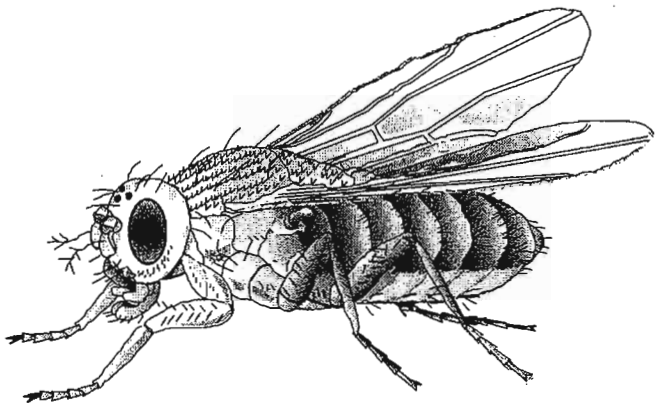
Drosophila Information Service



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Preface

Drosophila Information Service was first printed in March, 1934. Material contributed by *Drosophila* workers was arranged by C.B. Bridges and M. Demerec. As noted in its preface, which is reprinted in DIS 75, Drosophila Information Service was undertaken because, "An appreciable share of credit for the fine accomplishments in *Drosophila* genetics is due to the broadmindedness of the original *Drosophila* workers who established the policy of a free exchange of material and information among all actively interested in *Drosophila* research. This policy has proved to be a great stimulus for the use of *Drosophila* material in genetic research and is directly responsible for many important contributions." During the more than 60 years following that first issue, DIS has continued to promote open communication.

The production of DIS 77 could not have been completed without the generous efforts of many people. Stanton Gray, Merl Kardokus, and Eric Weaver helped prepare and proof manuscripts; Lou Ann Lansford, Shalia Newby, and Caroline Tawes maintained key records; and Coral McCallister advised on artwork and computer graphics. This year we changed the computer software used to prepare DIS and, as one might have predicted, problems arose in the late stages of preparation that delayed this issue somewhat. But that kind of difficulty simply challenges us to work harder on the next issue.

We are grateful for the continued support of 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 melanogaster* 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 a source of communication among *Drosophila* researchers.

James N. Thompson, jr.

Jenna J. Hellack

Drosophila Information Service

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Stock List

National *Drosophila* Species Resource Center

This stock list was provided by Jong Yoon, Director of the National *Drosophila* Species Resource Center, Bowling Green State University, Bowling Green, Ohio 43403-0212. Inquiries can be made by writing K.A. Yoon at this address or telephoning (419)-372-2742 or 372-2096. FAX, (419)-372-2024. e-mail, kayoon@bgsnet.bgsu.edu. The stock list is also accessible on FlyBase.

The National *Drosophila* Species Resource Center is supported by a grant from the National Science Foundation.

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HOW TO READ THE LIST

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A — 10000	GENUS DROSOPHILA			
B — 11000	SUBGENUS SCAPTODROSOPHILA			
11010	GROUP VICTORIA			
C — -0011.0	LEBANONENSIS CASTELLI	1865.3	VEYO, UTAH	B — G
D — -0021.0	LEBANONENSIS LEBANONENSIS	1733.1	BEIRUT, LEBANON	C or B
-0031.0	PATTERSONI	3359.I	BEIRUT, LEBANON	C or B
E — -0041.0	STONEI	GH3	TEHRAN, IRAN	B
-0041.1		T10	TEHRAN, IRAN	B
-0045.0	BROOKSAE		TUCSON, ARIZONA	B — H
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-0051.0	DIMORPHA	3338.1	SAO PAULO, BRAZIL	B
11030	GROUP LATIFASCIAEFORMIS			
-0061.0	LATIFASCIAEFORMIS	H75.4	HEREDIA, COSTA RICA	B
-0061.1		H79.2	BARRO COLORADO IS., CANAL ZONE, PANAMA	B
-0061.2		3012.1	MYRTLE BEACH STATE PARK, SOUTH CAROLINA	B
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12000	SUBGENUS HIRTODROSOPHILA			
-0072.0	PICTIVENTRIS	ORV 21	GREAT INAGUA, BAHAMAS ISLAND	B
-0072.1		ORV 28	LITTLE CAYMAN, CAYMAN ISLANDS	B
13000	SUBGENUS DORSILOPHA			

A: Code for genus
B: Code for subgenus
C: Code for group
D: Code for species and subspecies
E: Code for strains or mutants
F: Old stock number
G: Code for medium*
H: Original collection locality
#: Preserved in 100% alcohol

*Code for Drosophila medium
C: Cornmeal medium
CL: Cornmeal plus Liver medium
B: Banana medium
BC: Banana-Cactus medium
BL: Banana-Liver medium
BP: Banana plus Banana-Cactus medium
WC: Modified Wheller-Clayton medium
WI: Wheeler-Clayton medium plus Instant medium

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
10000		GENUS DROSOPHILA			
11000		SUBGENUS SCAPTODROSOPHILA			
11010		GROUP VICTORIA			
11010	-0011.0	LEBANONENSIS CASTEELI	1865.3	VEYO, UTAH	B
	-0021.0	LEBANONENSIS LEBANONENSIS	1733.1	BEIRUT, LEBANON	C or B
	-0031.0	PATTERSONI	3359.1	BEIRUT, LEBANON	C or B
	-0041.0	STONEI	GH3	TEHRAN, IRAN	B
	-0041.1		T10	TEHRAN, IRAN	B
	-0045.0	BROOKSAE		TUCSON, ARIZONA	B
11020		GROUP CORACINA			
11020	-0051.0	DIMORPHA	3338.1	SAO PAULO, BRAZIL	B
11030		GROUP LATIFASCIAEFORMIS			
11030	-0061.0	LATIFASCIAEFORMIS	H75.4	HEREDIA, COSTA RICA	B
	-0061.1		H79.2	BARRO COLORADO IS., CANAL ZONE, PANAMA	B
	-0061.2		3012.1	MYRTLE BEACH STATE PARK, SOUTH CAROLINA	B
12000		SUBGENUS HIRTODROSOPHILA			
	-0072.0	PICTIVENTRIS	ORV 21(HEED,1985)	GREAT INAGUA, BAHAMAS ISLAND	B
	-0072.1		ORV 28(HEED,1985)	LITTLE CAYMAN, CAYMAN ISLANDS	B
13000		SUBGENUS DORSILOPHA			
	-0081.0	BUSCKII	H75.18	HEREDIA, COSTA RICA	WI
	-0081.1		JDK-2 C C W Y		WI
	-0081.2		JDK-3 Y/T(Y;2R) RI		WI
	-0081.4		JDK-10 INS(1;?) SC		WI
	-0081.6		JDK-14 Y EC CT/IN(1) BLY		WI
	-0081.7		JDK-15 EC CT RAS/IN(1) BLY		WI
	-0081.8		JDK-17 T [DF(1);3R] N/ T(1;3R) W		WI
	-0081.9		JDK-21 IN(1-P)PM/IN(1) BLY		WI
	-0081.11		JDK-23 T(1;2R) SMS/IN(1) CE		WI
	-0081.12		JDK-30 CN		WI
	-0081.13		JDK-33 CD/IN(2LR) GO		WI
	-0081.14		JDK-36 IN(2L) DL/T(2L;2R;3L) LT		WI
	-0081.15		JDK-47 T[IN(1);3L] G;PI/IN(1) BLY		WI
	-0081.16		JDK-49 IN(1) 1570;Y PM/IN(1) BLY		WI
	-0081.17		JDK-5O IN(1) SMS/IN(1) BLY		WI
	-0081.18		JDK-53 W IN(1) BLY(273) IN(1)797		WI
	-0081.19		JDK-55 IN(1)(L942;R1349),SN PN/IN(1) BLY(273)		WI
	-0081.21		JDK-58 IN(2LR)926		WI
	-0081.22		JDK-59 ST SW CA		WI
	-0081.23		1894.9	BLUE MOUNTAIN LAKE, NEW YORK	WI
	-0081.24		2093.7	BYBLOS, LEBANON	WI

HOW TO READ THE LIST

STOCK	NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
13000	-0081.26	BUSCKII	3146.65	WULAI, TAIWAN	WI
	-0081.27		3269.1	AUSTIN, TEXAS	WI
	-0081.28#		3399.1	ACME STORE, PRINCETON, NEW JERSEY	WI
14000		SUBGENUS SOPHOPHORA			
14010		GROUP OBSCURA			
14011		SUBGROUP OBSCURA			
	-0091.1	AMBIGUA	1D	PORT COQUITLAM, B.C., CANADA	C
	-0095.0	GUANCHE	3265.1	TENERIFE, CANARY ISLANDS	C
	-0101.1	MIRANDA	M-76-13(1976)	MATHER, CALIFORNIA	C
	-0101.3		21M	PORT COQUITLAM, B.C., CANADA	C
	-0101.4		22M	PORT COQUITLAM, B.C., CANADA	C
	-0101.5		23M	PORT COQUITLAM, B.C., CANADA	C
	-0101.7		7M (ORANGE EYE)	PORT COQUITLAM, B.C., CANADA	C
	-0101.8			MATHER, CALIFORNIA	C
	-0111.0	PERSIMILIS	CC23	COLD CREEK, CALIFORNIA	C
	-0111.1		2529.6	QUESNEL, CANADA	C
	-0111.2		27/24 "ST"	MT. SAN JACINTO, CALIFORNIA	C
	-0111.3		JR #3A "ST"	MT. SAN JACINTO, CALIFORNIA	C
	-0111.13		MUTANT OR BL/OR +	(CHROM. 3)	C
	-0111.14		MUTANT DELTA;OR/OR		C
	-0111.15		MUTANT W(X)	McDONALD RANCH, CALIFORNIA	C
	-0111.16		MUTANT "RD"	WEOTT (REDWOODS FOREST), CA	C
	-0111.17		T9 "RD"	PORT TOUNSEND, WASHINGTON	C
	-0111.18		McD #4A "KL"	McDONALD RANCH, CALIFORNIA	C
	-0111.19		McD #7A "KL"	McDONALD RANCH, CALIFORNIA	C
	-0111.23		2B "KL"	VICTORIA, B.C., CANADA	C
	-0111.24		McD #7B "MD"	McDONALD RANCH, CALIFORNIA	C
	-0111.25		McD #16B "MD"	McDONALD RANCH, CALIFORNIA	C
	-0111.27		MD-5-2 "MD"	MT. SAN JACINTO, CALIFORNIA	C
	-0111.28		JR #1B "WT"	MT. SAN JACINTO, CALIFORNIA	C
	-0111.29		JR #12A "WT"	MT. SAN JACINTO, CALIFORNIA	C
	-0111.35		JR #3A/B "WT&ST"	MT. SAN JACINTO, CALIFORNIA	C
	-0111.36		JR #23/16A "WT&ST"	MT. SAN JACINTO, CALIFORNIA	C
	-0111.38		4-11 B "WE"	VICTORIA, B.C., CANADA	C
	-0111.39		R14 PA "PA"	PARKSVILLE, B.C., CANADA	C
	-0111.41		R14 CO "CO"	PARKSVILLE, B.C., CANADA	C
	-0111.42		M171 "PO"	PORT COQUITLAM, B.C., CANADA	C
	-0111.43		V30 "HU"	VICTORIA, B.C., CANADA	C
	-0111.44		41/32B "OR"	MT. SAN JACINTO, CALIFORNIA	C
	-0111.45		178 "OR"		C
	-0111.46		"OS"	MATHER, CALIFORNIA	C
	-0121.0	PSEUDOOBSCURA	A118.1	TUCSON, ARIZONA	C

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
14011	-0121.1	PSEUDOOBSCURA	SHB-1 TA	DEATH VALLEY, CALIFORNIA	C
	-0121.2		SHB-2 DY	DEATH VALLEY, CALIFORNIA	C
	-0121.3		SHB-3 BR	DEATH VALLEY, CALIFORNIA	C
	-0121.4		SHB-4 WTU	DEATH VALLEY, CALIFORNIA	C
	-0121.5		SHB-5 EY	DEATH VALLEY, CALIFORNIA	C
	-0121.6		SHB-6 Y	DEATH VALLEY, CALIFORNIA	C
	-0121.7		SHB-7 OH	DEATH VALLEY, CALIFORNIA	C
	-0121.8		WWA-3 CT SE LL SP TT		C
	-0121.9		WWA-5 V		C
	-0121.11		WWA-6 PT		C
	-0121.12		WWA-7 W		C
	-0121.13		WWA-10 CO SH		C
	-0121.14		WWA-11 SH		C
	-0121.15		WWA-12 SE		C
	-0121.16		WWA-37 UPT GL		C
	-0121.17		WWA-40 UPT BX BA GL(INV)/UBX GL^2 SELECT FOR BA		C
	-0121.18		WWA-42 OR		C
	-0121.21		WWA-76 OR PX		C
	-0121.22		WWA-78 OR BL PR CV (AR)/LETHAL (CU) SELECT FOR BL.		C
	-0121.23		WWA-79 OR SC PR (ST)/LETHAL SELECT FOR BL.		C
	-0121.24		WWA-80 OR L (SC)/OR + (ST);SPA SELECT FOR L.		C
	-0121.26		WWA-86 OR PR (ST)		C
	-0121.27		WWA-92 OR		C
	-0121.28		WWA-118 INC HK J		C
	-0121.29		WWA-119 INC HK J CY(INV)/LETHAL SELECT FOR CY		C
	-0121.31		WWA-128 SH OR (ST)		C
	-0121.32		2258.1	ZIMAPAN, HIDALGO, MEXICO	C
	-0121.33		2529.2	KELOWNA, B.C., CANADA	C
	-0121.34		2529.3	CUERNAVACA, MEXICO	C
	-0121.35		2542.2	BOGOTA, COLOMBIA	C
	-0121.36		3339.1 PP	MATHER, CALIFORNIA	C
	-0121.37		3339.2 PP	MATHER, CALIFORNIA	C
	-0121.38		3339.3 TL	MATHER, CALIFORNIA	C
	-0121.39		3339.4 TL	MATHER, CALIFORNIA	C
	-0121.41		3339.5 ST	MATHER, CALIFORNIA	C
	-0121.42		3339.6 AR	MATHER, CALIFORNIA	C
	-0121.43		3339.7 AR	MATHER, CALIFORNIA	C
	-0121.44		3339.8 CH	MATHER, CALIFORNIA	C
	-0121.45		3339.9 CH	MATHER, CALIFORNIA	C
	-0121.57		3339.20 CH	MATHER, CALIFORNIA	C
	-0121.64		3339.26 ST	MATHER, CALIFORNIA	C
	-0121.68		3389.1 SC(10)	BOGOTA, COLOMBIA	C
	-0121.69		3389.2 SC(8)	BOGOTA, COLOMBIA	C
	-0121.71		3389.3 SC(5)xTL(3)	BOGOTA, COLOMBIA	C

HOW TO READ THE LIST

STOCK	NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
14011	-0121.78	PSEUDOOBSCURA	SB22.1 A	DILLEY, TEXAS	C
	-0121.79		P75 ST	PORT COQUITLAM, B.C., CANADA	C
	-0121.81		P117 PP	PORT COQUITLAM, B.C., CANADA	C
	-0121.82		A34 TL	PORT COQUITLAM, B.C., CANADA	C
	-0121.83		S14 AR	SPRAY, OREGON	C
	-0121.84		P83 EP	PORT COQUITLAM, B.C., CANADA	C
	-0121.85		P47 OL	PORT COQUITLAM, B.C., CANADA	C
	-0121.87		"C(3) OR,PR" =("GST") (UNIV. OF CALF., DAVIS)		C
	-0121.88		2517.8	PACHUCA, MEXICO (RE-IDENTIFIED)	C
	-0121.89		2519.13	PACHUCA, MEXICO	C
	-0121.91		W^{ec} (LINE-1)		C
	-0121.92		W^{ec} (LINE-2)		C
	-0121.93		BSC-2351	TUSCON, ARIZONA	C
	-0131.3	SUBOBSCURA		MT. ST. HELENA, CALIFORNIA	C
14012		SUBGROUP AFFINIS			
	-0141.0	AFFINIS	2069.2	CRYSTAL LAKE, HASTINGS, NEBRASKA	C
	-0141.1		2528.7	NEBRASKA NATIONAL FOREST, HALSEY, NEBRASKA	C
	-0141.2		2528.8	WEEPING WATER, NEBRASKA NATIONAL FOREST, HALSEY, NEBRASKA	C
	-0171.0	AZTECA	2266.3	CHILPANCINGO, MEXICO	C
	-0171.1		3396.1	MOUNT BIGELOW, ARIZONA	C
	-0171.2		49-16	SAN JACINTO MTS., CALIFORNIA	C
	-0171.3		69-7	ANDREAS CANYON, CALIFORNIA	C
	-0171.4#		94-100	RINCON STATION, CALIFORNIA	C
	-0171.5		94-106	RINCON STATION, CALIFORNIA	C
	-0171.6		I10-13	MATHER, CALIFORNIA	C
	-0181.0	BIFASCIATA	3040.8	LAKE AKAN-KO, JAPAN	C
	-0191.0	NARRAGANSETT	2528.9	NEBRASKA NATIONAL FOREST, HALSEY, NEBRASKA	B
	-0191.1		3157.4A-D	BASTROP STATE PARK, TEXAS	B
14012	-0201.0	TOLTECA	H346.50	COROICO, BOLIVIA	C
14020		GROUP MELANOGASTER			
14021		SUBGROUP MELANOGASTER			
	-0224.0	ERECTA	(CAMBRIDGE UNIV. LAB. 1977)		C
	-0231.0	MELANOGASTER	2370.7	OAHU, HAWAII	C
	-0231.1		2394.9	ICA, PERU	C
	-0231.2		2401.2	KOLONIA, PONAPE, CAROLINE ISLANDS	C
	-0231.3		2404.11	TOONDA, QUEENSLAND, AUSTRALIA	C
	-0231.4		3033.8	KUALA LUMPUR, MALAYSIA	C
	-0231.5		3071.4	AGANA, GUAM, MARIANA ISLANDS	C
	-0231.6		3114.4	MYSORE, INDIA	C
	-0231.7		3160.7	KEN-TING, TAIWAN	C

STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY		MEDIUM
-0231.8#		SF1 AdH-Fr,b,pr,cn	CEDAR RAPIDS, IOWA		C
-0231.9#		ORE AdH-Fm,b,pr,cn	(FROM OREGON R)		C
-0231.11#		NCA23 AdH-Fs,b,pr,cn	NORTH CAROLINA		C
-0231.12#		SSS AdH-Sm,b,pr,cn	LAFAYETTE, NEW YORK		C
-0231.13#		GF31 AdH-Ss,b,cn,pr	GLEN FALLS, NEW YORK		C
-0231.14			ORLANDO, FLORIDA		C
-0231.15			BAHIA, BRAZIL		C
-0241.0	MAURITIANA	3400.1	RIVERE NOIRE, MAURITIUS, INDIAN OCEAN		C
-0241.1		wt(+)	DAVID 105	BSC-2252	C
-0241.2		wt(+)	DAVID 75	BSC-2278	C
-0241.3		wt(+)	72	BSC-2282	C
-0241.4		wt(+)	152	BSC-2308	C
-0241.5		wt(+)	207	BSC-2317	C
-0241.6		wt(+)	197	BSC-2318	C
-0241.7		wt(+)	102	BSC-2516	C
-0241.8		wt(+)	SYNTHETIC	BSC-3597	C
-0241.11		B[1]		BSC-2306	C
-0241.12		bg(prune)		Mid-Am	C
-0241.13		bg(prune) x		Mid-Am	C
-0241.14		bo[1]		BSC-2273	C
-0241.15		bw cu		Mid-Am	C
-0241.16		cn[1] bw[1]		BSC-2210	C
-0241.17		cn[1]; ir[1]		BSC-3595	C
-0241.18		cu(curved wing)		Mid-Am	C
-0241.19		dahmer[1]		BSC-1963	C
-0241.21		e		Mid-Am	C
-0241.22		f[2]		BSC-31	C
-0241.23		g[1]		BSC-2274	C
-0241.24		ir[1]		BSC-2228	C
-0241.25		j[1]		BSC-2216	C
-0241.26		j[1] bw[1]		BSC-2246	C
-0241.27		j[1] cn[1] bw[1]		BSC-2247	C
-0241.28		j[1] cn[1] bw[1]; ir[1]		BSC-2305	C
-0241.29		Net cy cn bw		Mid-Am	C
-0241.31		pn[1]		BSC-2316	C
-0241.32		pn[1] v[1]		BSC-2970	C
-0241.33		pn[1]; j[1]; ir[1]		BSC-3025	C
-0241.34		pr[1]		BSC-533	C
-0241.35		pr-3[1]		BSC-2520	C
-0241.36		ri		Mid-Am	C
-0241.37		ri-2[1]		BSC-2410	C
-0241.38		sn[1]		BSC-2519	C
-0241.39		sn[1]; j[1]; ir[1]		BSC-823	C

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STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
14021	-0241.41	MAURITIANA	sn[1]; ros[1]	BSC-2339	C
	-0241.42		v[1]	BSC-3182	C
	-0241.43		vi[1]	BSC-1927	C
	-0241.44		vi[1] sn[1]	BSC-1926	C
	-0241.45		w[1]	BSC-1955/2330	C
	-0241.46		w;e	Mid-Am	C
	-0241.47		w[pch]	BSC-2336	C
	-0241.48		ww[1]	BSC-3594	C
	-0241.49		w[1] f[2]	BSC-3596	C
	-0241.51		y[1]	BSC-2602	C
	-0241.52		y[1] f[1]	BSC-1957	C
	-0241.53		y[1] pm[1]	BSC-2323	C
	-0241.54		y[1] w[1]	BSC-1959	C
	-0241.55		y[1] w[1] f[1]	BSC-1960	C
	-0241.56		z[1]	BSC-2594	C
	-0241.61	MAURITIANA/SIMULANS	Dsim\se[1]	BSC-3466	C
	-0241.62	MAURITIANA/SIMULANS	Dsim\se[1] ir[1]	BSC-3074	C
	-0245.0	ORENA	(CAMBRIDGE UNIV. LAB. 1977)	Mid-Am	C
	-0248.0	SECHELLIA	(CAMBRIDGE UNIV. LAB. 1977)	Mid-Am	C
	-0248.1		wt(+)	BSC-3529 LINE 15	C
	-0248.2		wt(+)	BSC-3578 22	C
	-0248.3		wt(+)	BSC-3587 81	C
	-0248.4		wt(+)	BSC-3588 24	C
	-0248.5		wt(+)	BSC-3589 4	C
	-0248.6		wt(+)	BSC-3590 21	C
	-0248.7		wt(+)	BSC-3591 ROBERTSON	C
	-0248.8		wt(+)	BSC-2814 ss77 25x	C
	-0248.11		cn[1]	BSC-3577	C
	-0248.12		f[1]	BSC-128	C
	-0248.13		pur[1]	BSC-3592	C
	-0248.14		v[1]	BSC-3151	C
	-0248.15		w[1]	BSC-3235	C
	-0248.16		zn[1]	BSC-1912	C
	-0248.17		zn[1] f[1]	BSC-3160	C
	-0248.18		zn[1] v[1]	BSC-3225	C
	-0248.19		zn[1] v[1] f[1]	BSC-2423	C
	-0251.0	SIMULANS	H134.18	KENSCOFF, HAITI	C
	-0251.1		H231.2	GEORGETOWN, GUYANA	C
	-0251.2		H435.4	LETICIA, COLOMBIA	C
	-0251.3		H48.3	ZAMORANO, HONDURAS	C
	-0251.4		2372.17	AUSTRALIA	C
	-0251.5		2394.3	LIMA, PERU	C
	-0251.6		3015.8	NUEVA, CALIFORNIA	C
	-0251.7		3036.1	RAROTANGO, COOK ISLANDS	C

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY		MEDIUM
14021	-0251.8	SIMULANS	SB18.1A	TAMAZUNCHALE, S.L.P., MEXICO		C
	-0251.9		3028.8	GOROK, NEW GUINEA		C
	-0251.11		w^{501}	X-CHROMOSOME		C
	-0251.12		w[a]	X-CHROMOSOME	BSC-3598	C
	-0251.13		y[1] w[1]	X-CHROMOSOME	BSC-928	C
	-0251.14		y w f^{2}	X-CHROMOSOME		C
	-0251.15		m^{82} g^{8}	X-CHROMOSOME		C
	-0251.16		f[2]	X-CHROMOSOME	BSC-3275	C
	-0251.17		lz^{s}MALE/X^XY yw FEMALE X-CHROMOSOME			C
	-0251.18		"ORANGE"(X-LINKED)	X-CHROMOSOME		C
	-0251.19		X^XY yw FEMALE/+Y MALE X-CHROMOSOME			C
	-0251.21		GARNET	X-CHROMOSOME		C
	-0251.22		X^XY yw FEMALE;ywmf^{2}Y MALE X-CHROMOSOME			C
	-0251.23		Lhr[1]		BSC-1068	C
	-0251.24		stw	2-CHROMOSOME		C
	-0251.26		pm[1]	2-CHROMOSOME	BSC-2037	C
	-0251.27		net[1] pm[1]	2-CHROMOSOME	BSC-3119	C
	-0251.28		b[2]	2-CHROMOSOME	BSC-1095	C
	-0251.29		DIMPLED	2-CHROMOSOME		C
	-0251.31		nt[1] b[1] py[1] sd[1] pm[1]		BSC-923	C
	-0251.32		st[1]	3-CHROMOSOME	BSC-3388	C
	-0251.33		e	3-CHROMOSOME		C
	-0251.34		st[1] e[1]	3-CHROMOSOME		C
	-0251.35		ry[i83]	3-CHROMOSOME	BSC-2211	C
	-0251.36		or BEECHAM	3-CHROMOSOME		C
	-0251.37		upt	3-CHROMOSOME		C
	-0251.38		nt; st	MULTICHROMOSOME		C
	-0251.39		nt[1]; e[1]	MULTICHROMOSOME	BSC-3465	C
	-0251.41		f[2]; net[1] pm[1]; st[1] e[1]		BSC-2008	C
	-0251.42		nt pm;st e	MULTICHROMOSOME		C
	-0251.43		f^{2};nt pm	MULTICHROMOSOME		C
	-0251.44		f^{2};st e	MULTICHROMOSOME		C
	-0251.45		T(Y;2)3, net[1] b[1]/+		BSC-3579 (GRELL)	C
	-0251.46		T(Y;3)l, Ubx[m]/cutsy[1] ca[2]		BSC-1080	C
	-0251.47		T(Y;2)l/b[1]		BSC-1140	C
	-0251.48		T(Y;2)2/b[1]		BSC-1079	C
	-0251.49		T(Y;2)3/net[1] b[1]		BSC-1074	C
	-0251.51		T(Y;2)4/b[1] pm[1]		BSC-1069	C
	-0251.52		T(Y;2;3)1, Ubx[m]/b[1]		BSC-1077	C
	-0251.53		T(Y;2;3)2, Ubx[m]/b[2]; ca[2]		BSC-1097	C
	-0251.54		T(Y;2;3)3, Ubx[m]/b[2]; ca[2]		BSC-1072	C

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STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
14021 -0251.55	SIMULANS	T(Y;3)2, Ubx[m]/cutsy[1]	BSC-1082	C
-0251.56		T(Y;3)4, Ubx[m]/cutsy ca[2]	Mid-Am-0612	C
-0251.57		T(Y;3)5, Ubx[m]/cutsy[1]	BSC-1083	C
-0251.58		b[1]	BSC-915	C
-0251.59		bar-3[1]	BSC-602	C
-0251.61		b[2]; cutsy[1] ca[2]	BSC-1081	C
-0251.62		b[2]; H[3]ca[2]/Ubx[m] ca[2]	Mid-Am-0604	C
-0251.63		b^2 stw	Mid-Am-0614	C
-0251.64		bw[1]	BSC-916	C
-0251.65		Bx[1]	BSC-2327	C
-0251.66		ca[2]	BSC-1076	C
-0251.67		cd[B]	BSC-1768	C
-0251.68		car[2]	BSC-3304	C
-0251.69		clr[1]	BSC-1073	C
-0251.71		cn[1]	BSC-2209	C
-0251.72		cn[1]; e[D]	BSC-2167	C
-0251.73		C(1) RM, y[1] w[1]/+	BSC-3585	C
-0251.74		C(1) RM, y[1] w[1]/inc[1]	BSC-2582	C
-0251.75		C(1) RM, y[1] w[1]/stub[1]	BSC-3586	C
-0251.76		cu[S]	BSC-2117	C
-0251.77		cutsy[1]	BSC-1100	C
-0251.78		cutsy[1] ca[2]	BSC-1078	C
-0251.79		Cy[NC]	BSC-3582	C
-0251.81		dh[1] b[1] py[1] sd[1] pm[1]	BSC-917	C
-0251.82		dim[1]; osp-3[1]	BSC-2116	C
-0251.83		e[D]	BSC-2028	C
-0251.84		e[K]	BSC-1981	C
-0251.85		ey-2[1]	BSC-1977	C
-0251.86		f[66]	BSC-1084	C
-0251.87		fa[1] rb[1]	BSC-918	C
-0251.88		f[2]; nt[1] pr[1]; st[1] e[1]; ey[1]	BSC-3445	C
-0251.89a		g[1]	BSC-919	C
-0251.89b		g[1]	BSC-1773	C
-0251.91		g[1]; cn[1]; e[1]	BSC-3410/3035	C
-0251.92		g[1]; cn[1]; e[1]; ey[1]	BSC-3429	C
-0251.93		g[1]; cn[1]; e[1]; skew[1]	BSC-3451	C
-0251.94		H[3]/Ubx[m]	BSC-1102	C
-0251.95		jv[1] se[1]	BSC-920	C
-0251.96		jv[1] st[1] pe[1]	BSC-921	C
-0251.97		In(1)2, f[66]	BSC-1099	C
-0251.98		In(3R)Ubx, Ubx[m]/Dl[1]	BSC-1826	C
-0251.99		melon[1]	BSC-2110	C
-0251.101		m[65] f[65]	BSC-1087	C

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
14021	-0251.102	SIMULANS	net[1]	BSC-922	C
	-0251.103		net[1] dim[1]	BSC-1717	C
	-0251.104		np[1]	BSC-1716	C
	-0251.105		or-3[3]	BSC-1760	C
	-0251.106		osp-3[1]	BSC-447	C
	-0251.107		osp-3[1] st[1] e[1]	BSC-1895	C
	-0251.108		pk[1]	BSC-3361	C
	-0251.109		pn[2]	BSC-1085	C
	-0251.111		pr-3[1]	BSC-2212	C
	-0251.112		pur[1] osp-3[1]	BSC-3583	C
	-0251.113		pur[1] e[1]	BSC-3584	C
	-0251.114		py[1] Clr[1] pm[1]	BSC-1075	C
	-0251.115		rb[1]	BSC-2320	C
	-0251.116		ro[1] cutsy[1] ca[2]	BSC-1103	C
	-0251.117		se[1]	BSC-924	C
	-0251.118		sienna[1]	BSC-3385	C
	-0251.119		spd[1]/Ubx[m]	BSC-1101	C
	-0251.121		st[1] ca[1]	BSC-3409	C
	-0251.122		st[1] pe[1]	BSC-925	C
	-0251.123		stw clr	Mid-Am-0606	C
	-0251.124		tb[1]	BSC-2580	C
	-0251.125		ubx[m]/+	Mid-Am-0603	C
	-0251.126		v[1]	BSC-927	C
	-0251.127		v[2]; cutsy[1] ca[2]	BSC-1071	C
	-0251.128		v[1] f[2]	BSC-2443	C
	-0251.129		v[1] m[1]	BSC-3581	C
	-0251.131		v[1]; red[1] bar[1]	BSC-3580	C
	-0251.132		w[cr]	BSC-1086	C
	-0251.133		wf[2]	Mid-Am-1712	C
	-0251.134		w[le]	BSC-2191	C
	-0251.135		w[lt]	BSC-1090	C
	-0251.136		w[mky]	BSC-2206	C
	-0251.137		w[N]	BSC-2202	C
	-0251.138		w[R]	BSC-2166	C
	-0251.139		w[S]	BSC-2043	C
	-0251.141		y[1]	BSC-2180	C
	-0251.142		y[1] f[2]	BSC-2179	C
	-0251.143		y[2H]	BSC-2130	C
	-0251.144		y[e2]	BSC-1091	C
	-0251.145		y[e2] f[66]	BSC-1094	C
	-0251.146		y[2] v[2] f[66]	BSC-1089	C
	-0251.147		y[1] v[2] f[1] bb[1]	BSC-1627	C
	-0251.148		y[1] v[1] m[1] g[1] f[2]/C(1)RM, y[1] w[1]	BSC-2119	C

HOW TO READ THE LIST

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.		LOCALITY		MEDIUM
14021	-0251.149	SIMULANS	yw[am] v[2] f[66]			Mid-Am-0613	C
	-0251.151		y[2] w[am] m[65]			BSC-1092	C
	-0251.152		y[1] w[1] m[1] f[2]			BSC-2160	C
	-0251.161		wt(+)		GUATAMALA-3	BSC-911	C
	-0251.162		wt(+)		KUSHLA-F	BSC-912	C
	-0251.163		wt(+)		MORRO BAY	BSC-913	C
	-0251.164		wt(+)		SOUTH AFRICA	BSC-914	C
	-0251.165		wt(+)		FLORIDA CITY	BSC-1425	C
	-0251.166		wt(+)		ISLAMORADA	BSC-1093	C
	-0251.167		wt(+)		SOLWAY-HOCHMAN	BSC-1088	C
	-0257.0	TEISSIERI	(CAMBRIDGE UNIV. LAB.1977)			Mid-Am	C
	-0261.0	YAKUBA	2371.6		IVORY COAST, AFRICA		C
14022		SUBGROUP TAKAHASHII					
	-0271.0	LUTESCENS	3387.1		MITO, HONSHU, JAPAN		C
	-0271.1		3388.1		MITO, HONSHU, JAPAN		C
	-0281.0	PARALUTEA	3250.7		KHAO YAI, THAILAND		C
	-0291.0	PROSTIPENNIS	3146.61		WULAI, TAIWAN		C
	-0301.1	PSEUDOTAKAHASHII	3382.1		ATHERTON TABLELAND, NORTH QUEENSLAND, AUSTRALIA		C
	-0311.0	TAKAHASHII	2363.4		NEPAL, ASIA		C
	-0311.1		3072.1		KERALE, SOUTH INDIA		C
	-0311.2#		3075.4		WULAI, TAIWAN		C
	-0311.3#		3146.13		TAGAYTAY, LUZON, PHILIPPINES		C
	-0311.4		3146.14		LOS BANOS, LUZON, PHILIPPINES		C
	-0311.5		3146.16		YUN SHUI, TAIWAN		C
	-0311.6		3252.7		CHIANG DOA, THAILAND		C
14023		SUBGROUP SUZUKII					
	-0331.0	LUCIPENNIS	3068.3		CHI-TOU, TAIWAN		C
	-0331.1		3146.57		WULAI, TAIWAN		C
	-0331.2		3146.68		WULAI, TAIWAN		C
	-0341.0	MIMETICA	3033.29		KUALA LUMPUR, MALAYSIA		C
	-0351.0	PULCHRELLA	3040.11A		KIRISHIMA, JAPAN		C
	-0361.0	RAJASEKARI	3057.8A		ARI KSATR, CAMBODIA		C
	-0361.1		3057.8B		ARI KSATR, CAMBODIA		C
	-0361.2		3116.8B		BON CHAKKRARAT, THAILAND		C
	-0361.3		3253.9		HOSADURGA, INDIA		C
14024		SUBGROUP ANANASSAE					
	-0371.0	ANANASSAE	SB18.8C		TAMAZUNCHALE, S.L.P., MEXICO		C
	-0371.1		SB18.8D		TAMAZUNCHALE, S.L.P., MEXICO		C
	-0371.3		3045.1	(F-2)	HONOLULU, HAWAII		C
	-0371.4			(F-10)	TAPUTIMU, TUTUILA, SAMOA		C
	-0371.5			(F-14)	SURA, FIJI		C
	-0371.7#		2370.9	(F-16)	MAJURO		C

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.		LOCALITY	MEDIUM
14024	-0371.9	ANANASSAE		(F-25)	TONGA	C
	-0381.0	BIPECTINATA	2363.2		PATAN, NEPAL	C
	-0381.1		3054.3E		CABUYAO, LAGUNA, LUZON, PHILIPPINE ISLANDS	C
	-0381.2		3058.4		PAGO PAGO, AMERICAN SAMOA	C
	-0381.3		3160.18		CHIA-I, TAIWAN	C
	-0381.4		3256.4		SAMUT SONGKHRAM, THAILAND	C
	-0381.5#			(F-21)	UPOLU, SAMOA	C
	-0432.0	ERCEPEAE	164-14		DE REUNION, INDIAN OCEAN	C
	-0391.0	MALERKOTLIANA MALERKOTLIANA	3253.5		MYSORE, INDIA	C
	-0391.1		3256.6		SAMUT SONGKHRAM, THAILAND	C
	-0401.0	PARABIPECTINATA	3057.6A		ARI KSATR, CAMBODIA	C
	-0401.1#		3146.15		LOS BANOS, LUZON, PHILIPPINES	C
	-0401.2		3160.22		TAGAYTAY, PHILIPPINES	C
	-0401.3		3256.5		SAMUT SONGKHRAM, THAILAND	C
	-0434.0	PHAEOPLEURA	3044.4	(F-35)	SUVA, FIJI	C
	-0411.0	PSEUDOANANASSAE NIGRENS	3256.7		SAMUT SONGKHRAM, THAILAND	C
	-0421.0	PSEUDOANANASSAE PSEUDOANANASSAE	3251.8		CHIANG MAI, THAILAND	C
	-0431.0	VARIANS	3146.53		LOS BANOS, LUZON, PHILIPPINES	C
	-0433.0	PALLIDOSA		(F-5)	AOPO, SAVAII, SAMOA	C
	-0433.1			(F-7)	PAGO PAGO, SAMOA	C
	-0433.2			(F-8)	TAPUTIMU, TUTUILA	C
14025		SUBGROUP FICUSPHILA				
	-0441.0	FICUSPHILA	3075.8		KHAN-ING TONG, TAIWAN	C
	-0441.1		3075.9(ISO FEMALE)		KHAN-ING TONG, TAIWAN	C
14026		SUBGROUP EUGRACILIS				
	-0451.0	EUGRACILIS	3021.4		POPONDETTA, PAPUA, NEW GUINEA	C
	-0451.1		3056.5E		PALAWAN PHILIPPINES	C
	-0451.2		3057.5D		ARI KSATR, CAMBODIA	C
	-0451.3		3119.5C		SARAWAK, MALAYSIA	C
	-0451.4#		3251.7		CHIANG MAI, THAILAND	C
14027		SUBGROUP ELEGANS				
	-0461.0	ELEGANS	3140.2		BAGUIO CITY, LUZON, PHILIPPINES	CL
14028		SUBGROUP MONTIUM				
	-0471.0	AURARIA	3040.11B		KIRISHIMA JAPAN	C
	-0471.1		3040.15		NOPPORO, JAPAN	C
	-0481.0	BAIMAII	3033.24		KUALA LUMPUR, MALAYSIA	C
	-0481.1		3250.9		KHAO YAI, THAILAND	C
	-0491.0#	BARBARAE	3033.21		KUALA LUMPUR, MALAYSIA	C
	-0491.1		3033.3		KUALA LUMPUR, MALAYSIA	C
	-0491.2		3251.9		CHIANG MAI, THAILAND	C

