, n gives number of vials tested at each condition).

Under low illumination propionic acid showed a similar effect as acetic acid: it also heightened pupation sites (Figure 2c). On the other hand, high illumination (400 lx) lowered pupation sites (Figures 2a and 2b, see also Figure 1).

Pupation site is also dependent on light sources used. With a halogen 20W lamp (60 lx) pupation sites were rather low (Figure 2d). Addition of propionic acid to the food medium heightens pupation sites under identical conditions.

References: Bruins, B.G., W. Scharloo and G.E.W. Thörig 1991, Insect Biochemistry 21:535-539.

**Chang, Christine, and J.H. Williamson.** Department of Biology, Davidson College, Davidson, NC, USA. The effects of steroids on NADP-dependent enzymes of *Drosophila melanogaster*.

Levy, 1970). We surveyed the effects of several steroids on the activities of three NADP-dependent enzymes purified from the Oregon-R strain of *Drosophila*.

*Drosophila* (Oregon-R) G6PD, 6PGD and malic enzyme were purified 200-400 fold using affinity chromatography. Activities of these enzymes were determined by monitoring the reduction of NADP<sup>+</sup> at 340 nm at (Geer *et al.*, 1980; Williamson *et al.*, 1980). Steroids (Steraloids, Inc.) were dissolved in dioxane and all assays, except basic control assays, contained 4 mM steroid and 0.5% dioxane. All activities were recorded as percent of control activity in the presence of dioxane (see Table).

Mammalian 6PGD is inhibited by steroids, while spinach and yeast 6PGDs are not (Marks and Banks, 1960). Rat mammary gland G6PD is inhibited, in a non-competitive manner, by steroids possessing a single polar group, usually a keto group, at the C-17 or C-20 positions of the basic steroid structures (Rainieri and

Inhibitory effects of the 17- and 20- substituted steroids on G6PD activity were relatively small, being no greater than approximately 25%. None of the steroids tested inhibited 6PGD activity. These latter results are consistent with the suggestion of Marks and Banks that non-mammalian 6PGDs are not inhibited by steroids. One steroid had a major effect on malic enzyme activity, reducing the activity to only 16%. (See report of Wegulo).

References: Geer, B.W., et al., 1980, Comp. Biochem Physiol. 65B:25-34; Marks, P.A. and J. Banks 1960, Proc. Natl. Acad. Sci. USA 46:447-452; Raineri, R., and H.R. Levy 1970, Biochem. 9:2233-2243; Williamson, J.H., et al., 1980, Comp. Biochem. Physiol. 65B:339-343.

**Wegulo, Stephen, and J.H. Williamson.** Department of Biology, Davidson College, Davidson, NC, USA. Inhibition of *Drosophila* malic enzyme by 1,3,5(10)-Estratrien-2,3-diol-20-one.

*Drosophila*. Chicken liver malic enzyme was obtained from Sigma. *Drosophila* malic enzyme was purified from the Oregon R strain (Geer et al., 1980; Williamson et al., 1980). Malic enzyme activities were determined by monitoring the reduction of NADP<sup>+</sup> at 340 nm at 30°C for three minutes. Enzymes were incubated for three minutes with steroid prior to initiating the reaction by addition of substrate. Steroids were dissolved in dioxane and all assays, except basic controls, contained 0.05% dioxane. The final concentration of all steroids was 4 mM (see Table).

Compound name	Avian M E	Dros. ME
Control	110	109
Dioxane control	100	100
5a-Androstan-3b, 16a-diol	95	108
5a-Androstan-3b, 16b-diol	100	101
5a-Androstan-3b, 16a-diol-17one	95	104
5a-Androstan-3a-ol-17-one	100	103
1,3,5(10)-Estratrien-3,17a-diol	105	99
1,3,5(10)-Estratrien-3,17b-diol	83	85
1,3,5(10)-Estratrien-2,3-diol-17-one	0	0
5a-Pregnane	91	36
5a-Pregn-3b,17-diol-20-one	92	101

Compound name	G6PD	6PGD	ME
Control	82	110	82
Dioxane control	100	100	100
5a-Androstan-3b, 16a-diol	100	95	82
5a-Androstan-3b, 16b-diol	102	97	91
5a-Androstan-3b, 16a-diol-17one	76	95	94
5a-Androstan-3a-ol-17-one	83	106	82
1,3,5(10)-Estratrien-3,17a-diol	73	93	95
1,3,5(10)-Estratrien-3,17b-diol	74	88	88
1,3,5(10)-Estratrien-2,3-diol-17-one	89	98	16
5a-Pregnane	102	95	88
5a-Pregn-3b,17-diol-20-one	92	91	84
5a-Pregn-3b,20a-diol	84	129	97

In a study of the effects of steroids on activities of NADP<sup>+</sup>-dependent enzymes, Chang and Williamson observed strong inhibition of *Drosophila* malic enzyme by 1,3,5(10)-Estratrien-2,3-diol-17-one.

We compared the effects of several steroids on the activities of malic enzyme from a vertebrate and from *Drosophila*. *Drosophila* malic enzyme was purified from the Oregon R strain (Geer et al., 1980; Williamson et al., 1980). Malic enzyme activities were determined by monitoring the reduction of NADP<sup>+</sup> at 340 nm at 30°C for three minutes. Enzymes were incubated for three minutes with steroid prior to initiating the reaction by addition of substrate. Steroids were dissolved in dioxane and all assays, except basic controls, contained 0.05% dioxane. The final concentration of all steroids was 4 mM (see Table).

Seven of the nine tested steroids had little or no effect on either the avian or *Drosophila* malic enzyme. 1,3,5(10)-Estratrien-2,3-diol-17-one completely inhibited both enzymes and 5a-Pregnane had a major inhibitory effect on the *Drosophila* enzyme while having little or no effect on the avian malic enzyme. Further kinetic analysis revealed that 1,3,5(10)-Estratrien-2,3-diol-17-one inhibits *Drosophila* malic enzyme in a noncompetitive manner.

References: Geer, B.W., et al., 1980, Comp. Biochem Physiol. 65B:25-34; Williamson, J.H., et al., 1980, Comp. Biochem. Physiol. 65B:339-343.

**Srinivasan, Arjun, and J.H. Williamson.** Department of Biology, Davidson College, Davidson, NC, USA. Inhibition of *Drosophila* Glucose-6-Phosphate Dehydrogenase by Palmitoyl Coenzyme A.

The first step in the pentose phosphate pathway and is a major point of regulation of the pathway. Palmitoyl Coenzyme A has been suggested as a potential regulator, since this compound inhibits G6PD activity by enhancing proteolysis of the enzyme (Orstan and Gafni, 1990). *Drosophila* G6PD provides an invertebrate system with which to compare the mammalian system (Williamson, 1986); consequently, we compared the effects of palmitoyl Co-A as an inhibitor of *Drosophila* and bovine G6PD's.

G6PD was purified from the Oregon-R strain of *Drosophila* (G6PD-A) using affinity chromatography (Williamson et al., 1980). Bovine adrenal G6PD was obtained from Sigma Chemical Co. G6PD activities were determined by measuring the reduction of NADP<sup>+</sup> at 340 nm in one ml assays (Williamson et al., 1980).

Palmitoyl Co-A inhibited both forms of G6PD; the *Drosophila* enzyme is more sensitive than the bovine enzyme. In both cases, inhibition was non-competitive, consistent with the hypothesis that palmitoyl Co-A dissociates the multimeric native enzyme into inactive monomers. However, the mechanism of inhibition may be more complex, since addition of the coenzyme, NADP<sup>+</sup>, to the assay solution prior to the addition of palmitoyl Co-A protects the enzyme

The pentose phosphate pathway is a ubiquitous sequence of biochemical reactions generating reducing equivalents in the form of NADPH, as well as ribulose 5-phosphate which can, in turn, be converted into other sugars via the "pentose phosphate carbon shuffle". Glucose 6-phosphate dehydrogenase (G6PD) catalyzes

from denaturation.

References: Orstan, A. and A. Gafni 1990, Biochem. International 21:916-921; Williamson, J.H., 1986, *Drosophila G6PD*, pp 301-312, in *G6PD*, Horrie and Beutler, Academic Press; Williamson, *et al.*, 1980, Comp. Biochem. Physiol. 65B:339-343.

**Waddle, F., C. Moore, L. Hadley, and R. Hildebrand.** Fayetteville State University, Fayetteville, North Carolina. Stabilizer of Segregation Distortion.

produced results similar to those of Miklos in that there appear to be a large number of *SD* enhancers on the right arm of the second chromosome. Contrary to Miklos' claim that *St(SD)* is nothing more than the cumulative effect of many *SD* enhancers, however, we determined that *St(SD)* behaves as a unit entity located between *px* and *bw*. It appears to be at least as strong an enhancer as all the others combined.

In an attempt to map *St(SD)*, we mated *SD c St(SD)/cn px bw* females to *cn px bw* males and recovered 27 brown eyed male progeny. These were mated to *cn bw* females to establish lines for further testing. Each line carried *SD* and was kept by backcrossing heterozygotes to *cn bw* females. A minimum of 5 heterozygous *SD* males from each were tested for *St(SD)*. Five lines produced no *SD<sup>+</sup>* progeny. Three lines each produced one *SD<sup>+</sup>* progeny. Two lines produced one *SD<sup>+</sup>* progeny each from two of five males. Of the remaining 17 lines produced, most males of each line produced at least one *SD<sup>+</sup>* progeny (lowest average K = 0.92).

Two of the 3 lines producing only one *SD<sup>+</sup>* progeny were shown to carry *St(SD)* by testing them with an *SD* suppressing, marked Y chromosome (*y<sup>+</sup> sc Y*, introduced into the lines via *C(1)DX, Yf/y<sup>+</sup> sc Y; cn bw* females). Assumedly the third one does also. Since few suppressor-free *SD ST(SD)* heterozygous males produce *SD<sup>+</sup>* progeny at the test age (less than 4 days) and temperature (between 24° and 25°C), it is unlikely that lines producing more than one such progeny among five males carry *St(SD)*. Thus of the 27 crossovers tested, it is likely that 19 (70%) involved an exchange between the *px* and *St(SD)* loci while the remaining 8 (30%) involved an exchange between the *ST(SD)* and *bw* loci. This places *St(SD)* at approximately map position 2-103.3.

Acknowledgments: Supported by NIGMS Grant #GM08206.

References: Miklos, G.L.G. 1972, Genetics 70:405-418.

**Waddle, F., W. Williams, L. Turner, A. Cutno, B. Harris, K. Kelton, and A. Schenk.** Fayetteville State University, Fayetteville, North Carolina. Third chromosomal suppressors of Segregation Distortion in *Drosophila melanogaster*.

combination of two or more suppressors. Two moderately suppressing loci, here designated *Su(SD)3A* and *Su(SD)3BPi*, have been determined to reside in the left arm on either side of *h* (3-26.5).

In order to map *Su(SD)3A*, we mated *cn bw; ve Su(SD)3A h/+ + +* females to suppressor sensitive *SD bw/cn bw; ve h e* males. *SD*-bearing male progeny with crossovers in the *ve-h* region were testcrossed to *cn bw; ve h e* females to determine which carried *Su(SD)3A*. For males producing ambiguous results (k intermediate between high and low), several sons of the appropriate genotype were retested. Of 122 crossovers in the *ve-h* region, 81 were between *ve* and *Su(SD)3A*. This is 66.4% of the *ve-h* distance which placed *Su(SD)3A* at map position 17.7.

Acknowledgments: Supported by NIGMS Grant #GM08206.

**Parkash, R., J.N. Sharma, and A.K. Munjal.** M.D. University, Rohtak, India. Allozymic variation in some populations of *D. ananassae*.

studied in populations of the South Pacific islands (Johnson, 1971). The present communication deals with the allozymic

By use of the second chromosomal markers, *cn*, *c*, *px*, and *bw*, and by taking advantage of the crossover enhancement properties of nonhomologous chromosomal rearrangements, we have produced many *SD5* and *SD72* recombinant lines. Tests of these lines

Of 87 naturally occurring third chromosomes tested in combination with a suppressor sensitive *SD* chromosome, approximately a third carry suppressors of segregation distortion. Depending on the chromosome tested, the suppression effect ranges from slight to very strong. While the total number of loci has not been determined, strong suppression appears to be due to a

Electrophoretic analysis of genetic structure of diverse continental populations of *D. melanogaster* has helped in elucidating its biogeographical origin and the genetic potential for colonization (Endler, 1986). However, in *D. ananassae*, isozyme polymorphism was

polymorphism in three geographical populations of *D. ananassae* from the Indian subcontinent.

The data on allelic frequencies, heterozygosity values, application of G-test for fit to Hardy-Weinberg expectations at six polymorphic loci in these populations of *D. ananassae* have been represented in Table 1. The heterozygosity levels revealed significant differences and *Adh* and alpha-*Gpdh* loci while all the population samples depicted high heterozygosity values at most of the polymorphic loci. The patterns of deviations from Hardy Weinberg equilibrium were significant at most of the loci among the three populations. Thus, the genetic structure of geographical populations of *D. ananassae* was consistently heterogeneous and could be subjected to natural selection mechanisms.

Table 1. Data on distribution of allelic frequencies, sample size, heterozygosity (obs. / exp.) and G-values for loglikelihood chi square test for fit to Hardy-Weinberg expectations at six polymorphic loci in three geographical populations of *D. ananassae*.

Populations / Latitude	Genetic indices	Est-5	Adh	AO	Odh	$\alpha$ -GPDH	Mdh
Cochin / 9° . 58'N	F	—	—	—	.02	—	—
	F	.22	.49	.59	.72	.75	.13
	S	.78	.51	.35	.26	.25	.82
	S'	—	—	.06	—	—	.05
	N	90	120	128	102	102	80
	Het. o/e	.27 / .34	.23 / .50	.58 / .47	.44 / .43	.21 / .37	.25 / .35
	G-value	4.30*	38.33*	6.16*	2.09	18.90*	7.88*
Nagpur / 21° . 09'N	F	—	—	—	—	—	—
	F	.16	.54	.63	.74	.80	.18
	S	.84	.46	.31	.26	.20	.82
	S'	—	—	.06	—	—	—
	N	88	78	80	95	90	112
	Het. o/e	.22 / .27	.31 / .50	.50 / .50	.53 / .38	.27 / .22	.13 / .30
	G-value	3.52	15.18*	9.54*	18.23*	2.28	20.10*
Dehradun / 30° . 19' N	F	.18	.69	.66	.77	.86	.21
	S	.82	.31	.27	.23	.14	.79
	S'	—	—	.07	—	—	—
	N	100	90	115	105	105	102
	Het. o/e	.20 / .30	.22 / .47	.48 / .49	.46 / .35	.29 / .24	.29 / .33
	G-value	8.74	17.55	33.33*	14.29*	5.01*	.97

\* Significant at 5% level.

The changes in allelic frequencies patterns at *Adh*, alpha-*Gpdh*, *Odh* and *Est-5* loci were significant and indicated latitudinal variations in allelic frequencies. The frequency of *Adh*<sup>F</sup>, alpha-*Gpdh*<sup>F</sup>, *Odh*<sup>F</sup> and *Est-5*<sup>S</sup> positively correlated with the latitude while allele frequency of *Adh*<sup>S</sup>, alpha-*Gpdh*<sup>S</sup>, *Odh*<sup>S</sup> and *Est-5*<sup>F</sup> was found to be negatively correlated with latitude (Table 1). The observed clinal variation at *Adh* and alpha-*Gpdh* loci suggest adaptive changes in allelic frequencies along the climatically variable South-North gradient of the Indian subcontinent. The electrophoretic analysis of these populations revealed clinal varia-

Table 2. Comparison of allelic frequencies at four polymorphic loci in Indian versus South Pacific island populations of *D. ananassae*.

Populations	Adh		Odh			Mdh		Est-C (Est-5)		
	F	S	F	S	**	F	S	F	S	**
1. Mean values of South Pacific island populations*										
A.	0.995	0.005	0.982	0.018	0.002	0.97	0.03	0.735	0.225	0.04
B.	1.0	—	0.98	0.01	0.01	0.97	0.03	0.81	0.16	0.03
2. Indian Populations (mean values of three populations)										
	0.57	0.43	0.74	0.25	0.01	0.17	0.83	0.19	0.81	—

\* Data of island population from Johnson (1971)

\*\* Pooled allelic frequencies of rare alleles; The geographical and latitudinal data on island populations include: A. = Samoa and Fiji are located from 10°S to 16° S; B. = Yap, Palau and Philippines islands located from 5° N to 12° N.

tion at *Adh*<sup>F</sup>, alpha-*Gpdh*<sup>F</sup>, *Est-5*<sup>S</sup> and *Odh*<sup>F</sup> loci. The present observations concur with earlier reports on *D. melanogaster* (Oakeshott *et al.*, 1982; Singh and Rhomberg, 1987). The earlier studies on *D. ananassae* revealed moderate to higher level of polymorphism at *Est-C* loci, while *Mdh*, *Odh* and *Adh* loci were represented by homogenous patterns of one most frequent alleles (0.97) and one or more very rare alleles in different island populations. However, the data on allelic frequencies at six polymorphic loci in Indian populations significantly differed from the South Pacific island populations of *D. ananassae* (Table 2). Thus, the overall comparison of genetic variability indices in sub-continental populations from India and South Pacific island populations revealed the occurrence of genic differentiation at the loci examined.

The Indian populations of *D. ananassae* belong to the center of the species geographical distribution, while the South Pacific island populations constitute the marginal populations. The comparison of Indian data with South Pacific island populations revealed significant differences between central and marginal populations on the basis of latitudinal variation at *Adh*, alpha-*Gpdh* and *Odh* loci. The genetic endowment of South Pacific versus Indian populations seems to be significantly differentiated and adaptively maintained by ecogeographical conditions.

References: Endler, J.A., 1986, *Natural Selection in the Wild*, Princeton University Press, Princeton, New Jersey.

pp. 336; Johnson, F.M., 1971, Genetics 68:77; Oakeshott, J.G., J.B. Gibson, P.K. Anderson, W.R. Knibb, D.G. Anderson and G.K. Chambers 1982, Evolution 36:86; Singh, R.S. and L.R. Rhomberg 1987, Genetics 117:225.

**Hoelzinger, D. and W.W. Doane.** Department of Zoology, Arizona State University, Tempe, AZ 85287-1501. Site of *Amy* locus in chromosome 5 of *Drosophila hydei*.

from *Drosophila melanogaster* to polytene chromosomes of *D. hydei*. However, conditions that were optimal for specific hybridization of this interspecific probe were associated with poor banding morphology, making interpretation of the results tentative. Here we present new information obtained from the use of intraspecific probes. Excellent chromosomal banding patterns were obtained by modifying the treatments of chromosomal spreads prior to and after hybridization. Accordingly, we have re-defined the position of the *Amy* locus on the cytological map of *D. hydei*.

Clones containing *Amy* sequences were isolated from a lambda EMBL-4 genomic library for *D. hydei* (gift of D.T. Sullivan) using standard methods (Sambrook *et al.*, 1989). Following amplification on *Escherichia coli* LE 392, phage were screened by plaque hybridization under conditions of high stringency. The  $^{32}\text{P}$ -labelled probe employed was prepared from the cDNA clone pOR-M7 (provided by D.A. Hickey), which represents RNA from the proximal *Amy* gene of an Oregon-R strain of *D. melanogaster*. Although the number of phage screened was equivalent to 10 haploid genomes, the library was known to be incomplete. After four positive plaques were identified and rescreened, a restriction map was prepared for each isolate. Isolates fell into two classes of non-overlapping clones based on the length of their insert, *i.e.*, the inserted sequence was 14.65 kb in one class and 12.3 kb in the other. Each class contained two apparently identical *Amy* isolates. Isolates lambda-Dhy10 and lambda-Dhy17, representing the respective classes, were used to prepare biotin-labelled probes (BRL Bio-Nick Labeling System). Protocols employed in our earlier study of *in situ* hybridization to polytene chromosomes (Hoelzinger and Doane, 1992) were followed, except for the addition of modifications aimed at better preservation of chromosomal banding patterns. These were suggested by Johng Lim, whose advice we gratefully acknowledge. In particular, care was taken to avoid sudden temperature changes of the chromosomal squashes during pre- and post-hybridization treatments.

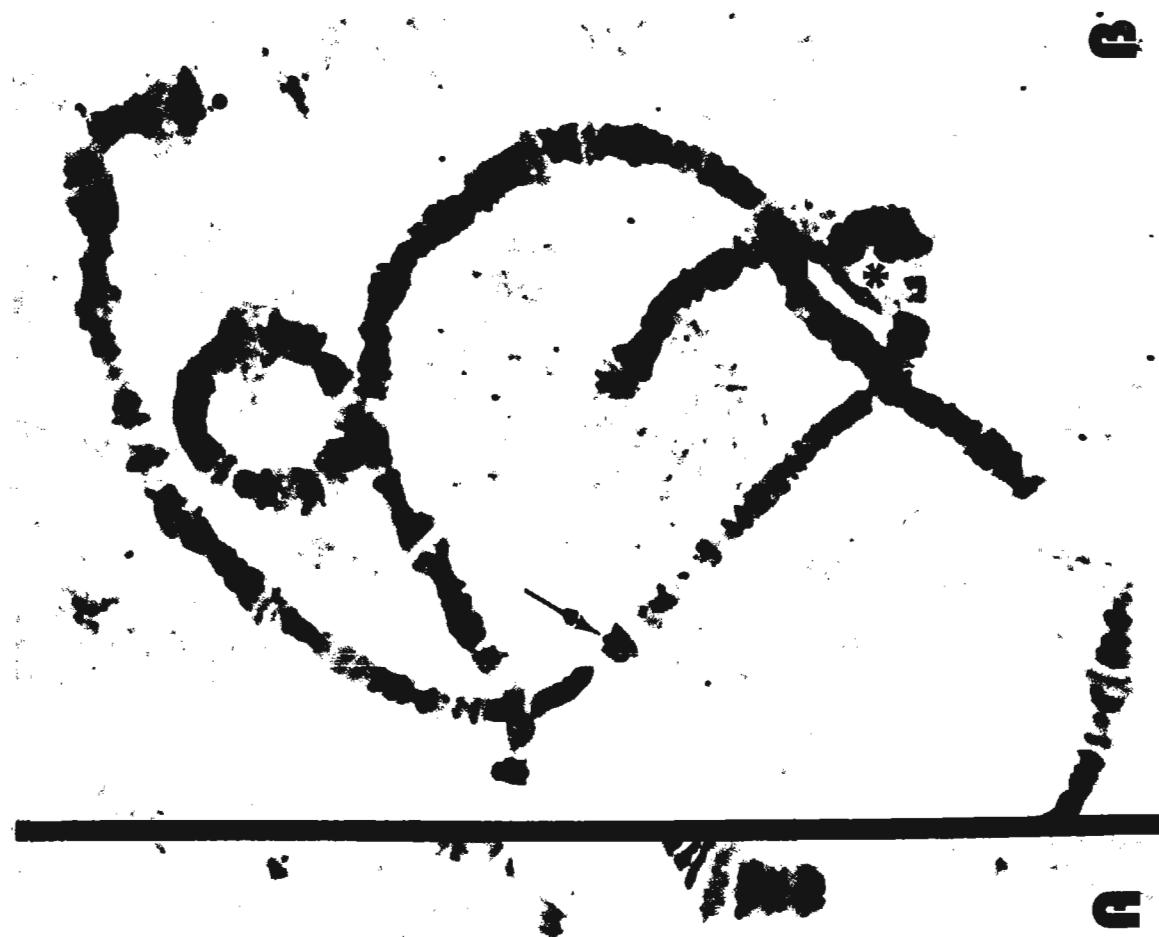
Chromosome squashes were prepared from late third instar larval salivary glands of three different genotypes of *D. hydei*: 1) *sca cn Amy<sup>7</sup>* vg homozygotes, 2) *In(5)36, Amy<sup>null</sup>/sca cn Amy<sup>7</sup>* vg heterozygotes, and 3) *In(5)15, cn Amy<sup>8</sup>/Amy<sup>7</sup>* (Zur) heterozygotes. The two chromosomal inversions had been induced by X-irradiation (Doane, 1971). They permitted rapid identification of chromosome 5 in squashes made from inversion heterozygotes. *In(5)36* has breakpoints at 107D and 110A, based on the revised cytological map of Ananiev and Barsky (1982). The *In(5)36* chromosome carries a recessive *vg* mutation, is lethal in homozygotes, and causes female-sterility in heterozygotes; it also contains an *Amy<sup>null</sup>* mutation which was derived from an *Amy<sup>8</sup>* allele and was X-ray-induced at the same time as the inversion (Doane, 1971 and unpublished data). *In(5)15* lies within the distal quarter of chromosome 5, which carries a *cn* marker; inversion homozygotes are viable and fertile. To produce genotype (3) above, *In(5)15* homozygotes were crossed to a Zurich (Zur) wild-type strain which is homozygous for *Amy<sup>7</sup>*.

The biotinylated *Amy* probes prepared from lambda-Dhy10 and lambda-Dhy17 hybridized to region 110B of chromosome 5 (Figure 1). When both probes were mixed together in equal amounts and co-hybridized to chromosomal squashes, a single site of hybridization was observed in 110B. This implies that the *Amy* sequences represented by these two probes are tightly linked in the genome of *D. hydei*. Whether or not each sequence is a functional *Amy* gene can be tested through interspecific somatic transformation experiments in which the amylase-null strain of *D. melanogaster* serves as the host strain (see Hawley *et al.*, 1990).

References: Ananiev, E.V. and V. Barsky 1982, Chromosoma (Berl.) 87:239-245; Doane, W.W., 1971, Dros. Inf. Serv. 4:46-48; Hawley, S.A., R.A. Norman, C.J. Brown, W.W. Doane, W.W. Anderson, and D.A. Hickey 1990, Genome 33:501-508; Hoelzinger, D. and W.W. Doane 1992, Dros. Inf. Serv. 71:233-234; Sambrook, J., E.F. Fritsch, and T. Maniatis 1989, *Molecular Cloning. A Laboratory Manual*, 2nd ed. Cold Spring Harbor Press, NY.

Figure 1 (next page). Hybridization of biotinylated *Amy* probes to polytene salivary chromosome 5 of *D. hydei*. The lambda-Dhy10 and lambda-Dhy17 probes were hybridized, respectively, to chromosomes from a *sca cn Amy<sup>7</sup>* vg homozygote (A) and an inversion heterozygote, *In(5)15 cn Amy<sup>8</sup>/Amy<sup>7</sup>* (Zur) (B). Arrows mark the site of hybridization to region 110B. The *In(5)15* inversion loop is indicated by an asterisk; the centromeric end of chromosome 5 is marked by a black dot.

We reported previously that the structural locus for alpha-amylase in *Drosophila hydei*, which contains at least two functional *Amy* gene copies, is located at a single site in chromosome 5 (Hoelzinger and Doane, 1992). This was based on *in situ* hybridization of a biotinylated cDNA probe containing *Amy* sequences



**Sardisco, A., G. Di Lemma, R.M. Ribaudo and A. Di Pasquale.** Dipartimento di Biologia cellulare e dello sviluppo "A. Monroy", Sezione di Genetica, Università di Palermo, Via Archirafi 22, 90123 Palermo (Italy). Following outcross high mutability in a strain of *Drosophila melanogaster*.

able elements (Ribaudo *et al.*, 1992). Following this hypotheses we have undertaken a genetic analysis aimed to determine if heterozygosity with the *tu-pb* genome may induce changes in the phenotype of homozygous non-*tu-pb* progeny. Males of a multi-marked 3rd chromosome strain (*ru cu ca*) were crossed to *tu-pb* females and F1 heterozygous males backcrossed to females from the multi-marked strain. Following generations were obtained by mating *ru cu ca* males and females. Offspring were scored for phenotypic modifications. At the third generation, in one of the lines, males were found exhibiting mutant *lozenge* eyes. In subsequent generations, other mutant phenotypes were detected and isolated. Somatic mosaicism was also observed. To ascertain possible dysgenic effects of the initial female *tu-pb* x male *ru cu ca* cross, we analyzed the gonads of heterozygous F1 *tu-pb/ru cu ca* individuals. No atrophied gonads were detected in this hybrid population. In this regard we have also studied the dysgenic properties (appearance of GD sterility) of the *tu-pb* strain, taking into consideration two known dysgenic systems, P-M and hobo. This analysis revealed that *tu-pb* has a P cytotype but uncertain P-dependent GD-sterility potential. Moreover, the *tu-pb* possesses full hobo repressor ability, while as regards its hobo induction potential some ambiguity remains. Furthermore, our results seem to exclude dysgenic effects due to mobilization of P and hobo elements in the germ cells of the progeny from the crosses performed in our investigation. Nevertheless, our data strongly suggest that the mutations we detected may be dysgenic-induced mutations. We suppose that mobile genetic elements, whose massive mobilization is not detectable by GD-sterility, presumably present in the *tu-pb* genome, might enter the line during the contamination generation and might have generated genetic instability.

References: Ribaudo R.M., G. Di Lemma and A. Di Pasquale 1992, Dros. Inf. Serv. 71:200-201.

**Chickering, M., H. Gardner, and J.G. Pelliccia.** Department of Biology, Bates College, Lewiston, Maine. Chlorate alters embryo morphology but not sperm motility in *Drosophila*.

ferase (TPST) (Baeuerle and Huttner, 1986).

In a previous report we showed that adult flies exposed to sodium chlorate at concentrations up to 100 mM show no obvious loss in viability, but these flies produce few progeny (Cusick and Pelliccia, 1992). Patterns of protein sulfation were quickly reversible when the adults were removed from chlorate containing medium. Mating experiments between chlorate treated adults and controls suggest that there was a reduction in fertility in both sexes. In an attempt to determine what aspects of reproductive physiology were being affected by chlorate, we chose to look at embryo morphology and sperm motility.

Adult males, 2-4 days old, were raised for 24 hours on Carolina Instant Drosophila Medium 4-24 reconstituted with 100 mM sodium chlorate. Their reproductive systems were dissected into Ringers solution and sperm motility was assayed under a compound microscope. The enlarged seminal vesicles were filled with spermatozoa whose long tails were clearly visible in bundles. Sperm tail movement was seen in 39 of 50 chlorate treated seminal vesicles and in 41 of 46 control vesicles. A chi square test produced a value of 2.71 with a p > 0.05, thus no significant difference in motility was observed (Ambrose and Ambrose, 1987). We assume that vesicles exhibiting no sperm bundle movement were probably damaged during dissection. Chlorate must, therefore, be exerting its effect in some other manner to reduce male fertility.

Embryo surface morphology was examined using a scanning electron microscope. Embryos were collected from agar laying plates from both control females and females raised on 100 mM sodium chlorate. Chorions were either left intact or removed by transferring to poly-L-lysine coated cover slips and submerged in 5% glutaraldehyde for 30 minutes. This was followed by two phosphate buffer rinses and a one hour fixation in 2% osmium tetroxide. The embryos were then rinsed again in phosphate buffer, transferred through a standard dehydration series, critically point dried, and sputter coated. They were examined in a JEOL JSM-6100 Scanning Electron Microscope at 4.0kV.

Previous cytological observations evidenced that salivary polytene chromosome configurations, peculiar to the *tu-pb* melanotic tumor strain of *Drosophila melanogaster*, can be assumed by *Oregon-R* polytene chromosomes which have passed through a generation of heterozygosity with the *tu-pb* ones. These findings seemed to suggest the possibility of "chromosomal contamination", therefore, the involvement of transposable elements (Ribaudo *et al.*, 1992).

Following this hypotheses we have undertaken a genetic analysis aimed to determine if heterozygosity with the *tu-pb* genome may induce changes in the phenotype of homozygous non-*tu-pb* progeny. Males of a multi-marked 3rd chromosome strain (*ru cu ca*) were crossed to *tu-pb* females and F1 heterozygous males backcrossed to females from the multi-marked strain. Following generations were obtained by mating *ru cu ca* males and females. Offspring were scored for phenotypic modifications. At the third generation, in one of the lines, males were found exhibiting mutant *lozenge* eyes. In subsequent generations, other mutant phenotypes were detected and isolated. Somatic mosaicism was also observed. To ascertain possible dysgenic effects of the initial female *tu-pb* x male *ru cu ca* cross, we analyzed the gonads of heterozygous F1 *tu-pb/ru cu ca* individuals. No atrophied gonads were detected in this hybrid population. In this regard we have also studied the dysgenic properties (appearance of GD sterility) of the *tu-pb* strain, taking into consideration two known dysgenic systems, P-M and hobo. This analysis revealed that *tu-pb* has a P cytotype but uncertain P-dependent GD-sterility potential. Moreover, the *tu-pb* possesses full hobo repressor ability, while as regards its hobo induction potential some ambiguity remains. Furthermore, our results seem to exclude dysgenic effects due to mobilization of P and hobo elements in the germ cells of the progeny from the crosses performed in our investigation. Nevertheless, our data strongly suggest that the mutations we detected may be dysgenic-induced mutations. We suppose that mobile genetic elements, whose massive mobilization is not detectable by GD-sterility, presumably present in the *tu-pb* genome, might enter the line during the contamination generation and might have generated genetic instability.

Chlorate is a sulfate analogue that can inhibit the sulfation of many acceptor molecules, as it inhibits the enzymatic formation of PAPS (3'-phosphoadenosine 5'-phosphosulfate), the activated sulfate donor in eukaryotic sulfate conjugations. One enzyme whose function is thus affected is tyrosyl protein sulfotrans-

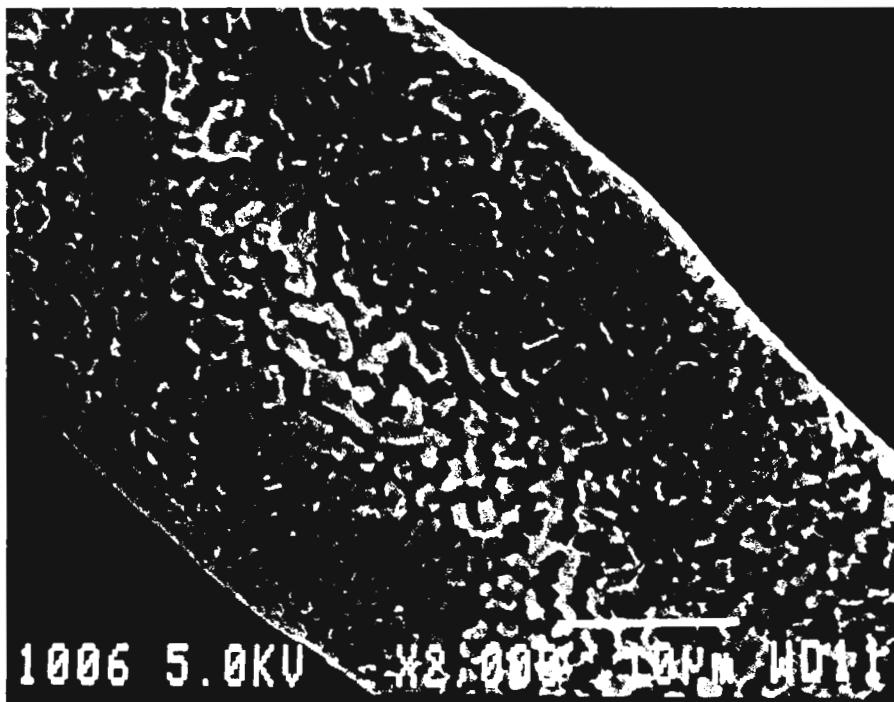


Figure 1: Scanning electron micrograph (x 2,000) of a normal embryo respiratory horn.

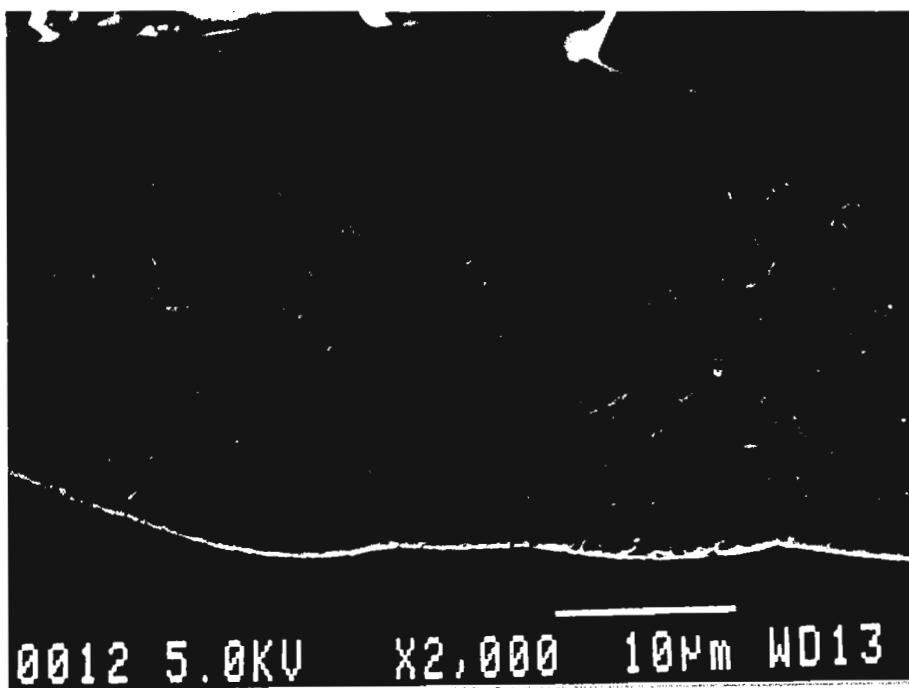


Figure 2: Scanning electron micrograph (x 2,000) of the respiratory horn of an embryo from a female treated with 100 mM sodium chlorate.

No reproducible differences were seen between control and treated embryos, either with or without the chorion, except for the appearance of the respiratory horns. The horns contain a meshwork structure into which air can diffuse (Evans, 1984). The developing embryo can exchange gases with its environment using the thin film of air held in this meshwork (Hinton, 1981). In the control embryos (Figure 1), there are large gaps in the outer surface of the horn for air to diffuse into the spongy mesh-like layer below. These gaps nearly disappear in the chlorate-treated embryos (Figure 2), thus reducing opportunity for gas exchange. These differences were reproducible, and the photographs show representative embryos.

Although the difference in morphology has not been shown to account for the reduced fertility, females removed from chlorate regained their fertility within 24 hours and produced embryos with normal looking respiratory horns. We are intrigued by this difference and would like to investigate further the nature of the material found in the horns so as to begin to understand if inhibition of sulfate conjugation is indeed involved in the reduction in fertility.

References: Ambrose, H.W. and K.P. Ambrose 1987, *A Handbook of Biological Investigation*, pp. 96-98; Baeuerle, P.A. and W.B. Huttner 1986, Biochem. Biophys. Res. Comm. 141:870-877; Cusick, S.M. and J.G. Pelliccia 1992, Dros. Inf. Serv. 71:187-188; Evans, H.E., 1984, *Insect Biology*, pp. 79-80; Hinton, H.E., 1981, *Biology of Insect Eggs*, pp. 724-762.

**Stark, W.S.<sup>1</sup>, M. Fesi<sup>1</sup>, H. Barnhill<sup>2</sup> and S.D. Carlson<sup>2</sup>**

<sup>1</sup>Saint Louis University, St. Louis, MO USA. <sup>2</sup>University of Wisconsin, Madison, WI USA. Morphology of the surface of the compound eye in *Drosophila melanogaster* and *D. virilis*: strains with and without R7.

The R7- *Drosophila virilis* (white-eyed) and a red-eyed R7+ control were from the Bowling Green Stock Center. The white-eyed *sevenless* (*w sev*, the LY3 allele) (Harris *et al.*, 1976) mutant of *Drosophila melanogaster* was also used. Red-eyed *boss*<sup>1</sup> (e.g. Basler and Hafen, 1991) and wild-type were also examined. For SEM, heads were dissected at the neck and dehydrated in an ascending ethanol series (30, 50, 70, 95, and 100%). After critical point drying using a Samdri-780A, heads were fixed to stubs with silver paint and gold coated on Biorad E5000 M sputter coater and viewed on a Hitachi S570 SEM.

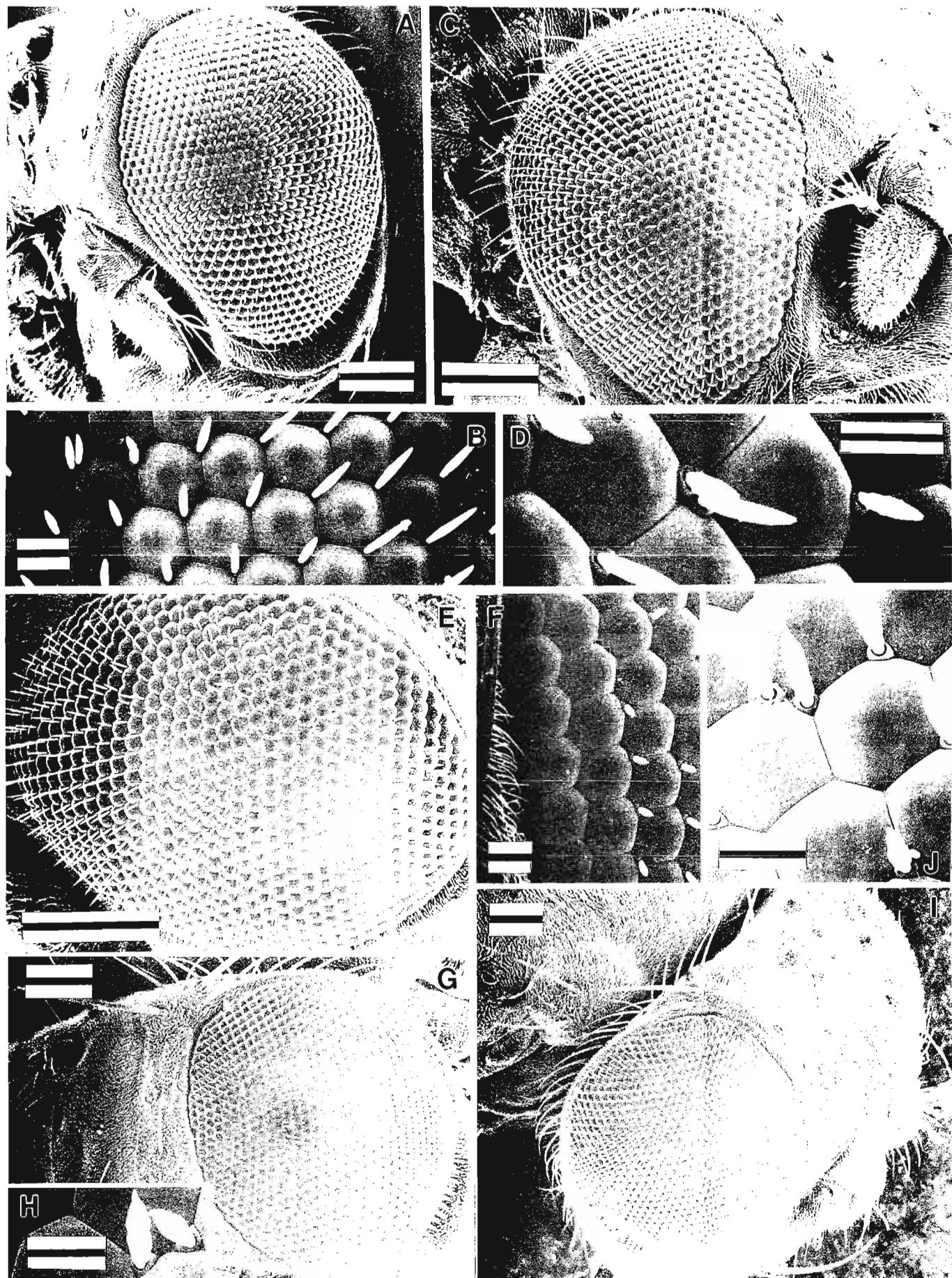
The surface of a typical wild-type eye is strikingly regular, with 3 hairs on every other corner of each hexagonal lens and with an anterior margin of facets lacking hairs (Figure A, accompanying plate, calibration bars on all low magnifications are 100 mm). Despite this overall regularity, there are just a few double hairs, one set enlarged in B (calibration bars on all high magnifications are 10 mm). By contrast, *sev* (C) has more irregularities, hairs on adjacent corners of the hexagons, more double hairs and missed corners; the latter two qualities are depicted in a higher magnification (D). The void of interfacetal hairs on the anterior margin is noted in both *sev* and wild-type. Although *boss* has a similar phenotype (R7 absence) as *sev*, double hairs are not seen (E), although missed corners (see also high magnification in F) and adjacent corner placement seems more common than in the LY3 allele of *sev*. Also, *boss* seems to have a wider anterior margin without hairs. In order to put our earlier observation (Stark, *et al.*, 1993) on the R7- *Drosophila virilis* into perspective, it was necessary to examine an R7+ control (G and H). As with *Drosophila melanogaster*, wild-type *Drosophila virilis* has an appealing regularity but several interfacetal hair flaws can be found. Such flaws (missed corners and adjacent corners but no doubles) are more frequent in the R7- mutant (I and J). *Drosophila virilis* has a naked margin like *Drosophila melanogaster*. The animal we examined was typical, although we have noticed some individual *Drosophila virilis* R7- specimens with substantially more disrupted surface morphology. Ocelli for all specimens were judged normal (shown only for wild-type *Drosophila melanogaster* (A) and for *Drosophila virilis* (G and I)).

In the oft-cited "neurocrystalline lattice" (Ready *et al.*, 1976), it seems possible that there would be a few flaws in wild-type surface morphology, though these have not, to our knowledge been emphasized before. Also, it is not surprising that a defect in assembly of 8 receptors into the normal ommatidial array might lead to additional flaws, since gross defects are frequently reflected in greatly distorted surface morphology (e.g., Stark and Carlson 1991; Stark *et al.*, 1989). It seems curious that we observe diagnostically different syndromes in surface aberrations in the three strains lacking R7 presented here.

Acknowledgments: Supported by NIH grant EY07192 to WSS.

References: Basler, K. and E. Hafen 1991, BioEssays 13:621-631; Harris, W.A., W.S. Stark and J.A. Walker 1976, J. Physiol. (Lond.) 256:415-439; Ready, D.F., T.E. Hanson and S. Benzer 1976, J. Dev. Biol. 53:217-240; Stark, W.S., G. Brown, D. Rodriguez and J.P. Carulli 1993, Dros. Inf. Serv. 72:164-167; Stark, W.S. and S.D. Carlson 1991, Dros. Inf. Serv. 70:217-219; Stark, W.S., R.J. Sapp and S.D. Carlson 1989, J. Neurogenet. 5:127-153.

Irregularities in the interfacetal hairs were reported in a *Drosophila virilis* stock which lacks R7 (R7-) (Stark *et al.*, 1993). We obtained, at low and high magnification, scanning electron micrographs (SEMs) to compare these flies with R7+ *Drosophila virilis* controls as well as wild-type, *sev* (= *sevenless*) and *boss* (*bride of sevenless*) *Drosophila melanogaster*.



**Juang, J.-L.<sup>1</sup>, W.S. Stark<sup>2</sup>, and S.D. Carlson<sup>1</sup>.**

<sup>1</sup>University of Wisconsin, Madison, WI USA, <sup>2</sup>Saint Louis University, St. Louis, MO USA. Scanning electron microscopy of the retina of *Drosophila*.

gate the retina of the housefly (Carlson and Chi, 1974; Carlson and Chi, 1979; Chi and Carlson, 1975), but there have been only a few SEM studies using *Drosophila*. For SEM and LVSEM, heads were dissected, fixed in the standard protocol for transmission EM [TEM] (Sapp *et al.*, 1991), dehydrated, critical point dried, fixed to stubs, fractured with a razor blade, gold coated and viewed on a Hitachi S570. For HRSEM, fixed tissue was freeze-cleaved (Hollenberg and Lea, 1988). For LVSEM, the coating was with platinum and the apparatus was the Hitachi 900.

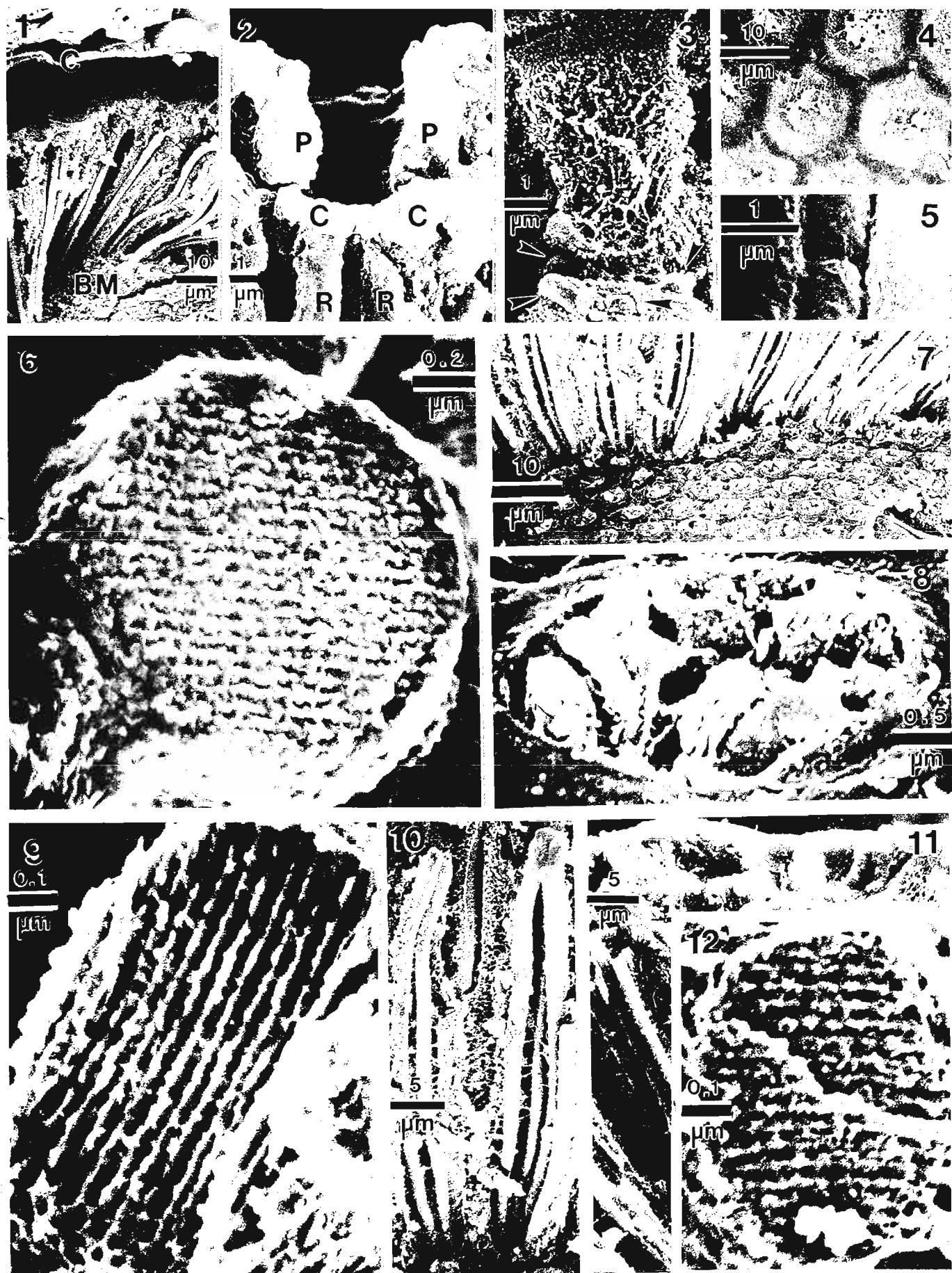
The accompanying plate highlights our observations using standard SEM [unless specialized SEM technique is specified] from vitamin A replete animals [unless deprivation is specified]. Figure 1 shows the retina in a survey from cornea [C] to basement membrane [BM]. Most features, fairly clear even in the low magnification of Figure 1, are displayed in greater detail and higher magnification. Figure 2 shows an opened chalice wherein the pseudocone formerly resided prior to being dissolved by preparative solvents. The primary pigment cells [P], cone [Semper] cells [C] and rhabdomeres [R] are prominent features. Figure 3 shows, at the top, where the secondary pigment cells separated from the cornea; in the central area, microvilli of primary pigment cells are revealed; rhabdomere caps [arrowheads] are also especially clear. Figure 4 is a glimpse down onto the hexagonal insertion sectors of the pigment cells which delimit and enclose the pseudocones. At the center of Figure 5 is the boundary between R7 and R8 rhabdomeres. Using HRSEM, cleaner fractures were achieved, and individual rhabdomeric microvilli can be easily discerned at the higher magnifications [Figure 6]. Figure 7 shows the bases of the rhabdomeres and an area where the massed photoreceptor cells with adhering basement membrane are fractured away from the more proximal visual projection areas. One can observe the fenestrations in the basement membrane through which axons of the ommatidia pass to the first synaptic neuropil, the lamina ganglionaris. An individual cross fractured pseudocartridge [the R1-8 axonal bundle] is enlarged in Figure 8, and some of the 8 axons are clearly delineated.

Figures 10 and 11 are SEMs at the same magnification from vitamin A replete and deprived flies, respectively. The size difference of the rhabdomeres in these two treatments [shown and quantified from TEMs in an earlier publication (Sapp *et al.*, 1991)] is quite clear. Figures 9 and 12 are LVSEMs, also at the same magnifications, to clearly show this size difference in replete vs. deprived *Drosophila*, respectively. Harris *et al.* (1977) used freeze fracture EM to show that P-face particle density [mostly rhodopsin molecules] in rhabdomeric microvilli was vitamin A dependent in *Drosophila*. Despite the thin coating of platinum used for LVSEM, membrane proteins are not visible in the microvilli. However, more recently, Suzuki *et al.* (1993) have demonstrated some success in visualizing rhabdomeric protein with a deep-etch quick-freezing technique. Our observations and those of Suzuki *et al.* (1993) extend high resolution SEM methodology to retinal characterizations. For those aspects of retinal structure which are well visualized using SEM, the greater ease of using SEM over TEM, together with the appealing three dimensional appearance and depth of focus, makes SEM an inviting technique for diagnosing retinal abnormalities in mutants.

**Acknowledgments:** Supported by NIH grant EY07192 to WSS and NSF grant BNS 8908081 to SDC. We thank the staff of the IMR [Integrated Microscopy Resource] at the University of Wisconsin - Madison [NIH Biotechnology Resource DRR 570], especially Mr. Alan R. Kutchera, for help with the LVSEM. Ms. Melissa J. Curtis and Mr. J. Scott Christianson assisted with the SEM work on the Hitachi S570 and Mr. Justin Lim helped in the dark room.

**References:** Carlson, S.D. and C. Chi 1974, Cell Tiss. Res. 149:21-41; Carlson, S.D. and C. Chi 1979, Ann. Rev. Entomol. 24:379-416; Chi, C. and S.D. Carlson 1975, Cell Tiss. Res. 159:379-385; Harris, W.A., D.F. Ready, E.D. Lipson, A.J. Hudspeth and W.S. Stark 1977, Nature (Lond.) 266:648-650; Hollenberg, M.J. and P.J. Lea 1988, Invest. Ophthalmol. Vis. Sci. 29:1380-1389; Sapp, R.J., J.S. Christianson, L. Maier, K. Studer and W.S. Stark 1991, Exp. Eye Res. 53:73-79; Suzuki, E., E. Katayama and K. Hirosawa 1993, J. Electron Microsc. 42:178-184.

The purpose of this study is to show particular key structures in the *Drosophila* retina exposed by fracture using scanning electron microscopy [SEM] and to test the limits of resolution afforded by Low Voltage [field emission] SEM [LVSEM] and High Resolution SEM [HRSEM]. SEM had been used extensively to investi-



**Band, H.T.** Michigan State University, East Lansing, MI 48824. More evidence that drosophilids overwinter in a preadult stage in mid-Michigan.

delayed capture of any adults until late May. Numbers coming to bait were also reduced. A total of 52 individuals (34 males and 18 females) representing 12 species were collected on over-ripe pears May 26-31.

Table 1. Species coming to bait in late May 1993 in East Lansing, MI.

Species	No. males	No. females
<i>D. affinis</i>	5	
<i>D. algonquin</i>	4	
<i>D. athabasca</i>	10	
<i>D. affinis group</i>		5
<i>D. paramelanica</i>	1	3
<i>D. macospina macrospina</i>		3
<i>D. falleni</i>	3	1
<i>D. tripunctata</i>	1	
<i>D. quinaria</i>	5	2
<i>D. immigrans</i>	4	2
<i>D. robusta</i>		1
<i>C. amoena</i>	1	1

one extreme and avoid the other by migration or hibernation (Judson, 1994). Few insects perform the spectacular migration of the monarch butterfly. Band and Band (1984, 1987) found that overwintering *C. amoena* larvae were physiologically and biochemically different from larvae developing in the breeding season, as expected from the dynamic nature of adaptations that can be manifest by insects in winter survival. Larvae taken from overwintering substrates showed fat body proteins and the heavy molecular weight salivary glue protein in the hemolymph (Band, 1993b). The population shifted genetically between summer and winter in the frequency of *Pgm* alleles (Band and Band, 1987). This suggests that insects in seasonal climates have to display adaptations both to the overwintering environment and to the breeding season environment in order for the population to exist in a seasonal climate.

References: Band, H.T., 1991, D.I.S. 70:29; Band, H.T., 1993a, Great Lakes Entomologist 26:237-240; Band, H.T., 1993b, Cryobiology News Notes (October) pp. 1-3; Band, H.T., and R.N. Band 1984, Experientia 40:889-891; Band, H.T., and R.N. Band 1987, Experientia 43:1027-1029; Judson, O.P., 1994, Trends in Ecol. and Evol. 9:9-14.

**Goerick, S., and W.-E. Kalisch.** Institut für Genetik, Ruhr-Universität Bochum, FR Germany. Dosage compensation and dosage effect in *D. nasuta*.

subgroup.

*D. nasuta nasuta* and *D. n. albomicans* are members of the *D. nasuta* subgroup of the *immigrans* species group (Wilson *et al.*, 1969). Their phenotypes and the band-interband patterns of the salivary gland chromosomes are almost identical. Genetic differences exist in the number of chromosomes (*D. n. nasuta*,  $2n = 8$ ; *D. n. albomicans*,  $2n = 6$ ) by fusion of chromosomes-3 with the gonosomes and by the amount of centromeric heterochromatin (Ranganath and Hägele, 1982). *D. n. nasuta* and *D. n. albomicans* are totally cross fertile. By this, both subgroup members have been interpreted as different species as well as chromosomal races (Nirmala and Krishnamurthy, 1972).

In SDS-PAGE (Figure 1), at least four major larval secretion protein fractions are found in *D. n. albomicans* (Figure 1 A; two tightly neighboured fractions of 36 kd; 27 kd and 26 kd) as well as in *D. n. nasuta* (Figure 1 N; two tightly neighboured fractions of about 40 kd; 29 kd and 28 kd) which are coded by an X-chromosomal gene cluster. Strong homologies (amount, glycoylation, electrophoretic mobility) between the major fractions in both protein patterns are confirmed. However, any homologies with *D. melanogaster* are missing (Ramesh and Kalisch, 1989).

The number of mutants known in the *D. nasuta* subgroup is insufficient so far. We found one spontaneous X-chromosomal mutant, *D. n. nasutasa* (Figure 1 S), indicating a different pattern of secretion protein fractions (Kalisch and Ramesh, 1988).

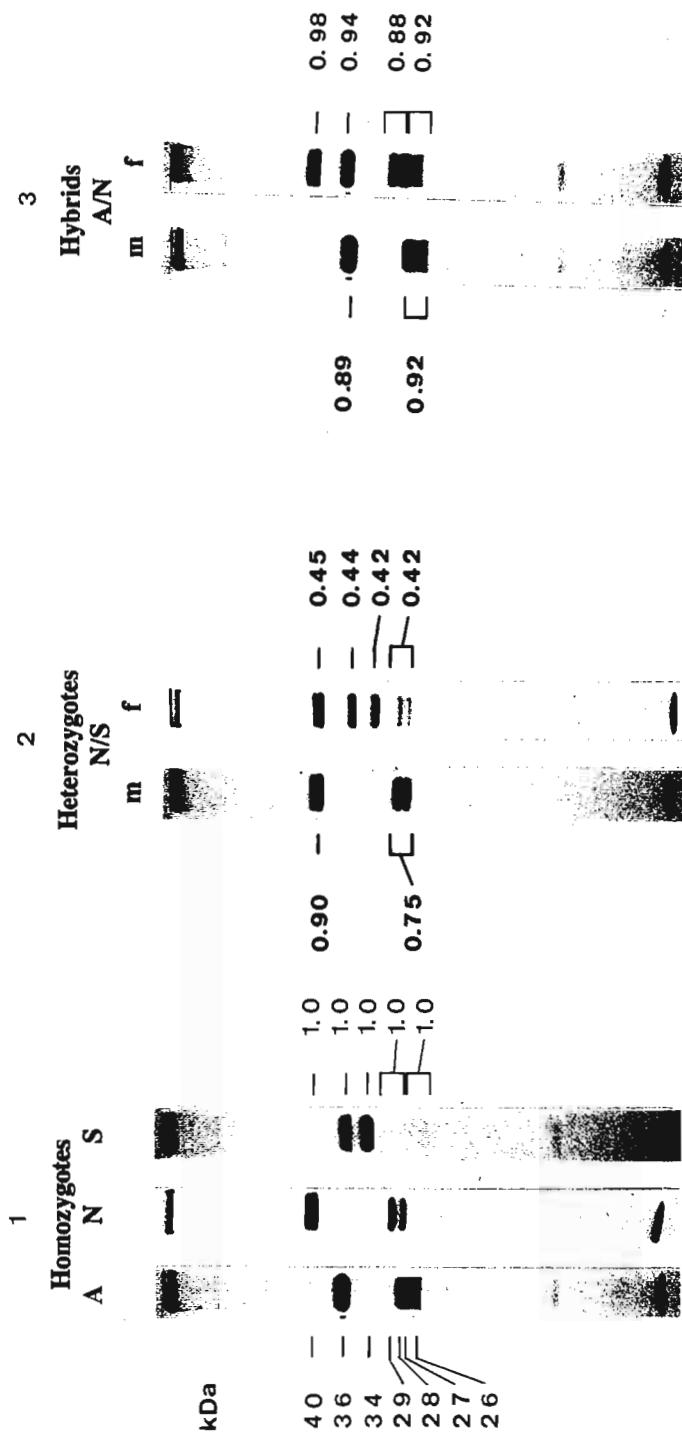
The two secretion protein plugs from a single pair of salivary glands in third instar larvae were used for each

Band (1993a) reported that drosophilids collected in spring had entire wings and were young looking in appearance. A total of 122 individuals (84 males and 38 females) representing 14 species were trapped between late April and late May 1992. In 1993 a cold spring

Table 1 lists the species and numbers captured. Again, all individuals coming to bait had entire wings. Ten of the species are identical between years and include both endemic and cosmopolitan species, and typical fruit, sap and mushroom breeding species. Earlier it was found that individuals in the *D. affinis* group can emerge from overwintered fruits (Band, 1991). This indicated *Drosophila* preadult overwintering in Michigan. Beninger (personal communication) has likewise found that *D. affinis* group species overwinter in Canada in a preadult stage. Whether overwintering is in the egg, larval or pupal stage remains to be determined. So far, only *C. amoena* is known to overwinter as a third instar larva (Band and Band, 1984, 1987).

Recently it has been argued that if a habitat is cold and has a high variance in temperature, animals will tend to be adapted to

The regulation of dosage compensation is still unclear in *Drosophila* (Sass and Meselson, 1991). To check whether dosage compensation is male- or female-regulated, we examined homozygous, heterozygous and hybrid larvae in the *D. nasuta*



Figures 1-3. X-chromosomal secretion proteins from individual pairs of glue plugs of third instar larvae. Homozygous (1), heterozygous (2) and hybrid larvae (3) of the same SDS-PAGE of species and mutants of the *D. nasuta* subgroup are shown. Densitometric data from individual measurements of the SDS-PAGE are presented. Relative densitometric data in (2) and (3) are based on the homologous fractions in (1). An autosomal fraction (not labelled) was used as internal control.

(1) X-chromosomal fractions (labelled by kDa) of individual homozygous *D. n. albomicans* (A), *D. n. nasuta* (N), and *D. n. nasutasa* (S) female larvae. Each densitometric amount of protein fractions (right) is set to 1.0. Homologous fractions in male larvae (data not shown) are compensated male specifically (*i.e.*, about 1.0 - 0.9 of the female fractions).

(2) Relative amount of X-chromosomal fractions in a *D. n. nasuta*/*D. n. nasutasa* heterozygous male (m) and female (f) larva. Dosage compensation is expressed by almost half the protein amount of the parental fractions in the heterozygous female compared with the homozygous female [N and S in (1)]. In reciprocal crosses, comparable values were obtained (data not shown).

(3) Relative amount of X-chromosomal fractions in a *D. n. albomicans*/*D. n. nasuta* F1 hybrid male (m) and female (f) larva. A dosage effect is expressed for all X-chromosomal fractions in the female. In reciprocal crosses, comparable values were obtained (data not shown).

SDS-PAGE in Figures 1-3. Fractions were stained with Coomassie Brilliant Blue R-250. A HOEFER GS300 densitometer was used for the quantitative comparison of homologous fractions. Storage, comparison, and plotting of data was achieved by the HOEFER GS365 computer programme.

Homozygous female and male larvae of *D. n. nasuta*, *D. n. albomicans*, and *D. n. nasutasa* indicate dosage compensation concerning the major X-chromosomal fractions (data of males are not shown in Figure 1).

In heterozygous *D. n. nasuta/D. n. nasutasa* F1 female larvae (Figure 2), the X-chromosomal fractions are also compensated. Each X-chromosomal fraction is expressed by half the amount compared with the homologous fraction in homozygous larvae (Figure 1). F1 male larvae, which bear a maternal *D. n. nasuta* X-chromosome, express comparable (male-specific) protein amounts as their homozygous mothers (Figure 2).

In *D. n. albomicans/D. n. nasuta* F1 hybrid female larvae, X-chromosomal fractions indicate a dosage effect (Figure 3). All parental fractions are expressed by almost the same amount as in homozygous female larvae. Hybrid male larvae, which bear a maternal *D. n. albomicans* X-chromosome, produce comparable (male-specific) amounts as their homozygous mothers. Therefore, hybrid female larvae produce almost double the amount of X-chromosomal protein fractions as hybrid males.

The increased amount of X-chromosomal fractions found in F1 hybrid females is not simply based on a hybrid effect: we found that all autosomal protein fractions of the glue and of the remaining larvae analysed did not indicate any dosage effects (data not shown). Additionally, data of X-chromosomal gene activities found in F2-female larvae, contradict a simple hybrid effect (Goerick and Kalisch, this issue).

Relative values of protein amounts found in several hundred heterozygous and hybrid female larvae indicate that dosage compensation in *D. n. nasuta* as well as *D. n. albomicans* is female-regulated by hypo-activity of both X-chromosomes. These data contradict the hyper-activity of the male-regulated X-chromosome assumed in *D. melanogaster* (Lucchesi and Manning, 1987).

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**Ivannikov, Andrey V. and Ilya K. Zakharov.** Institute of Cytology and Genetics of the Russian Academy of Sciences, Siberian Department, Novosibirsk, 630090, Russia. Synantropic species of *Drosophila* (Diptera: Drosophilidae) in some regions of Eurasia.

The flies were collected in and near by winery, fruit and vegetable preserving plants, vegetable stores, gardens, in living quarters and household housings in towns and countryside.

In total, 118 individual collections were carried out, in which more than 32,000 individuals were identified. The collection of flies was performed during the mass reproductive period, from August to October. The species composition is represented by 11 species from 4 subgenera of genus *Drosophila* (see the Table 1) (see also: Ivannikov, 1991; Ivannikov *et al.*, 1993).

In the period studied, our data made us conclude that the vast species variability is typical for Europe (11 species observed). Altai region is characterized by poor variability (4 species). Middle Asia (6 species) and Western Siberia (7 species) take an intermediate position.

Four species, namely, *D. melanogaster*, *D. funebris*, *D. immigrans* and *D. busckii* are present in all the regions. Among these obligatory species the "status" (*i.e.*, representability and the number) of *D. melanogaster* dominates in Europe and Middle Asia. In Altai and Novosibirsk regions, the leadership belongs to the more cold-resistant species, *D. funebris* and *D. immigrans*. *D. busckii* is rare and few everywhere except Europe. Among four obligatory species, parallel changes in regional status were observed for *D. funebris* and *D. immigrans*. Moreover, it is obvious that, as for *D. funebris* and *D. immigrans*, *D. busckii* prefers the temperate or even the cold climate, because these species are rare and few in the most warm region, Middle Asia.

One of the species studied, *D. hydei* was detected everywhere except Altai. The status of this species was higher in warm regions.

Two species, *D. virilis* and *D. mercatorum*, were noted in the Europe and Novosibirsk region. *D. virilis* was

The specific composition and the numerical strength of *Drosophila* were studied in anthropogenic landscapes of some regions of Eurasia at a period 1988-1993. Among the regions analyzed were: the East of Europe (Ukraine and Moldavia), Middle Asia (the Central Tadzhikistan), Western Siberia (environs of Novosibirsk), and the South of Western Siberia (Altai region).

common in the Ukraine and was observed only once in the Novosibirsk region in 1981.

*D. lebanonensis* was observed only twice, and only some individuals were present in both cases: first, in the neighborhoods of Yalta (Crimea, Ukraine) in 1990 and, second, in Dushanbe (Tadzhikistan). Three species were observed exclusively in the East of Europe. *D. bifasciata* previously was erroneously identified as *D. obscura* (Ivannikov *et al.*, 1993). This species is sporadically represented and few in number in Ukraine. In single cases, *D. simulans* and *D. repleta* were found in Kishinev and Uman, respectively.

To judge by published data on distribution of *Drosophila* species (including the synantropic ones) on the territory of the former Soviet Union (Stackelberg,

1970; Mitrofanov, 1977), three of the species studied, namely, *D. immigrans*, *D. lebanonensis*, and *D. mercatorum*, were found on this territory *de novo*. They differ dramatically in distribution and status. *D. lebanonensis* occurred only twice, whereas *D. immigrans* belongs to one of the four obligatory species. The distribution and status of *D. mercatorum* is worthy of notice. This species was found only once in Eastern Europe and it was represented by few individuals (Uman, 1990). In the Novosibirsk region, this species was first registered in the same year, but its status was significantly higher. In 1991, *D. mercatorum* was absent in the collections from Uman, and the status and representability of this species in Siberia were unchanged.

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**Yurchenko, N.N.** Institute of Cytology and Genetics, Novosibirsk, Russia. X<sup>Z</sup>-unstable chromosome from a natural population of *Drosophila melanogaster*.

indicated by the subsequent genetic analysis, this mutation marks an unstable X<sup>Z</sup>.

The instability of X<sup>Z</sup> was studied in the crosses of males, carrying various X<sup>Z</sup> derivatives to the C(1)DX, y w f/Y or C(1)RM, y f/Y females. The mutation rate was calculated as the ratio of the number of exceptional males (the mutant ones) to the total number of males. The sequential origin of analyzed stocks derived from the mutation of the initial sn<sup>mz</sup> chromosome is shown in Figure 1. These stocks appeared due to mutations in the y, w, sn, and g. The alleles of

In 1986, the samples of *D. melanogaster* captured from the natural population in Zaporozhie (the Ukraine) were studied. Having been mated in nature, a female from this population, heterozygous for N, gave rise to a new stock. In this stock, sn<sup>mz</sup> arose *de novo*. As was

these loci were designated as: y<sup>y1</sup>-has a similar appearance as y<sup>2</sup>; y<sup>mos</sup>-causes the mosaic coloration of array of bristles; w<sup>y1</sup>-looks like w<sup>1</sup>; sn<sup>s</sup>-strong alleles of the sn. In addition, another three visible mutations (vm) were isolated (their allelism was not detected): vm1-engravings of the inner side of the wings, with 95% penetrance (1 - 53.6±); vm2-lace-like cutting of the wings, with 100% penetrance (1 - 48.4±); vm3-lowered wings, with 57% penetrance (1 - 53.6±).

Table 1. X-linked visible mutation rate of X<sup>Z</sup> derivatives.

X <sup>Z</sup> derivatives	Mutation rate of X chromosome loci (X 10 <sup>-4</sup> )						No. of males tested
	y	w	sn	g	vm1	vm2	
sn <sup>mz</sup>	0	0	4.4	0	0	0	9,012
In (1) T1, sn <sup>sZ</sup>	2.7	0	2.7	0	0	0	11,143
*y <sup>y1</sup> sn <sup>mz</sup>	0	0	0	0	0	0	17,245
sn <sup>+Z</sup>	5.8	0	0	0	0	0	5,132
In (1) T2, y <sup>mos</sup> sn <sup>+Z</sup>	0	0.4	0	0	0.4	0	24,556
In (1) T2, y <sup>mos</sup> vm1	0	0	0	0	0	0	7,065
In (1) T2, y <sup>mos</sup> w <sup>y3</sup>	2.8	0	0	0	0	0	3,561
In (1) T2, y <sup>mos</sup> w <sup>Y1</sup>	0	20.4	0	0.4	0	0.4	26,922
In (1) T2, y <sup>mos</sup> g <sup>Y1</sup>	0	0	0	0	0	0	14,424
In (1) T2, y <sup>mos</sup> w <sup>Y1</sup> vm2	0	34.0	0	0	0	0	12,435

\* - Cytological analysis was not performed

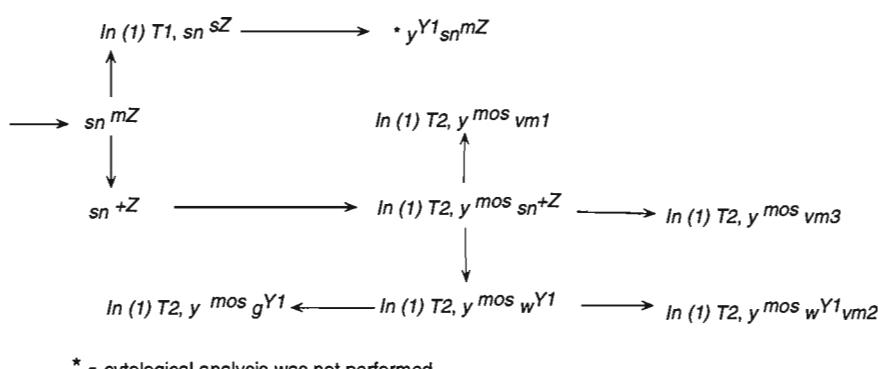


Figure 1. The origin of analysed  $X^Z$  derivatives.

Localizing the  $vm1$ ,  $vm2$ , and  $vm3$ , it was observed that recombination in a vast distal region of X chromosomes of 42+ map units length was blocked. After cytological analysis, it appeared that two different terminal ( $T$ ) inversions took place at the first stages of  $X^Z$  mutation. The inversion of the region 1A-

7D was found in the  $In(1)T1, sn^{sZ}$  and the inversion of the region 1A-10A was observed in the  $In(1)T2, y^mos sn^{+Z}$ .

The mutation rates of genes in the stocks that originated from  $X^Z$  are given in Table 1. All the mutations observed were of non-clustered nature (the only mutation per a vial); this means that they occurred most likely in meiosis. The mutation rates were rather low, of order of  $10^{-4}$ , but in the case of the  $w$ , the mutation rate was much higher, compared to the other genes. Some cases of simultaneous mutation of two genes were noted, for example,  $y^+ sn^{sZ} \rightarrow y^Y1 sn^{mZ}$  and  $y^mos w^Y1 gY1$ . Some stocks,  $y^Y1 sn^{mZ}$ ,  $In(1)T2, y^mos vm1$ , and  $In(1)T2, y^mos gY1$ , showed no evidence of mutation.

In the mating with  $sn^{mZ}$  males, a mutation that was phenotypically close to the  $N$  was found in  $C(1)RM, y f/Y$  female. In the following analysis of the  $C(1)RM, y Nf/Y$  stock, a female with the diluted apricot eye color was observed. Cytological analysis of these two mutant stocks failed to reveal some additional chromosomal rearrangements except the 4D-11E inversion of the initial compound.

There are some published data on isolation of unstable line as from the natural population (Tinyakov, 1939) as in the result of EMS-induced mutagenesis- $Uc$  chromosome, which accumulates rearrangements and lethal mutations (Lim, 1979, 1981). Although the concrete mobile elements responsible for the instability of  $X^Z$  are still unknown,  $X^Z$  and  $Uc$  are alike in some aspects. Two mobile elements are known to be responsible for the instability of  $Uc$ . Hobo was localized in the sites of chromosomal rearrangements, whereas gypsy was found in the hot spots for lethal mutations in chromosomes without rearrangements (Lim, 1988; Sheen, 1993). In the case of  $X^Z$ , mutation of some genes, for example the  $y$  and  $sn$ , is a result of the inversions. On the contrary, the high level of the  $w^Y1$  reversion is not correlated with visible chromosomal rearrangements. For  $Uc$ , a phenomenon of homologue destabilization was described (Lim *et al.*, 1983; Simmons *et al.*, 1986). In the article cited, the destabilization of X chromosome of  $C(1)RM, y f/Y$  females in chromosomal background received from the males carrying  $X^Z$  was described. Whereas  $Uc$  is characterized by a hot spot for the  $ct$  lethal mutations (Sheen, 1993), in  $X^Z$  a hot spot of mutagenesis is the  $w$ . In some stocks derived from  $X^Z$ , the stabilization was observed by analogy with the derivatives of  $Uc$  (Simmons *et al.*, 1985).

The interpretation of the data obtained is based on a model of a fluid eukaryotic nucleus. In this model, mobile elements serve as a liquid component of the genome and may flow from the compressed chromatin into the stretched one. This process leads to the instability of the chromosome studied. Probably, the site-specific stretching or compression of  $X^Z$  chromatin occurs in meiosis due to the interaction  $X^Z$  with other chromosomes. In this connection, it is pertinent to note that unstable mutations in maize were established by McClintock (1954) in the studying of chromosomal breakages in meiosis preceded by the stretching of the chromatin. When the site-specific tension of chromatin is relaxed due to the transposition of mobile elements, the chromosome may eventually stabilize as was observed in some  $X^Z$  derivatives.

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**Voloshina, M.A., and M.D. Golubovsky.** Institute of Cytology and Genetics of the Russian Academy of Sciences, Siberian Department, Novosibirsk, 630090, Russia. Instability of *lozenge* allele from the Far East natural population.

allele mutated with a high frequency of order  $10^{-2}$ ) to wild type and to other phenotypically different conditions, as was described in our previous report (Golubovsky, 1978). In this paper we continue the study of instability of  $lz^{75V}$  and its mutant derivatives.

To test the mutation rate,  $lz^*$  males ( $lz^*$  denotes  $lz^{75V}$  or some other mutant alleles derived from  $lz^{75V}$ ) were individually crossed to  $C(1)DX, y w f/Y$  or  $C(1)RM, y f/Y$  females with attached X-chromosomes and male progeny were recovered. The mutation rate was calculated as the ratio of the number of "non- $lz^*$ " males to the total number of males. To maintain the mutant stocks in the laboratory over prolonged period, we used a balancer stock *Basc*.

The 18-year long observations of  $lz^{75V}$  allele allow us to describe the following characteristics of its instability.

1. A wide variety of arising mutant phenotypes. Table 1 represents phenotypic classes, each including a set of phenotypically similar alleles independently appearing due to mutations of  $lz^{75V}$  or its mutant derivatives. The alleles within each phenotypic class may differ as in stability (see below) as in some secondary phenotypic traits, e.g., the interaction with other genes or the fertility of homozygous females. Consider the latter trait in some detail. The sterility or weak fertility of females is one of the known pleiotropic effects of many *lozenge* mutants (Lindsley and Zimm, 1990). In our case the females homozygous in *lz*-mutations belonging to three more extreme phenotypic classes  $lz^B$ ,  $lz^{75V}$ , and  $lz^{ex}$  were always sterile. But we also revealed that some of phenotypically wild type ( $lz^+$ ) mutants gave sterile homozygous females, too. The analysis of alleles of three phenotypically less extreme classes  $lz^+$ ,  $lz^n$ , and  $lz^{sl}$  for female sterility gives the following results. Among 25  $lz^+$  strains we detected fifteen strains with fertile females, two strains with weakly fertile females and eight strains with sterile females. Among 17  $lz^n$  strains there were nine strains with fertile, six with weakly fertile and two with sterile females. And out of two alleles  $lz^{sl}$  one led to female sterility and one to weak fertility. These results indicate that similar eye phenotype mutants may differ in such a phenotypic trait as female sterility and that at least some of the  $lz^+$ -derivatives are not true revertants.

To determine the dominance relations between mutants of different phenotypic classes, phenotypes of heterozygous females were analyzed. For all pairs of alleles dominance or semidominance of the phenotypically less extreme allele over the phenotypically more extreme allele was demonstrated.

It should be noted that our previous suggestion (Golubovsky, 1978) explaining the  $lz^{ex}$  phenotype by action of recessive enhancer located at the other site of X-chromosome was not supported. As well as the *lz* mutants of other phenotypic classes listed in Table 1,  $lz^{ex}$  is a mutant allele of the *lozenge* locus itself.

2. The second characteristic of the unstable system in question is that in each phenotypic class both unstable and stable alleles are available. The exception is only provided by the class of wild-type derivatives  $lz^+$ . All independently emerged  $lz^+$  mutants were stable. Single mutations from  $lz^+$  to  $lz^B$  and  $lz^{sl}$ -conditions were detected in special experiments, where recombination or hybrid dysgenesis were used, to destabilize the *lozenge* mutants. All the other phenotypic classes include the unstable alleles as well as the stable (no mutants were recovered among approximately 1,000 offspring) ones. The unstable alleles mutated to different mutant conditions with a rate on the order of  $10^{-3}$  to  $10^{-2}$ . The alleles of each phenotypic class mutated to limited set of new mutant conditions, which are represented in the third column of Table 1.

For each class a most preferential direction of mutagenesis (given first in Table 1) is available. The mutation rate at this direction is the highest. For most mutants this preferential direction is complete reversion ( $lz^+$ ), but for  $lz^n$  mutants this is the switching to the  $lz^B$  condition, and for  $lz^{ex}$  mutants - to the  $lz^{75V}$  condition. It should be noted that although the  $lz^n$  phenotypic class slightly differs from the wild type (only by yellow color of claws), most  $lz^n$  mutants by contrast to the  $lz^+$  mutants are unstable. All our *lozenge* alleles failed to produce large clusters of mutants as it had been described for the previously studied unstable alleles of the *singed* and the *yellow* loci, also isolated from natural populations (Golubovsky *et al.*, 1987; Zakharov, 1984). Most common was appearance of one or two "non- $lz^*$ " mutants in the progeny of one *lz* male, clusters including more members were rare. Nevertheless, offspring of two or even three different "non- $lz^*$ " phenotypes were sometimes found in the progeny of one *lz* male.

3. The observations over a period of years for the mutation rates of the same *lz* alleles allowed us to reveal spontaneous temporal changes of instability resulting in stabilization of previously unstable alleles. The stabilization period may be very different as the data of Table 2 show. For example, the stabilization of  $lz^{75V}$  allele of  $lz^{75V} v f$  strain took place within a few months after the stain arose. On the contrary, the *y ct lz<sup>sl</sup>* strain retained the supermutability

In 1975 males of the "fine bristle", *lozenge*<sup>+</sup> phenotype from a natural population from the Far East were individually crossed to females from laboratory stock  $C(1)DX, y w f/Y$ . Among the  $F_1$  progeny of one male, the mutant  $lozenge^{75V}$  ( $lz^*: 1 - 27.7$ ) arose *de novo*. The results of individual crosses of  $lz^{75V}$  male and its offspring to  $C(1)DX, y w f/Y$  females indicated that this

Table 1. Phenotypes and mutational spectrum of *lz<sup>75V</sup>* derivatives (arranged in the severity of their eye phenotype).

Allele	Phenotype	Mutation spectrum
<i>lz<sup>+</sup></i>	wild type: eyes and claws are absolutely normal, alleles are variable in female fertility	stable
<i>lz<sup>n</sup></i>	normal: eyes size and shape are normal, tarsal claws are yellow, alleles are variable in female fertility	<i>lz<sup>B</sup>, lz<sup>+</sup>, lz<sup>75V</sup>, lz<sup>ex</sup></i>
<i>lz<sup>sI</sup></i>	slight: eyes are almost normal in size and shape with slight facet disorganization at the posterior rim, claws are yellow, alleles are variable in female fertility	<i>lz<sup>+</sup>, lz<sup>B</sup>, lz<sup>75V</sup>, lz<sup>ex</sup></i>
<i>lz<sup>B</sup></i>	eyes size is slowly reduced, the colour is brownish, facet disorganization at one half of the eye, claws are yellow and reduced, females are sterile	<i>lz<sup>+</sup>, lz<sup>n</sup>, lz<sup>sI</sup>, lz<sup>75V</sup>, lz<sup>ex</sup></i>
<i>lz<sup>75V</sup></i>	eyes size is moderately reduced, oval, no true facets, the colour is red brown, claws are vestigial, females are sterile	<i>lz<sup>+</sup>, lz<sup>sI</sup>, lz<sup>B</sup>, lz<sup>ex</sup></i>
<i>lz<sup>ex</sup></i>	extreme: eyes size is about one half of normal, facets are fused and the whole eye has glossy surface, the colour is yellow brown with a darker rim, claws are vestigial, females are sterile	<i>lz<sup>75V</sup>, lz<sup>+</sup></i>

Table 2. Changing of mutation frequency of *lz* alleles over the period of the maintenance in the laboratory conditions.

Strain	Year	<i>lz<sup>+</sup></i>	<i>lz<sup>sI</sup></i>	<i>lz<sup>ex</sup></i>	<i>lz<sup>B</sup></i>	Total number
<i>lz<sup>75V</sup></i>	1975	$2.6 \cdot 10^{-2}$	$1.5 \cdot 10^{-3}$	$3.4 \cdot 10^{-4}$	0	5,919
	1986	$8.0 \cdot 10^{-4}$	0	$2.6 \cdot 10^{-4}$	0	3,832
	1987	$1.9 \cdot 10^{-4}$	$1.9 \cdot 10^{-4}$	0	0	5,163
	1989	0	0	0	0	1,772
	1993	0	0	0	0	2,697
<i>lz<sup>75V</sup> v f</i> *	Jan 1987	$7.4 \cdot 10^{-3}$	$1.0 \cdot 10^{-2}$	0	0	1,617
	Mar 1987	$5.0 \cdot 10^{-3}$	0	0	0	776
	Apr 1987	$9.0 \cdot 10^{-4}$	0	0	0	1,105
	Dec 1987	$3.0 \cdot 10^{-3}$	0	0	0	2,028
<i>y ct lz<sup>sI</sup></i> *	1980	$1.0 \cdot 10^{-2}$	—	0	$1.4 \cdot 10^{-3}$	1,387
	1983	$2.0 \cdot 10^{-2}$	—	$6.0 \cdot 10^{-4}$	$6.0 \cdot 10^{-4}$	8,282
	1987	0	—	0	0	1,074
<i>lz<sup>B</sup></i>	1986	$1.8 \cdot 10^{-3}$	0	0	—	7,071
	1987	$1.2 \cdot 10^{-3}$	0	0	—	5,032
	1989	$9.0 \cdot 10^{-4}$	0	$1.3 \cdot 10^{-4}$	—	7,684
	1993	$1.9 \cdot 10^{-3}$	0	$5.0 \cdot 10^{-4}$	—	2,046

\* Strain was derived by the recombination of *lz<sup>75V</sup>* with marker *y ct v f* stock.

some seems to be a full length element capable to induce the GD sterility in standard test. Second, numerous outcrosses of *lz<sup>\*</sup>* males with M-cytotype females of laboratory stocks *C(1)DX* and *Basc* did not lead to destabilization of stabilized *lz<sup>\*</sup>* alleles. To take an account of these facts we suppose that the regulation resulting in stabilization of alleles is achieved at the intrachromosomal level. The results of some experiments on the recombination substitutions of different *lz<sup>75V</sup>*-X-chromosome regions indirectly confirmed this assumption. In these experiments the wide variations of mutation rates were sometimes observed. To take an example, that was the way of the occurrence of the high mutable *lz<sup>75V</sup> v f* strain (see Table 2) from the relatively stable to 1987 *lz<sup>75V</sup>* allele.

If the *lz<sup>75V</sup>* instability was due to the movement of P element or other transposable elements, the increased mutagenesis at the other genes of the same X chromosome should be expected. Actually, during an 18 year observation period we found in the X chromosome carrying *lz<sup>75V</sup>* the arising of three stable *white* mutants in 1986, 1988, and 1989, two apparently stable (mutability was not tested) *yellow* mutants (*y<sup>2</sup>* in 1976 and *y<sup>1</sup>* in 1990) and two unstable mutants (*sn<sup>49</sup>* in 1975 and "rough eyes", designated as *rou<sup>1</sup>* and mapped to the region of 6-8 m.u. in 1986). As it follows from these data, the increased level of mutagenesis in other loci is really peculiar to *lz<sup>75V</sup>*-X-chromosome. Nevertheless, the most frequently mutating locus in the strains originating from *lz<sup>75V</sup>* is *lozenge*.

To summarize, the unstable *lz<sup>75V</sup>* allele exhibits a wide phenotypic variety of derivative mutant alleles both stable and unstable, temporal stabilization of some alleles and occasionally arising the mutant alleles of some other loci of the same X chromosome.

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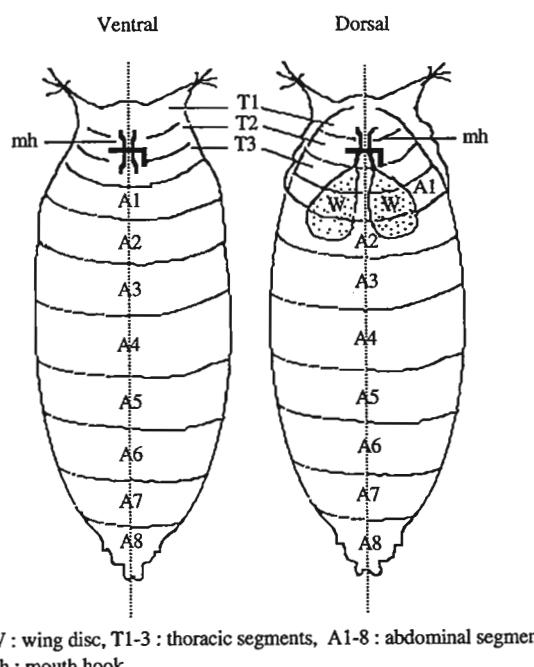
(the mutation rate was approximately  $10^{-2}$ ) during the observation from 1980 to 1983 but it became stable by 1987. Gradual stabilization over a period of years of the original *lz<sup>75V</sup>* allele was observed (see in detail Table 2). There are, however, some unstable alleles retained the constant mutation rate over the total period of observation (such as *lz<sup>B</sup>* presented in Table 2). There was not any case of opposite process of spontaneous destabilization of previously stable strains.

All the described traits of *lz<sup>75V</sup>* instability allow us to consider it to be due to insertion. The data of *in situ* hybridization of a P element probe indicated the presence of P elements at numerous sites of *lz<sup>75V</sup>*-X-chromosome including the cytological location of *lozenge* (8D4) (Golubovsky and Belyaeva, 1985). We assume it is the P element insertion that induces the unstable *lz<sup>75V</sup>* allele.

From this point of view the process of stabilization of unstable alleles can be most simply explained by the establishment of P-cytotype-like regulation. But at least two facts are opposite to this interpretation. First, no one of the P elements located in the *lz<sup>75V</sup>*-X-chromo-

**Gendre, N. and R.F. Stocker.** Institute of Zoology, University of Fribourg, Pérolles, CH-1700 Fribourg, Switzerland. Surface transplantation of imaginal discs for generating ectopic legs and wings on the thorax.

fifth abdominal segment of prepupal hosts. Grafting on thoracic segments was believed more difficult because of a risk of damaging the heart and because of a possible rejection of the graft by the everting discs of the host fly. For a study of the role of disc-associated myoblasts in the formation of thoracic muscles (K. VijayRaghavan *et al.*, in preparation), we have tried to modify the transplantation technique in order to enable the generation of supernumerary midlegs and wings on the thorax. This would allow one to determine the contribution of the donor's myoblasts to the host's muscle development. Using the protocol of Schmid *et al.* (1986), a series of new potential grafting sites on the thorax (as well as on more anterior and more posterior abdominal segments) were explored.



W : wing disc, T1-3 : thoracic segments, A1-8 : abdominal segments  
mh : mouth hook

Figure 1. Incision sites (indicated as L-shape) for transplantation on the ventral and dorsal surface of light brown prepupae.

Surface transplantation of imaginal discs in *Drosophila* was first established by Schubiger (1982). The method was improved later by Schmid *et al.* (1986) who succeeded in generating everted supernumerary wings, halteres, legs and even eyes and antennae at high frequencies. Discs were transplanted laterally on to the

As hosts light brown prepupae (2-3 hr after puparium formation: APF) of the *Minute* strain  $M(3)w^{124} Ki Sb^{63b}/TM2$  and of the wild type strain Sevelen were used. Choosing *Minute* strains as hosts provides the donor  $M^+$  discs with a growth advantage resulting in enlarged ectopic appendages. *Ki* and *Sb* markers allow an easy recognition of the graft border in the adult. Wing and midleg imaginal discs were from white prepupae (*i.e.*, up to 1 hr APF) of the donor lac-Z strains MHC-lacZ, IFM Actin88F-lacZ and Twist-lacZ (Fernandes *et al.*, 1991).

For transplantation the host was covered with Ringer's solution and a small L-shaped incision was made into the pupal case with a sharp microscalpel (Schubiger, 1982; Schmid *et al.*, 1986). The disc was then placed under the flap using a "constriction glass capillary" (Ursprung, 1967). Examination of different thoracic sites revealed that supernumerary legs or wings may be generated both dorsally or ventrally. For dorsal grafts, the cut was made across the dorsal midline near the mouth hooks, just anterior to the host wing discs which are well visible when the pupa is examined under the dissecting microscope (Figure 1). This site is well anterior to the pulsating heart. It corresponds approximately to the border between second and third thoracic segments which is difficult to be seen through the concave prepupal operculum. After evagination, ectopic appendages were often found slightly more posterior, *i.e.*, at the thoracico-abdominal border, probably because they were displaced by the host's discs. Transplantation directly at the thoracico-abdominal border failed because of damage to the heart.

For leg or wing grafts on the ventral thorax, the incision was made across the ventral midline at the border between second and third thoracic segments, which

is more distinct than in the dorsal thorax (Figure 1). Ventral ectopic appendages usually evaginated slightly dorsal to the host's legs except for a few cases that grew at the ventral midline pushing the host's legs aside. Successful leg and wing transplants were also made at the ventral midline of abdominal segments 1 to 3, and there is no reason to believe that grafting on the remaining segments would fail. Likewise, lateral transplantation seems to be possible on all abdominal segments.

In all types of transplantations, the rate of eclosing flies was always considerably more than 50%. Among the survivors, a large majority of the leg and wing discs evaginated very well (Table 1, Figure 2). However, the small leg

Table 1. Evagination success of the various types of surface transplants.

transplantation site	transplanted imaginal disc	no. survived animals	evagination state		
			very good	good	poor
T2 dorsal	wing	13	5	6	2
	2nd leg	2	2	0	0
T2 ventral	wing	12	4	6	2
	2nd leg	7	0	6	1

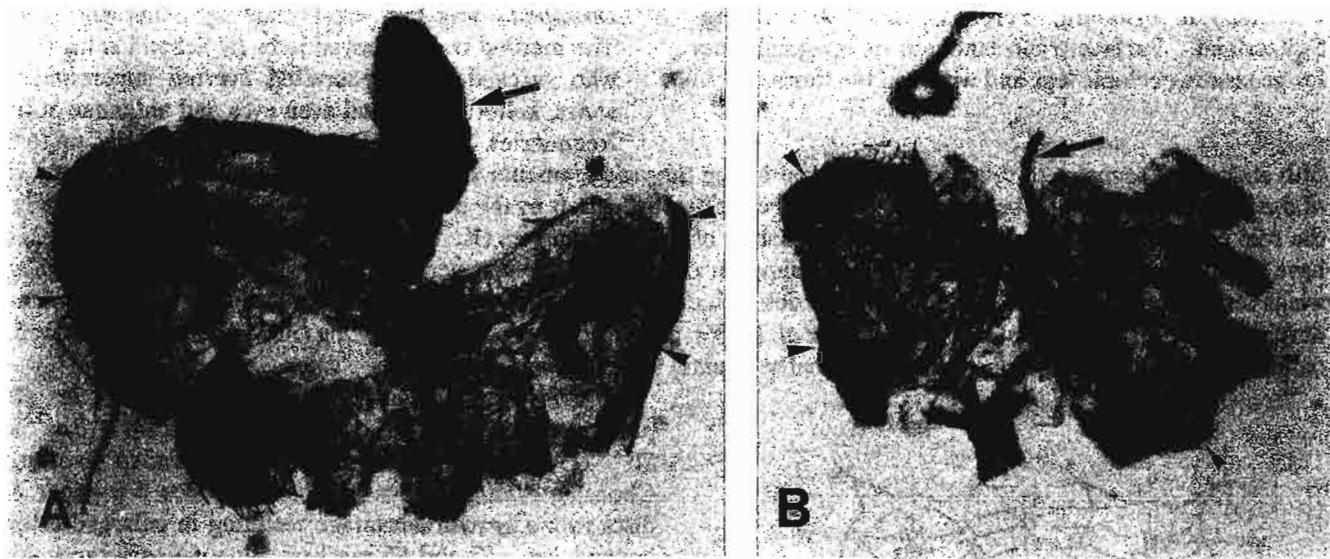


Figure 2. Ventral thoracic supernumerary wing (A; arrow) and midleg (B; arrow). The host thorax was cut and opened along the dorsal midline (arrowheads), and the host's wings and legs were removed. The host was either wild type (A) or  $M(3)w124 Ki Sb^{63b}$  (B).

discs integrate much better into the host's environment with its own evertting discs than the large wing discs.

Ectopic or supernumerary grafts are powerful tools for studying developmental interactions between peripheral and internal tissues, as well as between an appendage and its surrounding epithelium. The role of position may be studied along the anterior-posterior axis (*i.e.*, across segment boundaries) as well as along the dorsal-ventral direction. The following applications are conceivable: (1) By tracing sensory afferents, ectopic grafts allow one to study the role of the position of a particular sensory epithelium in the establishment of its central connections (Schmid *et al.*, 1986). (2) Likewise, by following the migration of myoblasts from genetically marked ectopic donor discs it may be explored how the location of a particular imaginal disc on the body affects the development and specification of muscles (K. VijayRaghavan *et al.*, in preparation). (3) Ectopic transplantation is particularly attractive since it allows one to deliberately choose the genotype of host and donor and to generate mosaics in a site specific manner that is not feasible with genetic techniques. (4) Supernumerary transplants are excellent tools for studying the role of competition, for example in the establishment of the central connectivity of afferents. (5) Surface transplantation may also allow one to follow how the various imaginal discs fuse with each other during metamorphosis in order to construct the adult epithelium. The establishment of our new technique of generating ectopic appendages on the thorax represents a substantial improvement for all the applications mentioned.

**Acknowledgments:** This work was supported by grants from the Swiss National Funds (No. 31-32479.91) and from the Sandoz Foundation.

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**Band, H.T.** Michigan State University, E. Lansing, MI 48824. A breeding site for drosophilids in Iron Mountain, MI.

Repeated visits to Iron Mt., Michigan (lat. 45.49N, long. 88.04W) in summer had failed to uncover breeding sites for drosophilids despite continued sampling of fruits (apples, ornamental crabapples) and nuts (acorns) in scattered locations over the past several years. This

contrasted with the ease of collecting *D. athabasca*, *D. busckii* and *D. immigrans* on/from melon and *C. amoena* on apples in neighboring Sagola in the 1980s (Band and Band, 1983) and of trapping *Chymomyza* on cut wood at various locations (12 *Chymomyza aldrichi*: lumber yard, August 1987; 2 *Chymomyza* spp.: birch logs, September 1992) in the Iron Mountain-Sagola area. Since the drosophilid collection in the Entomology Museum of Michigan State University

listed only *C. amoena* as having been collected in Dickinson Co., the aim has been twofold: 1) to be able to obtain *C. amoena* from the 45° N latitude for research and 2) to determine what other drosophilids can be collected in this northern location.

In 1993 collections of fruits were made in a garden in August and September in Iron Mountain near Lake Antoine which had a group of fruit trees (apples, 1 plum). Table 1 shows the species emerging and numbers. *Drosophila melanogaster* F1s were sent to John Ringo. The emerging *C. amoena* were used directly in crosses with Swiss *C. amoena*, with East Lansing, MI *C. amoena* and with Virginia *C. amoena*, and to establish a stock of Iron Mountain *C. amoena* for additional future work. Millar and Lambert (1985) had argued for the stability of mate recognition systems because of the interfertility of New Zealand *D. pseudoobscura* with recently established stocks of *D. pseudoobscura* from various West Coast locations. Although the results will be reported in detail elsewhere, the interfertility of Iron Mountain *C. amoena* with Swiss *C. amoena* and other newly emerging Michigan *C. amoena* supports the argument that *C. amoena* is a recent immigrant to Europe (Burla and Bächli, 1992; Máca and Bächli, 1994). *Chymomyza amoena* may be a weedy colonizing species which is now rapidly expanding its range in Europe, especially Eastern Europe (Máca and Bächli, 1994).

Table 1. Substrates collected in Iron Mountain, Michigan in 1993, month collected, drosophilid species emerging and numbers.  
f = females; m = males

Substrate	Month collected	Species	Number
apples	August	<i>Scaptomyza adusta</i>	1
		<i>Chymomyza amoena</i>	52
apples	September	<i>Drosophila falleni</i>	1 f
		<i>D. melanogaster</i>	5 f
		<i>C. amoena</i>	38
plums	September	<i>D. melanogaster</i>	1 m

The emergence of *D. falleni* from apples is in agreement with the fact that this species in spring in Michigan can be captured on both mushrooms and fruits (Band, 1993, 1994).

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**Joly, Dominique.** Laboratoire de Biologie et Génétique Evolutives, CNRS 91198 Gif sur Yvette Cedex, France. Number of spermatocytes per cyst in six species of the *Drosophila melanogaster* subgroup.

According to the species, the number of gonial divisions (n) and hence of secondary spermatocytes varies. As a result the number of spermatids within a bundle may differ from one species to another. All spermatogonia within one cyst are clonally derived and interconnected by a system of cytoplasmic bridges which are persistent until the individualization process at the end of the spermatogenesis (Hackstein, 1987). Development and differentiation of all cells within this syncytium are highly synchronous. The number of spermatogonia after the latest mitotic division and just before the meiosis is  $N = 2n$ .

Common development of all spermatocytes within a cyst could explain the existence of sperm with only a part of the normal chromosomal constitution. Other hypotheses emphasize the synchronous differentiation within the cysts; the synchronous divisions are able to insure the same development of all cells by exchanging proteins from one cell to another (King and Büning, 1985). This process is highly efficient during the RNA synthetic activity. All biochemical data show that the transfer of developmental information from genome to RNA is restricted to the primary spermatocyte stage (*i.e.*, the diploid stage) of germ cell development. It is obvious that the full genetic complement is present only premeiotically (Hackstein, 1987). Considering that the interconnected  $2n$  spermatocytes are the mitotic product of one single cell they can be compared to a clone.

Hihara (1976) showed that 4 mitotic divisions are expressed in both males and females just before meiosis and followed by an incomplete cytokinesis in species like *D. melanogaster*, *D. tumiditarsus* and *D. sordidula*. However, other species of the Drosophilidae family do not exhibit the same number of mitotic divisions between males and

However, years ago Throckmorton (personal communication) raised the possibility that North American populations which were sufficiently geographically distant might be reproductively isolated. Nevertheless, populations from Michigan's Lower Peninsula, Missouri and the mid-South remained interfertile (Band, 1988a,b) and populations from the Virginia mountains and eastern part of the state likewise were interfertile. Virginia populations also were similar in mating duration (Band, 1992). Further work is underway on *C. amoena* from Michigan, Virginia and Switzerland.

Like other Diptera, the process of spermatogenesis starts in *Drosophila* with the formation of primary spermatogonia by an unequal division of a stem cell. These stem cells occupy an apical position at the tip of the testis where they are anchored. The spermatogonium becomes invested by two cyst cells.

females. Analysis of 82 species shows that 3, 4, 5, or 6 mitotic divisions are expressed in males (Table I). Consequently, the number of first spermatocytes per cyst is 23 (= 8), 24 (= 16), 25 (= 32) and 26 (= 64), respectively. The two subsequent meiotic divisions generate 32, 64, 128 and 256 spermatids per cyst, respectively (Dobzhansky, 1934; Kurokawa and Hihara, 1976; Lindsley and Tokuyasu, 1980; Hanna *et al.*, 1982; King and Büning, 1985).

Table 1. Number of first spermatocytes per cyst ( $2^n$ ), and spermatids per bundle ( $2^n \times 4$ ) during either normal (left column) or abnormal development (right column) in 82 species of the Drosophilidae family (after Dobzhansky, 1934, Kurokawa and Hihara, 1976; Lindsley and Tokuyasu, 1980; Hanna *et al.*, 1982; King and Bünning, 1985).

$2^n$	$2^3 = 8$		$2^4 = 16$		$2^5 = 32$		$2^6 = 64$	
$2^n \times 4$	32	17 - 31	64	33 - 63	128	65 - 127	256	
s. Family Steganinae								
G. <i>Amiota</i>			2					
G. <i>Leucophenga</i>			4					
s. Family Drosophilinae								
G. <i>Drosophila</i>								3
sg. <i>Scaptodrosophila</i>								
sg. <i>Sophophora</i>								
gr. <i>obscura</i>						6		
gr. <i>melanogaster</i>								
sg. <i>Drosophila</i>	10	4	14	1				
sg. <i>Hirtodrosophila</i>	2		17	4			3	
sg. <i>Dorsílopha</i>			1			3	1	
G. <i>Microdrosophila</i>							2	
G. <i>Chymomyza</i>			1					
G. <i>Liodrosophila</i>			1					
G. <i>Scaptomyza</i>			3					
Total Drosophilidae	12	4	43	5	11	4		3

Table 2. Number of first spermatocytes per cyst ( $2^n$ ) and spermatids per bundle ( $2^n \times 4$ ) during either normal (right column) or abnormal development (left column). In 6 species of the *D. melanogaster* subgroup. The Taï (Ivory Coast) and Silinda (Zimbabwe) isofemale lines of *D. teissieni* are characterized by two main morphs of relatively short cysts and one main morph of relatively long cysts respectively.

$2^n$	$2^3 = 8$ 32	$2^4 = 16$ 64	$2^5 = 32$ 33 - 63	$2^6 = 64$ 128	$2^7 = 128$ 65 - 127
$2^n \times 4$					
<i>D. simulans</i>		17			
<i>D. mauritiana</i>	1	12	10		
<i>D. erecta</i>		34	1		1
<i>D. oreana</i>		17	1		1
<i>D. yakuba</i>		4			
<i>D. teissieri</i> Taï	1	27			1
Silinda		17	5		3
Total	2	128	17		6

The data presented here analyse this relationship in species of the *melanogaster* subgroup where sperm length is species-specific (Joly, 1987). This taxon is appropriate because of the existence of *D. teissieri* which is characterized by sperm heteromorphism (intra-individual variation), like species of the *D. obscura* group, but unlike its close relatives (Joly *et al.*, 1991). The number of first spermatocytes per cyst was counted from pupae at the distal part of the testis in 6 species of the *D. melanogaster* subgroup. The great majority of individuals show 16 first spermatocytes per cyst, indicating 4 mitotic divisions, consistently with most Diptera (Table 2). However, in a few cases, it is possible to find a variable number of first spermatocytes per cyst. Undeniably, in *D. teissieri* the existence of two different categories of cysts (short and long) does not result from a different number of mitotic divisions. However, it is worth noting that the number of first spermatocytes per cyst is much more variable in *D. mauritiana* than in any other relatives with 50% of the cysts analysed having less than 16 spermatocytes per cyst. This species is, therefore, characterized by a narrow cyst length distribution. Moreover, all the species of this subgroup can be considered as homogenous for the number of mitotic divisions but not for cyst length which varies from one to two millimeters (Joly, 1987; Joly *et al.*, 1989). Finally, the results do not support the hypothesis of a close relationship between the number of first spermatocytes per cyst and sperm length in this taxon.

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Table 1 shows that there is partial asynchronous divisions of spermatogonia in some species with a variable number of spermatids per cyst ranging from  $(2n-1 \times 4)$  to  $(2n \times 4)$ . This variability is observed between either species, individuals, or cysts in a same testis and between developmental stages (adults versus larvae; Hanna, 1981; Hanna *et al.*, 1982). There are two different hypotheses explaining the origin of asynchrony: 1) all cells but one further divide within a cyst, or 2) one cell

degenerates while not the others. Of interest is the possibility that the number of spermatocytes per cyst could be correlated to sperm length. Assuming the number of first spermatocytes per cyst is variable from one cyst to another in a testis, and meiosis goes normally, the number of spermatids should be variable to the same extent. In *D. neohydei* 16, 20 or 24 sperm are observed per bundle (Hanna *et al.*, 1982). One could expect a relation between short and long cysts and the number of spermatocytes per cyst in heteromorphic species like those of the *obscura* group (Joly *et al.*, 1989).

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**Krejci, Carmen M., Katherine G. Rendahl and Jeffrey C. Hall.** Department of Biology, Brandeis University, 415 South Street, Waltham, MA 02254-9110, (617) 736-3170, FAX (617) 736-3107. Rescue of behavioral phenotypes by a *nonA*-null transgene is influenced by chromosomal position effects.

formation (Jones and Rubin, 1990; Rendahl *et al.*, 1992).

An open reading frame was identified that appears to be necessary and sufficient to rescue visual defects in the hypomorphic allele, *nonA*<sup>H2</sup> (Jones and Rubin, 1990), and courtship song abnormalities caused by the *nonA*<sup>diss</sup> allele (Rendahl *et al.*, 1992). Mutations affecting single amino acids in this open reading frame have been found in both of these alleles (Rendahl and Hall, 1992). Flies transformed with a construct containing a stop codon inserted in this open reading frame showed no rescue of the above behavioral phenotypes (Jones and Rubin, 1990; Rendahl *et al.*, 1992).

A second mutation was made in this open reading frame that deleted two base pairs in exon 1 of the genomic fragment, creating a construct called 235R11-SacII (Jones and Rubin, 1990). The *nonA*<sup>H2</sup> flies containing this mutant construct failed to exhibit rescue of the visual phenotype (Jones and Rubin, 1990). Similarly, *nonA*<sup>diss</sup> flies carrying this transgene were optomotor blind. Yet, two of the five transformed lines in the *nonA*<sup>diss</sup> background showed complete rescue of the courtship song phenotype, while the other three lines showed the expected mutant courtship behavior associated with the *nonA*<sup>diss</sup> allele (data not shown). Similar results were obtained when these constructs were tested for phenotypic rescue in a *nonA* null background (Stanewsky *et al.*, 1993). Both of the rescuing transformant lines, P[(*ry*+)]235R11-SacII]B and P[(*ry*+)]235R11-SacII]E exhibited an intermediate, although not completely wild-type, courtship song phenotype in this genetic background, showing no significant differences in Cycles Per Pulse (CPP) scores (see Table legend) from either the rescuing genomic 235R11 lines, or the mutant *nonA* null control. The non-rescuing lines, P[(*ry*+)]235R11-SacII]C and P[(*ry*+)]235R11-SacII]D, on the other hand, were mutant in their courtship song, showing significantly different CPP scores from the wild-type genomic 235R11 line and no significant difference from the *nonA* null (Stanewsky *et al.*, 1993). The fifth transformant line, P[(*ry*+)]235R11-SacII]A, was determined to carry two copies of the 235R11-SacII construct and therefore was not analyzed further.

These results suggest the existence of a second product, perhaps the result of a cryptic splicing event or a translational frameshifting of the original gene product, which would rescue *nonA* locus related courtship song abnormalities in the P[(*ry*+)]235R11-SacII]B and P[(*ry*+)]235R11-SacII]E lines. A position effect that would be responsible for such a cryptic event in these two lines, seems to be operating.

The following genetic manipulations and behavioral analyses were undertaken to determine if the cause of the phenotypic rescue of courtship song in the P[(*ry*+)]235R11-SacII]B and P[(*ry*+)]235R11-SacII]E lines was indeed due to position effects on the inserted DNA. The chromosomal insertion sites of the 235R11-SacII constructs in these lines were genetically mapped and found to be on the second chromosome. These lines were then crossed to a strain containing the transposase source, *Sb delta-23/TM2* (Robertson *et al.*, 1988), which was removed in the next generation by crossing to *ry*<sup>506</sup>. Lines containing putative mobilization events were crossed to the *TM2/MKRS* and subsequently to *ry*<sup>506</sup> strains in order to detect hops of the P-element to the third chromosome. Lines that indicated such transposition events were then crossed to the *nonA* null genetic background and the progeny analyzed behaviorally.

The mobilization rate of the P-elements from the second to the third chromosome was calculated from multiple trials of the genetic crosses. A rate of 1 transposition event in every 54 lines scored (1.9%) was observed for the P[(*ry*+)]235R11-SacII]E line (total of 214 lines screened). A rate of only 1 hop in 285 lines (0.35%) occurred in crosses involving the P[(*ry*+)]235R11-SacII]B line (total of 285 lines screened). A control experiment was conducted using the

The *dissonance* allele at the *nonA* locus leads to a mutant courtship song, optomotor blindness, and an electroretinogram (ERG) deficit (Kulkarni *et al.*, 1988). The *nonA* null allele also exhibits these phenotypes, as well as a decrease in viability (Stanewsky *et al.*, 1993). The 11 kb genomic fragment 235R11 containing the *nonA* gene has been shown to rescue both sight and pulse song in the *nonA*<sup>diss</sup> allele by P-element transformation (Stanewsky *et al.*, 1993).

same transposase source and a line containing a different P-element located on the X chromosome, which allowed detection of twice the number of hops (*i.e.*, to either the second or third chromosome) as in the above cases. This control gave 1 transposition event in 21 lines (5%, total of 126 lines screened). The mobilization rate for the control data was similar to that seen in the  $P[(ry^+)]235R11-SacII]E$  line. The control differed from the low transposition rate observed in the  $P[(ry^+)]235R11-SacII]B$  line by a factor of 7.

Overall, the phenotypic pattern of the mobilized transformant lines containing the SacII construct showed more severe mutant courtship songs, consistently mutant optomotor scores and a general decrease in viability, relative to the parental lines. As indicated in Table 1, a decrease in viability was observed in all of the mobilized lines. As expected, the visual defect was not rescued in any of the mobilized lines; optomotor scores ranged from  $46 \pm 7\%$  to  $65 \pm 6\%$  (*c.f.* Kulkarni *et al.*, 1988). Optomotor blindness was also seen in all of the original  $P[(ry^+)]235R11-SacII]$  lines and the *nonA* null controls, 211S12A and B (Table 1, this work; Stanewsky *et al.*, 1993). All of the newly transposed lines, generated from  $P[(ry^+)]235R11-SacII]B$  and  $P[(ry^+)]235R11-SacII]E$ , gave more severely mutant CPP scores than the parental lines. However, only the  $P[(ry^+)]235R11-SacII]E$  Hops I and IV were significantly different from the  $P[(ry^+)]235R11-SacII]E$  parental line. The  $P[(ry^+)]235R11-SacII]B$  and all of the  $P[(ry^+)]235R11-SacII]E$  mobilized lines were significantly different from the 235R11A and 235R11B rescuing lines containing the wild-type genomic clone. None of these mobilized lines had significantly different CPP values than those of the *nonA* null genetic background or the  $P[(ry^+)]235R11-SacII]C$  and  $P[(ry^+)]235R11-SacII]D$  lines (Stanewsky *et al.*, 1993). Southern blot analysis indicates that the 235R11-SacII construct showed the expected removal of the SacII restriction site in all of the original lines.

These data indicate that there is indeed a position effect operating on insertions of the 235R11-SacII construct. This supports the notion that a single identified open reading frame at the *nonA* locus is necessary and sufficient for the rescue of the mutant behavioral phenotypes, because 9 of 11 lines containing the frameshifted 235R11-SacII construct fail to show phenotypic rescue. This new information helps to clarify, in a formal manner, how the rescue of courtship song phenotype occurred in two strains, although the mechanism by which this occurred is unknown.

Table 1. Behavioral analysis of mobilized lines and control genotypes

Genotype	Viability (n = 100)	Optomotor (n = 4)	Song regression (CPP) (n = 5 or 8)	p value
<b>rescued controls</b>				
235R11A	*96%	ND	* $0.02 \pm 0.15$	
235R11B	*81%	ND	* $0.22 \pm 0.05$	
<b><i>nonA</i> null controls</b>				
211S12A	14%	ND	* $2.00 \pm 0.49$	0.0030
211S12B	16%	ND	* $3.35 \pm 0.44$	0.0001
<b>SacII lines</b>				
$P[(ry^+)]235R11-SacII]B$	46%	52 ± 21	* $1.05 \pm 0.23$	0.1519
$P[(ry^+)]235R11-SacII]B$ Hop I	22%	49 ± 4	1.34 ± 0.28	0.0349
$P[(ry^+)]235R11-SacII]E$	44%	65 ± 6	* $0.87 \pm 0.13$	0.2560
$P[(ry^+)]235R11-SacII]E$ Hop I	30%	46 ± 7	2.87 ± 0.46	0.0001
$P[(ry^+)]235R11-SacII]E$ Hop II	38%	55 ± 10	1.75 ± 0.46	0.0048
$P[(ry^+)]235R11-SacII]E$ Hop III	36%	46 ± 6	1.40 ± 0.25	0.0277
$P[(ry^+)]235R11-SacII]E$ Hop IV	19%	52 ± 3	2.02 ± 0.26	0.0010
$P[(ry^+)]235R11-SacII]C$	*28%	ND	* $1.77 \pm 0.46$	0.0090
$P[(ry^+)]235R11-SacII]D$	*34%	ND	* $1.97 \pm 0.57$	0.0034

Table 1: Individual 3-7 day old, heterozygous males were placed with 1-3 day old attached-X *y* *f/Y* virgins in the recording chamber of an Insectavox (Gorczyca and Hall, 1987). Courtship song and behavior were recorded on Hi-8 video cassettes (Maxell) for 10 minutes per fly, using a Sony Hi8 Pro video recorder. Song was then digitized with a MacAudios11 digitizer and transferred to a Macintosh II computer. Pulse song was logged using Lifesong software (Bernstein *et al.*, 1992). A pulse train represents one bout of male fly song and each pulse contains several cycles. Linear

regression was performed to determine Cycles Per Pulse (CPP) values. Higher CPP regression scores are indicative of a mutant phenotype. One way ANOVA and Fisher's protected least significant difference (FPLSD) *post hoc* tests were done to determine the significance of the genotypic differences ( $\alpha = 0.05$ ), using Abacus Statview 4.0<sup>TM</sup> Software.

Optomotor analysis to determine blindness was also conducted. Individual flies were dark-adapted for 4 hours and tested using the methods described by Greenspan *et al.* (1980) and Kulkarni *et al.* (1988). Viability data were collected by scoring the number of hemizygous *nonA* null males per 100 heterozygous females containing a given transgene, as in Stanewsky *et al.* (1993).

Viability and song scores indicated by asterisks (\*) are taken from Stanewsky *et al.* (1993). The optomotor scores for genotypes marked ND (not done) were tested with a different apparatus and are reported in Stanewsky *et al.* (1993).

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**Budnik, M., and L. Cifuentes.** Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Casilla 70061 Stgo. 7 Santiago, Chile. Larval viability and rate of development of *Drosophila subobscura* competing simultaneously with two local species of *Drosophila*.

of larval interspecific competition in combination of three species. Preadult viability and egg-to-adult development time were studied.

#### Material and Methods

The following stocks were used: a) Chilean stock of *D. subobscura* Collin, b) *D. pavani* endemic Chilean species of the *mesophragnatica* group (Brncic and Koref-Santibanez, 1957), c) *D. funebris*. All the strains came from La Florida, (in the South eastern zone of Santiago) and they were maintained in mass culture for several months in the laboratory before the experiments.

The methodology used to competition on the survival was similar to the one used to study the effects of preadult and development time (in days) reported by Budnik *et al.* (1982, 1983). Thirty vials were used, each containing 10 cc of basic cornmeal-yeast agar medium, into which 50 fertilized eggs of the same age of *D. subobscura* were put together with 50 fertilized eggs of *D. pavani* and 50 eggs of *D. funebris*, with a total of 1,500 eggs sown per experimental group. As a control, 30 vials were established with the same amount of medium, into each of which 150 fertilized eggs of *D. pavani* or either of *D. subobscura* or *D. funebris* were transferred. All the eggs represented a random sample of those laid by 150 inseminated females from each stock.

The eggs were allowed to hatch and to develop at constant temperature of 19°C, and the emerging adults were counted and recorded every day in order to estimate the viability and development time of species.

#### Results and Discussion

Table 1 shows that the preadult viability of *D. subobscura* decreased when grown under competitive conditions simultaneously with two other species, and the differences with respect to the control group are statistically significant. Table 2 shows that the egg-to-adult development time of *D. subobscura* and *D. funebris* are significantly shortened, but *D. pavani* is significantly lengthened in these experimental conditions when compared with the control groups.

Results obtained in previous experiments in interspecific competition between *D. subobscura* and *D. pavani* (Budnik *et al.*, 1982) and *D. subobscura* with *D. funebris* (Budnik, unpublished data), showed that this type of larval interaction is modified when a third competitor is introduced. Table 3 shows a summary of these conclusions.

When *Drosophila subobscura* was first detected in Chile (Brncic and Budnik, 1980), various articles have been published of "exploitation" and "interference" competition between the colonizing species and the native Chilean species (for a full revision, see Budnik and Brncic, 1982; Brncic and Budnik, 1987). All these studies were done in combination of two species.