HOW TO READ THE LIST

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
14028	-0491.3	BARBARAE	3256.12	SAMUT SONGKHRAM, THAILAND	C
	-0501.0	BIAURARIA	3255.1	KA-ARI, KOREA	C
	-0511.0	BICORNUTA	3146.18	KEN-TING, TAIWAN	C
	-0511.1		3146.9	KEN-TING, TAIWAN	C
	-0521.0	BIRCHII	3007.1	CAIRNS, AUSTRALIA	C
	-0521.1		3020.4	PAPUA, NEW GUINEA	C
	-0521.2		3028.9	GOROKA, NEW GUINEA	C
	-0586.0	DIPLACANTHA	188.7	BAFUT NGEMBA, CAMEROUN	C
	-0712.0	GREENI	168.5	LAMTO, IVORY COAST	C
	-0531.0	JAMBULINA	3116.11	BON CHAKKRARAT,THAILAND	C
	-0531.1		3117.6	CHANGWAT, THAILAND	C
	-0531.2		3120.5	SIEM REAP, CAMBODIA	C
	-0541.0	KANAPIAE	3138.6	TAGAYTAY, LUZON, PHILIPPINES	C
	-0561.0	KIKKAWAI	H436.64	LETICIA, COLOMBIA	C
	-0561.2#		3014.4	HERON ISLAND, AUSTRALIA	C
	-0561.3#		3014.5	OAHU, HAWAII	C
	-0561.4		3028.4	GOROKA, NEW GUINEA	C
	-0561.5		3046.6	WULAI, TAIWAN	C
	-0561.7		3256.11	SAMUT SONGKHRAM, THAILAND	C
	-0571.0	LACTEICORNIS	3364.1	OKINAWA, JAPAN	C
	-0581.0	LINI	3146.1	YUN-SHUI, TAIWAN	C
	-0591.0	MAYRI	3007.2	LAE, NEW GUINEA	C
	-0591.1		3020.6	PAPUA, NEW GUINEA	C
	-0601.0	NIKANANU	2371.5	IVORY COAST, AFRICA	C
	-0611.0	OROSA	3250.17	KHAO YAI, THAILAND	C
	-0621.0	PARVULA	3033.9	KUALA LUMPUR, MALAYSIA	C
	-0631.0	PENNAE	3028.1(29.1E)	GAROKA, NEW GUINEA	C
	-0641.0	PUNJABIENSIS	3033.4	KUALA LUMPUR, MALAYSIA	C
	-0651.0	QUADRARIA	3075.1	CHI TOU, TAIWAN	C
	-0661.0	RUFA	1736.3	HANGCHOW, CHINA	C
	-0661.1		3040.16	KIRISHIMA, JAPAN	C
	-0671.0	SEGUYI	3254.1	SALISBURY, RHODESIA	C
	-0671.1		st-2	ISLAMABAD, PAKISTAN	C
	-0681.0	SERRATA	2372.8	QUEENSLAND, AUSTRALIA	C
	-0681.1#		2404.6	QUEENSLAND, AUSTRALIA	C
	-0681.2		3018.1	RABAUL, NEW BRITAIN, AUSTRALIA	C
	-0681.3		3019.7	WAU, NEW GUINEA	C
	-0681.4		3022.1	MATARANKA, NORTHERN TERRITORY, AUSTRALIA	C
	-0691.0	TRIAURARIA	1736.1	HANGCHOW, CHINA	C
	-0691.1		3365.1	SHIZEN-EN, MEGURO, TOKYO, JAPAN	C
	-0701.0	TSACASI	2371.4	IVORY COAST, AFRICA	C
	-0711.0	VULCANA	3254.2	MOUNT SELINDA, RHODESIA	C
14030		GROUP WILLISTONI			

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
14030	-0721.0	CAPRICORNI	H442.22	PALMIRA, COLOMBIA	C
	-0721.1		3015.3	CANAL ZONE, PANAMA	C
	-0721.2		3015.9	CANAL ZONE, PANAMA	C
	-0741.0	EQUINOXIALIS	H50.4B	LA HINA, HONDURAS	C
	-0741.1		2533.3	TEFFE, BRAZIL	C
	-0751.1	FUMIPENNIS	H331.1	ARIMA VALLEY, TRINIDAD	C
	-0761.0	NEBULOSA	H101.14	FACULTAD DE AGRONOMIA, PALMIRA, COLOMBIA	C
	-0761.1		H176.10	SAN JOSE, COSTA RICA	C
	-0761.3		H63.2	MERIDA, YUCATAN	C
	-0761.4		2373.9	TINGO MARIA, PERU	C
	-0761.5#		2374.4	SAO PAULO, BRAZIL	C
	-0771.0	PAULISTORUM	163 PN	MESITAS	C
	-0771.1#		164 W	MESITAS	C
	-0771.2		165 Y	MESITAS	C
	-0771.3		166 W	LANCETILLA, HONDURAS	C
	-0771.4		168 W	LLANOS	C
	-0771.5		3366.1	GEORGETOWN, GUYANA	C
	-0771.6		H66.1C	INSTITUTO TROPICAL, SAN SALVADOR, SALVADOR	C
	-0771.7#		3397.I	PLAYA, YUCATAN PENINSULA	C
	-0771.8		3397.2	PLAYA, YUCATAN PENINSULA	C
	-0771.9		3397.3	LAGO ESCONDIDO, YUCATAN PENINSULA	C
	-0771.11		AMAZONIAN(AM)		C
	-0771.12		ANDEAN-SOUTH BRAZIL(AN)		C
	-0771.13		CENTROAMERICA(CA)		C
	-0771.14		ORINOCAN(OR)		C
	-0771.15		TRANSITIONAL(TR)		C
	-0791.0	SUCINEA	H316.7A	MEDELLIN, COLOMBIA	C
	-0791.1		H49.15	MONTE CEYUCA, HONDURAS	C
	-0791.2		H62.39	LA PALMA, EL SALVADOR	C
	-0791.3#		2263.4B	TEZIUTLAN, PUEBLA, MEXICO	C
	-0801.0	TROPICALIS	H65.2	INSTITUTO TROPICAL, SAN SALVADOR, EL SALVADOR	C
	-0811.0	WILLISTONI	H57.30	SANTA MARIA, DE OSTUNA, NICARAGUA	C
	-0811.1		H66.1A	INSTITUTO TROPICAL, SAN SALVADOR, EL SALVADOR	C
	-0811.2		1156.4	ROYAL PALM PARK, FLORIDA	C
	-0811.3		1802.2	ATLIXCO, MEXICO	C
	-0811.4#		2268.17	CUERNAVACA, MEXICO	C
	-0811.5#		SBI9.11A	HUICHIHUAYAN, S.L.P., MEXICO	C
	-0811.6		H414.4	FAIRCHILD GARDENS, S. MIAMI, FL	C
	-0814.0	WILLISTONI QUECHUA		LIMA, PERU	C
14040		GROUP SALTANS			

HOW TO READ THE LIST

STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
14041	SUBGROUP CORDATA			
-0831.0	NEOCORDATA	2536.7	MINAS GERAIS, BRAZIL	C
14042	SUBGROUP ELLIPTICA			
-0841.0#	EMARGINATA	H158.2	TURRIALBA, COSTA RICA	C
-0841.1		H176.1	SAN JOSE, COSTA RICA	C
-0841.2		H191.49	BUCARAMANGA, COLOMBIA	C
-0841.3#		H407.86	BOQUETE, PANAMA	C
-0841.4		H62.51	LA PALMA, EL SALVADOR	C
-0841.5#		H91.20	MEDELLIN, COLOMBIA	C
-0841.6		2262.8	HUATUSCO, VERACRUZ, MEXICO	C
-0841.7		H328.3	QUITO, ECUADOR	C
14043	SUBGROUP STURTEVANTI			
-0854.0	DACUNHAI	H409.6A	PETIONVILLE, HAITI	C
-0861.0	MILLERI	H129.17	EL YUNQUE, PUERTO RICO	C
-0871.0#	STURTEVANTI	H161.6	TURRIALBA, COSTA RICA	C
-0871.1		H191.23	BUCARAMANGA, COLOMBIA	C
-0871.2#		H252.16	VOLCAN SOUFRIERE, GUADELOUPE, LESSER ANTILLES	C
-0871.3#		H282.3	STORM CREEK, BRITISH HONDURAS	C
-0871.4		H358.30	HERMITAGE RESERVOIR, JAMAICA	C
-0871.5		H66.2	INSTITUTO TROPICAL, SAN SALVADOR, EL SALVADOR	C
-0871.6#		2264.9	TUXTLA, VERACRUZ, MEXICO	C
-0871.7		2374.3	MATO GROSSO, BRAZIL	C
-0871.8#		SB19.1OA	HUICHIHUAYAN, S.L.P., MEXICO	C
-0871.9		3327.2	MARTINIQUE, WEST INDIES	C
14044	SUBGROUP PARASALTANS			
-0872.0	SUBSALTANS	2536.2	BALEM, BRAZIL	C
14045	SUBGROUP SALTANS			
-0881.0	AUSTROSALTANS	2536.4	PIRASSUNUNGA, BRAZIL	C
-0891.0	LUSALTANS	H411.20	PETIONVILLE, HAITI	C
-0901.0#	PROSALTANS	H163.13	TURRIALBA, COSTA RICA	C
-0901.1		H191.68	BUCARAMANGA, COLOMBIA	C
-0901.2		H29.6	SAN SALVADOR, EL SALVADOR	C
-0901.3#		H303.3	BALBOA, PANAMA	C
-0901.4#		H436.5	LETICIA, COLOMBIA	C
-0901.5#		1401.4	HUICHIHUAYAN, S.L.P., MEXICO	C
-0901.6		1911.5	GUATEMALA CITY, GUATEMALA	C
-0901.7		2536.9	EL DORADO, BRAZIL	C
-0911.0	SALTANS	H180.40	SAN JOSE, COSTA RICA	C
15000	SUBGENUS DROSOPHILA			
15010	GROUP VIRILIS			
-0951.0	AMERICANA AMERICANA	3367.1 (standard)	ANDERSON, INDIANA	C
-0951.1		1760.8 POOL	POPLAR, MONTANA	C

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15010	-0951.2	AMERICANA AMERICANA	1761.9A	CHINOOK, MONTANA	C
	-0951.3		1882.66	MILLERSBURG, PENNSYLVANIA	C
	-0951.4		1893.10	KEELERS BAY, LAKE CHAMPLAIN, VERMONT	C
	-0951.5		1901.5A	JACKSON, MICHIGAN	C
	-0951.6		2067.1M	CHADSON, NEBRASKA	C
	-0951.7		2068.6D	OAKDALE, NEBRASKA	C
	-0951.8		2515.3	HALSEY, NEBRASKA	C
	-0951.9		3013.1	MYRTLE BEACH STATE PARK, SOUTH CAROLINA	C
	-0951.14		INDEPENDENCE(THROCKMORTON'S LAB.)		C
	-0961.0	BOREALIS	2077.4B (standard)	ITASCA PARK, MINNESOTA	C
	-0961.1#		2077.4D	ITASCA PARK, MINNESOTA	C
	-0961.2#		2077.4L	ITASCA PARK, MINNESOTA	C
	-0961.3		1950.1H	CHESTER, IDAHO	C
	-0961.4		1974A	JAMESTOWN, N. DAKOTA	C
	-0961.5		1973A	LYTTON, QUEBEC	C
	-0961.6		1974A	WHITESHELL PROVINCIAL PARK, MANITOBA, CANADA	C
	-0961.7#		ISO FEMALE d (homo 105)	WHITESHELL PROVINCIAL PARK, MANITOBA, CANADA	C
15010	-0961.8#		ISO FEMALE ee (homo 109)	WHITESHELL PROVINCIAL PARK, MANITOBA, CANADA	C
	-0961.9#		ISO FEMALE c	AMERICAN FORK CANYON, UTAH	C
	-1091.0	CANADIANA		SMITHERS, BRITISH COLUMBIA	C
	-0971.0	EZOANA	2531.1	HOKKAIDO RAUS, JAPAN	C
	-0971.1		3040.35	SUGADAIRA, JAPAN	C
	-0971.2		1303	KJARUVENSI, FINLAND	C
	-0981.0	FLAVOMONTANA	1950.1C	CHESTER, IDAHO	C
	-0981.1#		1974.A	CRAIG, COLORADO	C
	-0981.2		1974.C	CRAIG, COLORADO	C
	-0981.3		1951.1	HAMILTON, COLORADO	C
	-0981.4		ISO FEMALE hh	AMERICAN FORK CANYON, UTAH	C
	-0981.5		ISO FEMALE a	POMPEY'S PILLAR, MONTANA	C
	-1061.0	KANEKOI		SAPPORO, JAPAN	C
	-0991.0	LACICOLA	ISO FEMALE A	SARANAC, NEW YORK 1973	C
	-0991.1#		ISO FEMALE A(POOL)	SIOUX NARROWS, MANITOBA, CANADA	C
	-0991.4		ISO FEMALE A(POOL)	WHITESHELL PROVINCIAL PARK, MANITOBA, CANADA 1974	C
	-0991.12		1756.26	FENSKE LAKE, MINNESOTA	C
	-0991.13		1974A	BEAVER CREEK CAMP GROUNDS, MANITOBA, CANADA	C
	-0991.14		1973.A	BRULE, WISCONSIN	C
	-0991.15#		ST.86 (1985)	(DICKENSON) UTAH	C

HOW TO READ THE LIST

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15010	-0991.16#	LACICOLA	ST.84+1O2	(DICKENSON) UTAH	C
	-0991.17		ESTERASE NULL	(DICKENSON) UTAH	C
	-1001.0	LITTORALIS	2000.1	MELLINGEN, SWITZERLAND	C
	-1001.1#		2000.3	VITZNAU, SWITZERLAND	C
	-1001.2#		2096.1	KUHN AARGAU, SWITZERLAND	C
	-1001.3		3115.5	GHAZVIN, IRAN	C
	-1001.4		3161.1	ROSCOFF NORD-FINISTERE, FRANCE	C
	-1001.5		3354.1 POOL	GEORGIA, USSR	C
	-1001.6#		3354.2 POOL	POKZOUKE, RUSSIA	C
	-1001.7		471	ROVANIEMI, FINLAND	C
	-1011.1	LUMMEI	3264.1	MOSCOW, USSR	C
	-1011.2		478	OVERHALIX, SWEDEN	C
	-1011.3#		1102	KUOPIO, FINLAND	C
	-1011.4		1108	KUKKOLA, FINLAND	C
	-1011.5		1109	KARESJOKI, FINLAND	C
	-1011.6#		1136	PALTAMO, FINLAND	C
	-1011.7		1138	OULU, FINLAND	C
	-1011.8			SAKATA, HOKKAIDO, JAPAN	C
	-1021.0#	MONTANA	ISO FEMALE A	MOOSONEE, ONTARIO, CANADA	C
	-1021.6		ISO FEMALE A	BLACKSANDS, ONTARIO, CANADA	C
	-1021.9		ISO FEMALE A	GOTHIC, COLORADO	C
	-1021.11		ISO FEMALE B	LYTTON, QUEBEC	C
	-1021.13		ISO FEMALE	KAWASAKI, JAPAN	C
	-1021.14		1218.80	COTTONWOOD CANYON, UTAH	C
	-1021.15#		1767.5 POOL	SALMON RIVER, IDAHO	C
	-1021.16		1769.1A	GRAND TETON NATIONAL PARK, WY	C
	-1021.17#		1942.6F	VERDI, NEVADA	C
	-1021.18		1942.6J	VERDI, NEVADA	C
	-1021.19		2193.4A	MOUNT HOOD NATIONAL FOREST, OR	C
	-1021.21		2501.2A	YUKON, CANADA	C
	-1021.22#		3000.1 POOL	ANCHORAGE, ALASKA	C
	-1021.23		3003.1	BETHEL, ALASKA	C
	-1021.24		3334.1	LYTTON, QUEBEC	C
	-1021.25#		ST 81+93+106	(DICKENSON) UTAH	C
	-1021.26#		ST 100+118	(DICKENSON) UTAH	C
	-1021.27#		renull #3	(DICKENSON) UTAH	C
	-1021.28#		renull #4	(DICKENSON) UTAH	C
	-1021.29#		renull #5	(DICKENSON) UTAH	C
	-1023.0	MONTANA OVIVORORUM	479	KARESUANDO, SWEDEN	C
	-1031.0	NOVAMEXICANA	ISO FEMALE B	GRAND JUNCTION, COLORADO	C
	-1031.4		1975A	MOAB, UTAH	C
	-1031.7		A2.13	PATAGONIA, ARIZONA	C
	-1031.8		1714.4	SAN ANTONIO, NEW MEXICO	C
	-1031.11#		1720.7 POOL	GILA, NEW MEXICO	C

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15010	-1031.12	NOVAMEXICANA	1952.2	ANTLERS, COLORADO	C
	-1031.13		2358.6	PATAGONIA, ARIZONA	C
	-1041.0	AMERICANA TEXANA	ISO FEMALE A	ST. FRANCISVILLE, LOUISIANA	C
	-1041.2		NO.l CN;DIM(SPONTANEOUS)		C
	-1041.3		N0.2 CN;ROUGH-4		C
	-1041.4		N0.4 CN;(HK)(2-3)		C
	-1041.5		NO.5 MALE/A CN(2-3)		C
	-1041.6		N0.40 WIDE		C
	-1041.7		N0.43 DARK EYE		C
	-1041.8		N0.45 IND. SPR. MALE/J ROUGHENED 2-3		C
	-1041.9		N0.52 (IND. SPR. MALE) 141 SHORT VEINS-C		C
	-1041.11		N0.58 (IND. SPR. MALE/D) SHORT VEINS-B		C
	-1041.12		N0.60 (IND. SPR. MALE/C) F2(C) NH-LIKE		C
	-1041.13		N0.108 IND. SPR. MALE/F ROUGHENED B		C
	-1041.14		N0.109 CN-GP (2-3)		C
	-1041.15		NO.111 CN;WIDE		C
	-1041.16		N0.112 (IND.SPR.MALE/C)		C
	-1041.17		N0.113 MALE/C	SMOKY MTS., NORTH CAROLINA	C
	-1041.18		N0.114 CN RD(2-3)		C
	-1041.19		NO.116 MALE/C MAHOGANY DARK EYE		C
	-1041.21		N0.117 (IND.SPR.MALE/2H)-1041.22		
	-1041.22#		1128.10	NEW ORLEANS, LOUISIANA	C
	-1041.23		1591.1	MORRILTON, ARKANSAS	C
	-1041.24		1719.4	NEW ORLEANS, LOUISIANA	C
	-1041.25		1880.6A	SWIFT CREEK, SOUTH RICHMOND, VIRGINIA	C
	-1041.26#		2007.6	TALLAHASSEE, FLORIDA	C
	-1041.27#		2021.4A	GOLDENHEAD BRANCH STATE PARK, FLORIDA	C
	-1041.28		2301.5	CRESTVIEW, FLORIDA	C
	-1041.29		2320.2A	JAMESTOWN, SOUTH CAROLINA	C
	-1041.31		2326.1	HOLLANDALE, MISSISSIPPI	C
	-1041.32		NO.115 HK(2-3)		C
	-1051.0	VIRILIS	ORIGINAL	PASADENA, CALIFORNIA	C
	-1051.3		NO.14 V^{40D}; ES		C
	-1051.5		NO.26 CD, ES		C
	-1051.6		NO.38 CD		C
	-1051.7		NO.42 DT II		C
	-1051.8		NO.44	TRUCKEE, CALIFORNIA	C
	-1051.9		NO.47	SENDAI, JAPAN	C
	-1051.12		NO.51 ES, PE		C
	-1051.13		NO.53 ST, PE		C
	-1051.14		NO.55 Y^{40A}, SI, DY		C

HOW TO READ THE LIST

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15010	-1051.l5	VIRILIS	NO.57 SE, y^1, SC, CV		C
	-1051.16		NO.60 Y, MT, SB		C
	-1051.17		NO.61 W^{EO}		C
	-1051.18		NO.62 Y, CH^2 MT		C
	-1051.19		NO.63 CV, V, DY		C
	-1051.21		NO.64 v^{48-6}, ES		C
	-1051.22		NO.65 CD, ES (DE)		C
	-1051.23		NO.68 R, GP, CD, PE		C
	-1051.24		NO.71 B, T, CD, PE		C
	-1051.25		NO.74 B/2, (TB, GP)/3, CD/4, PE/5		C
	-1051.26		NO.75 CH^2 AP		C
	-1051.27		NO.76 CH^2, DY		C
	-1051.28		NO.80 Y^{40A}, EC, AP, 40E		C
	-1051.29		NO.81 SE, Y^P, SC, EC		C
	-1051.31		NO.84 ST B^3		C
	-1051.32		NO.85 B^4		C
	-1051.33		NO.86 ST PE		C
	-1051.34		NO.89 RU, ST, B^3, PE, (ES)		C
	-1051.35		NO.98 W^E PE		C
	-1051.36		NO.101 W^E, ST, PE		C
	-1051.37		NO.102 TB, GP^2, ST, 1		C
	-1051.38		NO.104 W^E	JAPAN	C
	-1051.39		NO.105 Y^{40A}, EC, MT		C
	-1051.41		NO.106 Y^{40A}, W^E, AP		C
	-1051.42		NO.119 CD, IV		C
	-1051.43		NO.121 SE, Y^1, SC, CV, X:I		C
	-1051.45		NO.124 Y^{40A} EC CV V SI^2 DY W AP^{40E}		C
	-1051.46		NO.125 B, TB, GP, CD, PE		C
	-1051.47		1736.7	HANGCHOW, CHINA	C
	-1051.48		1801.1	TEXMELUCAN, PUEBLA, MEXICO	C
	-1051.49		1999.1	CHACO, ARGENTINA	C
	-1051.51		2375.8	SANTIAGO, CHILE	C
	-1051.52		3355.1 POOL	U.S.S.R.	C
	-1051.53		CHROM. X w^{50112}		C
	-1051.54		2 b bk dt		C
	-1051.55		va		C
	-1051.56		3 gp^2		C
	-1051.57		sv t tb gp^2		C

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15010	-1051.58	VIRILIS	5	B3	C
	-1051.59			B3 pe	C
	-1051.61			pe	C
	-1051.62			ru	C
	-1051.63			ru st mh	C
	-1051.64			ru st mh pe	C
	-1051.65			st es pe^{JAP}	C
	-1051.66		X;5	v^{48a}, pe	C
	-1051.67		Y;5	T(Y;5)pe^{m1}; pe	C
	-1051.68		X/X/Y:pe^{m1}; pe(FEM.) x X/Y:pe^{m1};pe(MALE)		C
	-1051.69			T(Y;5)pe^{m15}; pe	C
	-1051.71		3;5	cn; pe	C
	-1051.72			gp^2; pe	C
	-1051.73			gp^2S/gp^2 +;pe(select for Star periodically)	C
	-1051.74			gp^2S/gp2+;ru st mh pe (select for Star periodically)	C
	-1051.75			T(3;5) pe^{m4} sv gp^2 pe^{m4}	C
	-1051.76			T(3;5) pe^{m51}	C
	-1051.77			T(3;5) pe^{m51} t tb gp^2 pe	C
	-1051.78		4;5	cd; pe	C
	-1051.79			T(4;5) pe^{m3}	C
	-1051.81		5;6	pe;gl	C
	-1051.82		2;3;5	b;T(3;5) pe^{m4}, cn sv gp^2 ru pe^{m4}	C
	-1051.83		2;3;4;5	b; tb gp^2; cd; pe	C
	-1051.84			b; sv t tb gp^2; cd; pe	C
	-1051.85			T(Y;)Ubx[223]/+ BSC-3439	C
15020		GROUP ROBUSTA			
	-1101.0	LACERTOSA	3040.41	KIRISHIMA, JAPAN	C
	-1111.0	ROBUSTA	1752.2 (vermilion)	TOMBIGBEE RIVER, ALABAMA	C
	-1111.1		1893.3	KEELERS BAY, LAKE CHAMPLAIN,VT	C
	-1111.2		1894.3	BLUE MOUNTAIN LAKE, NEW YORK	C
	-1111.3		2069.3	CRYSTAL LAKE, HASTINGS, NEBRASKA	C
	-1111.4#		2320.7	JAMESTOWN, SOUTH CAROLINA	C
	-1111.5		2547.19	UNIVERSITY ARBORETUM, UNIV. ALABAMA, BIRMINGHAM, ALABAMA	C
	-1111.6		3383.1	HARDWOOD FOREST, SOUTHERN IL	C
	-1111.7#		SB30.3A	AUSTIN, TEXAS	C
	-1111.8		ORANGE EYE		C
	-1121.0	SORDIDULA	2531.3	SAPPORO, HOKKAIDO, JAPAN	B

HOW TO READ THE LIST

STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15020 -1121.1	SORDIDULA	3040.38	SUGADAIRA, JAPAN	C
15030	GROUP MELANICA			
-1131.0	EURONOTUS	45B	ST. LOUIS, MISSOURI	C
-1141.0	MELANICA	2008.9	MYAKKA HEAD, FLORIDA	C
-1141.1		2075.2A	CLIFF, NEW MEXICO	C
-1141.2		2158.16	PATAGONIA, ARIZONA	C
15030 -1141.3		SB37.3A	AUSTIN, TEXAS	C
-1151.0	MICROMELANICA	2160.12	MADERA CANYON, SANTA RITA MTS., ARIZONA	C
-1151.1		3368.1	SMITHVILLE, TEXAS	C
-1161.0	PARAMELANICA	1891.6	GUARETTE, MAINE	B
-1161.1		1893.4	KEELERS BAY, LAKE CHAMPLAIN, VT	B
-1161.2		2017.9	SMOKEMONT, NORTH CAROLINA	B
15040	GROUP ANNULIMANA			
-1171.0	ARACATACAS	H161.10	TURRIALBA COSTA RICA	C
-1171.1		H186.38	SANTA MAR;A, MAGDELENA, COLOMBIA	C
-1171.2		H42.38	VOLCAN BOQUERON, EL SALVADOR	C
-1181.0	GIBBEROSA	3369.1	MEXICO	C
-1181.1		A526	ZAPILOTE CANYON, GUERRERO	C
-1191.0	TALAMANCANA	H66.12	SAN SALVADOR, EL SALVADOR	C
15050	GROUP CANALINEA			
-1201.0	CANALINEA	H378.4	MINATITLAN, VERACRUZ, MEXICO	BP
-1201.1		SBI9.9A	HUICHIHUAYAN, S.L.P., MEXICO	BP
-1201.2		SBI9.9B	HUICHIHUAYAN, S.L.P., MEXICO	BP
15060	GROUP DREYFUSI			
-1221.0	CAMARGOI	H154.32	TURRIALBA, COSTA RICA	B
-1221.1		H164.12	TURRIALBA, COSTA RICA	B
-1221.2		H231.10	GEORGETOWN, BRITISH GUYANA	B
15070	GROUP MESOPHRAGMATICA			
-1231.0	GAUCHA	2403.2	CHILE	C
-1231.1		3164.2	LA PAZ, BOLIVIA	C
-1231.2		3164.4 w	LA PAZ, BOLIVIA	C
-1231.3		3164.6	CAMPOS DO JORDAN, BRAZIL	C
-1241.0	PAVANI	3164.13	MENDOZA, ARGENTINA	C
15080	GROUP REPLETA			
15081	SUBGROUP MULLERI			
-1251.0	ALDRICHI	E51.3 POOL	FRANCISCO MEDRANO, TAMAULIPAS, MEXICO	BC
-1251.1		E5.1	WESLACO, TEXAS	BC
-1251.2		W-5	ACATLAN, PUEBLA, MEXICO	BC
-1251.3#		W-8	TEHUANTEPEC, OAXACA, MEXICO	BC
-1251.4		113.4 POOL	TEHUANTEPEC, OAXACA, MEXICO	BC
-1261.0	ANCEPS	E13.1A	PACHUCA HIDALGO, MEXICO	BC
-1261.1		E13.2	PACHUCA HIDALGO, MEXICO	BC

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15081	-1261.2	ANCEPS	A964	HUAHUAPAN DE LEON, OAXACA	BC
	-1261.3		A960	METZTITLAN HIDALGO, MEXICO	BC
	-1271.0	ARIZONAE	A116.3C	SAN LUIS POTOSI, MEXICO	C
	-1271.1#		A130.2	RIO BAVISPE, SONORA, MEXICO	C
	-1271.2#		A131.2	CABORCA, SONORA, MEXICO	C
	-1271.3#		A236.2	LOS MOCHIS, SINALOA, MEXICO	C
	-1271.4		E2.2	NAVOJOA, SONORA, MEXICO	C
	-1271.5		E28 POOL	VENADOS, HIDALGO, MEXICO	C
	–	-1271.6			E30.2A
	-1271.7#		E34.13 POOL	NAVOJOA AIRPORT,HIDALGO,MEXICO(?)	C
	-1271.8#		E36.3 POOL	EMPALME SONORA, MEXICO	C
	-1271.9#		E52.1 POOL	GUAYALEJO, TAMAULIPAS, MEXICO	C
	-1271.12		3358.1 POOL	GUAYALEJO, TAMAULIPAS, MEXICO	C
	-1271.13		A806(=816.1)	TOMATLAN JALISCO, MEXICO	C
	-1271.14		810.8(a,b+d)	TUXTLA GUTIERREZ, CHIAPAS	C
	-1281.0	BORBOREMA	3403.2	CAFARNAUM, MORRO DO CHAPEN, BAHIA, BRAZIL	BC
	-1281.1		3403.4	CAFARNAUM, MORRO DO CHAPEN, BAHIA, BRAZIL	BC
	-1281.2		3403.6	CAFARNAUM, MORRO DO CHAPEN, BAHIA, BRAZIL	BC
	-1281.3		3403.9(B17.2i)	CAFARNAUM, MORRO DO CHAPEN, BAHIA, BRAZIL	BC
	-1281.4		3403.10(B17.2j)	CAFARNAUM, MORRO DO CHAPEN, BAHIA, BRAZIL	BC
	-1291.0#	BUZZATII	H345.3	COCHABAMBA, BOLIVIA	B
	-1291.1		H345.13	COCHABAMBA, BOLIVIA	B
	-1291.2		H347.9	CORDOBA, ARGENTINA	B
	-1291.3#		H347.10	SAN RUIZ, ARGENTINA	B
	-1291.4#		1732.4	BYBLOS, LEBANON	B
	-1291.5		2093.10	AIN ANUB, LEBANON	B
	-1291.6		3340.1 POOL	SAN RAPHAEL, ARGENTINA	B
	-1291.7		3340.2	NEW SOUTH WALES, AUSTRALIA	B
	-1291.8#		3340.3	QUEENSLAND, AUSTRALIA	B
	-1291.9	BUZZATII (MUTANT)	ABNORMAL ABDOMEN		B
	-1291.11		ANTENNAPEDIA		B
	-1291.12		ARISTATARSH		B
	-1291.13		BLACK		B
	-1291.14		BOBBED		B
	-1291.15		BOW-WING		B
	-1291.16		BROWN		B
	-1291.17		BURGUNDY		B
	-1291.18		CADINAL		B
	-1291.19		CHERUB		B

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STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15081 -1291.21	BUZZATII (MUTANT)	CHOPPED		B
-1291.22		CINNABAR		B
-1291.23		CINNABAR CURLED		B
-1291.24		CRUMPLED		B
-1291.25		CURLY		B
-1291.26		DELTA 2		B
-1291.27		DELTA 5		B
-1291.28		DROOPED		B
-1291.29		JAUNTY		B
-1291.31		KARMOISIN		B
-1291.32		ORANGE		B
-1291.33		PINK		B
-1291.34		POINTY		B
-1291.35		PURPLE		B
-1291.36		RADIUS INCOMLETUS		B
-1291.37		RED		B
-1291.38		ROSY		B
-1291.39		ROUGH EYE		B
-1291.41		RUBY		B
-1291.42		RUDIMENTARY-LIKE		B
-1291.43		RUDIMENTARY-LIKE XX		B
-1291.44		RUSSET		B
-1291.45		SCARECROW		B
-1291.46		SHRIVELLED WING		B
-1291.47		SINGED		B
-1291.48		SPINELESS		B
-1291.49		VERMILION		B
-1291.51		VESICULATED		B
-1291.52		WAVY		B
-1291.53		WHITE		B
-1291.54		WHITE APRICOT		B
-1291.55		WING VEIN 5-LESS		B
-1291.56		WINGS NARROW		B
-1291.57		XX		B
-1291.58		YELLOW 1		B
-1291.59		YELLOW 2		B
-1291.61		YELLOW MINATURE WHITE		B
-1292.0	EREMOPHILA	E52.7 POOL	GUAYALEJO, TAMAULIPAS, MEXICO	BC
-1292.1		A955	ZAPOTITLAN SALIWAS, PUEBLA, MEXICO	C
-1301.0#	HAMATOFILA	E2.6 POOL	TUCSON, ARIZONA	C
-1301.1		E32.2	PORTAL, ARIZONA	C
-1301.4		A841 (1985)	SANTA RITA EXPT.RANGE,TUCSON, AZ	C
-1302.0	HEXASTIGMA	A739 (1985)	TEHUACAN, PUEBLA, MEXICO	BC
-1302.1		A842 (1985)	ZAPOTITIAN, PUEBLA, MEXICO	BC

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15081	-1302.2	HEXASTIGMA	A955A	ZAPOTITIAN, PUEBLA, MEXICO	BC
	-1302.3		A960	METZTITLAN HIDALGO, MEXICO	BC
	-1305.0	KOEPFERAE	890.1	VIPOS, ARGENTINA	B or BC
	-1305.1		705.1	PALO LABRADO, ARGENTINA	B or BC
	-1395.0	LEONIS	A804	GUADALAJARA, JALISCO, MEXICO	BC
	-1395.1		A964/966	HUAHUAPAN DE LEON/IZU DE MATAMOROS	BC
	-1311.0	LONGICORNIS	E25.1F (PEACH EYE)	AUSTIN, TEXAS	BC
	-1311.1		E26.12	SAN PEDRO MINES, HIDALGO, MEXICO	BC
	-1311.2		E30.6 POOL	SAN LUIS POTOSI, S.L.P., MEXICO	BC
	-1311.3#		E67.6 POOL	CUATRO CIENEGAS, COAHUILA, MEXICO	BC
	-1311.4#		E67.6 D	CUATRO CIENEGAS, COAHUILA, MEXICO	BC
	-1311.5#		E67.6 G	CUATRO CIENEGAS, COAHUILA, MEXICO	BC
	-1311.6		E67.6 K	CUATRO CIENEGAS, COAHUILA, MEXICO	BC
	-1311.7		2513.1 "TYPE"	AUSTIN, TEXAS	BC
	-1311.8#		SB21.1W	SABINAS, HIDALGO, N.L., MEXICO	BC
	-1311.9#		286	TUCSON, ARIZONA	BC
	-1311.11		A961A	S. MARIA DE REGLA, HIDALGO	BC
	-1315.0	MAINLANDI	A956	CATALINA ISLAND, LOS ANGELES, CA	BC
	-1321.0	MARTENSIS	H208.1	BARQUISIMETO, VENEZUELA	BC
	-1321.1		511.3	BARCELONA, VENEZUELA	BC
	-1397.0	MAYAGUANA	ORV 8 (1985)	BEEF ISLAND, TORTOLA, BWI	BC
	-1397.1		ORV 29 (1985)	GRAND CAYMAN ISLAND	BC
	-1397.2		ORV 21 (1985)	GREAT INAGUA ISLAND, BAHAMAS	BC
	-1397.3		901.20	FOND PARISIEN, HAITI	BC
	-1331.0	MERIDIANA MERIDIANA	E64.1 POOL	PALMETTO STATE PARK, TEXAS	BC
	-1341.0#	MERIDIANA RIOENSIS	E28.190	VENADOS, HIDALGO, MEXICO	C
	-1341.1#		E30.80	SAN LUIS POTOSI, S.L.P., MEXICO	C
	-1341.2		E37.4C	ALAMOS, HIDALGO, MEXICO	C
	-1341.3		H305.5B	MADDEN ROAD, CANAL ZONE, PANAMA	C
	-1341.4		H381.8 POOL	ACATLAN, PUEBLA, MEXICO	C
	-1341.5#		1802.4	ATLIXCO, PUEBLA, MEXICO	C
	-1341.6#		1813.10	MONTERREY, NUEVO LEON, MEXICO	C
	-1341.7		2251.7	GOMEZ FARIAS, TAMAULIPAS, MEXICO	C
	-1341.8		SB51.5A	DILLEY, TEXAS	C
	-1341.9#		SB56.3A	BASTROP COUNTY, TEXAS	C
15081	-1344.0	MERIDIANALIS	2507.21	ANGRA DOS REIS, BRAZIL	C
	-1502.0	METTLERI	A850 (1985)	ORGAN PIPE NATIONAL MONUMENT, ARIZONA	BC
	-1502.1		A855 (1985)	CERRO COLORADO SONORA	BC
	-1502.2		A956	SANTA CATALINA ISLAND, LOS ANGLES, CAIFORNIA	BC
	-1346.0	MICROMETTLERI	951.	BARAHONA, DOMINICAN REPUBLIC	BC
	-1346.1		ORV 24	PORT HENDERSON, JAMAICA	BC
	-1346.2		ORV 33	GUANTANAMO BAY, CUBA	BC

HOW TO READ THE LIST

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15081	-1351.0#	MOJAVENSIS BAJA	A116.4	ESPERANZA, SONORA, MEXICO	C
	-1351.1		A122.1	TIBURON ISLAND, GULF OF CAL.	C
	-1351.2#		A130.1	RIO BAVISPE, SONORA, MEXICO	C
	-1351.3		A132	SAN ESTABAN ISLAND, GULF OF CAL.	C
	-1351.4		A202	CUNANO, BAJA CALIFORNIA	C
	-1351.5#		A211	SAN FELIPE, BAJA CALIFORNIA	C
	-1351.6#		A240	HERMOSILLO, SONORA, MEXICO	C
	-1351.7#		A319	CABORCA, SONORA, MEXICO	C
	-1351.8		A337	LOS MOCHIS, SINALOA, MEXICO	C
	-1351.9		A350	SAN BORJA, BAJA CALIFORNIA	C
	-1351.11#		A352	PUNTA CONCEPCION, BAJA CALIFORNIA	C
	-1351.12		A367	SAN IGNACIO, BAJA CALIFORNIA	C
	-1351.13#		A372	PUNTA PESCADERO, BAJA CALIFORNIA	C
	-1351.14		A374	TODOS SANTOS, BAJA CALIFORNIA	C
	-1351.15#		E10.1 POOL	ENSENADA, BAJA CALIFORNIA	C
	-1351.16#		E2.3 POOL	NAVOJOA, SONORA, MEXICO	C
	-1352.0	MOJAVENSIS MOJAVENSIS	2533.1	CHOCOLATE MTS., RIVERSIDE CO., CA	C
	-1352.1		3340.4 POOL	ANZA BORREGO DESERT, CALIFORNIA	C
	-1352.2		A956	CATALINA ISLAND, CALIFORNIA	C
	-1352.3	MOJAVENSIS (MUTANT)	y(YELLOW BODY)	PUNTA ONAH, SONORA, MEXICO	C
	-1352.4		t(TAN BODY)	PUNTA ONAH, SONORA, MEXICO	C
	-1352.5		w(WHITE EYE)	PUNTA PRIETA, BAJA, CALIFORNIA	C
	-1352.6		v(VERMILLION EYE)	CHOCOLATE MT., CALIFORNIA	C
	-1352.7		ad(ADOBE EYE)	CERRO COLORADO, SONORA, MEXICO	C
	-1352.8		br(BROWN EYE)		C
	-1352.9		ru(RUBY EYE)	SANTA ROSA MT., NEAR TUCSON	C
	-1352.11		y-ad(YELLOW BODY/ADOBE EYE)		C
	-1352.12		yv(YELLOW BODY/VERMIION EYE)		C
	-1352.13		a(APRICOT EYE)		C
	-1371.0	MULLERI	E1.14 POOL	LAKE TRAVIS, TEXAS	C
	-1371.1		E45.1 POOL	PANUCO, VERACRUZ, MEXICO	C
	-1371.2		E46.1A VG	PANUCO, VERACRUZ, MEXICO	C or B
	-1371.3#		E52.5 POOL	GUAYALEJO, TAMAULIPAS, MEXICO	C
	-1371.4		G207 (CH. 4 INV.)		C
	-1371.5#		H409.16	PETIONVILLE, HAITI	C
	-1371.7		3358.2 POOL	GUAYALEJO, TAMAULIPAS, MEXICO	C
	-1371.8		3370.1	ROY FARM, AUSTIN, TEXAS	C
	-1371.9#		SB56.2A	BASTROP COUNTY, TEXAS	C
	-1371.11#		SB58.2A	AUSTIN, TEXAS	C
	-1371.12#		—	MONTANA	C
	-1371.13#		ORV 21 (1985)	GREAT INAGUA ISLAND, BAHAMAS	C
	-1371.14#		ORV 25	DISCOVERY BAY, JAMAICA	C
	-1371.15#		ORV 27	CAYMAN BRAC, CAYMAN ISLANDS	B