In this note, the authors wish to report the results

Table 1. Differences in egg-to-adult viability of *D. subobscura*, *D. pavani*, and *D. funebris* in intra and interspecific competition at 19°C (each group consisted of 30 vials).

Eggs per vial	Viability per vial (X ± S.E.)	T-test	p (58 d.f.)
50 <i>D. subobscura</i> (100 <i>D. subobscura</i> )	21.14 ± 0.685		
50 <i>D. subobscura</i> (50 <i>D. pavani</i> ) (50 <i>D. funebris</i> )	13.86 ± 1.315	4.90	<0.0001
50 <i>D. pavani</i>	18.82 ± 0.562		
50 ( <i>D. pavani</i> ) (50 <i>D. subobscura</i> ) (50 <i>D. funebris</i> )	20.90 ± 0.986	1.83	0.072
50 <i>D. funebris</i> (100 <i>D. funebris</i> )	18.94 ± 1.835		
50 ( <i>D. funebris</i> ) (50 <i>D. subobscura</i> ) (50 <i>D. pavani</i> )	18.56 ± 2.350	0.12	0.900

Table 2. Differences in egg-to adult development time (in days) of *D. subobscura*, *D. pavani* and *D. funebris* in intra and interspecific competition at 18 °C (each group consisted of 30 vials).

Eggs per vial	Development time (in days) (X ± S.E.)	T-test	p (∞ d.f.)
50 <i>D. subobscura</i> (100 <i>D. subobscura</i> )	210.42 ± 0.122		
50 <i>D. subobscura</i> (50 <i>D. pavani</i> ) (50 <i>D. funebris</i> )	18.73 ± 0.118	9.95	< 0.0001
50 <i>D. pavani</i> (100 <i>D. pavani</i> )	24.96 ± 0.271		
50 ( <i>D. pavani</i> ) (50 <i>D. subobscura</i> ) (50 <i>D. funebris</i> )	25.63 ± 0.148	2.16	< 0.05
50 <i>D. funebris</i> (100 <i>D. funebris</i> )	21.97 ± 0.162		
50 ( <i>D. funebris</i> ) (50 <i>D. subobscura</i> ) (50 <i>D. pavani</i> )	21.05 ± 0.163	3.99	< 0.001

References: Brncic, D. and S. Koref-Santibañez 1957, Evolution 11:300-310; Brncic, D. and M. Budnik 1980, Dros. Inf. Serv. 55:20; Brncic, D. and M. Budnik 1987, Genética Ibérica 39:249-267; Budnik, M. and D. Brncic 1982, Actas V Congres. Latinoam. Genética 177-188; Budnik, M. and D. Brncic 1983, Oecologia 58: 137-140.

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Storage of short and long sperm in *D. subobscura*.

These two morphs of sperm seem to differ solely by the length of their flagella (Hauschkeck-Jungen and Rutz, 1983; Takamori and Kurokawa, 1986), and two discrete or contiguous distributions of length can be observed (Joly *et al.*, 1989). Evidence has formerly been provided that both morphs are stored (Bressac *et al.*, 1991a). Central to the question here is how they are stored and what are the implications of positioning within the seminal receptacle. Our hypothesis upon the evolutionary significance of such sperm dimorphism is based on the context of fertilization, that is early versus late fertilization, and single versus multiple mating, in which each morph is alternatively favored (Bressac *et al.*, 1991a, b; Joly *et al.*, 1991). We propose that short sperm are devoted to immediate fertilization, just after mating, while long to delayed fertilization. In order to test this assumption further, we used the results of re-embedding experiments consisting of successive optical and Transmission Electronic Microscopic (TEM) observations of the ventral receptacle of newly inseminated females (Pignot-Paintrand and Bressac, 1992). This original technique allows us to precisely determine the positions of heads, flagella and distal ends of sperm within the ventral receptacle. From serial sections observed at the optical level we made the tridimensional reconstruction of the lumen of the sperm-containing receptacle (Figure 1). Then, the TEM pictures give the localization of sperm cross sections (Figure 2) in each part of the receptacle. Basically, three different segments were recognized in the lumen of the receptacle, depending on the content in sperm cross sections (Pignot-Paintrand and Bressac, 1992):

- proximal segment: toward the uterine cavity; this part contains sperm heads and distal parts.
- intermediate segment: contains flagellas and distal parts.
- distal segment: contains sperm heads and distal parts.

The total length of the sperm-containing lumen is similar to that of long sperm, that is 185 um and 199 um (Joly *et al.*, 1989), respectively.

Moreover, sperm heads are more numerous in the proximal than in the distal segment. Sperm sections are mostly perpendicular to the major axis, and the lumen of the receptacle seems to be fully replenished. From these observations, we conclude that all sperm are stored longitudinally in the receptacle. Heads and distal parts observed in both proximal and distal segments may be assigned to long sperm. They are therefore assumed to be stored "head to tail", some being directed toward uterine cavity, others toward the cul-de-sac end. Extra-numerous heads and distal

Table 3. Differences in egg-to adult viability of *D. subobscura*, *D. pavani*, and *D. funebris* in bi and tri-interspecific competition.

Species	Viability	Development time (in days)
<i>D. subobscura</i> / <i>D. pavani</i>	- / +	- / +
<i>D. subobscura</i> / <i>D. funebris</i>	- / +	0 / 0
<i>D. subobscura</i> / <i>D. pavani</i> / <i>D. funebris</i>	- / 0 / 0	- / + / -

+ = increasing; - = decreasing; 0 = no effect

Regarding *D. subobscura*, the results always shows that this species is a bad competitor either in combination of two or three species. These findings are important to be considered in further studies on preadult competition and in the understanding of the colonization of *D. subobscura* in Chile.

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*Drosophila subobscura*, like any other species within the *obscura* group, is characterized by the presence of two sperm types simultaneously elaborated in testis (Joly *et al.*, 1989) and transferred into the uterine cavity of females (Beatty and Sidhu, 1970).

Figure 1. Shape of the lumen of the ventral receptacle in *D. subobscura* drawn from a 3-dimensional reconstruction obtained by serial sections examined under a light microscope. PS = Proximal segment, IS = Intermediate segment, DS = Distal Segment. Arrows indicate head location of both short (s, dashed line) and long (L, dotted line) sperm, identified from electron microscopic examinations. Long sperm may be either one or the other direction.

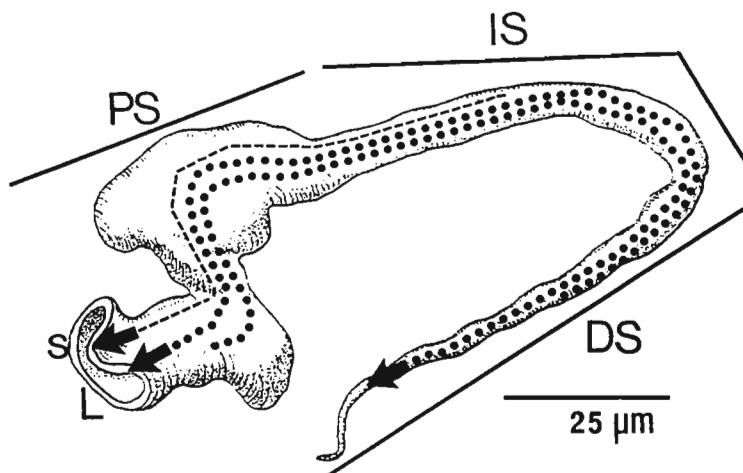
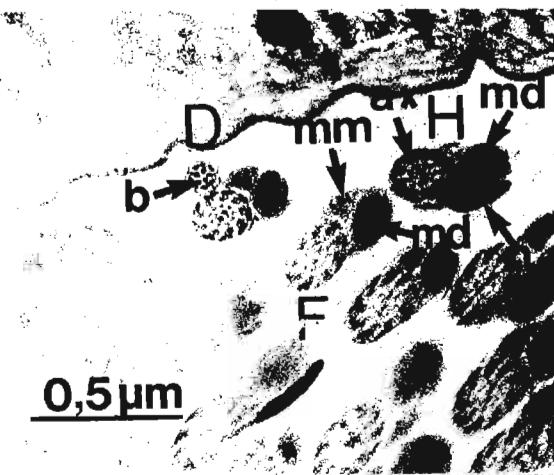


Figure 2. High power electron micrograph view showing together the three typical parts of cross-sectioned spermatozoa contained in the ventral receptacle: H, head region; F, flagellar region; D, tip of the tail. Such a picture is uncommon because the three levels are rarely seen together in the same segment of the receptacle. (n = nucleus, ax = axoneme, md = major mitochondrial derivative, mm = minor mitochondrial derivative, b = basal body).



parts localized in the proximal and intermediate segments, respectively, could correspond to short sperm; Hence, they are seemingly stored in only one direction: toward the uterine cavity (Figure 1).

These results are consistent with our previous hypothesis: being stored head toward uterine cavity, short sperm are most likely devoted to immediate fertilization, and prone to eviction in case of remating. In contrast, long sperm are stored in both directions, like in sperm monomorphic species (*D. melanogaster*, Lefevre and Jonsson, 1962). Such a position may result in better retention in the storage organ through successive remating of females and possibly in longer survival and delayed fertilization (Bressac *et al.*, 1991a, b; Joly *et al.*, 1991).

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**Mohapatra, S., A. Das, and B.B. Parida.** Genetics Laboratory, Department of Zoology, Utkal University, Bhubaneswar - 751 004, India. Starvation tolerance in natural populations of *Drosophila bipectinata* from India.

Two different abiotic stresses have been mainly studied in *D. melanogaster* and *D. ananassae*, i.e., starvation and desiccation tolerance and both physiological and genetic significance have been proposed.

India is a tropical country and several species of *Drosophila* are found in different seasons of the year. Intra- and interpopulational variations to starvation and desiccation tolerance have been detected in several natural populations of *D. melanogaster* and *D. ananassae* (Das *et al.*, 1994a, b, c). *Drosophila bipectinata* is originally described from Darjeeling, India, by Duda (1923) which belongs to the *bipectinata* species complex of the *ananassae* subgroup of the *melanogaster* species group (Bock and Wheeler, 1972). This species is of common occurrence in Indian subcontinent and mainly found in nature during the rainy season of the year. The effect of abiotic environmental factors on the life process has not been studied in this species.

In the present study, we have collected population samples of *D. bipectinata* from five localities in India and maintained them as isofemale lines. The F1 male and female individuals of each isofemale line were subjected to starvation stress at 25°C as described in Das *et al.* (1994a). The results are shown in Table 1, which depicts the mean survival time  $\pm$  s.e. for both male and female individuals in different isofemale lines of all the natural populations of *D. bipectinata*. The difference in survival time between male and female has been calculated by Student's t-test, and, in all populations except Berhampur, it was found to be statistically significant. This signifies that under starvation conditions the survival duration of male and female differs significantly. In each population, in general the female individuals were found to be more tolerant than their male counterparts. Intrapopulational variation to the tolerance was measured by carrying out analyses of variance (ANOVA), and, in all populations except Berhampur, it was found to be significant

Research on the significance of different abiotic environmental stresses to the evolutionary biology of different species of *Drosophila* has proved that different stresses could be manifested by the basic physiological processes (David *et al.*, 1983; Parsons, 1983, 1987; Hoffmann and Parsons, 1989; Da Lage, *et al.*, 1990).

Table 1. Mean survival time of male and female *Drosophila bipectinata* in different isofemale lines of five natural population samples subjected to starvation stress.

Population	Isofemale line	No. of individuals analysed	Male		Female	
			Mean Survival Time (in Hrs.) $\pm$ SE.	No. of individuals analysed	Mean Survival Time (in Hrs.) $\pm$ SE.	No. of individuals analysed
1.	2.	3.	4.	5.	6.	
Bhubaneswar	1	10	63.1 $\pm$ 4.4	10	93.87 $\pm$ 2.13	
	2	10	54.4 $\pm$ 1.61	10	73.0 $\pm$ 1.88	
	3	10	54.5 $\pm$ 4.9	10	74.7 $\pm$ 2.5	
	4	10	53.5 $\pm$ 1.68	10	66.7 $\pm$ 3.54	
	5	10	71.2 $\pm$ 1.8	10	71.9 $\pm$ 7.6	
	6	10	66.4 $\pm$ 2.8	10	76.0 $\pm$ 3.8	
	7	10	62.4 $\pm$ 2.6	10	65.7 $\pm$ 3.03	
	8	10	69.0 $\pm$ 3.6	10	71.2 $\pm$ 4.19	
	9	10	50.8 $\pm$ 3.75	10	61.6 $\pm$ 3.6	
	10	10	68.2 $\pm$ 2.32	10	85.0 $\pm$ 4.31	
<i>t</i> = 3.278, df = 18, P < 0.01 significant; <i>F</i> = 10.944, P < 0.005*						
Cuttack	1	10	24.0 $\pm$ 3.67	10	48.7 $\pm$ 4.26	
	2	10	41.0 $\pm$ 2.99	10	57.7 $\pm$ 2.61	
	3	10	57.2 $\pm$ 2.0	10	66.1 $\pm$ 3.1	
	4	10	22.9 $\pm$ 0.35	10	25.0 $\pm$ 1.15	
	5	10	43.4 $\pm$ 2.02	10	59.16 $\pm$ 2.13	
	6	10	43.2 $\pm$ 3.80	10	49.27 $\pm$ 5.8	
	7	10	51.7 $\pm$ 2.4	10	69.4 $\pm$ 4.8	
	8	10	34.0 $\pm$ 0.52	10	46.2 $\pm$ 0.75	
	9	10	39.3 $\pm$ 0.89	10	59.3 $\pm$ 0.87	
	10	10	44.4 $\pm$ 1.2	10	61.4 $\pm$ 2.4	
<i>t</i> = 2.678, df = 18, P < 0.02, significant; <i>F</i> = 7.118, P < 0.025*						
Berhampur	1	10	52.8 $\pm$ 2.14	10	57.1 $\pm$ 2.0	
	2	10	31.4 $\pm$ 2.74	10	45.0 $\pm$ 2.77	
	3	10	46.1 $\pm$ 3.03	10	60.9 $\pm$ 0.31	
	4	10	45.2 $\pm$ 2.50	10	58.7 $\pm$ 3.54	
	5	10	46.0 $\pm$ 2.09	10	56.9 $\pm$ 2.71	
	6	10	61.4 $\pm$ 1.19	10	81.5 $\pm$ 1.92	
	7	10	21.4 $\pm$ 1.74	10	24.18 $\pm$ 2.30	
	8	10	22.2 $\pm$ 1.70	10	38.33 $\pm$ 4.5	
	9	10	64.2 $\pm$ 3.87	10	65.2 $\pm$ 2.50	
	10	10	39.2 $\pm$ 3.21	10	79.8 $\pm$ 2.70	
<i>t</i> = 1.899, df = 18, P > 0.05, not significant; <i>F</i> = 3.608, P > 0.05						
Puri	1	10	50.6 $\pm$ 3.3	10	52.2 $\pm$ 3.1	
	2	10	50.4 $\pm$ 3.8	10	68.8 $\pm$ 2.3	
	3	8	43.73 $\pm$ 3.5	10	54.9 $\pm$ 4.6	
	4	10	52.0 $\pm$ 3.4	10	83.7 $\pm$ 2.8	
	5	10	53.8 $\pm$ 2.4	10	63.8 $\pm$ 4.1	
	6	9	44.8 $\pm$ 2.5	10	70.9 $\pm$ 3.6	
	7	8	51.0 $\pm$ 2.8	10	79.1 $\pm$ 3.7	
	8	10	46.9 $\pm$ 3.6	10	52.8 $\pm$ 3.7	
	9	10	65.3 $\pm$ 3.6	10	78.7 $\pm$ 5.0	
	10	10	61.4 $\pm$ 2.0	10	87.1 $\pm$ 2.8	
<i>t</i> = 3.920, df = 18, P < 0.01, significant; <i>F</i> = 13.779, P < 0.025*						
Calcutta	1	10	65.9 $\pm$ 2.17	10	94.8 $\pm$ 2.85	
	2	10	50.2 $\pm$ 1.59	10	46.2 $\pm$ 0.99	
	3	10	44.4 $\pm$ 1.99	10	77.3 $\pm$ 1.68	
	4	10	45.2 $\pm$ 2.21	10	69.4 $\pm$ 2.06	
	5	10	44.1 $\pm$ 1.71	10	76.6 $\pm$ 2.15	
	6	10	53.9 $\pm$ 2.48	10	54.4 $\pm$ 2.42	
	7	10	31.4 $\pm$ 0.95	10	30.5 $\pm$ 2.30	
	8	10	46.5 $\pm$ 0.96	10	59.5 $\pm$ 1.09	
	9	10	54.3 $\pm$ 2.35	10	74.3 $\pm$ 2.32	
	10	10	35.5 $\pm$ 2.10	10	55.5 $\pm$ 3.1	
<i>t</i> = 2.535, df = 18 P < 0.05, significant; <i>F</i> = 6.494, P < 0.025*						

\* Significant

statistically (Table 1). However, the degree of variation is different in different populations. Thus, the tolerance also shows interpopulational variation.

The observation of all these results is very interesting in an endemic species like *D. bipectinata*. Earlier studies on Indian *D. melanogaster* and *D. ananassae* present very interesting observations. While in both these species intra- and interpopulational variations are reported (Das *et al.*, 1994a, b, c), sexual variations for this phenomenon are detected only in the case of *D. ananassae* and *D. bipectinata* (Das *et al.*, 1994c and the present study). Both these species also share a common property; females survive longer than males in starvation conditions. These results contradict the observations made on French and India *D. melanogaster* where males were found to survive better than females under the same conditions (Da Lage *et al.*, 1989; Das *et al.*, 1994a, b).

It is often concluded that the greater difference in starvation tolerance sometimes observed between geographic populations of *D. melanogaster* and *D. ananassae* has a genetic basis (Da Lage *et al.*, 1990; Das *et al.*, 1994a, b) which can equally be applied to the present study. However, detailed research is needed in order to prove this phenomenon in this species. Since India is the homeland of *D. bipectinata* and also this species is only available for collection in nature during the rainy season, this type of study will prove the effect of starvation on evolution and adaptation of this species in India.

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**Offenberger, M., and A.J. Klarenberg.** Zoologisches Institut der Universität, München, Germany. Fitting parasitoid wasps to their drosophilid hosts: An overview of collecting methods with a checklist of hymenopteran parasitoids and their drosophilid hosts in European temperate woodlands.

about *Drosophila* hymenopteran parasitoids is still far from complete (Carton *et al.*, 1986).

There are different techniques for sampling *Drosophila* parasitoids. A straightforward technique is to net them over or rear them out of baits or natural substrates infected with *Drosophila* larvae or pupae (Carton *et al.*, 1986; Driessen *et al.*, 1990; Offenberger, 1994). This method may not always be very efficient because of low drosophilid and/or parasitoid density; it could be very productive, however, at locations with high parasitism rates (see Janssen *et al.*, 1988). The disadvantage of collecting parasitoids in this way is that it gives no information on parasite-host relationships; it only gives an indication of the number of *Drosophila* species that potentially can be parasitized (Hardy *et al.*, 1992; Offenberger, 1994). Parasitized *Drosophila* species may be identified by their pupae (Baker, 1979). However, not all drosophilid species have been described yet in this way.

A new technique, *i.e.* baits composed of rotten plants or mushrooms which were previously infected in the laboratory with a single *Drosophila* species and then set out in the field for one day, overcomes the disadvantage of the direct method (Offenberger, 1994; Offenberger and Klarenberg, in prep.). The wasps can only parasitize larvae or pupae of the *Drosophila* species in question. Other species which are attracted to these baits can be excluded with 100% certainty, since the larvae of newly laid eggs cannot hatch before parasitoid eclosion of the infected *Drosophila* species. With this method, six parasitoid hymenopteran species could be assigned unequivocally to their host(s), *D. limbata* and *D. phalerata*, in the field (Offenberger and Klarenberg, in prep.). A new pupal parasitoid, *Trichopria aequata* (Diapriidae), was reared from baits composed of rotten ramsons, *Allium ursinum* (Liliaceae), which were inoculated with *D. limbata* and set out in a flood plain forest near Munich in southern Germany. In spring, *Allium ursinum* is found in large patches in woodlands and is a natural breeding substrate of *D. limbata*, which is a specialist on decaying plants (Offenberger and Klarenberg, 1992a; Offenberger, 1994). In the laboratory, *T. aequata* was reared from a total of twelve *Drosophila* species. Developmental time for *T. aequata* in *D. limbata* was shorter than in the other potential host

A prerequisite in assigning hymenopteran parasitoid species to their hosts is an outstanding knowledge of the *Drosophila* natural breeding substrates. Although there has been an increasing interest in drosophilids specialized on fungi and decaying plant material (James *et al.*, 1988; Courtney *et al.*, 1990; Van Alphen *et al.*, 1991; Davis and Jenkinson, 1992; Offenberger and Klarenberg, 1992 a, b), information

species tested, e.g. the other European species of the *quinaria* group *D. kuntzei*, *D. phalerata* and *D. transversa*. These data support the assumption that *D. limbata* is the principal host for *T. aequata*. The baiting method also created the opportunity to experiment. Vet *et al.*, (1984) have demonstrated that female *Asobara* and *Leptopilina* species are attracted by substances released from the breeding substrate. Wiskerke *et al.*, (1993) showed that in searching for hosts, *L. heterotoma* makes use of the adult *Drosophila* aggregation pheromone. In order to determine the priority of the host species relative to its breeding substrate in attracting parasitoids, different combinations of fungal and decaying plant baits, each experimentally infested with larvae and pupae of a single *Drosophila* species, were tested in the field. These experiments with *D. limbata* showed that for successful parasitism, the type of substrate is more important than the host larvae and pupae themselves, which suggests that volatile compounds released by the substrate are involved in attracting the parasitoids (Offenberger, 1994). Baits with fixed numbers of *Drosophila* larvae or pupae could also be used in monitoring the population density of parasitoids. Moreover, different strains of a single *Drosophila* species or mixtures of different *Drosophila* species could be tested under field conditions. A final problem is species identification and taxonomy (Schmiedeknecht, 1907; Graham, 1969; Nordlander, 1980). Problems still remain nonetheless, as specialist knowledge is required for the identification of hymenopteran parasitoids. In many instances, comparison with reference material is required or the material has to be sent to specialists.

Table 1. The *Drosophila* species and their known larval and pupal hymenopteran parasitoids in European temperate woodlands. This list includes only those host-parasitoid relationships which have been established in nature. Abbreviations in bold print indicate pupal parasitoids; all others are larval parasitoids. Data were extracted from Carton *et al.* (1986), Van Alphen *et al.* (1991), Hardy *et al.* (1992) and Offenberger (1994).

Drosophilid Species	Hymenopteran Parasitoid Species
<i>D. busckii</i>	BIL; DUB; ERY; FIM; HET; TAB
<i>D. fenestrarum</i>	RUF
<i>D. funebris</i>	HET; MIN; TAB
<i>D. immigrans</i>	DUB; CLA; HET; SCA
<i>D. kuntzei</i>	DUB; ERY; HET; PSI; TAB
<i>D. limbata</i>	AEQ; AUS; BIL; HET; SCA
<i>D. melanogaster</i>	DUB; ERY; HET; MIC; TAB
<i>D. obscura</i>	DUB; HET; PUN; TAB
<i>D. phalerata</i>	BIC; BIL; CLA; DOL; DUB; ERY; FID; HET; PSI; TAC
<i>D. subobscura</i>	AUS; CLA; DUB; ERY; FIM; HET; MIC; RUF; TAB
<i>Scaptomyza pallida</i>	FIM; SCA; RUF

**AEQ:** *Trichopria aequata*; **AUS:** *Leptopilina australis*; **BIC:** *Kleidotoma bicolor*; **BIL:** *Tanycarpa bicolor*; **CLA:** *Leptopilina clavipes*; **DOL:** *Kleidotoma dollichocera*; **DUB:** *Pachycrepoideus dubius*; **ERY:** *Spalangia erythromera*; **FID:** *Vrestovia fidena*; **FIM:** *Leptopilina fimbriata*; **HET:** *Leptopilina heterotoma*; **MIC:** *Trichomalopsis micropterus*; **MIN:** *Aphaereta minuta*; **PSI:** *Kleidotoma psiloides*; **PUN:** *Tanycarpa punctata*; **RUF:** *Asobara rufescens*; **SCA:** *Aphaereta scaptomyzae*; **TAB:** *Asobara tabida*; **TAC:** *Phaenocarpa tacita*.

We have shown that assigning hymenopteran parasitoid species to their hosts is hampered in several ways by methodological problems. We recommend the use of our new method described above as the most effective strategy for collecting hymenopteran parasitoids.

Table 1 shows a checklist of larval and pupal hymenopteran parasitoids and their drosophilid host species in European temperate woodlands. Data were extracted from Carton *et al.* (1986), Van Alphen *et al.* (1991), Hardy *et al.* (1992) and Offenberger (1994). Only those *Drosophila* species are listed whose parasite-host relationship has been ascertained in nature. Potential parasite-host relationships as recorded by collecting parasitoids by netting and rearing out baits or those established under laboratory conditions have been omitted. Although these data may be indicative of a host-parasitoid relationship, for instance by measuring survival of a parasitoid

species in its drosophilid host, there is still a chance for artefacts. Field observations are required for certainty. One has to be cautious with any conclusion drawn from these data as they are still incomplete and in no way suited for proper statistical analysis. However, some interesting questions can be formulated. Does parasitoid diversity correlate with the abundance of their host species? *D. phalerata* and *D. subobscura* which belong to the most abundant species in European temperate woodlands (Herting, 1955; Shorrocks, 1982; Burla and Bächli, 1991; Offenberger and Klarenberg, 1992a) have ten and nine known parasitoids, respectively. *D. kuntzei*, *D. limbata*, *D. obscura* and *D. immigrans*, which are in most instances less abundant, have only four or five known parasitoids. It is important to determine whether this difference is real or due to differences in sampling intensities. Another interesting problem is whether hymenopteran parasitoids have co-evolved with their drosophilid hosts in substrate specialisation (Van Alphen *et al.*, 1991). Cosmopolitan drosophilids such as *D. busckii*, *D. immigrans* and *D. melanogaster* which invaded European temperate woodlands a shorter or longer time ago seem equally well suited hosts as the original drosophilid community. The diversity in larval parasitoids appears larger than in pupal parasitoids. There is a shortage of information, in particular with respect to the *Drosophila* pupal parasitoids. The check-list covers only some of the *Drosophila* species known in European temperate woodlands. Other host-parasitoid relationships remain undiscovered as a challenge for future investigations.

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**Klarenberg, A.J., and L. Kersten.** Zoologisches Institut der Universität, München, Germany. Nematode parasitism in *Drosophila quinaria* and *D. testacea* species groups.

mushrooms, are parasitized by the nematode *Howardula aoronymphium* (Allantonematidae; Nematoda). This species and its biology were first described by Welch (1959) in England on the basis of earlier observations by Basden and Goodey (1955). It is assumed that *H. aoronymphium* represents a single species (see Jaenike, 1993). Its life cycle (see Welch, 1959; Jaenike, 1992) is briefly described here. *Drosophila* larvae feeding on mushrooms are infected by one or more fertilized *Howardula* females. They come into *Drosophila* larvae through the mouth or anus. From here they move into the haemocoele. Around 400-600 juvenile nematodes are produced in the abdomen of a single adult *Drosophila*. The juveniles are released by the adult *Drosophila* through the intestinal or genital tract when they visit mushrooms for feeding or oviposition. After becoming sexually mature, the *H. aoronymphium* females mate and start searching for new host larvae. In this paper we are presenting the results of an investigation on nematode parasitism in wild populations of adult *Drosophila* in Southern Bavaria near Munich for comparison with earlier data described by Welch (1959) in England. In addition, we have compared these data with rates of parasitism in *Drosophila* species of the *quinaria* and *testacea* groups from the Eastern part of the U.S.A. (Jaenike, 1992) and Japan (Kimura and Toda, 1989). Biological details of our study will be presented elsewhere (Klarenberg and Kersten, in prep.). There are four *quinaria* species in Europe: *D. kuntzei*, *D. limbata*, *D. phalerata* and *D. transversa* (Shorrocks, 1982). *D. kuntzei*, *D. limbata* and *D. phalerata* are abundant in our collections, while *D. transversa* is rare (Offenberger and Klarenberg, 1992). The fungus-breeding *D. kuntzei* and *D. phalerata* are both parasitized by *H. aoronymphium*. *D. limbata*, which breeds in decaying herbage, was however, not infected. A third fungus-breeding drosophilid of the *testacea* species group, *D. testacea*, was also parasitized. All other species (e.g., *D. immigrans*, *D. subobscura* and *Scaptomyza pallida*) collected near Munich were free of nematodes. Parasitism in the three infected *Drosophila* species varies considerably: 34.7% of *D. phalerata* and 16.5% of *D. kuntzei* were parasitized, while only 4.9% of *D. testacea* was. In our 1993 collection *D. testacea* was numerically the most abundant species. *D. kuntzei* and *D. phalerata* showed lower percentages of infection in England, i.e. 4.3% and 20.1%, respectively (Welch, 1959). Note that these numbers are mean percentages of parasitization. For instance, we found that 50% of the *D. phalerata* males were infected with *H. aoronymphium* in September 1993.

Table 1 gives an overview of nematode parasitism in *Drosophila quinaria* and *testacea* species groups, both of which have a holarctic distribution. Parasitism in the *quinaria* species group ranges from 3.9 to 34.7%, and in the *testacea* group from 4.5 to 31.0%. Of the *quinaria* species, *D. falleni*, *D. kuntzei* and *D. phalerata* all show high parasitism rates (16.5 - 34.7%), while *D. recens* and the three Japanese *quinaria* species, *D. brachynephros*, *D. curvispina* and *D. unispina*, have relatively low rates of parasitism (3.9 - 6.5%). In the *testacea* species group, both *D. testacea* and *D. orientacea* have low parasitism rates (4.5 - 5.0%). As mentioned above, these values may show considerable seasonal and geographic variation. Jaenike (1992) has found one site where the fungus-breeding drosophilid community was free of nematodes. One important conclusion is that *H. aoronymphium* infects only fungus-breeding *Drosophila* species. In contrast, species breeding in decaying herbage are not parasitized in nature. *D. quinaria*, which breeds in decaying skunk cabbage (*Symplocarpus foetidus*), was, like *D. limbata*, not infected by *Howardula*. Transmission experiments showed that both species had no resistance to *H. aoronymphium* (Jaenike, 1988 and 1992; Jaenike, pers. comm.; Kersten, 1993). From the data in Table 1 it can be deduced that parasite pressure in many *Drosophila* species is relatively low ( $\pm 5\%$ ), which

Ecological and evolutionary studies have shown that nematode parasitism is an important factor influencing female fecundity in some fungus-breeding *Drosophila* (Jaenike and Anderson, 1992). North American, Japanese and European *Drosophila* of the *quinaria* and *testacea* species groups, which breed in

suggests that no severe effects of nematode parasitism are to be expected at the population level. This is in contrast to other *Drosophila* species which have high infection rates (+16-30%). Here, *H. aoronymphium* may have a significant influence at the population level. Apparently, infection rates vary within each species group. Welch (1959) described a second nematode species, *Parasitenchus diplogenius* (Allantonematidae; Nematoda), which parasitized three European *Drosophila* species of the *obscura* group (*D. subobscura*, *D. obscura* and *D. silvestris*) at a rate of about 5%. He was able to transmit *P. diplogenius* experimentally from these species to *D. melanogaster*, but not to *D. kurtzei* and *D. phalerata*. Transmission of *H. aoronymphium* from these last two species of the *quinaria* group (subgenus *Drosophila*) to those *Drosophila* species of the *obscura* group (subgenus *Sophophora*) was not successful. Welch (1959) has suggested that the restricted infectivity of these two parasitic nematode species is due to the evolutionary divergence of these species groups which belong to separate subgenera of the genus *Drosophila*. Why *Drosophila* species differ and how horizontal transmission between *Drosophila* species using the same substrate affects the rate of parasitism in each species is open to further research. In this context Herre's (1993) study on parasitic nematodes in fig wasps is very interesting. More information on *Drosophila* species other than the *quinaria* and *testacea* species groups will be required before conclusions can be drawn about the evolution of the host specificity of parasitic nematodes in *Drosophila*.

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**Umbetova, G.H., I.E. Vlassova, and I.F. Zhimulev.**  
Institute of Cytology and Genetics, Novosibirsk 630090, Russia. Immunofluorescent localization of DNA-RNA hybrids in polytene chromosomes of *Drosophila melanogaster* during development.

chromosomes have shown fluorescence occurs in large puffs (Rudkin and Stollar, 1977; Alcover *et al.*, 1982; Buesen *et al.*, 1982) and in the majority of small puffs (Vlassova *et al.*, 1985), but transcriptional activity of small puffs have been shown only for 0 hour (0 h) prepupae of *gt* mutants. In the present study we have analysed fluorescence in the regions of small puffs during development.

Table 1. A comparison of nematode parasitism in the holarctic *Drosophila quinaria* and *testacea* species groups.

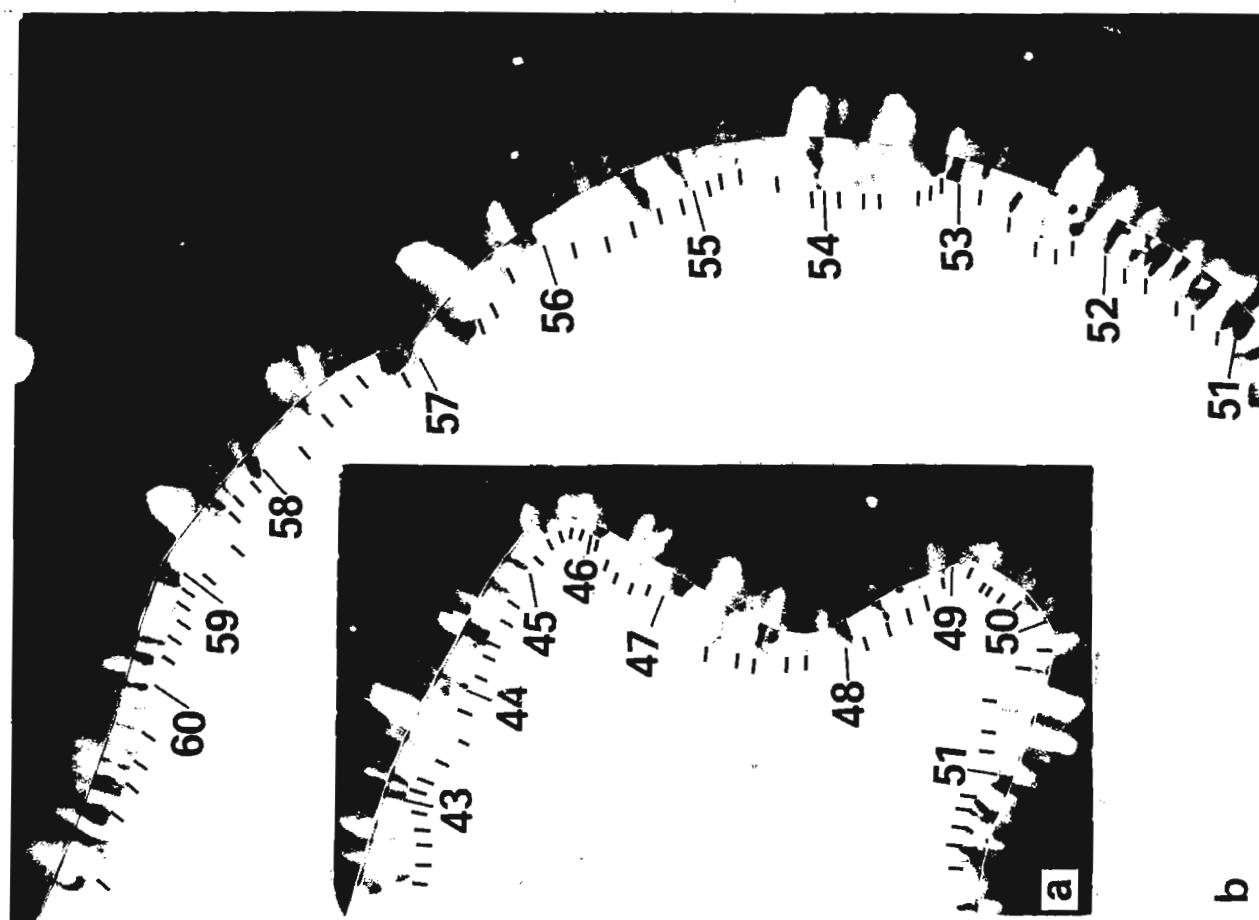
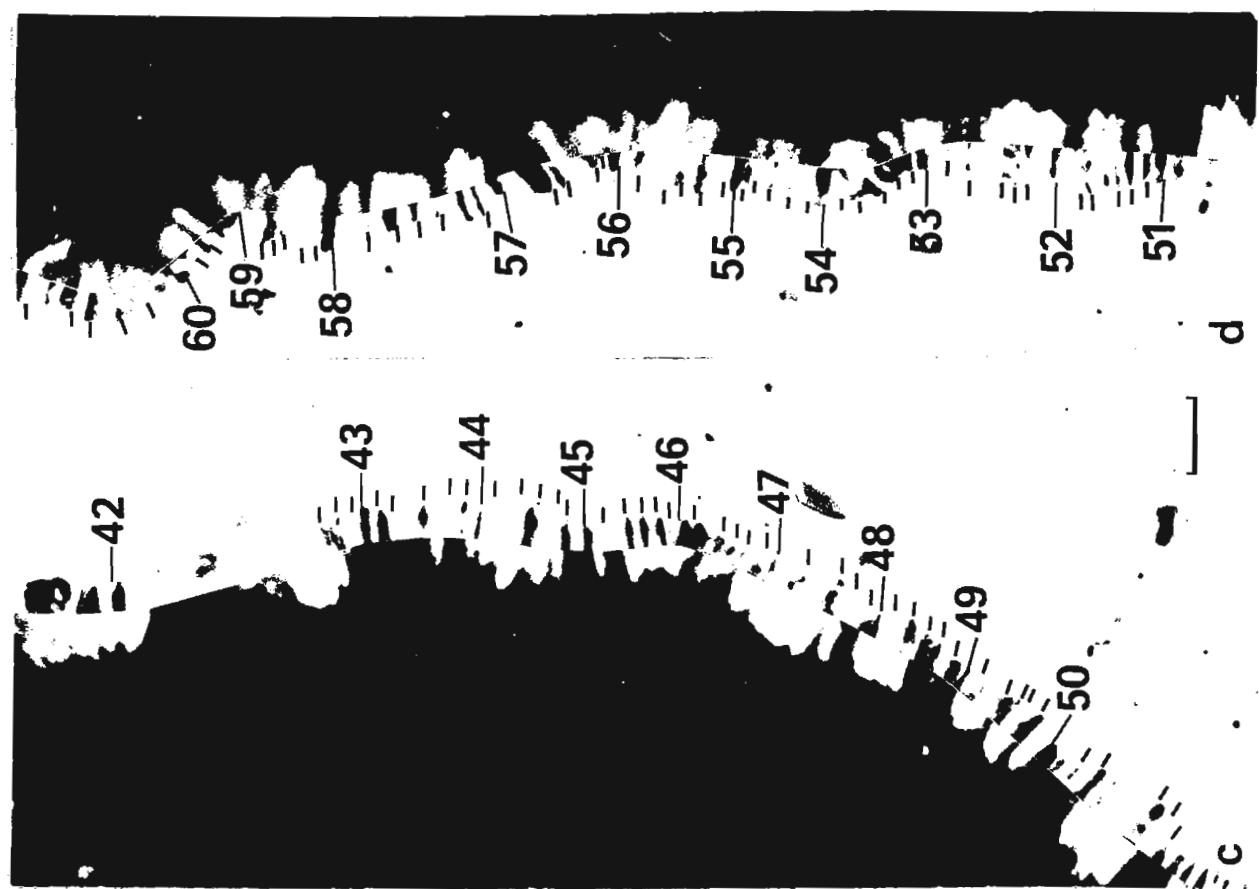
Site	Species Group	Drosophila species	% parasitized	n	
<b>Asia</b>	Japan <sup>1</sup>	<i>quinaria</i>	5.9	458	
		<i>D. brachynephros</i>	3.9	233	
		<i>D. curvispina</i>	4.7	85	
	<i>testacea</i>	<i>D. orientacea*</i>	5.0	516	
<b>Europe</b>	England <sup>2</sup>	<i>quinaria</i>	4.3	799	
		<i>D. phalerata</i>	20.1	1347	
	Germany <sup>3</sup>	<i>quinaria</i>	16.5	164	
		<i>D. phalerata</i>	34.7	597	
<b>North America</b>	U.S.A. <sup>4,5</sup>	<i>testacea</i>	4.9	1800	
		<i>D. kurtzei</i>			
		<i>D. falleni</i>	22.0	677	
		<i>D. recens</i>	6.5	46	
		<i>D. neotestacea*</i>	26.5	3040	
		<i>D. putrida</i>	31.0	476	

<sup>1</sup> Kimura and Toda (1989); <sup>2</sup> Welch (1959); <sup>3</sup> Kersten (1993); <sup>4</sup> Montague and Jaenike (1985); <sup>5</sup> Jaenike (1988, 1992). \* Taxonomy according Grimaldi *et al.* (1992).

Among polytene chromosome puffs two types have been found, big prominent puffs which are mainly ecdysterone-dependent (Ashburner, 1975), and small puffs which as a rule show only little developmental changes (Belyaeva *et al.*, 1974; Zhimulev, 1974).

Extensive studies on immunofluorescent localization of DNA-RNA hybrids in Dipteran polytene

Figure 1 (opposite page). Immunofluorescent localization of transcriptionally active regions of chromosome 2R in the larval development: a, b, PS-I; c, d, PS8-9. Matched phase contrast and fluorescent pictures of the chromosomes are shown. Bar corresponds to 5 microns.



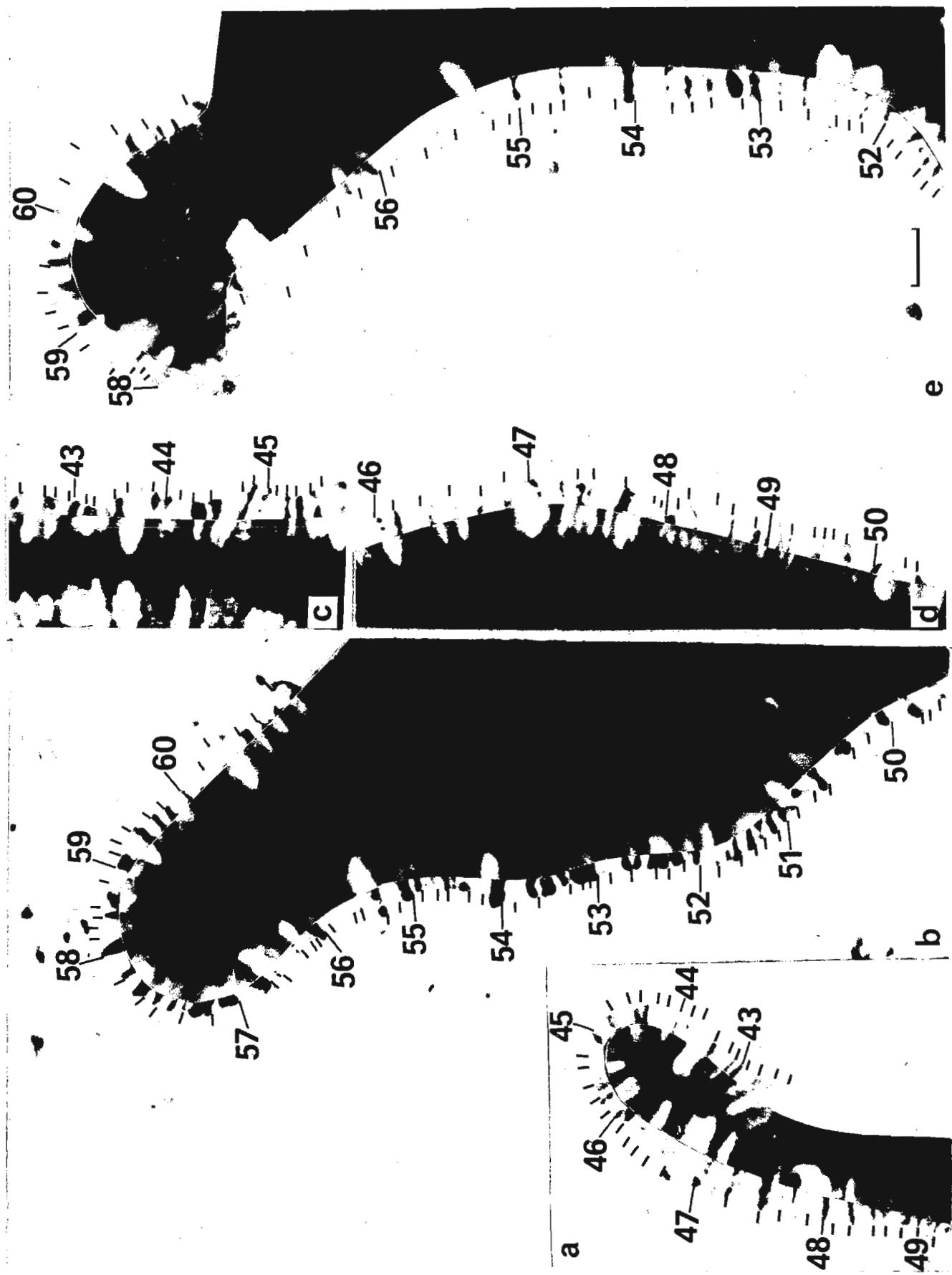


Figure 2 (opposite page). Immunofluorescent localization of transcriptionally active regions of chromosome 2R in prepupal development: a, b, PS 10-11; c, e, PS 14-16. Bar corresponds to 5 microns.

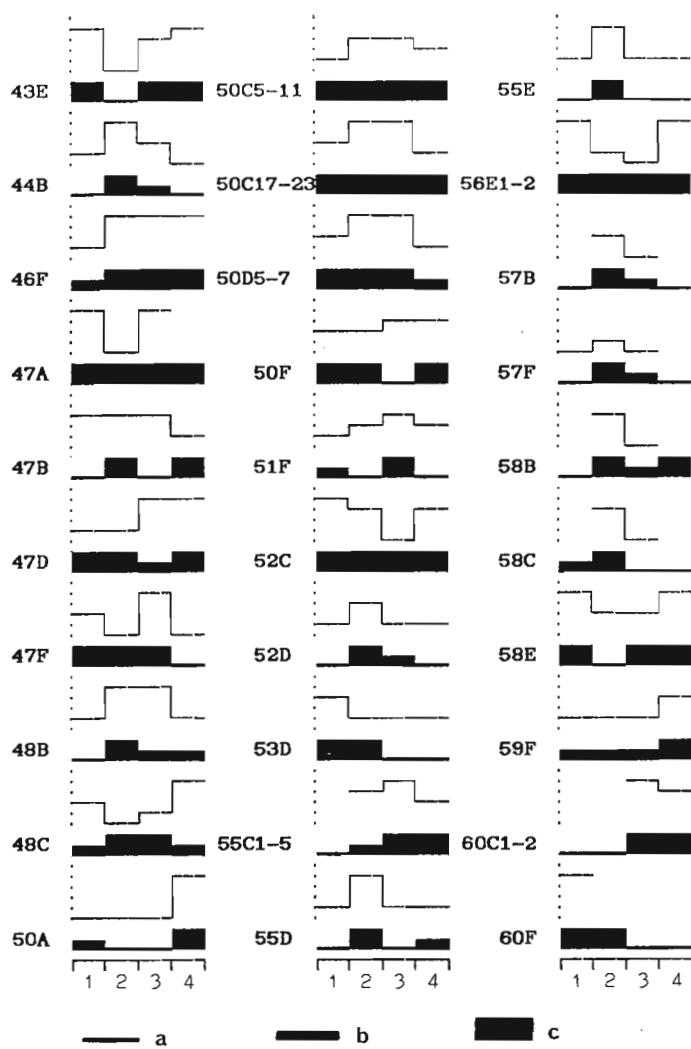


Figure 3. Changes in the degree of chromosome decondensation in the chromosome 2R regions forming prominent or intermediate puffs (thin upper lines) and the intensity of DNA/RNA hybrid immunofluorescence (solid bars). Abscissa, developmental stages: 1 - PS1, 2 - PS 8-9, 3 - PS 10-11, 4 - PS 14-16. Ordinate, the degree of region decondensation in 4-6 scale and immunofluorescence intensity: a - weak, b - moderate, c - bright.

pancies are in somewhat varying intensity of fluorescence. In the remainder both the morphology and fluorescence intensity are constant.

Except for the puffs of chromosome 2R, also the fluorescence changes of the 2B puff has been studied because this locus contains a key gene of ecdysterone induction - *BR-C* (Belyaeva *et al.*, 1987). According to the electron-microscopical data the early ecdysterone-inducible puff 2B3-5 is formed from the proximal part of the large

Third instar larvae from Puff Stage 1 (PS1) to PS 10 and 0, 4-6 and in some cases 10 h prepupae of Batumi-L wild strain were used. Aging of larvae (for PS, see Ashburner, 1975) was performed by morphology of the salivary gland duct (Zhimulev *et al.*, 1981). The age of prepupae was scored in hours (at 25°C) beginning with puparium formation. Puff sizes were estimated visually on the aceto-orcein preparations of salivary glands under a phase contrast microscope (see Zhimulev, 1974; Belyaeva *et al.*, 1974).

Preparations for immunofluorescent analysis were made and DNA-RNA hybrids revealed as described in detail earlier (Vlassova *et al.*, 1985). Fluorescence intensity of DNA-RNA hybrids in every chromosome region was estimated arbitrarily as bright, moderate, weak or absent. In each case, on the average 8 photographs of a chromosome region have been analysed. The comparative data on RNA synthesis and morphology of chromosome 2R regions for some developmental stages (PS1, PS8-9, PS10-11, PS14-16) during 12 h are presented in Figures 1 and 2 as matched fluorescent and phase contrast photographs. The alternation of the differentially fluorescent regions is likely to reflect the stage-specific changes in transcription during *Drosophila melanogaster* development. Visual estimates have yielded from 100 to 110 sites of fluorescence on the right arm of chromosome 2 at every developmental stage studied. The analysis of morphology of chromosome 2R during 12 h of development has yielded the two classes of regions: i) 30 regions of large or intermediate puffs appearing at definite stages of development (Figure 3) and ii) 90 regions of small morphologically stable puffs (Figure 4). Analysis of the data shown permits the conclusion that a strict correlation between the changes in fluorescence intensity and degree of puffing development exists. In some cases degree of the puffing development (decondensation of chromosomal material) at different developmental stages does not completely coincide with fluorescence intensity (47A, 52C, 56E regions). When analysing the small puffs, the morphology of which is more or less stable at all developmental stages, in half of the regions the discrepancies are in somewhat varying intensity of fluorescence. In the remainder both the morphology and fluorescence intensity are constant.

Except for the puffs of chromosome 2R, also the fluorescence changes of the 2B puff has been studied because this locus contains a key gene of ecdysterone induction - *BR-C* (Belyaeva *et al.*, 1987). According to the electron-microscopical data the early ecdysterone-inducible puff 2B3-5 is formed from the proximal part of the large

**Figure 4.** Changes in the intensity of DNA/RNA hybrid immunofluorescence in the regions of small puffs of chromosome 2R during normal development (morphology is stable or varies insignificantly, the degree of decondensation ranges from 1 to 3 points). Immunofluorescence intensity: a - absent, b - weak, c - moderate, d - bright. Developmental stages: 1 - PS1, 2 - PS 8-9, 3 - PS 10-11, 4 - PS 14-16.

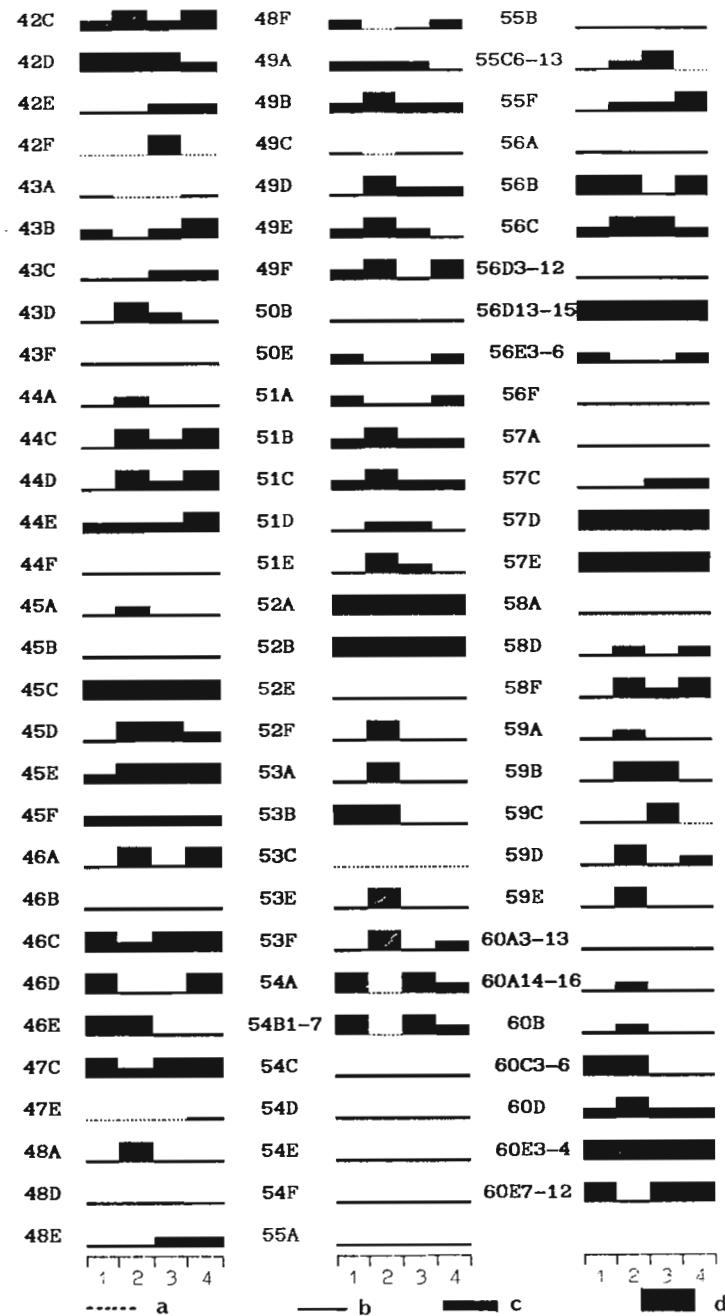
**Figure 5 (opposite page).** DNA/RNA hybrid immunofluorescence in the X chromosome regions 1E1-4 and 2B3-5 during normal development: a, a', PS-1; b, b', PS 8-9; c, c', PS 10-11; d, d', PS 14-16; e, e', PS20. Arrow points at the region of the 1E1-2/1E3-4 interband, and the arrowhead at the region of the 2B3-5 puff. Bar corresponds to 3 microns.

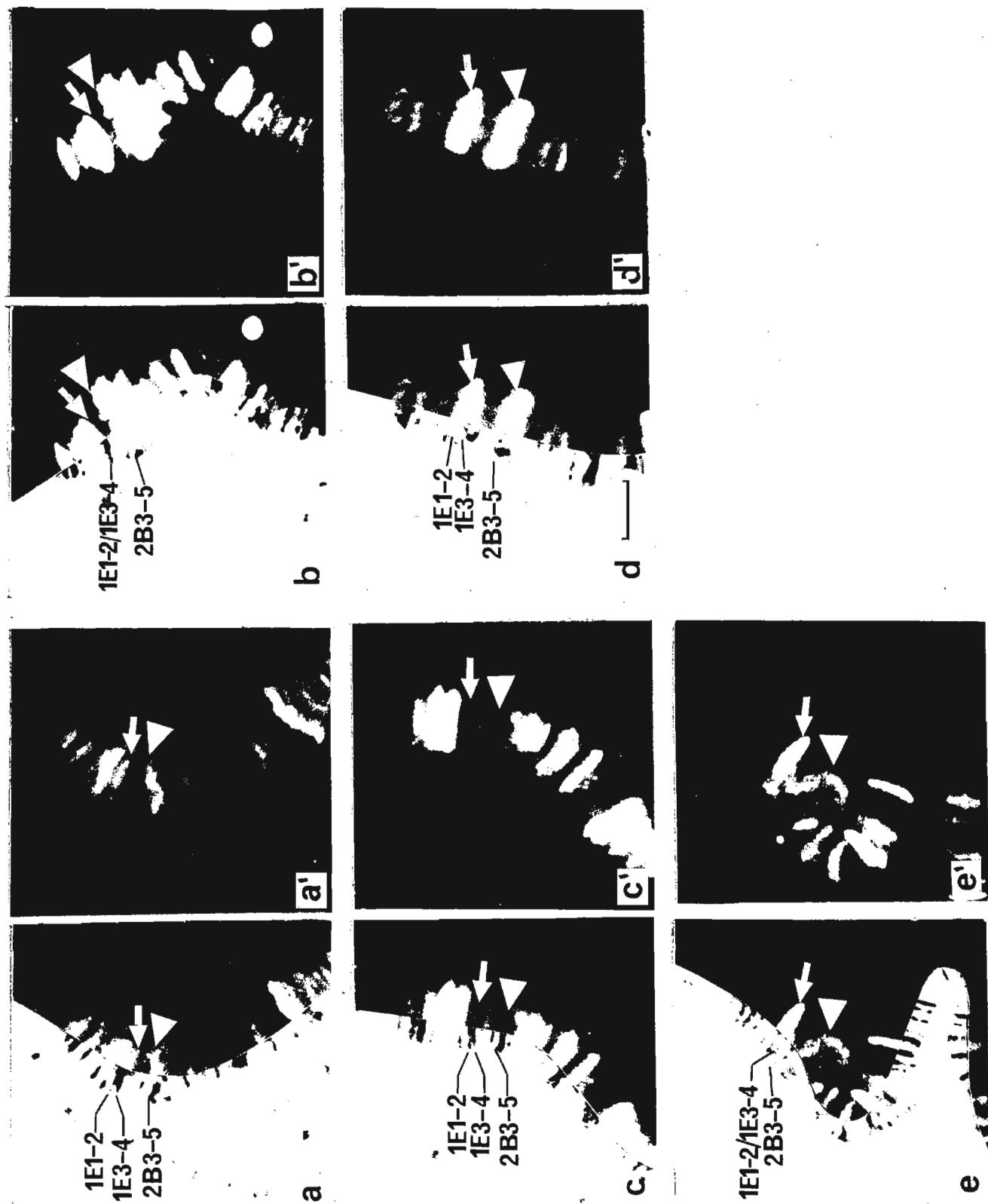
chromosome band, 2B3-5. Figure 5 shows the small puff 2B3-5 fluorescing moderately at PS1 (a, a') and at PS8-9 (b, b'), which practically ceases fluorescence at PS 10-11 (c, c') and again fluoresces brightly at PS 14-16 despite the small degree of puffing (d, d'). At PS20 the puff is large and fluoresces moderately (e, e').

The 1E1-4 region of the X chromosome has been shown by EM to comprise two dense bands, 1E1-2 and 1E3-4 (Semeshin *et al.*, 1985). In some photographs a fluorescing stripe of an interband can be noticed (Figure 5a', c'). In 4-6 h prepupae this interband elongates and turns into a micropuff by decondensation of the distal part of the 1E3-4 bands. The region incorporates  $^{3}\text{H}$ -uridine as shown by EM autoradiography (Zhimulev *et al.*, 1983) and fluoresces brightly (Figure 5d, d', e, e').

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**Madhavan, Kornath and Alan P. Picarillo.** Department of Biology, College of the Holy Cross, Worcester, MA 01610. Differential *lac<sup>Z</sup>* expression in the diploid and polytene cells of *Drosophila melanogaster*.

transposants, the reporter construct responds to enhancers and other regulatory elements in its vicinity (Bellen *et al.*, 1989; Bier *et al.*, 1989). Since the enzyme is thought to contain a nuclear targeting signal when fused with the P-transposase (Rio *et al.*, 1986), and since it has a huge molecular weight and fairly long half-life, one can expect in the tissues, depending on the presence or absence of nuclear membrane breakdown following DNA replication, differences in the localization of the enzyme. Thus, one could see the staining in the nucleus or in the nucleus and cytoplasm, respectively. In our effort to identify strains that shown interesting localization of the enzyme, we were able to see its activity in different ectodermal, mesodermal, and endodermal tissues of the larva of *Drosophila*. In the following we report this staining pattern and show that it supports our prediction. We believe that this is the first documentation that explains the differential staining of *lac<sup>Z</sup>* in the cells to their mitotic behavior.

Late third instar larvae, ready to pupariate within the next 1-2 h, from 100 transposants, kindly supplied by Drs. Allan Spradling, Anne Schneiderman, and Corey Goodman, were used for staining for *lac<sup>Z</sup>* activity. The larvae were cut into two halves and each half was further cut longitudinally. These preparations were fixed in 0.75% glutaraldehyde in 100 mM sodium cacodylate buffer, pH 7.3, for 15 minutes. The specimens were washed in phosphate buffered saline (PBS), and stained for enzyme activity for 24 - 36 h as described in Ashburner (1989). Subsequently, the preparations were rinsed in PBS, the desired tissues were dissected out and mounted in Aqua-mount for observations.