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15081	-1371.16	MULLERI	927.3	CAYMAN BRAC, CAYMAN ISLANDS	C
	-1371.17#		901.12	FOND PARISIEN, HAITI	C
	-1371.18		920.3	CONCEPTION ISLAND	C
	-1371.19		942.3	GONAIVES, HAITI	C
	-1371.21#		921.5M	GREAT INAGUA, BAHAMAS	C
	-1371.22		940.6	MONTECRISTI, DOM. REPUBLIC	C
	-1371.23		923.5A	DISCOVERY BAY, JAMAICA	C
	-1374.0	NAVOJOA	E2.1	NAVOJOA, SONORA, MEXICO	B
	-1374.1		811.11	TEHUANTEPEC, MEXICO	B
	-1374.2		A806	TOMATLAN, JALISCO	B
	-1381.0	NIGROCRURIA	H75.11 (1985)	HEREDIA, COSTA RICA	BC
	-1381.1		2263.15	TEZIUTLAN, PUEBLA, MEXICO	BC
	-1381.2		A966	IZUCA DE MATAMOROS PUEBLA, MEXICO	BC
	-1385.0	NIGRODUMOSA	514.8 "TYPE"	MERIDA, VENEZUELA	BC
	-1503.0	NIGROSPIRACULA	A42.2A	MAGDALENA, SONORA, MEXICO	BC
	-1503.1		3393.1	TUCSON, ARIZONA	BC
	-1503.2		A856	DESEMBOQUE	BC
	-1503.3		A866	CERRO COLORADO, SONORA	BC
	-1391.0	PACHUCA	E14.10A	CHAPINGO, MEXICO, MEXICO	BC
	-1391.2		E14.12A	CHAPINGO, MEXICO, MEXICO	BC
	-1391.3		E14.3 POOL	CHAPINGO, MEXICO, MEXICO	BC
	-1391.5		E16.1A(p-9a)	PACHUCA, HIDALGO, MEXICO	BC
	-1391.7		E26.15 POOL	SAN PEDRO MINES, HIDALGO, MEXICO	BC
	-1391.8		E26.3-1	SAN PEDRO MINES, HIDALGO, MEXICO	BC
	-1391.13		401.4U	PACHUCA, HIDALGO, MEXICO	BC
	-1392.0	PARISIENA	ORV 24	PORT HENDERSON, JAMAICA	BC
	-1392.1		ORV 33	GUANTANAMO BAY, CUBA	BC
	-1392.2		ORV 1	FOND PARISEIN HAITI	BC
15081	-1398.0	PEGASA	812.21D	0AXACA, OAXACA, MEXICO	BC
	-1398.1		626.1=E67.2	CUATROCIENGAS, COAHUILA	BC
	-1411.0	PROPACHUCA	E15.1 POOL stndard	TIANGUISTENCO, MEXICO	BC
	-1411.1		E16.2A(ISO FEMALE)	PACHUCA, HIDALGO, MEXICO	BC
	-1411.2		E30.7 POOL	SAN LUIS POTOSI, S.L.P., MEXICO	BC
	-1411.3		A960	METZTITLAN, HIDALGO, MEXICO	BC
	-1411.4		A961B	S. MARIA DE REGLA, HIDALGO, MEXICO	BC
	-1421.0	RICHARDSONI	E20.2	LA PARGUERA, PUERTO RICO	BC
	-1421.1		ORV 7 (1985)	MONTSERRAT AIRPORT	BC
	-1421.2		ORV 8	BEEF ISLAND, TORTOLA	BC
	-1471.2	RITAE	402.4 POOL	TEHUACAN, PUEBLA, MEXICO	BC
	-1471.3		A955C	ZAPOTITLAN SALINAS PUEBLA	BC
	-1431.1	SERIDO	3405.3	RIO PARAGUASSA, AEGOON, BAHIA, BRAZIL	BC
	-1431.2		3405.5	MILAGRES, BAHIA, BRAZIL	BC

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STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15081	-1431.3	SERIDO	3405.6	MILAGRES, BAHIA, BRAZIL	BC
	-1431.4		3405.9	CAFARNAUM, MORRO DO CHAPEN, BAHIA,BRAZIL	BC
	-1431.6		3405.11	CAFARNAUM, MORRO DO CHAPEN, BAHIA,BRAZIL	BC
	-1441.0	SPENCERI	E310.1	CUNANO, BAJA CALIFORNIA	BC
	-1441.1		A882	SAN CARLOS SONORA, MEXICO	BC
	-1441.2		A875	GUAYMAS, SONORA, MEXICO	BC
	-1451.0	STALKERI	2213.1	SAINT PETERSBURG, FLORIDA	BC
	-1451.1		ORV 25 (1985)	DISCOVERY BAY, JAMAICA	BC
	-1451.2		ORV 26 (1985)	CAYMAN BRAC, CAYMAN ISLANDS	BC
	-1451.3		961.11	SIGUA BEACH, CUBA	BC
	-1461.0	STARMERI	W-17	PUERTO CABELLO, VENEZUELA	BC
	-1461.1		501.1A	BARQUISIMETO, VENEZUELA	BC
	-1461.2#		501.30A	CORO, VENEZUELA	BC
	-1461.3		504.30H (ISO)	CORO, VENEZUELA	BC
	-1461.4		513.2	CARUPANO, VENEZUELA	BC
	-1461.5#		514.12	MERIDA, VENEZUELA	BC
	-1461.6#		302.1	LOS PALMARES, VENEZUELA	BC
	-1462.0	STRAUBAE	ORV 22	NAVASSA ISLAND, CARIBBEAN SEA	BC
	-1462,1		ORV 30	GUANTANAMO BAY, CUBA	BC
	-1462.2		ORV 1	FOND PARISIEN HAITI	BC
	-1496.0	VENEZOLANA	702.1	LA ESMERALDA, VENEZUELA	BC
	-1501.0	WHEELERI	A519.1	ARROYO SOCORRO, BAJA CALIFORNIA	BC
	-1501.1		A751 "TYPE"	ARCADIA, CALIFORNIA	BC
	-1501.2		A754	URUAPAN, MEXICO	BC
	-1501.3		A826	SANTA CATALINA ISLAND	BC
15082		SUBGROUP MERCATORUM			
	-1511.0	MERCATORUM	E9.I	PRAIA GRANDE, SAO PAULO, BRAZIL	C
	-1521.0	MERCATORUM MERCATORUM	H343.12	SANTA CRUZ, BOLIVIA	C
	-1521.1		H347.13	VALPARAISO, CHILE	C
	-1521.2		H442.15(yellow abd.)	PALMIRA, COLOMBIA	C
	-1521.4#		K96G8	LANAI INN, LANAI, HAWAII	C
	-1521.5#		1413.18	SAN JOSE, COSTA RICA	C
	-1521.7		170 Q VD BR 10	PUPUKEA, OAHU	C
	-1521.8		171 K23 BR 6	KAMUELA, HAWAII	C
	-1521.9		173 SL^Y W M (visible marker)		C
	-1521.11		174 V PM VL (visible marker)		C
	-1521.12		175 BR PM (visible marker)		C
	-1521.15#		2393.1	COCHABAMBA, BOLIVIA	C
	-1521.16		2507.7	ANGRA DOS REIS, BRAZIL	C
	-1521.18#		3011.1	ROCHESTER, NEW YORK	C

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15082	-1521.21#	MERCATORUM	3398.2	PUPUKEA, OAHU	C
	-1521.22		3398.3	KAMUELA, HAWAII	C
	-1521.23		3398.8	ROCHESTER, NEW YORK	C
	-1521.24#		3398.9	MINIZALES, COLOMBIA	C
	-1521.25		3404.1	RIO PARAGUASSA,ARGOIN,BAHIA,BRAZIL	C
	-1521.26#		3404.2	MILAGRES, BAHIA, BRAZIL	C
	-1521.28#		3404.5	RIO PARAGUASSA,ARGOIN,BAHIA,BRAZIL	C
	-1521.29#		3404.6	SAO PAULO, BRAZIL	C
	-1521.32#		3404.8	PRAIA GRANDE, SAO PAULO, BRAZIL	C
	-1531.0	MERCATORUM PARAREPLETA	E60.1	CAMPO GRANDE, BRAZIL	C
	-1531.2		H341.3	VILA ATLANTICA, BRAZIL	C
	-1531.3		1412.9A	JACAREPAQUA,FEDERAL DIST., BRAZIL	C
	-1531.4		1412.9B	SAO PAULO, BRAZIL	C
	-1541.0	PARANAENSIS	H194.44	VILLAVICENCIO, COLOMBIA	C
	-1541.1		H332.11	MARAVEL, TRINIDAD	C
	-1541.2		H378.6	MINATITLAN, VERACRUZ, MEXICO	C
	-1541.3#		H75.10	HEREDIA, COSTA RICA	C
	-1541.4		3335.1	CAMPO GRANDE, MATO GROSSO DO SUL, BRAZIL	C
	-1541.5		H435.60	LETICIA, COLOMBIA	C
	-1541.6#		4 B9	BARRO COLORADO IS., CANAL ZONE, PANAMA	C
15083		SUBGROUP FASCIOLA			
	-1551.0	ELLISONI	H336.9	BELEM, PARA, BRAZIL	BC
		SUBGROUP REPLETA			
	-1554.0	CANAPALPA	1402.17(1943)	JACALA, HIDALGO, MEXICO	BP
15084	-1561.0	FULVIMACULA FLAVOREPLETA	H194.28	VILLAVICENCIO, COLOMBIA	BP
	-1561.1		H51.13	LANCETILLA, HONDURAS	BP
	-1571.0#	FULVIMACULA FULVIMACULA	H181.8	BARRO COLORADO IS., CANAL ZONE, PANAMA	BP
	-1571.1		H302.28	LAS CRUCES TRAIL, CANAL ZONE, PANAMA	BP
	-1571.2		H403.46	CHANGUINOLA, PANAMA	BP
	-1571.4		H435.7	MARKO, BRAZIL	BP
	-1571.6		H8O.4	BARRO COLORADO IS., CANAL ZONE, PANAMA	BP
	-1571.7#		1808.37	OAXACA, OAXACA, MEXICO	BP
	-1571.8#		21B24	BARRO COLORADO IS.,CANAL ZONE, PANAMA	B
	-1571.9#		SB19.7B	HUICHIHUAYAN, S.L.P., MEXICO	BP
	-1581.0	FULVIMACULOIDES	A963	JALAPA, VERACNUZ, MEXICO	BP

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STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15084	-1591.0	LIMENSIS	1529.2A	LIMA, PERU	C
	-1594.0	MELANOPALPA	A9.15	TUCSON, ARIZONA	BP
	-1601.2	NEOREPLETA	1351.7	LAGUNA CAUSECAURO, MICHOACAN, MEXICO	C
	-1601.3		2160.15	MADERA CANYON, SANTA RITA MTS. AZ	C
	-1601.4		2358.16A	PATAGONIA, ARIZONA	C
	-1601.5		1402.17	JACALA, HIDALGO, MEXICO	C
	-1601.6		2251.8	GOMEZ FARIAS, COAHUILA, MEXICO	C
	-1401.0	PENINSULARIS	H130.0	RIO PIEDRAS EXPERIMENTAL STATION, PUERTO RICO	C
	-1401.1#		H260.20	MAYAGUEZ, PUERTO RICO	C
	-1401.2#		H268.4	MARICAO, PUERTO RICO	C
	-1401.3		H269.7 POOL	EL YUNQUE, PUERTO RICO	C
	-1401.4		H270.12	TREASURE ISLAND, PUERTO RICO	C
	-1401.5		H279.9	BATH BOTANICAL GARDENS, BATH, JAMAICA	C
	-1401.6#		H350.1	MAUTRA BRAE WOODS, JAMAICA	C
	-1401.7#		H352.2	OCHO RIOS, JAMAICA	C
	-1401.8		H411.22	PETIONVILLE WOODS, HAITI	C
	-1401.9#		2303.3	RIVERVIEW, FLORIDA	C
	-1401.12		2382.1	ILE DE PINOS, CUBA	C
	-1401.13		3371.2	TARPON SPRINGS, FLORIDA	C
	-1401.14#		ORV 25	DISCOVERY BAY, JAMAICA	C
	-1611.0	REPLETA	H118.1	BARBADOS, BRITISH WEST INDIES	C
	-1611.1		H17.8	IZALCO, EL SALVADOR	B
	-1611.2		H63.7	MERIDA, YUCATAN, MEXICO	C
	-1611.3		1	SUVA, FIJI	B
	-1611.5		3054.7C	CABUYAO LAGUNA,LUZON,PHILIPPINES	B
	-1611.6		960.7	SIBONEY, CUBA	B
	-1611.7		961.9A	SIGUA BEACH, CUBA	B
15085		SUBGROUP HYDEI			
	-1621.0	BIFURCA	A960	METZTITLAN HIDALGO	C
	-1621.1		DK FEMALE x LT MALE		C
	-1621.2		LT FEMALE x DK MALE		C
	-1631.0	EOHYDEI	H186.58	SANTA MARTA MTS., COLOMBIA	C
	-1631.1		H191.47	BUCARAMANGA, COLOMBIA	C
	-1631.2		H191.67	BUCARAMANGA, COLOMBIA	C
	-1641.0	HYDEI	1735.2	ZURICH, SWITZERLAND	C
	-1641.2#		E5O.I	FRANCISCO MEDRANO, TAMAULIPAS, MEXICO	C
	-1641.3		H194.23	VILLAVICENCIO, COLOMBIA	C
	-1641.4		H338.7	SAO PAULO, BRAZIL	C
	-1641.6#		H62.28	LA PALMA, EL SALVADOR	C
	-1641.7		T4		C

STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY		MEDIUM
15085 -1641.11	HYDEI	133 VF			C
-1641.12		135 Y TU-D			C
-1641.13		136 W^A			C
-1641.14		137 T0-2			C
-1641.15		139 689/9			C
-1641.16		140 689/21			C
-1641.17		141 CN; TU-P			C
-1641.18		142 V-I			C
-1641.22		145 340/1			C
-1641.23		146 340/10			C
-1641.24#		148 X-Y TRANSLOCATION 340/2			C
-1641.25		149 V, SC, SN, Y, MN, CH, BB			C
-1641.26		150			C
-1641.28		1854.10	PATAGONIA, ARIZONA		C
-1641.29#		2372.13	AUSTRALIA		C
-1641.31		2395.8	PISCO, PERU		C
-1641.32		3146.50	I-LAN, TAIWAN		C
-1641.33		3371.3	NADARIVATU, FIJI		C
-1641.34#		3398.7 (KH36-D-BI)	KAMUELA, HAWAII		C
-1641.35#			ORLANDO, FLORIDA		C
-1641.36		cn[1] sca[1] Stw[1]		BSC-1889	C
-1641.37		C(1)RM, w[hg] y[Lt]/R(Y)1, w[m] & w[iv]/R(Y)1, w[m]		BSC-1647	C
-1641.38		C(1)RM, y[1] m[1], ch[1] & w[iv] sn[1] y[1] m[1]		BSC-1995	C
-1641.39		cu-x[78] w[hg] y[1]		BSC-1570	C
-1641.41		et[1] & Tp(2)Anp/Sp[2] Dl[59], Kf[1]		BSC-3564	C
-1641.42		f[1-1]		BSC-1881	C
-1641.43		In(Ax, y , m) f[3]/T(1;2)v[t3] & T(1;2)v[t3]		BSC-3568	C
-1641.44		In(1) f[3] v[1]		BSC-2202	C
-1641.45		In(1) f[3] N[Ax-66h] y[1] m[1] ch[1]/spr[3-77] & spr[3-77]		BSC-1300	C
-1641.46		In(5) Rsh-i/Gk[1]		BSC-3565	C
-1641.47		In(1)w[m2H] f[68]		BSC-2049	C
-1641.48		T(1;5)Ba[F]/T(1;5)Ba[F] & T(1;5)Ba[F]/M(5)		BSC-3567	C
-1641.49		T(1;5)Ba[F]/T(1;5)Ba[F] & T(1;5)Ba[F]/In(5)Rsh, pb[1] Sf[1] Rsh[1]		BSC-3566	C
-1641.51		T(2;3;4)De		BSC-3576	C
-1641.52		T(Y;2;3;5)Do/pb[1] sca[1] cn[1] vg[1]		BSC-3570	C
-1641.53		v[1] sc[1] sn[1] y[1]		BSC-1821	C
-1641.54		w[hg]		BSC-1978	C
-1641.55		w[iv] f[74]		BSC-1857	C

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STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15085 -1661.1	NIGROHYDEI	A952	PORTAL, ARIZONA	C
15088	GROUP BROMELIAE			
-1682.0	BROMELIAE	ORV29	GRAN CAYMAN ISLAND, CARIBBEAN SEA	BC
15090	GROUP NANNOPTERA			
-1693.0	ACANTHOPTERA	A584.2	TEHUANTEPEC, OAXACA, MEXICO	BC
-1692.0	NANNOPTERA	H381.6 (1985)	ACATLAN, PUEBLA, MEXICO	BC
-1692.1		A836	TEHUACAN, PUEBLA, MEXICO	BC
-1692.2		800.6	GUERRERO, MEXICO	BC
-1697.0	WASSERMANI	A973	TEHUANTEPEC, OAXACA, MEXICO	BC
-1697.1		A974	TUXTLA-GUTIERREZ, CHIAPAS, MEXICO	BC
15100	GROUP POLYCHAETA			
-1701.0	ASPER	3330.1	HWALIEN, TAIWAN	C
-1711.0	POLYCHAETA	H435 44	LETICIA COLOMBIA	C
-1711.1		3035.1	SAMOA	C
-1711.2#		3117.3A	BAN PHRA BANGKOK, THAILAND	C
-1711.3		3117.3B	BAN PHRA BANGKOK, THAILAND	C
-1711.4		3300.6A	HAWAII	C
-1711.5		ORV 4	HATILLO, DOMINICAN REPUBLIC	C
15110	GROUP IMMIGRANS			
15111	SUBGROUP IMMIGRANS			
-1721.0	FORMOSANA	3033.44	KUALA LUMPUR, MALAYSIA	C
-1721.1		3116.53	BON CHAKKRARAT, THAILAND	C
-1721.2#		3146.22	WULAI, TAIWAN	C
-1721.3		3160.13	CHI-PENG, TAI-TUNG, TAIWAN	C
-1721.4		3160.9	TOROKO, TAIWAN	C
-1731.0	IMMIGRANS	H101.5	PALMIRA, COLOMBIA	C
-1731.1		H204.16	CARIPE, VENEZUELA	C
-1731.3		2363.1	PATAN, NEPAL	C
-1731.4		2395.2	LIMA, PERU	C
-1731.5		3066.4	CENTRAL HONSHU, JAPAN	C
-1741.0	SIGNATA	3120.4-1	SIEM REAP, CAMBODIA	C
-1741.1		3121.4-4	SEMONGOK FOREST RESERVE,SARAWAK, MALAYSIA	C
15112	SUBGROUP NASUTA			
-1751.0	ALBOMICANS	3045.11	OKINAWA	C
-1751.1#		3048.2	ALISHAN, TAIWAN	C
-1751.2		3066.11	MAKUNG, PENG HU IS., PESCADORES	C
-1751.3		3066.2	NAKANG, TAIWAN	C
-1751.4		3116.3-44	BON CHAKKRARAT, THAILAND	C
-1751.5		3155.2	KOMI, ISHIGAKI IS., JAPAN	C
-1751.6#		3160.36	PULI, NAN-TOU, TAIWAN	C
-1751.7#		3252.2-3	CHIANG DOA, THAILAND	C

STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15112 -1761.1	KEPULAUANA	3121.3-2	SEMONGOK FOREST RESERVE, SARAWAK MALAYSIA	C
-1761.2		3122.3-2	BRUNEI, BORNEO	C
-1771.0#	KOHKOA	3116.3-55	BON CHAKKRARAT, THAILAND	C
-1771.1		3121.3-1	SARAWAK, MALAYSIA	C
-1771.2#		3167.2	MOUNT MAKILING, LUZON, PHILIPPINES	C
-1771.3		3256.3	SAMUT SONGKHRAM, THAILAND	C
-1771.4		3266.1-2	RIZAL, PHILIPPINES	C
-1771.5		3344.1	KOTA KINABALU, MALAYSIA	C
-1781.0	NASUTA	3253.1(ISO F)	MYSORE, INDIA	C
-1781.1		3257.1B	SEYCHELLES IS., AFRICA	C
-1781.2#		3345.1	SEYCHELLES IS., AFRICA	C
-1781.6		3345.5	MOMBASA, KENYA	C
-1781.7#		3345.6	MOMBASA, KENYA	C
-1781.8#		3345.7	TANANARIVE, MADAGASCAR	C
-1781.9		3345.8	TANANARIVE, MADAGASCAR	C
-1781.11		3345.9	KANDY, SRI LANKA, INDIA	C
-178I.12#		3345.10	KANDY, SRI LANKA, INDIA	C
-1801.0	PULAUA	3121.5	SEMONGOK FOREST RESERVE, SARAWAK, MALAYSIA	C
-1811.0	SULFURIGASTER ALBOSTRIGATA	3033.17	KUALA LUMPUR, MALAYSIA	C
-1811.1#		3057.2-9	ARI KSATR, CAMBODIA	C
-1811.2#		3116.2A	BON CHAKKRARAT, THAILAND	C
-1811.3#		3119.2B	SEMONGOK FOREST RESERVE, SARAWAK, MALAYSIA	C
-1811.4		3120.2A	SIEM REAP, CAMBODIA	C
-1811.5		3122.2-13	BRUNEI, BORNEO	C
-1811.6		3138.2	TAGAYTAY, LUZON, PHILIPPINES	C
-1811.7		3347.1	SINGAPORE, INDONESIA	C
-1811.8		3347.2	KANDY, SRI LANKA, INDIA	C
-1821.0	SULFURIGASTER BILIMBATA	3045.1B	TANTALUS, OAHU, HAWAII	C
-1821.1		3045.2	PAGO PAGO, TUTUILA, AMERICAN SAMOA	C
-1821.2		3045.6	PALMYRA ISLAND, LINE ISLANDS	C
-1821.3#		3045.7	AOPO, SAVAII, WESTERN SAMOA	C
-1821.4		3045.8(BRIGHT EYE)	APIA, UPOLU, WESTERN SAMOA	C
-1821.5		3045.10	TONGATAPU, TONGA ISLANDS	C
-1821.6#		3063.2	NADARIVATU VITI LEVU, FIJI	C
-1821.7		3065.2	SAVUSAVUITANGGA, VANUA LEVU, FIJI	C
-1821.8		3071.6	GUAM, MARIANA ISLANDS	C

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STOCK NO.	SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
-1831.0	SULFURIGASTER SULFURIGASTER	2372.16	QUEENSLAND, AUSTRALIA	C
-1831.1		3017.4	KAVIENG, NEW IRELAND	C
-1831.2		3019.8	WAU, NEW GUINEA	C
-1831.4		3381.1	KAVIENG, NEW IRELAND	C
15114	SUBGROUP LINEOSA			
-1861.0	LINEOSA	3253.3	MYSORE, INDIA	C
15115	SUBGROUP HYPOCAUSTA			
-1871.0	HYPOCAUSTA	2535.3	KOLONIA, PONAPE, CAROLINE ISLANDS	C
-1871.1		3071.7	AGANA, GUAM, MARIANA ISLANDS	C or B
-1871.2		3134.4	MOUNT VIEW COLLEGE, BUKIDON, PHILIPPINES	C or B
-1871.3		3324D.1	WAU, NEW GUINEA	C or B
-1881.0	NEOHYPOCAUSTA	3351.1	I-LAN, TAIWAN	C
-1891.0	PARARUBIDA	3146.45	LOS BANOS, LUZON, PHILIPPINES	C
-1891.1		3146.69	TAGAYTAY, LUZON, PHILIPPINES	C
-1901.0	RUBIDA	3017.3	KAVIENG, NEW IRELAND	B
-1901.1		3019.1	WAU, NEW GUINEA	C
-1901.2		3020.7	PAPUA, NEW GUINEA	C
15120	GROUP FUNEBRIS			
-1911.0	FUNEBRIS	1042.4 VIRMILLION	STURGIS, KENTUCKY	C
-1911.1		1387.4A	MEXICO CITY, MEXICO	C
-1911.2#		2082.1	MINNEAPOLIS, MINNESOTA	C
-1911.3		2093.18	AIN ANUB, LEBANON	C
-1911.5		2351.8	ESPANOZA, NEW MEXICO	C
-1911.6		2503.2	BIG LAKE, ALASKA	C
-1911.7		3115.3	TEHRAN, IRAN	C
-1921.0	MACROSPINA LIMPIENSIS	2158.3	PATAGONIA, ARIZONA	C
-1921.1		2165.6	COCONIA NAT. FOREST, ARIZONA	C
-1931.0	MACROSPINA MACROSPINA	A2.1	PATAGONIA, ARIZONA	C
-1931.1		1241.5	RADIUM SPRINGS, NEW MEXICO	C
-1931.2		1261.1	HERMOSILLO, SONORA, MEXICO	C
-1931.3		1509.4	ALDRICH FARM, AUSTIN, TEXAS	C
-1931.4		1784.12	DURANGO, MEXICO	C
-1931.5		2073.4	ALBUQUERQUE, NEW MEXICO	C
-1931.6		2319.3(ISO F)	MILLER, GEORGIA	C
-1931.7		2321.13A(ISO F)	CROSS ANCHOR, SOUTH CAROLINA	C
-1941.0	MULTISPINA	3068.11	SAPPORO, HOKKAIDO, JAPAN	B
-1951.1	SUBFUNEBRIS	2181.3	WILLOW CREEK, CALIFORNIA	B
15130	GROUP QUINARIA			
-1961.0	FALLENI	3390.1	ITHACA, NEW YORK	B
-1971.0	GUTTIFERA	3151.3	AUSTIN, TEXAS	B

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15130	-1971.1	GUTTIFERA	3157.1	BASTROP STATE PARK, TEXAS	B
	-1971.2		3373.1	FAYETTEVILLE, ARKANSAS	B
	-1971.3		3373.2	UNIV. OF ARKANSAS, FAYETTEVILLE, ARKANSAS	B
	-2001.0	PALUSTRIS	1757.13	LAKE BEMIDJI, MINNESOTA	B
	-2031.0	PHALERATA PHALERATA	1951.1	BEIRUT, LEBANON	B
	-2071.0	SUBPALUSTRIS	1877.9	GEORGETOWN, SOUTH CAROLINA	B
	-2071.1		3012.2	MYRTLE BEACH STATE PARK, SOUTH CAROLINA	B
15150		GROUP TESTACEA			
	-2101.0	PUTRIDA	2067.3	CHADRON, NEBRASKA	B
	-2101.1		2539.1	AUSTIN, TEXAS	B
15160		GROUP CALLOPTERA			
	-2121.0	ORNATIPENNIS	H252.5	MATOUBA, GUADALUPE	B
	-2121.2		2378.2	VALE DE VINALES, ST.VICENTE, CUBA	B
15170		GROUP GUARANI			
15171		SUBGROUP GUARAMUNU			
	-2131.3	GRISEOLINEATA	H91.28	FACULTAD DE AGRONOMIA, MEDELLIN, COLOMBIA	B
15172		SUBGROUP GUARANI			
	-2151.0	GUARANI	H343.22	MONTERO, BOLIVIA	B
	-2151.1		2211.2	FELIZ, RIO GRANDE DO SUL, BRAZIL	B
	-2161.0	SUBBADIA	2262.24	HUATASCO, VERACRUZ, MEXICO	B
	-2161.1		3008B.2	EL NARANGO, MEXICO	B
15180		GROUP CARDINI			
15181		SUBGROUP CARDINI			
	-2171.1	ACUTILABELLA	H413.7 (MULTIPLE)	PETIONVILLE, HAITI	B
	-2171.2		2378.3	SAINT VICENTE, CUBA	B
	-2171.4#		H137.5	STONY HILL, JAMAICA	B
	-2171.7		H358.1	HERMITAGE RESERVOIR, JAMAICA	B
	-2171.8		H414.8A(LIGHT ISO F)	MIAMI, FLORIDA	B
	-2171.9		H415.6A(LIGHT IOS F)	EVERGLADES, FLORIDA	B
15181	-2181.1#	CARDINI	H207.29	CARPENTARO, VENEZUELA	B
	-2181.3		H340.37	MOGI DAS CRUZES, BRAZIL	B
	-2181.4		H409.15A(ISO F)	PETIONVILLE, HAITI	B
	-2181.8#		2263.6	TEZIUTLAN, PUEBLA, MEXICO	B
	-2181.9		2389.5	CONTRAMAESTRE, CUBA	B
	-2181.11		2395.6	PISCO, PERU	B
	-2181.12#		SB54.2 POOL	WESLACO, TEXAS	B
	-2181.13		ORV 29	GRAND CAYMAN	B
	-2181.14#		(FROM H. CARSON ,UNIV. OF HAWAII)		B
	-2191.0	CARDINOIDES	H188.23(FEW BRIST.)	SANTA MARTA, MAGDALENA, COLOMBIA	B
	-2191.2		H191.21	BUCARAMANGA, COLOMBIA	B
	-2191.3		H27.9	LAGO, PICHICHUELA, EL SALVADOR	B

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STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15181	-2191.6	CARDINOIDES	H428.44	EL REAL, DARIEN, PANAMA	B
	-2191.7#		2253.2	VALLES, S.L.P., MEXICO	B
	-2191.8#		2256.2(MORE BRIST.)	TAMAZUNCHALE, S.L.P., MEXICO	B
	-2201.0	NEOCARDINI	H339.I	SAO PAULO, BRAZIL	B
	-2221.0	PARTHENOGENETICA	A337	SINALOA, MEXICO	B
	-2221.1		1802.17	ATLIXCO, PUEBLA, MEXICO	B
	-2221.2		A877	LOS HORNOS, MEXICO	B
	-2231.0	POLYMORPHA	A396 (LIGHT)	DUACA, VENEZUELA	B
	-2231.1		A78.227(INTERMEDIATE)	PORTO ALEGRE, BRAZIL	B
	-2231.2		H333.32(LIGHT)	MARAVAL, TRINIDAD	B
	-2241.0	PROCARDINOIDES	H346.8	COROICO, BOLIVIA	B
15182		SUBGROUP DUNNI			
	-2251.0	ANTILLEA	H122.1	SAINT LUCIA, WINDWARD ISLANDS	B
	-2261.0	ARAWAKANA ARAWAKANA	H252.7	GUADALOUPE, LESSER ANTILLES	B
	-2261.1		3270.8	MONTSERRAT, LEEWARD ISLANDS	B
	-2261.2		3270.9	MONTSERRAT, LEEWARD ISLANDS	B
	-2271.1	BELLADUNNI	H356.3F(ISO F)	HARDWARE GAP, JAMAICA	B
	-2281.0	CARIBIANA	H248.1	FORTE DE FRANCE, MARTINIQUE	B
	-2291.0	DUNNI DUNNI	H254.21	MAYAGUEZ, PUERTO RICO	B
	-2301.0	DUNNI THOMASENSIS	H253.5	SAINT THOMAS, WEST INDIES	B
	-2311.0	NIGRODUNNI	H116.2	TURNER HALL WOODS, BARBADOS	B
	-2311.1		H247.1	MONKEY HILL, BARBADOS	B
	-2321.0	SIMILIS GRENADENSIS	H239.6	GRENADA, WEST INDIES	B
15210		GROUP PALLIDIPENNIS			
	-2331.0	PALLIDIPENNIS PALLIDIPENNIS	H183.17	CERRO LA CAMPANA, PANAMA	CL
	-2331.1		H191.48	BUCARAMANGA, COLOMBIA	CL
	-2331.2		H203.51	CARIPE, VENEZUELA	CL
	-2331.3		H341.5	VILA ATLANTICA, BRAZIL	CL
	-2331.4		H62.58(CLEAR EYE)	LA PALMA, EL SALVADOR	CL
15220		GROUP TRIPUNCTATA			
	-2341.0	CROCINA	H131.2	RIO PIEDRAS, PUERTO RICO	B
	-2341.I		H166.9	LA LOLA, COSTA RICA	B
	-2341.3		H437.10	MARKO, BRAZIL	B
	-2351.0	MEDIODIFFUSA	H130.6	RIO PIEDRAS, PUERTO RICO	B
	-2351.3		H260.1	MAYAGUEZ, PUERTO RICO	B
	-2351.4		H267.4	MARICAO, PUERTO RICO	B
	-2371.0	MEDIOPICTOIDES	H407.32	BOQUETE, PANAMA	B
	-2391.0	MEDIOSTRIATA	H107.11 X H109.21	ARIMA VALLEY, TRINIDAD	B
	-2391.1		H341.I3	VILA ATLANTICA, BRAZIL	B
	-2391.2		H403.3	CHANGUINOLA, PANAMA	B
	-2391.3		H435.12	LETICIA, COLOMBIA	B
	-2391.4		H442.5	PALMIRA, COLOMBIA	B

STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15220	-2401.0#	TRIPUNCTATA	1878.6	MORROW MT. STATE PARK, NORTH CAROLINA	B
	-2401.1#		1910.4	DEXTER, MISSOURI	B
	-2401.2		2003.3	NEW ORLEANS, LOUISIANA	B
	-2401.3		2010.7	EVERGLADES NATIONAL PARK, FLORIDA	B
	-2401.4		2300.6	DESOTO NATIONAL FOREST, MISSISSIPPI	B
	-2401.5		2512.1	STEPHEN FULLER AUSTIN EXP. FOREST, NACOGDOCHES, TEXAS	B
	-2411.0	UNIPUNCTATA	H192.29 X H192.17	RIO NEGRO, COLOMBIA	B
	-2411.1		H203.12	CARIPE, VENEZUELA	B
	-2411.2		H400.19	PALMARDE NORTE, COSTA RICA	B
	-2411.4		H91.14	MEDELLIN, COLOMBIA	B
15250		GROUP REPLETOIDES (=TUMIDITARSUS)			
	-2451.0	REPLETOIDES	1736.6	HANGCHOW, CHINA	C
	-2451.1		3040.43	SUGINAMI, TOKYO, JAPAN	C
15270		GROUP HISTRIO			
	-2461.0	STERNOPLEURALIS	3040.5	KIRISHIMA, JAPAN	B
15280		GROUP PICTURE-WINGED			
15283		SUBGROUP PLANITIBIA			
	-2491.0	PICTICORNIS	M11J3	KOKEE, KAUAI	WC
15284		SUBGROUP HAWAIIENSIS			
	-2501.0	GYMNOBASIS	M64G2	AUWAHI, MAUI	C
	-2511.0	SILVARENTIS	K18N4	HUMUULA SADDLE, HAWAII	WC
15287		SUBGROUP GRIMSHAWI			
	-2531.0	CRUCIGERA	C63.4	PUPUKEA, OAHU	WC
	-2531.1		C63.4 ST	PUPUKEA, OAHU	WC
	-2531.2		WH29A.7	KOKEE, KAUAI	WC
	-2531.3		WH29A.7B BROWN BODY MUTANT		WC
	-2541.1	GRIMSHAWI	PK-9	PUU KOLEKOLE, MOLOKAI	WC
15290		GROUP MODIFIED MOUTHPARTS			
15291		SUBGROUP HYSTRICOSA			
	-2551.0	BISERIATA	M63L26	MAKALEHA, OAHU	WC
	-2551.1		R43Y100	MOUNT KAALA, OAHU	WC
	-2551.2		R43Y7	MOUNT KAALA, OAHU	WC
15292		SUBGROUP MIMICA			
	-2561.0	MIMICA	K85P1	KIPUKA KI, HAWAII	WC
	-2561.1		S3Y42	KIPUKA PUAULU, HAWAII	WC
	-2561.2		T113Y1	PAPA, SOUTH KONA, HAWAII	WC
	-2561.3		T176Y6	BIRD PARK, HAWAII	WC
	-2561.4		T176Y12	BIRD PARK, HAWAII	WC
	-2561.5		T176Y15	BIRD PARK, HAWAII	WC
	-2561.6		T178Y8	BIRD PARK, HAWAII	WC
	-2561.8		T176Y15 ST	BIRD PARK, HAWAII	WC

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STOCK NO.		SPECIES SUBSPECIES	OLD STOCK NO.	LOCALITY	MEDIUM
15292	-2561.9	MIMICA	T1Y1	MOHANALOA, KAUAI	WC
15290		MODIFIED MOUTHPARTS GROUP NOT IN SUBGROUP			
	-2581.0	EURYPEZA	WH31.14D	KUMUWELA, KAUAI	WC
	-2591.0	SOONAE	C129.2	PAWAINA, HAWAII	C
15000		SPECIES (UNCERTAIN CLASSIFICATION) WITHIN SUBGENUS DROSOPHILA			
	-0656.0	PONERA		ILE DE LA REUNION	C
	-2595.0	IRI	FROM TSACAS	CONGO	C
	-2597.0	FRABURU	FROM TSACAS	CONGO	CL
20000		GENUS CHYMOMYZA			
	-2621.0	AMOENA	3392.1	LAKE CHARLEVOIX, EAST JORDAN, MI	CL
	-2631.1	PROCNEMIS	2370.3	OAHU, HAWAII	B
30000		GENUS SCAPTOMYZA			
31000		SUBGENUS PARASCAPTOMYZA			
	-2641.0	ADUSTA	2368.1	AUSTIN, TEXAS	BC
	-2651.3	ELMOI	K22A107	PALIKU, HALEAKALA, MAUI	BC
33000		SUBGENUS BUNOSTOMA			
	-2661.0	ANOMALA	G32C1	DRY FOREST, LANAI	BC
	-2681.1	PALMAE	3379.3	WAIMEA, HAWAII	BC
50000		GENUS ZAPRIONUS			
	-2741.0	TUBERCULATUS	3030.3	ALEMAYA, ETHIOPIA	C
	-2742.0	BADYI	194-4		C
	-2743.0	GHESQUIEREI	119-1		C
	-2744.0	SEPSOIDES	242-7 (1983)		C
50000	-2746.0	INERMIS		CAMEROUN, KOUTABA	C
60000		GENUS LIODROSOPHILA			
	-2751.0	AEREA	3040.51	SUGADAIRA, JAPAN	BC
80000		GENUS SAMOAIA			
	-2761.0	LEONENSIS	3058.5B	PAGO PAGO, TUTUILA, AMERICAN SAMOA	WC
	-2761.3		3060.5	AFIAMALU ROAD, UPOLU, WESTERN SAMOA	WC

Research Notes

Arnault, C.[1], **and C. Terzian**[2]. [1] Université Claude Bernard, URA CNRS 2055, 69622 Villeurbanne Cedex, France, and [2] Centre de Génétique Moléculaire, CNRS, 91198 Gif sur Yvette Cedex, France. Variability of segregation events on X chromosomes in *Drosophila melanogaster* inbred lines.

Segregation events resulting from mitotic recombinations contribute to create variability not only in somatic tissues but also in germline stem cells which produce clones genetically distinct from the surrounding tissues of the females. In *Drosophila melanogaster*, such clones can be identified by the dominant female sterile mutation ovo^{D1}, which allows one to detect changes concerning the X chromosome in lines where no morphological marker is available (Busson *et al.*, 1983; Perrimon and Gans, 1983). After crosses between ovo^{D1} males and wild type females, F_1 females able to lay fertile eggs normally appear with low frequencies. Their fertility may result from events occurring in maternal gametes (nondisjunction of X chromosomes) or in germ cells of heterozygous ovo^{D1} /+ females. In this latter case restoration of fertility may result from mitotic recombinations in the germ line (producing clones genetically distinct from the surrounding tissues of the female), or from recessive mutations at the ovo^{D} gene (Mével-Ninio *et al.*, 1989).

In this study we used 14 highly-inbred lines of *D. melanogaster* in which a polymorphism between generations was described by the insertion patterns of transposable elements (Biémont and Gautier, 1988). This polymorphism can be attributed either to a low rate of active transpositions followed by genetic drift, or to unequal recombinations generating excisions or new insertion sites (Biémont, 1986). Polymorphic sites were still detectable after a very elevated number of generations of inbreeding. In order to learn the precise nature and the importance of segregation events concerning their X chromosomes, the lines were crossed with the ovo^{D1} strain, and checked for the rate of fertile F_1 females. Their progenies were observed and tested by genetic and molecular methods.

The line K 1237 bears the dominant female-sterile allele ovo^{D1} at the site 4E, on the X chromosome marked also by the vermilion recessive gene, *v*. The ovo^{D1} allele is maintained in the male genome by crosses with X-linked females (Busson *et al.*, 1983). The ovo^{r} line, with a recessive allele of the *ovo* gene (balanced by the FM3 complex), was used for allelism test, eventually to detect a situation of true reversion of the dominant female-sterile gene to its recessive form. The highly-inbred lines, initiated from a wild population (Biémont, 1986), had been maintained by sib-crosses and then by small mass mating, and were from 171 to 196 generations old. They are wild type for the *ovo* gene, and they present

Table 1. Crosses between females of 14 highly-inbred lines and males from the ovo^{D1} strain of *D. melanogaster*. Presence of fertile females in F1 indicates occurrence of segregation events involving the X chromosomes of the lines.

Line No.	Number of individual crosses	F1 sex ratio males/ females	F1 females	Fertile F1 females				
				total	with 2 ovaries	with 1 ovary	non-identified	with males *v* in F2*
2	15	0.92	1175	5	—	—	5	1
4	9	—	678	8	—	+	+	2
5	89	1.01	8380	68	16	15	37	4
7	8	—	325	2	0	2	0	1
8	24	1.09	873	2	—	—	2	0
9	8	—	882	7	—	—	7	+
10	34	1.19	1230	3	—	—	3	0
11	5	—	394	3	—	—	3	0
13	33	0.99	4621	23	3	0	20	0
14	19	0.96	296	1	—	—	1	0
15	48	0.90	4223	71	17	1	53	0
16	15	0.89	1776	7	—	—	7	0
17	34	1.02	1143	9	5	0	4	0
18	17	1.17	626	2	1	0	1	0

+ = presence, 0 = absence, — = not determined

* proportions of male phenotypes: 50% *v*, 50% wild-type

the red-wild colour of eyes. All the lines were M' concerning the P element, and I for the I/R system, thus not able to be submitted to dysgenic conditions when crossed with the *ovo* strain. The experiments were performed at 25°C, in standard medium. The crosses were initiated with three ovo^{D1} males and three virgin females per vial for each inbred line. After 48 h, each female was isolated in a vial containing fresh medium for larval development. The F_1 flies were then transferred into a new vial. If F_2 eggs were observed, the ovaries were examined through the cuticle of the abdomen of each F_1 female. Sometimes no ovary was visible whereas few eggs had been laid on the medium: in this case we considered that one fertile unidentified female was present. The female fertility was calculated in percent of the total F_1 females.

In the case of non-disjunction of the X chromosomes, the F_1 females have two ovaries and their offspring is wild type. In the case of the reversion of ovo^{D1} into its recessive form by mutation, the F_1 females have only one ovary and the *v* phenotype is observed in 50% of the F_2 males. Fertile females found in F_1 after mitotic recombinations also have only one ovary, but the *v* phenotype in the F_2 males appears very scarcely, only after recombination between *v* and *ovo* (which are 22 CM apart). When a *v* F_2 male was found, it was crossed with X-linked females, to keep its X chromosome in male progeny. This new subline was used to study the kind of event having originated the fertility of the F_1 female, by allelism test with the ovo^r strain, and molecular analyses. To detect any insertion at the *ovo* locus, according to Mével-Ninio *et al.* (1989), the male larvae were dissected for *in situ* hybridization on the salivary gland polytene chromosomes with biotinylated probes (Biémont, 1994), and adult DNA was extracted for Southern blots and probed by the *ovo* sequence.

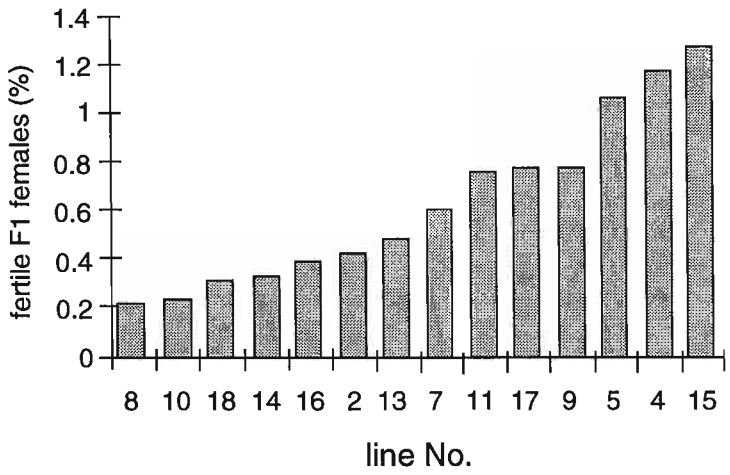

Figure 1. Classification of the 14 inbred lines of *Drosophila melanogaster* according to the percentage of fertile female progeny, after crosses with males ovo^{D1}. The numbers of individual crosses for each line are indicated in the Table.

The Figure illustrates the variability among the 14 inbred lines for fertility of the F_1 females, which ranges from 0.2 to 1.3%. Seven lines show a very elevated rate of fertile females, over 0.5% (3 of them >1%). The Kruskal-Wallis non-parametric test of multiple comparisons (analysis of variance by ranks) has been used to discriminate the importance of events. It indicates a highly-significant variability among the lines ($H = 51$, 13 df, $p < 10^{-4}$). The Table summarizes the data collected for the 14 lines with the description of the fertile F_1 females. The proportions of females with one or two ovaries is very variable, suggesting that several kinds of events (meiotic non-disjunction, mitotic recombinations or transpositions) can originate fertility in a line. Sometimes it was not possible to identify the fertile female, in spite of the presence of larvae, but this progeny was always very small (<10 fertilized eggs); this could be interpreted as tardive event in the germ line, resulting in a few fertile genotypes.

The analysis of the F_2 issued from the fertile F_1 females (sib crosses) reveals that most often no *v* males appear, thus most of the observed events may result from a crossing-over between the centromere and the *v* gene. In the few cases of an apparition of the *v* phenotype, it is attributed to a crossing-over between *v* and ovo^{D1}, or to an *ovo* recessive mutation. Indeed, in the lines 2, 4, 5, 7 and 9, some fertile F_1 females produced 50% of males *v* in F_2. The lines 4 and 5 (which also produced elevated proportion of fertile females) retained our attention because they presented a low but detectable polymorphism for the insertion patterns of the copia and mdg1 retrotransposons, which had been regularly analyzed through generations (Arnault and Loevenbruck, 1993). The *v* males of both lines were analyzed to look for ovo^{D1} 'reversion'. After *in situ* hybridization for copia and gypsy (transposable elements susceptible to induce reversion), according to Mével-Ninio *et al.* (1989), no insertion was detected in the 4E site on the X polytene chromosomes. Moreover, the DNA restriction analysis showed the normal size for the *ovo* *Eco*RI fragment. Thus, no new sequence was inserted into the gene. However, after digestion by *Pst*I, the Southern blot may suggest that some non-meiotic changes had occurred in the flanking sequence of the *ovo* locus, or that a *Pst*I polymorphism exists. The allelism test by crosses with the ovo^r line produced very variable results according to the males tested, even for those issued from the same mother. We observed either 100% of female sterility (suggesting apparition of a recessive *ovo* allele in the genome of the

inbred line), or total fertility (presence of an ovo^+ allele), or intermediate situations. The causes of restoration of fertility can thus be different among gametes, and several events can take place in the germ line.

The detection of variability following the ovo^{D1} sterility test of the inbred lines is in agreement with the mosaic state of the reproductive cells after somatic recombinations. The high rates of events, appearing here spontaneously in 50% of the inbred lines, may be of different origins and may occur simultaneously in the same individual. Such rates are unusual according to the literature, but can be compared with the disorders induced after X-ray treatments (Perrimon, 1984). A variable dose-effect of the expression of the *ovo* gene could also exist, like in other X-linked sterility genes (Banga *et al.*, 1995). The female fertility restored with the inbred lines after crosses with the ovo^{D1} strain can be attributed in some cases to abnormal pairings of the X chromosomes in the maternal gametes of the inbred line. According to our observations, this case exists but is not the most frequent, since no deficit appeared in the proportion of F_1 males. In this situation, two maternal X chromosomes in the gamete may restore fitness, when deleterious mutations occur on the X of the father, and thus may allow the line to remain viable. Hence, the non-disjunction could represent an adaptive advantage in highly-inbred lines. The majority of reversions observed in our experiments are attributed to mitotic exchanges, taking place in germline cells of early embryos, mostly in the centromeric region as usually described, but sometimes between the *ovo* and *v* loci. These exchanges may occur any time during mitosis and are not linked to sexual reproduction.

Acknowledgments: We thank N. Prud'homme and C. Biémont for comments and discussions, and M. Mével-Ninio for having kindly provided the *ovo* strains and probe. This work was supported by the Centre National de la Recherche Scientifique.

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Zajonz, M., S.R. Ramesh and W.-E. Kalisch. Ruhr-Universität Bochum, FR Germany. Chromosomal linkage of 130 kD secretion protein fractions in *D. sulfurigaster sulfurigaster* of the *D. nasuta* subgroup.

Secretion (glue) proteins in *D. nasuta* subgroup members (Wilson *et al.*, 1969) have the following features:

[1] The SDS-PAGE patterns of secretion proteins are similar in the whole *nasuta* subgroup. However, concerning prominence, electrophoretic mobility, presence of double or multiple bands instead of single bands, and PAS reaction of individual fractions, we could show by analyzing 30 wild type strains of eight *nasuta* members that the SDS-PAGE patterns are species-specific as well as wild type strain-specific (Ramesh and Kalisch, 1989a).

[2] Major (prominent) protein fractions in all members can be grouped (according to the domains of electrophoretic mobilities) into domains I - V of which domain II and III fractions are all X-chromosomal linked. By different experimental data we can assume that the genetic information of domain II and III fractions in all members are clustered within the huge puff of polytene X-chromosome division 10 (Ramesh and Kalisch, 1988a, 1989a).

[3] Most of domain II and III secretion fractions are composed of closely migrating double or multiple bands (Ramesh and Kalisch, 1988a). We assume that domain II as well as domain III fractions are the product of duplications of ancestral chromosome sections during evolution.

[4] Domain I, IV, and V fractions indicate almost no variations (exceptions: *D. s. albostrigata* and *D. s. sulfurigaster*) concerning the electrophoretic mobilities in different members of the *nasuta* subgroup. As a result, secretion fractions of domain I, IV, and V could not be analyzed so far by interspecific hybridization technique involving different members of the *nasuta* subgroup to distinguish between autosomal and gonosomal linkage (Ramesh and Kalisch, 1988b, 1989a).

Domain I bands include 130 kD fractions, which are located in the separating gel next to the border of the stacking gel, if our standard SDS-PAGE technique is used (l0 mm long 5.6% stacking gel together with a 13.4% separating gel; Ramesh and Kalisch, 1989b). We rechecked the 130 kD (domain I) fractions in the present study by changing the proportions of the different gel parts. We used alternatively 25 mm as well as 75 mm long stacking gels instead of our standard 10 mm long stacking gel. In the SDS-PAGE with a 25 mm long stacking gel (Figure lA) the 130 kD fractions were allowed just to pass the border of the separating gel. In the SDS-PAGE with a 75 mm long stacking gel (Figure 1 B) the 130 kD fractions were stopped just before passing the border of the separating gel.

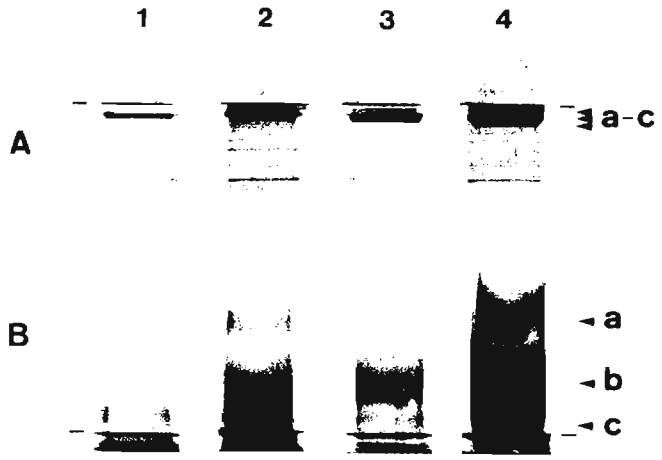

Figure 1. Chromosomal linkage of 130 kD secretion fractions. (1) *D. n. nasuta* female larva; (2) F_1 hybrid female larva of the cross: *D. n. nasuta* female × *D. s. sulfurigaster* male; (3) F_1 hybrid male larva of the same cross; (4) *D. s. sulfurigaster* male larva. (A) Homologous parts of SDS-PAGE gels composed of a 25 mm long 5.6% stacking gel and a 13.4% separating gel. Borders between the stacking gels and the separating gels are labeled. The 130 kD fractions (a - c) are located in the separating gel. (B) Homologous parts of SDS-PAGE gels composed of a 75 mm long 5.6% stacking gel and a 13.4% separating gel. The 130 kD fractions (a - c) are located in the stacking gel. (a) *D. s. sulfurigaster* specific X-chromosomal fraction; (b) *D. s. sulfurigaster*-specific autosomal fraction; (c) *D. n. nasuta*-specific fraction of unknown linkage (see text). Salivary glands were fixed for 3 min in ethanol; the two plugs of individual larvae were used for each lane; SDS-PAGE; Ag/CBB-staining.

We checked two cross-fertile members of the *D. nasuta* subgroup, which indicate a difference in the 130 kD fractions: *D. n. nasuta* (Mysore 1) and *D. s. sulfurigaster* (Bowling Green code of species stock list 1990: 15112-1831.1). Both members can only be hybridized in one direction: *D. n . nasuta* females with *D. s. sulfurigaster* males.

D. n. nasuta indicates single fraction-c (lane A1 and B1 in Figure 1) whereas *D. s. sulfurigaster* indicates more prominent fraction-a and fraction-b (A4 and B4), which could be identified as closely migrating double bands by PAS reaction (Ramesh and Kalisch, 1989b). Fraction-c in *D. n. nasuta* is not identical concerning prominence and electrophoretic mobility with fraction-b in *D. s. sulfurigaster* (B1 and B4 in Figure 1). Furthermore, one cannot distinguish from (B4) whether or not fraction-c of *D. n. nasuta* is also to be found beside fraction-a and fraction-b in *D. s. sulfurigaster*.

F_1 female hybrids (A2 and B2) and F_1 male hybrids (A3 and B3) in Figure 1 can be used for chromosomal linkage: Data of A1 - A4 in Figure 1 indicate that the hybrid F_1 female larva (lane A2) presents a double fraction whereas the hybrid F_1 male larva (lane A3) only presents a single fraction. By these data one could assume that all the 130 kD fractions are X-chromosomally linked.

However, B1 - B4 in Figure 1 indicate that the situation is somewhat more complicated. *D. n. nasuta* shows one 130 kD fraction (fraction-c), which is to be found in both F_1 hybrids (B2 and B3). However, by the data presented in Figure 1 and additional experiments not depicted, we could not be sure whether fraction-c also exists in *D. s. sulfurigaster*. If fraction-c would be present and covered by the more prominent fraction-b in *D. s. sulfurigaster*, the data would indicate an autosomal linkage. However, so far fraction-c could not be chromosomally localized by the data presented.

Fraction-a and fraction-b in *D. s. sulfurigaster* belong to different chromosomes. Fraction-a is only present in the hybrid F_1 female larva (lane B2), which indicates X-chromosomal linkage, whereas fraction-b is to be found in both sexes of F_1 hybrids (lane B2 and B3), which indicates autosomal linkage. Further linkage experiments are planned to show whether or not fraction-a in *D. s. sulfurigaster* can also be localized in the huge puff of X-chromosomal division 10 of polytene chromosomes.

The interesting part of the simple data presented is the fact that domain I double bands in *D. s. sulfurigaster* are not linked and, therefore, hardly can be the result of a duplication of an ancestral chromosome section during evolution. This is in contrast to our assumption concerning so far investigated domain II and domain III fractions in the whole *nasuta* subgroup.

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Padmaja Mandalaparthy, Sharon Jiang†, Greg Schneider°, and Carol J. Chihara. Department of Biology, University of San Francisco, 2130 Fulton St., San Francisco, CA 94117. The cuticle proteins of *Drosophila melanogaster*: Sequence of Lcp5 in the third chromosome cluster.

Drosophila melanogaster produces five cuticles during its life cycle, one for each of the three larval instars followed by the pupal and finally the adult cuticle. The nine cuticle proteins of the third larval instar are unique and are numbered 1 - 8 in the order in which they migrate on a denaturing gel, including a minor protein designated 2a.

The genes of the third instar larval cuticle proteins have been mapped to two clusters within the *Drosophila* genome. The genes for Larval cuticle proteins 1-4 and a pseudogene (1) have been placed in a cluster within 7 kb of each other at 44D on the right arm of chromosome two. The genes for Lcps 5,6,8 and an induced mutant protein Lcp10 (Rho) have been mapped to approximately 11 on the left arm of chromosome three (del Puerto and Chihara, 1988; Kimbrell *et al.,* 1988). Lcps 5 and 6 are first detected during the late second instar stage (presumably when the third instar cuticle proteins are first being made, prior to the molt of the second instar cuticle (Kimbrell *et al.*, 1988). Many of the identified insect larval and pupal cuticle protein genes display considerable sequence similarity throughout their carboxy terminal regions, these include larval *Drosophila melanogaster* proteins, Lcp -14 of *Manduca sexta*, SC 1 of *Sarcophaga bullata*, and HCCP-12 from *Hyalophora cecropia.*

An early third instar cDNA library in lambda gt 10 was screened with a degenerate 20 base pair oligo probe made from DNA sequences that coded for amino acid 13 to amino acid 19 of the N-terminal end of the mature Larval cuticle protein 5. We report the first characterization of a positive cDNA clone encoding the fifth cuticle protein of the third larval instar of *Drosophila melanogaster*. In order to make a probe for the *Lcp5* gene we isolated and sequenced the N-terminal end of *Lcp5.* We report here the N- terminal sequence of protein 5 and the complete sequence of a cDNA clone for *Lcp5.*

An early third instar larval gt10 cDNA library of *Drosophila melanogaster* was generously donated by Dr. Thomas Kaufman of Indiana University. The phage were grown in E. coli C - 600 hfr host.

All culture media were from DIFCO laboratories. Reagents were from either Sigma chemical company or J.T. Baker. Restriction enzymes and DNA standards were from New England Biolabs, Pharmacia or IBI. The fifth larval protein was purified by cutting the protein band from a 12% non-denaturing acrylamide gel. Protein five was isolated from wild-type cuticle and also from protein five in the omega mutant (Chihara and Kimbrell., 1986). The bands were electro-blotted onto Immobilon - P paper and sent to the facility at University of California, San Francisco for sequencing. The degenerate oligonucleotide probe was commercially synthesized by The Microchemical Facility at University of California, Berkeley. The lambda gt10 library was screened with the degenerate oligo probe labelled with ^{32}P on the 5' end. The insert DNA was amplified by PCR from the lambda gt10 clone. A single pure plaque of the phage vector containing the cDNA insert was dissolved in 100 ml of sterile water and vortexed. The reaction mix contained 5µl of this preparation as template DNA, with 1 µl gt10 forward and reverse primers (from New England Biolabs) at a concentration of 25 picomoles /µl, 1 µl of 10 mM dNTPs, 5 µl Taq buffer (10X) and 0.5 µl Taq enzyme in a total volume of 50 µl. The first cycle of PCR was 10 minutes at 95°C, followed by 35 cycles

```
1    GATCAAACAG TTCCAAGTTT TCTAACAAAC ACCACACAGC
41   TCCAACATGA AATTCCTCAT CGTCTTCGTC GCCCTCTTCG
           M K  F L I  V F V  A L F A
81   CCATGGCAGT GGCCCGCCCC AACCTTGCCG AGATCGTGAG
      M A V  A R P N L A E  I V R
121  GCAGGTCTCC GATGTTGAGC CCGAGAAGTG GAGCTCCGAC
      Q V S  D VE P E K W S  S D
161  GTGGAGACCA GCGATGGCAC CAGCATCAAA CAGGAGGGTG
      V E T S  D G T  S I K  Q E G V
201  TCTCAAGAAC GTGGCACTGC AAACGAGTGC GTGTGTCACG
       S R T  W H C?  K R V R  V S R
241  GATCCTTCAC CTGGGTGGAT GAGAAGACCG GCGAGAAGTT
       I L H  L G G  * E D R  R E V
281  CACCGATCAC ATACGTGGCT GATGAGAACG GATACCAGCC
       H R S H  T W L  M R T  D T S P
321  CCAGGGCGCC CATCTGCCCG TGGCACCAGT TGCTTAAGAT
        R A P  I C P  W H Q  L L K M
361  GTTTTCCAAA TCGATCAAAG AGTTTAAAAT AAATCAAAAT
       F S K  S I K E  F K I  N Q N
401  GCTTTAAATT AAATCAAAAT GCTTTAAATT
       A L N *
```

Figure 1. The signal sequence is underlined by letter, the sequence of amino acids which match those of the N-terminal end of Lcp5 are double underlined and the amino acids used for the degenerate probe are in bold letters. The putative poly A signal is underlined. The molecular weight of protein 5 is approximately 15,000D by SDS-PAGE (unpublished data). Thus we assume that codon AAT at position 407 is the C terminal amino acid. (The termination codon at position 260 is speculated to be an error incorporated during sequencing).

of 30 sec at 95°C, 30 sec at 52°C, 1 min at 72°C, and then 1 cycle of 10 minutes at 72°C. 10 µl of the PCR product was analyzed on a 0.8% agarose gel (modified from Maniatis, Fritsch and Sambrook, 1982).

The amplified insert DNA was subcloned into the plasmid vector pCRTM II of the TA cloning kit by Invitrogen. The vector was used to transform One ShotTM competent cells from the kit. White colonies were picked and screened for the insert by PCR. The reaction mix contained 1µl dNTPs, 1µl Sp6 / T7 primers, 5 µl Taq buffer (10X), 0.5 µl Taq enzyme, 42.5 µl water. A toothpick was used to suspend the template DNA containing cells into the 50 µl of the PCR reaction mix. The amplification program used was 1 cycle for 2 minutes at 95°C, followed by 35 cycles of 30 sec at 95°C, 45 seconds at 45°C, 1 min at 72°C, and then 1 cycle of 10 minutes at 72°C. The amplified DNA was analyzed on a 0.8% agarose gel (modified from Maniatis, Fritsch and Sambrook, 1982). Pure insert DNA for use as template DNA for sequencing was obtained from the positive white colony identified by PCR, by using the Qiagen kit for plasmid DNA extractions.

Sequencing was done using Sequenase (Version 2). Primers used were Sp6 and T7. Labelling was done with ^{35}S deoxynucleotides (^{35}S dATP). Sequences were analyzed using DNA StriderTM software program. Sequence comparisons and alignments were done with NCBI databases using Blast network services (Altschul *et al.*, 1990). Protein sequence analysis was also done with BLAST. Sequences were also compared to the Swiss Protein database. Molecular weight and hydrophobic indices were calculated using MacVectorTM software.

Protein band 5 of wild type and omega mutants was blotted from an acrylamide gel and sent to the sequencing facility at UCSF. Twenty amino acids were sequenced from the N-terminal end of the omega unmodified Lcp 5 and the first 14 amino acids from N-terminal end of the wild-type Lcp 5 protein.
The sequences were:

```
Wild Type      NLAEIVRQVSDVEP
                |||||||||||||
omega      RPNLAEIVRQVSDVEP?KKS
```

The two proteins were identical with the exception of the first two N- terminal residues. This is consistent with the observation that the omega mutation is in a gene which in some way modifies the 5th larval protein (Chihara and Kimbrell., 1986). Omega protein 5 has N - terminal Arginine-Proline-Asparagine, whereas the wild type is N- terminal Asparagine. It is difficult to assess the meaning of this difference at this time as preliminary data show that the MW (from SDS gels) of the unmodified omega protein 5 is about 1800D smaller than the wild type (gels not shown). A degenerate probe was synthesized from amino acids # 13 through 19 using the universal codon table. The probe sequence was:

GAYGTNGARCCNGAYAARAA

Using this probe we screened a lambda gt10 cDNA library and isolated 13 putative clones. The clone with the largest insert (S13) was subcloned into the pCRTM II plasmid vector of the TA cloning kit by Invitrogen. The sequence of the insert is shown in Figure 1. The insert is 430 base pairs long and includes the initiation codon AUG at the 5' end and a poly A tail signal sequence AATAAA about 15 nucleotides upstream from the putative UUA termination codon (Mandalaparthy, 1995). The calculated Molecular Weight is 14,691 which is close to the value reported in earlier estimates by SDS PAGE (approximately 15,000). The sequence includes a 16 amino acid signal sequence which seems to be cleaved somewhat differently in wild type and omega. The wild type has two amino acids more cleaved just after (or as part of) the signal sequence.

```
292   TACGTGGCTGATGAGAACGGATACCAGCCCCAGGGCGCCCATCTGCCC  339  cDNA  Lcp5:
      |||   ||||| ||||||||||||||||||||| | | ||  ||||||
2926  TACAAGGCTGACGAGAACGGATACCAGCCCCAGAGTGACCTGCTGCCC   2973   Lcp III:
```

a: Match with part of the 44D cuticle gene cluster encoding cuticle genes I, II, III, IV and a pseudogene I of *Drosophila melanogaster*. The match is to Lcp III within clone DMCUT 2. (Genbank).

```
286   TCACATACGTGGCTGATGAGAACGGATACCAGCCCCAGGGCGCCCATCTGCCC  339 cDNA Lcp5
      |||| ||||| ||||| |||||||||||||||||||| |  | |   || |||
277   TCACCTACGTCGCTGACGAGAACGGATACCAGCCCCAAGCTGACTTCCTCCCC  329  LCP 14:
```

b: Match with the part of the gene encoding *Manduca sexta* LCP 14

Figure 2. High scoring nucleotide sequence matches obtained by searching the nucleotide sequence databases at NCBI using the program DNA Strider.[TM] The sequence matches shown are in the plus strand.

```
3  FLIVFVALFAMAVARPNLAEIVRQVSDVEPEKWSSDVETSDGTSIKQEG Lcp 5:   51
   F I+ V   A  VA     E+   V+DV+P+ + S +   DG++    G
2  FKILLVCSLAALVAANANVEVKELVNDVQPDGFVSKLVLDDGSASSATG Lcp III: 50
```

a). Match with the protein sequence of *Drosophila melanogaster* larval cuticle protein III.

```
3     FLIVFVALFAMAVARPNLAEIVRQVSDVEPEKWSSDVETSDGTSIKQEG 51  Lcp 5:
      F I+     A  VA    AE+   V++V P+ + + V  SDG++ +  G
2     FKILLVCALAALVAANENAEVKELVNEVNPDGFKTVVSLSDGSASQASG 50  Lcp IV:
```

b). Match with the protein sequence of *Drosophila miranda* larval cuticle protein IV.

Figure 3. Protein sequence matches obtained by using the Swiss Protein database. (The exact matches are represented by the 'amino acid residue' and the' + 'represents similar but not identical amino acid residues.)

We searched the DNA sequence database at NCBI and retrieved two high scoring matches shown in Figure 2. A Swiss Protein database search gave partial matches with larval cuticle protein III of *Drosophila melanogaster* and larval cuticle protein IV of *Drosophila miranda*. (Figure 3). As expected most of the similarity is at the C terminal end of the cuticle protein genes. Since the sequence we have is a perfect match for the first 16 amino acids and is of the correct molecular weight we believe that we have identified the clone of *Lcp5* (or a closely related gene) from the third chromosome cluster of genes. In situ hybridization of the cDNA clone to Canton-S wild type salivary chromosomes places the gene at 65A(5-6) which is consistent with earlier meiotic mapping of the gene (Chihara and Kimbrell, 1986).

Acknowledgments: Funds for the early part of this research were provided by a Bristol-Myers Company Grant of Research Corporation to CJC, and by the Lily Drake Fund of USF. We would like to thank Dr. Thomas Kaufman of Indiana University for the generous donation of the early third instar cDNA library, and Dr. Bruce Elder for his help and guidance to PM. Fly stocks were provided by the MidAmerica Stock Center at Bloomington.

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Noor, M.A.F. Department of Ecology and Evolution, University of Chicago, Chicago, IL, U.S.A. (e-mail: mnoo@midway.uchicago.edu). Activity level of *Drosophila pseudoobscura* and *D. persimilis* hybrid males.

F1 hybrid males of *Drosophila pseudoobscura* and *D. persimilis* are sterile. While investigating the mating behavior of these males, I noticed that F1 hybrid males with an X-chromosome from *D. persimilis* appeared larger and less-active than those with an X-chromosome from *D. pseudoobscura*. In this short experiment, I quantified the activity level of these hybrid males. Strains hybridized to produce the F1 hybrid males were the Flagstaff strain of *D. pseudoobscura* and the Mount St. Helena strain of *D. persimilis* (Noor, 1995). Stocks were kept at 21° C on cornmeal/ Karo syrup/ yeast/ agar food under a light:dark cycle of 12:12h. Newly hatched males were kept for 8 days to reach maturity. Carbon dioxide was used for initial anesthetization, and no males were anesthetized within 7 days of this experiment.

Flies were aspirated into a food-containing vial partitioned into half-inch squares (see Figure 1), and the number of lines that the fly walked across in two minutes was recorded. Table 1 presents the number of lines crossed by pure-species and F1 hybrid flies.

These results suggest that F1 hybrid males with an X-chromosome from *D. persimilis* are approximately 80% as active as those with an X-chromosome from *D. pseudoobscura*. This difference in activity is significant (Mann-Whitney U-test, $p = 0.0005$).

References: Noor, M.A., 1995, Nature 375:674-675.

Figure 1.

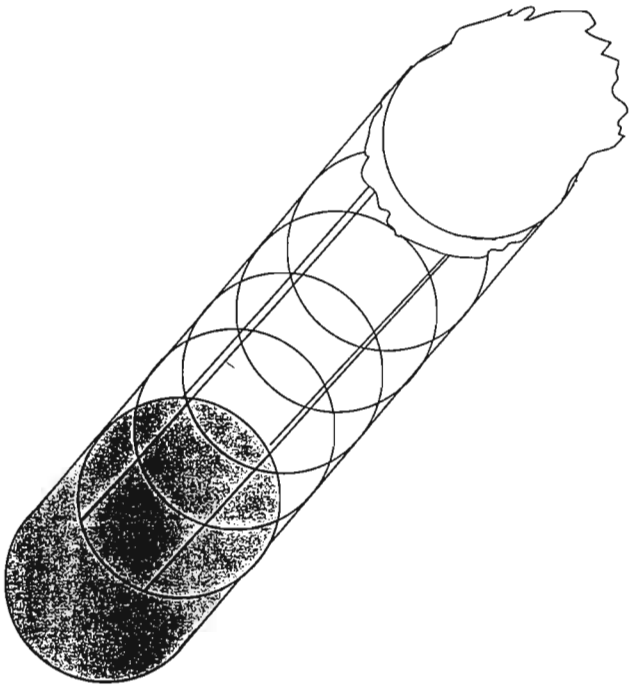

Table 1. Number of lines crossed by F1 hybrid males in two minute confinement.

Male	N	Line mean	Standard Error
$X^{pseudo}\,Y^{per}$	25	125	4
$X^{per}\,Y^{pseudo}$	25	100	6

Budnik, M.[1], L. Cifuentes[1], and M. Benado[2]. [1]Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Casilla 70061, Santiago 7, Chile. [2]Departamento de Estudios Ambientales, Universidad Simón Bolívar, Caracas, Venezuela. Preadult viability of *Drosophila subobscura, D. simulans*, and *D. pavani* under tri-specific competitive conditions.

In 1980, *D. subobscura* was reported in Chile for the first time (Brncic and Budnik, 1980). Since then, the species successfully colonized the country, covering a 3000 km expanse in a few years (Budnik and Brncic, 1982; Brncic and Budnik, 1987). We have found it pertinent to study the preadult viability of *D. subobscura, D. simulans*, and *D. pavani* under tri-specific competition conditions. The three species are commonly found in the same collecting sites and exploit the same substrates (Brncic, 1987), so the experiments reported below try to mimic the conditions faced by the species in the wild, and thus to contribute further to the understanding of the colonization success of *D. subobscura*. The null hypothesis is that the preadult viability is the same under mono- and tri-specific competition experimental set-ups.

Material and Methods: All the strains came from La Florida, Santiago, Chile, and were kept in mass cultures for several months before the experiments were started. Seven vials per group were used, each containing 10 cc of basic cornmeal-yeast agar medium, into which fixed numbers of fertilized eggs of the same age were sown, as indicated in Table 1. The eggs were considered a random sample of those laid by 150 inseminated females from each stock.

The eggs were allowed to hatch and to develop at 19°C, and the emerging adults were counted and discarded every day.

Preadult viability was defined as the ratio: #adults emerged/# eggs sown. Vials did not differ significantly for the number of emerged adults, and we pooled the data. Appropriate standard errors for binomial proportions were used. A simultaneous *a posteriori* multiple comparison procedure for proportions (Zar, 1984) was used to test the null hypothesis that there were no differences between the viabilities under the different competition conditions.

Table 1. Experimental design: # of eggs sown per vial under mono- and tri-specific conditions. SU: *D. subobscura*; SI: *D. simulans*, PA: *D. pavani*. *: controls.

	SU	SI	PA	SI +PA	SU + PA	SI + SU
SU	200*			66 + 66 + 66		
SI		200*			66 + 66 + 66	
PA			200*			66 + 66 + 66

Table 2. Preadult viability of *D. subobscura*, *D. simulans*, and *D. pavani* under mono- and tri-specific conditions. Pooled data for 7 vials. The viabilities differ significantly at the 5% level by a posteriori test. In parenthesis, competitor (s). Symbols as for Table 1.

Experiment	Viability ± S.E.
100SU (100SU)	0.48 ± 0.03
66SU (66SI + 66 PA)	0.02 ± 0.01
100SI (100SI)	0.57 ± 0.02
66SU (66SI + 66PA)	0.67 ± 0.01
100PA (100PA)	0.25 ± 0.01
66PA (66SU + 66SI)	0.28 ± 0.02

Results and Discussion: Table 2 indicates that the viability of *D. subobscura* was strongly decreased under tri-specific competition conditions. For *D. simulans* and *D. pavani*, it increased. This can be interpreted as *D. sub- obscura* being a bad competitor when interacting with the other species. We suggest that *D. subobscura* successfully colonized Chile because, among others, it found sites where competitors were absent. Data on abundances and seasonality support this interpretation (Budnik and Brncic, 1982; Brncic and Budnik, 1987; Benado and Brncic, 1994).

Acknowledgments: Funded by FONDECYT Grant #1930769.

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Cerda, Hugo[1], Moritz Benado[2], and Antonio Fontdevila[3]. [1]Plague Control Laboratory, Universidad Simón Rodríguez. PO Box. 47.925, Caracas 1041-A, Venezuela. [2]Department of Environmental Studies, Universidad Simón Bolivar, Caracas, Venezuela; [3]Department of Genetics and Microbiology, Universidad Autonoma de Barcelona, Bellaterra, Barcelona, Spain. Distribution of breeding substrates of the cactophilic *Drosophila martensis* cluster in Venezuela (*mulleri* complex).

Introduction: The cluster *Drosophila martensis* (*mulleri* complex, *repleta* group) is a group of four closely-related species that breed and live on fermenting cactus tissues (rot pockets) in the arid zones of the north of South America (Venezuela, Colombia, and the neighboring islands). The cluster species are: *D. martensis* Wasserman and Wilson (1957), *D. starmeri* Wasserman, Koepfer and Ward (1973), *D. uniseta* Wasserman, Koepfer and Ward (1973), and *D. venezolana* Wasserman, Fontdevila and Ruiz (1983).

These species were previously reported in the Venezuelan arid biomes (Wasserman *et al.*, 1973; Ruiz and Fontdevila, 1981; Cerda and Benado, 1986; Benado *et al.*, 1984; and Benado, 1989).

This paper completes the previous data by providing a detailed description of the geographic distribution of *Drosophila* in 14 desert localities in mainland Venezuela and in Venezuelan islands and by specifying the *Drosophila* emergence substrate in 8 of these 14 localities. The null hypothesis is proved that the *Drosophila* species emerge from the same cactus and are present in all of them.

Materials and Methods: *Localities of the study:* During 1983-1984 and 1985-1986, samples were taken in the 14 localities of the coastline deserts, the Andean highlands and the neighboring islands in the Venezuelan coast. The *Drosophila* records correspond to both a *Drosophila* sample from the emergence cactus and to net captures using fermented bananas and beer as bait. Figure 1 describes the localities of this study.

Emergence of the Drosophila *from the cactus*: The cactophilic species were identified according to Benado *et al.* (1984). Rotten pieces of cactus were placed in half gallon glass bottles with a paper or sand bed. Rotten cactuses were left in the laboratory at normal temperature for 30 days; daily inspections were made and the emerged *Drosophila* were collected.

Males were distinguished by observing the form of the claspers and females were identified for the abdominal band pattern. If doubts existed about their species, they were identified by means of their male descendants.

Results and Discussion: *Geographic distribution of the* Drosophila martensis *cluster in Venezuela*: Figure 1 shows the presence of the species of the *Drosophila martensis* cluster in Venezuela. Three groupings may be identified: continental localities (Piritu, Guaca, Oricao, Cata, Padre Diego and Prudencio); island localities (Gran Roque, Mal Pais, Coche, Los Frailes and La Blanquilla); and Andean localities (El Anis, Quirague and Sanare). In continental localities the four species of *martensis* cluster were observed: *D. martensis, D.starmeri, D. uniseta* and *D. venezolana*. The islands

were characterized by having only one of the *Drosophila* species. In Coche island, *D. uniseta* was observed; in Los Frailes island, La Blanquilla island and Gran Roque island, *D. venezolana* was observed. The exception is Mal Pais, in Curacao island, in which the four species of *martensis* cluster were observed, thus exhibiting the same behavior as in continental localities, a fact that may be explained in terms of the large size of the island and its closeness to the continent. Andean localities such as El Anis, Sanare and Quirague are small arid pockets which present a lower number of species of the *Drosophila martensis* cluster as compared to continental localities: three species were observed in El Anis (*D. martensis, D. uniseta* and *D.starmeri*) and just one species (*D. venezolana*) was observed in Sanare and Quirague.

Emergence of cactophilic Drosophila *in Venezuela:* Drosophila emerge from seven cactus: *Stenocereus griseus, Pilosocereus lanogenosus, Subpilocereus repandus, Opuntia wentiana, Opuntia elatior, Melocactus amoenus* and *Acanthocereus tetragonus* (Table I).

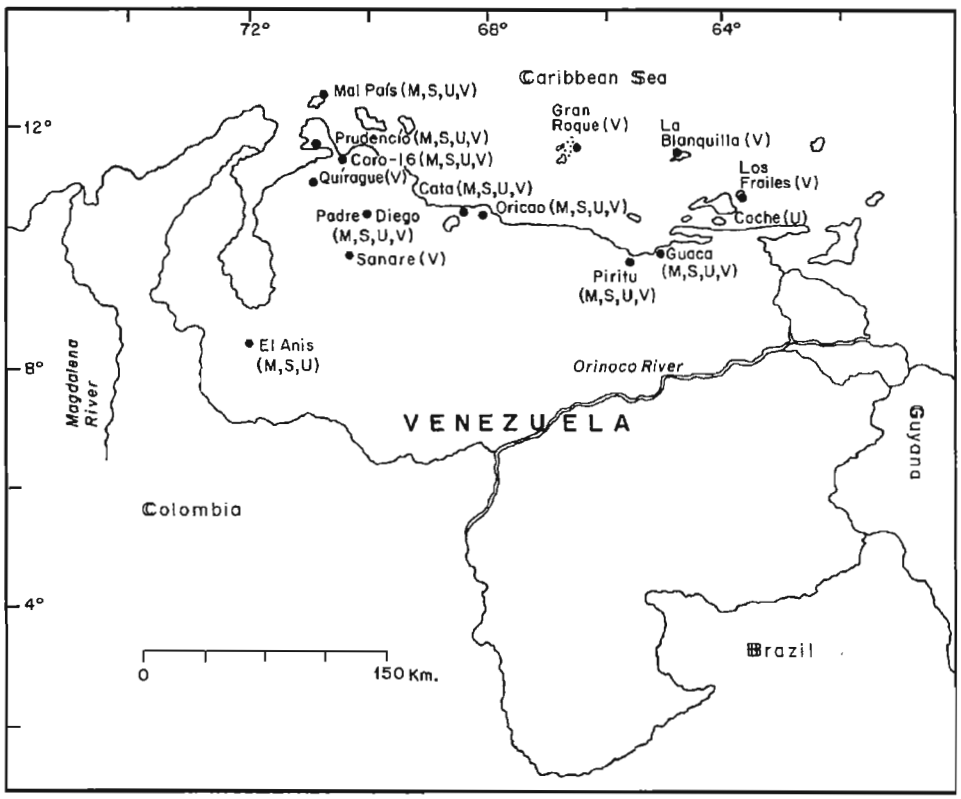

Figure 1. Geographic distribution of the *Drosophila martensis* cluster in Venezuela.

D. martensis emerges from the *S. repandus, O. wentiana* and *Melocactus amoenus.*

All individuals sam- pled from *D. uniseta* emerged from just one cactus, *S. griseus.* These findings confirm *D. uniseta* as a specialist species.

In most localities, *D. starmeri* emerges from the *S. repandus* and *P. lanoginosus* cacti. In some localities, this species also emerges from the *A. tetragonus, O. elatior, O. wentiana* and *M. amoenus* cacti.

In continental local-ities, *D. venezolana* emerges from the *Opuntia* genus cacti (*O. wentiana* and *O. elatior*), and in some localities it also emerges from *P. lanoginosus, A. tetragonus* and *M. amoenus*. In Gran Roque island, *D. venezolana* emerges from three cacti: *O. wentiana, M. amoenus* and *S. griseus* that are the three cacti present in the island.