Table 1 shows the *lac<sup>Z</sup>* expression pattern in the different tissues. From this table, it is clear that the polytene cells show only nuclear staining, while the diploid cells which undergo mitotic divisions show both nuclear and cytoplasmic staining. We interpret this pattern in the following way. Since the cells of the polytenic tissues of the larva, during their life, undergo only DNA replication without concomitant cyclic nuclear membrane breakdown and reconstruction (Ashburner, 1970), the nuclear targeted enzyme remains within the nucleus. This will also hold good for the diploid cells of imaginal anlagen that do not undergo mitosis during larval life. An example of this type of tissue is the different histoblast nests (Madhavan and Madhavan, 1980). Unfortunately, in the present study, we were not able to uncover a line that showed enzyme activity in these precursors of epidermal cells of the adult abdomen. On the other hand, the imaginal discs we have examined, all contain diploid cells and undergo active mitosis during the last larval instar (Madhavan and Madhavan, 1977). Similarly, the imaginal islands of the larval midgut (Bodenstein, 1965), several specific regions of the brain hemispheres and the fused ventral ganglion (Truman and Bate, 1988) show mitotic activity. Thus, in these tissues, during the prometaphase of each round of mitosis, due to the breakdown of the nuclear envelope, the initially nuclear-bound *lac<sup>Z</sup>* is now localized in the cell's cytoplasm. Since the volume of the cells of many of these tissues is several magnitudes smaller compared to that of the polytenic cells, the entire cell of the mitotically active tissue appears intensely stained.

The availability of transposants with differential staining pattern in the nucleus and cytoplasm provides a quick and convenient marker to isolate stocks that show abnormal nuclear membrane morphology. Such lines could provide an opportunity to analyse the role of genes and their products on nuclear membrane breakdown during prometaphase and reconstitution following anaphase; processes which are intimately associated with mitosis and which are still poorly understood (Hiroka *et al.*, 1990). In this context, the maternal effect lethal mutation, *gnu*, of *Drosophila* is valuable in that the nuclei of the cellular blastoderm show continuous DNA replication but fail to show nuclear division. As a consequence of this, the blastoderm contains a small number of cells with giant nuclei (Freeman *et al.*, 1986). How this mutation uncouples DNA synthesis from nuclear envelope breakdown remains to be studied. The information on the coordinated events leading to breakdown and reconstitution of the nuclear membrane during mitosis in somatic cells could also be extended to similar processes occurring during pronuclei formation, an important event in sexual reproduction (Yamashita *et al.*, 1990). Another use for this

One of the current and powerful analytical tools in developmental biology is the enhancer detection method. In *Drosophila*, this method entails simple genetic crosses by which single copies of a transposon with a reporter gene *lac<sup>Z</sup>* and a weak promoter are introduced randomly into the fly's genome. In these

Table 1. Histochemical localization of  $\beta$ -galactosidase activity in the polytene (P) and diploid (D) cells of the different tissues of the late third larval instar of transposants of *Drosophila melanogaster*.

Tissues	Localization of enzyme activity	
	Type	Nuclear Nuclear and cytoplasmic
Salivary gland	P	+
Proventriculus	P	+
Esophagus	P	+
Gastric caeca	P	+
Midgut	P	+
Fat body	P	+
Malpighian tubules	P	+
Epidermis	P	+
Oenocytes	P	+
Ring gland [prothoracic gland part]	P	+
Eye-antennal disc	D	+
Wing disc	D	+
Haltere disc	D	+
Leg disc	D	+
Brain	D	+
Ventral ganglion	D	+
Midgut imaginal islands	D	+

differential localization of the enzyme in the cells is to determine when, during embryonic development of *Drosophila*, cells choose the polytenic, larval (most of them perished during metamorphosis) or diploid, imaginal pathways. Such studies will help to determine whether there are distinctly different imaginal and larval developmental pathways, involving the expression of specific sets of genes.

Acknowledgments: We thank Drs. Allan Spradling, Anne Schneiderman and Corey Goodman for providing the transposants. A.P. also thanks the Pfizer, Inc., for the award of an Undergraduate Summer Fellowship.

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**Krebs, R.A., and J.S.F. Barker.** Department of Animal Science, University of New England, Armidale, NSW, Australia. Crosses between *D. aldrichi* from Australia and northwest Mexico suggest that they are different species.

Their frequency in this genus, many of which never would have been discovered had *Drosophila* not been the focus of so much research, suggests that cryptic species exist in other insect groups.

Research on the cactophilic *D. buzzatii* in Australia led to the discovery of *D. aldrichi* in that country in the early 1970's (Mulley and Barker, 1977). *Drosophila aldrichi* is presumed to have entered Australia in the 1930's during biological control program for *Opuntia*, as did *D. buzzatii* (Barker and Mulley, 1976). The true origin of the Australian *D. aldrichi* is unknown. However, this knowledge would be useful for studies of evolutionary changes that may have occurred during the 60 or more years separation between the new and ancestral populations, as was done for Australian and Argentinean populations of *D. buzzatii* (e.g., Barker *et al.*, 1985).

Ten isofemale lines of *D. aldrichi* were prepared from flies collected at Dixalea, Queensland, Australia (lat. 23°56 S, 150°47 E) on banana bait. These lines were maintained ca. 15 generations after which equal numbers from each line were pooled in ten half pint bottles of cactus-yeast-sucroseagar medium for three generations to form a mass population. Samples of two collections of *D. aldrichi* from northern Sinaloa, Mexico, were provided by William B. Heed of the University of Arizona (stock numbers 955c and 955m), and these also were maintained as a mass population. Flies from these latter two collections were fully interfertile, and therefore results for them were pooled for comparing fertility between *D. aldrichi* from Australia and Mexico.

Virgin males and females from the two populations were collected under carbon dioxide anesthesia. All flies were held in food vials with live yeast on the surface until the flies were sexually mature. Single pairs of the desired cross (Table 1) were then placed in vials for a minimum of one week after which adults were removed. The ability to produce offspring was recorded subsequently.

Under the conditions used, over 90% of single pairs from the same population produced offspring, and crosses between the Australian and Mexican populations produced offspring at a similar frequency as within population crosses (Table 1). No sex ratio effects were observed. There was a total of 672 males and 630 females counted from 38 between-population vials. However, only four of 80 vials of the F2 crosses produced offspring (Table 1), providing a total of only 12 flies. Back-crosses indicated that the low progeny production of the F2 crosses was due to F1 males. Hybrid females back-crossed to males either from the Mexican population or the Australian population generally produced progeny, although those paired with Australian males were significantly less likely to produce offspring than were those paired to males from the Mexico population or were control pairs (2 x 2 contingency analysis, P < 0.05 for both comparisons). Additionally, only 35% of male back-cross progeny were fertile, although most female back-cross progeny produced offspring if paired with control males (Table 1).

Table 1. The percentage of vials from which offspring were obtained, control vials (single pairs of flies from each population), vials of F<sub>1</sub> population crosses, vials of F<sub>2</sub> population crosses and vials of F<sub>1</sub> progeny backcrossed to parentals, between *D. aldrichi* from Queensland, Australia and Sinaloa, Mexico.

Cross	Males	Females	Vials (N)	Percent vials with offspring
Control	Australia	Australia	20	100
Control <sup>1</sup>	Mexico	Mexico	66	91
F <sub>1</sub> AM	Australia	Mexico	19	100
F <sub>1</sub> MA	Mexico	Australia	19	95
F <sub>2</sub>	F <sub>1</sub> AM	F <sub>1</sub> AM	40	0
F <sub>2</sub>	F <sub>1</sub> MA	F <sub>1</sub> MA	40	10
Back-cross	Australia	F <sub>1</sub> AM	20	85
Back-cross	Australia	F <sub>1</sub> MA	20	75
Back-cross	Mexico	F <sub>1</sub> AM	20	100
Back-cross	Mexico	F <sub>1</sub> MA	20	95
Back-cross	F <sub>1</sub> AM	Australia	20	0
Back-cross	F <sub>1</sub> MA	Australia	20	5
Back-cross	F <sub>1</sub> AM	Mexico	20	10
Back-cross	F <sub>1</sub> MA	Mexico	20	0
Test of back-cross progeny	mixed	mixed	40	35
	Australia or Mexico	mixed back-cross	24	92

<sup>1</sup> includes crosses between two nearby collections from Sinaloa, Mexico

analyses. *D. aldrichi* utilizes a variety of necrotic cactus species in northwest Mexico (Ruiz and Heed, 1988) and has been collected around Austin, Texas, on *Opuntia* species (Richardson and Smouse, 1976). Perhaps the form from Texas is the same as that from Australia. To answer this question, we request that anyone in possession of *D. aldrichi* from this region (or who could collect it), contact Prof. Stuart Barker. Strains from other localities also would be of interest.

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**Sapunov, V.B. and Legkov, V.V.** Peasant Academical University, Kirova, 73, Luga of Leningrad District, 188266, Russia. Effect of ultrasound on egg development in *D. melanogaster*.

concerning time distribution of hatching out the eggs. Some important characters of embryogenesis may become clear under nocuous effect from the environment.

The aims of present work were: 1. Study of polymorphism of wild *D. melanogaster* populations on embryonic development time under normal conditions, 2. Study of ultrasonic treatment effect of characters of embryonic development. We used Canton-S stock bred in a population box under standard laboratory conditions (Medvedev, 1969). Synchronous egg deposition was obtained during 30 minutes. The number of eggs was about 2,000. Half of them were used as a control. The second half was used for experiments with ultrasonic treatment. This shock was applied to eggs of age 30 minutes (early treatment) and 10 hours (late treatment). The time of ultrasonic exposure was 5, 10, 15, 20, 25, and 30 min. Ultrasonic intensity was from 0.2 to 1 watt/cm<sup>2</sup>. Dynamics of hatching out eggs was studied during 48 hours. Frequency was 0.88 ± 0.04 MHz.

Distribution of hatching time is demonstrated in Figure 1.

First larvae were born 2-3 hours after oviposition. In control data (Figure 1A), there appeared to be 2 peaks of hatching out eggs. Their modes are 8.2 and 32.2 hours. Hence, literature data on mean 20 hours must be corrected. The sense of mean is relative. The nature of the

Postzygotic reproductive isolation is present between the two populations of *D. aldrichi* used in this study. Cursory observations of rapid mating between the two forms and the high frequency of between-population pairs producing offspring is suggestive that prezygotic isolation is minimal. William Heed suggests that the name "*D. aldrichi*" may actually encompass a number of species, as a species swarm, and that species differences are unidentifiable by morphological traits (pers. comm.). We could discern no means of discriminating between the Mexican and Australian forms visually, even by external or internal aspects of the genitalia. However, the nearly complete male sterility of hybrids between the Australian and Mexican forms of *D. aldrichi*, and the low fertility of male offspring of females back-crossed to parental stocks, would limit gene flow between these forms if they were sympatric. As the exact origin of the Australian form is not known, the ancestry is unclear, but it is highly unlikely that so dramatic a change has occurred between the two forms in the short time since *D. aldrichi* has entered Australia. No postzygotic isolation was indicated between *D. buzzatii* from Australia and Argentina in a similar

Despite the big number of insect embryogenesis studies (e.g., Hinton, 1921; Regulation of Insect Reproduction, 1989), some important characters of embryogenesis are obscure. There is data on mean time of egg development in *D. melanogaster* - 20 hours (Medvedev *et al.*, 1969). But there is a deficiency of data

Table 1. Percents of undeveloped eggs in dependence of ultrasonic intensivity.

Variant	Ultrasonic intensivity, watt / cm <sup>2</sup>				
	0.05	0.2	0.4	0.7	1.0
Control	2.9 ± 0.5				
Earlier application	5.9 ± 1.4	10.5 ± 1.8	5.0 ± 1.3	12.5 ± 1.9	11.0 ± 1.8
Late application	14.3 ± 3.4	13.7 ± 3.4	12.0 ± 3.2	28.6 ± 4.5	53.5 ± 4.9

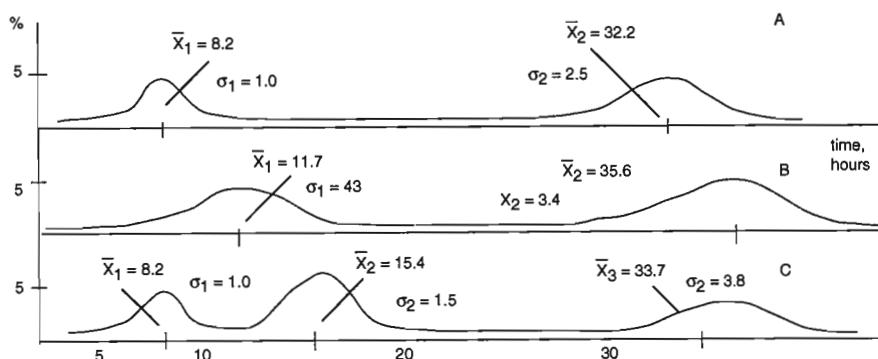


Figure 1. Distribution of hatching out time. A, control; B, early treatment; C, late treatment.

detected dimorphism is unclear. It may be that there are 2 morphogenic types of embryos. The second possibility is that physiological characters of female organism divide eggs into two groups. The first of them deposits immediately. The second group is delayed within mother organism, where they have first half of embryogenesis.

The next experiments dealt with ultrasonic influence on eggs. There were no effects of time of exposure (from 5 to 30 minutes). Hence, the biological effect is complete during some first minute. Data on different time exposures are summarized together. The first effect of ultrasound is an increase of percent of undeveloped eggs (Table 1).

Egg mortality in the control is low, about 3%. After treatment the mean increased. In the first variant (earlier treatment), there was no connection between pressure of ultrasound and lethal result. In the second variant of experiment the connection took place (coefficient of correlation is  $0.6 \pm 0.1$ ). Late exposure induces additional peak of hatching in age 15.1 hours. In every case, variability of hatching time increased after ultrasonic pressure (see Figure 1, B and C). This effect is accorded to data on increase of population variability under stress conditions (Sapunov, 1991).

Hence, ultrasonic treatment has a significant effect on embryonic development of *Drosophila*. These data may have a big practical importance for ecology because of present activity of industry producing ultrasounds, having effect on both animals and man.

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Department of Ecology and Genetics, University of Aarhus, Ny Munkegade, Bldg. 540, DK-8000 Aarhus C, Denmark. Adult nutrition and the relationship between body size and fecundity in *D. melanogaster*.

(Markow, 1985; Partridge *et al.*, 1987a, b; Wilkinson, 1987; Pitnick, 1990). With the many available references purporting an advantage to larger flies, the proposal that bigger is better is perhaps a common first assumption when measuring fitness. However, an advantage for larger adult body size has not always been shown nor should it be expected, as there is no indication of an increase in body size over evolutionary time. For example, no relationship to mating success or the rapidity of mating was found for adult body size in *D. mojavensis* (Krebs and Markow, 1989). While *D. buzzatii* males out-competed smaller males two to one in mating success (note also an advantage to larger males in the field, Santos *et al.*, 1988), smaller males mated more often than larger males in *D. aldrichi* (Krebs and Barker, 1991). Further, neither *D. aldrichi* nor *D. buzzatii* showed a significant relationship between body size and the number of eggs laid (Krebs and Barker, 1991). Besides, results not reporting a relationship between adult body size and fitness likely are under-reported in the literature.

For a study of the effect of short term non-lethal temperature stress on fitness (Krebs and Loeschke, in review), we estimated fecundity in eight independent groups of female *D. melanogaster*. Females (40 vials with 10 individuals per vial) were first partitioned into four groups, three (groups 1, 2 and 3) of which were exposed to 36°C for 75 min in an incubator. Females in one no-treatment group (group 0), and exposed females at all other times, were held at 25°C. All females then were placed individually in vials of sucrose-agar-yeast medium along with two males. Live yeast was

In *Drosophila*, adult body size, generally estimated from thorax length, wing length, or weight, is a commonly studied character that is related to components of fitness (reviewed in Partridge and Fowler, 1993). Larger *Drosophila* have been shown to possess higher fecundity (Robertson, 1957; Atkinson, 1979; Partridge, 1988) and greater mating success

sprinkled across the surface of the medium in half of the vials in each group, and in another half, no live yeast was added. While group 1 females were exposed only once to the mild stress, females in groups 2 and 3 were exposed a second time after 48 hours, and females of group 3 also were exposed a third time another 48 hours later. All females were 3-days-old when they were first exposed to 36°C and/or placed with two males. Flies were transferred to fresh vials every second day over the following six days, and then every third day for the next nine days. Fecundity (offspring produced) was counted over 15 days. Thorax length (anterior margin of the thorax to the posterior tip of the scutellum, dorsal side up) was measured at the end of the experiment. Females that died during the 15 days were not measured. The total number of progeny per female was log transformed for analysis.

A significant positive relationship between size and fecundity within only two of the eight independent groups of females was found (Table 1). However, the power of the individual analyses was not great. For a sample size of 30, a correlation coefficient of 0.37 (explaining ca. 14% of the variance) was required to obtain significance. Where measurements exceeded 100 individuals, the correlation coefficient required for significance was reduced to about 0.20 (or 4% of the variance explained). Pooling data gave sample sizes in excess of 100 for each nutrition treatment, but a significant relationship between body size and fecundity was obtained only for females held on vials with live yeast (Table 1). Mean offspring number of the with-yeast ( $618 \pm 23$ ) and no-yeast ( $142 \pm 7$ ) groups were very different. Therefore, pooling all females in the analysis resulted in a non significant correlation coefficient between body size and fecundity ( $0.12$ ,  $N = 231$ ,  $P = 0.07$ ).

Table 1. Correlation coefficients between thorax length and the number of progeny produced by females (log transformed). Females were treated by exposure to 36°C for 75 min, non, one, two, or three times, and were held on vials with or without live yeast added to the surface of the medium. Following treatment, the progeny produced over 15 days were counted.

Number of exposures to 36°C	with-yeast	no-yeast
0	0.14 (N = 30)	-0.12 (N = 30)
1	0.51** (N = 32)	0.16 (N = 34)
2	0.02 (N = 29)	0.47* (N = 26)
3	0.36 (N = 30)	0.30 (N = 20)
Pooled treatments	0.30** (N = 121)	0.14 (N = 110)

presence or absence of yeast on the medium had a larger effect on the number of eggs laid than any difference in female body size, and the variance in fecundity also differed between nutritional treatments. The potential effect of adult nutrition on the variance in oviposition therefore could confound tests of the relationship between body size and fecundity, or other components of fitness estimated in the field.

Further, only differences in adult nutrition were considered here, without considering that flies of different size may have been genetically different. The presence of genotype by environment (nutritional) interactions will contribute to variation in fecundity within any particular size class. Genetic correlations between female fecundity and body size have been found in some studies, but not in others (Partridge and Fowler, 1993). Here we found a surprisingly large variation in the phenotypic correlation between body size and fecundity among independent groups of females despite that, for this species, a size/fecundity relationship commonly is observed.

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Regression analysis and comparison of the coefficients of variation suggested that the difference between nutrition treatments in the size-fecundity relationship was due, at least in part, to greater variation in fecundity when nutrition was limited. If regression coefficients for each nutrition treatment were obtained separately (with fecundity log transformed), the coefficient for the no-yeast treatment was larger than that of the with-yeast treatment. In the no-yeast treatment, log fecundity =  $1.71$  (s.e. = 0.38) + 0.56 (s.e. = 0.39) thorax length (mm); and with-yeast, log fecundity =  $2.40$  (s.e. = 0.14) + 0.49 (s.e. = 0.15) thorax length (mm). The regression coefficients for the two treatments were not significantly different from each other, but only that for the with-yeast treatment was significantly different from zero. The coefficient of variation for fecundity also was much larger for the no-yeast treatment than for the with-yeast treatment (69.8 versus 49.4, a ratio of 1.41, with  $F_{100,100} = 1.39$  at the 5% significance level).

This difference in variation due to a different nutrition demonstrates one of the major difficulties with estimating fitness from the number of offspring produced. Many environmental variables affect fecundity, especially where environmental extremes are considered (Hoffmann and Parsons, 1991; Krebs and Loeschke, 1994). Effects of these factors first should be partitioned in an analysis before effects of the character of interest may be tested. The basic difference of the

Chandler 1987a, Anim. Behav. 35:555-562; Partridge, L., A.A. Hoffmann and J.S. Jones 1987b, Anim. Behav. 35:468-476; Pitnick, S. 1990, Anim. Behav. 41:735-745; Robertson, F.W. 1957, J. Genet. 55:428-443; Santos, M., A. Ruiz, A. Barbadilla, J.E. Quazada-Diaz, E. Hasson and A. Fontdevila 1988, Heredity 61:255-262; Wilkinson, G.S., 1987, Evolution 41:11-21.

**Parkash, R., A.K. Munjal and J.N. Sharma.** M.D. University, Rohtak, India. Ethanol tolerance in *D. melanogaster* and *D. bipectinata*.

alcohol dehydrogenase of *D. melanogaster* converts a wide range of alcohols to aldehydes and more than 90% of the external alcohols are metabolized in a pathway initiated by ADH (Geer *et al.*, 1989). Most studies on ADH polymorphism and ethanol tolerance have been made on U.S. and Australian populations of *D. melanogaster* while Asian populations remain unexplored (David and Capy, 1988). It was, therefore, considered worthwhile to look at the extent of genic divergence at the *Adh* locus and the extent of ethanol tolerance in latitudinally varying natural Indian populations of *D. melanogaster* and *D. bipectinata*.

Table 1. Data on *Adh<sup>F</sup>* allelic frequencies and ethanol tolerance indices (ethanol threshold values, LT<sub>50</sub> max. / LT<sub>50</sub> control, LC<sub>50</sub> ethanol concentration and larval ethanol threshold values) of seven latitudinally varying Indian populations of *Drosophila melanogaster*.

Population	Latitude	<i>Adh<sup>F</sup></i> Freq.	Adult ethanol		Adult LC <sub>50</sub> (Ethanol conc.)	Larval ethanol (threshold values)
			(threshold values)	LT <sub>50</sub> max. / LT <sub>50</sub> control		
Cochin	9°. 58' N	0.11	9.0	1.75	9.25	6.0
Hyderabad	17°. 20' N	0.21	9.4	2.0	10.8	8.0
Nagpur	21°. 09' N	0.30	11.2	2.3	11.8	9.0
Bhopal	23°. 16' N	0.56	11.4	2.6	12.0	9.5
Rohtak	28°. 58' N	0.74	12.4	2.95	12.0	11.0
Saharanpur	29°. 58' N	0.78	13.0	3.0	13.5	13.0
Dehradun	30°. 18' N	0.80	13.2	3.1	14.0	13.0

Table 2. Comparison of *Adh<sup>F</sup>* allele frequency and ethanol tolerance indices (increase in longevity, LT<sub>50</sub> hrs, LT<sub>50</sub> max. / LT<sub>50</sub> control values at 1% ethanol, ethanol threshold concentrations in larvae as well as adults and LC<sub>50</sub> ethanol concentrations) in six geographical populations of *D. bipectinata*.

Population	Latitude	<i>Adh<sup>F</sup></i> Freq.	Increase in longevity		Ethanol threshold		
			LT <sub>50</sub> (hrs)	LT <sub>50</sub> max. / LT <sub>50</sub> control	Larval	Adult	LC <sub>50</sub>
Cochin	9°. 58' N	0.56	72	1.35	3.0	2.5	2.7
Bangalore	12°. 58' N	0.60	74	1.39	3.4	2.5	2.8
Nagpur	21°. 09' N	0.64	82	1.40	4.0	3.0	3.0
Calcutta	22°. 34' N	0.66	83	1.41	4.6	3.3	3.8
Hasimara	26°. 18' N	0.77	88	1.42	5.0	3.5	4.0
Rohtak	28°. 54' N	0.80	96	1.48	6.0	4.0	4.3

southern to northern localities (Table 1). The ethanol concentrations up to 10% ethanol concentration could be utilized by southern Indian populations of *D. melanogaster* while a maximum of 10% ethanol concentration could be utilized by northern Indian populations. The LC<sub>50</sub> ethanol concentrations were calculated from mortality data of adults after 6 days of ethanol treatment. These data revealed clinal variation in the range of 9.25% to 14.0% in *D. melanogaster* populations.

In *D. bipectinata*, the longevity data revealed that the Cochin population represented lesser longevity (72 hours) as compared with the higher longevity (96 hours) in Rohtak populations. The ethanol concentrations in the range of 3.0% to 4.0% served as a resource for north Indian populations of *D. bipectinata* while lower ethanol concentration (2.5%) could be utilized by south Indian populations (Table 2). The LC<sub>50</sub> values revealed clinal variation in the range of 2.7% to 4.3%. Since all the six Indian populations of *D. bipectinata* could utilize ethanol as a resource up to 1%, comparative longevity effects at 1% revealed inter-populational divergence (Table 2).

The ethanol utilization indices in larval as well as adult individuals were found to vary latitudinally in different populations of *D. melanogaster* and *D. bipectinata* (Tables 1-2). The statistical correlations were found to be significantly high among latitude versus adult and larval ethanol tolerance. The *Adh-F* allelic frequency also revealed significant correlation with latitude. Thus, ethanol utilization seems to be adaptively maintained by natural selection

*Drosophila* species populations exploit a wide array of fermenting and decaying fruits, vegetables, and other organic materials. Ethanol is the end product of fermentation and ethanol vapors provide a normal energy source in *D. melanogaster* (Parsons, 1983). The

The allelic frequency patterns at the *Adh* locus in *D. melanogaster* and *D. bipectinata* revealed significant clinal variation along a south-north axis among different geographical populations. The extent of clinal variation at the *Adh* locus was found to be signifi-

cantly higher in *D. melanogaster* (3% with 1° latitude; r = 0.97) than in *D. bipectinata* (1% with 1° latitude; r = 0.96) (Tables 1-2). The data on LT<sub>50</sub> control, the measures of resource versus stress, for seven *D. melanogaster* populations have revealed latitudinal variation (Table 1). The adult ethanol threshold values were found to vary clinally in the range of 9.0% to 13.2% among seven Indian populations from 13% served as resource for northern

populations of *D. melanogaster* while a maximum of 10% ethanol concentration could be utilized by southern Indian populations. The LC<sub>50</sub> ethanol concentrations were calculated from mortality data of adults after 6 days of ethanol treatment. These data revealed clinal variation in the range of 9.25% to 14.0% in *D. melanogaster* populations.

In *D. bipectinata*, the longevity data revealed that the Cochin population represented lesser longevity (72 hours) as compared with the higher longevity (96 hours) in Rohtak populations. The ethanol concentrations in the range of 3.0% to 4.0% served as a resource for north Indian populations of *D. bipectinata* while lower ethanol concentration (2.5%) could be utilized by south Indian populations (Table 2). The LC<sub>50</sub> values revealed clinal variation in the range of 2.7% to 4.3%. Since all the six Indian populations of *D. bipectinata* could utilize ethanol as a resource up to 1%, comparative longevity effects at 1% revealed inter-populational divergence (Table 2).

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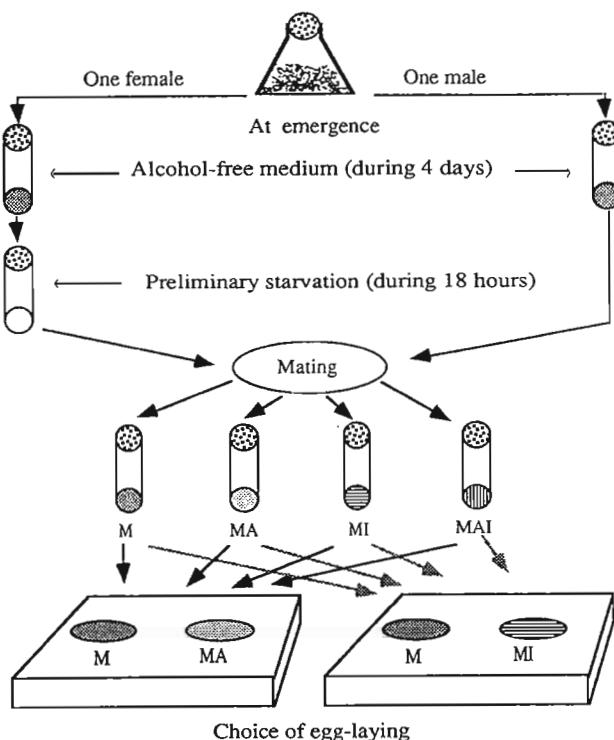
mechanisms.

The present data on clinal variation at the *Adh* locus in Indian populations of *D. melanogaster* and *D. bipectinata* further support and validate the hypothesis that occurrence of parallel or complementary latitudinal clines across different continental populations provide strong evidence of natural selection maintaining such clinal allozymic variations (Lemeunier *et al.*, 1986; Watada *et al.*, 1986; Singh and Rhomberg, 1987; David, 1982). The northern and southern populations revealed significant genetic divergence in their potential to utilize ethanol. The LT<sub>50</sub> ethanol/LT<sub>50</sub> control, adult threshold values, and LC<sub>50</sub> values for ethanol were found to be significantly higher in northern populations of *D. melanogaster* and *D. bipectinata* as compared with the southern populations. The lower threshold values of ethanol utilization in southern populations of *D. melanogaster* and *D. bipectinata* seem to be correlated with the lower levels of alcohol in diverse types of fermented sweet fruits in the tropical parts of the Indian sub-continent.

References: David, J.R., 1982, Biochem. Genet. 20:747; David, J.R. and P. Capy 1988, Trends in Genetics 4:106; Geer, B.W., P.W.H. Heinstra, A.M. Kapoun and A. Van Der Zel 1989, In: *Ecological and Evolutionary Genetics of Drosophila*, (Barker, J.S.F. and W.T. Starmer, eds.), Plenum Press, New York, pp. 231; Lemeunier, F., J.R. David, L. Tsacas, and M. Ashburner 1986, In: (Ashburner, M., H.L. Carson and J.N. Thompson, jr., eds.), *The Genetics and Biology of Drosophila*, vol. 3e, pp. 147; Parsons, P.A., 1983, *The Evolutionary Biology of Colonizing Species*, Cambridge University Press, Cambridge, pp. 262; Singh, R.S. and L.R. Rhomberg 1987, Genetics 117:255; Watada, M., Y.N. Tobari and S. Ohba 1986, Japan J. Genetics 61:253;

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University P. Sabatier Lab. d'Ethologie et Psychologie Animale, URA CNRS.1837, IREB.94/01 31062 Toulouse, France. Importance of *Adh* gene in ovipositional choice of alcoholized medium: a study using an ADH inhibitor.

the success of their offspring. The FF females homozygous for the *Adh* gene in ovipositional choice of alcoholized medium (Hougouto, 1982; Van Delden, 1990; El Amrani, 1991; El Amrani *et al.*, 1991).



In *D. melanogaster*, the individuals differing by the Fast and the Slow alleles of the alcohol dehydrogenase gene (*Adh*), exhibit a level of preference for an alcoholized medium in accordance with their ability to use ethanol as food (Cavener, 1979; Gelfand *et al.*, 1980; Van Herrewege *et al.*, 1980; Depiereux *et al.*, 1985; El Amrani *et al.*, 1991). In the fruit fly, the choice of ovipositional site by a female seems to be decisive for the fast allele of *Adh* gene preferentially lay their eggs on alcoholized medium (El Amrani, 1991; El Amrani *et al.*, 1991).

By utilization of an inhibitor of ethanol metabolism, the 4-methylpyrazole (4-MP), we attempt to induce an avoidance for ethanol in *Drosophila*. We search how a previous either beneficial or harmful experience following alcohol ingestion may act upon the choice by FF females between an alcoholized or an alcohol-free egg-laying medium.

The flies were reared at 25°C, on an alcohol-free nutritive medium, until the imaginal age of 4 days. Then we individually transferred, after a starvation of 18 hours, to various substrata: either deprived of alcohol and inhibitor (M), or supplemented with 3% ethanol (MA), or supplemented with inhibitor at the  $3\text{ M }10^{-3}$  concentration (MI), or supplemented with both alcohol and inhibitor (MAI).

All these groups of *Drosophila* were maintained on these substrata for 24 hours. Then the flies of each group were distributed into two sets of pairs (Figure 1).

In the first set ( $N = 120$  pairs): egg-laying choice between an alcoholized medium (MA) and an alcohol-free medium (M).

Figure 1. Method used to study egg-laying choice by FF females.

In the second set ( $N = 120$  pairs): between an alcohol and inhibitor deprived medium (M) and medium supplemented with inhibitor (MI).

Parametric tests were used to analyze the results where permitted by the distribution of the values: analyses of variance (Anova), followed by the Fisher test. Choice percentages were compared to the reference value of 50% (Schwartz, 1963).

No preference or repulsion for alcohol was shown in females reared on an alcohol-free medium (M). On the other hand females reared on an alcoholized medium (MA) preferentially laid their eggs on this medium. The ovipositional preference for alcohol in FF flies results from their individual experience.

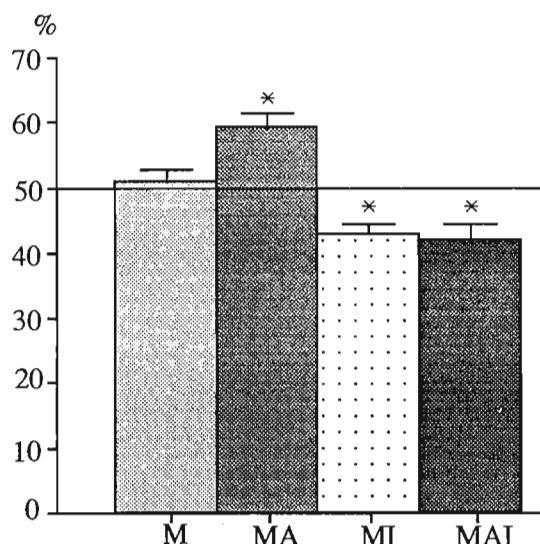


Figure 2. Egg-Laying percentage on medium supplemented with alcohol by females according to conditions of rearing before choice of ovipositional site. The (\*) indicates a significant difference ( $p < 0.05$ ) compared to the reference value of 50%.

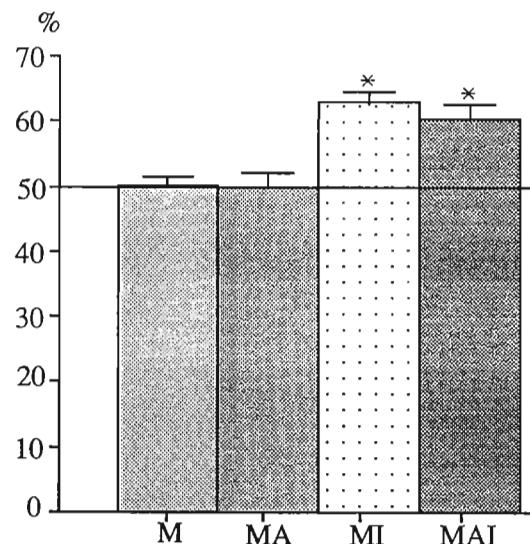


Figure 3. Egg-laying percentage on medium supplemented with inhibitor by females according to conditions of rearing before choice of ovipositional site. The (\*) indicates a significant difference ( $p < 0.05$ ) compared to the reference value of 50%.

Flies which previously ingested inhibitor associated (MAI) or not (MI) to alcohol, avoided ethanol when they laid their eggs (Figure 2). On the contrary they tended to lay on the medium supplemented with inhibitor (Figure 3).

The alcohol avoidance in flies which previously ingested inhibitor is not, therefore, imputable to ethanol association with a repellent substance as the 4-methylpyrazole. In this case, avoidance of alcohol seems to result from some disruption of ethanol metabolism induced by the inhibition of the ADH enzyme activity.

Incomplete metabolic transformation of ethanol appears as a negative reinforcement, inducing aversion for ethanol.

The choice of an alcoholized medium as ovipositional site by the FF females seems to result from a beneficial experience following the ingestion of ethanol and its metabolism by the ADH enzyme.

References: Cavener, D., 1979, Behav. Genet. 9(5):359-366; Depiereux, E., N. Hougouto, L. Lechien, M. Libion-Mannaert, M-C. Lietaert, E. Feytmans and A. Elens 1984, Behav. Genet. 15(2):181-188; El Amrani, A., 1991, Thèse. C.R.B.C., U.P.S. Toulouse; El Amrani, A., N. Cadieu, and J.C. Cadieu 1991, Behav. Proc. 24:153-165; Gelfand, L.J. and J.F. McDonald 1980, Behav. Genet. 10 (3):237-244; Hougouto, N., M.C. Liétaert, M. Libion-Mannaert, E. Feytmans, and A. Elens 1982, Genetica 58:121-128; Schwartz, D., 1963, Flammarion. Paris:57-65; Van Delden, W. and A. Kamping 1990, Behav. Genet. 20(5):661-674; Van Herrewege, J. and J. David 1980, Heredity 44:229-235.

**Chakrabarti, C.S.**, Department of Zoology, University of North Bengal, Siliguri-734-430, Darjeeling, India. SEM study on the anterior spiracular structures of the larva and pupa of *Drosophila ananassae*.

Under the SEM, I (Chakrabarti, 1990) have noticed very distinct anterior spiracles with neatly arranged finger-like spiracular papillae in the case of third instar larvae. The spiracle appears as a hypodermal elevation from which 11 to 12 finger-like processes protrude out. These papillae are provided with a broad base and gradually tapering apex. The tip of each papillae is provided with a knob-like ending (Figure 2). The papillae are of unequal length and diameter. Average length of the papillae are 50  $\mu\text{m}$ .

In each third instar *Drosophila* larva, a pair of anterior spiracles are found at the end of the great lateral tracheal trunk, opening into the prothoracic segment (Figure 1). Important information about the anterior spiracles appeared in the paper of Bodenstein (1950).

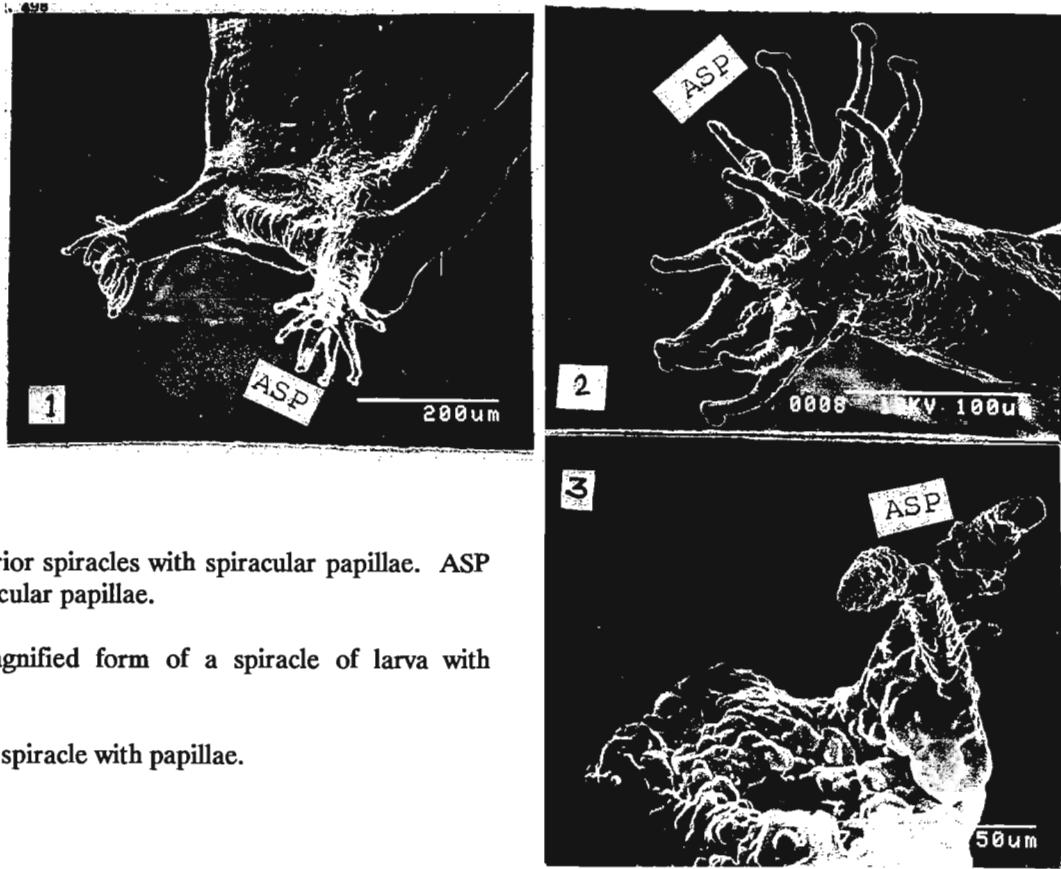


Figure 1. Anterior spiracles with spiracular papillae. ASP = anterior spiracular papillae.

Figure 2. Magnified form of a spiracle of larva with papillae.

Figure 3. Pupal spiracle with papillae.

In the case of prepupa and pupa, although the spiracular papillae are numerically identical to that of the third instar larvae, they are structurally reduced in size and are provided with wart-like cuticular elevations (Figure 3).

The instar-wise development of the anterior spiracles would be a parameter for the identification of different instars of *Drosophila* larvae. Since the anterior spiracles are absent in first instar larvae and also not distinctly visible in the case of second instar larvae, SEM observation can play a vital role in this direction because more detailed information could be achieved through this process of study. For instance, earlier observers (see Bodenstein, 1950) have mentioned the presence of 6-9 finger-like projections around the spiracular opening in the case of third instar larvae, but I (Chakrabarti, 1990) have seen as many as 12 projections (papillae), which are arranged encircling the opening (Figure 2).

References: Bodenstein, D., 1950, In: *Biology of Drosophila*, (M. Demerec, ed.), J. Wiley and Sons, New York, pp. 275-364; Chakrabarti, C.S., 1990, Ultrastructural and genetical studies on the preadult and adult of *Drosophila ananassae*, Ph.D. thesis, University of Burdwan (unpublished).

**Chakrabarti, C.S.** Department of Zoology, University of North Bengal, Siliguri 734 430, Darjeeling, India. SEM observation on the proboscis of the adult *Drosophila ananassae*.

basiproboscis, which includes the clypeus and the area of submentum containing the maxillary palpi; second the

Ferris (1942) described the structure of the proboscis of *Drosophila* in great detail. Ultramicroscopic observations on the sensory structures of the proboscis of *Drosophila ananassae* were made to fill the gap of information on this particular insect.

Proboscis is divided into three main parts: first the

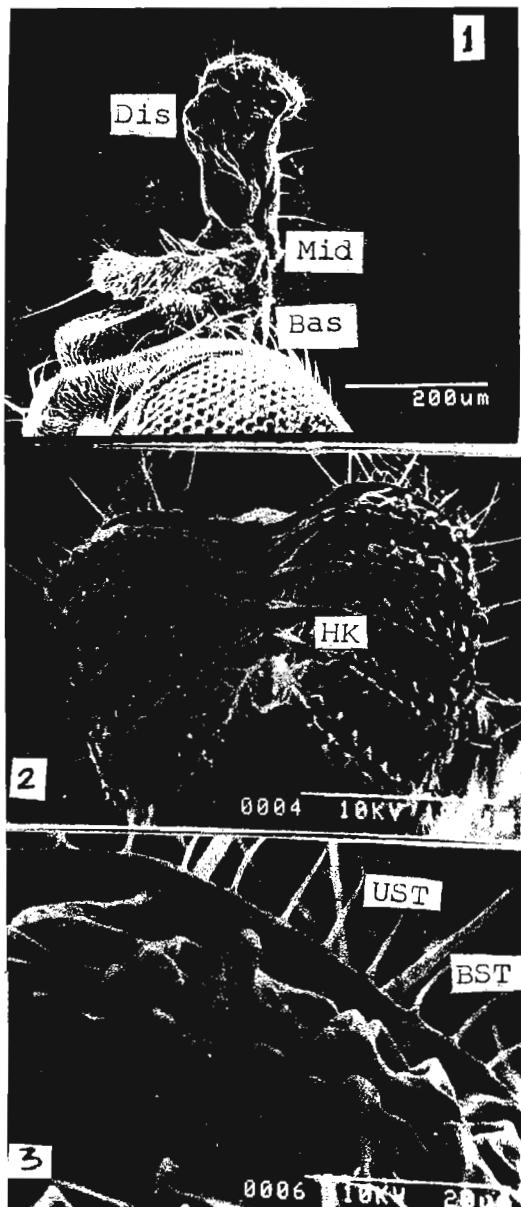


Figure 1. Extended proboscis showing three main parts.

Figure 2. Apical region of the proboscis showing sclerotized plates.

Figure 3. Hooklets of the sclerotized band.

Dis = Distiproboscis, Mid = Mid proboscis, Bas = Basiproboscis, HK = Hooklet, BST = Bifurcated Sensilla trichoda, UST = Undivided sensilla trichoda, SH = Small hairs, DST = Divided sensilla trichoda



Figure 4. Enlarged form of the apical region of the proboscis showing small hairs.

Figure 5. Tip of the proboscis showing sensilla trichoda.

Figure 6. Sensilla trichoda with divided arms.

mediproboscis; and third the distiproboscis, which is made up of the swollen labial palpi in the case of *Drosophila* (Figure 1).

The apical region of the proboscis forms two large lobes, commonly known as labella. This area is highly ornamental. On the dorsal surface of this zone, two sclerotized plates are present, which articulate with the elongated dorsal labial plates (Figure 2). From each of these plates, five or six furrows radiate on either side. The furrows are kept open by narrow transverse sclerotized bands of variable shapes. The bands are provided with a number of hooklets (Figure 3). At the rostral part of the labial plate several small hairs are found, which radiate out from the margin of a dome shaped structure (Figure 4). The hairs may be sensory in nature.

The main sensory structures of the proboscis are sensilla trichoda. Some of them are long and stout with bifurcated tips, others are short and thin with undivided tips (Figures 3 and 5).

Kankel, et al., (1980) reported that both the long and short sensilla trichoda are with bifurcated tip but in the case of *D. ananassae*, I, (Chakrabarti, 1990) have noticed only the long sensilla trichoda with bifurcated tip. Albeit, some hairs with divided arms and a common stalk were noticed at the apical end of the proboscis (Figure 6).

References: Chakrabarti, C.S., 1990, Ultrastructural and genetical studies on the preadult and adult of *Drosophila ananassae*. Ph.D. Thesis. University of Burdwan (unpublished); Ferris, G.F., 1942, Microentomology 5(3):87-90; Kankel, D.R., A. Rerrus, S.H. Garen, P.J. Harte, and P.E. Lewis 1989, In: *The Genetics and Biology of Drosophila*, Vol. 2d (M. Ashburner and T.R.F. Wright, eds.), pp. 295-368, Academic Press, London.

**Chakrabarti, C.S.** Department of Zoology, University of North Bengal, Siliguri 734 430, Darjeeling, India. SEM observation on the segmental spinules of *Drosophila ananassae* larvae.

external surface of the cuticle. In the collar region the spinules are very delicate, papillae-like, and arranged in four to five rows (Figure 1). The papillae are broad based with pointed tips, whose tapering ends are backwardly directed (Figure 2). The entire thoracic part of the third instar larva is beset with segmental spinules. Thoracic spinules are triangular projections, arranged in parallel rows. Tips of all these spinules are backwardly directed (Figure 3). On the ventral surface of each of the eight abdominal segments, patches of spinules are present (Figure 4). The spinules are dwarf hook-like projections with broad bases, curved body and pointed tips (Figure 5). In each segment, the spinules are found in a number of batches (Figure 6).

There are seven rows of spinules in a batch (Figure 6). In comparison to the thoracic spinules, abdominal spinules are arranged in different directions, the spinules of the two outermost rows being prominently arranged in opposite directions (Figure 6). In each batch, the spinules are different in size and are oriented in different directions.

Therefore, in consonance with the proposition of Godoy-Herrera and Araneda (1987), I, (Chakrabarti, 1990) would like to suggest that like *D. melanogaster* the larvae of *D. ananassae* utilize the spinules of thoracic segments for burrowing purposes and the abdominal spinules for locomotory activities. The spinules of the collar region might be sensory in function.

References: Chakrabarti, C.S., 1990, Ultrastructural and genetical studies on the preadult and adult of *Drosophila ananassae*, Ph.D. Thesis, University of Burdwan, (Unpublished); Godoy-Herrera, R., and J.C. Araneda 1987, Dros. Inf. Serv. 66:67.

Figure 1 (opposite page). Anterior part of the larva showing collar papillae. CP = Collar papilla, TS = Thoracic spinule, AS = Abdominal spinule.

Figure 2 (opposite page). Magnified view of the collar papillae.

Figure 3 (opposite page). Unidirectional segmental spinules of the thorax.

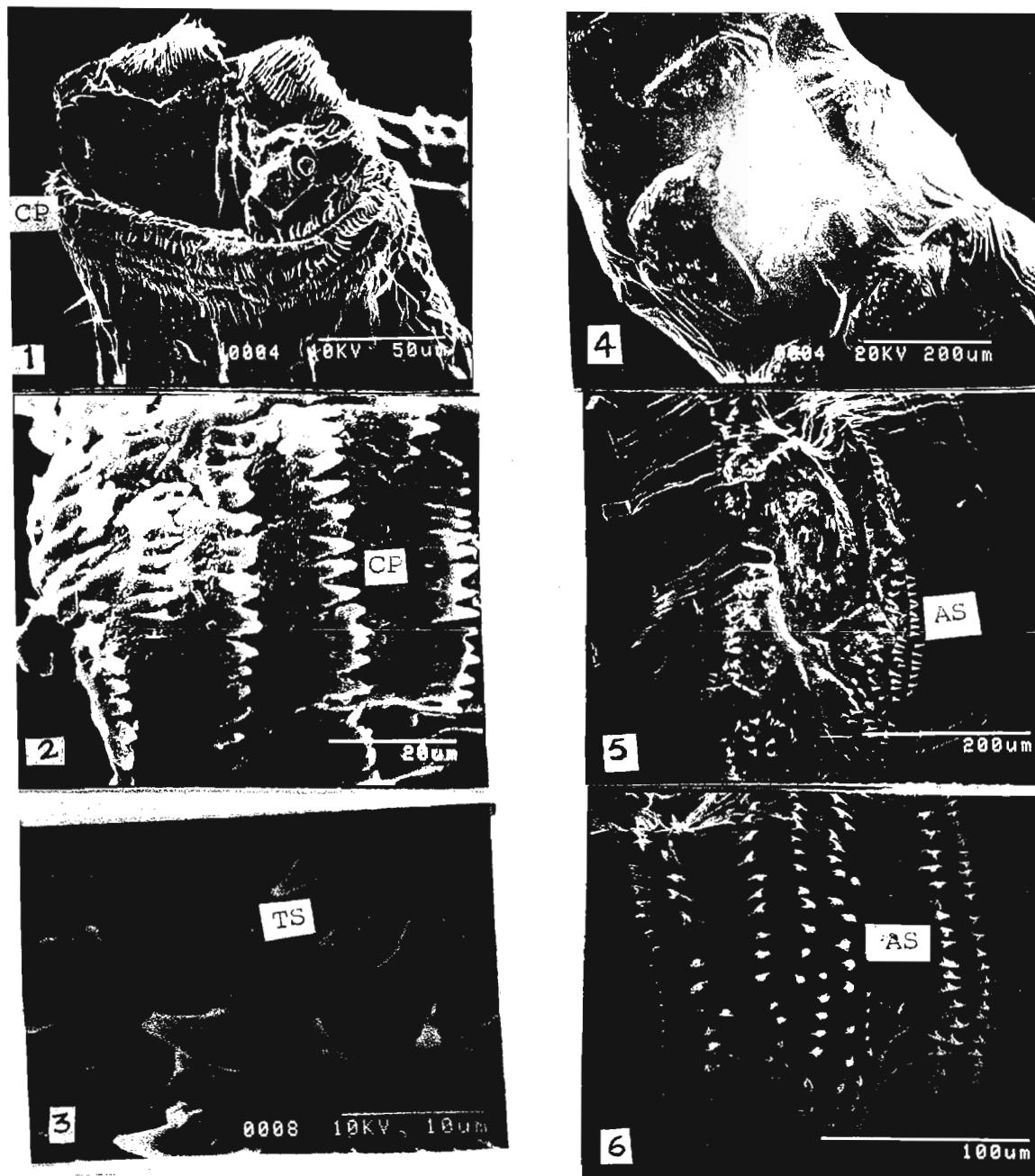
Figure 4 (opposite page). Larval abdomen showing patches of spinules.

Figure 5 (opposite page). Abdominal spinules of one area.

Figure 6 (opposite page). Rows of abdominal spinules.

Godoy-Herrera and Araneda (1987) described the structures and orientations of the segmental spinules of *Drosophila melanogaster*.

In the case of *Drosophila ananassae*, I (Chakrabarti, 1990) have noticed different types of spinules arranged in various forms throughout the



**Chakrabarti, C.S.** Department of Zoology, University of North Bengal, Siliguri 734 430, Darjeeling, India. SEM observations on the posterior spiracular structures of the larva and pupa of *Drosophila ananassae*.

SEM studies on the posterior spiracles of the third instar larvae of *Drosophila ananassae* clearly displays four groups of hairs radiating out from the cuticle of the spiracular plate (Figure 1). These hairs alter their morphology and orientation with the aging of the third instar larvae. In the early third instar, the hairs are flat and leaf-like, without distinct branching (Figure 2). In the mid-third instar, the hairs are much branched. Each bunch of hair starts radiating from a central axis. The axis divides into four or five main branches. Among these, the median branches are longer and thicker than the peripheral branches. The longer branches again divide dichotomously, thus forming a rosette (Figure

Posterior spiracles are found in *Drosophila* larvae of all stages, opening on the dorsal side of the eighth abdominal segment. The posterior spiracles of first instar larvae have only two openings but the second and third instar larvae have three openings (Bodenstein, 1950).

3). In the late third instar, the branching system gradually diminishes (Figure 4). Spiracular hairs disappear in the case of prepupa and pupa (Figure 5). Moreover the pupal posterior spiracles undergo distinct morphological changes with the development of uneven cuticular folding (Figure 6).

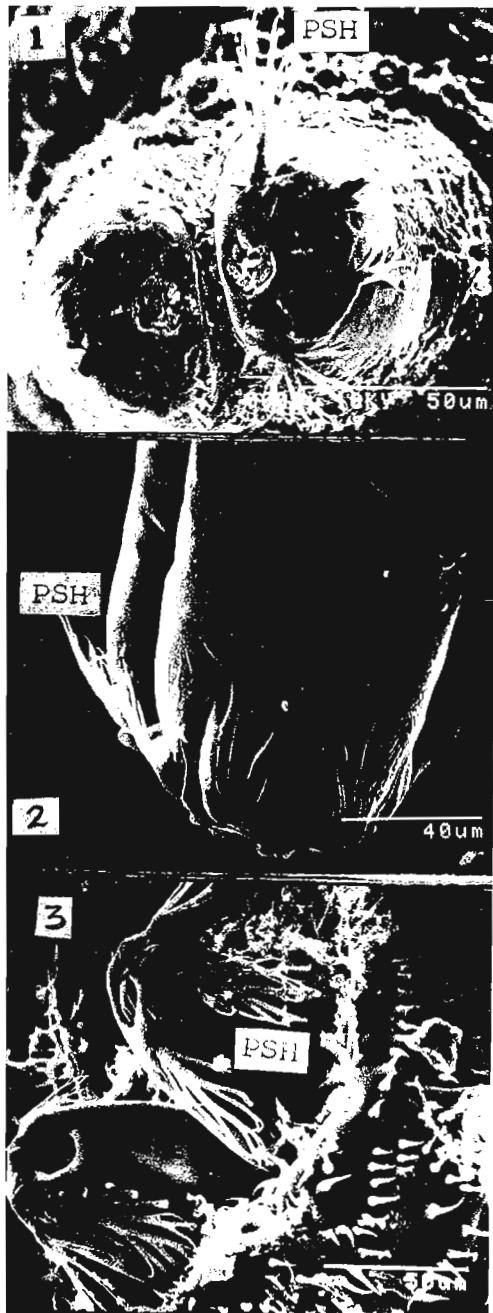


Figure 1. Four groups of hair originating from the spiracular plate. PSH = Posterior spiracular hair.

Figure 2. Leaf like spiracular hair.

Figure 3. Branched spiracular hairs.

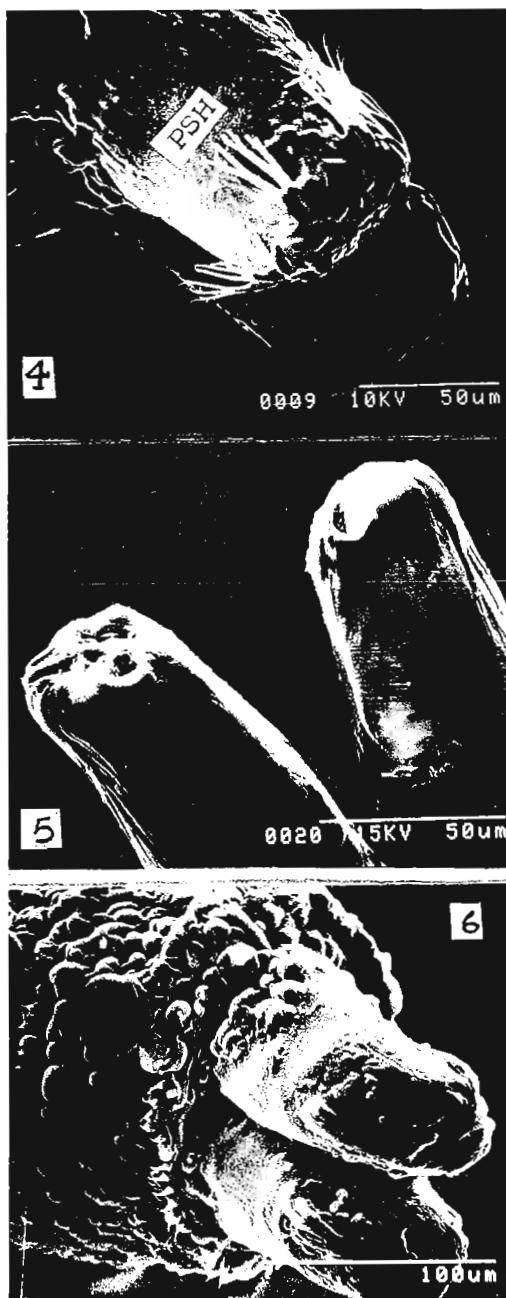


Figure 4. Reduced spiracular hairs.

Figure 5. Prepupal posterior spiracles.

Figure 6. Pupal posterior spiracles.

SEM study on the posterior spiracles clearly show gradual transformation of the spiracular hairs with the aging of the larvae. Possibly these hairs perform sensory functions because they are much branched in the most active phase of

the third instar larvae (Chakrabarti, 1990).

References: Bodenstein, D., 1950, In: *Biology of Drosophila*, (M. Demerec, ed.), J. Wiley and Sons, New York, pp. 275-364; Chakrabarti, C.S., 1990, Ultrastructural and genetical studies on the preadult and adult of *Drosophila ananassae*, Ph.D. thesis, University of Burdwan (unpublished).

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longing to 9 genera and 2 subfamilies, which have so far been collected from Sakha SSR, the East Siberia.

Sakha, with about 3.1 million km<sup>2</sup>, is a cold territory characterized by huge taiga and arctic tundra. The faunal survey was made at five localities, Tiksi (72°N, 128 E), Verkhoyansk (68° N, 144 E), Jigansk (66° N, 123 E), Yakutsk (62° N, 130 E) and Olekminsk (60° N, 119 E), along the River Lena and the River Yana. Tiksi is located in the arctic tundra, but the other four localities are in the forest zone (taiga). Fly collections were made by fermenting malt baits (Lakovaara *et al.*, 1969) and by net-sweeping on herbaceous plants and mushrooms in July to August, 1992, and in July, 1993.

Numbers of collected individuals of 31 drosophilid species are shown separately for each locality in Table 1. Of the 31 collected species, 2 are new to science (double-asterisked in Table 1), though one of them, *Amiota* sp. like *conifera takadai*, has been collected also from the Russian Far East and northeastern China, 21 are new to Siberia (single-asterisked in Table 1), and 1 is an undetermined species. In Tiksi, only a domestic species, *Drosophila melanogaster*, was collected in a heated fruit shop. Seven species were obtained each from two localities, Verkhoyansk and Jigansk, within the Arctic Circle. All of them were collected by malt traps, except for *Scaptomyza pallida* collected by net-sweeping. In the further south, collections not only by malt traps but also by net-sweeping on mushrooms and herbaceous plants yielded samples more abundant in number of species: 21 spp. from Yakutsk and 16 spp. from Olekminsk.

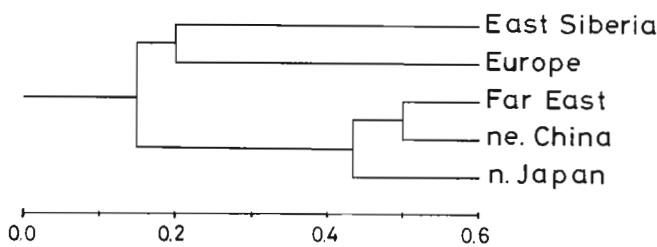


Figure 1. Comparison of drosophilid faunas among five subregions of the Palearctic Region, based on the Jaccard's coefficient similarity.

The drosophilid fauna of the East Siberia was compared with those of four neighboring regions, based on the following data sources: Europe (101 spp.; Baechli and Rocha-Pite, 1984), the Russian Far East (87 spp.; Sidorenko, 1989, 1990, 1993, etc.), northeastern China (87 spp.; Watabe *et al.*, 1993; Toda, unpubl.), and northern Japan (149 spp.;

The East Siberia is an important area for tracing the evolution of drosophilid species distributed at high latitudes in the Palearctic and/or Nearctic Regions and for studying their adaptations to an extremely cold climate. However, little is known about its drosophilid fauna. Only 13 species have so far been recorded from Siberia except the Far East Region (Duda, 1935; Sidorenko, 1993; etc.).

We just started a Siberian *Drosophila* study in 1992, and the preliminary report here is a list of 31 species belonging to 9 genera and 2 subfamilies, which have so far been collected from Sakha SSR, the East Siberia.

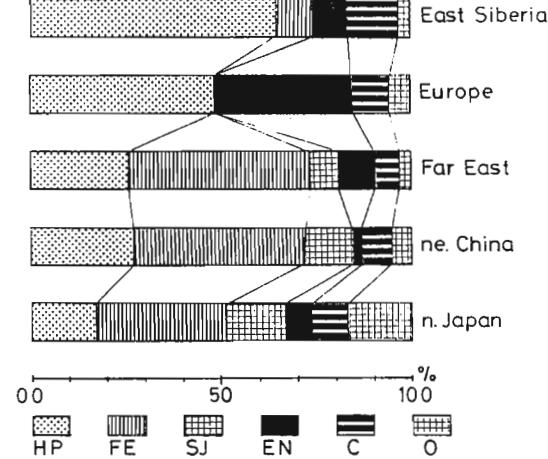


Figure 2. Drosophilid faunal composition of chorological elements in five subregions of the Palearctic Region.

Table 1. Drosophilids collected from Sakha SSR, the East Siberia.

Species	Tiksi	Verkhoyansk	Jigansk	Yakutsk	Olekmansk	Total
<b>Subfamily Steganinae</b>						
Genus <u>Gitona</u>						
* <u>G. distigma</u>	-	-	-	1	-	1
Genus <u>Amiota</u>						
Subgenus <u>Amiota</u>						
* <u>A. neochungi</u>	-	-	-	1	-	1
Subgenus <u>Phortica</u>						
** <u>A. sp. like conifera takadai</u>	-	-	-	9	5	14
Genus <u>Leucophenga</u>						
* <u>Le. quinquemaculipennis</u>	-	-	-	3	-	3
<b>Subfamily Drosophilinae</b>						
Genus <u>Scaptodrosophila</u>						
* <u>Sd. rufifrons</u>	-	-	-	5	-	5
Genus <u>Chymomyza</u>						
* <u>C. caudatula</u>	-	-	-	2	-	2
* <u>C. costata</u>	-	7	1	68	20	96
* <u>C. distincta</u>	-	-	-	-	1	1
* <u>C. fuscimana</u>	-	-	-	2	-	2
Genus <u>Hirtodrosophila</u>						
* <u>H. subarctica</u>	-	19	3	11	-	33
Genus <u>Drosophila</u>						
Subgenus <u>Sophophora</u>						
* <u>D. alpina</u>	-	11	1	-	-	12
<u>D. bifasciata</u>	-	-	-	1880	3	1883
<u>D. melanogaster</u>	1	-	-	157	-	158
Subgenus <u>Dorsilopha</u>						
* <u>D. busckii</u>	-	-	-	15	-	15
Subgenus <u>Drosophila</u>						
* <u>D. ezoana</u>	-	2	17	-	-	19
* <u>D. littoralis</u>	-	-	3	-	1	4
* <u>D. lummei</u>	-	-	27	14	-	41
<u>D. funebris</u>	-	-	-	192	6	198
* <u>D. immigrans</u>	-	-	-	2	-	2
* <u>D. phalerata</u>	-	-	-	-	1	1
* <u>D. metakuntzei</u>	-	6	-	17	-	23
* <u>D. transversa</u>	-	3	1	95	45	144
<u>D. testacea</u>	-	-	-	3	1	4
Genus <u>Lordiphosa</u>						
* <u>Lo. hexasticha</u>	-	-	-	-	3	3
Genus <u>Scaptomyza</u>						
Subgenus <u>Hemiscaptomyza</u>						
* <u>Sc. okadai</u>	-	-	-	-	5	5
<u>Sc. unipunctum unipunctum</u>	-	-	-	-	6	6
** <u>Sc. sp.1 like unipunctum unipunctum</u>	-	-	-	2	37	39
Subgenus <u>Parascaptomyza</u>						
<u>Sc. pallida</u>	-	1	-	2	3	6
Subgenus <u>Scaptomyza</u>						
<u>Sc. flava</u>	-	-	-	-	7	7
* <u>Sc. polygonia</u>	-	-	-	-	10	10
<u>Sc. sp.SB1</u>	-	-	-	1	-	1
Total number of species	1	7	7	21	16	31
Total number of individuals	1	49	53	2482	154	2739

\*\* New to science, \* new to the Siberia

Okada, 1988; Toda, unpubl.). Faunal similarity between two regions was evaluated by Jaccard's coefficient of similarity:  $S = c/(a + b - c)$ , where  $c$  is the number of species common to both regions and  $a$  or  $b$  is the number of species occurring in each region. The similarity matrix resulting from pair-wise calculations was then subjected to a cluster analysis.

Three regions, the Russian Far East, northeastern China and northern Japan, constitute a compact group in the dendrogram (Figure 1), indicating that these Asian parts of Eurasia share many species in common. On the other hand, East Siberia has a closer similarity in the species composition with Europe than with the three regions of eastern Eurasia.

Furthermore, the faunas of five regions were compared with each other for the composition of chorological elements (Figure 2). The component species were classified into six elements for their geographic distribution patterns: HP) Holarctic or Palearctic, FE) Far Eastern, SJ) Sino-Japanese, EN) Endemic, C) Cosmopolitan, and O) others. In the composition of chorological elements, too, the East Siberia is more similar to Europe than to the other regions of eastern Eurasia. Of the 31 drosophilid species recorded presently, 20 HPs and 4 Cs are commonly distributed in Europe, especially Scandinavia. On the other hand, the fauna of East Siberia includes only 4 FEs and no SJ elements: FE elements are major components in cool temperate regions of eastern Eurasia, and SJ elements are distributed in warm temperate regions from Nepal through southern China to Japan.

In conclusion, East Siberia possesses a close relation to Europe, especially Scandinavia, in the drosophilid biogeography.

**Acknowledgments:** We wish to express our hearty thanks to the following persons for their great help in this study: Prof. N.G. Solomonov, Dr. B.I. Ivanov, Dr. T.K. Maximov, Dr. A.I. Averensky and many staff members of the Biological Institute of Yakutsk. We also thank Mr. G. Takaku for his help in collecting the samples. This work is supported by Grant-in-Aid for Overseas Scientific Study from the Ministry of Education, Science and Culture of Japan (No. 04041014).

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**Sugimura, Isamu, Fumiaki Maruo, Yukiko Iwai and Masukichi Okada.** Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan. On the allelism of *fs(1)K1274* and *Sex-lethal* mutations.

cause of tumorous egg chambers); at 25°C, have egg chambers containing 16 nurse cells and no oocyte; but, at 23°C, produce fertile eggs. The genetic map position is located between *cv* and *ct* (Komitopoulou *et al.*, 1983), and cytological location previously reported is 5D5-6E1 (Perrimon *et al.*, 1986).

M. Mével-Ninio and M. Gans kindly provided us with *fs(1)K1274*, so that we can repeat the observation of this strain. Different from the original description, all homozygous females had ovaries filled with tumorous egg chambers. The phenotype was stable under any tested temperature conditions (18°C - 29°C) and unaffected by genetic backgrounds.

We noted that the tumorous egg chamber phenotype of *fs(1)K1274* mutation resembles that of *Sxl* germline clone. Furthermore, the region 5C-6E in the X chromosome is deduced to include a sex determining activity from the previous report (Steinmann-Zwicky and Nöthiger, 1985). The available information allowed us to build a working hypothesis that *fs(1)K1274* is a sex determination gene like *fs(1)A1621* (= *snf, liz*). To test this hypothesis, we carried out a pilot study to see if *fs(1)K1274* is in dominant interaction with *Sxl*, in which *fs(1)K1274* was crossed to *Sxl<sup>f1</sup>* or *Df(1)HA32* (= *Df(1)6E4-5;7A6*, a chromosome uncovering the *Sxl* locus (6F5)). Resulting flies of the genotype *fs(1)K1274/Sxl<sup>f1</sup>* and *fs(1)K1274/Df(1)HA32* were both completely lethal (over 500 progeny counted for each cross).

The *fs(1)A1621* locus is involved in germ line sex determination cascade (Oliver *et al.*, 1988; Steinmann-Zwicky, 1988). Females of *fs(1)A1621/fs(1)K1274* were occasionally observed to have egg chambers with 16 nurse cells. Double homozygous females of *fs(1)A1621* and *fs(1)K1274* were semilethal with survivors having tumorous egg chambers.

The *fs(1)K1274* mutation was isolated from the screen of X-linked female sterile mutations by Komitopoulou *et al.* (1983). According to the original description, this mutation causes temperature sensitive recessive female sterility: ovaries of homozygous females, at 29°C, are severely atrophied (probably be-

Genetic mapping using *cv*, *cm*, *ct* markers in X chromosome revealed that *fs(1)K1274* was mapped at 1 - 19.2. This map position coincides with that of *Sxl*. Moreover, *Df(1)HA32* deletes the region including this locus. If the lethality of *fs(1)K1274/Sxl<sup>f1</sup>* females is caused by dominant interaction of two different loci, *Df(1)HA32* should be dominant lethal. Our observation showed that it is not the case. From these results, we discard our working hypothesis and conclude that the dominant interaction between *fs(1)K1274* and *Sxl* is an allelism rather than synergistic interaction.

**Acknowledgments:** Stocks of *Sxl<sup>f1</sup>*, *Df(1)HA32*, *cm* *ct* were obtained from the Bloomington Stock Center, and *fs(1)A1621* stock was provided by the Mid-America *Drosophila* Stock Center. We thank M. Mével-Ninio, M. Gans and the two stock centers for stocks and N. Perrimon for his comment.

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**Kamping, A. and W. van Delden.** Department of Genetics, Biology Centre, University of Groningen, The Netherlands. Differences in restoration of male fertility among *Inversion (2L)t* and Standard karyotypes in *Drosophila melanogaster* after high temperature exposure.

geographic distribution of *In(2L)t*, with temperature as a selective agent. Karyotypic differences in viability and productivity in *D. melanogaster* were also observed for *In(2L)B* karyotypes (Watanabe and Watanabe 1973, 1977).

In addition to decreased viability, exposure to high temperature will lead to complete or partial sterility of both females and males (Young and Plough, 1926). When exposure time is not extremely long and damage is restricted, a change to normal temperatures may result in restoration of fertility.

We examined the restoration of fertility of *In(2L)t* and Standard homo- and heterokaryotypes. Sixty males (four days old and raised at 25°C) of each of the three karyotypes were individually exposed to 33°C for a period of two days. After exposure for two days at 33°C no adult mortality was observed; however, all males of the three karyotypes were completely sterile. After a subsequent recovery period of four days at 25°C, each of the males was combined with two 6 days old virgin females of the same karyotype raised at 25°C. The number of matings in a 3 hours testing period at 25°C were scored. Males and females were separated and the number of matings producing offspring was recorded.

About 90% of all exposed males mated, with no significant difference in the number of matings among karyotypes. However, the difference in number of matings producing offspring was highly significant among karyotypes ( $\chi^2 = 19.3$ ,  $df = 2$ ,  $p < 0.001$ ). Restoration of male fertility of *In(2L)t* homo- and heterokaryotypes was 61.5% and 53.4%, respectively, but only 21.2% for Standard homokaryotypes. Similar results were obtained when egg-to-adult development was accomplished at 33°C and the emerged males were immediately placed at 25°C. So both juvenile and adult stages are affected by high temperature exposure resulting in temporary male sterility.

These karyotypic differences in restoration of male fertility may play a significant role in the maintenance of the latitudinal distribution of *In(2L)t*, characterized by high inversion frequencies in tropical regions and a negative correlation of inversion frequencies with latitude on both hemispheres (Mettler *et al.*, 1977; Knibb *et al.*, 1981; Knibb, 1982).

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In previous papers, we observed a significant superiority of *D. melanogaster* karyotypes containing *In(2L)t* for egg-to-adult survival at high temperatures (Van Delden and Kamping 1989, 1991). It was concluded that these differential viabilities together with observed karyotypic differences in body weight and development time (Van Delden and Kamping 1989, 1991) play a significant role in the maintenance of the

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cDNA libraries were constructed by using a cDNA synthesis system plus (Amersham), and ligating to Lambda ZAP vector DNA (Stratagene, USA) (Akaboshi and Howard-Flanders, unpublished). cDNAs corresponding to the mRNAs induced by MMS treatment were enriched by the subtraction method. <sup>32</sup>P labeled cDNAs prepared from the cells treated with MMS were hybridized twice with an excess of mRNAs of untreated cells. After each hybridization reaction the sample was incubated with Avidin-DN and chromatographed over a copper-chelate resin in order to get the enriched cDNAs (Welcher *et al.*, 1986).

Table 1. cDNAs induced by MMS treatment

Name of cDNA clone	Length of inserts (kb)	Cytological location	Comments
4	1.2	62C	
23	1.9	4F, 48D/E	
35	1.9	86E/F	ribosomal protein S11 (RATRPS11, HUMRPS11, 65% in 197 bp)
42	1.2		D.m. Glutathione S-transferase 1-1 (DRODST, 78% in 451 bp)
43	2.3	91C/D	Ubiquitin
53	2.1	55C	
82	1.2	22F	
91	1.4	42C/D	
127			
128	1.9	98F	DROHSP82 Human cystic fibrosis mRNA (HUMCFTRM, 53% in 1046bp) D.m. p-glycoprotein (DROMDR49, 52% in 733bp)
145	0.65	8B/C	
158	2.2	7A	
163	0.9	77E/F	
217	4.9	6C, 8E, 29A, 43C, 49D, 55A, 59F, 85D, 88E, 91A	
BS1*	1.1	29C	
BS3*	1.2	46B/C	
BX203*	1.7	21A	

\* These clones were confirmed to be inducible in the search for homologous genes of human IL-6 genes.

were classified as follows. Insert DNA fragments from several randomly selected clones were purified and confirmed that they were the sequences transcribed when treated with MMS by either northern blots or spot test hybridization. Then they were hybridized to a mutagen sensitive gene library. Clones which showed positive signals were considered as one group. These procedures were repeated with clones which did not show appropriate signals.

Nucleotide sequences of the inserted DNA of a clone of a classified group were determined and searched for homology with the GenBank DNA database by using the FASTA program (Pearson and Lipman, 1988). Their cytological locations were mapped by *in situ* hybridization to the salivary gland chromosomes of Oregon R. The results are summarized in Table 1.

**Acknowledgments:** We thank Drs. J.T. Lis and E.A. Craig for the gifts of plasmid DNAs, aDm 4.46, p70X 2.6 and aDm 202.7, and T6BV1, respectively. We are very grateful to Dr. M.M. Green for reading the manuscript.

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Prokaryotic and eukaryotic cells respond to the stresses caused by agents that damage DNA and many genes are activated (Little and Mount, 1982; Fornace *et al.*, 1988; Akaboshi and Howard-Flanders, 1989). This is a report of cloning and cytological mapping of genes expressed in *Drosophila melanogaster* cultured cells when treated by an alkylating agent, methyl methane sulfonate (MMS). The *Drosophila* cells were treated with 0.025% MMS for 3 or 6 hours as described previously (Akaboshi and Howard-Flanders, 1989). The mRNA was isolated from treated cells using a guanidium-CsCl method followed by poly(A) selection.

Two hundred twenty clones, which showed signals stronger in plaque hybridization to the <sup>32</sup>P labeled-enriched cDNAs than to <sup>32</sup>P labeled-cDNAs control, were isolated from 2 x 10<sup>4</sup> plaques. 40% of the clones were hybridized to the DNAs known to correspond to the heat shock proteins (hsp) and were confirmed to be hsp's 22K, 26K, 70K, 83K and either 21K or 23K (Craig *et al.*, 1979; Lis *et al.*, 1981; Hackett and Lis, 1983). They were eliminated from the mutagen sensitive gene library. The remaining clones

**Davis, A.J. and K.E. Jones.** Drosophila Population Biology Unit, Pure and Applied Biology, The University, Leeds, West Yorkshire LS2 9JT UK. *Drosophila* as indicators of habitat type and habitat disturbance in tropical forest, central Borneo.

Because complete faunal surveys are laborious, expensive and potentially disruptive, it is useful to find indicator species that can offer a means of answering these two questions. Indicator species should be short-lived so that they represent the effects of the current habitat without historical effects, mobile so that the absence of a species is caused by the unsuitability of the habitat and not a failure to colonize, and numerous to improve their ability to detect subtle differences between habitats. The individuals of these species should also be abundant and easily caught. The genus *Drosophila* has all these attributes and, therefore, to investigate their utility as indicators of faunal diversity and species composition, we undertook a study of the *Drosophila* in lowland tropical forest in Borneo.

Table 1. Identification and description of the six forest habitat types from which *Drosophila* were collected.

Habitat type	Habitat description	No. baits successful
dipterocarp C	primary forest with large 35 - 40 m <i>Dipterocarpus krii</i> trees	10
dipterocarp D	primary forest with large 35 - 40 m Dipterocarpus krii trees	19
heath forest	primary forest dominated by relatively short <i>Alstonia</i> and <i>Hopea</i> trees	14
secondary forest	30 yr-old regrowth <i>Cratoxylum</i> , <i>Macaranga</i> and <i>Tristania</i> dominant, dense herb and understory layers	16
edge habitat	edge of cleared and 30 yr-old secondary forest, <i>Cratoxylum</i> , <i>Macaranga</i> and <i>Tristania</i> nearby	14
cleared forest	maximum tree height 20 m center of base camp in garden dominated by banana and cassava	14

Table 2. Mean total species (species richness) and Shannon diversity indices with standard deviations (in brackets) for six tropical habitats in central Borneo

Habitat type	mean total species	Shannon diversity
dipterocarp C	9.9 (3.81)	2.03 (0.35)
dipterocarp D	6.9 (3.02)	1.39 (0.54)
heath forest	3.2 (1.62)	0.88 (0.43)
secondary forest	2.8 (1.45)	0.83 (0.56)
edge habitat	5.0 (3.49)	1.27 (0.79)
cleared forest	5.5 (2.68)	1.37 (0.57)

Table 3. Qualitative Sorenson estimates (means above and standard deviations below the diagonal) of species of *Drosophila* shared by pairs of tropical forest habitats in central Borneo.

	dipt-C	dipt-D	heath	2nd ry	edge	cleared
dipterocarp C	—	.568	.367	.356	.625	.498
dipterocarp D	.026	—	.367	.390	.461	.397
heath forest	.027	.023	—	.576	.266	.467
secondary forest	.017	.023	.029	—	.304	.302
edge habitat	.037	.018	.011	.021	—	.583
cleared forest	.027	.014	.023	.012	.021	—

Tropical forests appear to regain much of their vegetative diversity within a few decades of exploitation for timber or small-scale farming. However, environmentalists need to be able to answer two important questions; is the apparent recovery of floral diversity matched by recovery of faunal diversity and, does the fauna recover the same species composition.

Because complete faunal surveys are laborious, expensive and potentially disruptive, it is useful to find indicator species that can offer a means of answering these two questions. Indicator species should be short-lived so that they represent the effects of the current habitat without historical effects, mobile so that the absence of a species is caused by the unsuitability of the habitat and not a failure to colonize, and numerous to improve their ability to detect subtle differences between habitats. The individuals of these species should also be abundant and easily caught. The genus *Drosophila* has all these attributes and, therefore, to investigate their utility as indicators of faunal diversity and species composition, we undertook a study of the *Drosophila* in lowland tropical forest in Borneo.

The study took place within 430 ha of lowland tropical rainforest Barito Ulu, Kalimantan tengah, Indonesian Borneo, 114°E 0°. The study habitats included two types of primary and three grades of exploited forest all within 1000 m of each other (Table 1). In July 1992, banana baits were exposed in each habitat for nine days and the resulting larvae reared out. Because there are no complete keys to the drosophilids of Borneo (Okada, 1988; M. Toda, pers. comm.), the adult flies were assigned to Recognizable Taxonomic Units (RTUs) using conventional morphological characteristics.

The baits produced 1604 individuals in 89 drosophilid RTUs, most of which were *Drosophila*. Non-drosophilids were very infrequent and are omitted from this analysis.

We assessed species diversity as species richness, the mean number of species at each site considering baits as replicated samples, and by the Shannon diversity index. Species richness was significantly different between sites (Kruskal-Wallis  $n = 87$ ,  $H_5 = 34.11$ ,  $p < 0.0001$ ) (Table 2). The two primary dipterocarp habitats had the greatest species richness, followed by the cleared and edge habitats. The sites with fewest species were heath forest and secondary forest. Shannon diversity indices for each habitat were also significantly different (Kruskal-Wallis  $n = 87$ ,  $H_5 = 20.37$ ,  $p < 0.002$ ) (Table 2). The highest diversities were for the two primary dipterocarp forest sites although that for dipterocarp (C) was significantly greater (MW  $n_1 = 10$   $n_2 = 19$ ,  $z = 4.114$ ,  $p < < 0.001$ ). Secondary forest diversity, however, was significantly lower than that of dipterocarp habitat (minimum difference; MW  $n_1 = 19$   $n_2 = 16$ ,  $z = -2.667$ ,  $p < < 0.001$ ) and similar to that for primary heath forest. In the dendrogram (Figure 1) based on the relative diversities of all habitats, we show that heath and secondary forest habitats have similar diversities and that the diversities of cleared, edge and dipterocarp (D) habitat are similar. The high diversity of dipterocarp (C) forest is, however, more divergent.

We calculated qualitative Sorenson indices, a measure of the number of species shared by two habitats, for each pair of sites (Table 3). The largest values, and thus the greatest similarities, occurred between dipterocarp (C) and



Figure 1. Dendrogram based on relative Shannon diversity indices for six tropical forest habitats in central Borneo.

Figure 2. Dendrogram based on qualitative Sorenson indices of the *Drosophila* species shared by six tropical forest habitats in central Borneo.

edge, cleared and edge, and between the two dipterocarp habitats. The dendrogram (Figure 2) shows that the cleared, edge and dipterocarp habitats share a large part of their faunas and are faunally distinct from the heath and secondary forest.

The evidence from this study indicates that *Drosophila* faunas do differ between habitats. Primary dipterocarp forest has high species richness and high diversity. In contrast, secondary forest has low species richness and low diversity suggesting that recovery towards the floristic diversity of primary forest does not indicate recovery of faunal diversity. Furthermore, the *Drosophila* fauna of secondary forest differs markedly from that of primary dipterocarp forest indicating that secondary forest does not recover the same *Drosophila* species and is structurally distinct from the original primary forest. It is something of a surprise to find that secondary forest and heath forest resemble each other with low species richness, low diversity and similar species composition.

None of the three measures can be used as simple indicators of habitat disturbance. The cleared and edge habitats have relatively diverse *Drosophila* faunas. They contain a number of RTUs that are probably cosmopolitan commensals like *D. albomicans* and *D. melanogaster* as well as sharing many of the RTUs found in primary dipterocarp forest.

Our study reveals interesting differences between the *Drosophila* faunas of tropical forest habitats despite the imprecision introduced by our inability to identify our material fully. More precise use of *Drosophila* as indicator species in tropical forests is dependent on adequate taxonomic work in these areas.

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**References:** Okada, T., 1988, Entomol. scand. Suppl. 30:109-149.

**Law, G.R., R.C. Parslow, A.J. Davis, and L.S. Jenkinson.** Drosophila Population Biology Unit, Pure and Applied Biology, The University, Leeds, West Yorkshire, LS2 9JT UK. Drosophilidae exploiting decaying herbage and fungi in a northern European wetland habitat.

restricted to fermenting substrates, but 16 breed in decaying herbage, fungi, or both (Shorrocks, 1972; Shorrocks, 1982). These species may be considered closer to the ancestral lineage of the *Drosophila* and thus their study might provide useful clues to the ecology, biology and genetics of the entire genus. As yet, however, there have been few studies of non-fruit feeding *Drosophila* in Europe, particularly not of those that exploit decaying herbage. As part of a study of these species, we report here the species reared from fungi and from decaying herbage at a site in central Scotland. Fungi have been little investigated in Scotland (Basden, 1954), and there has been no investigation of decaying herbage.

Baits were set out and samples collected at Doune Ponds, a 25 ha site with ponds and marshes near Stirling in Scotland, UK ( $56^{\circ} 15'N$ ,  $4^{\circ} 7'W$ ; elevation 46 m). The annual precipitation is 1500-2000 mm and average monthly temperatures  $3-15^{\circ}C$ .

The *Drosophila* are among the most intensively studied of organisms, but most studies concentrate on those that breed in fermenting substrates, such as fruit, and in particular on *D. melanogaster*. Many *Drosophila*, however, breed on substrates other than fruit and in temperate areas, where fruit is an extremely seasonal resource, the fauna is dominated by these species. In the British Isles only five of the 33 recorded species are

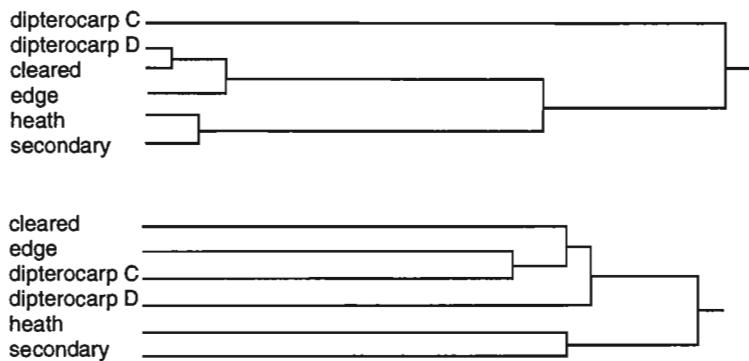


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Decaying herbage and fungi together yielded in 1992 and 1993 approximately 6410 individual Diptera. The 336 decaying herbage baits yielded 5929 Diptera of which 5107 (86%) were drosophilids. Only six additional specimens, all *D. subobscura*, were obtained from the samples collected at the reserve. The other plant samples failed to yield any flies because they dried out and did not decay. The 83 fungal samples in 1992 and the 106 collected in 1993 yielded, respectively, approximately 175 and 622 individual *Drosophila*. The drosophilids *S. pallida* and *L. andalusiaca* were reared only from decaying herbage and four species, *D. busckii*, *D. camheraria*, *D. funebris* and *D. phalerata* only from fungi. *Drosophila subobscura* and *D. transversa* occurred on both substrates but in lower numbers from fungi than from decaying herbage. Very few non-diptera were reared apart from numerous small wasps, *Leptopilina heterotoma* (Hymenoptera: Eucoilidae), parasitic in drosophilid larvae, from decaying herbage alone. The drosophilid species most frequently reared from decaying herbage was *Scaptomyza pallida* (2949 individuals) followed by *D. subobscura* (1992). The numbers of *D. transversa* (158) and *L. andalusiaca* (8) were much lower. The most frequent species from fungi was *D. camheraria* (approximately 446), followed by *D. busckii* (251), *D. transversa* (69), *D. subobscura* (25), *D. phalerata* (18) and *D. funebris* (7) (Table 1).

The eight drosophilid species reared from decaying herbage or fungi at Doune comprise almost half of the 18 species reared from these substrates in Europe. The most abundant species at Doune, *S. pallida*, is frequently one of the most abundant species recorded elsewhere (Davis and Jenkinson, 1992; Janssen *et al.*, 1988; Offenberger and Klarenberg, 1992). *Drosophila subobscura*, the second most abundant species at Doune, has been reared from both decaying herbage and fungi in several parts of Europe. At Doune decaying herbage is a major resource for this species but it produced relatively few individuals in Germany (Offenberger and Klarenberg, 1992). In northern England and the Netherlands, *D. subobscura* is reared consistently from decaying herbage at frequencies between those found in Germany and Scotland (Janssen *et al.*, 1988; Davis and Jenkinson, 1992; van Alphen *et al.*, 1991). The abundance of this species thus appears to be greatest in communities where there are few species breeding in decaying herbage. Fungi produced low numbers of *D. subobscura* throughout Europe (Baker, 1979; Basden, 1954; Driessen *et al.*, 1990; Shorrocks and Charlesworth, 1980) and it is absent from this resource in Hungary (Dely-Draskovits and Papp, 1973), Finland (Hackman and Meinander, 1979) and Switzerland (Burla and Bächli, 1968; Burla *et al.*, 1991). Nevertheless, *D. subobscura* is abundant throughout Europe, in Greece and the Balkans (Triantaphyllidis and Tsacas, 1981; Kekic and Bächli, 1991), England (Shorrocks, 1975) and Scandinavia (Gahne, 1959; Hackman, 1971) suggesting that it uses resources other than fungi. It breeds and is active between February and October and does not diapause like its similar congener *D. obscura* (Begon, 1976). During this period none of its alternative breeding substrates are consistently available in Europe, and the species must use decaying herbage. The ability to use this resource may be crucial in allowing *D. subobscura* to maintain itself in northern Europe. Since it must pass more generations on decaying herbage than on other substrates, we feel that *D. subobscura* must be considered an opportunist species breeding, in northern Europe particularly, primarily in decaying herbage. Begon and Shorrocks (1978) contended that rotting vegetation was not an important breeding substrate for *D. subobscura*, but they attempted to rear the species out of decaying herbage collected from forks and holes in trees not from the ground.

The large numbers of *D. transversa* we reared from Doune conforms with findings that it is the most abundant member of the *quinaria*-group in areas with cool climates. It is increasingly frequent with increasing altitude in the Swiss Alps (Bächli and Burla, 1992) and more common than *D. phalerata* in the Arctic (Basden, 1956). Apart from small numbers of *D. phalerata*, *D. transversa* was the only *quinaria* species at Doune, suggesting that it does replace other members of the group, in particular *D. kuntzei* and *D. phalerata*, where mean January temperatures are very low (Shorrocks, 1977). The numerous *D. transversa* we reared from decaying herbage were most surprising. This species is widely considered to be an obligate fungal breeder (Burla and Bächli, 1991; Shorrocks, 1982; Shorrocks and Charlesworth, 1982), and it has previously been reared from decaying herbage only in Germany, in small numbers (Offenberger and Klarenberg, 1992). At Doune, however, fewer specimens were reared from fungi than from decaying herbage. Another *quinaria* species, *D. phalerata*, was reared only from fungi although it was common among the *Drosophila* species reared from decaying herbage in Germany (Offenberger and Klarenberg, 1992) and in northern England. Other European *quinaria* species are also classified as fungal breeders (Burla and Bächli, 1968, 1991; Shorrocks, 1977; Baker, 1979) but several also breed in decaying plants (van Alphen *et al.*, 1991; Driessen *et al.*, 1990; Hummel, van Delden and Drent, 1979). In Japan (Kimura *et al.*, 1977) and in North America (Jaenike, 1978) species of this group predominantly exploit decaying herbage. The group as a whole can, therefore, be justifiably seen as breeding in decaying herbage with some species using fungi in addition. It is obvious, however, that there are marked differences in the breeding site preferences of populations of the *quinaria*-group species. These are mediated by physiological and behavioural differences between and within species (Wolff *et al.*, 1991; Offenberger and Klarenberg, 1992; A.J. Klarenberg, pers. comm.). *Lordiphosa andalusiaca* has been reared from decaying herbage in Germany (Offenberger and Klarenberg, 1992) and northern England (LSJ and AJD) but, as in Doune, only in small numbers. We are the first

to rear this species from Scotland, although adults have been collected there on several occasions (BM(NH) Drosophilidae database).

The generalist *D. busckii* is frequently reared from fungi and decaying herbage (Basden, 1954; Shorrocks and Wood, 1973; Shorrocks, 1975; Janssen *et al.*, 1988; Driessen *et al.*, 1990; Burla and Bächli, 1991). It was slightly more abundant at Doune, however, than in many of these studies and, as a generalist like *D. subobscura*, may be more abundant where there are few competing species. However, *D. busckii* was absent from decaying herbage at Doune and so might differ in its use of the two substrates.

*Drosophila cameraria* was the most abundant species from fungi at Doune and is found in many parts of Europe. Shorrocks (1977) characterises this species as predominantly southern European, making up more than 50% of the *Drosophila* exploiting fungi only in collections from the Iberian peninsula and the extreme south of France. At Doune, however, well over half of the individuals reared from fungi were *D. cameraria*, demonstrating that this species may be locally dominant even in northern Europe.

We reared only small numbers of *D. funebris* at Doune primarily because, although capable of breeding in both fungi and decaying herbage, this species is synanthropic and uncommon in woodland habitats (Frydenberg, 1956; Burla and Bächli, 1991).

The differences between the drosophilid faunas of decaying herbage and of fungi at Doune and those elsewhere in Europe are unlikely to be because our survey was too short or used too few baits, since studies drawing on several years' data or on large numbers of samples do not necessarily yield many species. We surveyed in the same season as did Offenberger and Klarenberg (1992) at a time of year when *D. limbata* (Hardy *et al.*, 1992), *D. confusa* and *D. kuntzei* (Baker, 1979) are active. Our failure to rear several drosophilid species found elsewhere in Europe might reflect the number and identity of plant species sampled, but this is unlikely. We used eight fewer plant species than were used in Germany, but these additional plants did not produce any species in addition to those reared from plant species common to both studies. *Rheum rhabonticum* and *H. mantegazzianum* both support the larvae of several drosophilid species (Davis and Jenkinson, 1992; Janssen *et al.*, 1988) and *Cirsium* species yielded seven drosophilid species in Germany (Offenberger and Klarenberg, 1992). Therefore, had any of the species we failed to rear, five found on *H. mantegazzianum* (van Alphen *et al.*, 1991) and six species from *Cirsium* (*D. immigrans*, *D. limbata*, *D. kuntzei*, *D. phalerata*, *D. testacea* or *L. fenestrarum*) (Offenberger and Klarenberg, 1992) been present and exploiting decaying herbage at Doune we would have expected to rear them from the baits we used. We also collected a range of fungi from genera that have yielded more than the five drosophilid species found at Doune (Buxton, 1960; Hackman and Meinander, 1979; Shorrocks and Charlesworth, 1980; Janssen *et al.*, 1988). The absence from Doune of several drosophilids frequently reared from decaying herbage or fungi elsewhere in Europe is thus likely to result from ecological factors not sampling method.

Our collections from fungi and decaying herbage at a northern European site indicates that the *Drosophila* exploiting these substrates differ in substrate use within species from different parts of Europe as well as between species at Doune and may well present interesting physiological and genetic differences. The fungal and decaying herbage faunas are now under investigation as part of a Europe-wide project on species diversity in the *Drosophila*.

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Table 1. Numbers of adult Diptera reared from fungi and from decaying herbage collected at Doune Ponds reserve, Stirlingshire, Scotland. (Drosophilidae; S = *Drosophila subobscura* Collin, T = *D. transversa* Fallen, A = *Lordiphosa andalusiana* (Strobl) (= *D. andalusiana*), P = *Scaptomyza pallida* (Zetterstedt), C = *D. cameraria* Haliday, B = *D. busckii* Coquillett, Q = *D. phalgrata* Meigen, *D. funebris* Fabr.) Eucoilidae; L = *Leptopilina heterotoma* (Hartig); a, b, c, d, e = collection dates, respectively 07/07/92, 21/07/92, 04/08/92, 31/08/92, and 30/08/93; MF = mixed unidentified fungi.

	S	T	A	P	L	species	C	B	Q	F
<i>Rheum rhabonticum</i>	a 424 b 153 c 230	— 17 4	— — —	36 504 230	— 2 9	— — —	— — —	— — —	— — —	— — —
<i>Heracleum mantegazzianum</i>	a 608 b 127 c 130	9 18 20	— 2 —	500 397 224	15 65 53	— — —	— — —	— — —	— — —	— — —
<i>Cirsium arvense</i>	a 315 b 1 c 41	67 10 13	— 4 2	364 464 230	35 23 20	— — —	— — —	— — —	— — —	— — —
<i>Heracleum sphondylium</i>	c 4	—	—	—	—	—	—	—	—	—
<i>Chrysanthemum vulgare</i>	c 2	—	—	—	—	—	—	—	—	—
<i>Urtica dioica</i>	c —	—	—	—	—	—	—	—	—	—
<i>Cirsium vulgare</i>	c —	—	—	—	—	—	—	—	—	—
<i>Epilobium palustre</i>	c —	—	—	—	—	—	—	—	—	—
<i>Tussilago farfara</i>	c —	—	—	—	—	—	—	—	—	—
Grasses	c —	—	—	—	—	—	—	—	—	—
<i>Russula</i> sp.	c 1 MF d 7	6 —	—	—	—	—	-100	-50	-10	—
<i>Lyophyllum decastes</i>	e 7	—	—	—	—	—	—	—	—	—
<i>Lactarius terminosus</i>	e —	—	—	—	—	1	—	—	—	—
<i>Laccinum scabrum</i>	e 5	30	—	—	—	—	201	1	7	—
<i>Laccinum aurantiaca</i>	e 4	—	—	—	—	124	—	—	—	—
<i>Cortinarius</i> sp.	e —	—	—	—	—	45	—	—	—	—
<i>Russula</i> spp.	e —	—	—	—	—	166	—	4	—	—

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**Zuzick, P.H., and D. Dapkus.** Winona State University, Winona, Minnesota. The effects of density and DDT on an inversion polymorphism in *Drosophila melanogaster*.

This project studied a *Drosophila* population which exhibits a persistent inversion polymorphism. The population, called J#2, has been subjected to long-term selection for DDT resistance since 1959 (Johnson, 1974).

Originally an aldehyde oxidase (AO) polymorphism was

detected and was found to be in complete linkage disequilibrium with an inversion, *In(3R)P* (Laurie-Ahlberg and Merrell, 1979). High inversion frequencies in several different DDT-resistant populations led to the hypothesis that DDT was involved in the maintenance of the polymorphism (Laurie-Ahlberg and Merrell, 1979). Laurie-Ahlberg and Merrell (1979) studied the fitness of inversion heterozygotes by means of viability tests. They found the inversion heterozygote to be most fit in the presence of DDT. In the absence of DDT, the inversion was selected against. Barnes (1983) continued these studies at three different inversion frequencies and again found a strong heterozygote advantage in the presence of DDT. Contrary to Laurie-Ahlberg and Merrell, Barnes found a heterozygote advantage in the absence of DDT. However, the magnitude of the heterozygous advantage was diminished in the absence of DDT.

The purpose of this project was to study further the role of DDT in the maintenance of the inversion polymorphism. Our method was to perturb the inversion frequency in several synthetic populations and follow their return to equilibrium in the presence and absence of DDT.

Two AO alleles are present in J#2: a fast (F) allele (AO4 of Laurie-Ahlberg and Merrell 1979) and a slow (S) allele (AO1). We determined that the equilibrium frequency of the F allele is currently about 0.55. The frequency has increased since the previous studies when it was about 0.46 (Laurie-Ahlberg and Merrell, 1979; Barnes, 1983).

Nine lines homozygous for the F allele and eight lines homozygous for the S allele were established by setting up random single pair matings and electrophoresing the parents. To verify the inversion type, each line was crossed to a strain with the standard sequence. Salivary gland squashes showed that all F lines had *In(3R)P* and all S lines had the standard arrangement. Pure F (inversion) and S (standard sequence) populations were created by mixing all the F or all the S lines in approximately equal numbers. These populations were maintained in population bottles (Dapkus, 1976) in the presence of DDT. Virgin flies from the F and S populations were collected and mixed to produce three pairs of populations at high (0.95), equilibrium (0.55) and low (0.05) frequencies of the inversion. These six populations were placed in population bottles. One of each pair received a filter paper strip containing 150 mg of DDT (Aldrich Company, 99+ % purity). Samples of 100 to 300 adults from each population were assayed for AO genotype, a convenient marker for inversion type, at approximately three week intervals for a period of 18 weeks. Agarose gels (0.8%) were run using Tris-boric acid buffer. Gels were stained for AO using a modification of the method of Dickinson (1970).

Changes in inversion frequency in the six populations are presented in Figure 1. The following trends were noted: 1) Both high populations (HD = high with DDT and HN = high non-DDT) and both low populations (LD and LN) returned to values near equilibrium within 13 weeks. We were surprised by the rapidity of the response. 2) There seemed to be a tendency for the non-DDT populations to return to equilibrium faster than the DDT populations. This was most apparent in the three to nine week samples, and is contrary to expectations if DDT were the main selective

agent. Comparing HD inversion frequency to HN and LD to LN gives a statistically significant difference only for the high populations at week 9 ( $2 \times 2$  contingency  $X^2 = 4.64$ ,  $p = 0.03$ ). However, five of the six comparisons show the postulated trend. 3) There were three unexplained, sudden decreases in the frequency of the inversion. One "dip" occurred in each of the non-DDT populations. 4) The final inversion frequency in the three non-DDT populations is lower than that in the three DDT populations. The difference is highly significant ( $2 \times 2$  contingency  $X^2 = 20.06$ ,  $p = 0.00$ ). The difference remains significant if the aberrant HN value is excluded ( $X^2 = 8.90$ ,  $p = 0.003$ ).

Figure 1. Changes in the frequency of *In(3R)P* in six synthetic populations over time. H, E, and L refer to high (0.95) equilibrium (0.55) and low (0.05) initial frequencies of the inversion. D and N refer to the presence and absence of DDT-containing strips in the population bottles. Each point represents a sample of 100 to 300 flies tested.

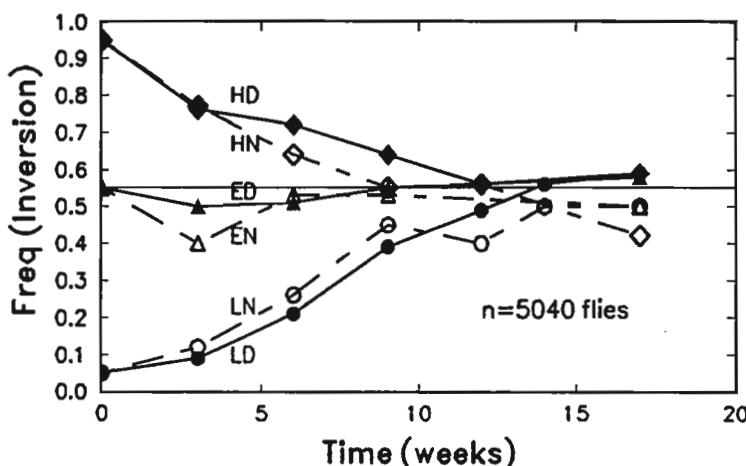
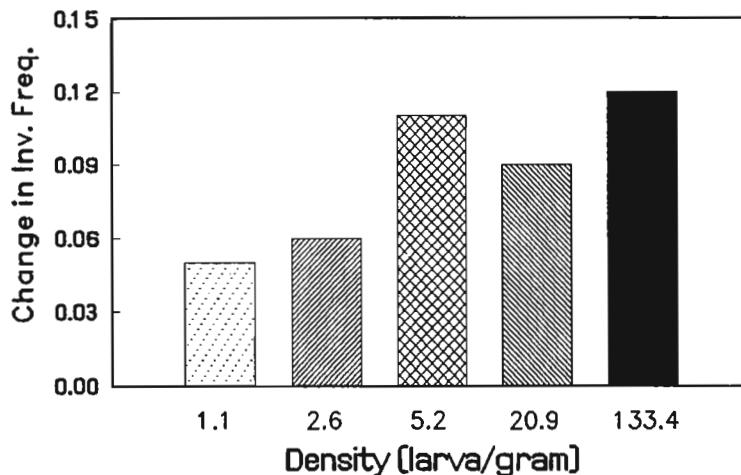


Figure 2. Changes in the frequency of the inversion in populations with differing larval densities. All five populations were established with an initial inversion frequency of 0.84. One hundred forty to 400 flies at each density were tested to determine the one generation change in frequency toward the equilibrium value of 0.55.



We also examined the level of heterozygosity in each of the populations over the course of the experiment. Heterozygous genotypes were 23% to 43% in excess of Hardy-Weinberg expectations for each of the six populations. Two  $\chi^2$  tests were calculated for each population. All were greater than  $\chi^2 = 23.62$  with  $p = 0.000$ .

These results were generally consistent with the maintenance of the inversion polymorphism by balancing selection related to a heterozygous advantage. However, the role of DDT seemed to be in question. We asked what might account for the slower rate of return of the DDT populations to equilibrium? We hypothesized that DDT might actually lower the relative advantage of heterozygotes by lowering larval density. If this were true, we predicted that 1) the DDT populations should have a lower larval density and 2) artificially lowered larval densities should reduce the rate of return to equilibrium. We tested these predictions as follows: First, we measured larval density in the DDT and non-DDT populations. Three weighed samples were taken from each of the six populations. Each sample was dissolved in a 3.0M NaCl solution in a separatory funnel. At this concentration, larva float to the surface while the medium sinks to the bottom (Ashburner, 1989). The solution containing the medium was then drawn off and the larva were filtered through Whatman #4 filter paper in a Buchner funnel. Larva on the filter paper were chilled and counted with a dissecting microscope. The mean number of larva per gram of food  $\pm$  S.E. in the DDT and non-DDT populations was  $61.2 \pm 7.5$  and  $103.0 \pm 10.8$ , respectively. Thus the DDT populations had about a 40% decrease in larval density. This difference

was highly significant (One way ANOVA  $F(1,16) = 10.14$ ,  $p = 0.006$ ).

Next we determined if larval density influenced the rate of return to equilibrium. To perform this experiment we set up five different sets of two to four half-pint bottles with different larval densities. This was accomplished by allowing 100 pairs of flies with an inversion frequency of  $F = 0.84$  to lay eggs for different lengths of time, ranging from 2 hr to 72 hr. The larval densities were determined using the method above. Changes in the inversion frequencies (from the 0.84 starting frequency) were determined by electrophoresis of the adults emerging from the bottles. The results are presented in Figure 2 which shows an obvious trend: the less dense bottles exhibited the smallest shifts toward equilibrium (*i.e.*, the weakest balancing selection). Inversion frequencies in the 72 hr bottles had the greatest change of 12% while the 2 hr sample had the smallest shift of 5%. A 2x2 contingency  $X^2$  test was carried out comparing the highest density and the combined two lowest densities (The 2 hr samples had few flies). The difference was highly significant ( $X^2 = 7.49$ ,  $p = .006$ ). From these results we conclude that selective intensities may differ with larval density in these populations and that DDT may slow the return to equilibrium by reducing larval density.

In conclusion, we, like Laurie-Ahlberg and Merrell (1979) and Barnes (1983) found evidence that the *In(3R)P* inversion polymorphism is maintained by strong balancing selection involving a heterozygous advantage. Contrary to their conclusions, our perturbation experiments did not show a strong role for the action of DDT. In fact, perturbed populations maintained without DDT seem to return to equilibrium faster than those with DDT. Further experiments indicated that this result may be due to an indirect effect of DDT through its effects on larval density. We do not know the reason(s) for the difference between our results and previous studies. One possibility is that Laurie-Ahlberg and Merrell (1979) probably performed their studies at lower densities. Another possibility is that the population has undergone significant genetic changes over the past 10-15 years. Perhaps detrimental alleles have accumulated and become fixed by genetic drift in these small populations in which numbers fluctuate widely. These genetic changes may have increased the relative advantage of the heterozygotes, even in the absence of DDT. At the same time the level of DDT resistance may have risen, making genetic differences in DDT resistance associated with this inversion less important.

We are continuing to study these populations in order to better understand the factors responsible for the sudden drops in the inversion frequency in the non-DDT populations. Barnes (1983) noted that inversion frequency fluctuations were more frequent in the absence of DDT. This instability may be due to the high larval density of the populations. We also wish to determine if the inversion frequency in non-DDT populations will continue to drop or will stabilize at a frequency below the DDT equilibrium frequency. In this way we hope to be better able to assess the role of DDT in maintaining the inversion polymorphism in the J#2 population.

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**Downie, Dee Dee.** Ninth grade, Winona Senior High School, Winona, Minnesota USA. Tobacco Whole Smoke Condensate treatment of larva causes sex-linked recessive lethal mutations in *Drosophila melanogaster*.

research cigarettes in a cigarette smoking machine. All combustion products, including tar and nicotine, were collected in a cold trap and stored frozen. Canton-S males were treated with TWSC. The *Basc* strain was used to detect SLRL mutations. Both strains were obtained from the Mid-America *Drosophila* Stock Center.

TWSC was dissolved in 95% ethyl alcohol and added to molten Pearl's medium (2% agar, 3% Brewer's yeast, 4% sucrose and 0.1% Tegosept). Eggs were collected and placed directly on TWSC-containing medium in order to expose all larval stages. Preliminary tests using various TWSC dilutions showed that 16.2 mg TWSC/ml of Pearl's medium allowed approximately 50% of eggs placed on the medium to survive. Forty eggs were treated yielding ten surviving males. Each male was crossed individually to ten *Basc* females. F1 females from these crosses were mated individually with two *Basc* males in one ounce Solo cups (Solo Cup Co., Urbana, IL) containing 5 ml Pearl's medium (without TWSC). The control group was subjected to the same procedure except that an equivalent amount of 95% ethyl alcohol was added to the medium. Ethyl methane sulfonate (EMS), (0.025 M in 1% sucrose), was used as a positive control treatment according to the procedure of Lewis and Bacher (1968). F2 offspring emerging from each cup were scored. The cross was scored as a lethal if at least five *Basc* males and no red-eyed males were present.

The results are shown in Table 1. A total of 2308 F2 crosses were set up of which 1454 produced sufficient

The purpose of this experiment was to determine if Tobacco Whole Smoke Condensate (TWSC) caused sex-linked recessive lethal (SLRL) mutations. TWSC was purchased from the Tobacco and Health Research Institute, University of Kentucky, Lexington, Kentucky. It is a viscous, black fluid produced by burning standard

offspring to be scored. The lethal mutation rate was 0% in the control group, 1.55% in the TWSC-treated group and 10.87% in the EMS-treated group. These results were tested statistically using 2 x 2 contingency tests. When the TWSC group was compared to the control group, the difference was statistically significant ( $X^2 = 8.12$ ,  $p = 0.004$ ) indicating that TWSC increases the SLRL mutation rate. Although only 46 chromosomes were tested, comparison of the EMS group to the control group also gave a significant result ( $X^2 = 57.03$ ,  $p = 0.000$ ).

Table 1. Comparison of Tobacco Whole Smoke Condensate sex-linked recessive lethal mutation rates.

	Total set up	Total counted	Number of lethals	Lethal mutation rate (%)
Control	805	520	0	0
TWSC	1377	906	14	1.55
EMS	126	46	5	10.87

larvae, pupae, and adults to heavy doses of aerosol smoke. All three stages had significant increases in SLRL mutation rates over the control. Finally, Jansson, *et al.* (1991) tested the mutagenic effects of an oral snuff extract fed to adults. A small, significant increase in the SLRL mutation rate resulted. Thus, the present study is consistent with previous studies on the mutagenic effects of tobacco products as assessed by increases in the SLRL mutation rate in *Drosophila*.

References: Jansson, T., *et al.*, 1991, Mut. Res. 261:101-115; Kale, P.G., and J.W. Baum 1982, *Mutation Research*. Elsevier Biomedical Press; Lewis, E.B., and F. Bacher 1968, Dros. Inf. Serv. 43:193; Pescitelli, A.R., 1979, Environmental mutagenesis 1:118.

**Kim, D.I.** Department of Biology, Mok-Won University, Taejon 301-729, S. Korea. P activity of flies with *In(3L)P* by hybrid dysgenesis in natural populations of *Drosophila melanogaster* in Korea.

strain was higher in flies with *In(3L)P* from TP(91) and TY(91).

**Materials and Methods:** *Drosophila melanogaster* was collected from two Taejon locations: Pan-am dong and Yu-sung between early September and mid November, 1991 and 1992. The collections of the 541 inseminated females in wild populations were transferred to vials containing corn-meal-molasses-yeast-agar medium and 0.5% propionic acid, and were kept at room temperature,  $25 \pm 1^\circ\text{C}$ , and  $65 \pm 1\%$  humidity.

The smears of salivary gland chromosomes were prepared with F1 third instar larvae which were selected randomly from each isofemale line. The salivary gland cells of the F1 larvae from each isofemale line were examined. The salivary chromosome preparations were made by the lactic-acid-orcein method using siliconized slides and then the cover slips were sealed with nail polish.

The salivary chromosomes were observed with a BH2 Olympus microscope for the presence of heterozygous inversions. The standard chromosomal map of Bridges (1935) and revised map of Lefevre (1976) were employed to identify the breakage points of the chromosomal inversions.

Two tester strains (a strong P strain,  $\pi_2$  and the standard M strain (Canton S) were used to assay the GD sterility of the wild strains.

The cross A was carried out using two females of Canton-S with one wild type unknown male. The cross A° was carried out using one wild unknown female with two males of strong P factor ( $\pi_2$ ). The vials with these flies were kept for a week at  $29^\circ\text{C}$  for the cross and then parents were discarded. The F1 flies emerging by the 11th day were transferred to fresh vials with medium at  $25^\circ\text{C}$ . After flies had matured for four additional days, 24 F1 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%.

**Results:** The overall mean frequency in cross A° showed 0.97% from the cross of F1 males with Canton-S females. TP populations decreased from the

From this experiment, it was concluded that treating larvae with TWSC caused a significant increase in the SLRL mutation rate. A search of the literature revealed several other studies of the mutagenic effects of tobacco products. For example, Pescitelli (1979) fed a crude smoke condensate to adults and larvae. He also exposed adults and larvae to aerosol smoke from a smoking machine. Only the treated larvae had significantly increased SLRL mutation rates. Kale and Baum (1982) exposed

GD sterility frequency of flies with *In(3L)P* was only in M' and Q strains. Though the normal flies collected in wild populations showed a little GD sterility in true M and P(M) strains, the flies with *In(3L)P* were not observed. P activity in M' strains was mostly higher in flies with *In(3L)P* with TP(92) and TY(92), and Q



Figure 1. Common cosmopolitan inversion *In(3L)P*.

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

Table 1. Frequency (%) of GD sterility strain tested from two local populations

strains tested	Cross A no. ovaries per female (%)				Cross A° no. ovaries per female (%)			
	2	1	0	GD	2	1	0	GD
TP (91)	98.90	0.24	0.86	1.10	63.84	0.90	35.25	36.16
TP' (92)	99.70	0.10	0.10	0.20	57.45	0.07	42.48	42.55
TY (91)	99.20	0.05	0.75	0.80	62.50	0.40	37.10	37.50
TY' (92)	98.21	0.06	1.73	1.80	66.30	0.16	33.56	33.72
means	99.03	0.11	0.86	0.97	62.52	0.38	37.10	37.48

TP: Taejon Pan-am dong populations

TY: Taejon Yu-sung populations

Table 2. Strain identified with GD frequencies in strain tested from two local populations of functional properties for GD sterility

strains tested	Strains of functional properties for GD sterility						$\chi^2$
	N	M	M'	Q	P(M)	P(S)	
TP (91)	96	0.0208	0.4167	0.5521	0.0104	0.0000	
TP' (92)	165	0.0182	0.4485	0.5333	0.0061	0.0000	6.0805
TY (91)	113	0.0177	0.4159	0.5664	0.0000	0.0000	
TY' (92)	167	0.0299	0.3532	0.6048	0.0119	0.0000	
means	135.25	0.0217	0.4086	0.5642	0.0056	0.0000	

Table 3. Sterility frequencies of flies with *In(3L)P* of Hybrid Dysgenesis of P-M system

strains tested	In(3L)P						$\chi^2$
	N	M	M'	Q	P(M)	total	
TP (91)	96	0.0000	0.0104	0.0313	0.0000	0.0417	
TP' (92)	165	0.0000	0.0364	0.0303	0.0000	0.0667	1.7442
TY (91)	113	0.0000	0.0088	0.0265	0.0000	0.0354	
TY' (92)	167	0.0000	0.0539	0.0599	0.0000	0.1138	
means	135.25	0.0000	0.0274	0.0370	0.0000	0.0644	

Table 4. The relative frequencies of strains for GD sterility of flies without *In(3L)P* and flies with *In(3L)P*

strains tested	M		M'		Q		P(M)	
	N	N(ln)	n	ln	n	ln	n	ln
TP (91)	96	4	0.0208	0.0000	0.4167	0.2500	0.5521	0.7500
TP' (92)	165	11	0.0182	0.0000	0.4485	0.5455	0.5333	0.4545
TY (91)	113	4	0.0177	0.0000	0.4159	0.2500	0.5664	0.7500
TY' (92)	167	19	0.0299	0.0000	0.3532	0.4739	0.6048	0.5263
means	135.25	9.5	0.0217	0.0000	0.4086	0.3799	0.5642	0.6202

N: tested individuals; ln: frequencies of flies with *In(3L)P*; n: frequencies of flies without *In(3L)P*; N(ln): tested individuals of flies with *In(3L)P*

previous year, and TY populations increased more than the previous year. In the cross A°, 261 TP and 280 TY isofemale lines were tested, and the mean sterility frequencies of both populations were 39.36% and 35.61%, respectively (Table 1). Each strain was tested with cross A and cross A° according to Kidwell's criteria (1986). Distribution of M cytotype 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. P(S) strain was not found in all populations. M' strain of the TP population was increased more than the previous year (1991), but the TY population was decreased more than previous year. Chi square tests ( $\chi^2$ ) among annual differences in four populations were not significant ( $\chi^2 = 6.0805$ ,  $P > 0.005$ ) (Table 2).

P activity of flies with *In(3L)P* in isofemale lines was observed from M' and Q strains by the results of hybrid dysgenesis, but not from others. Though GD sterility frequency in Q strains with *In(3L)P* was mostly higher than in M' strains, except for TP(92), the difference in each population was not significant ( $\chi^2 = 1.7442$ ), sterility frequencies of each population were mostly increased from the previous year. Flies of P(M) and true M strains with *In(3L)P* were never observed in each isofemale (Table 3).

The M' and Q strain of flies with *In(3L)P* in wild flies were found only from the results of hybrid dysgenesis of the P-M system. The mean frequencies of the M' strain and Q strain of flies with *In(3L)P* were 0.3942 and 0.5721, respectively. Mean sterility frequency of true M strains was found with 0.0217 on the flies without *In(3L)P* in these populations, and P(moderate) strain was 0.0056. Sterility frequency of flies with *In(3L)P* was observed to be concentrated

completely in M' and Q strains. Sterility frequency of true M and P(M) strains was not found on the flies with *In(3L)P* in these populations. M' strains of the flies with *In(3L)P* showed only higher GD sterility on the TP(92) and TY(92) populations, but the GD sterility frequency of Q strains was rather greater in flies without than in flies with *In(3L)P* in TP(92) populations (Table 4). The Q strain with *In(3L)P* was observed with higher frequency than M' strain in these

populations except for TP(92).

**Discussion:** The sterility frequencies of F1 males tested from 261 TP and 280 TY isofemale lines were 0.65% and 1.30% in cross A, respectively. P activity of F1 males tested in four populations were observed to be with each one individual in two populations. GD sterility of Taegoo and Cheju populations was 0.18% and 0.17% (Paik *et al.*, 1989). GD sterility frequencies of TP and TY populations were 1.03 and 0.94 (Kim, 1992). In cross A<sup>o</sup>, the mean sterility frequencies in tested 261 TP and 280 TY isofemale lines were 39.3550 and 35.6100. In TP and TY populations, Kim (1992) reported that average sterility in the same populations was 38.84% and 38.63%. Paik *et al.*, (1989) reported that average sterility in Taegoo and Cheju populations was 31.98 ± 2.59 and 32.84 ± 3.39.

Each strain from crosses of cross A and cross A<sup>o</sup> was classified according to Kidwell's criteria (1983, 1986). Most strains in TP and TY populations were found to be M' and Q strains. True M strain was in low frequency in four populations and P(M) strains were rarely found in three populations (Table 2). The P-M hybrid dysgenesis cline in eastern Australian *Drosophila melanogaster* is nearly contiguous from discrete P, Q and M regions (Boussy *et al.*, 1987). Chi square test ( $\chi^2$ ) of each strains between four populations was not significant difference ( $\chi^2 = 6.0805$ ,  $P > 0.05$ ). The sterility frequency of flies with *In(3L)P* was only in M' and Q strains. The Q strain was mostly in higher frequency than the M' strain in three populations (Table 3). True M and P(M) strains were not found to have P activity in flies with *In(3L)P*. The relative frequencies of strains for GD sterility of flies without and flies with *In(3L)P* were observed mostly from M' strains and Q strains of isofemale lines. Though the normal flies collected in wild populations were observed lightly GD sterility in true M and P(M) strains, the flies with *In(3L)P* were not observed. P activity in M' strains was mostly higher in flies with *In(3L)P* from TP(92) and TY(92), and Q strain was higher in flies with *In(3L)P* from TP(91) and TY(91) (Table 4).