In short, there is one specialist species, *D. uniseta* that only emerges from *S. griseus,* and the other species of the *Drosophila martensis* cluster emerge from three or more cacti. The use made by these species of the cactus may depend on the interspecific

Table 1. Emergence of *Drosophila martensis* cluster of cactus in eight (8) localities in Venezuela. means there is no *Drosophila* emergence register in that cactacea; **m** means *D. martensis*; **s** means *D. starmeri*; **v** means *D. venezolana*; **u** means *D. uniseta*; **Sg** means *Stenocereus griseus*; **Pl** means *Pilosocereus lanogenosus*; **Sre** means *Subpilocereu repandus*; **Ow** means *Opuntia wentiana*; **Oe** means *Opuntia elatior*; **Me** means *Melocactus amoenus*; **At** means *Acanthocereus tetragonus.*

	Cactaceas							
Localities	Sg	Pl	Sre	Oe	Ow	Ma	Me	At
Guaca	u	s,v	s,m	s,v			m,v	
Piritu	u	s,v	s,m	v	s,v			
Padre Diego	u		s					
Prudencio	u	s,v	s		v,s		s	s,v
Gran Roque	v				v		v	
Mal Pais					v			
El Anis	u	s	s,m		m			
Quiragua				v	v			

competence and the cactus community present in each locality, as the case of Gran Roque island in which the emergence of *D. venezolana* from *S. griseus* has been explained by Benado (1989) in terms of the absence of *D. uniseta* in the island.

References: Benado, M. 1989, Ecotropicos, 2 (1): 45-48; Benado, M., A. Fontdevila, H. Cerda, G. Garcia, A. Ruiz, and C. Montero 1984, Biotropica, 16: 120-124; Cerda, H. and M. Benado 1986, Acta Cientifica Venezolana, 34: 148-150; Ruiz, A. and A. Fontdevila 1981, Acta Científica Venezolana, 32: 338-345; Wasserman, M. and F.D. Wilson 1957, Texas Univ. Pub. 5721: 132-156; Wasserman, M., H.R. Koepfer, and B.L. Ward 1973, Ann. Ent. Soc. America 66: 1239-1242; Wasserman, M, A. Fontdevila, and A. Ruiz 1983, Ann. Ent. Soc. America, 76: 675-677.

Roote, J., D. Gubb, and M. Ashburner. Department of Genetics, University of Cambridge, Downing Street, Cambridge, CB2 3EH, U.K. Aberrations in the *l(2)34Fc – l(2)35Bb* region of chromosome arm 2L.

This report is a supplement to Roote, *et al.*, 1996 (in prep.). It describes the genetics and cytology of all aberrations characterised in Cambridge with at least one breakpoint in the *l(2)34Fc – l(2)35Bb* region of chromosome arm 2L. The numbers in the left column refer to the groups of averrations shown in Figure 1 (from Roote, *et al.*, 1996, in prep.).

	Aberration	Cytology	Extent†
1	*Df(2L)b80e3*	Df(2L)34C4;35A4	*l(2)34Da⁻—l(2)35Aa⁻*
1	*Df(2L)b84h1*	Df(2L)34D3;35A4	*Sos⁻—l(2)35Aa⁻*
—	*Df(2L)b88h49* **a**	Df(2L)34C5-6;35A2	*l(2)34Da⁻—elB⁻*
2	*In(2L)b83d29a*	In(2L)34C7.8;35D5-7	*l(2)34Db⁻—pu⁻*
2	*Ts(YSt;2Lt)G74+Ts(YSt;2Rt)A80* **b**	Df(2L)34C3;35A3.4	*l(2)34Da⁻—pu⁻*
—	*Df(2L)b84a2*	Df(2L)34C3;35A4	*l(2)34Da⁻—elA⁻*
—	*In(2LR)D20^{L}noc^{4R}*	Df(2L)34E4-F1;35B1.2	*rk⁻—noc⁻*
3	*Df(2L)TE35B-300* **b**	n.d.	*wb⁻—noc⁻*
3	*Df(2L)TE35B-203; Dp(2;3)17* **b**	Df(2L)34D3.4;35B1.2 §	*l(2)34De⁻—noc⁻*
3	*Df(2L)TE35B-407* **b**	n.d.	*l(2)34Eb⁻—noc⁻*
3	*Ts(2Lt;4Lt)GT6+Ts(2Rt;4Rt)TE35B-101* **c**	Df(2L)34F3;35B1.2 §	*l(2)34Fc⁻—noc⁻*
3	*Ts(2Lt;3Lt)H118+Ts(2Rt;3Rt)TE35B-8* **b**	Df(2L)34C;35B1.2 §	*l(2)34Db⁻—noc⁻*
3	*Ts(2Lt;3Lt)H147+Ts(2Rt;3Rt)TE35B-8* **b**	Df(2L)34C3-6;35B1.2 §	*wb⁻—noc⁻*
4	*Df(2L)b81a1*	Df(2L)34D3;35B1.2	*l(2)34Db⁻—noc⁻*
4	*Df(2L)b84a5*	Df(2L)34D3.4;35B2.3	*l(2)34Db⁻—noc⁻*
4	*Df(2L)TE35B-4*	Df(2L)34F1.2;35A2	*l(2)34Fa⁻—noc⁻*
4	*Df(2L)TE35B-10*	Df(2L)34F5;35B2	*wb⁻—noc⁻*
4	*In(2LR)b81a2^{L}DTD43^{R}* **b**	Df(2L)34D5;35B1.2 §	*b⁻—noc⁻*
5	*Df(2L)b84a4*	Df(2L)34D3;35B1.2	*l(2)34Db⁻—noc⁻*
5	*Df(2L)b84a8*	Df(2L)34D3;35B1.2	*l(2)34Db⁻—noc⁻*
5	*Df(2L)GT4*	Df(2L)34F3;35B2	*l(2)34Fa⁻—noc⁻*

—	*Df(2L)TE35B-8*	Df(2L)34E4.5;35A2-4	*l(2)34Fa⁻—osp⁻*
6	*Df(2L)A47*	Df(2L)34E1;35B2	*l(2)34Eb⁻—Adhr⁻*
6	*Df(2L)A217*	Df(2L)34F5;35B3	*l(2)34Fc⁻—Adhr⁻*
6	*Df(2L)AdhnBR41*	Df(2L)34F3.4;35B3	*l(2)34Fa⁻—Adhr⁻*
6	*Df(2L)AdhnBR100* **d**	n.d.	*wb⁻—Adhr⁻*
6	*Df(2L)b81l1*	Df(2L)34C1;35B4 **1**	*l(2)34Db⁻—Adhr⁻*
6	*Df(2L)b84a1*	Df(2L)34D3;35B1.2	*l(2)34Db⁻—Adhr⁻*
6	*Df(2L)b84a7*	Df(2L)34C1.2;35B1.2	*l(2)34Da⁻—Adhr⁻*
6	*Df(2L)b88c25* **a**	Df(2L)34B12-C1;35B2-3	*l(2)34Da⁻—Adhr⁻*
6	*Df(2L)el17*	Df(2L)34F1.2;35A4	*wb⁻—Adhr⁻*
6	*Df(2L)el77*	Df(2L)35A1-3;35B3 **2**	*wb⁻—Adhr⁻*
6	*Df(2L)el81i1*	Df(2L)34F5;35B1.2	*wb⁻—Adhr⁻*
6	*Df(2L)el90* **b**	n.d.	*l(2)34Db⁻—Adhr⁻*
6	*Df(2L)fn7*	Df(2L)34E1.2;35B3.5	*j⁻—Adhr⁻*
6	*Df(2L)fn36*	Df(2L)35A3;35B4	*wb⁻—Adhr⁻*
6	*Df(2L)noc10*	Df(2L)34F1.2;35B1.2	*l(2)34Fa⁻—Adhr⁻*
6	*Df(2L)noc13*	Df(2L)35A1.2;35B2	*wb⁻—Adhr⁻*
6	*Df(2L)noc20*	Df(2L)34F1.2;35B2	*l(2)34Fa⁻—Adhr⁻*
6	*Df(2L)osp141*	Df(2L)34F5;35B5	*l(2)34Fa⁻—Adhr⁻*
6	*Df(2L)TE35B-3*	Df(2L)34F5;35B3	*l(2)34Fa⁻—Adhr⁻*
6	*Df(2L)TE35B-9*	Df(2L)34F1;35B2	*l(2)34Fa⁻—Adhr⁻*
6	*Df(2L)TE35B-15*	Df(2L)34F3;35B2	*wb⁻—Adhr⁻*
6	*Df(2L)W*	Df(2L)35A2.3;35B3-5	*l(2)34Fa⁻—Adhr⁻*
6	*In(2L)75c^{L}C158.1^{R}*	Df(2L)35A1.2;35B3 **3**	*wb⁻—Adhr⁻*
—	*Ts(2Lt;3Rt)GT10+Ts(2Rt;3Lt)TE35B-209* **c**	Df(2L)35A1-4;35B1.2 **§ 4**	*l(2)34Fa⁻—noc⁻*
—	*Df(2L)TE35B-7*	Df(2L)35A3.4;35B2	*l(2)35Aa⁻—Adhr⁻*
7	*Df(2L)A400*	Df(2L)35A1-4;35B10	*elB⁻—stc⁻*
7	*In(2L)TE35B-13*	In(2L)35B1.2;40	*elB⁻—l(2)35Ea⁻*
—	*Ts(Y;2Lt)GT2+Ts(YSt;2Rt)A80* **c**	Df(2L)35A1.2;35A3-4 **§**	*elB⁻—pu⁻*
—	*Ts(Y;2Lt)el^{4}+Ts(YLt;2Rt)R15* **b**	Df(2L)35A2.3;35B8.9-C1 **§**	*elB⁻—l(2)35Bg⁻*
—	*Ts(2Lt;3Lt)dpp^{s19}+Ts(2Rt;3Rt)el^{24}* **b**	**5**	*elB⁻—elA⁻*
8	*Ts(Y;2Lt)el^{4}+Ts(YSt;2Rt)A80* **b**	Df(2L)35A2.4;35A3.4	*elB⁻—pu⁻*
8	*Ts(Y;2Lt)el^{5}+Ts(YSt;2Rt)A80* **b**	n.d.	*elB⁻—pu⁻*
—	*In(2LR)el^{6L}A379^{R}* **b**	**6**	*pu⁻—Adhr⁻*
—	*Df(2L)TE35B-12*	n.v.	*pu⁻—noc⁻*

9	*Df(2L)ARR1*	Df(2L)35A3.4;35B8.9-C1	*elA⁻—l(2)35Bg⁻*
9	*Df(2L)el28*	Df(2L)35B3;35D4	*elA⁻—l(2)35Ea⁻*
9	*Df(2L)TE35B-1*	Df(2L)35B1;35C4.5	*elA⁻—esg⁻*
9	*Df(2L)TE35B-6*	Df(2L)35B1;35C1	*elA⁻—stc⁻*
9	*Df(2L)TE35BC-8*	Df(2L)35B1.2;35E1.2	*elA⁻—l(2)35Ea⁻*
—	*Df(2L)fn2*	Df(2L)35A3;35B2	*elA⁻—Adhr⁻*
10	*Df(2L)el14* **b**	n.v.	*elA⁻—noc⁻*
10	*Df(2L)el16*	n.v.	*elA⁻—noc⁻*
11	*In(2LR)DTD128^{L}TE35B-226^{R}* **b**	n.d.	*elA⁻—noc⁻*
11	*Ts(YLt;2Lt)A80+Ts(Y;2Rt)TE35B-18* **b**	Df(2L)35A3.4;35B1.2 §	*elA⁻—noc⁻*
12	*Df(2L)AdhnBR102* **d**	n.d.	*noc⁻—esg⁻*
12	*Df(2L)AdhnBR118* **d**	n.d.	*noc⁻—esg⁻*
12	*Df(2L)fn3*	Df(2L)35B1;35B3.4	*noc⁻—l(2)35Bb⁻*
12	*Df(2L)n78l3*	Df(2L)35B1;35D5-7	*noc⁻—l(2)35Ea⁻*
12	*Df(2L)TE35D-24* **b**	Df(2L)35B1.2;35E1.2	*noc⁻—l(2)35Ea⁻*
12	*Ts(Y;2Lt)TE35B-18+Ts(YLt;2Rt)R15* **b**	Df(2L)35B1.2;35B8.9-C1	*noc⁻—l(2)35Bg⁻*
13	*Df(2L)A245*	Df(2L)35A4;35B2	*noc⁻—Adhr⁻*
13	*Df(2L)A260*	Df(2L)35B1.2	*noc⁻—Adhr⁻*
13	*Df(2L)A266*	Df(2L)35B2.3	*noc⁻—Adhr⁻*
14	*Df(2L)A446*	Df(2L)35B1.2;35F1.2 **7**	*noc⁻—Ca-a1⁻*
14	*Df(2L)AdhnBR129* **d**	n.d.	*noc⁻—l(2)35Cd⁻*
14	*In(2LR)noc^{4L}D6^{R}* **e**	Df(2L)35B1.2;35D	*noc⁻—l(2)35Ea⁻*
14	*In(2LR)noc^{4L}Sco^{rv9R}*	Df(2L)35B1.2;35D1.2	*noc⁻—l(2)35Da⁻*
—	*Df(2L)A178*	n.v.	*noc⁻—Adhr⁻*
15	*Df(2L)TE35B-54^{L}* **b**	Df(2L)35B1.2;35C	*noc⁻—lac⁻*
15	*In(2L)TE35B-210^{L}C163.41^{R}* **b**	Df(2L)35B1.2;35E1.2 **8**	*noc⁻—BicC⁻*
15	*In(2LR)TE35B-15^{L}Sco^{rv1R}*	In(2LR)35B1.2;44C4.5 **9**	*noc⁻—l(2)35Da⁻*
15	*In(2LR)TE35B-6^{L}Sco^{rv9R}*	Df(2L)35B1.2;35D1	*noc⁻—l(2)35Da⁻*
15	*In(2LR)TE35B-14^{L}Sco^{rv1R}* **b**	Df(2L)35B1.2;35D1.2 **10**	*noc⁻—l(2)35Da⁻*
15	*T(2;3)TE35B-2*	T(2;3)35B1.2;80	*noc⁻—esg⁻*
15	*T(2;3)TE35B-5*	T(2;3)35B1.2;80-81	*noc⁻—vasa⁻*
15	*Ts(2Lt;3Lt)TE35B-3+Ts(2Rt;3Rt)G16* **b**	Df(2L)35B1.2;35D5.6 **11**	*noc⁻—CycE⁻*
15	*Ts(2Lt;3Lt)TE35B-4+Ts(2Rt;3Rt)Sco^{rv13}* **b**	Df(2L)35B1.2;35D1.2	*noc⁻—l(2)35Da⁻*
15	*Ts(2Lt;4Lt)TE35B-50+Ts(2Rt;4Rt)DTD22* **b**	Df(2L)35B1.2;35E1.2	*noc⁻—twe⁻*
16	*Df(2L)TE35B-11*	n.v.	*noc⁻—Adhr⁻*
16	*Df(2L)TE35B-48*	n.v.	*noc⁻—Adhr⁻*

16	*Df(2L)TE35B-50*	Df(2L)35B1.2	*noc*$^{-}$—*Adhr*$^{-}$
16	*Df(2L)TE35B-54*	n.v.	*noc*$^{-}$—*Adhr*$^{-}$
16	*Df(2L)TE35B-400* **b**	n.d.	*noc*$^{-}$—*Adhr*$^{-}$
16	*Df(2L)TE35B-401* **b**	n.d.	*noc*$^{-}$—*Adhr*$^{-}$
16	*Df(2L)TE35B-402* **b**	n.d.	*noc*$^{-}$—*Adhr*$^{-}$
16	*Df(2L)TE35B-403* **b**	n.d.	*noc*$^{-}$—*Adhr*$^{-}$
17	*Ts(2Lt;3Lt)TE35B-28+Ts(2Rt;3Rt)osp*^{90} **b**	Df(2L)35B1.2;35B3.4 **12**	*noc*$^{-}$—*Adhr*$^{-}$
17	*Ts(2Lt;3Lt)TE35B-28+Ts(2Rt;3Rt)pb*^{3} **b**	Df(2L)B1.2 **13**	*noc*$^{-}$—*Adhr*$^{-}$
18	*Df(2L)A267*	Df(2L)35B2.3;35B10	*noc*$^{+/-}$—*vasa*$^{-}$
18	*Df(2L)AdhnBR106* **d**	n.d.	*noc*$^{+/-}$—*ck*$^{-}$
18	*Df(2L)AdhnBR126* **d**	n.d.	*noc*$^{+/-}$—*BicC*$^{-}$
18	*Df(2L)fn27*	Df(2L)35B1;35D1.2	*noc*$^{+/-}$—*lac*$^{-}$
18	*Df(2L)nNXF1*	Df(2L)35B3;35B10	*noc*$^{+/-}$—*stc*$^{-}$
18	*Df(2L)nNXF2*	Df(2L)35B3;35B10	*noc*$^{+/-}$—*stc*$^{-}$
18	*Df(2L)TE35BC-7*	Df(2L)35B3;35B9.10	*noc*$^{+/-}$—*ck*$^{-}$
18	*Df(2L)TE35D-20* **b**	Df(2L)35B1.2;35D4	*noc*$^{+/-}$—*CycE*$^{-}$
18	*In(2L)Scorv4*	complex **14**	*noc*$^{+/-}$—*stc*$^{+/-}$
18	*Ts(2Lt;3Lt)Mpe+Ts(2Rt;3Rt)G16* **b**	Df(2L)35B1.2;35D5-7 **15**	*noc*$^{+/-}$—*CycE*$^{-}$
19	*Df(2L)A445*	n.v.	*noc*$^{+/-}$—*Adhr*$^{-}$
19	*Df(2L)AdhnBR119* **d**	n.d.	*noc*$^{+/-}$—*Adhr*$^{-}$
19	*Df(2L)PA4*	Df(2L)35B1.3 **16**	*noc*$^{+/-}$—*Adhr*$^{-}$ **17**
19	*Df(2L)Sco*^{rv10}	like *Sco*	*noc*$^{+/-}$—*Adhr*$^{-}$ **18**
19	*Df(2L)Sco*^{rv14}	like *Sco*	*noc*$^{+/-}$—*Adhr*$^{-}$ **19**
19	*Df(2L)Sco*^{rv18}	like *Sco*	*noc*$^{+/-}$—*Adhr*$^{-}$ **20**
19	*In(2LR)A379*	Df(2L)35B1.3 **21**	*noc*$^{+/-}$—*Adhr*$^{-}$
19	*T(2;3)Sco*^{rv7}	T(2;3)35D1.2;93F11-14	*noc*$^{+/-}$—*Adhr*$^{-}$ **22**
20	*Df(2L)osp144*	n.v.	*noc*$^{+/-}$—*osp*$^{-}$
20	*Tp(3;2)osp204*	Tp(3;2)89A4;86D4;35B3 **23**	*noc*$^{+/-}$—*osp*$^{-}$
21	*Df(2L)A48*	Df(2L)35B2.3;35D5-7	*osp*$^{-}$—*lac*$^{-}$
21	*Df(2L)A72*	Df(2L)35B2.3;35B7.8	*osp*$^{-}$—*stc*$^{-}$
21	*Df(2L)A220*	Df(2L)35B1.2;35B9	*osp*$^{-}$—*stc*$^{-}$
21	*Df(2L)AdhnBR105* **d**	n.d.	*osp*$^{-}$—*stc*$^{-}$
21	*Df(2L)AdhnBR107* **d**	n.d.	*osp*$^{-}$—*ck*$^{-}$
21	*Df(2L)AdhnBR122* **d**	n.d.	*osp*$^{-}$—*stc*$^{-}$
21	*Df(2L)AdhnBR128* **d**	n.d.	*osp*$^{-}$—*stc*$^{-}$
21	*Df(2L)osp18*	Df(2L)35B1.2;35C4.5	*osp*$^{-}$—*esg*$^{-}$
21	*Ts(Y;2Lt)GT1+Ts(YLt;2Rt)R15* **c**	Df(2L)35A4-B1;35B9-C1	*osp*$^{-}$—*l(2)35Bg*$^{-}$
21	*Ts(2Lt;3Lt)GT7+Ts(2Rt;3Rt)Sco*^{rv13} **c**	Df(2L)35B3;35D1.2	*osp*$^{-}$—*l(2)35Da*$^{-}$
22	*Df(2L)A63*	n.v.	*osp*$^{-}$—*Adhr*$^{-}$

22	*Df(2L)AdhnBR121* **d**	n.d.	*osp⁻—Adhr⁻*
22	*Df(2L)AdhnBR127* **d**	n.d.	*osp⁻—Adhr⁻*
22	*Df(2L)fn52*	n.v.	*osp⁻—Adhr⁻*
—	*Df(2L)osp29*	Df(2L)35B1.3;35E6	*Adh⁺, osp⁻—BicC⁻*
23	*Df(2L)TE35BC-4*	Df(2L)35B4;35C3	*l(2)35Bb⁻—esg⁻*
23	*Df(2L)TE35BC-28*	Df(2L)35B2;35B7	*l(2)35Bb⁻—ck⁻*
23	*Df(2L)TE35BC-34*	Df(2L)35B4;35D4	*l(2)35Bb⁻—l(2)35De⁻*
23	*Df(2L)TE35BC-35*	Df(2L)35B2;35D4	*l(2)35Bb⁻—lac⁻*
23	*Df(2L)TE35D-5*	Df(2L)35B3;35E1.2	*l(2)35Bb⁻—l(2)35Ea⁻*
23	*Df(2L)TE35D-10* **b**	Df(2L)35B1.3;35E1.2	*l(2)35Bb⁻—*
ms(2)35Eb⁻			
23	*Df(2L)TE35D-12* **b**	Df(2L)35B4;35D3.4	*l(2)35Bb⁻—l(2)35Ea⁻*
23	*Df(2L)TE35D-23* **b**	Df(2L)35B4.5;36C7	*l(2)35Bb⁻—l(2)36Ac⁻*
23	*In(2L)C158.1^{L}Sco^{rv11R}*	Df(2L)35B3;35D1 **24**	*l(2)35Bb⁻—l(2)35Da⁻*
23	*In(2L)C158.1^{L}Sco^{rv17R}*	Df(2L)35B3;35D1 **25**	*l(2)35Bb⁻—l(2)35Da⁻*
23	*Ts(2Lt;3Lt)TE35BC-3+Ts(2Rt;3Rt)Sco^{rv13}* **b**	Df(2L)35B5;35D1.2	*l(2)35Bb⁻—l(2)35Da⁻*
—	*Ts(2Lt;4Lt)TE35B-101+Ts(2Rt;4Rt)GT6*	Dp(2;2)34F3;35B1.2 **§**	*l(2)34Fc⁺—elA⁺*
—	*Ts(YLt;2Lt)A80+Ts(YLt;2Rt)G74* **b**	Dp(2;2)34C3;35A3.4	*l(2)34Da⁺—pu⁺*
—	*Ts(YLt;2Lt)R15+Ts(Y;2Rt)el^{4}* **b**	Dp(2;2)35B1;35B9-C1	*pu⁺—l(2)35Bg⁺*
—	*Ts(YLt;2Lt)A80+TS(Y;2Rt)el^{4}* **b**	Dp(2;2)35A3.4	*pu⁺*
—	*Ts(YLt;2Lt)R15+Ts(YSt;2Rt)A80* **b**	Dp(2;2)35A3.4;35B9-C1	*elA⁺—l(2)35Bg⁺*
—	*In(2LR)TE35B-226^{L}DTD128^{R}* **b**	n.d.	*elA⁺*
—	*Ts(Y;2Lt)TE35B-18+Ts(YSt;2Rt)A80* **b**	n.d.	*elA⁺*
—	*Ts(YLt;2Lt)R15+Ts(Y;2Rt)TE35B-18* **b**	Dp(2;2)35B1.2;35B9-C1	*osp⁺—l(2)35Bg⁺*
24	*Dp(2;2)TE35B-54^{L}* **b**	Dp(2;2)35B1.2;35C;38F	*osp⁺—lace⁺*
24	*In(2L)C163.41^{L}TE35B-210^{R}* **b**	Dp(2;2)35B1.2;35E1.2 **26**	*osp⁺—BicC⁺*
24	*In(2LR)Sco^{rv1L}TE35B-15^{R}*	Dp(2;2)35B;35D1.2	*osp⁺—l(2)35Da⁺*
24	*In(2LR)Sco^{rv1L}TE35B-14^{R}*	Dp(2;2)35B1.2;35D1.2 **§ 27**	*osp⁺—l(2)35Da⁺*
24	*In(2LR)Sco^{rv1L}TE35B-4^{R}* **b**	Dp(2;2)35B1.2;35D1.2 **§ 28**	*osp⁺—l(2)35Da⁺*
24	*Ts(2Lt;4Lt)DTD22+Ts(2Rt;4Rt)TE35B-50* **b**	Dp(2;2)35B1.2;35E1.2	*osp⁺—l(2)35Fa⁺*
24	*Ts(2Lt;3Lt)G16+Ts(2Rt;3Rt)TE35B-3* **b**	Dp(2;2)35B2;35D5-7 **29**	*osp⁺—lace⁺*
24	*Ts(2Lt;3Lt)G40+Ts(2Rt;3Rt)TE35B-28* **b**	Dp(2;2)35B2;35F4.5 **30**	n.d.
—	*Tp(2;3)osp^{3}*	Tp(2;3)35B3.4;36C11; 98E1.2-F1.2	*l(2)35Bb⁺—twe⁺*

—	*In(2L)C163.41^{L}C158.1^{R}*	Dp(2;2)35B3;35E1.2 **31**	*l(2)35Bb^{+}—BicC^{+}*
—	*T(2;3)GT10*	T(2;3)35A1-4;76A5-7	*l(2)34Fc|l(2)34Fd*
—	*T(Y;2)GT2*	T(Y;2)Y;35A1.2	*l(2)35Aa|elB*
—	*Tp(2;3)GT3*	complex **32**	*l(2)35Aa|elB*
—	*In(2L)el^{9}*	In(2L)34A2.3;35A3.4	*elB^{-}*
—	*T(Y;2)a15*	T(Y;2)Y;35A4-B1.2	*elB^{-}*
—	*T(Y;2)el^{4}*	T(Y;2)Y;35B1	*elB^{-}*
—	*T(2;3)dpp^{s19}*	T(2;3)22F;35A;97A	*elB^{-}*
—	*In(2LR)DTD128*	In(2LR)35A3.4;48C6-8	*pu|elA*
—	*In(2LR)el^{6}*	In(2LR)35B1-3;57C3-9	*elA^{-}*
—	*T(Y;2)A80*	T(Y;2)YS;35A3.4	*pu|elA*
—	*T(2;3)el^{24}*	35B1.2;93C3-7	*elA|noc*
—	*T(Y;2)TE35B-18*	T(Y;2)Y;35B1.2	*elA|noc*
—	*In(2LR)noc^{4}*	In(2LR)35B1.2;41	*noc^{-}*
—	*T(2;3)GT8*	T(2;3)35A1-4;62F3-6 **33**	*noc^{-}*
25	*In(2)TE35B-219*	In(2)35B;40-41 **34**	*noc^{-}*
25	*In(2)TE35B-101*	In(2)35B;het	*noc^{-}*
25	*In(2)TE35B-1a*	In(2)35B;het	*noc^{-}*
25	*In(2L)TE35B-1*	In(2L)35B;39AB	*noc^{-}*
25	*In(2L)TE35B-8*	In(2L)35B;35C4	*noc^{-}*
25	*In(2L)TE35B-9*	In(2L)35B1.2;40	*noc^{-}*
25	*In(2L)TE35B-11*	In(2L)35B1.2;40	*noc^{-}*
25	*In(2L)TE35B-16*	In(2L)35B1.2;40	*noc^{-}*
25	*In(2L)TE35B-27*	In(2L)35B1.2;38C1.2	*noc^{-}*
25	*In(2L)TE35B-53*	In(2L)35B1.2;40	*noc^{-}*
25	*In(2L)TE35B-55*	In(2L)35B1.2;36C3-11	*noc^{-}*
25	*In(2L)TE35B-56*	In(2L)35B1.2;40	*noc^{-}*
25	*In(2L)TE35B-63*	In(2L)35B1.2;40	*noc^{-}*
25	*In(2L)TE35B-210*	In(2L)28B12-D1.2;35B	*noc^{-}*
25	*In(2L)TE35B-220*	In(2L)35B;39D	*noc^{-}*
25	*In(2L)TE35B-225*	In(2L)35B;40	*noc^{-}*
25	*In(2L)TE35B-227*	In(2L)35B;40 **35**	*noc^{-}*
25	*In(2L)TE35B-400* **b**	In(2L)35B3-5;36D1.2	*noc^{-}*

25	*In(2LR)TE35B-4*	In(2L)35B1.2;43B3-C1	*noc*⁻
25	*In(2LR)TE35B-6*	In(2LR)35B1.2;41	*noc*⁻
25	*In(2LR)TE35B-14*	In(2L)35B1.2;42F1-4	*noc*⁻
25	*In(2LR)TE35B-15*	In(2LR)35B1.2;44D1	*noc*⁻
25	*In(2LR)TE35B-50*	In(2LR)35B1.2;het	*noc*⁻
25	*In(2LR)TE35B-205*	In(2LR)35B;60B8-13 **36**	*noc*⁻
25	*In(2LR)TE35B-222*	In(2LR)35B;41B-C	*noc*⁻
25	*In(2LR)TE35B-223*	In(2LR)35B;41C-D	*noc*⁻
25	*In(2LR)TE35B-226*	In(2LR)35B;47B10-14;58F	*noc*⁻
25	*T(Y;2)TE35B-51*	T(Y;2)Y;35B1.2	*noc*⁻
25	*T(Y;2)TE35B-102*	T(Y;2)Y;35B	*noc*⁻
25	*T(Y;2)TE35B-201*	T(Y;2)Y;35B	*noc*⁻
25	*T(Y;2)TE35B-204*	T(Y;2)Y;35B	*noc*⁻
25	*T(Y;2)TE35B-206*	T(Y;2)Y;35B	*noc*⁻
25	*T(Y;2)TE35B-211*	T(Y;2)Y;35B **37**	*noc*⁻
25	*T(Y;2)TE35B-213*	T(Y;2)Y;35B	*noc*⁻
25	*T(1;2)TE35B-217*	T(1;2)20;35B	*noc*⁻
25	*T(1;2;3)TE35B-100*	complex **38**	*noc*⁻
25	*T(2;3)TE35B-3*	T(2;3)35B1.2;86F12-87A1	*noc*⁻
25	*T(2;3)TE35B-3a*	T(2;3)35B1.2;80-81 **39**	*noc*⁻
25	*T(2;3)TE35B-4*	T(2;3)35B1.2;81	*noc*⁻
25	*T(2;3)TE35B-7*	T(2;3)35B1.2;80-81	*noc*⁻
25	*T(2;3)TE35B-8*	T(2;3)35B1.2;81 **40**	*noc*⁻
25	*T(2;3)TE35B-25*	T(2;3)35B1.2;70B1.2	*noc*⁻
25	*T(2;3)TE35B-26*	T(2;3)35B1.2;81 **41**	*noc*⁻
25	*T(2;3)TE35B-28*	T(2;3)35B1.2;90C3-6	*noc*⁻
25	*T(2;3)TE35B-58*	T(2;3)35B1.2;94A4.5	*noc*⁻
25	*T(2;3)TE35B-200*	T(2;3)35B;94A1.2	*noc*⁻
25	*T(2;3)TE35B-202*	T(2;3)35B;81	*noc*⁻
25	*T(2;3)TE35B-207*	T(2;3)35B;80 **42**	*noc*⁻
25	*T(2;3)TE35B-208*	T(2;3)35B;80-81	*noc*⁻
25	*T(2;3)TE35B-209*	T(2;3)35B;80	*noc*⁻
25	*T(2;3)TE35B-212*	T(2;3)35B;67A7-15;het	*noc*⁻
25	*T(2;3)TE35B-215*	T(2;3)35B;82B	*noc*⁻
25	*T(2;3)TE35B-216*	T(2;3)35B;80-81	*noc*⁻
25	*T(2;3)TE35B-218*	T(2;3)35B;86E12.13	*noc*⁻
25	*T(2;3)TE35B-221*	T(2;3)35B;80-81	*noc*⁻
25	*T(2;3)TE35B-224*	T(2;3)35B;67F1.2;68A1.2	*noc*⁻
25	*T(2;3)TE35B-229*	T(2;3)35B;80-81	*noc*⁻
25	*T(2;3)TE35B-230*	T(2;3)35B;62A3-12 **43**	*noc*⁻
25	*T(2;3)TE35B-401* **b**	T(2;3)35B3.4;66B9.13	*noc*⁻
25	*T(2;4)TE35B-50*	T(2;4)35B1.2;101-102	*noc*⁻
25	*T(2;4)TE35B-101*	T(2;4)35B;101-102	*noc*⁻
25	*T(2;4)TE35B-214*	T(2;4)35B;102C	*noc*⁻

25	*Tp(2;2)TE35B-54*	Tp(2;2)35B1.2;35C1;38F	*noc*$^{-}$
25	*Tp(2;3)TE35B-203*	Tp(2;3)34C1.2;35B;80-81	*noc*$^{-}$
26	*Df(2L)Scorv25*	like *Sco*	*noc*$^{+/-}$
26	*In(2L)noc*^{2}	In(2L)35B1.2;36D3	*noc*$^{+/-}$
26	*In(2L)Sco*^{rv8}	In(2L)34C1.2;*Sco*;35D1.2	*noc*$^{+/-}$
26	*In(2L)Sco*^{rv24}	In(2L)34A7-B1.2;*Sco*;35D1	*noc*$^{+/-}$
26	*In(2LR)noc*^{7}	In(2LR)35A1.4;46B1.2; 40;48C6 **44**	*noc*$^{+/-}$
26	*In(2LR)Sco*^{rv1}	In(2LR)*Sco*;35D1.2;44C3-5	*noc*$^{+/-}$
26	*In(2LR)Sco*^{rv9}	In(2LR)*Sco*;35D1.2;41	*noc*$^{+/-}$
26	*T(2;3)Mpe*	T(2;3)35B2.3;86C1.2	
26	*Tp(2;2)Sco*	Tp(2;2)35A4-B1;35B3; 35B10-C1;35D1.2	*noc*$^{+/-}$
26	*Tp(2;2)Sco*^{rv12}	Tp(2;2)34A8.B1;*Sco*;34F4.5	*noc*$^{+/-}$
—	*In(2L)Sco*^{rv11}	In(2L)24C3-9;*Sco*;35D1.2	*noc|osp*
—	*In(2L)Sco*^{rv17}	In(2L)25D3-7;*Sco*;35D1.2	*noc|osp*
—	*T(Y;2)GT1*	T(Y;2)Y;35A4-B1	*noc|osp*
—	*T(2;3)GT7*	T(2;3)35B3;81;92F1.2	*noc|osp*
—	*T(2;3)H47*	T(2;3)35A-B;65F	*osp*$^{-}$
—	*In(2L)osp*^{22}	In(2L)35B3;38D3-5	*osp*$^{-}$
—	*In(2L)osp*^{59}	In(2L)35B3;38B3-6	*osp*$^{-}$
—	*In(2L)dpp*^{H86}	In(2L)22F;26C;35B3.5	*osp*$^{-}$
27	*T(2;3)osp*^{90}	T(2;3)35B3.4;89B9-11	*osp*$^{-}$
27	*T(2;3)pb*^{3}	T(2;3)35B3;83E2-8 **45**	*osp*$^{-}$
—	*In(2L)C158.1*	In(2L)26D1.2;35B3	*osp|l(2)35Bb*
—	*T(2;3)TE35BC-3*	T(2;3)35B5.10;81	*osp|l(2)35Bb, ck*$^{-}$

n.v., not cytologically visible. n.d., not determined. † genetically determined limits of aberrations (predicted limits of duplications). § predicted cytology.

1 induced on *In(2LR)Gla*

2 induced on *Cu*^{57}

3 also Dp(2;2)26D1.2;27D1.2

4 also Dp(3;3)76A5-7;80 §

5 predicted new order: 21—22F|35A—22F|97A—61A; 100—93C3-7|35B1.3—60

6 predicted new order: 21—35B1.3|57C3.9—35B1.3|57A8.11—60

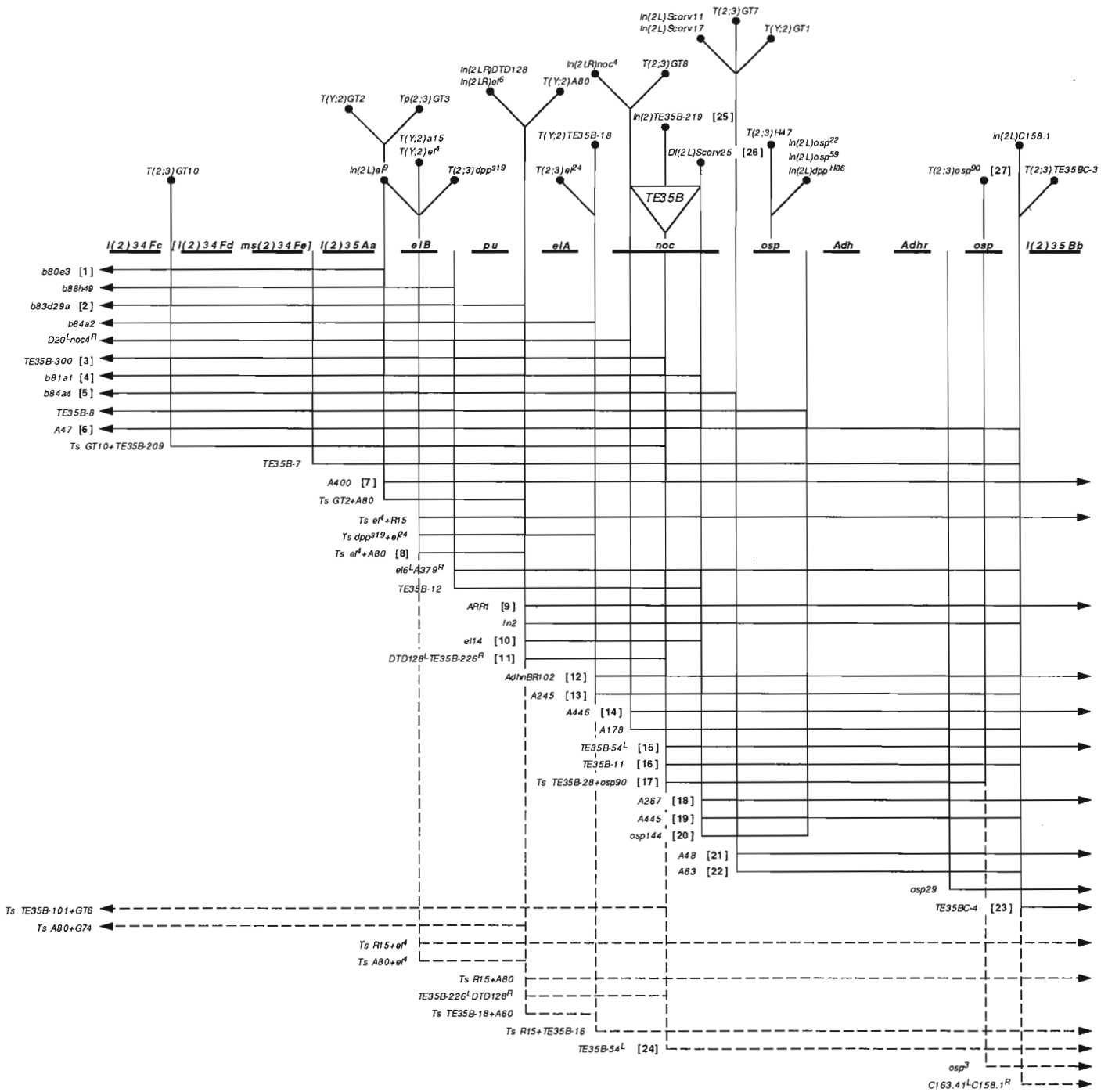

Figure 1. A genetic map of the el-noc region (from Roote et al., 1996, Genetics, in prep.). This map is based on complementation data. Deletions are indicated by solid lines and duplications by dashed. Other genetically mapped breakpoints of chromosomal aberrations are shown above the map.