**Reference:** Bridges, C.B., 1935, J. Heredity 26:60-64; Boussy, I.A., and M.G. Kidwell 1987, Genetics 115:737-745; Kidwell, M.G., 1983, Proc. Natl. Acad. Sci. USA. 80:1655-1659; Kidwell, M.G., 1986, In: *Drosophila: A Practical Approach* (D.B. Roberts, ed.), IRL Press, Washington DC, pp. 70; Kim, D.I., 1992, Korean J. Genetics 14-3; 203-211; Paik, Y.K., M.S. Lyu and C.G. Lee, 1989, Korean J. Genetics 11:47-55.

**Fritsch, P.S., M. Murray<sup>1</sup>, and D. Dapkus.** Winona State University, Winona, Minnesota, and <sup>1</sup>Mayo Clinic, Rochester, Minnesota. Lipid concentration in an artificially-selected, anesthetic-resistant population of *Drosophila melanogaster*.

Interaction holds that anesthetics act to fluidize the membrane lipid bilayer, thus interfering with impulse transmission (Koblin, 1990). This research found an increased lipid concentration in flies selected for halothane resistance.

## Materials and Methods

Approximately 200 wild flies were collected near Rochester, Minnesota, to found a base population. This initial population was then divided into two groups. The first group was maintained by distributing 100 pairs of flies each generation among four half pints of cornmeal-molasses-agar medium as an unselected control population (QHC). The second group was selected over 13 generations for halothane resistance. The selection was performed by placing batches of one to two thousand flies in an inebriometer (Weber, 1988). The 100 most resistant males and 100 most resistant females from each generation were selected to serve as the parents for the next generation. The last generation to be selected was used to found the resistant population (QHR).

Shane Heckes (unpublished data), using the bowl test (Dapkus and Murray, 1992), found the ED<sub>50</sub> (the estimated amount of anesthetic needed to produce anesthesia in one-half of the flies tested) of QHR to be about 2.3 times that of QHC.

Lipid concentrations were determined using a gravimetric technique (Bligh and Dyer, 1959). The lipids were extracted from samples of QHR and QHC using a modification of the technique of Folch (1957). Samples of about 500 mixed sex flies (approximately 0.5 g) were analyzed. Pairs of samples, one QHR and one QHC, were taken from bottles set up at the same time and maintained side by side. The weighed fly samples were stored under nitrogen at -20°C until analyzed. Flies were ground in a Waring Blender with 10 ml of 2:1 chloroform:methanol (v/v). The blender was rinsed twice by blending additional 10 ml and 5 ml aliquots of 2:1 chloroform:methanol. The three extracts were then pooled and filtered. Seven ml of Folch's multiple salt solution (1957) were added to the filtrate and the contents were mixed. The tubes were centrifuged for 10 min at 2,400 rpm. The upper aqueous layer was removed and the lower organic layer

## Introduction

This project studied the relationship of lipids to anesthetic resistance using *Drosophila melanogaster* as the model system. This work was based on one of the two prevailing theories of anesthetic action, that anesthetics interact with neuron membrane lipids rather than proteins. One concept related to lipid-anesthetic

was transferred to a preweighed erlenmeyer flask. The solvent was evaporated under vacuum to a constant weight (about 24 hrs). The weight of the lipids extracted was determined by re-weighing the flask. The concentration of lipids in the fly sample was determined by dividing the weight of extracted lipids by the live weight of flies analyzed. The concentration was expressed as a percent of live weight.

To test the hypothesis that the QHR population had an altered lipid composition, the results from four pairs of QHR and QHC samples were compared using a two-way analysis of variance (ANOVA). The variables in this test were test days and strains (QHR or QHC).

### Results

Using a gravimetric technique, it was determined that the mean percentage of total lipids  $\pm$  standard error for the resistant population was  $8.73 \pm 0.08$  and  $7.57 \pm 0.03$  for the control population. This is a 15.3% increase in total lipids in the resistant population as compared to the control population.

A two-way ANOVA showed that the difference in the means was significant ( $F_{(1,7)} = 301.01, p = 0.0004$ ). This test also showed that the day the sample was tested was not a significant source of variation ( $F_{(3,7)} = 1.88, p = 0.31$ ).

### Discussion

The data suggest that there is an increase in the total lipid concentration in the halothane resistant population. To our knowledge, there have been no other studies testing the lipid concentration of artificially-selected, anesthetic-resistant strains.

Further studies will be required to establish that the change in lipid concentration is related to anesthetic resistance. This connection might be pursued by associating the chromosomal pattern of inheritance of halothane resistance and of lipid concentration differences.

In this study, testing was done on the entire organism. Since anesthesia involves the central nervous system (Krishnan and Nash, 1990), the sensitivity of the test might be improved by testing an area rich in neuronal tissue, such as the head. Studies done on these populations using just the heads might yield different results.

Further, this study extracted all of the lipids. Further work could be done by individually analyzing the lipid classes (phospholipids, sterols, triglycerides, and free fatty acids). It is possible that the increased lipid concentration in the QHR population is due to an increased concentration of only one lipid class.

In conclusion, this study detected a significant increase in lipid concentration in an anesthetic-resistant population. Chromosomal analysis needs to be performed to determine if there is a link between this increase and anesthetic resistance. Further analysis of this interesting population may shed light on the mechanism of anesthetic action.

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**Stoltenberg, S.F., and J. Hirsch,** University of Illinois, Urbana-Champaign, Illinois USNA. Evidence for a Y chromosome effect on geotaxis.

study on the sterility of XO males, the sexual behavior of 54 such males was observed by Safir (1920) and found to be "normal" (p. 478) which supported earlier work by Bridges.

A study by Aslund *et al.* (1978) appears to be one of a few that do provide evidence for a Y chromosome effect on behavior. The main finding of Aslund *et al.* (1978) is that alterations in the number of Y chromosomes in males in either direction from the normal complement (*i.e.*, XYY or XO) results in similar decrements in mating activity, mating capacity, and mating competition ability relative to XY males.

Lines of *D. melanogaster* that have evolved stable, extreme expressions of geotaxis provide a unique opportunity to examine the effects of the Y chromosome on a relatively well studied behavior (see Ricker and Hirsch, 1985 for a description of the lines). In general, Y chromosome effects on geotaxis were either ignored for technical reasons (Ricker and Hirsch, 1988; Pyle, 1978) or not consistently detected (Ricker, 1984). However, Ricker (1984) did find that, in particular backcrosses where the males differed only with respect to the origin of their Y chromosome (Low female  $\times$  (High female  $\times$  Low male) F1 male [Low Y], and Low female  $\times$  (Low female  $\times$  High male) F1 male [High Y]), significantly more males with the Y chromosome from the Low line were found in the lowest three categories of the

There has been little experimental evidence for Y chromosomal effects on behavior in *Drosophila*. Early work examined the phenomenon of primary nondisjunction in *D. melanogaster* that resulted in XXY females and XO males (*e.g.*, Safir, 1920). As part of the

maze compared to males with the Y chromosome from the High line.

In the present study, we performed five generations of backcrosses in order to clarify our understanding of the relationship between the Y chromosome and geotactic behavior. By successively backcrossing hybrid males each generation to females from the High or Low lines we constructed four sublines. In the following notation the first letter indicates the geotaxis line of origin for females (H or L). The two letters after the 'x' indicate the particular F1 reciprocal cross that generated the male for the crosses (*i.e.*, LH = Low female x High male, HL = High female x Low male). First we crossed reciprocally individuals from the High and Low geotaxis lines at generation 777. Male F1 offspring from the two crosses were then used to establish four separate backcross sublines: HxHL, HxLH, LxHL and LxLH. Males from the HxLH backcross subline possess cytoplasm, most autosomes, and an X and Y chromosomes from the High line. Males from the HxHL backcross subline possess cytoplasm, most autosomes and an X chromosome from the High line, but a Y chromosome from the Low line. Males from the LxHL backcross subline possess cytoplasm, most autosomes, and X and Y chromosomes from the Low line. Males from the LxLH backcross subline possess cytoplasm, most autosomes and an X chromosome from the Low line, but a Y chromosome from the High line.

By comparing geotaxis score distributions of sublines with the same background (*i.e.*, cytoplasm, X chromosome and autosomes), but different Y chromosomes (*i.e.*, HxLH-HxHL and LxLH-LxHL) the effect of the Y chromosome should be seen. Table 1 presents such comparisons for five generations of backcrosses. Probability values that indicate the likelihood that the two distributions are drawn from the same population were generated by Monte Carlo Contingency Table Tests (kindly provided by W. Engels, University of Wisconsin, Madison; see Lewontin and Felsenstein, 1965). Figure 1 shows the mean geotaxis scores of the backcross sublines for five generations of backcrosses. Sample sizes for each generation are around 200.

Geotaxis score distributions of flies with Low line cytoplasm, X chromosome and autosomes, but different Y chromosomes are significantly different. The line of origin of the Y chromosome makes a difference in a Low line background. Geotaxis score distributions of flies with High line cytoplasm, X chromosome and autosomes, but different Y chromosomes are not significantly different. In a High line background the line of origin of the Y chromosome does not make a difference.

Lines with dominant morphological markers on the second, third and fourth chromosomes are currently being used in a chromosome substitution experiment to gain additional insight into the effect of the Y chromosome on geotaxis.

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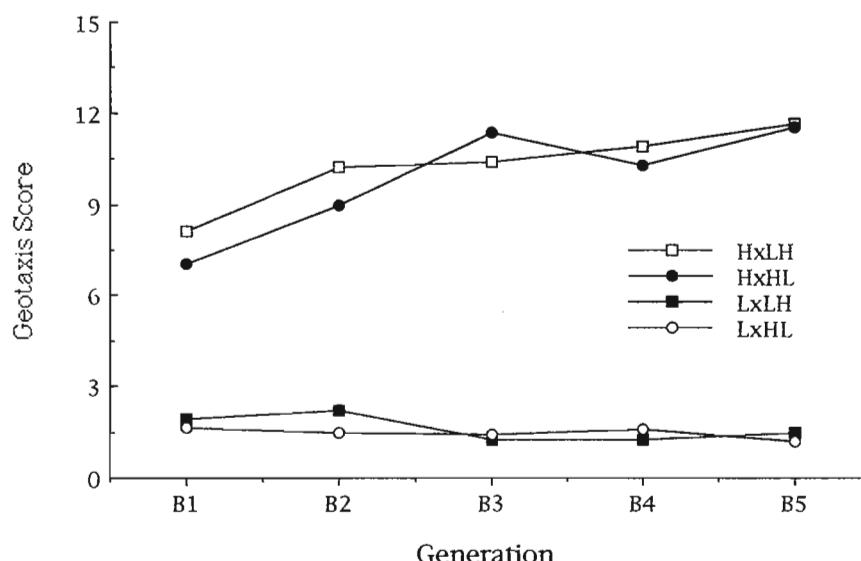


Figure 1. Mean geotaxis scores for samples of males from the four backcross sublines are given for five generations. Sample size in each case is approximately 200.

Table 1. Comparisons of sublines that have cytoplasm, X chromosome and autosomes from the same geotaxis line, but Y chromosomes from different geotaxis lines for five backcross generations.<sup>a</sup>

Generation	Comparison	p	± s.e.	No. of Trials
B1	H x LH - H x HL	0.20681	0.003668	12,190
B1	L x LH - L x HL	0.49173	0.004415	12,820
B2	H x LH - H x HL	0.03920	0.001256	23,880
B2	L x LH - L x HL	0.002076	0.000415	12,040
B3	H x LH - H x HL	0.43042	0.004514	12,030
B3	L x LH - L x HL	0.02002	0.000932	22,580
B4	H x LH - H x HL	0.35934	0.003689	16,920
B4	L x LH - L x HL	0.02471	0.000981	25,050
B5	H x LH - H x HL	0.51910	0.002303	47,070
B5	L x LH - L x HL	0.03052	0.000577	88,890

<sup>a</sup> Probability values and standard errors were generated with Monte Carlo Contingency Table Tests. Number of trials indicates the number of Monte Carlo simulations used to generate given values.

**Stoltenberg, S.F., P. Chopra, J. Wang and J. Hirsch,**  
University of Illinois, Urbana-Champaign, Illinois  
USNA. Replicating reverse selection in lines of *D. melanogaster* that have evolved stable, extreme geotaxis performance.

extreme expressions of geotaxis (Ricker and Hirsch, 1985). Successful reverse selection for geotaxis in sublines derived from these lines indicated that genetic variation for geotaxis remained after 514 generations of intermittent forward selection (*i.e.*, the lines were not fixed for alleles influencing geotaxis; Ricker and Hirsch, 1985). Fifty four generations later (generation 568), reverse selection for geotaxis was successful in a subline derived from the Low line (LR), but not in a subline derived from the High line (HR; Ricker and Hirsch, 1988). These results may indicate that genetic variation for geotaxis remains in the Low line, but may not remain in the High line.

Following reverse selection, Ricker and Hirsch (1985; 1988) relaxed selection allowing natural selection to reassert itself (see Dobzhansky and Spassky, 1969) and observed a regression of the geotaxis means of the LR sublines toward the positive extreme. Apparently in the LR subline, fitness was associated with positive geotaxis, which is not the "normal" *D. melanogaster* behavioral response.

In an attempt to replicate these findings we are presently applying reverse selection pressure on sublines derived from the High (generation 770) and Low (generation 771) geotaxis lines. See Ricker and Hirsch (1985) for general culturing and testing procedures.

Flies are collected and separated by sex under ether anesthesia within 4 h of eclosion. Generally, for each subline, separate sex samples of 100-200 individuals (in two cases samples of approximately 30 were tested) are given a day to recover from anesthesia before testing at 2-5 days old. Following geotaxis testing (Hirsch, 1959) the 50 highest scoring males and 50 highest scoring females are positively assortatively mated based on their geotaxis scores in the LR subline. In the HR subline, the lowest scoring 50 males and females are also mated positively assortatively.

To test whether geotaxis score distributions for the most recent generation of reverse selection differed significantly from the geotaxis score distributions of the parental lines we compared them using Monte Carlo Contingency Table Tests (kindly supplied by W. Engels, University of Wisconsin, Madison; see Lewontin and Felsenstein, 1965). These tests supply Monte Carlo estimates of the probability that the two distributions came from the same population and the standard errors (s.e.) of those estimates. At least 10,000 trials were performed in each case to arrive at the reported p-value.

For LR females, the geotaxis distribution of the LR 0 generation differed significantly from that of the LR 9 generation ( $p = 0.012$ , s.e. = 0.001). The mean geotaxis scores of generations LR 0 and LR 9, however, show that nine generations of reverse selection have resulted in an even lower mean score (2.07, 1.44, respectively; see Figure 1). Therefore, no response to reverse selection has been seen in the LR females. For LR males, the geotaxis score distributions of generations LR 0 and LR 9 are not significantly different ( $p = 0.277$ , s.e. = 0.004; see Figure 2). No response to reverse selection has been observed in LR males in the present experiment.

In the HR sublines it appears that reverse selection may be affecting the geotaxis score distributions of both the HR females ( $p = 0.051$ , s.e. = 0.002) and the HR males ( $p = 0.007$ , s.e. = 0.001). The mean geotaxis scores of the HR females, however, have remained relatively constant from generation HR 0 to generation HR 12 (14.30, and 14.14 respectively; see Figure 3). The mean geotaxis scores of the HR males have, on the other hand, become more extreme over the 12 generations of reverse selection (10.52 and 11.73; see Figure 4).

In the present round of reverse selection the LR subline has not responded as LR sublines have in the past (Ricker and Hirsch, 1985; 1988). This may indicate that additive genetic variation for geotaxis has been lost in the approximately 200 generations since the last round of reverse selection. The lack of response to reverse selection in the HR subline is consistent with the results of Ricker and Hirsch (1988). Stoltenberg, Hirsch and Berlocher (1992) reported that no allozyme variation was detected in either the High or Low lines which may be consistent with the lack of selection response seen in the present experiment. Further reverse selection will be carried out in an attempt to clarify the situation.

References: Dobzhansky, T., and B. Spassky 1969, Proc. Nat. Acad. Sci. USNA 62:75-80; Erlenmeyer-Kimling, L, J. Hirsch, and J.M. Weiss 1962, J. Comp. Physiol. Psych. 55:722-731; Hirsch, J., 1959, J. Comp. Physiol. Psych. 52:304-308; Lewontin, R.C., and J. Felsenstein 1965, Biometrics 21:19-33; Ricker, J.P., and J. Hirsch 1985, J. Comp. Psych. 99:380-390; Ricker, J.P., and J. Hirsch 1988, J. Comp. Psych. 102:203-214; Stoltenberg, S.F., J. Hirsch, and S.B. Berlocher 1992, Dros. Inf. Serv. 71:185-186.

A statistically significant response to artificial selection indicates the presence of variation within and among the gene correlates of the selected trait. Lines of *D. melanogaster* that have been intermittently divergently selected for negative (High) and positive (Low) geotactic performance since 1958 (Erlenmeyer-Kimling *et al.*, 1962) have evolved stable,

Low Reverse Males

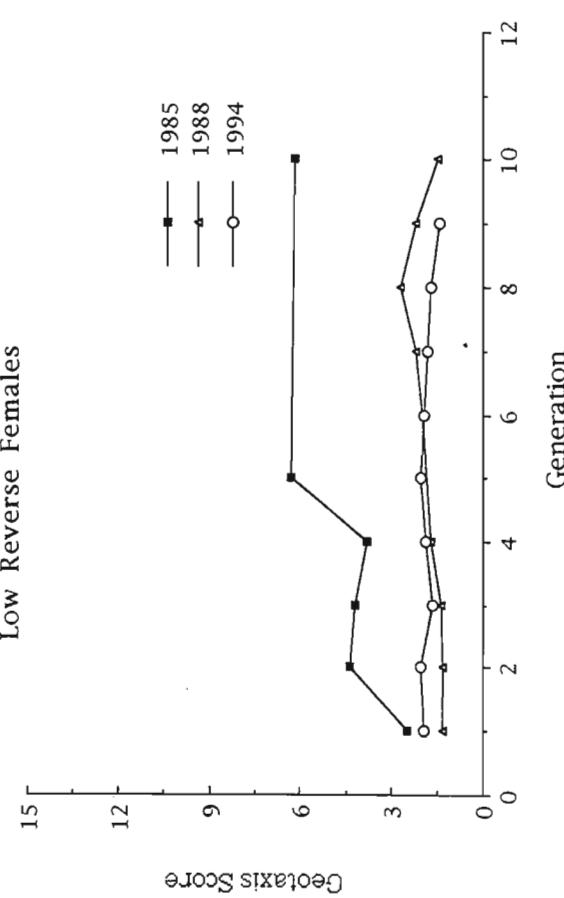


Figure 1. Mean geotaxis scores of females from Low Reverse (LR) sublines over nine (1994) and ten (1985, 1988) generations of reverse selection.

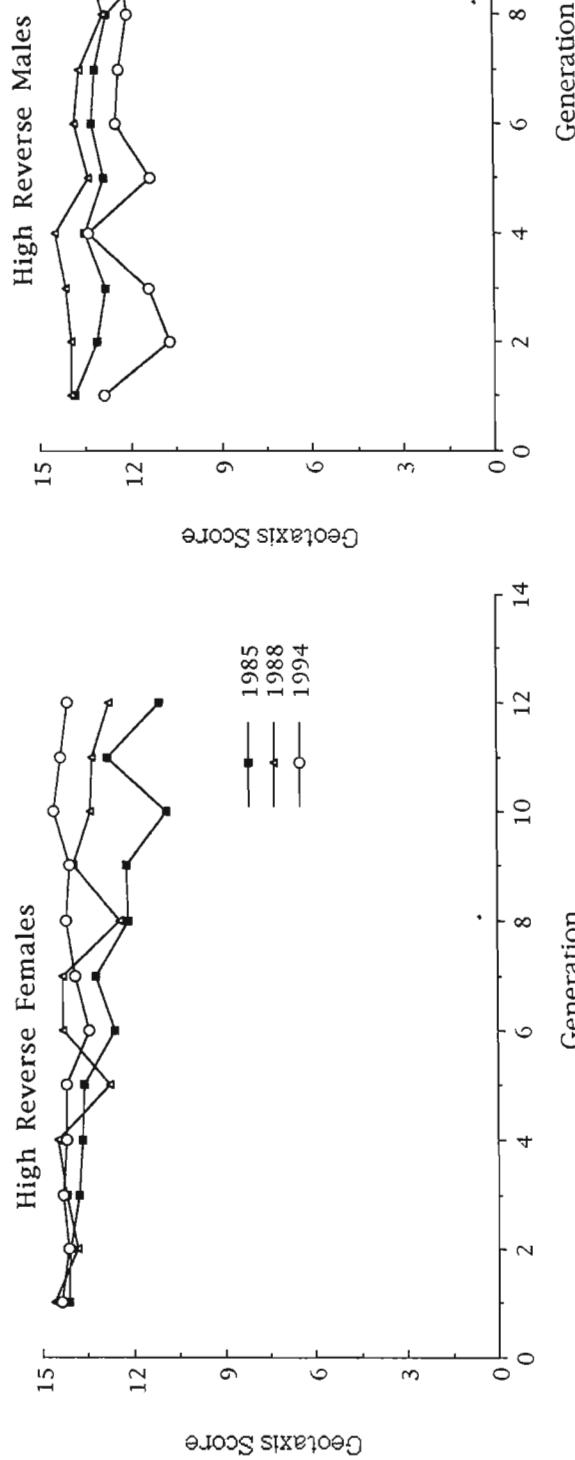


Figure 3. Mean geotaxis scores of females from High Reverse (HR) sublines over twelve generations of reverse selection.

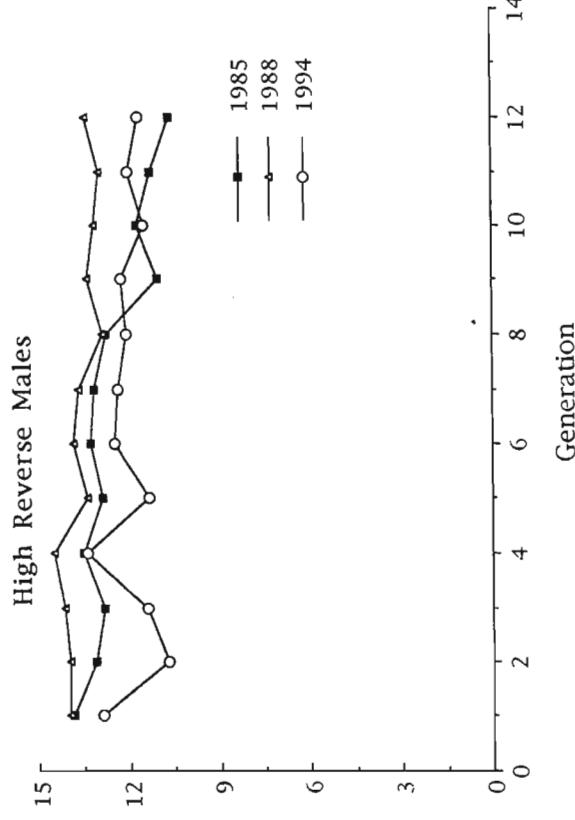


Figure 2. Mean geotaxis scores of males from Low Reverse (LR) sublines over nine (1994) and ten (1985, 1988) generations of reverse selection.

**Choo, Jong Kil, Bong Ki Kim and Hyun Cho.**  
Department of Biology, Chung-Ang University, Seoul 156-756, Korea. Intra- and interspecific variations of mitochondrial DNA in natural populations of *Drosophila*.

Mitochondrial DNA (mtDNA) polymorphism was investigated in eight geographical populations of *D. melanogaster* and *D. simulans* in Korea and Japan. mtDNA was isolated from 35 and 8 isofemale strains in *D. melanogaster* and *D. simulans*, respectively, and were analysed by eight restriction endonucleases.

With eight restriction endonucleases, a total of 30 and 27 restriction cleavage sites were revealed, respectively, in 35 isofemale strains of *D. melanogaster* and in 8 isofemale strains of *D. simulans*. On the basis of mtDNA fragment differences, site variation appeared in two restriction endonucleases, HaeIII and ScaI, and four haplotypes were identified in *D. melanogaster* (Table 1).

Table 1. Restriction pattern for the four mt DNA haplotypes in *D. melanogaster*

Haplotype	Restriction enzyme pattern							Strains		
	Hpa II	HaeIII	Pvu II	Xba I	EcoR1	Hind III	Sca I	Pst I	Number	Percent
M1	a	a1	a	a	a	a	b1	a	20	57.1
M2	a	a2	a	a	a	a	b1	a	12	34.3
M3	a	a1	a	a	a	a	b2	a	1	2.9
M4	a	a2	a	a	a	a	b2	a	2	5.7

Table 2. Estimates of nucleotide divergence and shared sites between mtDNA types in *D. melanogaster* and *D. simulans*

Haplotype	<i>D. melanogaster</i>				<i>D. simulans</i> si II
	M1	M2	M3	M4	
M1	(20 + 7)	0.0059	0.0034	0.0088	0.0525
M2	20 + 6	(20 + 8)	0.0088	0.0032	0.0789
M3	18 + 7	18 + 6	(19 + 7)	0.0061	0.0513
M4	18 + 6	18 + 8	19 + 6	(19 + 8)	0.0783
si II	12 + 2	12 + 1	12 + 2	12 + 1	(21 + 6)

Average substitution rate of *D. melanogaster*: 0.0033

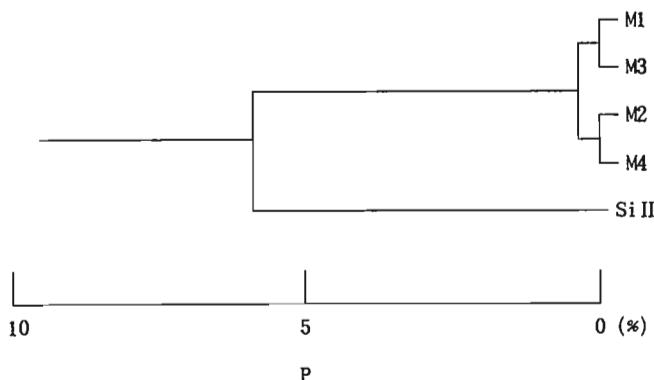


Table 2 shows the results of nucleotide substitution rate, which employs equations of Nei and Li (1979, 1981). Nucleotide substitution rates among four haplotypes were ranging from 0.0032 to 0.0088 (average 0.0033). Figure 1 shows the dendrogram of mtDNA haplotypes, which has been constructed from the data in Table 2 by UPGMA (Sneath and Sokal, 1973).

A possible explanation of low divergence was that mtDNA variation of eight geographical populations in *D. melanogaster* could not be accumulated sufficiently owing to recent divergence of few individuals, and that sequence divergence was prevented by frequent migration.

References: Nei, M., and W.H. Li 1979, Proc. Natl. Acad. Sci. 76:5269-5273; Nei, M., and F. Tajima 1981, Genetics 97:145-163; Sneath, P.H.A. and R.R. Sokal 1973, *Numerical Taxonomy*. W.H. Freeman, San Francisco.

Figure 1. The dendrogram of *D. melanogaster* and *D. simulans* derived by UPGMA method from the nucleotide distance matrix.

**Terzic, Tatjana, Marko Andjelkovic and Marina Stamenkovic-Radak.** Department of Genetics, Institute of Biological Research, University of Belgrade, Yugoslavia. Effect of fluorescent dust, as a marker, on the frequency of mating in *Drosophila melanogaster*.

observation time and conditions, etc. (Spiess, 1968; Manning, 1970), distinguish the genotypes of flies in those experiments, e.g. wing clipping, could also affect the results (Bryant *et al.*, 1980; Kence, 1981).

The phenomena of variability in mating success among different genotypes has been the subject of many studies in population genetics of *Drosophila* (Ehrman, 1966; Ehrman and Spiess, 1969; Petit *et al.*, 1980). Numerous factors can affect mating: different frequencies of males and females, multiple matings, previous mating experience of flies, female receptivity, (1967; Petit *et al.*, 1980; Sharp, 1982). Methods used to

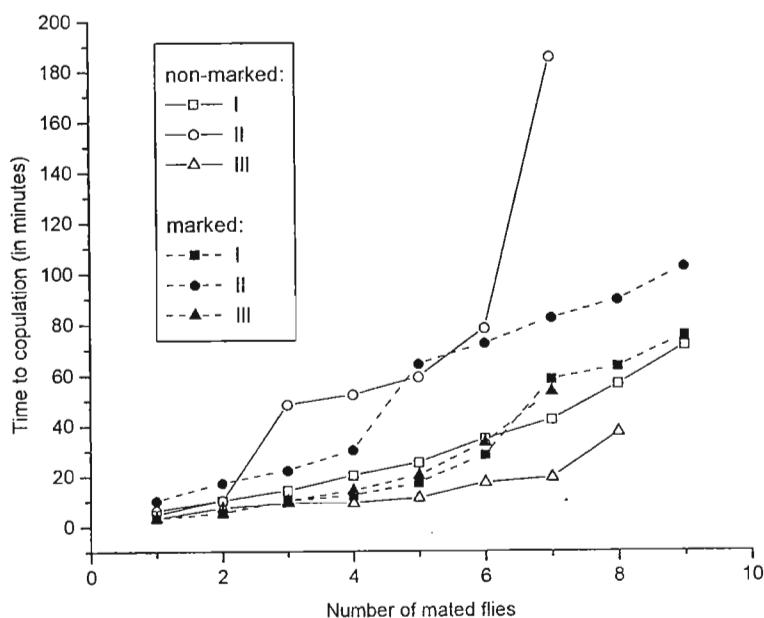


Figure 1. Number of marked and non-marked flies mated in different time intervals; I, II, and III are replicas.

On each occurrence of copulation, the time to the beginning of copulation was recorded and the mated pair removed.

Results given in Figure 1 show that 70% of unmarked and 60% of marked males mated in the first hour. Until the end of observation period 80% of marked and 83% of unmarked males began copulation. Statistical testing of the above proportions (z-statistics) shows the absence of significant difference between marked and unmarked flies in any replica ( $z = 1.11$ ;  $p > 0.05$ ). According to these results, fluorescent dust does not affect mating behavior in *Drosophila* and can be used as a convenient marker in studies of differential mating success and in experiments that involve different genotypes, without affecting their reproductive success.

References: Begon, M., 1978, Ecol. Entomol. 3:1-12; Bryant, E.H., A. Kence and K.T. Kimbal 1980, Genetics 96:975-993; Crumpacker, D.W., and J.S. Williams 1973, Ecol. Monogr. 43:499-538; Dobzhansky, Th., J.R. Powell, C.E. Taylor and M. Andregg 1979, Am. Nat. 114:325-334; Ehrman, L., 1966, Anim. Behav. 14:332-339; Ehrman, L. and E.B. Spiess 1969, Am. Nat. 103(934):675-680; Kekic, V., C.E. Taylor and M. Andjelkovic 1980, Genetika 12(2):219-225; Kence, A., 1981, Am. Nat. 117:1027-1028; Manning, A. 1967, Anim. Behav. 15:239-250; Nigro, L., and B. Shorocks 1982, Dros. Inf. Serv. 58:115; Petit, C., P. Bourgeon and H. Mercot 1980, Heredity 45:281-292; Sharp, P.M., 1982, Genetical Res. 40:201-205; Spiess, E.B., 1968, Am. Nat. 102:363-379.

**Shivanna, N. and S.R. Ramesh.** Department of Studies in Zoology, University of Mysore, Manasagangotri, MYSORE - 570 006, INDIA. A preliminary comparative analysis of paragonial secretions in four species of the *Drosophila immigrans* group.

after mating (David, 1963; Perotti and Hihara, 1981; Chen, 1984; Chen *et al.*, 1985). Electrophoretic analysis of secretory proteins in different species of *Drosophila* (Chen, 1976; Fuyama, 1983; Stumm-Zollinger and Chen, 1985) has revealed that the pattern is species specific.

The present study on the analysis of paragonial secretions was undertaken to analyse the following 3 aspects (a) the extent of qualitative variation (b) the extent of quantitative variation between different species during the development of the adults and (c) the extent of variation in the quantity of secretions transferred from males to the females during mating; in four related species of the *Drosophila immigrans* group namely, *Drosophila nasuta nasuta*, *D. sulfurigaster neonasuta* of the *nasuta* subgroup and *D. rubida* and *D. pararubida* of the *hypocausta* subgroup.

Fluorescent dust (visible under UV light) has been a well-known marker in numerous studies on dispersion and density of *Drosophila* populations in the wild (Crumpacker and Williams, 1973; Begon, 1978; Dobzhansky *et al.*, 1979; Kekic *et al.*, 1980; Nigro and Shorocks, 1982). We have considered fluorescent dust as a useful marker in multiple choice mating experiments related, for examples, to rare male mating phenomena. The present work is a test whether there is a difference in the frequency of mating between marked and unmarked flies.

One half of collected virgin males of a *Drosophila melanogaster* laboratory population (Slankamen, 1989) were dusted with red fluorescent dust, 24 hours before testing. Both marked and unmarked males were kept individually prior to mating. Matings were observed in  $50 \text{ cm}^3$  empty vials, simultaneously, for both groups (marked and unmarked), in three replicas, ten pairs in each. All the pairs were observed for 3 hours.

The accessory glands (paragonia) of the male reproductive system in adult *Drosophila* play specific major roles in reproduction by synthesizing a complex of secretory proteins. This tissue secretory protein is shown to participate to various extents in oviposition, inhibition of female receptivity, sperm transfer, storage, displacement and to maintain the function in the female

For the qualitative and quantitative analysis of the accessory gland secretory proteins, the glands from the flies aged 1 to 10 days were dissected and the samples were prepared by following the procedure of Ramesh and Kalisch (1988) for larval salivary gland secretions of *Drosophila*.

The electrophoretic protein patterns of accessory gland secretions in *D. melanogaster* on 10% SDS gels has revealed the presence of 40 protein fractions of molecular weight ranging from 15 to 175 kd (Stumm-Zollinger and Chen, 1985). The present study involving analysis of secretions obtained from 7 day old adults on 13.4% SDS polyacrylamide gels showed the presence of 10, 11, 8 and 9 protein fractions in *D. n. nasuta*, *D. s. neonasuta*, *D. rubida* and *D. pararubida*, respectively. The molecular weight of these protein fractions range from 12 to 120 kd (Figure 1). The protein patterns in *D. n. nasuta* and *D. s. neonasuta* are identical except for only one extra protein fraction in *D. s. neonasuta* with a molecular weight of 92 kd. A comparison of the protein patterns between *D. rubida* and *D. pararubida* reveals that *D. rubida* differs from *D. pararubida* by only one fraction having a molecular weight of 58 kd. Whereas *D. pararubida* differs from *D. rubida* in having two exclusive protein fractions of molecular weight 56 and 60 kd. Further, except for the low molecular weight protein fraction (14 kd), all the other fractions are glycoproteins, as evidenced by their PAS positivity. Thus, the extent of similarity in protein patterns between the species under study is found to be greater than the extent of similarity reported (Chen, 1976) between *D. melanogaster* and *D. simulans* of the *melanogaster* subgroup.

The quantity was estimated by the spot analysis method as described by Neuhoff (1985). The O.D. of the samples was measured at 630 nm and the percentage of secretions was calculated by taking into consideration the amount of protein present in a pair of glands containing secretions and secretions alone. To estimate the amount of protein transferred during mating, the males were separated from the females after mating and the quantity of accessory gland protein remaining in the gland was estimated following the above mentioned procedure. The difference in the quantity of secretions between the mated and the unmated males gives the amount of secretions transferred during mating. The secretory proteins accumulated in the gland lumen of 7-10 day old *D. melanogaster* males accounts for about 80% of the total soluble protein (Chen, 1984), and one third of the accessory gland stored secretions are transferred to the female reproductive tract during mating (Baumann, 1974). The present analysis revealed that the secretions accumulate gradually from the first day after eclosion and reaches a maximum on the 7th day after eclosion. The secretory protein in 7 day old adults constitutes about 72% in *Drosophila nasuta nasuta*, 70% in *D. s. neonasuta*, 50% in *D. rubida* and 54.2% in *D. pararubida*. The amount of protein transferred into the female genital tract during mating in different species analysed in the present study was found to be higher as compared to *D. melanogaster*. The males of *D. n. nasuta* and *D. s. neonasuta* transfer 8 ug of secretion per mating, which amounts to 55% and 57.14% of stored secretions, respectively, while the males of *D. rubida* and *D. pararubida* transfer 5 and 4.7 ug per mating, which amounts to 66.66% and 62.5% of stored secretions, respectively.

It is evident from the present investigations that both the quantity and the complexity of accessory gland secretory protein fractions are lesser in the species under study as compared with that of *D. melanogaster*.

**Acknowledgment:** We thank the Chairman of the department for providing facilities. S.R.R is grateful to the DAAD and GTZ of Germany for providing Chemicals and Gift equipment. N.S. is thankful to the University Grants Commission, New Delhi for award of scholarship.

**References:** Baumann, H., 1974, J. Insect Physiol. 20:2347-2362; Chen, P.S., 1976, Experientia 32:549-551; Chen, P.S., 1984, Ann. Rev. Entomol. 29:233-255; Chen, P.S., E. Stumm-Zollinger, and H. Caielari 1985, Insect Biochem.

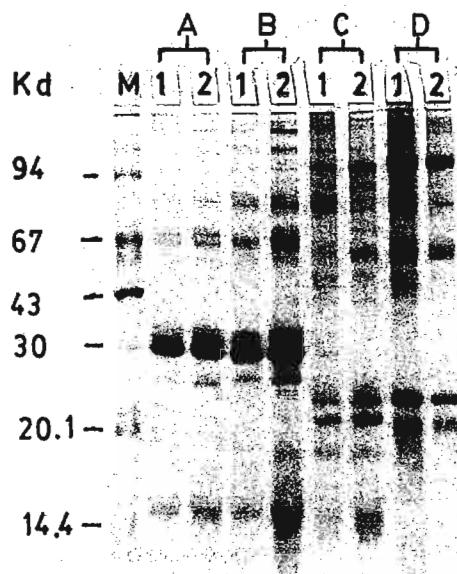


Fig. 1

Figure 1. Hale accessory gland proteins of *Drosophila* separated by 13.4% SDS-PAGE stained with Coomassie Brilliant Blue R-250. A: *D. n. nasuta*, B: *D. s. neonasuta*, C: *D. rubida*, D: *D. pararubida*, M = Marker proteins, 1 = Glands, 2 = Only secretions.

15:385-390; David, J., 1963, J. Insect Physiol. 9:13-24; Fuyama, Y., 1983, Experientia 39:190-192; Hihara, F., 1981, Zool. Mag. 90:307-316; Neuhoff, V., 1985, In: *Modern Methods in Protein Chemistry*. (Tschesche, H., ed.), Vol. 2, Walter de Gruyter, Berlin, N.Y.; Perotti, M.E., 1971, J. Submicrosc. Cytol. 3:255-282; Ramesh, S.R., and W.-E. Kalisch 1988, Biochem. Genet. 26:527-541; Stumm-Zollinger, E., and P.S. Chen 1985, Insect Biochem. 15:375-383.

**Futch, David G.** Department of Biology, San Diego State University, San Diego, CA 92182-0057. Genetic mosaic daughters of parthenogenetic *D. ananassae* and *D. pallidosa* females.

Futch, 1973; Futch, 1979). All of these impaternal genetic mosaics have been females and most have been basically like the fly shown in Figure 1. This fly was the asexually produced daughter of a *D. pallidosa* female that was heterozygous for the X-linked recessive alleles *white* (*w*) and *forked* (*f*) and also for the 3rd chromosome recessive allele *abrupt* (*abt*), which causes an early termination of longitudinal wing vein V. The left side of the head and thorax of the fly in Figure 1 exhibits the phenotype of the two X-linked mutant alleles. The right wing has the abrupt phenotype.

From time to time I have found an interesting kind of genetic mosaic among the asexually produced offspring of heterozygous parthenogenetic females of *D. ananassae* and *D. pallidosa*. Selected unisexual strains of the two species have been maintained in my laboratory for a number of years now (see Futch, 1972;

The mother of the fly pictured in Figure 1 was produced by a cross between a female from a mutant (*w*, *f*, *abt*) unisexual strain and a wild-type male from a bisexual bridge strain. Bridge strains are maintained by backcrossing males each generation to females from their conspecific wild-type unisexual strain. *D. ananassae*, *D. pallidosa* and interspecific hybrid females produced this way are fertile asexually.

Not all of the mosaics are symmetrical as the one shown in Figure 1. Some are "spotty" with patches of mutant and non-mutant tissue on the same side; e.g., part of an eye white and the rest wild-type, forked bristles on part of one side of the head, but none on the thorax, etc. Mosaics are always female and are fertile as virgins or when mated. They occur infrequently. The incidence observed so far in *D. ananassae* is 12 of 7136 (0.00168) offspring examined and in *D. pallidosa* 21 of 5574 (0.00377). No count has been made yet of the offspring of interspecific hybrid mosaics, although they do occur.

I have been attempting to analyze such mosaic offspring by test crossing them, but the results so far have been mixed and often confusing. Since external structures are mosaic genetically, internal structures, including ovaries must also be

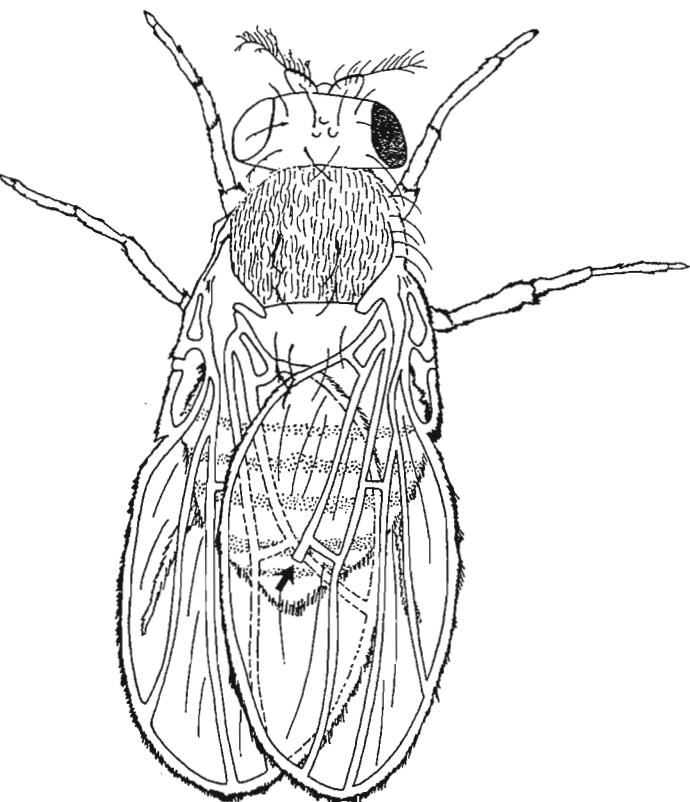


Figure 1. Mosaic *D. pallidosa* female with *white* and *forked* on the left side of the head and thorax and *abrupt* (arrow) on the right wing.

mosaic. Undoubtedly mosaics must be the result of some type of aberration in the process that leads to the diploid unfertilized eggs responsible for the asexual fertility of the females in these unisexual strains. The usual process seems to be the same as that responsible for the asexual fertility of females in the unisexual strains of *D. mercatorum* analyzed by Carson *et al.* (1969). In this model, meiosis is completed in the unfertilized egg and diploidy is restored either by the fusion of two of the haploid products of the meiosis or by the mitotic doubling and subsequent fusion of one of these nuclei, probably the egg pronucleus. The latter happening is by far the more common since about 80% (*D. pallidosa*) to 90% (*D. ananassae*) of the impaternal offspring of heterozygous females are isogenic. The mosaics are perhaps the result of simultaneous fusions of two separate pairs of haploid nuclei resulting in two genetically different diploid nuclei

in the same unfertilized egg.

References: Carson, H.L., I.Y. Wei and J.A. Niederkorn, Jr., 1969, Genetics 63:619-628; Futch, D.G., 1972, Dros. Inf. Serv. 48:78; Futch, D.G., 1973, Genetics 74:s86-s87; Futch, D.G., 1979, Genetics 91:s36-s37.

**Doane, W.W.** Department of Zoology, Arizona State University, Tempe, AZ 85287-1501. The *adipose* gene is located in section 55A of chromosome 2R.

phenotypes were uncovered in flies carrying *Df(2R)PC4*, which has breakpoints in 55A and 55F, but not in heterozygotes carrying *Df(2R)P29*, whose breaks are in 55C1-2 and 56B1-2.

More recently, I tested the two *adp* mutant alleles over *Df(2R)Pc111B* (= *Df(2R)54F6-55A1;55C1*), *Df(2R)Pcl-W5* (= *Df(2R)55A-55B;55C*) and *In(2R)Pcl<sup>W4</sup>*, whose breakpoints are in 55A and 57A (see Lindsley and Zimm, 1992). All of these rearrangements were originally isolated as *Polycomb like* (*Pcl*) mutants. All three of them uncovered *adp*. *Pcl* is located at 2-84 on the genetic map, closely linked to *adp* (Lindsley and Zimm, 1992). Since neither *Pcl* nor *adp* can be located in region 57A, where the distal breakpoint of *In(2R)Pcl<sup>W4</sup>* is found, it appears that the proximal break caused by this inversion produced a small deletion that includes both the *adp* and *Pcl* gene loci. Accordingly, both of these genes are located in 55A.

References: Doane, W.W., and F.E. Dumapias 1987, Dros. Inf. Serv. 66:49; Lindsley, D.L. and G.G. Zimm 1992, *The Genome of Drosophila melanogaster*. Academic Press, New York.

**Foehr, E.D. and W.W. Doane.** Department of Zoology, Arizona State University, Tempe, AZ 85287-1501. Analysis of the protein phosphatase Y gene in *Drosophila melanogaster*.

probe. The cDNA nucleotide sequence for *PpY-55A* and its predicted amino acid sequence are known (Dombradi *et al.*, 1989), but no mutant phenotype has been described. PP-Y is a member of the PP-1/2A/2B family of phosphatases, which function in carbohydrate and lipid metabolism (Cohen, 1989; Bollen and Stalmans, 1992). It is most closely related to PP-1 phosphatases that regulate glycogen synthase, oocyte maturation, and muscle contractility in mammals. Of the 4 known PP-1 genes in *D. melanogaster*, the best characterized is *PP1 87B*, mutants of which block mitosis (Axton *et al.*, 1990), alter heterochromatin, and cause pupal lethality (Dombradi and Cohen, 1992).

We have characterized *PpY-55A* on the grounds that it may be synonymous with *adipose* (*adp*, 2-84.3). The *adp* gene lies within section 55A of the polytene map for the second chromosome (Doane, 1994), wherein *PpY-55A* is located. The abnormal phenotypic traits expressed by *adp* mutants are what might be expected from reduction in serine threonine protein phosphatase activity. For example, one week old flies homozygous for either of the known mutant alleles, *adp<sup>60</sup>* or *adp<sup>fs</sup>*, display approximately a 2-fold increase in total lipid content (Doane, 1960b; Teague *et al.*, 1986) and about half the wild-type levels of total carbohydrate or glycogen (Doane, 1980). Also, eggs laid by *adp<sup>fs</sup>* homozygotes show meiotic or mitotic arrest at metaphase (Doane, 1960a).

Glycogen synthase, a key enzyme in glycogen metabolism, is regulated by PP-1 in mammals, and PP-Y in *D. melanogaster* is most closely related to PP-1, as noted earlier. We postulated that the abnormally low glycogen levels displayed by *adp* mutants might reflect deficiencies in glycogen synthase activity which, in turn, could be due to reduced PP-Y activity. Therefore, both of these enzymes were assayed in crude extracts of flies homozygous for *adp<sup>60</sup>*, *adp<sup>fs</sup>* and *adp<sup>+</sup>*, the wild-type allele in an Oregon-R strain. Glycogen synthase activity was assayed by combining and modifying the methods of Bogardus *et al.* (1984) and Leloir and Goldemberg (1971). A modified assay (Freymond *et al.*, 1988) was used to test the activity of glycogen synthase phosphatase. Results indicate that glycogen synthase activity is significantly lower in both *adp* mutants - on the order of about 60% of wild-type activity in *adp<sup>60</sup>* homozygotes and 48% in *adp<sup>fs</sup>* homozygotes. Preliminary data also suggest that the level of glycogen synthase phosphatase activity in flies homozygous for *adp<sup>60</sup>* or *adp<sup>fs</sup>* is approximately 60-65% that of Oregon-R wild-type flies, the levels being somewhat lower in *adp<sup>fs</sup>/adp<sup>fs</sup>* extracts (with some as low as 40%). These results are consistent with PP-Y playing a role in the regulation of glycogen synthase in *D. melanogaster*, assuming that *adp* mutants produce a defective PP-Y protein. (Unpublished data of E. D. Foehr reveal no significant difference between the levels of *PpY-55A* RNA in *adp* mutants and wild-type,

The *adipose* gene (*adp*, 2-84.3) was broadly located by deletion analysis to the region between 55A and 55C1-2 of the polytene chromosome map for the second chromosome in *Drosophila melanogaster* (Doane and Dumapias, 1987). Thus, the *adp<sup>60</sup>* and *adp<sup>fs</sup>* mutant

phenotypes were uncovered in flies carrying *Df(2R)PC4*, which has breakpoints in 55A and 55F, but not in heterozygotes carrying *Df(2R)P29*, whose breaks are in 55C1-2 and 56B1-2.

The *PpY-55A* gene in *Drosophila melanogaster*, which encodes serine threonine protein phosphatase Y (PP-Y), was mapped to polytene chromosome region 55A1-3 by *in situ* hybridization (Dombradi *et al.*, 1989). *Drosophila* head cDNA with homology to rabbit serine threonine protein phosphatase 1 (PP-1) served as the probe. The cDNA nucleotide sequence for *PpY-55A* and its predicted amino acid sequence are known (Dombradi *et al.*, 1989), but no mutant phenotype has been described. PP-Y is a member of the PP-1/2A/2B family of phosphatases, which function in carbohydrate and lipid metabolism (Cohen, 1989; Bollen and Stalmans, 1992). It is most closely related to PP-1 phosphatases that regulate glycogen synthase, oocyte maturation, and muscle contractility in mammals. Of the 4 known PP-1 genes in *D. melanogaster*, the best characterized is *PP1 87B*, mutants of which block mitosis (Axton *et al.*, 1990), alter heterochromatin, and cause pupal lethality (Dombradi and Cohen, 1992).

We have characterized *PpY-55A* on the grounds that it may be synonymous with *adipose* (*adp*, 2-84.3). The *adp* gene lies within section 55A of the polytene map for the second chromosome (Doane, 1994), wherein *PpY-55A* is located. The abnormal phenotypic traits expressed by *adp* mutants are what might be expected from reduction in serine threonine protein phosphatase activity. For example, one week old flies homozygous for either of the known mutant alleles, *adp<sup>60</sup>* or *adp<sup>fs</sup>*, display approximately a 2-fold increase in total lipid content (Doane, 1960b; Teague *et al.*, 1986) and about half the wild-type levels of total carbohydrate or glycogen (Doane, 1980). Also, eggs laid by *adp<sup>fs</sup>* homozygotes show meiotic or mitotic arrest at metaphase (Doane, 1960a).

Glycogen synthase, a key enzyme in glycogen metabolism, is regulated by PP-1 in mammals, and PP-Y in *D. melanogaster* is most closely related to PP-1, as noted earlier. We postulated that the abnormally low glycogen levels displayed by *adp* mutants might reflect deficiencies in glycogen synthase activity which, in turn, could be due to reduced PP-Y activity. Therefore, both of these enzymes were assayed in crude extracts of flies homozygous for *adp<sup>60</sup>*, *adp<sup>fs</sup>* and *adp<sup>+</sup>*, the wild-type allele in an Oregon-R strain. Glycogen synthase activity was assayed by combining and modifying the methods of Bogardus *et al.* (1984) and Leloir and Goldemberg (1971). A modified assay (Freymond *et al.*, 1988) was used to test the activity of glycogen synthase phosphatase. Results indicate that glycogen synthase activity is significantly lower in both *adp* mutants - on the order of about 60% of wild-type activity in *adp<sup>60</sup>* homozygotes and 48% in *adp<sup>fs</sup>* homozygotes. Preliminary data also suggest that the level of glycogen synthase phosphatase activity in flies homozygous for *adp<sup>60</sup>* or *adp<sup>fs</sup>* is approximately 60-65% that of Oregon-R wild-type flies, the levels being somewhat lower in *adp<sup>fs</sup>/adp<sup>fs</sup>* extracts (with some as low as 40%). These results are consistent with PP-Y playing a role in the regulation of glycogen synthase in *D. melanogaster*, assuming that *adp* mutants produce a defective PP-Y protein. (Unpublished data of E. D. Foehr reveal no significant difference between the levels of *PpY-55A* RNA in *adp* mutants and wild-type,

suggesting regulation of gene expression is not involved here.)

We have cloned and sequenced the *PpY-55A* gene from our Oregon-R strain, as well as from homozygous *adp*<sup>60</sup> and *adp*<sup>fs</sup> flies, and found no introns. The predicted amino acid sequence of PP-Y in all three genotypes was compared. PP-Y in *adp*<sup>60</sup> mutants has an asn147 --> ser147 amino acid change when compared to the Oregon-R wild-type sequence. PP-Y in homozygous *adp*<sup>fs</sup> flies, which display the more severe mutant phenotype of maternal effect embryonic lethality (Doane, 1960a), shows the same asn --> ser difference from wild-type as the *adp*<sup>60</sup> mutant, plus three additional changes: gln186 --> glu186, arg229 --> gln229, and ser280 --> asn280. While any functional significance of these sequence differences remains to be demonstrated, their nature and numbers do not refute our view that *adp* and *PpY-55A* are synonymous. Definitive evidence for this, pro or con, must come from experiments designed to either clone and sequence the *adp* gene by tagging it with a defective P element, or to rescue the *adp* mutant phenotypes with a wild-type *PpY-55A* allele through P-mediated germ line transformation. These experiments are in progress.

Acknowledgments: Support for this work came from research incentive and graduate research funds, Arizona State University and from the Center for Insect Science, University of Arizona.

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**Kim, W.\*, M.F. Wojciechowski, L. Bieling and M.G. Kidwell,** Department of Ecology and Evolutionary Biology, University of Arizona, Tucson AZ 85721 U.S.A. Genetic transformation with P transposable vectors carrying the *rosy* gene in *Drosophila melanogaster*.

*melanogaster* (O'Hare and Rubin, 1983). The *rosy* (*ry*<sup>+</sup>) gene sequence, contained on a 7.2 kb HindIII fragment, is positioned in a noncoding region of the element, downstream from the unique Sall site at sequence position 2410. This element combines a marker gene and a source of P transposase in a single transposon construct. The second construct consists of a nonautonomous derivative of *Pc[ry]B*, designated *P[ry]Δ SX9*, that was created by deleting approximately 1680 bp between the *XhoI* site at position 727 and the *Sall* site at position 2410. The source of transposase for microinjection and transformation was provided by a P element helper plasmid designated *p-Δ 2-3hs π* (a gift from Dr. Y.K. Kim, SUNY, Purchase), which was co-injected with *P[ry]Δ SX9* into a true M strain carrying the *ry*<sup>506</sup> mutation.

Table 1. Results of microinjection experiments with *Pc[ry]B* and *P[ry]Δ SX9*

Construct	No. G <sub>0</sub> injected	No. (%) G <sub>0</sub> eclosed	No. (%) G <sub>0</sub> <i>ry</i> <sup>+</sup> flies	No. (%) fertile flies	No. G <sub>1</sub> <i>ry</i> <sup>-</sup> flies	No. G <sub>1</sub> <i>ry</i> <sup>+</sup> flies	No. independent transformants
<i>Pc[ry]B</i>	523	55	25	45	3,836	38	11
<i>P[ry]Δ SX9</i>	620	142	53	102	7,294	114	24
Total	1143	197 (17.2)	78 (39.6)	147 (74.6)	11,130	152	35 (23.8)

Table 2. The distribution of G<sub>1</sub> flies with *ry*<sup>+</sup> eye color from 35 G<sub>0</sub> transformed lines (see Table 1)

% <i>ry</i> <sup>+</sup> progeny in G <sub>1</sub>	1 - 2	3 - 4	5 - 6	7 - 9	10 - 19	20 - 30
No. of G <sub>0</sub> transformed lines	16	7	5	4	2	1

We have used two kinds of P element constructs for genetic transformation by microinjection of *D. melanogaster* in studies of the population dynamics of P element transposition. The first construct was *Pc[ry]B* (Karess and Rubin, 1984), constructed from the P-*rosy* vector Carnegie 20 (Rubin and Spradling, 1982) and the 2907 bp autonomous P element from p 25.1 from *D.*

*melanogaster* (O'Hare and Rubin, 1983). The *rosy* (*ry*<sup>+</sup>) gene sequence, contained on a 7.2 kb HindIII fragment, is positioned in a noncoding region of the element, downstream from the unique Sall site at sequence position 2410. This element combines a marker gene and a source of P transposase in a single transposon construct. The second construct consists of a nonautonomous derivative of *Pc[ry]B*, designated *P[ry]Δ SX9*, that was created by deleting approximately 1680 bp between the *XhoI* site at position 727 and the *Sall* site at position 2410. The source of transposase for microinjection and transformation was provided by a P element helper plasmid designated *p-Δ 2-3hs π* (a gift from Dr. Y.K. Kim, SUNY, Purchase), which was co-injected with *P[ry]Δ SX9* into a true M strain carrying the *ry*<sup>506</sup> mutation.

Embryos were collected on an agar plate (2%) plus enough grape juice to color the solution deeply. We used a non-dechorination method in the first round of the microinjection experiment, but considerable difficulties were experienced in obtaining successful transformation. Since it was difficult to produce embryos with chorion membranes that were sufficiently desiccated, penetration by a needle resulted in leakage of cytoplasm. However, survival and successful transformation were subsequently achieved when a dechorination method was adopted. Embryos collected from eggs laid within a 20 min period were rinsed and transferred on double-coated tape (2 x 20 mm) attached to a slide. Slides

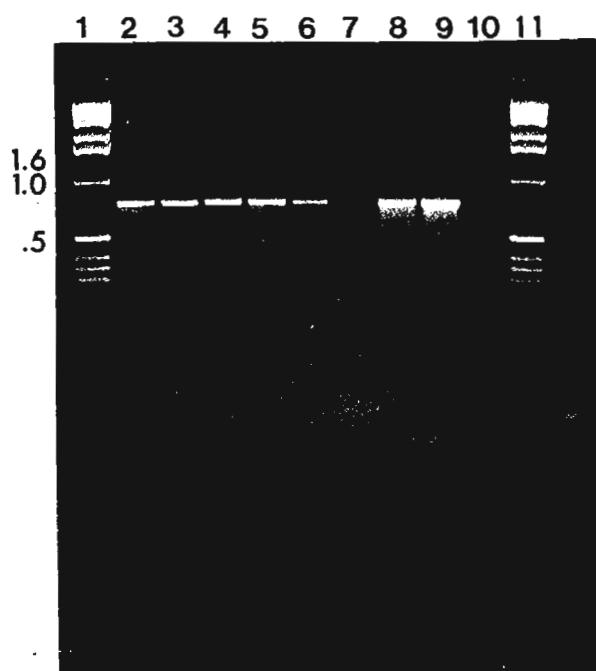


Figure 1. The presence of P element sequences (Pc[*ry*]B construct) in the genomic DNA of individual transformed flies using PCR with P specific oligonucleotide primers. Lanes are as follows: (1 and 11) DNA size markers, Kb ladder; (2-5) independent G<sub>1</sub> transformants; (6) G<sub>0</sub> transformants; (7) *ry*<sup>506</sup>; (8) Harwich-w; (9) p 25.1; (10) blank.

containing the embryos were chilled on ice for 5-10 min in order to make the embryos more rigid and easier to handle during the dechorination process. After chilling, we dechorinated the embryos by rolling them over the tape on the slide. The dechorinated embryos were then placed onto another strip of double-sided tape with the posterior ends of the embryos hanging off the tape. The embryos were lined up and desiccated in a box containing calcium chloride on ice for about 5 min and covered with water-saturated mineral oil. Injection followed immediately.

Needles were made from FHC capillaries (Omega Dot Fiber for rapid filling, catalog #30-31-1). They were siliconized prior to being pulled on a vertical pipette puller (KOPE, model 720). One of the most

important steps in microinjection is to make a fine needle that has a sharp bevelled tip. The tip of the needle was broken off under an inverted microscope using a syringe needle (B-D 22 gauge) by hand. The DNA solution containing 0.5 mg/ml of the relevant constructs was injected into the cleft space of the posterior pole of the embryo and the needle was quickly removed. After injection, a thin strip of culture medium was set alongside the oil to supply food for the hatching larvae. The slides were placed into a humidity box containing wet paper towels to allow the development of the

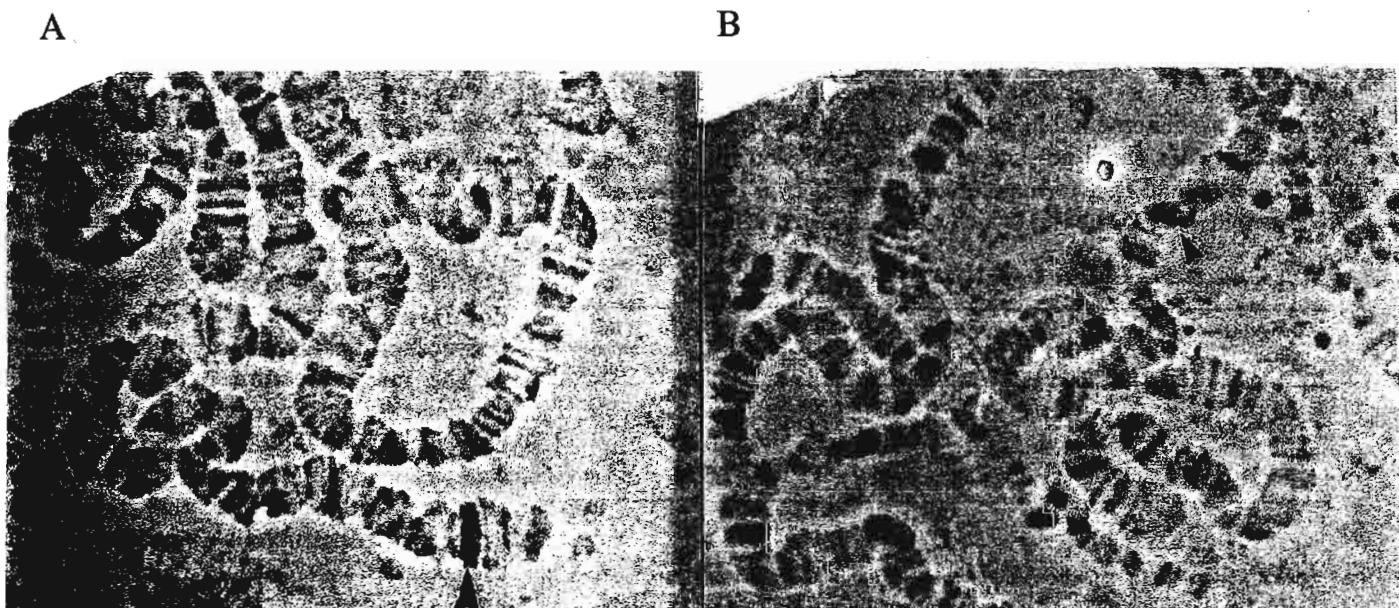


Figure 2. Photographs of the polytene chromosome squashes of individual larvae from the original B1-8 (A panel) and SX31-1 (B panel) transformed lines in which the Pc[*ry*]B and P[*ry*] SX9 transposons are inserted in chromosomes 3L (61F) and 2L (36B), respectively.

embryos on the tape under the oil at 23°C. Within 15 hours of emergence, the larvae were transferred, together with the strip of culture medium, into vials containing regular food. Flies were mated singly with several virgin females or males to produce the G1 generation.

The presence of P element sequences in the genomic DNA of individual transformed flies was first confirmed using PCR (polymerase chain reaction) with oligonucleotide primers that specifically hybridize to the P element sequence. The four lines with independent G1 transformants (lanes 2 - 5 in Figure 1) obviously contain the *Pc[ry]B* construct in their genomes, and one G<sub>0</sub> transformed line (lane 6 in Figure 1) also gave a positive result for the presence of the construct in the cytoplasm or genome.

Further, the P element copy number and cytological position of the *Pc[ry]B* or *P[ry]ΔSX9* transposon insertions in the polytene chromosomes of the initial transformed flies was determined by *in situ* hybridization, using the plasmid p $\pi$ 25.1 as probe. A non-radioactive method (Kim and Kidwell, this volume) was used for labeling and detection; DNA probes were labeled using PCR by incorporation of a nucleotide analog, digoxigenin-11-dUTP. Hybridization was detected by enzyme-linked immunoassay, using an antibody conjugate and subsequent enzyme-catalyzed color reaction. Each of the initial transformed lines were found to carry only one insertion of the P element construct as shown, for example, in Figure 2.

A summary of transformation results using the dechorination method is presented in Table 1.

As shown in Table 1, about 20% of the injected embryos eclosed as adults. Among G<sub>0</sub> eclosed flies, approximately 40% exhibited eye color that was similar to wild-type (*ry*<sup>+</sup>), presumably the result of transcription and translation of the *rosy* gene sequences in these constructs (Spradling, 1986). A total of 31 putative G<sub>0</sub> transformed lines were found to have no G<sub>1</sub> transformants (50 lines among 78 G<sub>0</sub> transformed lines were fertile in our experiment). Therefore it is unlikely that G<sub>0</sub> expression requires integration of the *rosy* transposon into chromosomes. Overall our transformation frequency was 3.1% of injected embryos (35/1143). The survival frequency of eclosed flies is similar to that of other reports, but the transformation frequency was somewhat higher.

Spradling (1986) reported that usually only a small fraction of the germline cells of a G<sub>0</sub> individual contain integrated *rosy* transposons and gave rise to *ry*<sup>+</sup> G<sub>1</sub> progeny. Our results are also consistent with his report. The frequencies of G<sub>1</sub> *ry*<sup>+</sup> progeny produced by 35 transformed lines is summarized in Table 2.

The results from Table 2 indicate that the frequency of G<sub>1</sub> progeny with *ry*<sup>+</sup> eye color was quite low from those G<sub>0</sub> individuals that produced transformed progeny. For example, in almost 50% of the 35 G<sub>0</sub> transformed lines *ry*<sup>+</sup> flies represented less than 3% of the total progeny recovered.

We backcrossed the transformed G<sub>1</sub> progeny with *ry*<sup>506</sup> in order to establish a series of G<sub>2</sub> iso-lines. Seven independent lines among a total of 35 were found to carry X-linked insertions. One of these seven was also found to carry a recessive lethal mutation and a second carried a recessive mutation affecting wing shape. Based on progeny tests and *in situ* hybridization experiments, none of the G<sub>1</sub> transformed lines contained more than a single insertion of the P element construct.

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**References:** Karess, R.E., and G.M. Rubin 1984, Cell 38:135-146; O'Hare, K., and G.M. Rubin 1983, Cell 35:25-35; Rubin, G.M. and A.C. Spradling 1982, Science 218:348-353; Spradling, A.C., 1986, In: *Drosophila: A Laboratory Approach* (D.B. Roberts, ed.). IRL Press.

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**Kopantseva, T.I., L.F. Maximovsky and I.F. Zhimulev.**  
Institute of Cytology and Genetics, Novosibirsk, Russia.  
Increase of RNA quantity in salivary gland nuclei of nonpupariating larvae homozygous for the *l(3)tl* mutation in *Drosophila melanogaster*.

To check the possibility of a general increase of RNA content in the nuclei, the method of microanalysis was used. Salivary glands were dissected in a drop of saline, fixed, and cells and nuclei were dissected with the help of a micromanipulator as described by Edstroem and Daneholt (1967). RNA from nuclei or cytoplasm (the rest of the cell) was digested with RNase in separate drops which then were scanned in UV, and RNA quantity was calculated according to Edstroem (1964) and Maximovsky (1969).

*Drosophila melanogaster* larvae homozygous for *l(3)tl* do not pupariate at the end of the third larval instar and survive as larvae about 30 days. During this time in the salivary gland nuclei big masses of nucleolar-like material appear (Zhimulev and Lytchev, 1972) and polytene chromosomes become very short (Zhimulev *et al.*, 1976).

Data of Table 1 show that in the mutant nuclei RNA quantity grows gradually with increasing the period of larval life. In 17 days *l(3)tl* larvae nuclear RNA content is more than twice greater than in normal 0h prepupae which show most active puffing in polytene chromosomes during observable period of larval development.

In the same cells the increase in the RNA quantity in cytoplasm is not more than 10%. Data obtained seem show that in *l(3)tl* mutants increasing the RNA quantity is related to a disturbance of RNA transport rather than to additional synthesis. It was shown earlier that polytene chromosomes shorten in great extent and this shortening is related to compaction of long pieces of chromatin (inactivation of transcription according to all known theories). This may mean that in nuclei of *l(3)tl* larvae mainly ribosomal RNA accumulates.

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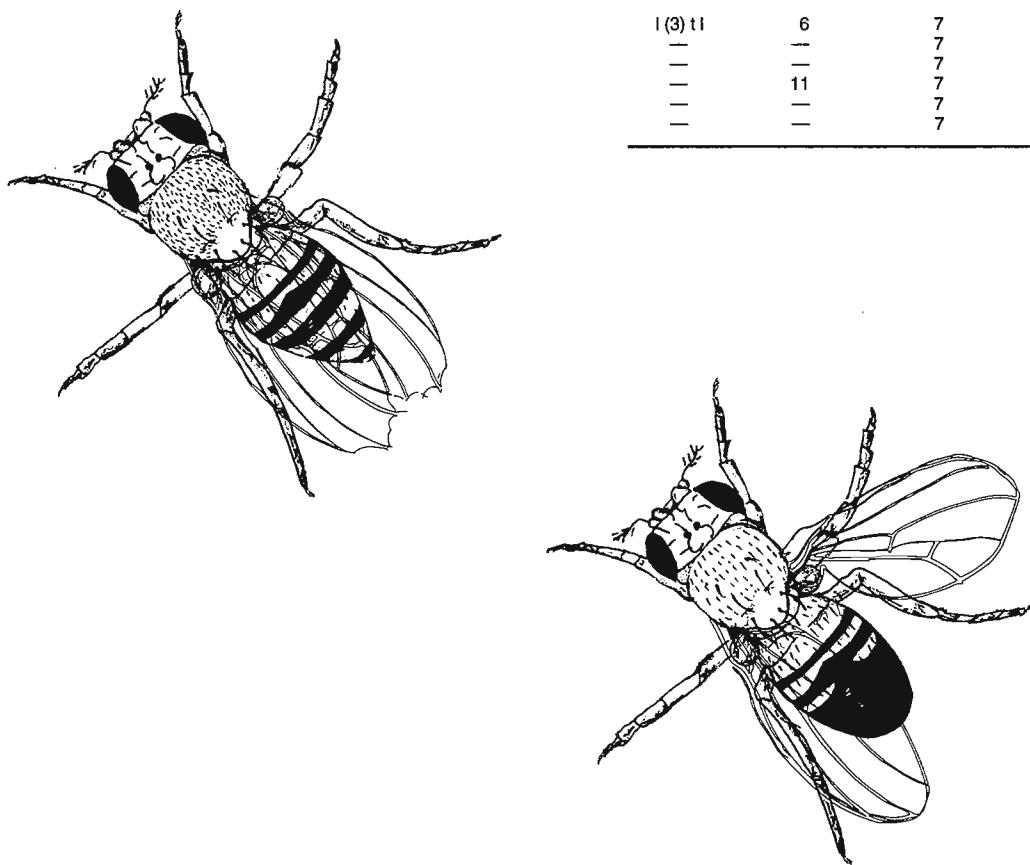


Table 1. RNA content in salivary gland nuclei of *l(3)tl* larvae and wild type Batumi-L 0 hr prepupae.

Strain	Age (days)	No. of samples studied	RNA content in one nucleus (picograms)
Batum-L	0 hr. Prepupae	20	340 ± 7
—	—	10	337 ± 11
—	—	10	332 ± 13
—	—	10	348 ± 21
<i>l(3)tl</i>	6	30	500 ± 4
—	6	20	514 ± 6
—	6	10	512 ± 10
—	6	10	504 ± 11
—	6	10	515 ± 10
—	7	20	557 ± 26
—	11	30	632 ± 5
—	11	10	644 ± 15
—	11	10	621 ± 23
—	11	10	632 ± 14
—	17	20	755 ± 24

Table 2. RNA content in larval salivary gland cells of *l(3)tl* mutant and 0 hr prepupae of wild type Batumi-L.

Strain	Age (days)	No. of cells studied	RNA quantity in one cell (picograms)
Batum-L	0 hr. Prepupae	7	3200 ± 64
—	—	7	3125 ± 62
—	—	7	3008 ± 71
<i>l(3)tl</i>	6	7	3317 ± 102
—	—	7	3467 ± 64
—	—	7	3453 ± 90
—	11	7	3352 ± 108
—	—	7	3608 ± 129
—	—	7	3458 ± 134

## 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 from research conferences whenever space allows. The editor invites conference organizers or participants to submit copies of meeting programs and a brief description of the theme, location, and time of the conference. DIS will endeavor to publish authors, affiliation of the senior or corresponding presenter, and title of the talks or posters. This can then be used by readers of DIS to locate individuals pursuing problems of common interest, locate possible postdoctoral researchers, and find sources for materials or information. Even if space does not permit publishing all proceedings, we hope that a listing of regional and national conferences will be useful.

### 35th Annual *Drosophila* Research Conference 20 to 24 April 1994, Chicago, Illinois, U.S.A.

The 35th Annual *Drosophila* Research Conference for the U.S.A. was held at the Sheraton Chicago Hotel, and the 1994 Program Chair was Victoria Finnerty, Emory University. The conference was sponsored by The Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814-3998. Approximately 700 slide and poster presentations were given. The plenary session lectures are listed here, grouped by topic as in the conference program.

- Gelbart, William. (Dept. of Cell and Developmental Biology, Harvard University, Cambridge, MA). @FlyBase: An electronic database of *Drosophila*.
- Pick, Leslie. (Brookdale Center for Molecular Biology, Mt. Sinai School of Medicine, New York, NY). Regulating stripes in the *Drosophila* embryo.
- Shearn, Allen. (Department of Biology, Johns Hopkins University, Baltimore, MD). Killer of Prune, AWD/NDP kinase and invasive tumors.
- Leptin, Maria. (Abteilung Genetik, Max Planck Institut fur Entwicklungsbiologie, Tübingen, Germany). Determination and morphogenesis of endoderm and mesoderm.
- Wharton, Kristi. (Division of Biology and Medicine, Brown University, Providence, RI). TGF-beta-type signaling in *Drosophila* development.
- Artavanis, Spyros. (Department of Biology, Yale University, New Haven, CT). The role of the *Notch* pathway in cell fate determination.
- Aquadro, Chip. (Department of Genetics and Development, Cornell University, Ithaca, NY). Why are levels of DNA variation correlated with rates of recombination in *Drosophila*?
- Salz, Helen. (Department of Genetics, Case Western Reserve University, Cleveland, OH). Sex determination and the regulation of pre-mRNA splicing.
- Heisenberg, Martin. (Lehrstuhl für Genetik, Universität Würzburg, Würzburg, Germany). Learning at the torque meter: from psychophysics to behavioral neurogenetics.
- Theurkauf, Bill. (Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY). Analysis of cytoskeletal functions during oogenesis and early embryogenesis.
- Berg, Celeste. (Department of Genetics, University of Washington, Seattle, WA). Establishing polarity in the *Drosophila* egg.

#### WORKSHOPS:

- DNA Repair: Metabolism and Mutagenesis. Chair, Christopher Osgood (Department of Biological Sciences, Old Dominion University, Norfolk, VA).
- Spermatogenesis. Chair, Margaret Fuller (Department of Developmental Biology, Stanford University, School of Medicine, Stanford, CA).
- Molecular Biology Ecdysone Response. Chair, William Segraves (Department of Biology, Yale University, New Haven, CT).
- Development in Non-*Drosophila* Insects. Chairs, Rob Denell and Sue Brown (Division of Biology, Kansas State

University, Manhattan, KS).  
**Muscle Biology.** Chair, Bob Storti (Department of Biochemistry, University of Illinois College of Medicine, Chicago, IL).  
**Mitosis.** Chair, Margarete Heck (Department of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, MD).  
**Drosophila in the Classroom.** Chairs, Christopher Osgood (Department of Biological Sciences, Old Dominion University, Norfolk, VA) and Joe Pelliccia (Department of Biology, Bates College, Lewiston, ME).

### 13th European *Drosophila* Research Conference 12 to 17 September 1993, Crete

The 13th European *Drosophila* Research Conference was held in Crete, and information on the conference can be provided by Dr. K. Louis, Insect Molecular Genetics Group, Institute of Molecular Biology and Biotechnology, P.O. Box 1527, GR-711 10 Heraklion, Crete, Greece.

- Alifragis, P., D. Eastman, E. Skoula and C. Delidakis. 1993. Preliminary Analysis of Protein Interaction Involving Members of the E(spl) Complex. *Europ. Dros. Res. Conf.* 13. (FBrf0058190)
- Allen, M.J., K.G. Moffat, I.J.H. Roberts and C. O'Kane. 1993. The Effects of Both Native and Mutants of Ricin A-Chain in vivo and on Cultured Drosophila Cells. *Europ. Dros. Res. Conf.* 13. (FBrf0058191)
- Alves, G., E. Guillemet, F. Bihl, F. Chalvet, P. Therond, H. Tricoire, B. Limbourg-Bouchon, C. Lamour-Isnard, T. Preat and D. Busson. 1993. The Two Domains of the Fused Serine-Threonine Kinase and their Functional Role in the fused-suppressor of fused Interaction: Genetic and Molecular Dissection. *Europ. Dros. Res. Conf.* 13. (FBrf0058192)
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Joint Meeting with the Australian Society for Biochemistry and Molecular Biology  
7 to 10 July 1993**

This joint conference was held in Adelaide, and the following talks, taken from a copy of the provisional programme, are on *Drosophila* or related insect research. The programme copy was provided by John Gibson, Molecular and Population Genetics Group, RSBS, Australian National University, GPO Box 475, Canberra City, ACT 2601, Australia. Other talks on *Drosophila* research may have been overlooked by this reference just to titles, and a copy of the programme can be obtained from the DIS editor.

Charles, Robin, Kerrie Medveczky, John Oakeshott, and Robyn Russell. Evolution of an esterase gene cluster implicated in organophosphate resistance in insects. (1) Organization and sequence of the cluster in *Drosophila melanogaster*.

Claudianos, Charles, and Hugh Campbell. The *Caenorhabditis elegans* homologue of the novel *Drosophila flightless-I*

- gene: a study in the evolution of the gelsolin gene family.
- Court, Leon, John Oakeshott, and Robyn Russell. Evolution of an esterase gene cluster implicated in organophosphate resistance in insects. (2) Correlation of the cloned genes with biochemical phenotypes in *Drosophila melanogaster*.
- Davies, A.G., A.Y. Game, S. Goodall, J. Yen, J.A. McKenzie, and P. Batterham. Evidence that an allele of the *Notch* homologue of the Australian sheep blowfly is an asymmetry modifier.
- Goode, S. M. Morgan, and A. Mahowald. Cloning of *brainiac*: a neurogenic gene of *Drosophila* which is required for multiple follicle cell functions during oogenesis.
- Healy, Marion, Mira Dumancic, Anh Cao, and John Oakeshott. Evolution of regulatory sequences conferring sex specific expression on *D. melanogaster EST 6*
- Jermiin, Lars S., and Ross Crozier. Patterns of evolutionary change in insect mtDNA AT content.
- Jones, Lynn, Helena Richardson, and Robert Saint. Genomic and genetic characterization of the cyclin E gene of *Drosophila melanogaster*.
- Lawler, Susan. Artificial selection for development time and the correlated response in the ribosomal genes of *Drosophila hydei*.
- Lonie, Andrew, Stanley Robert, Richard D'Andrea, and Robert Saint. The *Polycomblike* gene of *Drosophila melanogaster* is required for the correct expression of the homeotic genes of the *Antennapedia* and *Bithorax* complexes.
- Morrish, B.C., M.J. Healy, and J.G. Oakeshott. Complexities in *Est6* regulation uncovered using *D. melanogaster* and *D. yakuba* *Est6* promoters.
- O'Neill, Michael. Enhanced germ cell specific transcription of *Tctex-1* due to deletion of repressor sequences.
- Patterson, B., P. Wigley, and R. Saint. Regulation of *string* gene during embryonic cell proliferation.
- Reed, Darryl, and John Gibson. TATA box and amino acid mutations in the *sn-glycerol-3-phosphate* gene in *Drosophila melanogaster*.
- Sved, J., L. Blackman, R. Colless, and Y. Svaboda. The mechanism of P element-induced recombination in *Drosophila melanogaster*.
- Warr, C., A. Phillips, and L. Kelly. Investigation of the *trp* and *trpl* phototransduction genes of *Drosophila melanogaster*.
- Wilanowski, Thomaz, and John Gibson. The effects of a retrotransposon insertion on the expression of the *Gpdh* gene in *Drosophila melanogaster*.

### The 1993 Midwest *Drosophila* Conference 8 to 9 October, Allerton Park, Illinois

The 1993 Midwest *Drosophila* Conference was organized by Hugh Robertson, Department of Entomology, University of Illinois, Urbana, IL 61801, U.S.A. The following presentations are taken from the program, which listed them according to general topic.

- Tom Flickinger and Helen Salz (Case Western Reserve U.) The sex determination gene *snf* is the fly homolog of the human U2B" snRNP protein.
- Marilyn Hart and Douglas Coulter (St. Louis U.) Similarities between *odd-skip* and a closely related homologue.
- Robert Brendza and William Saxton (Indiana U.) Clonal analysis of kinesin function during oogenesis.
- Thomas Blackburn, Kathy Miles, Rami Sweis and Deborah Keiko Hoshizaki (U. Illinois-Chicago) Fat cell determination and differentiation: identification of genes necessary for fat cell gene expression.
- Sally Amero (Loyola U.) The PEP ribonuclease complex.
- Michael Anderson, Greg Perkins, Rebecca Shrigley, Pat Chittick and Wayne Johnson (U. Iowa) Function of the POU-domain transcription factor, *Cfia*, in neuronal and tracheal cell determination.
- David Lampe and Hugh Robertson (U. Illinois-Urbana) The *mariner* transposons are horizontally jumping genes.
- James Skeath, Grace Panganiban and Sean Carroll (U. Wisconsin) Separate regulatory elements mediate proneural gene expression in proneural clusters and neuroblasts.
- Ron Lee and William Stark (St. Louis U.) Retinoid regulation of opsin gene expression.
- Daniel Mallin, Kristin Scott, Robin Roseman and Pamela Geyer (U. Iowa) Effects of the *suppressor of Hairy-wing* protein on interactions between enhancer and promoter elements.
- Lori Wallrath and Sarah Elgin (Washington U.) Heterochromatin and gene repression.
- Eric Cabot, Andrew Davis, Hope Hollocher, Mike Palopoli, Daniel Perez and Chung-I Wu (U. Chicago) The nature of

- species differences: Skepticisms toward beanbag genetics.  
Robert Miller and Billy Geer (Grand View and Knox Colleges) Long-chain fatty acids influence ethanol tolerance in larvae.  
Hiroyuki Kose, Craig Lassy and Tim Karr (U. Illinois-Urbana) Cytoplasmic incompatibility probed using monoclonal antibodies.  
Troy Zars, Young Jay Lee and David Hyde (U. Notre Dame) The role of a GTP-binding protein alpha subunit, dgq, in phototransduction.  
Karen Hales and Robert Storti (U. Illinois-Chicago) Transcriptional and post-transcriptional regulation of maternal and zygotic cytoskeletal tropomyosin mRNA during development correlates with specific morphogenetic events.  
Cynthia Kelsey Motzny, Diane Slusarski and Robert Holmgren (Northwestern U.) The role of the segment polarity gene, *cubitus interruptus*, in the *wingless* signaling pathway.  
Tom Breen (S. Illinois U.) Regulation of *engrailed* expression by *trithorax*.  
Thomas Kaufman (Indiana U.) Ectopic expression of homeotic loci.

POSTER SESSION:

- Beth Albrecht and Helen Salz (Case Western U.) Screening for enhancers of *snf*: A method to identify new sex determination genes.  
Rachel Brewster, Mirra Chung, Laura Maglott and Rolf Bodmer (U. Michigan) Characterization of *cut*-dependent genes involved in sensory organ differentiation.  
Xiaoyuan Chi and Joel Eissenberg (St. Louis U.) Molecular cloning of a dominant suppressor of position-effect variegation.  
Yu-me Eureka Wang and Allan Bieber (Purdue U.). Domains in neuroglian that mediate cell adhesion.  
Alice Gardner, Laura McCullough and Wendi Neckameyer (St. Louis. U.) Regulation of tyrosine hydroxylase and tryptophan hydroxylase.  
Krista Golden and Rolf Bodmer (U. Michigan) The *tinman* gene is necessary for heart and visceral muscle formation.  
Stephen Hall, Michael Hortsch and Allan Bieber (Purdue U. and U. Michigan) Identification and characterization of the novel 2A7 protein in the central and peripheral nervous system.  
Bassem Hassan, Kenneth Wallace and Harald Vaessin (Ohio State U.) Identifying *in vivo* target sites for DNA binding proteins involved in *Drosophila* neurogenesis.  
Abizar Lakdawalla, Kweon Yu and Eileen Underwood (Bowling Green State U.) Analyses of two maternal genes in 52A.  
Craig Lassy, Hiroyuki Kose, Lynn Chang and Tim Karr (U. Illinois-Urbana) Studies of mechanisms of cytoplasmic incompatibility.  
Quinn Lu, Lori Wallrath, Peter Emanuel, Sarah Elgin and David Gilmour (Washington U. and Pennsylvania State U.) Mutation of the TATA box alters the binding of TFIID and expression of *hsp26*, but not the chromatin structure.  
Chris Merli, David Bergstrom and Ronald Blackman (U. Illinois-Urbana) Independent regulation of two neighboring genes.  
Scott Milligan, Tom Vihtelic and David Hyde (U. Notre Dame) The *retinal degeneration-B* gene encodes a membrane-associated phosphatidylinositol transfer protein: analysis of functional domains and role in phototransduction.  
Helen Salz, Tom Flickinger, Beth Mittendorf, Alex Pellicina-Palle, J. Petschek and Beth Albrecht (Case Western Reserve U.) The maternal effect locus, *deadhead*, encodes a thioredoxin homolog and is required for female meiosis and early embryonic development.  
Ken Sorensen and Sally Amero (Loyola U.) RNA-binding characteristics of the hnRNP-associated protein PEP.  
Susan Tsunoda and Lawrence Salkoff (Washington U.) *Shal* encodes most transient K<sup>+</sup> currents in embryonic neurons: *shaw* may also be present.  
Carl Urbinati and Sally Amero (Loyola U.) The yeast homolog of the hnRNP-associated protein PEP.  
Lei Wang and Doug Coulter (St. Louis U.) Molecular and genetic characterization of *odd-related gene II*.  
Ellen Ward and Doug Coulter (St. Louis U.) Localization of the *odd-skipped* protein during development.  
Edward Wojcik, K. Dang-vu and Stuart Tsubota (U. Michigan and St. Louis U.) *mod(r)2*, a regulator of the *nudimentary* (*CAD*) gene.

## Drosophila Information Newsletter

The following material is reprinted from DIN volumes 11-15, appearing between July 1993 and July 1994. The complete tables of contents of all five issues are included here, but out-of-date material, or material expected to be covered elsewhere in this issue, has not been reprinted.

The Drosophila Information Newsletter has been established with the hope of providing a timely forum for informal communication among Drosophila workers. The Newsletter will be published quarterly and distributed electronically, free of charge. We will try to strike a balance between maximizing the useful information included and keeping the format short; priority will be given to genetic and technical information. Brevity is essential. If a more lengthy communication is felt to be of value, the material should be summarized and an address made available for interested individuals to request more information. Submitted material will be edited for brevity and arranged into each issue. Research reports, lengthy items that cannot be effectively summarized, and material that requires illustration for clarity should be sent directly to Jim Thompson (THOMPSON@AARDVARK.UCS.UOKNOR.EDU) for publication in Drosophila Information Service (DIS). Materials appearing in the Newsletter will be reprinted in DIS. Back issues of DIN are available from FlyBase in the directory flybase/news or in News/ when accessing FlyBase with Gopher. Material appearing in the Newsletter may be cited unless specifically noted otherwise.

Material for publication should be submitted by e-mail. Figures and photographs cannot be accepted at present. Send technical notes to Carl Thummel and all other material to Kathy Matthews. The e-mail format does not allow special characters to be included in the text. Both superscripts and subscripts have been enclosed in square brackets; the difference should be obvious by context. Bold face, italics, underlining, etc. cannot be retained. Please keep this in mind when preparing submissions. To maintain the original format when printing DIN, use Courier 10cpi font on a standard 8.5" x 11" page with 1" margins.

Drosophila Information Newsletter is a trial effort that will only succeed if a broad segment of the community participates. If you have information that would be useful to your colleagues, please take the time to pass it along.

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ANNOUNCEMENTS, VOL. 11-15

**RECENT ADDITIONS TO FLYBASE**

\* A major new FlyBase product has been released to the FlyBase server at Indiana, and is available either by Gopher (in Flybase/References) or by ftp (in flybase/ref directory). It is a unified list of publications concerning Drosophila. Drawn from a variety of sources (described in references-sources.txt) it includes 51552 entries, from 1684 to 1993. All are full references with as complete bibliographic information as is available.

You can search this reference list via Gopher. The reference file is available in a few formats including refer, as readable by many bibliographic programs including Endnote, Pro-Cite and Refer, and comma-separated-values (csv) as used by many spreadsheet and database programs.

It is impossible that this file is either 'complete' or free of errors. Mail [flybase-refs@morgan.harvard.edu](mailto:flybase-refs@morgan.harvard.edu) with additions and corrections.

\* The stock list of the National Drosophila Species Resource Center at Bowling Green is now available on FlyBase.

Three versions of the list are posted: 1) species-center.rtf contains the center's catalogue in rich text format, which retains formatting information from the original word-processing file provided by the stock center. If you have a word processing program that handles rtf you can print a copy of the center's catalogue from this file; 2) species-center.txt is a database/spreadsheet format file; 3) species-center.rpt is a file in report format more easily read by humans than the txt file (when searching the stock list with gopher, information from this report file is returned). See the document species-center.doc for further information about the species stock center and these files. All of these files are in the directory flybase/stocks/stock-centers.

#### BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 11

\* Second chromosome lethal P-inserted stocks from the Drosophila Genome Center will soon be available. Third chromosome inserts should be available sometime this fall. Less well characterized insertion strains or those containing less versatile constructs will be discarded at some point in favor of the new stocks. Individuals who contributed the stocks will be notified personally before stocks are discarded and a list of all stocks to be discarded will be posted on the BIOSCI/Binet Drosophila newsgroup (see DIN Vol. 10) six weeks before stocks are to be discarded. Discard lists will also be posted on FlyBase in the file stock-center-news.doc in the directory flybase/stocks/stock-centers/bloomington. If you would like to receive a copy of stocks on the discard list please order them as soon as possible after the list is made public.

\* HAVE YOU MOVED? If you have a new mailing address please EXPLICITLY state this when you order stocks, or let Kathy know as soon as you move so our files can be updated. Address labels are generated by computer from our master address file and are not checked individually against each order. Flies sent to the wrong address create delays for you and extra work for us so please help us keep our records current.

#### BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 12

\* A new set of ry[+]-marked P insertions from the Drosophila Genome Center are now available from the Bloomington Stock Center. Most of these stocks carry lethal inserts on 3; the rest are male sterile inserts on 2 or 3. All carry the PZ construct. A list of insertion sites has been posted on bionet.drosophila and the relevant stock lists on FlyBase (p-list.txt, p-list.rpt, p-by-location.txt and p-by-location.rpt2) have been updated to include these stocks.

\* There is mounting evidence that stocks carrying both hsFLP and FRTs are unstable. We will soon discard the hsFLP + FRT stocks from the Xu and Rubin set (Development 117:1223). If you aren't among the 5,007 people that have already received these stocks and would like to receive them despite the potential for problems, order them now. FRT-only and hsFLP-only stocks will continue to be maintained at the center.

#### BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 13

\* USE STATISTICS FOR 1993 -- 16,785 stocks were shipped from the Bloomington Stock Center in 1993. This represents a 36% increase compared to 1992, and a 401% increase over the past 5 years. Weekly averages for the last quarter of 1993 were 58 requests for stocks or information, 48 shipments, and 442 stocks shipped. 35% of those shipments went outside the USA: 23% to European Community countries, 3.6% to Canada, 3.6% to Japan, 2.4% to Israel, and 2.9% to an assortment of other countries. Deficiency stocks continue to be the most requested category.

\* USER SURVEY FOR NIH -- At present NSF is the only funding agency providing support for Drosophila stock centers. NIH has expressed an interest in sharing support for the Bloomington center with NSF if we can demonstrate that a large proportion of our users are funded by NIH. We will be distributing user surveys over the next few weeks to gather information about our users' sources of research support. We apologize for one more piece of paperwork/e-mail (but imagine how we feel!), and will very much appreciate your prompt response.

\* SEND US YOUR REFERENCES -- In response to Vice-President Gore's good-government activities, NSF is asking stock centers funded by its program to provide documentation of specific scientific advances that were supported by materials from the center. We have started maintaining a database of publications that made significant use of stocks received from the Bloomington Stock Center. It would be extremely helpful if you would send us references for your relevant papers (now and in the future) with a VERY brief description of the role of center stocks, for example, 'used P insert at 25F to clone gene x'. Send e-mail to MATTHEWK@INDIANA.EDU.

\* HELP US COPE -- As you can see from the statistics cited above, use of the center has increased dramatically over the past five years. It is increasingly important that all of our users use the center responsibly. Please help us maintain our current level of service by complying with the following requests:

1. We are funded as a research resource, not as a teaching resource. Please do not order stocks from us for teaching purposes unless those genotypes are not available elsewhere, and PLEASE!!! do not refer teachers, parents, and science fair advisors to us for help with their student projects.

2. If the same stocks are available from both Bloomington and the Umea Stock Center, workers in European labs should order stocks from Umea. It is quite time-consuming for us to routinely check European requests against the Umea stock list and we will soon stop doing this altogether. Requests from European labs for non-P stocks must note that the requested stocks are not available from Umea or the order will be returned by post unfilled.

3. Use e-mail if you have it (to MATTHEWK@INDIANA.EDU). Sending a FAX is very time-consuming compared to responding to an e-mail message and FAXes usually have to be trimmed or folded to fit into our file folders.

4. When you place your first order from a new address explicitly state that the address provided is a new one. Mailing labels are automatically filled in from our 'address' database (1,760 names and addresses) and your stocks may go to your old address if you count on us to notice that you have moved. We often catch these, but it creates extra work to correct them after your request has been entered into the database. Also, it is helpful if you always use the same form of your name when ordering. It would be very helpful if some of you Johnsons and Martins would change your last names (just kidding on that one).

5. Order efficiently. Whenever possible, order by stock number; check your request for typos in the stock numbers before sending it. If you do not have access to our stock list, include the gene symbol in your request (e.g., fzy instead of or in addition to fizzy). Learn to use FlyBase so you always have access to current stock lists (read stocks.doc before trying to search the stock lists). Don't order Bowling Green stocks from Bloomington, and vice versa. Don't order the same stocks from multiple stock centers 'just to be sure'. If you find yourself often ordering one or two stocks a week for several weeks in a row, consider delaying your next order for a week or two so you can order everything you will need for a while at once. For small orders, the processing and packaging time vastly outweighs the stock set up time, and up to 12 stocks can be shipped for the same postage as one. It is very helpful when workers in the same lab pool their orders. We try to identify multiple orders from the same lab and ship them together, but this takes extra time, and we aren't always aware of your lab affiliation.

6. Check the redbook or FlyBase for basic information about a gene or an aberration before calling the stock center for such information.

\* NO, IT WASN'T YOUR IMAGINATION -- Try to forgive us if you suffered from Kathy's even-crankier-than-usual disposition this fall. We were seriously oversubscribed and biting a head off now and again just felt too good to resist. Our five year renewal application is in, we are once again fully staffed, and spring isn't all that far off, so you should be safe for a while.

#### BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 14

\* NEW DEADLINE -- The weekly deadline for stock requests is now 11 AM on Thursday. Large orders and requests that don't include our stock numbers must be received on Wednesday to assure inclusion in the current week's order.

\* WRONG BREAKPOINTS -- The breakpoints shown in our stock list for Df(3R)p-XT103 #1962 were not correct. The reported breakpoints for this deficiency are 85A2;85C1-2 (we had the breakpoints for p-XT9 instead of p-XT103). Thanks to Hilary Ellis for catching and reporting this error. There are without doubt others. Typographical and transcription errors in the stock lists will be identified and corrected when stocks are added to the developing relational version of FlyBase. In the meantime, always check the breakpoints in our stock list against the new redbook or the aberrations file in FlyBase. We recommend that you check the cytology yourself before investing significant effort in any aberration stock.

\* USER SURVEY FOR NIH -- Thanks to everyone who responded to the user survey. At last count the response rate was 64%. 60% of respondents had research grants from NIH in 1993, another 8% had students or post-docs supported by NIH training grants, and 4% of the survey population were NIH employees. Among the 200 NIH research grants held by respondents in 1993, 61% were from NIGMS, 12% from NICHD, 12% from NINDS, and 10% from NEI. However, on average, groups with NEI grants were heavier users of the center than groups with NIGMS grants - 34% of stocks shipped to survey respondents went to groups with NEI funding, the same proportion that went to NIGMS-funded groups.

\* hsFLP+FRT COMBINATION STOCKS ARE UNSTABLE -- FRT sites are damaged over time when maintained in stock with hsFLP, even when stocks are kept at low temperature. It is very likely that you will get poor clone production if you use FRTs that have been kept with hsFLP for extended periods. We no longer maintain these

combination stocks. Everyone who received hsFLP+FRT stocks from the center before we discarded them was notified of the potential for problems. However, it appears that this information has not always been relayed to all potential users in each lab. If you continue to maintain these stocks in your lab we strongly recommend that you discard them. Three stocks are available from the center that can be used as a source of hsFLP: #6 y w[1118] hsFLP1; Adv/CyO, #7 y w[1118] hsFLP1; Dr[Mio]/TM3, and #279 w[1118]; MKRS, hsFLP3/TM6B. Make the necessary hsFLP+FRT combination genotype only when you are ready to generate clones.

#### BLOOMINGTON STOCK CENTER NEWS -- from DIN Volume 15

\* myc-tagged FRT stocks: Some people are having problems getting good staining of myc-tagged clones with the FRT system of Xu and Rubin (Development 117:1223). According to Gerry Rubin, the quality of the individual batch of antibody is a critical factor. He says that the Artavanis-Tsakonas lab, and others, have generated batches of antibody that give reliably good results by growing subclones of the monoclonal line (Myc 1-9E10.2) and selecting one that provides good staining.

\* Voice-mail is currently unavailable at 812-855-5783. Please place your stock requests by e-mail to MATTHEWK@INDIANA.EDU, by FAX to 812-855-2577, or phone 812-855-5782.

#### MID-AMERICA STOCK CENTER NEWS (BOWLING GREEN)

182 new stocks have been added to the collection. The update of our stock list that we provided to FlyBase this spring includes these new stocks. For further information, contact Ron Woodruff or Phyllis Oster, Dept. of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403. 419-372-2631, demlano@opie.bgsu.edu.

#### FLYBASE

A new release of FlyBase will appear on the server sometime in January. It will be announced on bionet.drosophila. If you don't have local access to the bionet.drosophila discussion group you can subscribe directly by sending an e-mail message to BIOSCI@NET.BIO.NET asking to subscribe to bionet.drosophila. A person will read your message, so it need not be in any specific format. You may post a message to the group by sending your message to DROS@NET.BIO.NET.

#### DROSOPHILA RESEARCH CONFERENCE

The 1995 US conference will be held April 5-9 in Atlanta, Georgia.

#### THE DEVELOPMENT OF DROSOPHILA MELANOGASTER

Edited by Michael Bate (University of Cambridge) and Alfonso Martinez Arias (University of Cambridge).

A monograph in two volumes, this reference work represents the most comprehensive synthesis of Drosophila developmental biology currently available. The text is complemented with a full-color Atlas for bench use, which graphically illustrates the day-by-day development of the Drosophila embryo.

##### Contents:

Developmental Genetics of Oogenesis (A. Spradling)

Spermatogenesis (M. Fuller)

Mitosis and Morphogenesis in the Drosophila Embryo (V. Foe, G. Odell, B. Edgar)

Maternal Control of Anterior Development in the Drosophila Embryo (W. Driever)

Pole Plasm and the Posterior Group Genes (D. St. Johnson)

The Terminal System of Axis Determination in the Drosophila Embryo (F. Sprenger, C. Nusslein-Volhard)

Maternal Control of Dorsal-Ventral Polarity and Pattern in the Embryo (R. Chasan, K. Anderson)

Gastrulation in Drosophila: Cellular Mechanisms of Morphogenetic Movements (M. Costa, D. Sweeton, E. Wieschaus)

Blastoderm Segmentation (M. Pankratz, H. Jackle)

Development and Patterning of the Larval Epidermis of Drosophila (A. Martinez Arias)

Development of the Drosophila Tracheal System (G. Manning, M. Krasnow)

The Terminal Regions of the Body Pattern (G. Jurgens, V. Hartenstein)

Imaginal Disc Development (S. Cohen)

The Metamorphic Development of the Adult Epidermis (D. Fristrom, J. Fristrom)  
 Hormones and Drosophila Development (L. Riddiford)  
 The Alimentary Canal (H. Skaer)  
 The Mesoderm and Its Derivatives (M. Bate)  
 Early Neurogenesis in *Drosophila melanogaster* (J. Campos-Ortega)  
 Embryonic Development of the *Drosophila* Central Nervous System (C. Goodman, C. Doe)  
 The Peripheral Nervous System (Y.N. Jan, L.Y. Jan)  
 Formation of the Adult Nervous System (J. Truman, B. Taylor, T. Awad)  
 Pattern Formation in the *Drosophila* Retina (T. Wolff, D. Ready)  
 Genetic Dissection of Eye Development in *Drosophila* (B. Dickson, E. Hafen)  
 The Development of the Optic Lobe (I. Meinertzhagen, T. Hanson) Epilogue (M. Ashburner)

Atlas of *Drosophila* Development (V. Hartenstein)  
 Poster: *Drosophila* Third Instar Eye Disc (T. Wolff)

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#### NIAAA PROGRAM ANNOUNCEMENT

The following is an excerpt from a National Institute on Alcohol Abuse and Alcoholism Program Announcement of particular relevance to Drosophilists. For the complete announcement see the Program Announcement, Genetic Studies in Alcohol Research (PA 93-086). Copies of this and other Program Announcements can be obtained from the National Clearinghouse on Alcohol and Drug Information (NCADI), P.O. Box 2345, Rockville, MD 20852, telephone: 1-800-729-6686.

Information on research grants can be obtained from: Robert W. Karp, Ph.D., Director, Genetics Program, Division of Basic Research, National Institute on Alcohol Abuse and Alcoholism, 5600 Fishers Lane, Room 16C-05, Rockville, MD 20857. E-mail: RKARP@AOAA1.SSW.DHHS.GOV

#### ALCOHOL-RELATED GENETIC STUDIES IN INVERTEBRATES

Because of their small size, short generation time, and high fecundity, the fruit fly *Drosophila melanogaster* and the soil nematode *Caenorhabditis elegans* lend themselves to large-scale systematic searches of tens to hundreds of thousands of individuals to find single-gene mutations conferring a specific phenotype of interest. For both of these invertebrate species sophisticated genetic and molecular methods are available which facilitate the cloning of genes based either on the phenotypes they confer, or on their known map locations (Ashburner, 1989; Herman and Shaw, 1987; Mello, et al., 1991; Coulson, et al., 1991). The combined power of these methods has led to important contributions to our understanding of development and functioning of the nervous systems of these species. Many of their genes critical for neurotransmission and central nervous system development (e.g., those encoding neurotransmitter biosynthetic enzymes and receptors, protein kinases, adenyl cyclases, G proteins, ion channel subunits, cell adhesion proteins, transcription factors) have homologues which function critically in the vertebrate central nervous system as well (Molecular Neurobiology of *Drosophila*: Cold Spring Harbor Laboratory meeting abstracts, 1991; Chalfie and White, 1988). In both of these species, single-gene mutants have been described which alter sensitivity to volatile anaesthetics (Krishnan and Nash, 1990; Sedensky and Morgan, 1991). Cloning of the mutated genes from these mutants will serve to identify gene products which participate in the physiology of anaesthetic sensitivity. The cloned genes can also be used to isolate mammalian (including human) homologues which will be invaluable for studying the mechanisms of action of anaesthetics in these higher species. This approach may well reveal targets for the action of anaesthetics not yet disclosed by direct genetic or biochemical studies on mammals. Although an approach based on systematic mutant searches of mammals (e.g., mice) would certainly be desirable, the impracticability of rearing a sufficiently large number of individuals renders studies in invertebrates more expedient. The example of volatile anaesthetics demonstrates how an approach based on invertebrate genetic studies provides an otherwise inaccessible entree to the elucidation of the

mechanism of action in vertebrates of a drug whose molecular targets have not yet been definitively identified.

It would be of great interest to characterize in detail the behavioral and developmental responses of *Drosophila* and *Caenorhabditis* to ethanol. If such responses as attraction to, consumption of, sensitivity to, tolerance to, and withdrawal from ethanol, as well as ethanol-induced developmental defects can be demonstrated, then systematic searches for single-gene mutations affecting these responses can greatly facilitate the elucidation of the entire chain of physiological events mediating these responses. Cloning of the mutant invertebrate genes discovered by these searches could then lead to cloning of homologous mammalian genes with important functions in responses to ethanol. It is difficult to predict in advance which (if any) invertebrate ethanol-related behaviors will prove relevant to human alcoholism. An objective test for true homology (based on shared underlying genetic or physiological mechanisms), as opposed to analogy (superficial behavioral similarity), is therefore essential for guiding this line of research. Such a test can be accomplished post hoc by testing human homologues of the invertebrate genes for linkage to alcoholism in human pedigrees.

**Areas needing further research include:**

Characterization of behavioral and developmental responses of *Drosophila* and *Caenorhabditis* to ethanol. Behavioral responses can include attraction to, consumption of, sedation by, motor impairment by, tolerance to, and withdrawal from ethanol.

Systematic searches (using either mutagens or wild populations) for mutants altered in the responses mentioned above. Mapping and cloning of the genes altered in mutants discovered in these screens.

Characterization of the products of the cloned genes.

Cloning of mammalian (including human) homologues of the cloned invertebrate genes.

Testing for linkage of the human homologues to alcoholism in human pedigrees.

**References:**

- Ashburner MA: *Drosophila: A Laboratory Handbook*. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1989
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- Herman RK, Shaw JE: The transposable genetic element Tc1 in the nematode *C. elegans*. *Trends Genet* 3:222-225, 1987
- Krishnan KS, Nash HA: A genetic study of the anesthetic response: Mutants of *Drosophila melanogaster* altered in sensitivity to halothane. *Proc Nat Acad Sci USA* 87:8632-8636, 1990
- Mello CC, Kramer JM, Stinchcomb D, Ambros V: Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J* 10:3959-3970, 1991
- Molecular Neurobiology of *Drosophila*: Cold Spring Harbor Laboratory meeting abstracts, Sept 25-29, 1991
- Sedensky MM, Morgan PG: Genetics of response to volatile anesthetics in *Caenorhabditis elegans*. *Ann NY Acad Sci* 625: 524-531, 1991
- Chalfie M, White J: The nervous system, in Wood WB (ed): *The Nematode Caenorhabditis elegans*. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1988, pp 337-395

**REQUESTS FOR MATERIALS, VOL. 11-15**

**INSERTS OR LETHALS IN 17AB**

Lee Fradkin, Nusse Lab, HHMI, CMGM B269, Stanford Medical Center, Stanford, CA 94305.  
FRADKIN@CMGM.STANFORD.EDU.

We would appreciate strains or information about strains with enhancer traps or lethals that map to the 17AB region. Thanks.

**DEFECIENCY**

Rob Jackson, Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545.  
JACKSON@SCI.WFEB.EDU.

I'm looking for Df(3L)Mg27 produced by Mglinetz. I would appreciate hearing from anyone who has this deficiency or knows of its whereabouts.

#### MALE STERILES

Peter Clyne, Dept. of Biology, Yale U., PO Box 6666, New Haven CT 06511, USA. 203-432-3542, Fax/5631, DRONGO@VENUS.CIS.YALE.EDU

I am seeking ms(2)E8 and ms(2)E9 flies which were first isolated by Edmonson in 1951. Both flies and information about whom to contact directly for the flies would be deeply appreciated. Thank you.

#### MATERIAL FOR FLY THESAURUS

Joanne Martinez, U. of Arizona School of Library Science/Dept. Management Information Systems. JPMARTIN@CCIT.ARIZONA.EDU

Our group is developing an automatic thesaurus for the Drosophila research community. We are gathering "object filters" (gene names, protein function names, researcher names, technique names, subjects and other keywords), as well as full text electronic documents for cluster analysis (semantic relationships). The flybase and online Redbook have been very helpful for the former, but we need more electronic full text documents for determining relationships between words.

I ask your assistance in providing us with a wide range of full text documents in \*electronic\* format, particularly abstracts and review-type articles. This is important, so that the terms in our thesaurus are as rich as possible, i.e. that the thesaurus covers as much of the terminology used in Drosophila research as possible.

The documents will \*not\* be used for their intellectual content, so there is no problem with copyright. We are only interested in the terms and their relationships to one another. The documents will be erased once we have extracted the objects and conducted the cluster analysis. Please send whatever files you can to: jpmartin@ccit.arizona.edu

Thank you very much.

#### BACKISSUES OF DIS

Kathy Matthews, Dept. of Biology, Indiana U., Bloomington, IN 47401. 812-855-5782, FAX/2577, MATTHEWK@INDIANA.EDU

The Bloomington Stock Center would like to obtain a complete set of Drosophila Information Service. We have all volumes from number 34 to the present. If you have any of the earlier issues that you are ready to part with we would be very happy to give them a good home.

#### OVARIAN cDNA LIBRARIES

Kathleen A. Fitzpatrick, IMBB, Simon Fraser U., Burnaby, B.C., Canada V5A 1S6. (604) 291-5931, KATHLEEF@SFU.CA

We need a good ovarian cDNA library as well as alleles of known tyrosine kinase loci, such as sevenless, hopscotch, breathless, and any others for which there is a scorable phenotype. We have some genes that interact with torpedo and want to determine whether they will interact with other tyrosine kinase genes or only with top. Thanks for any help you can give us.

#### MUTATIONS AT 68EF

Helen Benes, Dept. of Biochemistry/Molecular Biology, Slot 516, U. of Arkansas for Medical Sciences, 4301 W. Markham St., Little Rock, AR 72205. FAX 501-686-5782, HXBENES@LIFE.UAMS.EDU

We would appreciate any information on *D. melanogaster* stocks with enhancer trap insertions or lethals that map to, or reasonably near to, the 68E/F region. The lethals could be isolates of screens following mutagenesis by EMS, DEB, X-ray, P elements, etc. Thank you.

#### WILD-CAUGHT bb MUTANTS

Leonard G. Robbins, Genetics Program, S308 Plant Biology, Michigan State U., E. Lansing, MI 48824-1312.

517-355-0337, FAX/353-1926, 21675MGR@MSU.EDU or 21675MGR@MSU.BITNET.

For an attempt to find other instances of Rex, I would appreciate cultures of any wild-caught or spontaneous melanogaster bb mutants.

Dr. Robert E. Nelson, UCLA, Molecular Biology Institute, Room 459, Los Angeles, CA 90024, Tel: 310-825-5267, e-mail: nelson@ewald.mbi.ucla.edu.

Looking for stocks with transposable elements, mutations, and/or deletions that map within or around 62F. Thanks, in advance, Bob Nelson.

#### INSECTAVOX

Scott P. McRobert, Dept. of Biology, St. Joseph's University, Philadelphia, PA 19131. 215-660-1833, smcrober@sju.edu.

Does anyone still build INSECTAVOXes for fun and profit? I have the box, but I need someone who can install the "guts".

#### MATERIALS AVAILABLE, VOL. 11-15

#### CHROMOSOME III HOMOZYGOUS BY DESCENT LINES

Ananias Escalante and Francisco Ayala, Dept. Ecology and Evolutionary Biology, U. of California, Irvine, CA 92717. FAYALA@ORION.OAC.UCI.EDU, AESCALAN@DARWIN.BIO.UCI.EDU

We are currently developing around 400 lines of *D. melanogaster* homozygous by decent at the chromosome III using the lethal balanced TM3 strain. These flies were collected in northern California as part of a project directed to study polymorphism in natural populations. The lines were started with males collected directly from the field. If any person is interested in these lines please contact us. These lines will be discarded after we finish our project.

#### MONOClonAL ANTIBODY AGAINST EMBRYONIC CHORDOTONAL ORGANS

Beate Lichte, Thilo Schneider, and K.-F. Fischbach, Inst. fuer Biologie III, Schaezlestr. 1, D-79194 Freiburg, Germany. 0761-203-2730, FAX/2745, LICHTE@SUN1.RUF.UNI-FREIBURG.DE or KFF@SUN1.RUF.UNI-FREIBURG.DE

We recently produced (as a "by-product" of our current research) a mouse monoclonal antibody which recognizes exclusively all chordotonal organs of the *Drosophila* embryo in histochemical staining experiments. The staining is very strong without any background. So, if anyone is interested in using this antibody, e.g. as a marker, please contact us.

#### COMPILATION OF DROSOPHILA cDNA AND GENOMIC LIBRARIES

Carl Thummel, Howard Hughes Medical Institute, 5200 Eccles Institute of Human Genetics, Bldg. 533, Univ. of Utah, Salt Lake City, UT 84112 U.S.A. 801-581-2937, FAX/5374, CTHUMMEL@HMBGMAIL.MED.UTAH.EDU.

The following is an update of the listing of *Drosophila* cDNA and genomic libraries that are currently available and in common use. Please do not request shipment of a library unless you have an immediate use for it - many contributors are concerned about the time and money involved in mailing their libraries. Also, please inquire with local colleagues before requesting a library since many of these libraries are already widely distributed.

#### cDNA LIBRARIES

--Nick Brown, Wellcome/CRC Institute, Tennis Court Rd, Cambridge CB2 1QR United Kingdom Phone: 44-223-334128 FAX: 44-223-334089 Email: NB117@MB1.BIO.CAM.AC.UK

Vector/Insertion/Complexity/mRNA source

pNB40/see ref./3x10[5]/0-4 hr embryo

pNB40/see ref./3x10[6]/4-8 hr embryo

pNB40/see ref. 3x10[5]/8-12 hr embryo

pNB40/see ref./1x10[6]/12-24 hr embryo

pNB40/see ref./3x10[6]/imaginal discs

The Drosophila strain used is an isogenic second chromosome stock: dp cn bw, from the Gelbart lab. Ron Blackman has made a genomic library from this same strain (see below). The vector is a pUC based plasmid with a SP6 promoter at the 5' end of the cDNA and a T7 promoter at the 3' end of the cDNA. The cloning strategy was directional and designed to maximize the number of full-length cDNAs. A useful diagnostic of full-length cDNAs is a non-coding G nucleotide at the 5' end, after the polyC tract; the origin of this nucleotide is, however, unknown.

Reference: Brown, N.H., and F.C. Kafatos (1988) Functional cDNA libraries from Drosophila embryos. *J. Mol. Biol.* 203: 425-437.

--Steve Russell, Dept. of Genetics, University of Cambridge, Downing Street, Cambridge, CB2 3EH United Kingdom  
Phone: 44-223-337733 FAX: 44-223-333992 Email: sr120@mbfs.bio.cam.ac.uk

All libraries were made with RNA isolated from Oregon R strain

Vector/Insertion/Complexity/mRNA source

NM1149/RI/2x10[6]/Male 3rd instar larvae

NM1149/RI/6x10[5]/Female 3rd instar larvae

NM1149/Directional: RI-HIII/3x10[6]/Adult male heads

NM1149/Directional: RI-HIII/1x10[6]/Adult female heads

lambda gt11/RI/3x10[5]/Testes

--Charles P. Emerson, Jr. or Mary Beth Davis, Biology Dept., University of Virginia, Charlottesville, Virginia, 22901,  
USA Phone: 215-728-5283 (Emerson); 215-728-5284 (Davis) FAX: 215-728-2412 Email: emerson@castor.rm.fccc.edu  
or davis@castor.rm.fccc.edu

Vector/Insertion/Complexity/mRNA source/Titer

lambda gt10/RI/1x10[6]/late pupae/1x10[10]

Blunt-ended cDNA was ligated to EcoRI adaptors, then ligated to EcoRI digested gt10 lambda arms. We have isolated cDNA clones corresponding to MHC isoforms that were lengths of 5940 and 5500 bases.

Reference: George, E.L., M.B. Ober, and C.P. Emerson, Jr. (1989) Functional domains of the *Drosophila melanogaster* muscle myosin heavy-chain isoform are encoded by alternatively spliced exons. *Mol. Cell Biol.* 9: 2957-2974.

--Bruce Hamilton, Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142, USA Phone: 617-258-5174 FAX:  
617-258-6505 Email: hamilton@genome.wi.mit.edu

Library name/Vector/Insertion/Complexity/mRNA source

Head M/lambda EXLX/ApaI-SacI/1.1x10[7]/Oregon R adult heads

Head P/lambda EXLX/ApaI-SacI/9x10[6]/Oregon R adult heads

Head 1.2/lambda EXLX/ApaI-SacI/2.7x10[6]/Oregon R adult heads

Head 2.0/lambda EXLX/ApaI-SacI/1.2x10[6]/Oregon R adult heads

Adult/lambda EXLX/ApaI-SacI/>1x10[6]/Oregon R adults

0-24 mojo/lambda EXLX/ApaI-SacI/3.4x10[6]/Can S, 0-24 hr embryos

All libraries were cloned directionally into the ApaI-SacI sites of lambda EXLX, as described in ref. 1, with internal restriction sites protected. Lambda EXLX allows *in vivo* excision of plasmid DNA using a CRE/loxP site-specific recombination system. This vector also allows regulated expression of the insert DNA as a phage T7 gene 10 N-terminal/cDNA fusion protein, under the control of a T7 RNA polymerase promoter (1). The Head 1.2 library was prepared from cDNAs that were size-selected for molecules 1.2 kb or larger by fractionation through an agarose gel. Head 2.0 contains cDNAs that are 2 kb or larger. The cDNA for the Adult library was not size-fractionated.

The Adult and mojo libraries were published in ref. 1. The Head M and Head P libraries are unpublished, but I have asked people who use them to refer to ref. 1, since they were constructed in the same way and in the same vector. The

two size-selected libraries, Head 1.2 and Head 2.0 were published in ref. 2, which also describes a rapid screening procedure that is very straightforward.

#### References:

1. Palazzolo et al (1990) Gene 88, 25-36.
2. Hamilton et al (1991) Nucl. Acids Res. 19, 1951-1952

--Tom Kornberg, Department of Biochemistry, University of California, San Francisco, CA 94143 USA Phone: 415-476-8821 FAX: 415-476-3892 Email: tomk@ucsf.cgl.edu

Our cDNA libraries were prepared from RNA isolated from Oregon R animals, with the cDNA sequences inserted into the EcoRI site of lambda gt10. Libraries will be shipped by Federal Express. Requests should be accompanied by an appropriate Federal Express Authorization Number.

#### Stage/Library designation/Complexity

0-3 hr embryo/D/300,000  
3-12 hr embryo/E/500,000  
12-24 hr embryo/F/300,000  
1st and 2nd instar/G/200,000  
early 3rd instar/H/300,000  
late 3rd instar/I/300,000  
early pupal/P/300,000  
late pupal /Q/300,000  
adult male/R/300,000  
adult female/S/300,000

Reference: Poole, S., Kauvar, L.M., Drees, B., and Kornberg, T. (1985) The engrailed locus of Drosophila: Structural analysis of an embryonic transcript. Cell 40: 37-43.

--John Tamkun, Department of Biology, University of California, Santa Cruz, CA 95064, USA Phone: 408-459-3179 FAX: 408-459-3139

Vector/Insertion/Complexity/mRNA source  
lambda gt11/EcoRI/>6x10[5]/iso-1, 0-24 hr embryos

The iso-1 strain, constructed by Jim Kennison, is isogenic for all four chromosomes. Genomic libraries from this strain are also available.

Reference: Tamkun et al. (1986), Cell 46: 271-282.

--Pat Hurban and Carl S. Thummel, Dept. of Human Genetics, 5200 Eccles Institute, Bldg. 533, University of Utah, Salt Lake City, Utah, 84112 USA Phone: 801-581-2937 FAX: 801-581-5374 Email: chummel@hmbgmail.med.utah.edu

Vector/Insertion/Complexity/mRNA source  
lambda ZAPII/RI-XhoI/2x10[7]/larval tissues cultured in vitro with cycloheximide + ecdysone  
lambda ZAPII/RI-XhoI/3x10[6]/0-24 hr embryos  
lambda ZAPII/RI/3x10[5]/0-24 hr embryos  
lambda ZAPII/RI-XhoI/2x10[6]/mid-late third instar larvae  
lambda ZAPII/RI/2x10[6]/mid-late third instar larvae  
lambda ZAPII/RI-XhoI/2x10[5]/0-15 hr pupae  
lambda ZAPII/RI/2x10[6]/0-15 hr pupae

All RNA was isolated from Canton S animals. Two cDNA libraries were constructed from each of three stages: embryonic, late larval, and early pupal.