[7] also Tp(2;2)35F1.2;36C1.2;49B1-3 new order: 21—35B1.3|36C1.2—49B1-3|35F1.2—36C1.2|49B1-3—60

[8] also Dp(2;2)27D1.2;28B3-D1

[9] also Dp(2;2)44C4.5;44DE

10 also Df(2R)42F1-4;44C3-5 *nec*$^{-}$—*cn*$^{-}$

11 also Dp(3;3)85F6-8;86A1.2

12 also Dp(3;3)85F6-8;86C1.2

13 also Dp(3;3)89B9-11;90C3-6

14 new order: 21—28F1.2|33A1.2—35A4|28F3-5—32F5|37A1.2—38F6|35D1.2—36F11|39A1.2—60

15 also Dp(3;3)89A9.10;90C3-6

16 also Df(2L)35D1;36A1.2

17 also *sna*$^{-}$—*BicC*$^{-}$

18 also *sna*$^{-}$—*l(2)35Dh*$^{-}$, *l(2)35Ea*$^{-}$

19 also *sna*$^{-}$—*l(2)35Dh*$^{-}$, *l(2)35Ea*$^{-}$

20 also *sna*$^{-}$—*BicC*$^{-}$

21 also In(2LR)35B1.3;40-41;57A8-10 new order: 21—35B1.3|57A8-10—40.41|35B1.3—40.41|57A8-10—60

22 also *sna*$^{-}$—*l(2)35Dh*$^{-}$, *l(2)35Ea*$^{-}$

23 new order: 21—35B1.2|89A4—86D4.5|35B3—60; 61—86C1.2|89A7—100

24 also Dp(2;2)24C3-9;26D1.2

25 also Dp(2;2)25D3-7;26D1.2

26 also Df(2L)27D1.2;28B3-D1

27 also Dp(2;2)42F1-4;44C3-5 §

28 also Dp(2;2)43B3-C1;44C3-5 §

29 also Df(3R)85F6.8;86A1.2

30 also Dp(3;3)90C3.4;91E5.6

31 also Dp(2;2)26D1.2;27D1.2

32 Tp(2;3)22C;41;35B1.2;28BC;42F;98D new order: 21—22C|41—35B1.2|28BC—22C|42F—60; 61—98D|35B1.2—28BC|42F—41|98D—100

33 also T(2;3)50B;81 new order: 21—35A1-4|62F3-6—81|50B—60; 61—62F3-6|35A1-4—50B|81—100

34 also T(Y;2)Y;24F

35 also T(2;3)40;72BC + In(3R)81;88B

36 also T(2;3)45EF;75C + T(Y;3)Y;85E1.2

37 also In(3R)81;99B

38 new order: 21—25F|het; het|35B—40|het; het|25F—35B|het

39 also T(2;3)25A3-8;67C9 + In(2R)54F;59A

40 also In(2L)29D2;34D4

41 also In(3LR)69F6.7;81

42 also T(2;3)50AB;84AB

43 also Tp(2;3)21A4;26D1;68B3.4 new order: 21—21A4|26D1.2—35B|62A3-12—68B3.4|21A4— 26C1.4|68C1.2—100; 61—62A1.2|35B—60

44 new order: 21—35A1.4|46B1.2—48C6|40—46B1.2|35A1.4—40|48C6—60D7|Y; Y|60D7—60F

45 also T(2;3)50C14;80 + In(3R)83E2-8;89A9.10 new order: 21—35B3|83E2-8—89A9.10|83E2-8—80|50C14—60; 61—80|50C14—35B3|89A10—100

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Drosophila virilis linkage map revision: Cytological localisation of *Delta* and *ebony* loci.

Drosophila virilis is considered to be the most primitive ancestor of the large "virilis" group which includes such species as *D. americana, D. novamexicana, D. montana, D. lummei, D. ezoana, D. texana, D. borealis, D . flavomontana, D. imeretensis, D. littoralis, and D. lacicola* (Patterson and Stone, 1952; Alexander, 1976) widely used in experimental genetic investigations (Alexander, 1976; Gubenko and Evgen'ev, 1984). The *D. virilis* karyotype has 5 pairs of large telocentric chromosomes and a pair of microchromosomes (Patterson and Stone, 1952) for which some variants of cytological maps were constructed reflecting position of bands, interbands and puffs in polytene chromosomes (Fujii, 1936; Fujii, 1942; Patterson *et al.,* 1940; Hsu, 1952). There are also genetic maps of *D.virilis* chromosomes constructed by recombination analysis of mutants (Alexander, 1976; Gubenko and Evgen'ev, 1984).

Using these data a cytogenetic analysis of an "unusual" heat shock sensitive locus localized in the second chromosome was undertaken. This locus is equivalent to the 93D locus in *D. melanogaster* and to the 2-48B locus in *D. hydei* and was named as *heat shock response omega* locus (*hsr-*) (Pardue *et al.*, 1990). By heat shock or other stress conditions activation of *hsr-* locus take place: "unusual" heat shock puff appears in the 20CD region and develops mainly from the 20C6-7 band (Gubenko *et al.,* 1991). In parallel with data on organization and location of the *hsr-* gene *in D. virilis,* we obtained some new experimental data concerning location of two other genetic loci, namely *Delta* and *ebony*, and genetic distance between them was determined by recombination analysis.

Table 1. Data on *Dl* and *eb* in *D. virilis* mutants.

Symbol	Name	Chromosome, locus	Phenotype	Reference
Dl	Delta	2 - 45.0	Longitudinal veins are widened near to edge of wings. Lethal in homozygous	Lebedeff, unpublished data*
eb	ebony	2 - 83.5	Very black body	Chino 1935*

* Ref. of Alexander, 1976

Table 2. List of *eb* and *Dl* mutations with chromosome rearrangements (X-ray, 6000 R) used for cytological mapping.

	Rearrangement	Break point	Reference
A.	*Df (2) eb* $^{TG-52}$	20C; 20D	Gubenko *et al.* 1991
	In (2) eb $^{PC-19}$	20C; 28H	*-ibidem*
B.	*T(2;3) Dl* ^{78e}	21E-F; 39H	Gubenko & Baritcheva 1982
	T(2;3)Dl $^{6-SG}$	21F-G; 27C	Subbota & Gubenko, unpublished
	T(2;3)Dl $^{22-SG}$	21G; 32C	*-ibidem*
	T(2;3)Dl $^{18-SG}$	21E-F; 33C	*-ibidem*
	T(2;6?)Dl ^{SD}	21G-H; chr*	*-ibidem*
	T(2; chr)Dl* ^{AS}	21F; chr*	*-ibidem*
	T(2; chr)Dl* ^{GB}	21F-G; chr*	Gubenko & Subbota, unpublished
	In(2)Dl $^{7-SG}$	21F-G; 26C	Subbota & Gubenko, unpublished
	In(2)Dl ^{EE}	21EF; 29H	*-ibidem*

chr* - chromocenter

Initially three strains were used in this work: 1) strain 9, wild type, isolated from Batumi natural population; 2) strain 160, synthesized by M.B.Evgen'ev from Japanese and American strains and having the following markers in autosomes: *broken* (*b*, 2-188.0); *gap* L_2 (*gp*, 3-118.5); *cardinal* (*cd*, 4-32.5); *peach* (*pe*, 5-203.0) and *glossy* (6-1.0); 3) strain 140 with recessive markers *ebony* (*eb*, 2-83.5) and *varnish* (*va*, 2-231.5). Males of strains either 9 or 160 were irradiated by X-rays (6000R) and mated with virgin females of strain 140 (Figure 1) to isolate mutant flies with *Dl* and *eb* phenotype (Table 1, Figure 2). We have gotten a rather representative collection of mutants with defects of *Dl* and *eb* loci (Gubenko and Subbota, 1992).

Beginning with experiments on genetic location of the *hsr-* gene, we considered that the dominant mutation *Delta* should be the most suitable marker for cytogenetical analysis. First attempt to find out a correspondence between cytological and genetical chromosome maps was made by Fujii (1936, 1942) and according to his data the locus *Dl* (2-45.0) has to be situated near the *incomplete* locus (*in*, 2-45.2) just to the left of its position on the genetic map (Figure 2 **a,b**). In the photographic map (Figure 2**d**) the interband space between two large bands in the 20E region corresponds to this locus (Figure 2**b,d**). Figure 2**c** shows arrangement *Dl, in* and *eb* on the existing genetic map (Gubenko and Evgen'ev, 1984) built on the base of localization data summarized by Alexander (1976). Table 2**A,B** shows the results of cytological analysis of break points in mutants with chromosome rearrangements, which were obtained by X-ray irradiation. From these data we can conclude that the real arrangement of *Dl* and *eb* loci in chromosome 2 is completely different from those in existing genetic maps of *D. virilis* (Figure 2**b**). We

have found that in the case of *In(2)eb*$^{PC-19}$ a break point is situated in the 20CD region and in *Df(2)eb*$^{TG-52}$ it is localized in the border of subsections 20B and 20C (Table 2**A**), (Gubenko *et al.*, 1991). These results are in good agreement with the conclusion that *hsr-* and *eb* loci are in close vicinity also in the 93D region of *D. melanogaster* and in the 2-48B region of *D. hydei* (Caizzi *et al.*, 1987; Peters *et al.*, 1984; Ryseck *et al.*, 1987).

Mutants with *Dl* phenotype and rearrangements in chromosome 2 have break points mainly in the 21E-H region (Table 2**B**). We failed to localize *Dl* locus in cytological map more precisely: 7 translocations, 1 deficiency and 2 inversions have break points in a large interval which includes 4 subsections. Difficulties with precise localization of *Dl* in polytene chromosomes are due to close vicinity of break points to the chromocenter (*i.e.*, translocations *T(2;6)Dl*^{SD}; *T(2;chr)Dl*^{AS} and *T(2;chr)Dl*^{GB}) or to other chromosome heterochromatic regions (*i.e.*, translocation *T(2;3)Dl*^{78e} or inversion *In(2)Dl*^{EE} (Table 2**B**) and apparently such regions can be involved in heterochromatisation processes. That is why appearance of mutant *Dl* can be connected with partial or complete gene inactivation for account of heterochromatisation of the regions studied. Under such effect banding pattern in the region also can be changed dramatically, which does not permit us to localize the *Dl* locus precisely. More accurate mapping of the locus could be carried out by the *in situ* hybridization technique of cloned DNA from the *Dl* locus with polytene chromosomes of wild type strains.

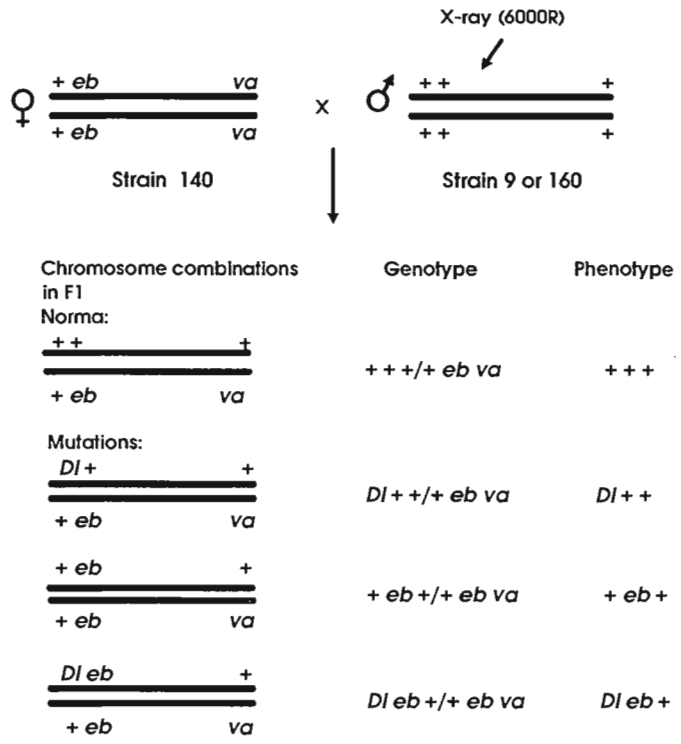

Figure 1. Scheme of mating used for selection of mutants with *Dl* and *eb* phenotypes after X-ray irradiation.

Nevertheless, it is obvious that existing information concerning localization of *eb* and *Dl* genes in the second chromosome is erroneous. Data of cytogenetic analysis show that the *eb* locus is situated more distal than the *Dl* locus but not on the contrary, as it was considered earlier (Alexander, 1976; Gubenko and Evgen'ev, 1984). We have determined also mitotic recombination frequency and evaluate that genetic distance between *eb* and *Dl* is 32.4 centimorgans (this value is somewhat less than those which could be found from Table 1 and Figure 2c). Because of the absence of corresponding genetic markers we could not fix distances between telomeric region and loci under investigation in order to ascertain their real position in the second linkage group relatively to the position of other genes.

In any case, the genetic map of chromosome 2 needs further revision. Now we can conclude that the arrangement of loci under investigation in the second chromosome is the following: *hsr-* (20C6-7 band) - *eb* (20C-D region, probably the border of C and D subsections) - *Dl* (21E-H region).

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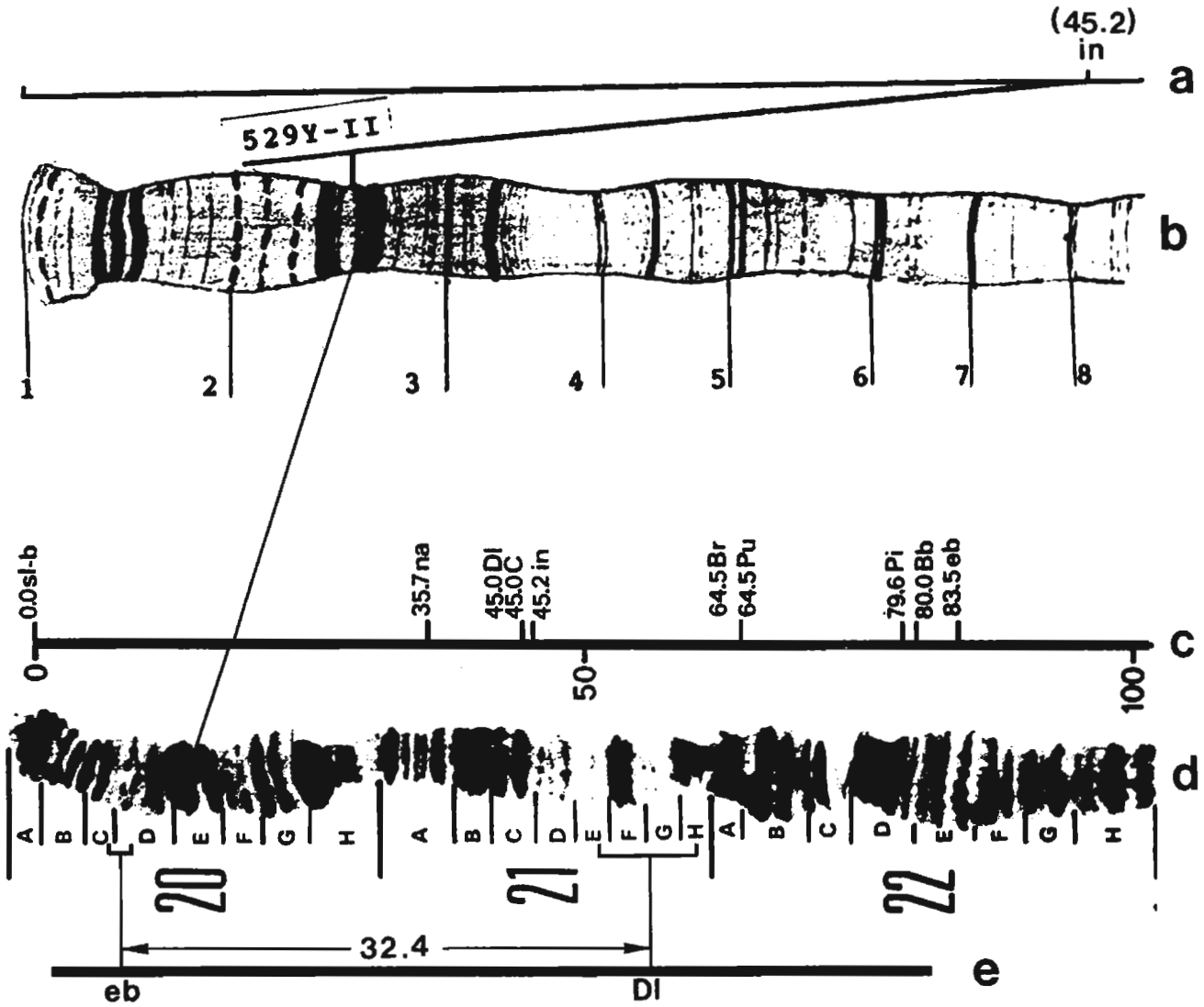

Figure 2. Maps of the distal part of the *D. virilis* chromosome 2. **a** - genetic, **b** - cytological maps (Fujii, 1936, 1942); **c** - genetic, **d** - cytological maps (Gubenko and Evgen'ev, 1984); **e** - genetic distance between *eb* and *Dl* loci. Line between cytological maps (**b** and **d**) shows the position of incomplete locus (*in*, 2-45.2). Brackets on the photographic map (**d**) shows cytological location of *eb* and *Dl* mutations determined in this work.

Barral, J.P., and H.F. Naveira. Departamento de Biologia Celular y Molecular, Facultad de Ciencias, Universidad de A Coruña, A Coruña, Spain. Contribution to the study of the evolution of the gene *Sex-lethal* in *Drosophila*.

The *Drosophila* gene *Sex-lethal* (*Sxl*) (X,19.1) plays key roles in the sexual determination of both somatic and germline cells and in dosage compensation (Baker, 1989; Cline, 1989; Steinmann-Zwicky *et al.*, 1990). *Sxl* is functionally expressed only in female flies. The sex-specific expression of this gene is regulated by alternative mRNA splicing which results in either the inclusion or exclusion of a translation stop codon (Sakamoto *et al.*, 1992). Female-specific mRNAs do not contain the sequence corresponding to the third exon in males, thereby having a long open reading frame that encodes a protein product with two potential RNA binding domains (Bell *et al.*, 1988). The female SXL product autorregulates *Sxl* pre-mRNA splicing by binding with some uridine-rich sequences included in this pre-mRNA (Sakamoto *et al.*, 1992).

The regions of the SXL product implicated in this splicing are RNA binding domains, which identify SXL as a member of a group of developmental regulators with, so called, ribonucleoprotein consensus sequence (RNP-CS) motifs.

These proteins contain a highly conserved octapeptide motif, termed RNP1 in *Sxl*, and frequently other(s) auxiliary segments unique to each type of protein (Bandziulis *et al.*, 1989).

The importance of the SXL protein suggests that its amino acid sequence should be highly conserved throughout the evolution of *Drosophila*, and fundamentally the regions implicated in the alternative splicing, the RNP-binding domains.

```
                                          RNP 2
mel_cDNA      NNSLNNLCGLSLGSGGSDDLMNDPRASNTNLIVNYLPQDMTDRELYALFRA
mel_Toonda    NNSLNNLCGLSLGSGGSDDLMNDPRASNTNLIVNYLPQDMTDRELYALFRA
mau_83        NNSLNNLCGLSLGSGGSDDLMNDPRASNTNLIVNYLPQAMTDRELYALFRA

                                  RNP 1
mel_cDNA      IGPINTCRIMRDYKTGYSFGYAFVDFTSEMDSQRAIKV
mel_Toonda    IGPINTCRIMRDYKTGYSFGYASVDFTSEMDSQRAIKV
mau_83        IGPINTCRIMRDYKTGYSFGYVFVDFTSEMDSQRPIKV
                                  *           *
```

Figure 1. Protein alignment of the analyzed regions. Variable positions are indicated by the asterisks.

In this paper, we present some evidence about the degree of conservation of these motifs, through the comparison of this region from one strain of *D. melanogaster* (Toonda, Australia) and one strain of *D. mauritiana* collected by David in 1983, with the previously published sequence of a *D. melanogaster* female cDNA (Bell *et al.*, 1988) (GenBank accession number M23636). The sequences from *D. melanogaster* Toonda and *D. mauritiana* comprised between nucleotide positions 771-1076 were amplified by PCR, sequenced, translated and aligned with the published sequence, as shown in Figure 1.

Table 1. Levels of variation for the RNP2-RNP1 region of the *Sxl* gene. The values in brackets are those corresponding for levels of variation in coding regions of *period* (data from Kliman and Hey, 1993).

	Proportion of differences by site (coding regions)		
	Total	Synonymous	Non-synonymous
Within *melanogaster*	0.0040 (0.0062)	0.0000 (0.0400)	0.0051 (0.0003)
melanogaster-mauritiana	0.0112 (0.0345)	0.0341 (0.1476)	0.0048 (0.0001)

Levels of variation of this region are shown in Table 1, together with those for the *period* gene (Kliman and Hey, 1993).

We found a high degree of divergence between *melanogaster* and *mauritiana* in the analyzed region involving both synonymous and non-synonymous differences (Table 1). Particularly remarkable are the differences observed in the RNP1 which bring about two amino acid replacements (Figure 1). Overall, the *mauritiana* - *melanogaster* comparison shows a considerable increment in the non-synonymous variation as compared with that observed for the *period* gene (Kliman and Hey, 1993). On the contrary, the number of synonymous differences per synonymous site is slightly smaller for *Sxl* than for *period*. A similar pattern of divergence has been observed for the *T4-scute* gene (Gandarela, Bouzada and Valadé, in prep.), another gene implicated in the same regulatory cascade, and it corresponds to what is usually expected from selection acting on nearby genes (hitchhiking effects) (Maynard Smith and Haigh, 1974; Stephan *et al.*, 1992), although a direct action of selection on *Sxl* evolution can not be discarded.

As regards the polymorphism in *D. melanogaster*, only one nucleotide change has been detected, which happens to be non-synonymous, consisting in a substitution of F by S close to the RNP1 (Figure 1). Sequences from other *melanogaster* populations are being obtained to get a more thorough picture of the extension of polymorphism in this region. The sequence of introns comprised between exons 5-6 and 6-7 have been sent to the GenBank (accession numbers *******-*******).

Further studies are necessary to explain the unexpected amino acidic divergence found in this gene in order to know the mechanisms that affect molecular evolution of *Sex-lethal*.

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Krishna, M.S., and S.N. Hegde. Department of Studies in Zoology, Manasagangotri, Mysore: - 570 006 India. Incipient sexual isolation in the bottleneck lines of *D. malerkotliana*.

The population size in nature often fluctuates and dwindles to the minimum leading to the loss of genetic diversity. It is suggested that the genetic diversity is regained in natural populations after certain generations of such bottlenecks in housefly (Bryant *et al.*, 1990). We have initiated bottleneck populations of *Drosophila malerkotliana* to check whether such rebouncing occurs in *Drosophila* or not, and we report here the effect of these bottleneck sizes on sexual isolation in *Drosophila malerkotliana*.

Bottleneck lines with one, four and eight pairs were initiated from F_2 generation of 150 naturally inseminated outbred females. The outbred population was used as a control line. After 10 generations of laboratory culture, virgin females and males were isolated within four hr of their eclosion from both control and bottleneck lines to study sexual isolation using the multiple choice method (15 males and 15 females of each line), male choice method (15 males of each of two lines introduced with 15 females of one of the two lines). The flies of one line were painted with Indian ink on the scutellum and were used to distinguish from the other. The experiments were carried out during morning hours at 22±1°C. Five sets of experiments were carried out for each method and their means were taken to estimate the isolation index using the formula of Merrell (1960). The isolation estimate between control and bottleneck lines and also between bottleneck lines using the multiple choice method are given in Table 1. We found more homogamic matings than heterogamic matings in three crosses out of six crosses made, and they were statistically significant. In the male choice experiment (Table 2), we noticed that in all the crosses, homogamic matings were higher than heterogamic matings. However, in two crosses the sexual isolation was asymmetric. The isolation estimate was close to one in one direction but in another direction it was significantly different. In the female choice experiment (Table 3), out of six crosses, five crosses showed asymmetric isolation where females of one line preferred its own male and discriminated against alien males in one direction, but in another direction there was no sexual isolation.

Table 1. Isolation estimates between control and bottleneck lines and also between bottleneck lines in *Drosophila malerkotliana* using multiple choice method.

Control and bottleneck lines	Number of homogamic and heterogamic matings				Isolation estimates	χ^2
	Homo	Hetero	Homo	Hetero		
CL x 1P	1P♂ x 1P♀	1P♂ x CL♀	CL♂ x CL♀	1P♀ x CL♂		
	76.8	38.8	76.6	40.8	0.52	23.37**
CL x 4P	4P♂ x 4P♀	4P♂ x CL♀	CL♂ x CL♀	4P♀ x CL♂		
	47.6	49.2	48.6	49.8	1.03	0.04
CL x 8P	8P♂ x 8P♀	8P♂ x CL♀	CL♂ x CL♀	8P♀ x CL♂		
	70.6	36.6	69.6	35.8	0.52	21.62**
8P x 1P	1P♂ x 1P♀	1P♂ x 8P♀	8P♂ x 8P♀	1P♀ x 8P♂		
	67.2	48.8	67.4	48	0.72	30.87**
4P x 1P	1P♂ x 1P♀	1P♂ x 4P♀	4P♂ x 4P♀	1P♀ x 4P♂		
	38	61.6	37.4	66.8	1.70	13.78**
8P x 4P	4P♂ x 4P♀	4P♂ x 8P♀	8P♂ x 8P♀	4P♀ x 8P♂		
	44.8	58.4	43.4	56.8	1.31	3.58

** Significant at 0.01 level, df=1, CL = control line, 1P = single pair bottleneck line, 4P = four pair bottleneck line, and 8P = eight pair bottleneck line.

Table 2. Isolation estimates between control and bottleneck lines and also between bottleneck lines in *Drosophila malerkotliana* using male choice method.

Crosses	Females	Male	Homogamic matings	Heterogamic matings	Isolation estimates	χ^2
1P x CL	1P + CL	1P	71	69	0.97	0.02
	1P + CL	CL	82	50	0.60	7.75*
4P x CL	4P + CL	4P	97.8	40.6	0.41	23.64**
	4P + CL	CL	89.2	29.8	0.33	29.65**
8P x CL	8P + CL	8P	89.4	59.2	0.66	6.13*
	8P + CL	CL	73.2	63.8	0.87	0.64
1P x 4P	1P + 4P	1P	64.4	52	0.81	1.32
	1P + 4P	4P	94.6	41.4	0.44	21.44**
1P x 8P	1P + 8P	1P	68	66	0.97	0.02
	1P + 8P	8P	79.8	32.2	0.40	20.23**
4P x 8P	4P + 8P	4P	91.4	45	0.49	15.78**
	4P + 8P	8P	80	38.4	0.48	14.61**

* Significant at 0.05 level, ** significant at 0.01 level, df = 1, CL = control line, 1P = single pair bottleneck line, 4P = 4 pair bottleneck line, 8P = eight pair bottleneck line

From these results it is evident that the incipient sexual isolation exists between the control line and bottleneck lines and also between bottleneck lines, which is in conformity with Kaneshiro (1980) and Meffert and Bryant (1991).

Table 3. Isolation estimates between control and bottleneck lines and also between bottleneck lines in *Drosophila malerkotliana* using female choice experiment.

Crosses	Males	Female	Homogamic matings	Heterogamic matings	Isolation estimates	χ^2
1P x CL	1P + CL	CL	47.4	91	1.91	13.73**
	1P + CL	P1	68.4	46.8	0.68	4.05*
4P x CL	4P + CL	CL	60.2	63.2	1.04	0.07
	4P + CL	4P	68.5	54.2	0.79	1.66
8P x CL	8P + CL	CL	59.4	74.6	1.25	1.72
	8P + CL	8P	61.4	67.4	1.09	0.27
1P x 4P	1P + 4P	4P	77	49.8	0.64	5.83*
	1P + 4P	1P	54.8	58.2	1.06	0.10
1P x 8P	1P + 8P	8P	66.2	71	1.07	0.16
	1P + 8P	1P	71.2	50.6	0.71	3.48
4P x 8P	4P + 8P	4P	61.4	69.6	1.13	0.51
	4P + 8P	8P	83.2	45.2	0.54	11.24**

* Significant at 0.05 level, ** significant at 0.01 level, df = 1, CL = control line, 1P = single pair bottleneck line, 4P = 4 pair bottleneck line, 8P = eight pair bottleneck line

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Preliminary study of the evolution of the bHLH and the leucine zipper domains of the gene *daughterless* in the *melanogaster* complex of *Drosophila*.

The gene *daughterless* (*da*) of *Drosophila* is a very promiscuous gene, whose functions can be separated in two groups. In the first group are the functions done by the maternal transcripts: sex determination and dosage compensation. In the second group are the functions done by embryonic transcripts: determination of the peripheral nervous system fate. This gene acts as a heterodimer with other gene products which are responsible for the functional specificity. Formation of these heterodimers is possible due to the presence of two dimerization domains in the *da* structure: the basic-Helix-Loop-Helix (bHLH) and the leucine zipper, whose evolution is studied in the present communication. Our work continues the studies on sex determination by O'Neil and Belote (1992) and Scott Whalthour and Schaffer (1994) using the gene *transformer* (*tra*). These studies are also interesting because genes implied in sex determination or embryonic development could be involved in speciation if their coevolution leads to the emergence of reproductive barriers.

We have studied the evolution of *da* in the *melanogaster* complex of *Drosophila*. In order to do it we used the already published sequence of this gene in the strain *Canton-S* of *D. melanogaster* (Caudy *et al.*, 1988) and we amplified (by PCR), cloned and sequenced a 315 bp region including the bHLH and leucine zipper domains in the other three species of the complex (*D. simulans, D. mauritiana* and *D. sechellia)* supplied by the National *Drosophila* Species Resource Center at Bowling Green.

Table 1. Nucleotide differences between species. Above the diagonal are values for nucleotide divergence between pairwise species per nucleotide site. Below the diagonal are values for non synonymous divergence between pairwise species per non synonymous site. The period data (Kliman and Hey, 1993; Hilton *et al.*, 1995) have a asterisk.

	D. melanogaster	*D. simulans*	*D. mauritiana*	*D. sechellia*
D. melanogaster		0.06032 0.03216*	0.01905 0.02858*	0.02539 0.02859*
D. simulans	0.03693 0.00155*		0.05397 0.01489*	0.05079 0.00119*
D. mauritiana	0.00410 0.00077*	0.04112 0.00077*		0.02222 0.01846*
D. sechellia	0.01228 0.00232*	0.04106 0.00387*	0.01641 0.00232*	

The obtained sequences were aligned using the multiple alignment option from the CLUSTAL V program (Higgins *et al.*, 1992). Predictions of the protein structures and hydrophobicity were performed using SEQAID II v. 3.81 (Roads and Roufa, 1991), which allows one to test the influence of the nucleotide changes on the

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SECHELLIA       ........C.......................................A.............
MAURITIANA      ........C.......T...............................A.............
SIMULANS        ........C.....A.................................A.............
MELANOGASTER    GCAAATAATGCGCGCGAACGTATCCGCATTCGGGATATTAACGAGGCGCTCAAGGAGCTGGGC
                ---------basic------------><--------Helix I--------------------
MELANOGASTER    AlaAsnAsnAlaArgGluArgIleArgIleArgAspIleAsnGluAlaLeuLysGluLeuGly
SIMULANS        ........................................................................
MAURITIANA      ..............Val.....................................................
SECHELLIA       ........................................................................

SECHELLIA       ..............................................A..............C...
MAURITIANA      ........................................................................C...
SIMULANS        ..............T..........G.................................CC...
MELANOGASTER    CGCATGTGCATGACGCACCTGAAGTCCGACAAGCCGCAGACGAAGCTGGGCATCCTGAATATG
                --------><-----Loop---------------------><------Helix II------
MELANOGASTER    ArgMetCysMetThrHisLeuLysSerAspLysProGlnThrLysLeuGlyIleLeuAsnMet
SIMULANS        ..............Tyr.....................................Thr...
MAURITIANA      ........................................................................
SECHELLIA       ........................................................................

SECHELLIA       ........................................................................
MAURITIANA      ........................................................................
SIMULANS        ................................A.............................C
MELANOGASTER    GCCGTCGAGGTGATCATGACGCTGGAGCAGCAGGTGCGCGAGCGCAACCTGAATCCGAAGGCG
                ---------------------------------><-----------Leucin zipper-----
MELANOGASTER    AlaValGluValIleMetThrLeuGluGlnGlnValArgGluArgAsnLeuAsnProLysAla
SIMULANS        ........................................................................
MAURITIANA      ........................................................................
SECHELLIA       ........................................................................

SECHELLIA       ..............................................T.........................
MAURITIANA      ........................................................................
SIMULANS        ..............A.........A.............................T....A...
MELANOGASTER    GCGTGCCTGAAGCGGCGCGAGGAGGAGAAGGCCGAGGATGGTCCCAAGCTAAGTGCCCAGCAT
                ----------Leucin zipper-----------------------------------------
MELANOGASTER    AlaCysLeuLysArgArgGluGluGluLysAlaGluAspGlyProLysLeuSerAlaGlnHis
SIMULANS        ..............His.....................................Ser......
MAURITIANA      ........................................................................
SECHELLIA       ..............................................Ser.....................

SECHELLIA       T.......................................................T.C..........
MAURITIANA      ................................T.....................C..........
SIMULANS        T....T.....................C..........C..............C.....T...T
MELANOGASTER    CACATGATACCGCAGCCGCAGCAGGTGGGCGGCACGCCCGGCAGCAGCTATCATAGCCAGCCAG
                ----------><---------------------5' region---------------------
MELANOGASTER    HisMetIleProGlnProGlnGlnValGlyGlyThrProGlySerSerTyrHisSerGlnPro?
SIMULANS        TyrIle.....................Ala.........Arg...............His...?
MAURITIANA      ........................................................................?
SECHELLIA       Tyr.....................................................Tyr.........?
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Figure 1. Comparison of the DNA and the amino acid sequences of the *da* genes from the *melanogaster* complex. The complete nucleotide and amino acid sequence is shown only for *D. melanogaster* Canton-S, while for the other species only bases and amino acids that differ from this sequence are shown.

two parameters.

Figure 1 presents the alignment of nucleotide and amino acid sequences. There are several non-synonymous changes present in the bHLH domain: one in the basic region of *D. mauritiana* and two in *D. simulans* (one in the loop and another in the Helix II). These changes are important considering that they modify the protein conformation of this

domain which could have an influence in its functionality (Pakula and Sauer, 1989). It is also possible to observe the large number of synonymous and non-synonymous substitutions in the leucine zipper region in *D. simulans.* Although the nucleotide substitutions do not affect leucine residues, they modify the conformation and the hydrophobicity in the leucine zipper domain. Overall, we observe (Table 1) a high number of non-synonymous nucleotide substitutions in the region analyzed compared to *period* data, whose evolution is thought to be essentially neutral (Kliman and Hey, 1992; Hilton *et al.*, 1995). It is also possible to see in Table 1 an unusually high evolutionary rate appearing in *D. simulans.* These findings could have important biological implications.

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References: Caudy *et al.*, 1988, Cell 55: 1061-1067; Hey and Kliman 1993, Mol. Biol. Evol. 10(4): 804-822; Higgins *et al.*, 1992, Comput. Appl. Biosci. 8: 189-191; Hilton *et al.*, 1995 Evolution 48(6): 1900-1913; O'Neil and Belote 1992, Genetics 131: 113-128; Pakula and Sauer 1989, Annu. Rev. Genet.: 289-310; Roads and Roufa 1991, Seqaid II 3.81: Molecular Genetics Laboratory, Center for Basic Research, Kansas State University, Manhattan, U.S.A.; Whalthour and Schaffer 1994, Genetics 136: 1367-1372.

Zajonz. M., S.R. Ramesh and W.-E. Kalisch. Ruhr-Universität Bochum, FR Germany. Homologous fractions of larval salivary secretion in various species of *Drosophila.*

One of the peculiarities concerning secretion proteins of the larval salivary glands (glue) in *Drosophila* is the species-specific variety of prominent and of non-prominent fractions, which in several cases is a subspecies-specification or even a wild type-specification (Ramesh and Kalisch,1989). By this, one can hardly imagine that there are any homologous fractions for the basic function, which is to fix the pupa to the substratum. In general, we have to realize that very little is known so far about function and interaction of the individual prominent fractions and almost nothing is known about the non-prominent ones.

We checked several strains of the *Sophophora* subgenus and the *Drosophila* subgenus for homologous non-prominent secretion fractions (Figure 1). In the *melanogaster* group we checked: (1) *D. melanogaster* (wild type Berlin), (2) *D. mauritiana* (Bowling Green code of species stock list 1990: 14021-0241.0) and (3) *D. simulans* (14021-0251.0). In the *virilis* group and the *repleta* group we checked: (4) *D. virilis* (15010-1051.0) and (5) *D. hydei* (Bochum). In the *immigrans* group we checked the *immigrans* subgroup: (6) *D. signata* (1 5111-1 741.0); the *nasuta* subgroup: (7) *D. nasuta nasuta* (Mysore 1), (8) *D. n. albomicans* (1 511 2-1751.0), (9) *D. sulfurigaster sulfurigaster* (15112-1831.1) and the *hypocausta* subgroup: (10) *D. rubida* (15115-1901.2).

We used SDS-PAGE together with a 13.4% separating gel covered by an elongated (25 mm) stacking gel of 5.6%. Silver-staining (Ansorge, 1985) was used; in lane 9 of Figure 1 silver-staining was used together with CBB staining. For more methodological details see Ramesh and Kalisch (1988, 1989). Glue plugs were prepared from the salivary glands after fixing the glands for 3 min in ethanol and separating the salivary gland cells with sterile needles. By this preparation technique, cell membranes and cell protein fractions keep attached to the plugs in various amounts. These cell fractions get visualized together with the (glue) secretion fractions by silver staining.

Figure 1 indicates data from different experiments. By the limitation of the technique used, we are not able to analyse homologous non-prominent secretion fractions in the separating gel from these data. However, data from one and the same experiment and with a more precise separation technique would hardly give us any more information. This is because cell proteins originally attached to the glue plugs mimic homologous non-prominent secretion protein fractions in different strains.

Because most of the cell fractions and most of the secretion fractions are small enough to pass through the stacking gel, we only find three types of fractions left in the stacking gels: (1) Fractions on top of each lane in the stacking gel, consisting of molecules which are obviously too big to enter the 5.6% stacking gel within the 4 h run of the SDS-PAGE. (2) Fractions indicating diffused bands, which are species-specific or even sex-specific (compare Zajonz *et al.,* this issue). (3) One or two fractions which include focused bands in the upper part of the stacking gels (labeled in Figure 1).

In the *Sophophora* subgenus (*D. melanogaster, D. mauritiana* and *D. simulans*, Figure 1, lanes 1-3), we only find single fractions or a single fraction with a very faint fraction on top of it (in *D. mauritiana* and *D. simulans*). In the subgenus *Drosophila*, however, there are always (beside *D. hydei*) two clearly separated fractions. But even in *D. hydei* we could show (compare Zajonz *et al.*, this issue) that the band is composed of two fractions.

These fractions (based on different experimental data) indicate two homologies: (1) Unique appearance of focused bands in the same or almost the same region of the stacking gel, and (2) comparable number and prominence of the bands.

Three simple methodological facts might have been the reasons why these fractions did not elicit interest so far: (1) The common way to separate the glue from salivary glands is to eluate with TCA and ethanol/chloroform mixture. However, these fractions get lost by preparation with TCA and ethanol/chloroform mixture. (2) Coomassie Brilliant Blue, which does not stain these bands, is commonly used instead of silver-staining. (3) Usually, stacking gels are much shorter, which probably does not bring about separation of these fractions from those on top of the stacking gels. Furthermore, stacking gels are not commonly published together with the separating gels.

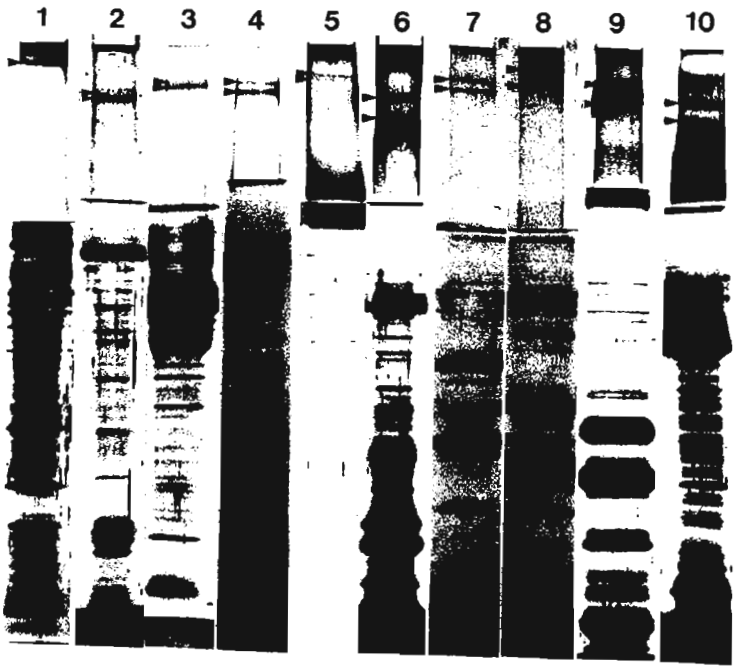

Figure 1. SDS-PAGE fraction pattern of salivary gland plugs (with attached salivary gland cell proteins) in: (1) *D. melanogaster*; (2) *D. mauritiana*; (3) *D. simulans*; (4) *D. virilis*; (5) D. *hydei*, (6) *D. signata*; (7) *D. n. nasuta*; (8) *D. n. albomicans*; (9) *D. s. sulfurigaster* and (10) *D. rubida*. Individual salivary glands (two plugs) from late third instar female larvae were used for each lane. Salivary glands were fixed for 3 min in 95% ethanol. Plugs were separated from the surrounding cells by sterile needles. 13.4% separating gel, 25 mm long stacking gel (5.6%); silver-staining [in (9) together with CBB]. Lanes 2, 6, and 10 as well as 7 and 8 are from the same gel, respectively. Fractions of interest are labeled.