One set of libraries was primed from the 3' end using an XhoI-oligo dT primer adapter. The cDNAs were directionally inserted between the RI-XhoI sites of lambda ZAPII, such that XhoI is at the 3' end of the insert and RI is at the 5' end. Most of these cDNAs should contain 3' end sequences. The other libraries were synthesized using random primers and the cDNAs were inserted into the RI site of lambda ZAPII. These libraries should have a better representation of 5' ends. Although the synthesis of these libraries went smoothly, none have yet been tested. We would thus like feedback on the results of any screens. The titers are all approximately 10[10] pfu/ml. Please send a Federal Express number to facilitate shipment.

--Peter Tolias, Public Health Research Institute, 455 First Ave., New York, New York, 10016 USA Phone: 212-578-0815 FAX: 212-578-0804 Email: tolias@wombat.phri.nyu.edu

Vector/Insertion/Complexity/mRNA source  
lambda gt22A/SalI-NotI/5x10[5]/Canton S ovaries, stages 1-14

This is a cDNA expression library in which the inserts are directionally cloned. A SalI site is present at the 5' end and a NotI site is at the 3' end.

--Kai Zinn, Division of Biology, 216-76, Caltech, Pasadena, CA 91125, USA Phone: 818-356-8352 FAX: 818-449-0679 Email: kai@seqvax.caltech.edu

Vector/Insertion/Complexity/mRNA source  
lambda gt11/EcoRI/1.2x10[6]/Oregon R, 9-12 hr embryos

The complexity is an underestimate for larger cDNAs, since it was >5X size-selected for cDNAs larger than 1.8 kb. The complexity could thus be as high as 6x10[6] for these larger inserts.

## GENOMIC LIBRARIES

--Winifred W. Doane, Department of Zoology, Arizona State University, Tempe, Arizona 85287-1501 USA Phone: 602-965-3571 FAX: 602-965-2012 Email: icwwd@asuacad

Vector/Insertion/Complexity/DNA source  
pWE15/BamHI/4x10[4]-1x10[6]/Amy[1,6] mapP[12] strain of *D. melanogaster*

This cosmid vector contains a T3 and T7 promoter on either side of the insertion site, to facilitate the preparation of end-specific probes for chromosomal walking.

Reference: Thompson, D.B., and Doane, W.W. (1989) A composite restriction map of the region surrounding the Amylase locus in *Drosophila melanogaster*. Isozyme Bull. 22: 61-62.

--Ron Blackman, Department of Cell and Structural Biology, 505 S. Goodwin Ave., Univ. of Illinois, Urbana, Illinois 61801 USA Phone: 217-333-4459 FAX: 217-244-1648 Email: Ron\_Blackman@qms1.life.uiuc.edu

Vector/Insertion/Complexity/DNA source  
lambda EMBL3/BamHI/1x10[6]/Adult *Drosophila virilis*  
lambda EMBL3/BamHI/1x10[6]/Embryonic *D. melanogaster*, see below

Both libraries were prepared by MboI partial digestion of the DNA and insertion into the BamHI site of lambda EMBL3. The inserts can be excised by digestion with SalI. Titer is approximately 5x10[9] pfu/ml. The *D. melanogaster* genomic library is made from animals that are isochromosomal for chromosome 2, dp cn bw. The same strain was used by Nick Brown for his cDNA libraries.

--Howard Lipshitz, Division of Biology, 156-29, California Institute of Technology, Pasadena, CA 91125, USA Phone: 818-356-6446 FAX: 818-564-8709 Email: lipshitzh@starbase1.caltech.edu

Vector/Insertion/Complexity/DNA source  
Charon 4/EcoRI/6x10[5]/Canton S embryos

This is the original Drosophila genomic library from the Maniatis lab. It has been amplified several times but is still useful for most purposes.

Reference: Maniatis et al., The isolation of structural genes from libraries of eucaryotic DNA. Cell 15: 687-701.

--Richard W. Padgett, Waksman Institute, Rutgers University, Piscataway, NJ 08855, USA Phone: 908-932-0251 FAX: 908-932-5735 Email: padgett@mbcl.rutgers.edu

Vector/Insertion/Complexity/DNA source/Titer  
lambda DASH II/Sau 3A/5x10[5]/dp cn cl bw/1x10[8]  
lambda DASH II/Sau 3A/5x10[5]/st e/1x10[8]

Libraries were constructed from adult DNA from dp cn cl bw and st e strains. The dp cn cl bw strain is the same one used by N. Brown in constructing his cDNA libraries. The st e strain is the same one used by Wieschaus, Nusslein-Volhard and co-workers in their screens for pattern mutants. Libraries will be sent if the requester provides a Federal Express number.

Reference: Finelli, A.L., C. A. Bossie, T. Xie and R. W. Padgett (1994). Antimorphic Alleles of the Drosophila tolloid Gene Contain Amino Acid Substitutions in the Protease Domain, Development, in press.

--John Tamkun, Department of Biology, University of California, Santa Cruz, CA 95064, USA Phone: 408-459-3179 FAX: 408-459-3139

Vector/Insertion/Complexity/DNA source  
lambda EMBL3/BamHI/>5x10[5]/iso-1; see ref.  
lambda EMBL3/BamHI/>5x10[5]/D. virilis  
NotBamNot-CoSpeR/BamHI/high/iso-1

The iso-1 strain, constructed by Jim Kennison, is isogenic for all four chromosomes. Two genomic libraries of iso-1 DNA are available, one in a lambda and one in a cosmid vector. Both are in wide use and have been used successfully by many labs. A cDNA library from iso-1 0-24 hr embryos is also available.

Reference: Tamkun et al. (1984), PNAS 81: 5140-5144.

#### TECHNICAL NOTES, VOL. 11-15

##### DOUBLE-SIDED STICKY TAPE FOR EMBRYO INJECTIONS

Mary Whiteley and Judith A. Kassis, Food and Drug Administration, 8800 Rockville Pike, Bethesda, Maryland, 20892. 301-496-9309, FAX /4684, KASSIS@HELIX.NIH.GOV

One of the most common problems associated with microinjection of Drosophila embryos is the toxicity of different batches of double-sided sticky tape. Recently, we have found that batches of 3M double-sided tape purchased at our local grocery stores is often toxic to embryos. This is evident the morning after microinjection with the embryos being noticeably caved in and hollow. We called the 3M company to see if they manufactured alternate kinds of double-sided tape, and the reply was yes .... some 200 kinds. We then spoke with someone in the 3M testing laboratory about which of these 200 kinds of tape was likely to be the least toxic. She sent us a sample of industrial double-sided stick tape that had been rigorously tested for toxicity (to what we are not sure). This tape is an acetate based tape (type 415, 1/4"). We obtained a trial sample of the tape and, in our hands, and others on the NIH campus, this tape is virtually non-toxic to Drosophila embryos - we now routinely obtain about five-fold more hatching larvae than before. We have not rigorously tested this tape versus the tape at the grocery stores, nor have we tried different batches of the tape, but we think that it is important to inform Drosophilists of our success so that others may hopefully benefit. Unfortunately,

3M does not supply this tape directly, so you must first call 3M to locate the distributor in your area (612-733-1110; ask for product information). Some distributors are reluctant to sell you less than 1 case (144 rolls). Although I have contacted some that will split up a case, the cost of a 36 yard roll is about \$8.00 versus \$3.00 a roll bought by the case.

#### AVAILABILITY OF MATERIALS FROM THE DROSOPHILA GENOME CENTER

Gerald M. Rubin, Dept. of Molecular and Cellular Biology, Life Science Annex Bldg., Box 539, U. of California, Berkeley, CA 94720. 510-643-9945, FAX /9947, FLYGENOME@MAILLINK.BERKELEY.EDU

The following is an excerpt from a text document now available on FlyBase, P1.doc - further information, as well as the list of P1 clones, are available on FlyBase.

The overall goal of the Center, that was funded by the NIH for three years starting August 1, 1992, is to build an integrated physical, genetic, and cytogenetic map of the Drosophila genome based on STS content mapping. We hope that the data we generate as the map is gradually assembled will be useful and widely available to Drosophila workers. In return, we ask for your help in bringing any errors, inconsistencies or independent confirmations of the data contained in these tables to our attention. Such feedback will improve the quality of the final map and speed its completion. Correspondence can be sent by email to flygenome@maillink.berkeley.edu or by FAX to 510-643-9947; alternatively correspondence can be directed to specific members of the Center as outlined below.

The generation of the data described in this database is supported by a Drosophila Genome Center Grant from the NIH (NIH grant HG00750, Principal Investigator Gerald M. Rubin; Co- Investigators: Daniel Hartl, Christopher Martin, Michael Palazzolo, and Allan Spradling), by the Howard Hughes Medical Institute through its support of the Rubin and Spradling laboratories and by the DOE through its support of the LBL Human Genome Center, the home of the Palazzolo and Martin laboratories. Please acknowledge the Drosophila Genome Center in publications using this information; additional acknowledgments that apply to specific subsets of the data are detailed below.

In addition to these data tables two types of materials are being made available to the Drosophila community: Clones of *D. melanogaster* DNA in the P1 vector and fly stocks carrying mapped single P element insertions that inactivate a vital gene. All clones, fly stocks and other information may be used for research purposes without restriction.

The average insert size in the P1 library is about 80kb. The following 16 laboratories have volunteered to store the 9,216 arrayed clones that comprise the basic P1 library (approximately 5-hit) and to make them available to other laboratories in their geographical areas. They are not being financially compensated for their effort and we all owe them our appreciation.

Sean Carroll, University of Wisconsin-Madison, Howard Hughes Medical Institute, Laboratory of Molecular Biology, 1525 Linden Drive, Madison, WI 53706, Tel: 608-262-3203, Fax: 608-262-4570

Allan Spradling, Carnegie Institute Washington, Howard Hughes Medical Institute, Department of Embryology, 115 West University Parkway, Baltimore, MD 21210, Tel: 410-554-1221, Fax: 410-243-6311

Hugo Bellen, Baylor College of Medicine, Howard Hughes Medical Institute, One Baylor Plaza, Room T634, Houston, TX 77030, Tel: 713-798-5272, Fax: 713-797-6718

Steven Henikoff, Fred Hutchinson Cancer Research Center, Howard Hughes Medical Institute, Department of Genetics, Room A1-111, 1100 Fairview Avenue North, Seattle, WA 98109, Tel: 206-667-4514, Fax: 206-667-5889

Thomas C. Kaufman, Indiana University, Howard Hughes Medical Institute, Department of Biology, Jordan Hall, Room A-507, Third Street and Faculty, Bloomington, IN 47405, Tel: 812-855-3033, Fax: 812-855-2577

S. Larry Zipursky, University of California at Los Angeles, HHMI/5-748 MacDonald Bldg., 10833 Le Conte Avenue, Los Angeles, CA 90024-1662, Tel: 310-825-2834, Fax: 310-206-3800

Carl Thummel, University of Utah, Howard Hughes Medical Institute, Department of Human Genetics, Eccles Institute of Human Genetics Bldg. 533, Room 2100, Salt Lake City, UT 84112, Tel: 801-581-2612, Fax: 801-581-5374

Tsuneyuki Yamazaki, Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan, Tel: (81)-92-641-1101, Fax: (81)-92-632-2741

Michael Ashburner, Department of Genetics, Cambridge University, Downing Street, Cambridge CB2 3EH, England, Tel: (44)-223-333969, Fax: (44)-223-333992

Spyros Artavanis-Tsakonas, Yale University, Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, 295 Congress Avenue, New Haven, CT 06536-0812, Tel: 203-737-4466, Fax: 203-787-3364

Gary H. Karpen, Salk Institute, Department of MBVL, PO Box 85800, San Diego, CA 92186-5800, Tel: 619- 453-4100, X473, Fax: 619-457-4765

Gerald M. Rubin, University of California at Berkeley, Howard Hughes Medical Institute, Department of Molecular &

Cell Biology, Room 539 LSA Bldg., Berkeley, CA 94720, Tel: 510-643-9945, Fax: 510-643-9947  
Daniel L. Hartl, Harvard University, Department of Organismic & Evolutionary Biology, 16 Divinity Avenue, Cambridge, MA 02138, Tel: 617-496-3917, Fax: 617-496-5540  
Matthew Scott, Stanford University School of Medicine, Department of Developmental Biology, Beckman Center, B300, Stanford, CA 94305-5427, Tel: 415-725-7680, Fax: 415-723-9878  
Paul Lasko, McGill University, Department of Biology, 1205 Ave Docteur Penfield, Montreal, PQ H3A 1B1, Canada, Tel: 514-398-6721, Fax: 514-398-5069  
Marek Mlodzik, European Molecular Biology Laboratory, Differentiation Programme, Meyerhofstr. 1, D-6900 Heidelberg, Germany, Tel: (49)-62-21-387-303, Fax: (49)-62-21-387-306

Drosophila stocks carrying mapped single P element lethal insertions are being deposited in the Bloomington Drosophila Stock Center. The first 500 lines will be available this summer or fall. More details on the P element lines will appear on FlyBase when the stocks are available.

#### CONSTRUCTION AND REPLICATION OF THE P1 LIBRARIES.

The two P1 libraries we are using were constructed by David Smoller in the Hartl laboratory. Source material for the P1 libraries consisted of Sau3A partial digests of adult genomic DNA from a highly inbred strain of genotype: y; cn bw sp. In order to minimize ambiguities in the PCR assays resulting from duplicate clones, the master library, of which copies are being distributed, was derived from the original libraries by streaking each of the 9216 clones for single colonies and then repicking individual colonies. The restreaking and library replication was carried out at LBL.

#### RETRIEVAL OF P1 CLONES FROM THE LIBRARY.

A common question about the library is about nomenclature. There are 96 microtiter plates in the library. In each microtiter plate there are 96 wells and they are labeled in an alphanumerical fashion. However, in our clone lists we describe the positions using two numbers. The first number in our clone identification refers to the microtiter plate. The second number is a conversion number for the alphanumerical position in the plates. The conversion system that we use can be most easily described as reading a book. Counting begins with the well in the upper left hand of the plate. Thus, position A1 becomes 1. The numbers increase as you move from left to right in the first row. A2 becomes 2, A3 is 3 etc. until you reach A12, which is 12. In the second row we begin counting at the left hand well at 13, thus B1 is 13. This continues across the row until B 12 is 24. This system continues down the plate with C1 as 25, etc. Ultimately, the bottom well on the right hand side H12 is 96. Using this system, a clone labeled 12-23 would be on the 12th plate in well B11.

Another common question concerns how to isolate the clones. All the plates have been covered with a plastic plate sealer. This plate sealer should stay on the plates at all times and the plates themselves should never be thawed. If you want to isolate a clone, it is possible to pierce the plate sealer over an individual well with a sterile needle, or a sterile eppendorf tip. The clones can then be streaked on LB kanamycin (50 micrograms/ml of kanamycin) and grown overnight.

The library was constructed in two different vectors. In each plate the clones in the wells 1 through 40 (A1 through D4) contain inserts in the P1 vector NS582 tet14ad10. In each plate the wells 41-96 (D5 through H12) contain inserts in the P1 vector ad10sacBII.

#### References:

- a. General reference for the P1 cloning system:  
N. Sternberg, 1990 Bacteriophage P1 cloning system for the isolation, amplification, and recovery of DNA fragments as large as 100 kilobase pairs. Proc. Natl. Acad. Sci. USA 87: 103-107.
- b. Construction of the Drosophila NS582 tet14 ad10 library and associated methods:  
D. A. Smoller, D. Petrov and D. L. Hartl, 1991 Characterization of bacteriophage P1 library containing inserts of Drosophila DNA of 75-100 kilobase pairs. Chromosoma 100: 487-494.
- c. Description and use of the ad10 sacBII vector:  
J. C. Pierce, B. Sauer, and N. Sternberg, 1992 A positive selection vector for cloning high molecular weight DNA by the

bacteriophage P1 system: Improved cloning efficacy. *Proc. Natl. Acad. Sci. USA* 89: 2056-2060.  
 Lozovskaya, E. R., D. A. Petrov and D. L. Hartl, 1993 A combined molecular and cytogenetic approach to genome evolution in *Drosophila* using large-fragment DNA cloning. *Chromosoma* 102:253-266.

**d. Amplification of clone ends in ad10 sacBII by PCR:**

Nurminsky, D. I. and D. L. Hartl, 1993 Rapid and efficient amplification of the ends of DNA fragments cloned in bacteriophage P1. *Biotechniques* (in press).

It is also worth noting that direct sequencing of the ends works in about 80% of the cases. Within the Center, we have been using standard cycle sequencing protocols for the ABI (dye terminator) or Pharmacia (labeled primer) automated fluorescent sequencers. For the ad10 sacBII vector we have been using the following primers:

SP6L (23 bases) 5' GGCGTCGACATTAGGTGACAC 3';  
 T7L (24 bases) 5' CCGCTAATACGACTCACTATAGGG 3'.

A template preparation protocol for direct radioactive sequencing of P1 ends is available from David Smoller, Genome Systems, Inc., 7166 Manchester Road, St. Louis, Missouri, 63143. It is also worth mentioning that David Smoller's company, for a reasonable price, is able to screen the same *Drosophila* library, as well as an additional 4000 clones with any single copy *Drosophila* probe.

**IN SITU LOCALIZATIONS OF P1 CLONES.**

A list of P1 clones localized to the euchromatin of *Drosophila melanogaster* by in situ hybridization with the salivary gland chromosomes (Oregon R) is provided. The extent of the hybridization signal is indicated by the 'starting band' and 'ending band' table entries. The cytology was carried out by Elena R. Lozovskaya and Robert W. Jones of the Hartl laboratory. The experimental procedures and cytological localizations have been carried out as carefully as possible, and we are confident that a very high percentage of the assignments are correct. However, in a project of this magnitude, there is an inevitable chance of error being introduced at any of a number of stages. There are also differences in judgment, for example, in deciding whether a clone with multiple sites of hybridization has one or more major sites of hybridization. In addition, there is the possibility that some mix-ups occurred in the restreaking and repicking of the library; many of the in situ hybridizations were done on the original library, prior to restreaking. There are enough cross-checks in the experimental design of the *Drosophila* Genome Project that any misplaced clones will be identified and corrected eventually, but users should always check for themselves. Therefore, prior to using any clone, it would be advisable to verify the cytological location by in situ hybridization. If there is any discrepancy with the assignment, please inform us immediately so that we investigate. Contact by email HARTL@MCZ.HARVARD.EDU or FAX 617-496-5854. In the event that a P1 clone you need does not match the description in the library as distributed, the Hartl laboratory can try to recover it from the original plates. In any event, we would appreciate any additional information about these clones that users could provide.

**A PROTOCOL FOR MAKING P1 DNA:**

1. Grow an overnight culture in 25 micrograms/ml kanamycin.
2. Inoculate 500 ml of LB (25 micrograms/ml kanamycin) with 0.5 ml of the overnight culture. Shake at 325 rpm at 37 degrees C for about 3 hours (OD550 = 0.15).
3. Add 5 ml of 0.1 M IPTG (dissolve 0.6 g in 25 ml of ddH<sub>2</sub>O and filter sterilize) to the 500 ml culture. Shake for another 3 hours at 37 degrees C until OD550 = 1.3 to 1.5.
4. Harvest the cells (5K in GSA rotor for 10 minutes), and proceed with the Qiagen maxi-prep according to the kit protocol.
5. Resuspend the DNA (10-30 micrograms) in a suitable volume of TE.

**DATA BASED ON CONTIG ASSEMBLY.**

Contigs of overlapping P1 clones are being assembled by STS content mapping. The principle of physical map construction based on STS content mapping is straightforward, as shown by the following example. Consider three P1-clones denoted A, B, and C that are close together in the genome, and suppose that their content of 7 STS markers is as follows: A contains STS markers 1, 2, and 3; B contains markers 4, 6, and 7; and C contains markers 2, 4, and 5. Then it is clear that the clones must overlap, and the unique ordering consistent with the data is A-C-B (or the reverse). The STS sites are ordered within the clones as (1 3) 2 5 4 (6 7), where the parentheses around any markers indicate

incomplete specification of the order. In addition, the STS content strategy requires that these single copy markers be both mapped and at least partially sequenced. In this way, in addition to identifying the overlaps between large cloned inserts, STS content mapping provides a mechanism for the introduction of biological content, flexibility and community access into the map as it is being constructed.

STSs derived from the ends of mapped P1 clones and P element insertion sites are being positioned by the mapping group at LBL. STSs derived from the sequences of Drosophila genes that have been deposited in GenBank are being mapped in the Hartl laboratory. The P1 data table presents information on STSs that have been derived from the ends of the insert DNA in the P1 vector. These sequences have been generated using the SP6 primer site (S\_STS) and T7 primer site (T\_STS) that flank the Drosophila insert in the ad10 sacBII vector. The table also presents data on which STSs from other P1s, P element insertion sites, or known genes have been mapped to a particular P1 (Hit\_by\_STS). By searching for all entries with a given STS you can obtain the data necessary to assemble contigs. We hope to be able to represent these graphically in a later version of the database.

The work at LBL is being jointly managed by Bill Kimmerly, Chris Martin, and Michael Palazzolo. Bill Kimmerly is the day to day supervisor of the research associates on the project. The research associates working on the mapping project at LBL are: Karen Stultz; Victor Stevko; Ami Richardson; Gail Shirley; and Dan Hong. Charles Yu is an undergraduate also working on the project.

#### LETHAL P ELEMENT INSERTIONS

The overall goal of this part of the project is the analysis of P element insertion sites that disrupt vital autosomal genes in order to cross-reference the physical, cytogenetic and genetic maps of the *Drosophila melanogaster* genome. By defining STS's within sequences adjacent to all those insertions disrupting different vital genes, this collection would serve as a versatile link between the genetic and physical maps of the *Drosophila* genome. There are thought to be about 4,000 autosomal *Drosophila* genes capable of mutating to lethality. Our original project involved a collection of 1,800 autosomal recessive lethal P insertion lines, that were expected to define approximately 1,100 vital genes on the physical map, or about 27% of the total. Our goal is to create a collection of 1,000-1,200 *Drosophila* strains meeting specific quality criteria. Lines should contain single P element insertions each defining a unique vital gene. A group of 1,800 candidate strains has been assembled; non-redundant, single-insert strains causing recessive lethality will be selected and mapped from among these lines through seven sequential steps:

1. Map the chromosome location of the insertion in each of the 1,800 lines by *in situ* hybridization to polytene chromosomes.
2. Identify and eliminate lines containing background lethal mutations from the initial collection of 1800 lethal single P insertion strains.
3. Identify and remove lines with two insertions, and also redundant lines, in which the same gene is mutated, from the collection of 1800 lethal single P insertion strains.
4. Identify and eliminate lines in which the P insertion has not caused a lethal mutation.
5. Plasmid rescue DNA flanking the P element insertion from each of the approximately 1,100 lines that are expected to remain after the criteria of specific aims 2-4 have been applied.
6. Determine the sequence of approximately 400 bp of genomic DNA immediately adjacent to the site of insertion in each of these lines to provide an STS for mapping onto the P1 library.

We have collected pre-existing lethal lines from several laboratories as the starting material for this project. These initially included the Spradling, Rubin, Scott and Jan laboratories. More recently we have initiated a collaboration with Istvan Kiss which will allow us to substantially increase the number of P-induced lethals available for the project. We would appreciate hearing from individuals with collections of P-induced lethal, sterile, or visible mutations that they would like to make available. Contact Allan Spradling (email: spradling@mail1.ciwemb.edu or FAX 410-243-6311).

The *in situ* hybridization localization of P element insertion sites (Step 1) is being carried out in the Rubin laboratory by Todd Laverty, Glenn Doughty, Wan Yu and Donna Nakahara. The Genetic verification tests (Steps 2 and 3) are being carried out in the Spradling laboratory by Allan Spradling and Dianne Stern. The first collection analyzed was that from the Spradling laboratory. Lines will be deposited in the Bloomington Stock Center when steps 1-3 above have been completed and will be posted here at that time. We expect the first 500 lines to be available from the Stock Center sometime this summer.

**References:**

The isolation of the P element lines from the Spradling laboratory was described in: Karpen, G.H. and Spradling A.C. (1992). Analysis of subtelomeric heterochromatin in the Drosophila minichromosome DP1187 by single-P-element insertional mutagenesis. *Genetics* 132: 737-753.

The PZ-enhancer trap element used to generate the Spradling lab lines is described in Mlodzik, M. and Hiromi, Y. (1991). The enhancer trap method in Drosophila: its application to neurobiology. In: *Gene Expression in neural tissues. Methods in Neuroscience*, Vol 9. P.M. C. Orlando, ed. Academic press.

**NEEDLE SHARPENING FOR EMBRYO INJECTIONS**

James A. Powers, HHMI, Dept. of Biology, Indiana U., Bloomington, IN 47405. 812-855-7674, FAX/2577, JPOWERS@BIO.INDIANA.EDU

For me, doing injections isn't so bad once I have a good needle. However, breaking needles to get a usable tip was often very frustrating. I have adapted a technique for sharpening needles for mouse embryo injections (Gundersen et al. (1993) *Biotechniques*, 14(3), 412-414) for use in Drosophila. The needles are ground in a slurry of "sand" to give a beveled tip that is sharp and has a large enough bore to resist clogging.

**A) Preparation**

1) Wash silicon carbide (Grit 120 from Buehler Ltd., Lake Bluff, Illinois. They have a \$50 minimum order so you get 5 lbs. which should last a very long time.) in several changes of MQ water until water remains clear with no fines floating on the surface. 2) Mix washed sand and MQ water (1:3).

3) Autoclave.

4) Store at 4C.

**B) Method**

1) Backfill needle

2) Place needle in microinjection holder.

3) Apply pressure with syringe to avoid backflow of the slurry solution into your needle. I use a 60ml syringe placed in a standard caulking gun.

4) Swirl slurry at medium to high speed by stirring with a stir bar at about "7"(out of 10) on a stir plate.

5) Hold needle steady in slurry at an angle for about 1 min. 45 sec. Adjust the time to get the bore size you want.

6) Maintain pressure and rinse needle with MQ H<sub>2</sub>O from a squirt bottle. Release pressure and blot excess water from needle (but don't touch the tip).

7) Mount needle and inject.

**C) Notes**

1) This method will eventually chew up your stir bar.

2) I rinse and reautoclave the sand about once a week. I'm not sure this is necessary, but it doesn't hurt.

3) After trying several containers, I have settled on a Pyrex storage dish (#3250-DO) 100 x 80 mm. I use about a 1cm layer of sand.

**PREPARATION OF DNA FROM SINGLE EMBRYOS FOR PCR**

Maryann Garozzo and Alan C. Christensen, Dept. of Biochemistry and Molecular Biology, Thomas Jefferson U., 233 S. 10th Street, Philadelphia, PA 19107, 215-955-5190, FAX/5393, CHRISTEN@CALVIN.JCI.TJU.EDU.

We have adapted the single fly PCR method of Gloor and Engels (DIS 71: 148-149, 1992, and DIN Vol. 1, 1991) to single embryos. We have also slightly modified their procedure for single fly PCR which gives less background in our hands. The ability to use single embryos for PCR allows one to determine the genotype of an embryo following phenotypic analysis or other manipulation. Since there are relatively few good visible phenotypic markers for embryos, polymorphic sequence tagged sites can be used as chromosome markers in individual embryos. Single P element inserts can also be used; in this context they serve as portable sequence tagged sites. The sex of the embryo could also be determined by this method.

1. **DNA PREPARATION FROM EMBRYOS.** Single embryos are squashed in 10 ul of Gloor and Engels' extraction buffer (10mM Tris pH 8.2, 1mM EDTA, 25mM NaCl, 200ug/ml proteinase K freshly diluted from a frozen 20mg/ml stock). This is most conveniently done in a 0.5 ml microfuge tube, using the pipettor tip to crush the embryo in the buffer. Care should be taken to avoid getting the embryo stuck inside the pipettor tip. The homogenate is incubated at 37[°]C for 30 minutes, then 95[°]C for 2 minutes, then stored at 4[°]C. It is easy to program a thermocycler for these incubations. We typically use 1 ul of this extract in a 15-50 ul PCR using standard conditions, as appropriate to the primers.

2. **NOTES ON THE EMBRYOS.** We have successfully amplified single copy sequences with this procedure using embryos 12 hours old and older. It also works with first instar larvae. The embryos may be dechorionated or not. If they are dechorionated, we have found (not surprisingly) that the bleach must be thoroughly rinsed off. We have also used this procedure on embryos that have been permeabilized with heptane, immersed in halocarbon oil or stained for programmed cell death with acridine orange (Abrams et al., Development, 117: 29-43, 1993) If the embryos have been in halocarbon oil, we wash the oil off with heptane, although this may not be necessary. None of these procedures appears to interfere with DNA extraction or PCR. We have not attempted the procedure with fixed embryos.

3. **MODIFICATIONS OF THE SINGLE FLY PCR PROCEDURE.** Generally, the procedure of Gloor and Engels works very well. However, we have occasionally had problems with spurious background bands, and these are often worse when the priming sites are absent in the fly being tested. This problem is alleviated with no loss of the bona fide amplification product by using less fly extract in the PCR. For example, if the fly was homogenized in 50 ul, we use 1 ul in a 50 ul reaction, rather than 1 in 15. Reducing the number of PCR cycles from 30 to 25 also reduces the amount of background with no loss of signal.

#### INJECTING UN-DECHORIONATED EGGS OF DROSOPHILA MELANOGASTER UNDER ETHANOL

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In using a standard method of injecting Drosophila eggs we encountered a serious problem with egg viability. Dechorionated eggs incubated under oil did not develop, while control, un-dechorionated eggs hatched normally. A method for injecting un-dechorionated eggs has been described (Robertson et al., 1988; Cockburn et al., 1991; K. Matthews, personal communication) which has the advantage that difficulties with controlling humidity and preventing desiccation are avoided. On the other hand, un-dechorionated and non-desiccated eggs accept smaller quantities of injected solution. As we first thought that an infection might be responsible for the reduced egg viability we tried immersing un-dechorionated eggs in ethanol. We found that we obtained good viability when un-dechorionated eggs were injected when covered with 100% ethanol instead of oil. As cytoplasm starts leaking after injecting it is precipitated by the ethanol, sealing the puncture. The treatment probably also dehydrates eggs to some extent, so it is possible to inject similar amounts of DNA as were injected into dechorionated and desiccated eggs. Another advantage of using ethanol is that post injection care is much easier. The use of ethanol would not be appropriate if it might affect the phenotype being investigated, e.g. ADH activity (Bijlsma-Meeles, 1979).

Below we give a detailed protocol for the technique:

1. With a paintbrush put about 100 eggs into a drop of water on a coverslip stuck to a slide. Mix with a dissecting needle. Suck out most of the water with a pipette and a tissue. Wash the eggs again if too much yeast remains.
2. Place eggs in small groups about 3-4 mm from the end of the coverslip in tiny drops of water. We usually arrange eggs in 10 groups each containing 5 eggs. Using the corner of a rolled tissue, suck out most of the water from a group of eggs, so that the eggs can be moved with a dissecting needle. Arrange the eggs so that they stick together, at an angle of about 45 degrees to the edge of the coverslip. Repeat with other groups of eggs. Using a tissue suck out remnants of water and discard the remaining embryos.
3. Arrange the slide on the microscope stage. Gently, drop ethanol onto the coverslip. Don't add too much ethanol, because if it seeps over the coverslip you have to dry the slide and the coverslip and mount them again. It is better to add more ethanol as it evaporates. Adjust a needle loaded with DNA so that it is close to the embryos. Under pressure adjust the needle so that it just pierces the tip of one of the eggs. Move the needle with the micro-manipulator horizontally in order to break the tip of the needle. This procedure gives a very fine broken end to the needle. The

needle we use is more sharply tapering than that used for dechorionated eggs, but a fine tip is essential. Start injecting slightly away from the tip of the egg, which is much harder than the rest of the chorion. Bending the eggs to one side helps to place the tip of the needle in the germ cytoplasm.

4. After injecting all of the eggs, suck out the ethanol and under a dissecting microscope destroy any eggs that are too old.

5. Put the coverslip with the embryos into a vial of a fly food. The coverslip is pushed into the food so that the eggs are in close proximity to the food surface but not in contact with it. A few drops of water are added to the cotton plug to keep the vial humid. The vial does not require any attention until adult flies start to eclose.

This method gives very good viability - in a recent experiment, 505 eggs were injected and 136 adult flies were obtained. This is similar to the standard method using dechorionated eggs, but the injecting and post-injection care takes us about half of the time required by the standard method.

References: Bijlsma-Meeles, E. 1979, Heredity 42, 79-89; Cockburn, A.F., Meier, H. and Benedict, M.Q. 1991, DIS 70, 240; Robertson, H.M., Preston, C.R., Phyllis, R.W., Johnson-Schlitz, D.M., Benz, W.K. and Engels, W.R. 1988, Genetics 118, 461-470.

#### GENETIC NOTES, VOL. 11-15

##### CORRECTIONS FOR THE REDBOOK

Dan Lindsley and Georgianna Zimm, Dept. of Biology, U. of California, La Jolla, CA 92093. 619-534-3109, FAX/0053, REDBOOK@JEEVES.UCSD.EDU, ZIMM@JEEVES.UCSD.EDU

(p=page; L=left; R=right)

p 215L, f<sub>j</sub> (cytology): "Df(2R)11B" to "Df(2R)Pcl11B"

p 215L, f<sub>j</sub> (cytology): "Df(2R)Pcl-w5" to "Df(2R)Pcl-W5"

p 548L, "Phb: Photophobe" to "Ppb: Photophobe"

p 548L, change all table and text entries from "Phb" to "Ppb"

p 549L, "Photophobe: see Phb" to "Photophobe: see Ppb"

p 702L: remove the references for the gene tbs

p 782L, wt (alleles): Insert this reference (after left parenthesis) "Mglinetz and Vikulova, 1977, Genetika 13: 1318-20;"

p 782R, wt (cytology): Second line should be "Df(2R)Pcl-W5 = Df(2R)55A-B;55C but not Df(2R)Pcl11b"

p 854L, Df(2R)Pcl (table): "Df(2R)Pcl11B[beta]" to "Df(2R)Pcl[beta gamma]"

p 854L, Df(2R)Pcl (table): "Df(2R)Pcl-W5" to "Df(2R)Pcl-W5[delta]"

Note: Page corrections made in Photophobe corrections of Ballinger.

Editor's note: A complete list of reported corrections to the Redbook and to Ashburner's Greybook are maintained on FlyBase. See flybase/greybook/errors.txt and flybase/redbook/update.txt. A more coherent version of the latter will be available soon as redbook/errors.txt. -- K.M.

#### Announcement

##### Request for Assistance from Robert Farkas.

Institute of Experimental Endocrinology, Slovak Academy of Science, Vlarska 3, 83306 Bratislava, Slovakia

I would be very interested in buying back volumes of *Drosophila* Information Service, volumes 58, 57, 52, and all earlier issues. I can be contacted at the address above or at: Phone (+ 42 7) 373-800; Fax (+ 42 7) 374-247; E-mail FARKAS@UEE.SAVBA.CS.

## Historical Perspectives

Since this is the 75th volume of *Drosophila* Information Service, it seemed appropriate to allocate some space to a retrospective of *Drosophila* Information Service, the annual U.S. *Drosophila* research conferences, and some of the ideas that have helped shape the open communication that characterizes the *Drosophila* research community. In this section are the editors of *Drosophila* Information Service, a reprint of the Foreword from Volume 1 (March 1934), a reprint of Larry Sandler's 1981 description of the origin of the U.S. *Drosophila* conferences (DIS 56), and an update on the research conferences prepared for this issue by Dan L. Lindsley. The Directory printed in Volume 1 listed 75 researchers, three of whom had or would receive the Nobel Prize for their contributions. The current FlyBase Directory lists 4,667 researchers. Submission of other historical materials of interest to the *Drosophila* community is welcomed.

### Editors of DIS

Volume 1 (March 1934) to Volume 24 (November 1950)	C.B. Bridges and M. Demerec
Volume 25 (November 1951) to Volume 33 (November 1959)	M. Demerec
Volume 34 (December 1960) to Volume 49 (November 1972)	E. Novitski
Volume 50 (December 1973)	E. and E. Novitski
Volume 51 (December 1974)	E. Novitski
Volume 52 (May 1977) to Volume 53 (June 1978)	E. and E. Novitski
Volume 54 (July 1979) to Volume 67 (June 1988)	P.W. Hedrick
Volume 70 (July 1991) to Present	J.N. Thompson, jr.

### SPECIAL ISSUES:

Volume 54 (July 1979)	Prepared by D.L. Lindsley and G.G. Zimm
Volume 62 (September 1985)	Prepared by D.L. Lindsley and G.G. Zimm
Volume 64 (October 1986)	Prepared by D.L. Lindsley and G.G. Zimm
Volume 65 (January 1987)	Prepared by D.L. Lindsley and G.G. Zimm
Volume 68 (January 1990)	Prepared by D.L. Lindsley and G.G. Zimm
Volume 69 (May 1991)	Prepared by D.L. Lindsley and G.G. Zimm
Volume 73 (June 1994)	Prepared by M. Ashburner; Edited by W. Gelbart
Volume 74 (June 1994)	Prepared by the FlyBase Consortium; Edited by W. Gelbart
	Prepared by the FlyBase Consortium; Edited by W. Gelbart

### Foreword from **Drosophila Information Service, Volume 1, 1934**

Material Contributed by Drosophila Workers  
and arranged by  
C.B. Bridges and M. Demerec

Department of Genetics  
Carnegie Institution of Washington  
Cold Spring Harbor, Long Island, N.Y.

The aim of this undertaking was presented in a letter sent to Drosophila geneticists on November 10, 1933, which read in part as follows:

"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. In over twenty years of its use no conspicuous abuse has been experienced.

At present, as it was in the past, stocks and information available in different Drosophila laboratories are at the disposal of all Drosophila workers. The number of laboratories engaged in Drosophila research, however, is now large and is becoming larger every year with the result that the intimate contact which existed between the Drosophila workers of the past has been lost. Due to this lack of contact much of the valuable material is not being used and a great deal of effort is being duplicated.

This undertaking is an attempt to establish a closer contact between different Drosophila laboratories and by doing that to aid investigators in their research.

For several years now workers on genetics of maize have been receiving mimeographed circulars prepared in Professor Emerson's laboratory, containing information contributed by various investigators. This service proved to be so useful that steps are being taken to extend it and make it a permanent institution.

It is planned to establish a similar service for Drosophila workers, viz. to issue a mimeographed circular once or twice yearly including information which is not ordinarily suitable for publication but which is of importance to a number of workers active in Drosophila research. Such circulars are to contain the following:

1. New mutant characters. This section will include names, symbols, locations, descriptions, data on origins, etc., of all new mutants, reoccurrences and alleles (including those not of sufficient interest to warrant a special paper).
2. Summaries of linkage data submitted by workers.
3. Chromosome maps, with valuation of loci indicated.
4. Stock list, with names of laboratories where each stock is kept.
5. Notes and news; improvements in cultural and genetical technique; requests and offers of material, etc.
6. Drosophila directory. Names and addresses of Drosophila laboratories with staffs.
7. Drosophila bibliography. First issue gives to date those not given in certain basic reference works. Current issue thus gives yearly additions and supplies omissions.
8. Suggestions; corrections; miscellaneous.

The circular should be the result of the cooperative effort of all Drosophila workers. Its usefulness will be proportional to the degree of cooperation by all Drosophilists."

This first number of the circular is based upon the material contributed in response to the above letter. Because of the desirability of prompt issue of this first number it has been found advisable to limit somewhat the material to be included. Hence the main emphasis is placed temporarily on the list of symbols of the mutants still alive, upon the stock lists which make it possible for workers to find which mutants and stocks are available and where, and upon the directory of workers most actively engaged in the Drosophila research. For the present the map positions given are those based upon the familiar frameworks or basic maps, but there going revisions are being made. The revisions of some of the maps should be available for the second number, planned for early in the fall.

This first issue is being mailed rather widely, but the second and succeeding issues will be sent mainly to those laboratories and investigators who are actively cooperating in the project.

#### The Directory listed the following Drosophila researchers:

S.I. Alihanian (Moscow)  
 Edgar Altenburg (Houston, TX)  
 E.G. Anderson (Pasadena, CA)  
 Charlotte Auerbach (Edinburgh)  
 H.S. Baker (Los Angeles, CA)  
 George W. Beadle (Pasadena, CA)  
 Sarah Bedichek (Austin, TX)  
 Catherine Beers (Los Angeles, CA)  
 Z.I. Berman (Moscow)  
 R.D. Boche (Pasadena, CA)

Weldon Brewster (Austin, TX)  
 Calvin B. Bridges (Pasadena, CA)  
 George P. Childs (NY Univ., NY)  
 F.A.E. Crew (Edinburgh)  
 V. Curry (Pasadena, CA)  
 M. Demerec (Cold Spring Harbor, NY)  
 Th. Dobzhansky (Pasadena, CA)  
 F.N. Duncan (Pasadena, CA)  
 L.C. Dunn (Columbia U., NY, NY)  
 S.H. Emerson (Pasadena, CA)

Boris Ephrussi (Pasadena, CA)	T.S. Painter (Austin, TX)
S. Gershenson (Moscow)	Hal B. Parks (Austin, TX)
Marc A. Graubard (Columbia U., NY, NY)	J.T. Patterson (Austin, TX)
M.A. Grosscurth (Pasadena, CA)	H.H. Plough (Amherst, MA)
Marie L. Harnly (NY Univ., NY)	D.F. Poulson (Pasadena, CA)
Morris H. Harnly (NY Univ., NY)	Morris Rabinowitz (NY Univ., NY)
A.H. Hersh (Cleveland, OH)	Helen Redfield (Pasadena, CA)
R.B. Howland (NY Univ., NY)	J. Schultz (Pasadena, CA)
Alfred F. Huettner (NY Univ., NY)	Morton D. Schweitzer (Columbia U., NY, NY)
Philip T. Ives (Amherst, MA)	A.S. Serebrovsky (Moscow)
V. Jollos (Madison, WI)	N.I. Shapiro (Moscow)
Margaret Kaylor (Cold Spring Harbor, NY)	M.F. Shenihova (Moscow)
P. Ch. Koller (Edinburgh)	N.P. Sivertzeva-Dobzhansky (Pasadena, CA)
R. Lamey (Edinburgh)	R.V. Smith (Los Angeles, CA)
Donald E. Lancefield (Columbia U., NY, NY)	W.P. Spencer (Wooster, OH)
G.A. Lebedeff (Cold Spring Harbor, NY)	Mary B. Stark (Flower Hosp., NY, NY)
Wilbur M. Luce (Urbana, IL)	Curt Stern (Rochester, NY)
Otto Mackensen (Austin, TX)	W.S. Stone (Austin, TX)
Otto S. Margolis (NY Univ., NY)	Florence D. Stuck (Columbia U., NY, NY)
W.G. Moore (Austin, TX)	A.H. Sturtevant (Pasadena, CA)
Thomas Hunt Morgan (Pasadena, CA)	Meta Suche (Austin, TX)
Lillian V. Morgan (Pasadena, CA)	Isabel Thomas (Austin, TX)
H.J. Muller (Austin, TX)	David H. Thompson (Urbana, IL)
Eleanor Nichols (Pasadena, CA)	K.V. Volkova (Moscow)
N.I. Noujdin (Moscow)	E.M. Wallace (Pasadena, CA)
C.P. Oliver (Minneapolis, MN)	Alexander Weinstein (Baltimore, MD)
	Eunice White (Cold Spring Harbor, NY)
	A.M. Winchester (Austin, TX)
	Charles Zeleny (Urbana, IL)

### On the Origin of the *Drosophila* Conferences

Reprinted from *Drosophila Information Service*, Volume 56 (March 1981)

... a noble storie  
And worthy for to drawen to memorie

Larry Sandler \*

Contemporary *Drosophila* geneticists feel that *Drosophila* studies occupy a position close to the cutting edge of modern biology, a conviction which must be evident to participants of recent Annual *Drosophila* Conferences. This feeling can be objectively validated. A comparison of the material presented one year ago in Bloomington with that reported this year in Salt Lake City shows very rapid progress in many aspects of the genetic biology of *Drosophila*.

A second striking feature of *Drosophila* studies that has been amply illustrated in recent *Drosophila* Conferences is the exploitation for experimental purposes of multiple biological aspects of the fly. From the classical role of *Drosophila* as a mere vehicle for its chromosomes and its traditional use as a manipulable object in population genetics, *Drosophila* studies now include biochemical, developmental, neurobiological, and behavioral attacks. The depth and sophistication of this exploitation of the biology of *Drosophila* is shown by the range of material now considered in the specialized concurrent workshops held during *Drosophila* Conferences of recent years.

This sense of vitality and centrality that has animated the Conferences lately, as well as the contemporary use of *Drosophila* in all of its important biological aspects, were not prominent features of the earliest *Drosophila* Conferences. Indeed, the *Drosophila* Conferences mirror precisely the renaissance in, and the evolution of, *Drosophila* studies themselves. Today the meetings are characterized as useful, perhaps even as important. But they started as small get-togethers that could have been called "genetic analysis for fun and recreation". The very first *Drosophila*

Conference, which took place in Madison, Wisconsin in the fall of 1958, was not a formal conference, nor did anyone there at that time imagine that it would give rise to a tradition which has now continued for over twenty years.

In 1958 Dan Lindsley, who was then at the Oak Ridge National Laboratories, flew to Chicago and joined Bill Baker, who was at the University of Chicago, and together they drove to the University of Wisconsin in Madison, where I was a postdoctoral fellow in the laboratory of Jim Crow. Ted Pittenger, a *Neurospora* geneticist now in Manhattan, Kansas, was then at Marquette. Because Ted had been one of the geneticists at Oak Ridge along with Bill and Dan (and, for a time, me), he joined the two of them in Milwaukee and also came to Madison. Thus, the first "*Drosophila* Conference", which lasted for two days, involved both *Drosophila* and *Neurospora* genetics.<sup>1</sup> The meeting, however, consisted primarily of Baker, Crow, Lindsley, Pittenger, and Sandler each speaking -- more or less endlessly -- about his current research. Of course, other members of Crow's laboratory, his students and postdoctoral fellows, also attended and participated in the sessions. Most notably, from my point of view, was that Yuichiro Hiraizumi, with whom I was just beginning to collaborate, was present. Also there -- this list is from my memory and therefore almost surely incomplete -- were Rayla Greenberg (now Temin), Elaine Johansen (now Mange), and Tom Gregg.

It was difficult in those days to find people both interested in and able to discuss the intricacies of formal genetic analysis. The whole group of us did nothing else for two whole days, and it was, to say the least, enormous fun. While the meeting could scarcely be characterized as important, there was enthusiastic agreement that we should do the same thing again the following year, and Bill Baker suggested Chicago as the site of that meeting. In 1959, therefore, Bill sent notices to Dan, Jim and me (*Neurospora* and corn being summarily dismissed as temporary aberrations), to attend the "Little Men's Chowder and Marching Society". Dan, however, both to explain his absence from Oak Ridge and in order to get reimbursed for the trip, adorned the event with the title of "Midwestern *Drosophila* Conference".<sup>2</sup> Attendance at this "Second Annual *Drosophila* Conference" at Chicago ballooned to about 15 or 20 (according to my best recollection), as there were rather a lot of Drosophilists at Chicago and some other workers had heard casually about the projected meeting during the year. This meeting followed the same format as the one at Madison, with individuals speaking whenever they felt they had something relevant to say.

The Chicago meeting was quite as much fun as the earlier one, but it created a considerable furor as news of its existence spread through the *Drosophila* community, both by word of mouth and through the medium of the bulletin put out by the Oak Ridge National Laboratories. The Christmas edition of that bulletin was received by most laboratories in the country, and contained a notice that Dan Lindsley had attended the Midwest *Drosophila* Conference. Ed Novitski (learning of the meeting from his new position in Eugene, Oregon) asserted that since Hawaii had become a state, Oregon was in the midwest, and he should therefore have been invited. H.J. Muller was angry that he had not been informed about it, when, after all, he worked in Bloomington, Indiana which was archetypically midwestern, while Dan Lindsley worked in Tennessee, which was the south.

In general, there developed a strong feeling about the impropriety of "exclusive congresses", like those of 1958 and 1959 (although, of course, neither had been congresses in any real sense), so that in 1960 the Third Annual *Drosophila* Conference, which was held in Bloomington, Indiana, had an enrollment open to anyone who knew of it and wished to come. It was, however, another year before the idea of *Drosophila* meetings as annual events, with all *Drosophila* workers formally invited, originated.<sup>3</sup>

At that conference, as well as at the next several following, the format was still primarily free-form. The entire group met in a single room, and there was no fixed program. People spoke as the spirit moved them, though, as enrollment grew, it became necessary to sign up to speak just before a session was held. However, by 1962 at the latest, enrollment had grown to a point where concurrent sessions had to be scheduled, although free-form plenary sessions were still the norm. At the 1965 Seattle meeting, for example, there was only one afternoon with concurrent sessions (cytogenetics, evolutionary genetics, and physiological genetics).

Following is a list of the Conferences from 1958 through 1980, as reconstructed in 1979 in Bloomington, Indiana and put together and spot-checked by Adelaide Carpenter. There are included, parenthetically, some highlights of early meetings. Also note that the first five meetings, from 1958 through 1962, were held in the fall; afterwards they were held in the spring, and one year (1963) was skipped in the change-over.

1. 1958 Madison I
2. 1959 Chicago I
3. 1960 Bloomington I
4. 1961 Oak Ridge
5. 1962 St. Louis
6. 1964 Madison II [There was a dinner honoring A. H. Sturtevant as he approached his 75th birthday]
7. 1965 Seattle [There was a first general showing of several films depicting courtship and mating in *Drosophila*,

including that classic example of erotica, "I Am Curious Yellow Forked"]

8. 1966 Chicago II
9. 1967 Texas [A eulogy to H.J. Muller, who had recently died, was delivered by C.P. Oliver]
10. 1968 Yale
11. 1969 Iowa
12. 1970 Pasadena
13. 1971 Ithaca College
14. 1972 North Carolina State (Raleigh)
15. 1973 DeKalb
16. 1974 Banff
17. 1975 Louisiana State University
18. 1976 Tempe
19. 1977 La Jolla  
1978 No meeting-- no coal
20. 1979 Bloomington II
21. 1980 Salt Lake City

\*The perspectives provided here are, of course, mine. However, I have included -- mostly without specific attribution -- current recollections of Bill Baker, Yuichiro Hiraizumi, Dan Lindsley, and Ted Pittenger. To them, my thanks, in part for their help here, but mostly for making me a participant in the events themselves. Those of you who have read any of my scientific works will surely wonder at the quality of the prose in this note. I haven't learned to write, merely to dictate to Ms. Barbara Hlavin, whose typewriter turns gibberish into English. You may ask, if Barbara Hlavin is responsible for the writing, and others have supplied the memories, why am I the author of this note? You may indeed ask.

<sup>1</sup>R.A. Brink dropped in one morning to talk about his current studies on maize, and Hiraizumi distinctly recalls an extended discussion about the chromosomal basis of the departure from equality of the human sex-ratio. As can be seen, Drosophilists in those days were rather more tolerant of work on other, less tractable, organisms than they are today.

<sup>2</sup>This story appears in Human and Mammalian Cytogenetics, An historical perspective by T.C. Hsu (1974). It is interesting that the *Drosophila* Conferences took on an official name, and, concomitantly, a certain measure of independent reality, in order to satisfy ORNL's bureaucracy!

<sup>3</sup>It is probably worth reprinting here a section of the letter Dan Lindsley sent out on September 19, 1961 as the invitation to the Fourth Annual *Drosophila* Conference held at Oak Ridge:

"Three years ago Bill Baker and I got together with Larry Sandler and Jim Crow in Madison and spent two days informally telling each other of our recent experimental results. This meeting was so successful that we decided to repeat it the following year in Chicago. In Chicago we were joined by George Brosseau and Stan Zimmering among others. Last year the meeting was held in Bloomington and the attendance was again higher than the year before.

"In previous years we have had no formal program and no official invitations; people simply agreed verbally to meet, and we took turns presenting material. I think that this year the group will be quite large, but we plan to follow much the same procedure as in previous discussions. In view of the annual increase in attendance, the time has come to make a decision whether we wish to formalize an annual *Drosophila* genetics meeting open to all investigators in the field or whether it would be preferable to disband into the originally conceived, small, regional, bull sessions."

On the copy sent to Bill Baker, Dan appends (by hand): "I decided this year we have to invite everyone -- as they all know about it and want to come -- or be chicken -- I chose the former."

## Recent History of the U.S. *Drosophila* Conferences

Dan L. Lindsley

The nearly decade and a half since Larry Sandler wrote his article on the history of the annual *Drosophila* Meeting has seen a further explosion in our science that none of us would have anticipated at that time. *Drosophila* has become the organism of choice for probing the basic molecular mechanisms responsible for a myriad of biological processes. The power of the *Drosophila* system has attracted the best and the brightest, and the results from *Drosophila* research have provided the tools and insights for investigations into other less tractable species. A measure of the health of the *Drosophila* enterprise is seen in the accelerating rate of publication of research findings: 27,859 papers since 1980 versus 34,912 in all the years before. The increased interest in *Drosophila* as a model experimental organism has resulted in substantial changes in the structure and content of our annual meeting. The venue, rather than being the home institution of the organizing group was changed to hotels or conference facilities; this was necessitated by the increased attendance and the desire to continue meeting during the academic year.

In 1983 the attendants were delighted with our first meeting at Asilomar, where the program was organized by John Merriam, and the logistics were handled for the first time by a professional meetings manager. It was decided to return to Asilomar in three years' time under the same arrangement. The Seattle group was in charge of the 1986 program, and it was the first meeting in which short abstracts of talks were provided with the program. In dealing with the Asilomar conference center, Larry Sandler blithely agreed to personally guarantee payment for several hundred rooms; when he realized that a blizzard, some other natural disaster or an airline strike could doom the meeting and present him with a financial obligation that he would be unable to meet, he recognized that local organizers could not be expected to assume such a responsibility in the future. Accordingly, Linda Hall agreed to explore solutions to the dilemma. At about the same time, the Genetics Society of America had established, in cooperation with the American Society of Human Genetics, a permanent office in Bethesda, under the capable supervision originally of Gerry Gurvitch and more recently of Elaine Strass, both professional executive officers. The GSA was at the time negotiating with the yeast genetics community the management of their biennial meeting, and the idea was considered that they could similarly manage and be the guarantor for the annual fly meeting.

A *Drosophila* Board was established in 1988. It comprises nine regional representatives, each serving a three-year term; a new president is chosen and three members are replaced each year. The Board meets at the time of the annual meeting. It interacts with the Genetics Society of America, decides on the venues for future meetings, and deals with other matters affecting the community at large.

In February of 1987 Larry Sandler died unexpectedly, just as many of his initiatives were coming to pass. At the spring meeting in Chicago, a group of Larry's former students and colleagues decided that a fitting memorial to his importance to the field was the establishment of a Larry Sandler Fund through donations from the membership to support an annual lecture by the recent Ph.D. graduate whose thesis was judged by a panel of judges to be the most outstanding submitted during the previous year; this was considered particularly fitting in view of the importance that Larry attached to graduate teaching. Jim Crow delivered a fine tribute to Larry at a plenary session. Also at the Chicago meeting the advisability of affiliation with GSA as our meetings manager was put to a vote. The motion passed despite reservations by some that the informal nature of the meeting would change as a result; it has changed, not because of GSA organization, but because of the burgeoning numbers of participants. In 1988, as a result of plans initiated earlier by Sandler, the first International *Drosophila* Meeting was held in association with the International Congress of Genetics in Toronto. Bruce Edgar presented the first Larry Sandler Memorial Lecture to open that meeting. This was the first meeting organized by the Genetics Society of America in the capable hands of meetings manager, Peggy Gardener. The logistics of all subsequent meetings have been handled by GSA, first by Peggy Gardener, then by Jean Francese, and most recently by Anne Marie Langevin. Meeting organization by GSA has been an unqualified success. 1990 was the last meeting to be held in Asilomar; we reluctantly concluded that we had outgrown that superb venue.

Some of the highlights of previous meetings are indicated in the following sentences: The seminal contribution to the 1982 Storrs meeting was the announcement of Rubin and Spradling of successful P-mediated germ-line transformation, leading initially to the possibility of transposon tagging and subsequently to the construction of ingenious specialized vectors for the insertion of varied types of genetic information into the *Drosophila* genome. Preceding the 1985 meeting, the National Science Foundation sponsored a workshop bringing together Drosophilists and cryobiologists to explore the possibilities of cryopreservation of *Drosophila*; the workshop stimulated two research grant applications, the successful, but as yet not implemented, results of which were reported at the 1993 San Diego meeting by Peter

Steponkas of Cornell and Peter Mazur of the Oak Ridge National Laboratory. At the New Orleans meeting, it was agreed that a comprehensive electronic data base of *Drosophila* science was required in order that the onslaught of information could be made available in an easily accessible format to workers in the field; As a result a meeting was held in December of that year in Washington D. C. under NIH auspices. A grant proposal was subsequently submitted and approved, and now, thanks to the heroic efforts of Michael Ashburner, Bill Gelbart, Thom Kaufman, John Merriam and their colleagues, FlyBase is a growing reality that places ever-increasing amounts of information at the fingertips of the *Drosophila* community. The 1992 meeting in Philly saw the unveiling of the new red book by Lindsley and Zimm, for which workers had been waiting for ten years. Orders were taken, and the authors returned home with personal volumes autographed by the attendants.

The following table details some of the relevant information concerning the twenty-second through the thirty-fifth annual *Drosophila* Research Conferences.

#	Year	Venue	Attendance	Program	Board President	Sandler Lecturer
22	1981	Chicago		Janice Spofford		
23	1982	Storrs		Arthur Chovnick		
24	1983	Asilomar		John Merriam		
25	1984	Chicago		Sally Elgin		
26	1985	Charleston		Joe Jack		
27	1986	Asilomar		Larry Sandler		
28	1987	Chicago	765	Bill Engels		
29	1988	Toronto	701	Ross Hodgetts		Bruce Edgar
30	1989	New Orleans	725	Ian Duncan	Linda Hall	Kate Harding
31	1990	Asilomar	883	Matt Scott	Dan Lindsley	Michael Dickinson
32	1991	Chicago	1021	Thom Kaufman	Ross Hodgetts	Maurice Kernan
33	1992	Philadelphia	1112	Bill Gelbart	Thom Kaufman	Russ Nichols
34	1993	San Diego	1160	Gerry Rubin	John Lucchesi	David Schneider
35	1994	Chicago	1248	Victoria Finnerty	Mariana Wolfner	Kendal Broadie

## Cumulative Subject Index DIS Volumes 1 to 71

The following key word index to *Drosophila* Information Service research and technique notes is intended to be a guide to the volumes in your laboratory or institutional library. Key words were drawn from article titles. Space does not allow a reprinting of all titles, although a listing of the titles of articles in volumes 50 (1973) to 71 (1992) was printed in DIS Volume 71 (pages 1-51). A diskette file in ASCII is being prepared which will contain the complete tables of contents for the research and technical notes in all volumes. This can be obtained from James Thompson, DIS Editor, for \$5.00 (check, payable in U.S. currency, made out to "Drosophila Information Service"; price includes postage; please specify your preference of Macintosh or IBM-compatible, 3.5" or 5.25" diskette). Please write to the Editor if you are unable to use a diskette version but would like to have a printed copy.

- 1,2-propylene oxide, sperm (1954, 28:155)
- 1,4-bisdiacetyl butane (1983, 59:12)
- 1-naphthalen acetic acid (1954, 28:167)
- 2 nitrofluorene (1973, 50:80)
- 2,2'-Dipyridyl (1973, 50:84)
- 2,4,5-T (1972, 48:102)
- 2<sup>14</sup>C-uracil (1972, 48:53)
- 2-chloroethyl methane sulphonate (1960, 34:103)

- 2-methoxyethanol (1991, 70:63)  
 2-propanol (1981, 56:121), (1987, 66:132)  
 2-X sperm (1949, 23:91)  
 20-OH-ecdysone (1983, 59:134)  
 2:4:6-tri (ethyleneimino)-1:3L5 triazine (1953, 27:89)  
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 2R (1984, 60:117)  
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 3C (1971, 46:141)  
<sup>3</sup>H-acetamide (1968, 43:101)  
<sup>3</sup>H-thymidine labeling (1977, 52:127), (1978, 53:188), (1981, 56:56)  
<sup>3</sup>H-uridine (1982, 58:35)  
<sup>3</sup>H-uridine, incorporation (1977, 52:138)  
<sup>3</sup>H-uridine, testes (1968, 43:166)  
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 4-nitroquinoline-N-oxide (1963, 37:80, 110)  
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 5 fluorouracil (1964, 39:102), (1968, 43:150)  
 5-Aza-2'-deoxycytidine (1985, 61:184)  
 5-bromodeoxyuridine, DNA (1966, 41:135)  
 5-bromouracil, facet number (1967, 42:71)  
 6-diazo-5-oxo-1-norleucine (1964, 39:103)  
 6-phosphogluconate dehydrogenase (1972, 48:93), (1973, 50:132), (1981, 56:53), (1982, 58:136), (1983, 59:48)  
 8-hydroxyquinoline sulfate (1971, 46:109)

**A**

- A, chromosomal arrangement (1984, 60:136)  
 a-amanitin (1982, 58:35), (1985, 61:53)  
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