We checked whole larvae (without salivary glands and mouth hooks) as well as separated fat bodies for homologous stacking gel fractions, but in neither of these experiments we could find them. This indicates (limited to the technique used) that these fractions are salivary gland-specific rather than common cell-specific fractions.

In an additional test we used PNGase F (18 h in 37°C) for deglycosylation of salivary gland secretion fractions in various species (see Tarentino *et al.*, 1989 for details of the procedure). After this treatment, the focused stacking gel fractions get lost.

Even these data are preliminary and nothing is known about the biochemical details of these fractions so far. We assume that these bands (by their low prominence) rather represent fractions of homologous function than non-homologous fractions of the same or almost the same molecular weight.

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Zajonz, M., S.R. Ramesh and W.-E. Kalisch. Ruhr-Universität Bochum, FR Germany. Sex-specific fractions in salivary plugs of *D. hydei*.

We checked salivary plugs from glands of late third instar larvae in different *D. hydei* wild-type strains (Alicante, Bochum, Düsseldorf, Münster, Tübingen and Zürich) and in the *D. hydei* mutant (Hess, 1970) by SDS-PAGE.

Secretion plugs of individual male as well as female larvae were prepared by fixing the glands for 3 min in ethanol and separating the plugs from the surrounding salivary gland cells by sterile needles. By the preparation technique used, cell membranes and cell protein fractions keep attached to the plugs in various amounts. These fractions get visualized

together with the (glue) secretion fractions by silver staining (Ansorge, 1985). We used a 13.4% separating gel covered by an elongated (25-75 mm) stacking gel of 5.6%. Further methodological details in Ramesh and Kalisch (1988, 1989).

Figure 1 indicates data of the two plugs of an individual wild type larva in each lane. Four different types of fractions can be distinguished: (a) comparable secretion fractions and cell fractions in all *D. hydei* wild-type strains tested, (b) strain-specific fractions (labeled by ✶ in lanes 3-8), (c) one male-specific fraction in the stacking gel (labeled by < in lane 12) and (d) several female-specific fractions in the stacking gel and in the separating gel (labeled by • in lane 3, 5, and 7).

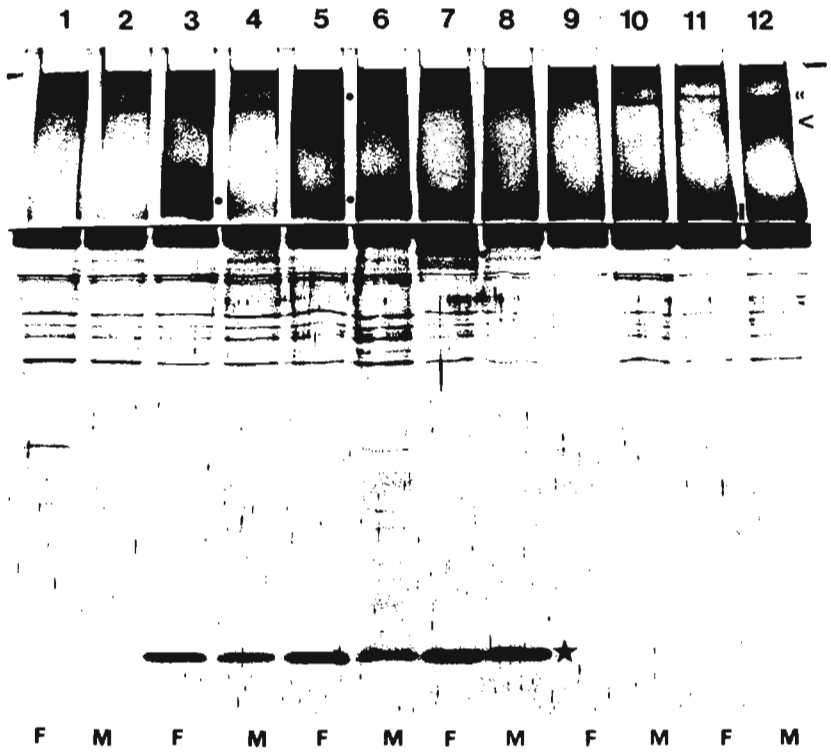

Figure 1. SDS-PAGE fraction patterns of salivary plugs (with attached salivary gland cell proteins) in *D. hydei* wild-type strains: (1-2) Münster, (3-4) Zürich, (5-6) Alicante, (7-8) Tübingen, (9-10) Düsseldorf, (11-12) Bochum. F = female larva; M = male larva. Individual salivary glands (two plugs) are used for each lane; 13.4% separating gel and elongated (25 mm) stacking gel of 5.6%; silver-staining. Labeled fractions: ✶ strain-specific fractions; < male-specific fraction; ● female-specific fractions; for « and ▮ see text.

Male- and female-specific fractions (lane 3, 5, and 12) are obviously large molecules, because they do not enter the 5.6% separating gel. Note that there is still a prominent fraction on top of each lane, which does not even enter the stacking gel.

The reason why sex-specific fractions are not reported so far could be based on three methodological details: (1) Silver-staining instead of the common CBB-staining is needed to visualize these fractions. (2) Ethanol fixation and ethanol washing procedures of salivary glands are needed. Sex-specific fractions get lost by the common TCA fixation and washing procedures in a mixture of methanol and chloroform. (3) Stacking gels are commonly short. By this, sex-specific fractions hardly get separated from the prominent fraction(s) on top of the stacking gel. Furthermore, usually the stacking gels are eliminated from the separating gel in published figures.

We checked whole larvae (without salivary glands and mouth hooks) as well as separated fat bodies for sex-specific fractions, but in neither of these experiments we succeeded. This indicates (limited to the technique used) that sex-specific fractions are not produced somewhere outside the salivary glands.

We also investigated the sex-specific fractions in an elongated (75 mm) stacking gel to get more detailed information about individual fractions. We compared a female and a male larva of the Bochum wild-type strain (lane 1 and 2 in Figure 2) with a female and a male larva of the COM TKS mutant (lane 3 and 4 in Figure 2). In comparison with the Bochum wild-type strain in Figure 1 (lane 11 and 12), fraction pattern of the Bochum wild-type strain in Figure 2 (1 and 2) indicates: Two fractions instead of one (labeled «) in the male. In the female (lane 11 in Figure 1) a faint double fraction is visible, whereas these fractions in lane 1 in Figure 2 probably are covered by the diffuse material, which has entered the stacking gel. In the same female (lane 1 in Figure 2), two fractions (labeled □) are found instead of the one fraction (labeled ▮) in lane 11 of Figure 1.

The *COM TKS* mutant in Figure 2 (lane 3 and 4) has a remarkably different pattern in both sexes compared with the Bochum wild type. *COM* mutants are composed of one translocation between the Y-chromosome and an autosome (A•Y) as well as one translocation between the remaining part of the Y-chromosome and the X-chromosome (X•Y). Females carry attached X-chromosomes and the A•Y translocation; whereas males carry the A•Y and the X•Y translocation (Hess, 1970). It is unknown in detail, which wild-type strains (in comparison to the strains we have tested) had been

Figure 2. Sex-specific fractions in the salivary plugs of the *D. hydei* Bochum wild-type strain (lane 1 and 2) and the *COM TKS* mutant (lane 3 and 4). F = female larva; M = male larva. The (75 mm) elongated stacking gel (5.6%) and the upper rim of the separating gel (13.4%) are depicted; for « and □ see text

involved in constructing these genotypes about 25 years ago. Therefore, the differences of sex-specific fractions shown in Figure 2 could be based on the wild-type strains used or could be based on the strain-specific X•Y translocations as well.

The data shown in Figures 1 and 2 are reproducible. However, due to the separation technique we used, data are still preliminary so far concerning the biochemical details.. Finally, we have no idea what is the functional significance of the larval (!) sex-specific fractions in *D hydei*.

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References: Ansorge, W. 1985, J. Biochem. Biophys. Methods 11: 13-20; Hess, 0. 1970, Molec. Gen. Genetics 106: 328-346; Ramesh, S.R. and W.- E. Kalisch 1988, Biochem. Genetics 26 (7/8): 527-541; Ramesh, S.R. and W.- E. Kalisch 1989, Biochem. Genetics 27 (9/10): 507-520.

Zajonz, M., S.R. Ramesh and W.-E. Kalisch. Ruhr-Universität Bochum, FR Germany. Deglycosylation of larval secretion protein fractions in the *D. nasuta* subgroup.

D. nasuta subgroup members are characterized by a strain-specific glycosylation pattern of the X-chromosomal secretion protein fractions of larval salivary glands and by a subgroup-specific glycosylation pattern of the 130kD and the 12kD fractions (Ramesh and Kalisch ,1988b; 1989a, b). In the present study we investigated the deglycosylation of the X-chromosomal fractions and the 130kD fractions in the following *D. nasuta* subgroup strains: *D. n. nasuta* (wild type Mysore I), *D. n. nasuta* (a spontaneous secretion protein mutant of Mysore 1; Kalisch and Ramesh, 1988), and *D. n .albomicans* (wild type Okinawa; Bowling Green code of species stock list 1990:15112-1751).

Figure 1 (lane la, 2a, and 3a) indicates the Coomassie Brilliant Blue R-250 patterns of protein fractions (CBB-staining; for details see Ramesh and Kalisch, 1988a). We overloaded the gels by using 30 (glue) secretion plugs, containing approximately 150 μg of protein, for each lane. Plugs were prepared by fixing the bloaded glands for 3 min in ethanol and separating salivary gland cells with sterile needles. By this simple technique, we cannot prevent cell proteins getting attached to the plugs. Therefore, cell protein fractions attached to 30 plugs get depicted together with secretion protein fractions in CBB-stained patterns of Figure 1.

Figure 2 (lane la, 2a, and 3a) indicates the patterns of secretion proteins in comparable experiments after PAS-staining as per the procedure of Segret and Jackson (1972). As already mentioned above, strain-specific glycosylation of X-chromosomal fractions (domains II and III; for details see Ramesh and Kalisch, 1989b) as well as subgroup-specific glycosylation of 130kD fractions (domain 1) and 12kD fractions (domain V) is found in comparison with the control data of the a-lanes depicted in Figure 1.

For deglycosylation of secretion protein fractions we used PNGase F (Sigma Chemicals, USA; 18h, 37°C; Tarentino, Gómez and Plummer,1985). The following is evident from Figure 1 (lane 1b, 2b, and 3b) which includes the SDS-PAGE patterns of deglycosylated salivary gland secretion protein samples after CBB-staining and from Figure 2 (lane 1b, 2b, and 3b) which includes the pattern of deglycosylated protein fractions after PAS-staining:

[1] Apparently, the complete deglycosylation of the 130kD fractions (domain I) in Figure 2 (lane 1b, 2b, and 3b) is accompanied by a complete disappearance of the CBB-stained 130kD protein fractions in Figure 1 (lane 1b, 2b, and 3b).

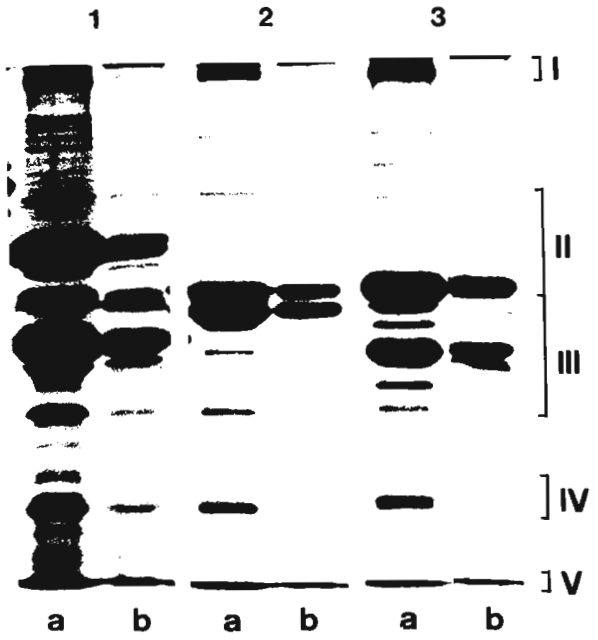

Figure 1. CBB-staining of secretion protein fractions in the *D. nasuta* subgroup: (1) *D. n. nasuta*; (2) *D. n. nasuta* [Sa] ; (3) *D. n. albomicans*. (a) Control. (b) Secretion (glue) plugs deglycosylated with PNGase F (18h; 37°C). Bloated salivary glands are fixed for 3 min in ethanol; 30 plugs are used for each lane; SDS-PAGE with a 5.6% stacking gel (not depicted) and a 13.4% separating gel; for details about domain I - V see Ramesh and Kalisch (1989b). The double band at the border between domain II and III in *D. n. nasuta* (1a and b) is not to be found as a prominent fraction in CBB-stained patterns from individual glue plugs. They could be the result of overloading the lanes with 30 glue plugs or could be the result of other reasons (Kalisch and Ramesh, 1988).

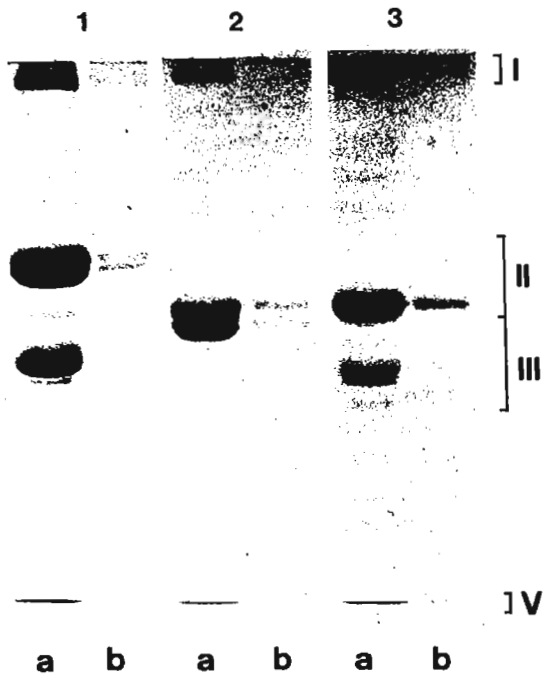

Figure 2. PAS-staining of secretion protein fractions in the *D. nasuta* subgroup: (1) *D. n. nasuta*; (2) *D. n. nasuta* [Sa] ; (3) *D. n. albomicans*. (a) Control; (b) Secretion (glue) plugs deglycosylated with PNGase F (18h; 37°C). Same method used as in Figure 1.

[2] Deglycosylation of prominent X-chromosomal fractions (domain II and III) in Figure 2 (lane 1b, 2b, and 3b) is not complete for most of the fractions, but could be correlated with the quantity of sugar originally attached to individual fractions.

[3] Furthermore, deglycosylation of PAS-stained X-chromosomal fractions in [2] is correlated with a reduction of protein quantities of the CBB-stained X-chromosomal fractions in Figure 1 (1b, 2b, and 3b). This seems to be obvious from the data depicted, even though CBB is only known as a semi-quantitative staining technique.

[4] Comparing the a- and the b-lanes in Figure 1 and Figure 2, there is no fraction that has changed significantly its electrophoretic mobility after deglycosylation.

[5] There are no additional fractions found after deglycosylation in the b-lanes in comparison with the controls (a-lanes in Figure 1 and Figure 2).

In general, data [1] - [5] indicate that the quantity of protein fractions (and not the molecular weight) seems to be reduced during the process of deglycosylation. The following three hypotheses could explain these findings:

(A) Reduction of protein quantities simply could have methodological reasons. This would explain that even most of the cell protein fractions (a-lanes in Figure 1) are reduced in their quantities after deglycosylation (b-lanes in Figure 1).

(B) Glycosylated secretion proteins could be composed of short amino acid sequences which undergo posttranslational modification together with sugar molecules (Ramesh and Kalisch, 1989b). By this, protein fractions depicted in Figure 1 (1b, 2b, and 3b) would represent the remaining fractions without deglycosylation. This would explain the unchanged electrophoretic mobility.

(C) Deglycosylation as described in hypothesis (B) may yield several fractions which are not prominent enough to get localized by the low-sensitive CBB-staining technique used.

Figure 3. Ag-staining of secretion protein fractions in *D. n. nasuta*. (1) 130kD fractions were cut out of unstained SDS-PAGE gels, homogenized, deglycosylated (PNGase F for 25-30h; 37°C) and used for the second SDS-PAGE (5.6% stacking gel; 13.4% separating gel). (2) Ag-stained pattern of an individual deglycosylated salivary gland as a control. The genetic backgrounds of protein fractions depicted in this lane and in Figure 1(1b) are identical. Differences are mainly based on the more sensitive Ag-staining technique used. Arrow head indicates the position of the original 130kD fraction.

Hypotheses (A) - (C) have not to be alternative, but could also be additive to each other. For the strategy of further experiments, we checked hypothesis (C) using the following technique: We selected 130kD fractions by cutting out gel blocks (the separation gel areas next to the border of the stacking gels) from overloaded and unstained SDS-PAGE gels of *D. n. nasuta*. As a control, we used CBB-staining after cutting out the 130kD fractions to be sure that fractions adjacent to the 130kD bands are not mixed up. We homogenized the 130kD gel blocks and deglycosylated the protein fractions by using PNGase F (25 - 30h; 37°C in a deglycosylation buffer; Tarentino, Gómez and Plummer, 1985). We used this deglycosylated probe for a second SDS-PAGE. In this experiment we used the more sensitive silver-staining (Ansorge, 1 985) instead of CBB-staining.

Figure 3 (lane 1) indicates that the 130kD fractions are not lost but become smaller fractions with their molecular weight decreased after deglycosylation. In contrast to Figure 1 (lane 1b, 2b, and 3b), a faint Ag-stained 130kD fraction is still found in Figure 3 (lane 1; labeled by an arrow head). This indicates: (1) even the 130kD fractions are not completely deglycosylated by PNGase treatment, and (2) deglycosylated fractions are not lost during the SDS-PAGE.

The pattern of Figure 3 (lane 1) is based on 30 glue plugs, whereas the pattern in Figure 3 (2) depicts an individual deglycosylated salivary gland. It is obvious that any further analysis of deglycosylated secretion protein fractions in the *D. nasuta* subgroup has exclusively to be based on Ag-stained patterns.

Acknowledgments: The second author (S.R.R., permanent address: Dept. of Zoology, University of Mysore, Mysore, India) is grateful to the DAAD, FR Germany, for the award of a scholarship. The authors wish to thank Mrs. C. Plehn for technical assistance.

References: Ansorge, W. 1985, J. Biochem. Biophys. Methods 11: 13-20; Kalisch, W.-E. and S.R. Ramesh 1988, Dros. Inf. Serv. 67: 51; Ramesh, S.R. and W.-E. Kalisch 1988a, Biochem. Genetics 26 (7/8): 527-541; Ramesh, S.R. and W.-E. Kalisch 1988b, Dros. Inf. Serv. 67: 107; Ramesh, S.R. and W.-E. Kalisch 1989a, Genetica 78: 63-72; Ramesh, S.R. and W.-E. Kalisch 1989b, Biochem. Genetics 27 (9/10): 507-519; Segrest, J.P. and R.L. Jackson 1972, In: Ginsburg (ed.) *Methods in Enzymology* 28: 54-63; Tarentino, AL., C.M. Gómez and T.H. Plummer 1985, Biochem. 24: 465-467.

Doane, W. W. Department of Zoology, Arizona State University, Tempe, AZ, U.S.A. 85287-1501. The *adp*^{fs} and *fs(2)lto* mutations of *Drosophila melanogaster* show allelic interaction.

The *adipose* (*adp*; 2-83.4) locus appears to represent the only known "obesity gene" in *Drosophila* (Lindsley and Zimm, 1992; *FlyBase*, 1996). Well-fed *adp* mutant flies, 6 days post-eclosion or older, are obese in that they weigh more than wild-type and have about twice the normal lipid content. Hypertrophied fat bodies packed with large lipid vesicles are visible through the body wall when these flies are submerged in ethanol or water. Both sexes also display reduced levels of glycogen, which is associated with lowered glycogen synthase and glycogen synthase phosphatase activities, at least in males (Foehr and Doane, 1994).

Although *adipose* was named for its abnormal lipid metabolism, the first mutant allele described for this locus, *adp*^{fs} (syn. *fs(2)adp*; Doane, 1960a), was actually screened from a natural population in Kaduna, Nigeria, on the basis of complete female sterility. Fertilized eggs laid by *adp*^{fs} homozygotes display maternal effect lethality: regardless of their paternal genotype, they fail to develop past the first few mitotic cycles and undergo meiotic or mitotic arrest. Sterility is autonomous to the ovary, and pleiotropic defects related to oogenesis are often expressed as a "yolk deficiency syndrome". Some of the abnormal traits included in this syndrome are reduction in egg production, small eggs (yolk deficient), and defects in the egg

chorion, chorionic filaments and/or vitelline membrane. The yolk deficiency syndrome can be rescued through manipulation of the residual genetic background (Doane, 1960b). In this situation, egg production appears normal, meiosis is completed, and the eggs appear normal. However, the embryos still fail to develop beyond the 5th to 6th mitotic cycles, their chromosomes remaining on the metaphase plate so that no anaphase movement to the poles occurs.

Soon after the initial work on *adp*^{fs} was published, an obese but fertile allele called *adp*^{60} was isolated from the same Kaduna population that yielded the first allele (Doane, 1961, 1963). This raised a possibility that the pleiotropic effects associated with female-sterility in *adp*^{fs} homozygotes are encoded by a different gene than are the defects related to their abnormal lipid and carbohydrate metabolism, although many attempts to separate these two general classes of defects through genetic recombination have failed (W. W. Doane, unpublished data). This possibility was recently strengthened, however, by the discovery that the female sterility trait of *adp*^{fs} mutants displays allelic interaction with one of the EMS-induced female-steriles listed by Schupbach and Wieschaus (1991), namely *fs(2)ltoDF6*. The latter mutation had been isolated by saturation screening for second chromosome female-steriles (Schupbach and Wieschaus, 1989) and is described in a note by T. Schupbach on page 237 of Lindsley and Zimm (1992) under the revised gene symbol *fs(2)lto5*. *FlyBase* (1996) lists it under the latter symbol.

The *adp* locus was assigned the cytological map position of 55A-C1, based on its inclusion in *Df(2R)PC4* but not in *Df(2R)P29* (Doane and Dumapias, 1987; Lindsley and Zimm, 1992, p. 21). More recently, its cytological location was refined to 55A (Doane, 1994). Several female sterility mutations besides *adp*^{fs} are uncovered by *Df(2R)PC4*, including *fs(2)lto5*, *early*, *halted*, *staufen*, and *subito* (Schupbach and Wieschaus, 1989, 1991). Since the sterility phenotypes of these mutants share some features in common with *adp*^{fs}, strongly expressed mutant alleles for each were tested in *trans* against *adp*^{fs} for complementation. Stocks containing these mutants were generously provided by Trudi Schupbach. They included: DF6 (*fs(2)lto5*), HL45 and QP71 (*early*), DB48 and P156 (*halted*), HL54 (*staufen*), HM26 and PF24 (*subito*). (*staufen* and several other female-sterile stocks had been tested earlier by Doane and Dumapias, 1987.) The only heterozygous females that failed to show complementation by producing lethal eggs had the genotype *fs(2)lto5/adp*^{fs}; all others were fertile. Therefore, it appears that *fs(2)lto5* is allelic to *adp*^{fs}.

Females homozygous for *fs(2)lto5* lay short eggs and their sterility defects appear in later stages of oogenesis, during yolk uptake, chorion synthesis, or egg laying (Lindsley and Zimm, 1992; *FlyBase*, 1996). While a detailed analysis has not been made of *fs(2)lto5*, these defects are clearly reminiscent of the "yolk deficiency syndrome" expressed by *adp*^{fs} female homozygotes. It is important to note that none of the above female sterility mutants, including *fs(2)lto5*, display the obesity phenotype that is characteristic of *adp*^{fs} and *adp*^{60} mutants and is visible through the body wall. This permits us, for the first time, to perform genetic fine structure analysis of the *adp* locus in order to determine the linkage distance between the recessive obesity trait displayed by both *adp* mutant alleles and the recessive female sterility trait expressed by *adp*^{fs} and *fs(2)lto5*. Such an analysis is in progress.

Fine structure recombination analysis may resolve the question of whether or not the pleiotropic defects caused by mutations in the *adp* obesity gene include female sterility. However, if these traits prove to be very tightly linked, structure/function analysis of DNA in the *adp* region will be needed to resolve this question. In the meantime, it would be futile to alter the gene symbols for *adp*^{fs} and *fs(2)lto5* to indicate their allelism.

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Aslanukov, A.R., V.A. Vasilyev, and V.N. Bashkirov. Institute of Gene Biology, Russian Academy of Sciences, Moscow 117334, Russia. A novel Giant insert into the white gene possibly having properties of mobile element.

Bashkirov *et al.* (1992) described a case of revealing in the females of the *C(1)RM, y w*/O; *Dp(1;3)w*^{vou} /+ stock a unique female, which has light brownish-orange uniformly colored eyes. This trait was not inherited with *Dp(1;3)w*^{vou} and instead demonstrated coupling with the attached-X chromosome. The new allele was called white-unstable-apricot (*w*^{ua}) because it demonstrated mutation transition to other allelic conditions at frequencies of 10^{-3} to 10^{-5}. Southern blot analysis revealed that the *w*^{ua} mutation was caused by insertion of an unknown sequence, and its length was very approximately evaluated by the authors as 7 kb.

Table 1.

Restrictase	Summary Length: According to map †	Summary Length: Observed	Net increase/ decrease	Possible number of sites in insert
Bam HI	10.1 + 3.4 = 13.5	10.1 + 12.0 + 6.5 = 28.6	+15.1	1
HindIII	9.1 + 5.3 + 1.2 = 15.6	9.1 + 9.8 + 10.6 + 1.2 = 30.7	+ 5.1	1
Eco RI	13.0 + 2.5 = 15.5	11.0 + 13.0 = 24.0	+ 8.5	>2
Sal I	10.0	12.0 + 6.0 = 18.0	+ 8.0	>2
Xba I	8.5	19.3 + 4.3 = 23.6	+ 15.1	1
Sac I	5.8 + 9.3 = 15.1	6.0 + 9.3 + 8.0 = 23.1	+ 8.0	>2
Xho I	15.0	30.1	+ 15.1	0

† Levis *et al.*, 1982

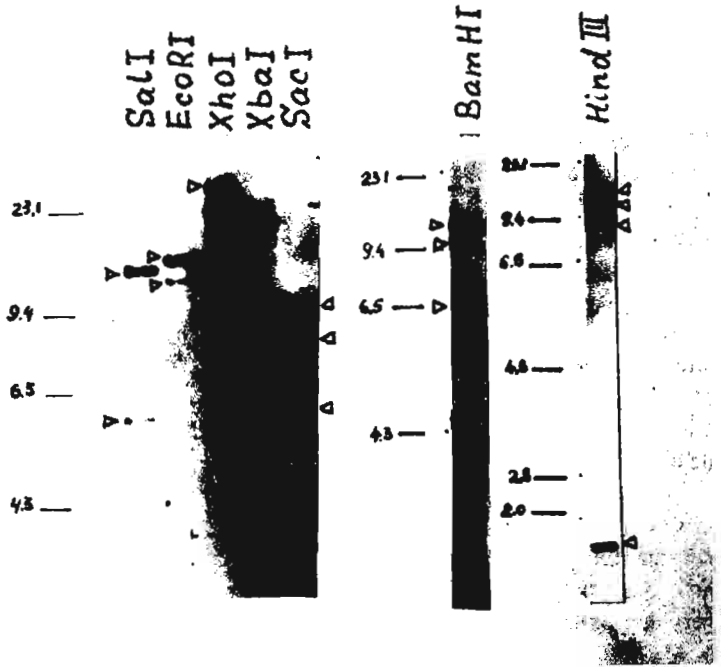

Figure 1. Southern blot of various w^{ua} genomic digests.

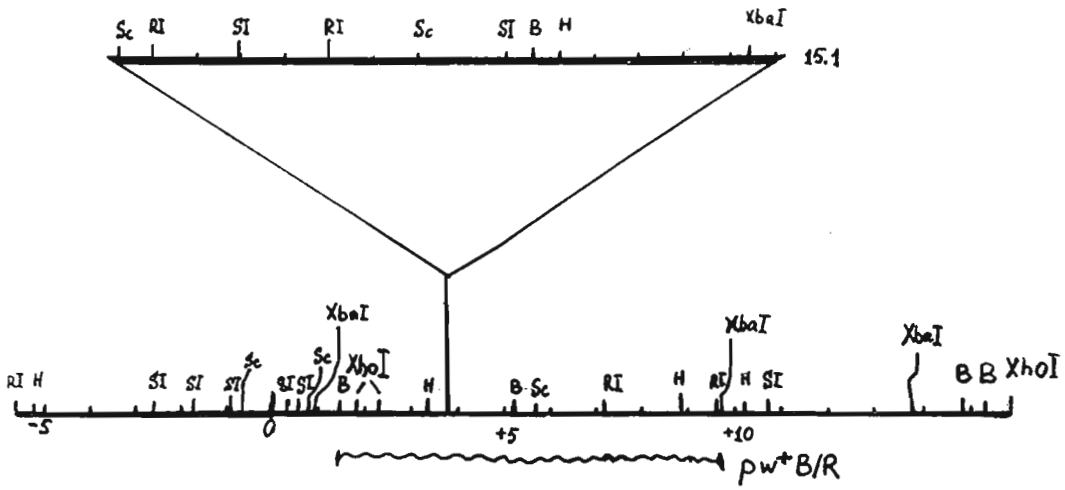

Figure 2. Restriction map of the w^{ua} allele based on genomic digests. Wavy line - structure of pw^+ B/R zond.

We widen the circle of using restriction endonucleases and use more prolonged gel running. So, it gives us the possibility to evaluate the w^{ua} insertion length more precisely. All data are presented in Figure 1 and Table 1. Our data show that really we have a giant insertion whose length is 15.1 kb. Usually the insertion of such a length range are various derivatives of the FB element (Finnegan, 1992). But as can be seen from our map (Figure 2), the w^{ua} insertion has another nature.

Acknowledgments: This work was supported by a grant of the Russian State Scientific-Technical Programme "Frontiers in Genetics".

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Benado, M. Departamento de Estudios Ambientales, Universidad Simón Bolívar, Caracas, Venezuela, and Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Santiago, Chile. Results of adult competition between *Drosophila subobscura* and *D. simulans* do not depend on the initial frequency in one-generation experiments.

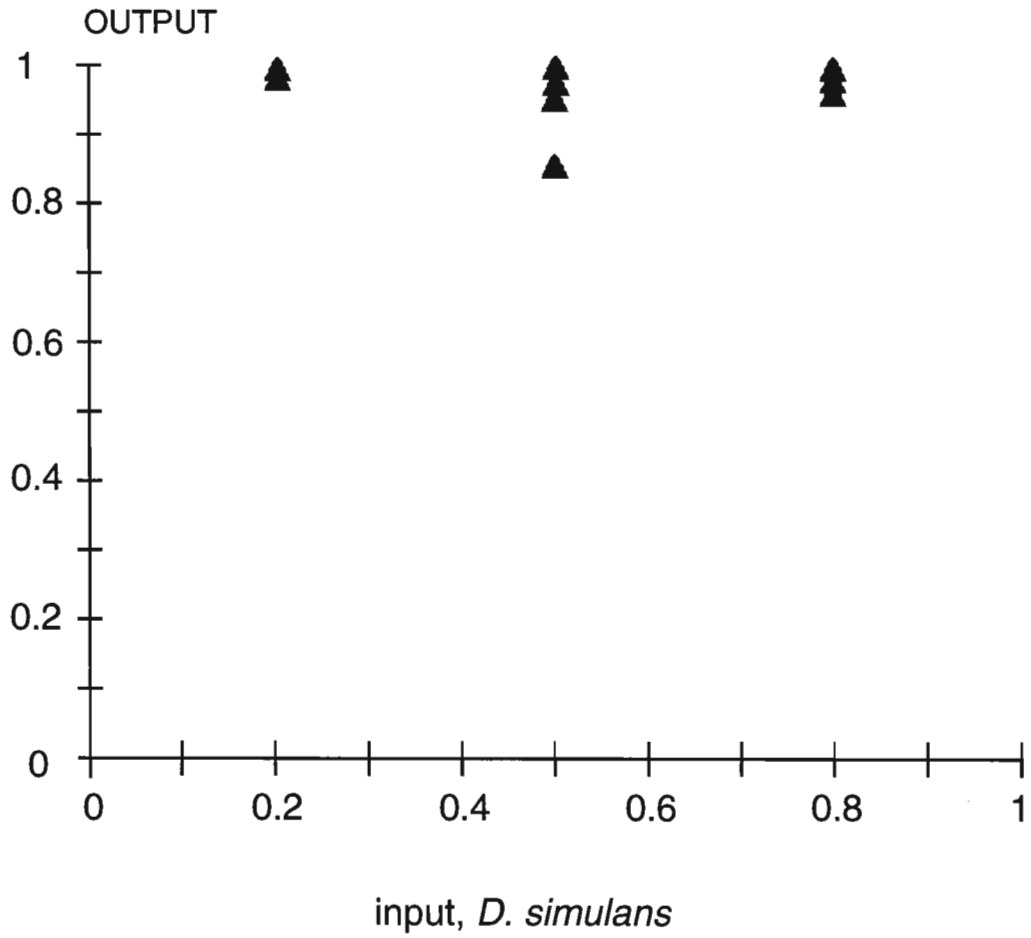
D. subobscura colonized Chile within a few years after 1980, when it was reported there for the first time (Brncic and Budnik, 1980). In Central Chile, the species is found with *D. simulans* in the same collecting sites, emerging simultaneously from several fermenting fruits (Brncic, 1987). In the experiment reported below, I study adult competition between the species in a single-generation set-up, to further the understanding of the colonization success of *D. subobscura*.

Material and Methods: The strains came from La Florida, Santiago, Chile, and were kept in mass cultures for several months before experiments were started. All the experiments were carried out at 19°C.

Vials with 10 cc of standard cornmeal-yeast agar medium were used, into which 100 fertilized females of the same age were put. The following *D. simulans*:*D. subobscura* ratios were used, 2:8, 5:5, 8:2 (input series), with four replicates per ratio. After 13 days, the flies were discarded and the number of adults counted for 26 days after they began emerging. The results were expressed as the ratio *D. simulans*:*D. subobscura* of the emerging adults (output series). After angular transformation, the output was regressed against the input to test the null hypothesis that there were no differences among the output ratios for the several inputs.

Results and Discussion: As can be seen in Figure 1, the output ratios were always near one, meaning that almost all the emerging flies were *D. simulans*. The regression analysis was not significant (slope ± se = -0.02 ± 0.13, t_{10} = -0.15). This can be interpreted as *D. subobscura* being at a competitive disadvantage when interacting with *D. simulans*. This result corroborates long-term experiments where *D. simulans* out-competed *D. subobscura* in serial-transfer systems at several starting ratios (Benado and Budnik, in press). It seems unlikely that *D. subobscura* colonized Chile through interactions like those of the present experiment.

Acknowledgments: Funded by FONDECYT, Grant #1930769.



References: Benado, M., and M. Budnik, in press, Evolución Biológica; Brncic, D., 1987, Medio Ambiente 8: 3-9; Brncic, D., and M. Budnik 1980, Dros. Inf. Serv. 55: 20.

Jones, C.D., and H.A. Orr. University of Rochester. Rochester NY. An attempt to rescue hybrid fertility by germline mitotic recombination in *Drosophila.*

Drosophila melanogaster does not form fertile hybrids with any other species. As a result, we cannot use the powerful genetic tools available in *D. melanogaster* to study the genetics of speciation. To circumvent this problem, we attempted to create fertile *D. melanogaster* x *D. mauritiana* hybrids through germline mitotic recombination. Our approach was analogous to the dominant-female sterile technique of developmental genetics (Ashburner, 1989). In our case, however, the "dominant-female steriles" were the wild type alleles causing reproductive isolation between *D. melanogaster* and *D. mauritiana.* By inducing recombination in the germline of F_1 hybrid females, some germ cells will be homozygous for regions from the *D. melanogaster* genome, and other germ cells will be homozygous for regions from the *D. mauritiana* genome. Ideally, we will "recombine away" the genes causing hybrid sterility in some of these germ cells, resulting in a few fertile F_1 hybrid females.

This strategy assumes that hybrid female sterility is autonomous. Fortunately, both ovary and pole cell transplant experiments prove that hybrid sterility depends solely on germline genotype (Monod and Poulson, 1937; Santamaria, 1977). Therefore, any germline genotype that would be fertile as a backcross or F_2 individual should be fertile as a germline clone within F_1 hybrids.

Materials and Methods: All *D. melanogaster* mutations are described in Lindsey and Zimm (1992). *D. mauritiana* Synthetic is derived from a pooled set of iso-female lines (Coyne, 1993).

Our experiment involved four crosses. First, we crossed *D. melanogaster* $w^{6613.4}$ females to *D. mauritiana* Synthetic males and collected F_1 hybrid females. This species cross yields sterile adult females and inviable males, with

males dying as pseudopupae. The F_1 females have fairly atrophied ovaries, but an otherwise normal reproductive tract (Santamaria,1977). A portion of these hybrids, while larvae, were exposed to gamma-radiation. Unirradiated larvae were used as a control. To test their fertility, we backcrossed irradiated and unirradiated F_1 females to males from both parental species. Because F_1 interspecies hybrids are sterile, we cannot directly test if recombination actually occurred in the hybrids. Thus, as a control for our irradiation technique, we crossed *D. melanogaster* $w^{66l3.4}$ females to *D. melanogaster* ovo^{D1} males (ovo^{D1} is a dominant female sterile). Again, a portion were irradiated and a portion were not. If radiation induced mitotic recombination is successful, then some of the irradiated F_1 ovo^{D1} females should be fertile.

We irradiated 30-40 hours old larvae with 1500 rads from a Cesium source (Ashburner, 1989). All crosses were performed at room temperature on cornmeal medium.

Table 1. The fertility of unirradiated *versus* irradiated female genotypes. An asterisk (*) indicates genotypes that were irradiated. N is the estimated number of females of each genotype tested (= number of vials X 12).

Genotype	N	Vials producing no progeny	Vials producing progeny
$ovo^{D1}/+$	408	32	2
$ovo^{D1}/+$ *	180	4	11
F_1 (mel/maur)	504	42	0
F_1 (mel/maur) *	1572	131	0

Results and Discussion: Not surprisingly, unirradiated $ovo^{D1}/+$ females are sterile: over 400 females produced only 10 offspring (Table 1). Irradiation dramatically increases the fertility of $ovo^{D1}/+$ females (Table 1), confirming the effectiveness of our irradiation technique.

As expected, the species crosses of *D. melanogaster* $w^{66l3.4}$ females x *D. mauritiana* Synthetic males produced only hybrid females. The unirradiated hybrid females were, of course, completely sterile (Table 1). Unfortunately, irradiated female F_1 hybrids remained sterile: although, we irradiated over 1500 hybrid female larvae, none of the resulting adults produced any eggs (Table 1).

In sum, fertile germline clones could not be created by mitotic recombination in *D. melanogaster* x *D. mauritiana* F_1 hybrids. Two possible explanations are: (1) any single homospecific chromosome region cannot restore hybrid fertility because the genetics of hybrid female sterility are too complex, or, (2) the homozygous regions created by recombination may contain recessive female steriles. Our approach, however, may prove useful in other species pairs, especially those that are more closely-related than *D. melanogaster* and *D. mauritiana*.

Acknowledgments: We thank B. Fleming for helpful discussions. This work was supported by NIH grant GM 51932 to H.A.O. and a Caspari Fellowship to C.D.J.

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Klarenberg, A.J.*, S. Remer, and W. Traunspurger. Zoologisches Institut der Ludwig-Maximillians-Universität, München, Germany. The potential use of hsp70-*lacZ* constructions in transgenic *Drosophila melanogaster* as a biomarker for heavy metals in ecotoxicological research.

The use of living systems in monitoring environmental pollution caused by human activities is growing steadily. *Drosophila* has been used in classical radiation and toxicity tests (Green *et al.,* 1986; Würgler, 1992). However, *Drosophila* is not listed in any modern environmental and ecotoxicological tests which make use of a variety of other organisms including luminescent bacteria, plants, nematodes and waterfleas (Rudolph and Boje, 1986; Van Kessel *et al.,* 1989; Ernst and Peterson, 1994; Gälli *et al.,* 1994). In monitoring the effects of soil and water contaminated with heavy metals, it seems that almost all organisms may respond in some way. Crucial, however is, the development of a system which reacts in a predictable manner and which is easy to use. A recent approach to cope with this problem was presented by Stringham and Candido (1994) using transgenic soil nematodes, *Caenorhabditis elegans*. They showed that hsp16-*lacZ* responded rapidly to heavy-metals (Cd^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, Pb^{2+}). This assay may be a more sensitive and rapid indicator of stress caused by heavy metals and other pollutants (e.g. paraquat) than current LC50 assays using *C. elegans* (Williams and Dusenberry, 1990). Moreover, the assay methods can be adapted to soil or sediment testing.

Heat-shock promotors respond in particular to environmental stress, such as heat, cold, ethanol and heavy metals (Schlesinger *et al.,* 1982). The *Drosophila* heat-shock effect has been described in detail (Lindquist and Craigh, 1988). Their genetic components, *hsp* genes, have been cloned and sequenced, and their regulation has been analysed. In contrast to investigations of the heat-shock response in relation to temperature (*e.g.* Bosch *et al.,* 1988; Gehring and Wehner, 1995; Coleman *et al.,* 1995), the induction of *hsp* genes in *Drosophila* due to heavy metals has not been

explored in much detail. It has been shown that *hsp* genes are induced by $CdCl_2$ in *Drosophila* cell cultures (Courgeon *et al.*, 1984) and embryonic cells (Bournias-Vardiabasis *et al.*, 1990). Most heavy metals are toxic for *Drosophila* at relatively low levels and may cause both mutations and teratogenic effects (Rasmusen, 1985). Wild-type strains of *D. melanogaster* show variations in their tolerance to heavy metals. Selection experiments with *Drosophila* on media containing Cu_2SO_4 (Wallace, 1982), $CdCl_2$ (Christie *et al.*, 1985; Maroni and Watson, 1985) and CH_3HgOH (Magnusson and Ramel, 1986) show a rapid response. Maroni *et al.* (1987) and Mokdad *et al.* (1987) demonstrated that metallothionein genes (*Mtn* and *Mto*) are involved in tolerating increased cadmium and copper concentrations.

We are presenting an outline of some initial results demonstrating the effects of heavy metals on ß-galactosidase expression in transgenic *Drosophila* larvae which contain hsp70-*lacZ* constructions (see Lis *et al.*, 1983) and a comparison with earlier data described by Stringham and Candido (1994). The original idea of using heavy metals arose from the need to detect hsp70-*lacZ* in wild-type populations of *D. melanogaster* without heat-shocking, as this treatment might kill or sterilize flies. Courgeon *et al.*, (1984) have shown that $CdCl_2$ induces *hsp*-response in *Drosophila* cell cultures. However, Cd^{2+} is very toxic for *Drosophila*. Therefore, some other less toxic heavy metal salts were tested. It turned out that both Zn^{2+} and Cu^{2+} provoked a response similar to that of Cd^{2+} and could be used for the purpose described earlier (Klarenberg, unpublished).

To detect ß-galactosidase activity caused by the induction of the hsp70-*lacZ* constructions due to heavy metals supplemented to the food media, a rapid and sensitive fluorescent assay was developed using methyl-umbelliferyl-ß-D-galactoside (MUG) as the substrate to detect variation in endogenic ß-galactosidase expression in the midgut of *Drosophila* larvae (Klarenberg *et al.*, 1991, 1994); it was also employed as a spot test on the whole-animal assay by Krefer (1993). In addition, we used the XGAL-chromogenic ß-galactosidase assay for a closer inspection of the hsp70-*lacZ* expression (Lis *et al.*, 1983). All other details of this study will be presented elsewhere (Klarenberg *et al.*, in prep.). One important aspect of the effect of heavy metals on hsp70-*lacZ* expression in the midgut is that some metals may react in a tissue-specific manner, as has been demonstrated for *C. elegans* by Stringham and Candido (1994). A first screen of hsp70-*lacZ* expression (using both MUG and XGAL for staining) in third-instar larval midguts grown on media contaminated with 10^{-3} M solutions of $CuCl_2$, $CdCl_2$ and $HgCl_2$, respectively, does indeed suggest differences in expression. For instance, $CdCl_2$ induced hsp70-*lacZ* expression mainly in the posterior midgut, $HgCl_2$ in the anterior and posterior midgut and $CuCl_2$ in the middle midgut (see Figure 1 for details). The maximum responses to these heavy metals were reached after exposing third-instar larvae to standard cornmeal food with 0.5×10^{-3} M of $CuCl_2$, $CdCl_2$ and $HgCl_2$, respectively, for 20 hrs. If treated carefully, controls without heat-shock or with 0.5×10^{-3} M NaCl and KCl, show very little or no activity. Wild-type strains with a high endogenous ß-galactosidase expression (Klarenberg, 1986; Klarenberg *et al.*, 1993 and in prep.) do not have an expression similar to the hsp70-*lacZ* constructs when grown as larvae on media with different heavy metals. Heat-shocked larvae (120 min.; 35°C) express hsp70-*LacZ* in all parts of the gut and the Malpighian tubules. Why different heavy metals show differential expression is unclear, but it may be related to differences in the uptake capacity of cells which line the inner midgut. Another interesting aspect is that there is an overlap in hsp70-*lacZ* expression due to $CuCl_2$ and Mt-expression due to Cu and Cd (Maroni and Watson 1985). The high activity of hsp70-*lacZ* expression due to CuCl2 coincides with a copper accumulating region in the middle midgut (Filshie *et al.*, 1971).

An important aspect for the development of an assay using transgenic *Drosophila* is the fact that the reaction of hsp70-*lacZ* to heavy metals can be detected at sub-lethal levels and may therefore be employed as an early warning sign in determining environmental contamination (Anderson, 1989; Welch, 1992). Consequently, it may be advantageous to apply these transgenic *Drosophila* in environmental monitoring. By adding more hsp70-*lacZ* copies to a single strain of *D. melanogaster*, one may enhance the sensitivity of the response to heavy metals. It is likely that similar or even better results can be obtained using constructions of *lacZ* as the reporter in conjunction with promotors of other *hsp* genes, e.g. *hsp26* (Simon and Lis, 1987). Perhaps the metallothionein genes *Mtn* and *Mto* (see Maroni, 1993), in which expression in the larval and adult midguts is induced by cadmium, copper, mercury, silver and zinc, are even more appropriate. In addition, it may be very useful to isolate strains with a low threshold by responding selectively to one single compound, e.g. cadmium. This may be problematic, as the *hsp70* promotor is sensitive to an array of heavy metals and other stress factors. Another important premise for such *Drosophila* biomarker strains is that they should respond to sub-lethal levels of a single heavy metal, i.e. far below the LC50 concentration for *D. melanogaster* larvae in the test medium (e.g. $< 10^{-3}$ M for $CdCl_2$). When these tests are completed with parallel observations on larval development time, the number of pupae formed and the number of eclosed flies, allowing estimations to be made for several ecotoxicological threshold parameters such as EC (effect concentration), LC (lethal concentration), LOEC (lowest observed effect concentration), MATC (maximum acceptable toxicant concentration) and NOEC (no observed effect concentration), a standardized, single species bio-test using *D. melanogaster* for aquatic, sediment and soil environments according to the OECD guidelines (OECD, 1984) could perhaps be developed.

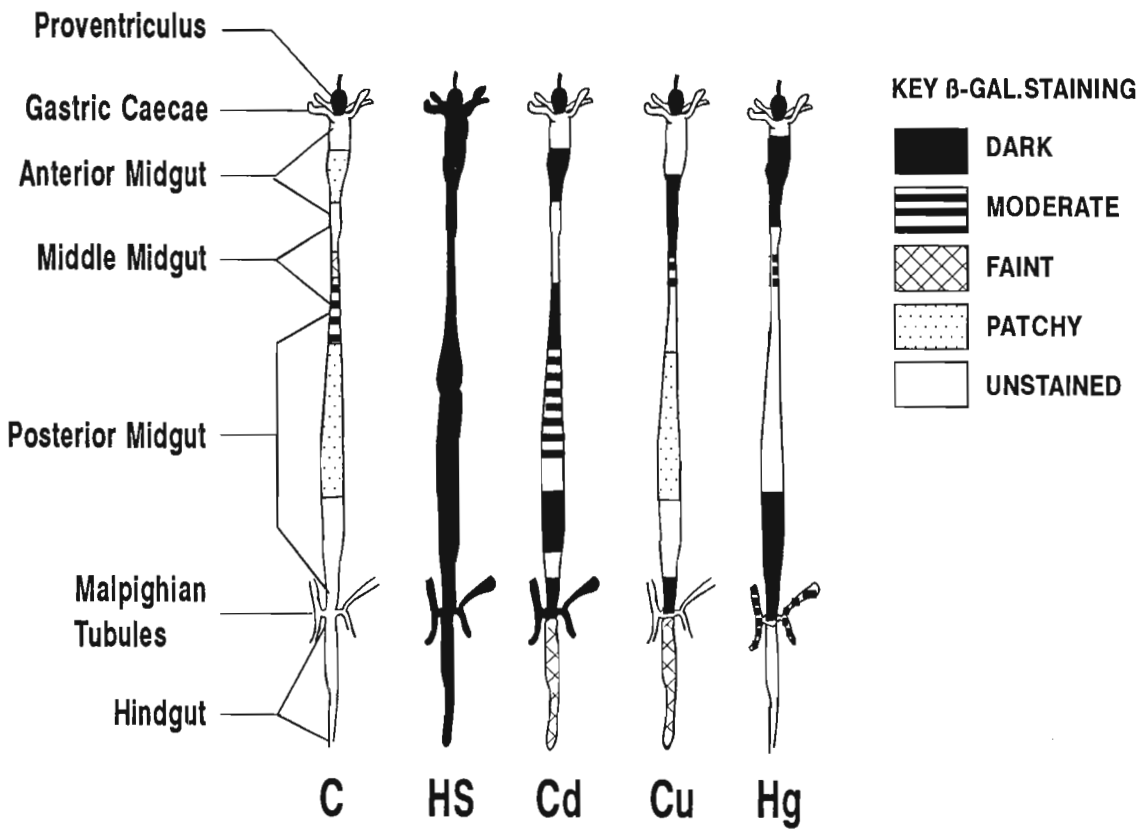

Figure 1. Effects of heavy metals on the midgut tissue-specific expression of hsp70-*lacZ* constructions in *Drosophila melanogaster* third-instar larvae. Differential response of hs70-*lacZ* shown by the in situ expression of *E. coli* ß-galactosidase enzyme activity (XGAL staining pattern) in the midgut of third-instar larvae of a transgenic strain of *D. melanogaster* (see Lis *et al.,* 1983). The midguts are oriented vertically with AMG on top for each treatment (n = 140). **C**. Control larvae raised on a standard cornmeal medium without heat shock at 22°C. **HS**. Control larvae raised on a standard cornmeal medium and heat-shocked for 2½ hr at 35°C. **Cd.** Third-instar larvae exposed for 20 hrs on standard cornmeal medium with 0.5×10^{-3} M $CdCl_2$. **Cu**. Third-instar larvae exposed for 20 hrs on standard cornmeal medium with 0.5×10^{-3} M $CuCl_2$. **Hg.** Third-instar larvae exposed for 20 hrs on standard cornmeal medium with 0.5×10^{-3} M $HgCl_2$.

The scenarios described above for *Drosophila* and earlier for *C. elegans* by Stringham and Candido (1994) using heat-shock promotors may be extended to a larger number of substances and other organisms. We conclude that transgenic *Drosophila* containing gene constructs which respond in a specific manner to heavy metals or other toxic substances offers a large, still unexplored, potential field for future practical applications in biomonitoring environmental pollutants.

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Ramachandra, N.B., and H. A. Ranganath. Department of Studies in Zoology, *Drosophila* Stock Centre, University of Mysore, Manasaganogtri, Mysore 570 006 India. Evolution of new karyotypic strains (Cytoraces) through hybridization in *Drosophila nasuta* system.

The process and pattern of speciation is an important event in understanding the evolutionary genetics of any organism. Raciation genetics is much concentrated on populations that have been only recently separated from each other and may not as yet qualify for the species status. The more advanced the stage of differentiation of two diverging populations, the more difficult to delineate the genetic events that have set the process into motion. Therefore, it will not be possible to understand and to dissect the process by looking at the finished products (Zouros, 1989). Evolution is a continuous and an ongoing process. It is being state that botanists have long recognized introgressive hybridization as important in the evolution of plants. Hybridization among animals is also common, yet a few zoologists have considered it evolutionarily important. Interestingly, our long range interracial hybridization experiments between *Drosophila nasuta nasuta* and *D. n. albomicans* have yielded very unique karyotypic strains (Cytoraces). Herein we report the evolution of these new karyotypic strains which are passing through the programme of Anagenesis.

The *Drosophila nasuta* subgroup of species consists of about 12 taxa that are classified within the *immigrans* species group. *D. nasuta* and *D. albomicans* are extensively distributed allopatric, sibling races that differ in their chromosomal configuration with very little morphological differentiation and are cross fertile (Wilson *et al.*, 1969; Ranganath *et al.*, 1974; Ramachandra and Ranganath, 1986). *D. nasuta* has 2n = 8, with one pair of metacentrics (chromosome 2), two pairs of acrocentrics (chromosome 3 and X) and a pair of small dots (chromosome 4). In males one of the X chromosomes is replaced by a submetacentric Y chromosome. *D. albomicans* has 2n = 6, with two pairs of metacentrics (chromosome 2 and X3,X3 or X3,Y3) and a pair of long dots (Wilson *et al.*, 1969; Wakahama and Kitagawa, 1972; Nirmala and Krishnamurthy, 1972; Ranganath *et al.*, 1974). Subsequently Ranganath and his coworkers have contributed significantly to the understanding of the cytological distinctness and chromosomal evolution between these two races (Ramachandra and Ranganath, 1986; 1987, 1988, 1990, 1994; Ranganath, 1974; Ranganath and Hagele, 1981, 1982; Ranganath *et al.*, 1974, 1982; Ranganath and Ramachandra, 1987, 1994).

The chromosomes of *D. n. nasuta* and *D. n. albomicans* can be easily identified with certainty in the hybrid populations. The sequence of events in the evolution of Cytoraces through interracial hybridization between strains of *D. n. nasuta* and *D. n. albomicans* are as follows:

1). The F1 hybrids of the cross between *D. n. nasuta* and *D. n. albomicans* has 2n = 7. In spite of the differences between these races in their karyotypic organization, the F1 and the succeeding hybrid progeny were fertile.
2). The analysis of the karyotypes of F2 and of the succeeding generation showed that the hybrid populations, karyotype-wise are extremely heterogeneous with different types of karyotypes. This was called 'karyotypic mosaicism', wherein one can see different chromosome combinations other than the parental and of the F1 hybrids.
3). The karyotypic mosaicism present in the hybrid populations declines over generations and during this process there was selective elimination of some parental chromosomes, while some parental chromosomes were retained.

4). By F20 the karyotypic variability disappears in most of the hybrid lines, but some hybrid populations took more generations to produce stable karyotypes.
5). Further, in the proceedings of the evolution of these Cytoraces, there was a transitory phase of 'karyotypic instability' due to karyotypic mosaicism. This ephemeral phase was preceded and succeeded by phases of 'karyotypic stability'. The karyotypes of the parental races and those of the Cytoraces represent phases of karyotypic stability. Hence, these events fit into "transilience mode of speciation' proposed by Templeton (1981).
6). Such an event has led to the emergence of new strains of hybrid populations, which differ from their parents as to the karyotypic composition and/or chromosome number and hence these newly derived hybrids have been called 'Cytoraces'.

The evolution of these Cytoraces was achieved in three phases. In the first phase, the Cytorace I was evolved by the hybridization between the males of the Coorg strain of *D. n. nasuta* and the females of Okinawa strain of *D. n. albomicans*. The males had 2n = 7 ($2^n\ 2^a\ Y^n\ X3^a\ 3^n\ 4^n\ 4^n$) and the females had 2n = 6 ($2^n\ 2^a\ X3^a\ X3^a\ 4^n\ 4^n$) (Ramachandra and Ranganath, 1986). The superscripts denote the parent from which that particular chromosome was inherited -n = *D. n. nasuta* and a = *D. n. albomicans*. The Cytorace II was the product of the hybridization between the males of Okinawa strain of *D. n. albomicans* and the females of Coorg strain of *D. n. nasuta*. Both males and females had 2n = 6 (male - $2^n2^a\ X3^aY3^a\ 4^a4^a$; female - $2^n2^a\ X3^aX3^a\ 4^a4^a$).

In the second experimental phase, hybridization between the females of Coorg strain of *D. n. nasuta* and the males of Thailand strain of *D. n. albomicans* has resulted in the formation of Cytorace III. The males were with 2n = 7 ($2^n2^a\ Y3^aX^n3^n\ 4^a4^a$) and the females were with 2n = 8 ($2^n2^a\ X^nX^n3^n3^n\ 4^a4^a$). The hybrid progeny of the reciprocal cross between the males of Coorg strain of *D. n. nasuta* and the females of Thailand strain of *D. n. albomicans* have given birth to a new stable hybrid called Cytorace IV. Both the males and females had 2n = 8 (male - $2^n2^a\ Y^nX^n3^n3^n\ 4^a4^a$; female - $2^n2^a\ Y^nX^n3^n3^n\ 4^a4^a$) (Ramachandra and Ranganath, 1990).

Recently, by utilizing these four Cytoraces and two parental races namely, Coorg strain of *D. n. nasuta*, Okinawa strain of *D. n. albomicans*, over 30 interstrain hybridizations were carried out and each hybrid population is being maintained separately for over five years. The karyotypic analysis of these hybrid populations has yielded twelve more new stabilized karyotypes. Of these five were new karyotypes which differ from their parents and the seven hybrid populations were similar to one of their parents in their karypotypic composition except chromosome 2 which is always in polymorphic condition. In addition to this, wherever the male karyotypes stabilize with 7 chromosomes does produce some aneuploids. The following are the karyotypic composition of newly stabilized races evolved during third phase of interstrain hybridization among *D. n. nasuta*, *D. n. albomicans* Cytorace I, Cytorace II, Cytorace III and Cytorace IV:

Cytorace 5: parents : Cytorace I male x *albomicans* female) The males had 2n = 7 ($2^n2^a\ X3^aY^n3^n\ 4^a4^a$) and the females had 2n = 6 ($2^n2^a\ X3^aX3^a\ 4^a4^a$) with aneuploids.

Cytorace 6: (parents: Cytorace IV male x Cytorace I female) The males with 2n = 7 ($2^n2^a\ Y3^aX^n3^n\ 4^n4^n$) and the females with 2n = 8 ($2^n2^a\ X^nX^n3^a3^a\ 4^n4^n$) with aneuploids.

Cytorace 7: (parents: Cytorace I male x Cytorace II female) The male with 2n = 7 ($2^n2^a\ X3^aY^n3^n\ 4^a4^a$) and the females with 2n = 6 ($2^n2^a\ X3^aX3^a\ 4^a4^a$) with aneuploids.

Cytorace 8: (parents: Cytorace I male x Cytorace IV female) The male had 2n = 7 ($2^n2^a\ X3^aY^n3^n\ 4^a4^a$) and the female had 2n = 6 ($2^n2^a\ X3^aX3^a\ 4^a4^a$) with aneuploids.

Cytorace 9: (parents: Cytorace II male x *nasuta* female) The males and females have 2n = 6 (male - $2^n2^a\ Y3^aX3^a\ 4^a4^a$; female - $2^n2^a\ X3^aX3^a\ 4^a4^a$).

Cytorace 10: (parents : Cytorace III male x *nasuta* female) The male and females had 2n = 8 (male - $2^n2^a\ Y^nX^n3^n3^n\ 4^a4^a$; female - $2^n2^a\ X^nX^n3^n3^n\ 4^a4^a$).

Cytorace 11: (parents: Cytorace II male x *albomicans* female) Both the males and females had 2n = 6 (male - $2^n2^a\ Y3^aX3^a\ 4^a4^a$; female - $2^n2^a\ X3^aX3^a\ 4^a4^a$).

Cytorace 12: (parents: *albomicans* male x Cytorace I female) Both the males and females had 2n = 6 (male -$2^n2^a\ Y3^aX3^a\ 4^a4^a$; female - $2^n2^a\ X3^aX3^a\ 4^a4^a$).

Cytorace 13: (parents : *albomicans* male x Cytorace II female) Both the males and females had 2n = 6 (male - $2^n2^a\ Y3^aX3^a\ 4^a4^a$; female - $2^n2^a\ X3^aX3^a\ 4^a4^a$).

Cytorace 14: (parents :Cytorace IV male x cytorace III female) The males had 2n = 7 (male - $2^n2^a\ Y3^aX^n3^n\ 4^a4^a$) and the females had 2n = 8 (female - $2^n2^a\ X^nX^n3^n3^n\ 4^a4^a$).

Cytorace 15: (parents: Cytorace III male x Cytorace Iv female) Both the males and females had 2n = 8 (male -2^n2^a

$Y^nX^n3^n3^n$ 4^a4^a; female - 2^n2^a $X^nX^n3^n3^n$ 4^a4^a).

Cytorace 16: (parents: *nasuta* male x Cytorace III female) Both the males and females had 2n = 8 (male - 2^n2^a $Y^nX^n3^n3^n$ 4^a4^a; female - 2^n2^a $X^nX^n3^n3^n$ 4^a4^a).

Thus, the chromosome dynamics witnessed in the evolution of each of these Cytoraces is different. The same parental race when it crossed to different Cytoraces of their own has led to different patterns of chromosomal selection and resulted in the evolution of new karyotypes, and hence 16 new Cytoraces. These events augment the opinion of Dobzhansky (1970) who felt that the course of events in an evolutionary path is unrepeatable and unpredictable. All these Cytoraces are in different transitional stages of raciation/speciation are of considerable interest precisely because they help to identify the sequences of events by which new cytologically differentiated populations arise. The newly evolved Cytoraces along with the parental races constitute the "Nasuta-Albomicans Complex" of the *nasuta* subgroup of the *immigrans* species group of *Drosophila*.

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Chatterjee, R.N., and J. Mukherjee. Department of Zoology, University of Calcutta, Calcutta - 700 019, India. The effect of intersex (*ix*) mutation on hemolymph vitellogenin synthesis of *D. melanogaster*.

In *Drosophila*, vitellogenins (yolk protein precursors) are synthesized by female fat body and secreted into hemolymph. In males, on the other hand, yp genes are not expressed. Studies of Ota *et al.* (1981) indicated that while the diplo X individuals homozygous for *tra* and *tra-2* do not synthesize any yolk proteins, in XY, *dsx/dsx* individuals, the expression of the three yp genes is not inhibited. Later, Baker *et al.* (1987) claimed that the yp genes which are expressed in the adult fat body in a female specific manner are under the direct control of the major sex regulatory loci, *e.g., dsx, tra, tra-2*, and so forth. However, very few workers have addressed the question whether *ix* gene function has any role in expression of yp genes. In fact, direct demonstration of the effect of the *ix* gene on the expression of the yp genes is yet lacking. In this report, we present the results of the levels of hemolymph vitellogenin of *ix* intersex flies.

For present experiments, the wild type strain Oregon R and B^SY, *pr cn ix* /SM5 were used. Flies were raised on standard Drosophila food medium containing cornmeal, molasses, yeast, and agar-agar. Propionic acid was added as a mold inhibitor. Adults and all developmental stages were reared at 24° ± 1°C.

For determination of presence or absence of vitellogenins in the fat body of mutant and control flies, fresh hemolymph samples (about 2 µl each) were collected from flies, using micro-injection pipettes, through the ventral side of the body. Then 20 µl of fresh SDS sample buffer was added to each sample. The sample buffer contained 10 µl v/v glycerol, 5% v/v β-mercaptoethanol, 2.3% w/v SDS and 10 ml of 0.0625M Tris HCl (pH 6.8). The mixture was then heated in a water bath at 90°C for 12-15 minutes and then subjected to SDS polyacrylamide gel electrophoresis in 10% running gel with 3% stacking gel at a constant current of 20 mA using the method of Laemmili (1970). The gels were thereafter stained with Coomassie Brilliant Blue.

The SDS polyacrylamide gel pattern of hemolymph proteins from wild type Oregon R female flies is presented in Figure 1 (lane 1). As it appears from the photomicrograph, three bands marked yp1-yp3 were present in females and represent hemolymph vitellogenins. The two other bands marked H1 and H2 were of unknown character (see Ota *et al.*, 1981). They were present in both sexes up to 4-5 days after eclosion. However, the H1 band decreased greatly in amount in older flies. Therefore, attempts have been made to collect the same volume from younger flies. These two bands serve as corresponding controls.

Although the leakiness of *ix* is noted in morphological characters, there was no correlation between the morphological appearance and hemolymph vitellogenins in all *ix* flies since all types of intersexes have vitellogenins in their hemolymph. The gross morphological appearance of rudimentary gonads was not completely correlated with presence or absence of hemolymph vitellogenins. For example, *ix* flies (which at a low frequency carry nearly mature sized ovaries) were separable into those with rudimentary ovaries and those with large ovaries, and the hemolymph samples were examined separately (see Figure 1, lanes 2 and 3). No difference in the amount of hemolymph vitellogenins was detected. On the other hand, in *ix/ix* males, the expression of yp genes was repressed.

The following conclusion is tentatively made on the basis of the results obtained so far. Intersex (*ix*) gene function is neither required for vitellogenin synthesis nor for maintaining proper level of dosage compensation of yp genes. Furthermore, in *ix* mutant males, the expression of the yp gene is repressed, which suggests that this gene is inactive at least in the fat body cells of the males.

Acknowledgments: This work was supported by CSIR research grant [37(850)/94/EMR II] to RNC.

References: Baker, B.S., R.N. Nagoshi, and K.C. Burtis 1987, BioEssays 6: 60-70; Laemmili, U.K., 1970, Nature New Biol. 227: 680-685; Ota, T., A. Fukunaga, M. Kawabe, and K. Oishi 1981, Genetics 99: 429-441.

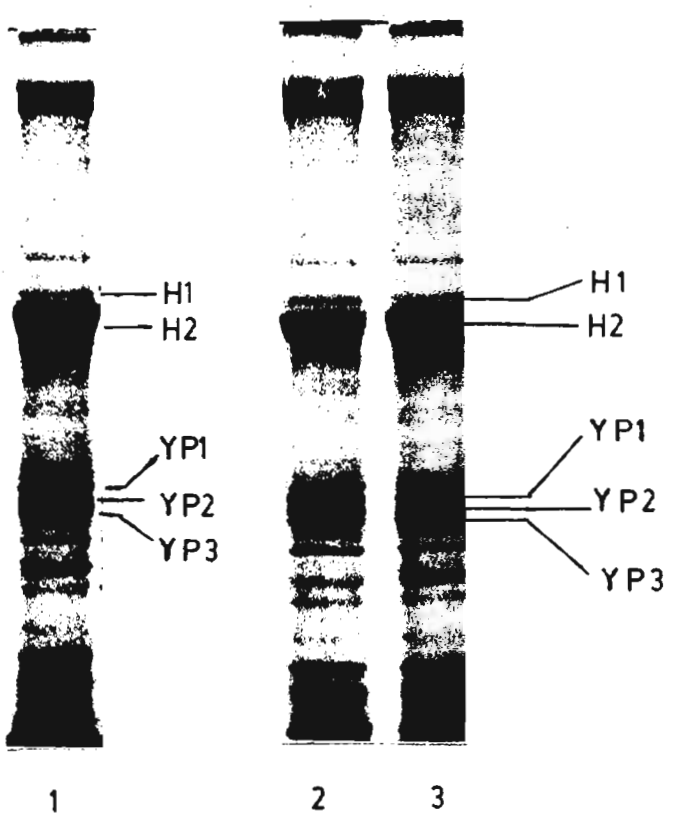

Figure 1. SDS polyacrylamide gel patterns of hemolymph proteins from Oregon R female (lane 1) and XX, *ix/ix* intersexes (lanes 2 and 3) of *D. melanogaster*. Note the presence of vitellogenins in all three types. All samples were taken from 5-6 day old flies. Bands yp1-3 are hemolymph vitellogenins and H1 and H2 are hemolymph proteins of unknown character.

Mukherjee, J., and R.N. Chatterjee. Department of Zoology, University of Calcutta, Calcutta - 700 019, India. Morphology of terminal body segments of triploid females of *D. melanogaster*.

To analyse the morphology of the terminal body segments of triploid females in detail, flies were generated from the cross of C(1)RM, *y v pn*/Y females to males whose major autosomes are in the form of four compound chromosomes or isochromosomes (y^2 /B^S Y; C(2L)RM, *dp*; C(2R)RM *px*; C(3L)RM, h^2 rs^2; C(3R)RM, +) (see Mann et al., 1986). The adult bodies were prepared for light microscopy as described by Szabad (1978). Scanning electron microscopy was made as described by Mukherjee and Chatterjee (1991).

Figure 1a shows the morphology of the terminal body segments of a triploid female. As evident from the photomicrograph, the triploid females were generally indistinguishable from diploid females. However, their body sizes were larger than their diploid sisters. Like diploid females, in triploid females also, each tergite (T2 to T6) is composed of an anterior portion of light colored unpigmented area and a posterior heavily pigmented black area. Thus, the dorsal side of the abdomen of a triploid female shows five black bands separated by unpigmented bands (Figure 1a). There is also a rudimentary seventh tergite (T7). The anus of the female is placed in between the dorsal and ventral plates.

When the terminal abdominal segments and genitalia of the triploid females were analysed using scanning electron microscopy, it was observed that in addition to normal female genital structures, and female type anal plates, a large number of chitinous outpushings of the exoskeleton appeared in the lateral and ventral part of the vulva (Figure 1b). This architecture was more common in 10-12 day old flies. It is possible that this effect in the adult flies are due to the presence of an extra set of chromosome complement in the genome of triploid flies.

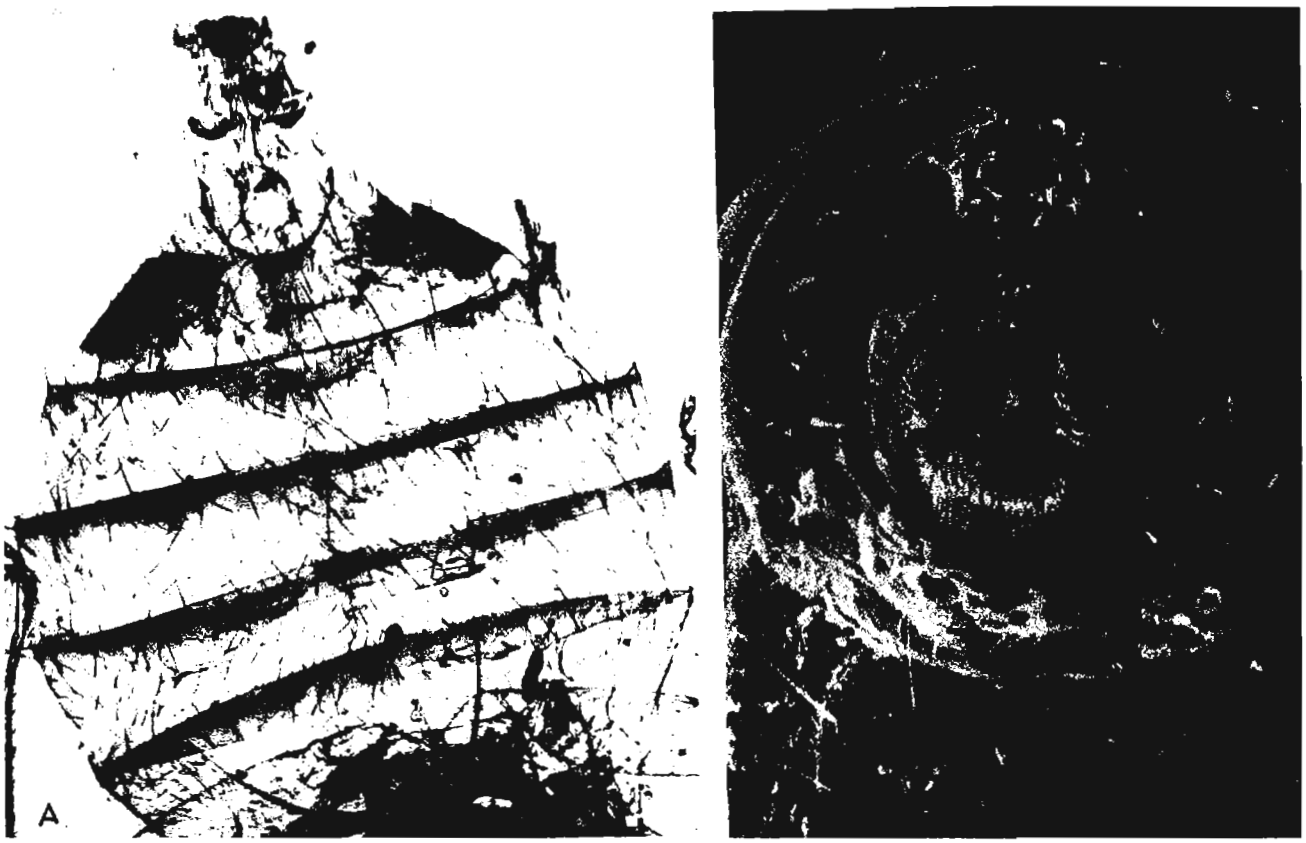

Figure 1. Two examples of abdomens from triploid females. (a) Triploid female abdomen under light microscope, (b) terminal abdominal segments and genitalia under scanning electron microscope. Note the presence of larger number of chitinous outpushings around the vulva. OP - outpushings, V - vulva, T8 - eighth tergite.

Acknowledgments: This work was supported by CSIR research grant [37(850)/94/EMR II] to RNC.

References: Mann, R., L. Hake, and J.C. Lucchesi 1986, Dev. Genet. 6: 247-255; Mukherjee, J., and R.N. Chatterjee 1991, Dros. Inf. Serv. 70: 255-256; Szabad, J., 1978, Dros. Inf. Serv. 53: 215.

Baek, Kwang-Hyun[1], and William P. Hanratty[2]. [1]Division of Genetics, Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Howard Hughes Medical Institute, 20 Shattuck Street, Boston, MA 02115 USA, [2]Department of Biology, Indiana University, Bloomington, IN 47405 USA. The characterization of temperature-sensitive *lethal(2)giant larvae* in *Drosophila melanogaster.*

The *lethal(2)giant larvae*, *l(2)gl*, gene was the first recessive oncogene described in *Drosophila.* Recessive mutant alleles of this gene result in the overgrowth of presumptive adult optic centers in the larval brain and of the imaginal discs (Gateff and Schneiderman, 1974). The critical evidence that demonstrates the neoplastic nature of the larval tissues was the ability to establish subculturable neoplasms when the affected tissues were cultured in adult hosts (Gateff and Schneiderman, 1974; Hanratty, 1984b). The purpose of our studies presented in this paper was to isolate temperature-sensitive mutant alleles at the locus of *l(2)gl* and begin to characterize three temperature *l(2)gl* alleles including two known alleles ($l(2)gl^{ts1}$ and $l(2)gl^{ts2}$; Hanratty, 1984a).

EMS-treated lethal free $dp^{ov}or^{49h}$ chromosomes were screened for the newly induced *l(2)gl* allele at 29°C by the scheme depicted in Figure 1. Using EMS mutagenesis (Procedure; Lewis and Bacher, 1968), three newly induced *l(2)gl* alleles were isolated among the 2,500 chromosomes tested. Of these one generated viable orange-eyed progeny when raised at 22°C. But, they failed to generate viable orange-eyed progeny at 29°C. These results indicate that the viability of this allele is temperature-sensitive.

Table 1. Viability of the heteroallelic *l(2)gl* alleles and controls at two different temperatures (22°C and 29°C).

Genotype	T (°C)	Types of offspring CyO	or /or	% expected
$l(2)gl^{ts1}$or/CyO	22°C	7295	3653	100
x				
$l(2)gl^{ts3}dp^{ov}$or/CyO	29°C	6143	298	9.7
$l(2)gl^{ts2}$or/CyO	22°C	3801	1758	92.5
x				
$l(2)gl^{ts3}dp^{ov}$or/CyO	29°C	1395	49	7.0
$l(2)gl^{+}dp^{ov}$or/CyO	22°C	5359	2362	88.2
x				
$l(2)gl^{ts3}dp^{ov}$or/CyO	29°C	5985	2477	82.8

Table 2. Fertility of the heteroallelic *l(2)gl* alleles and controls at two different temperatures (22°C and 29°C).

T (°C)	N days	Hatchability N	N_{unf}	N_h	%H
		$l(2)gl^{ts1}$or/$l(2)gl^{ts3}dp^{ov}$or x $l(2)gl^{+}dp^{ov}$or/Df(2L)net^{62}or			
22°C	5	267	20	240	97
29°C	5	60	14	0	0
		$l(2)gl^{ts1}$or/$l(2)gl^{ts3}dp^{ov}$or x $l(2)gl^{ts3}dp^{ov}$or/Df(2L)net^{62}or			
22°C	5	241	15	206	91
29°C	5	69	16	0	0
		$l(2)gl^{ts2}$or/$l(2)gl^{ts3}dp^{ov}$or x $l(2)gl^{+}dp^{ov}$or/Df(2L)net^{62}or			
22°C	5	146	16	120	92
29°C	5	4	6	0	0
		$l(2)gl^{ts2}$or/$l(2)gl^{ts3}dp^{ov}$or x $l(2)gl^{ts3}dp^{ov}$or/Df(2L)net^{62}or			
22°C	5	162	23	131	94
29°C	5	21	5	0	0
		$l(2)gl^{+}dp^{ov}$or/$l(2)gl^{ts1}$or x $l(2)gl^{+}dp^{ov}$or/Df(2L)net^{62}or			
22°C	3	310	18	282	97
29°C	3	430	10	375	89
		$l(2)gl^{+}dp^{ov}$or/$l(2)gl^{ts1}$or x $l(2)gl^{ts3}dp^{ov}$or/Df(2L)net^{62}or			
22°C	3	353	16	340	99
29°C	3	560	17	532	98

Note. Fertility was calculated as the percentage of hatched embryos: % hatching is 100 times the number of larva (N_h) divided by the number of fertilized eggs (N_f) {N_f = number of eggs (N) - number of unfertilized eggs (N_{unf})} (Perrimon, 1988).

The complementation analysis between balanced heterozygotes for the $l(2)gl^{ts}$ alleles were performed at 29°C and 22°C (Table 1). The results demonstrate that heteroallelic $l(2)gl^{ts}$ animals eclose with the viability of over 90% at 22°C and under 10% at 29°C. However, the cross of $l(2)gl^{ts3}$ with $l(2)gl^{+}dpor/CyO$ as a control resulted in similar viability at both temperatures (88.2% at 22°C and 82.8% at 29°C). Thus, the viability of the two heteroallelic combinations, $l(2)gl^{ts3}/l(2)gl^{ts1}$ and $l(2)gl^{ts3}/l(2)gl^{ts2}$ is temperature-dependent. And this suggests that *trans-allelic* (intragenic) complementation is occurring between $l(2)gl^{ts3}$ and $l(2)gl^{ts1}$, and $l(2)gl^{ts3}$ and $l(2)gl^{ts2}$ gene products. However, none of the progeny emerged from a cross between $l(2)gl^{ts1}$ and $l(2)gl^{ts2}$ alleles at 29°C.

The heteroallelic males raised at either 29°C or 22°C were fertile. To determine the effect of the $l(2)gl^{ts}$ genotypes on female fertility, heteroallelic females raised at either 29°C or 22°C were crossed separately to both $l(2)gl^{ts3}$ heteroallelic males or to wild type *l(2)gl* males, and the female fecundity and fertility were determined at the temperature at which the females were raised (Table 2). The numbers of eggs produced by the two heteroallelic genotypes were different at two different temperatures. Given that the control heterozygous females produced more eggs at 29°C than at 22°C, the difference in the fecundity of the heteroallelic females raised at two different temperatures represents a temperature effect on the fecundity of the females. At 22°C, the heteroallelic *l(2)gl* females as well as controls produced eggs which developed into normal embryos. However, at 29°C those females produced eggs with normal morphology, but they failed to hatch into viable larvae, suggesting that the *l(2)gl* function is required for the proper development during early embryogenesis. A detailed analysis of recessive mutation at the temperature-sensitive *l(2)gl* locus on both the genetic and molecular levels will help to clarify the function of this gene during embryonic development.

Acknowledgment: This is in memory of Dr. William P. Hanratty for his contribution to *Drosophila* community. The $Df(2L)net^{62}$ was kindly provided by M.D. Golumbovsky of the Institute of Cytology and Genetics, Novosibirsk, Russia.

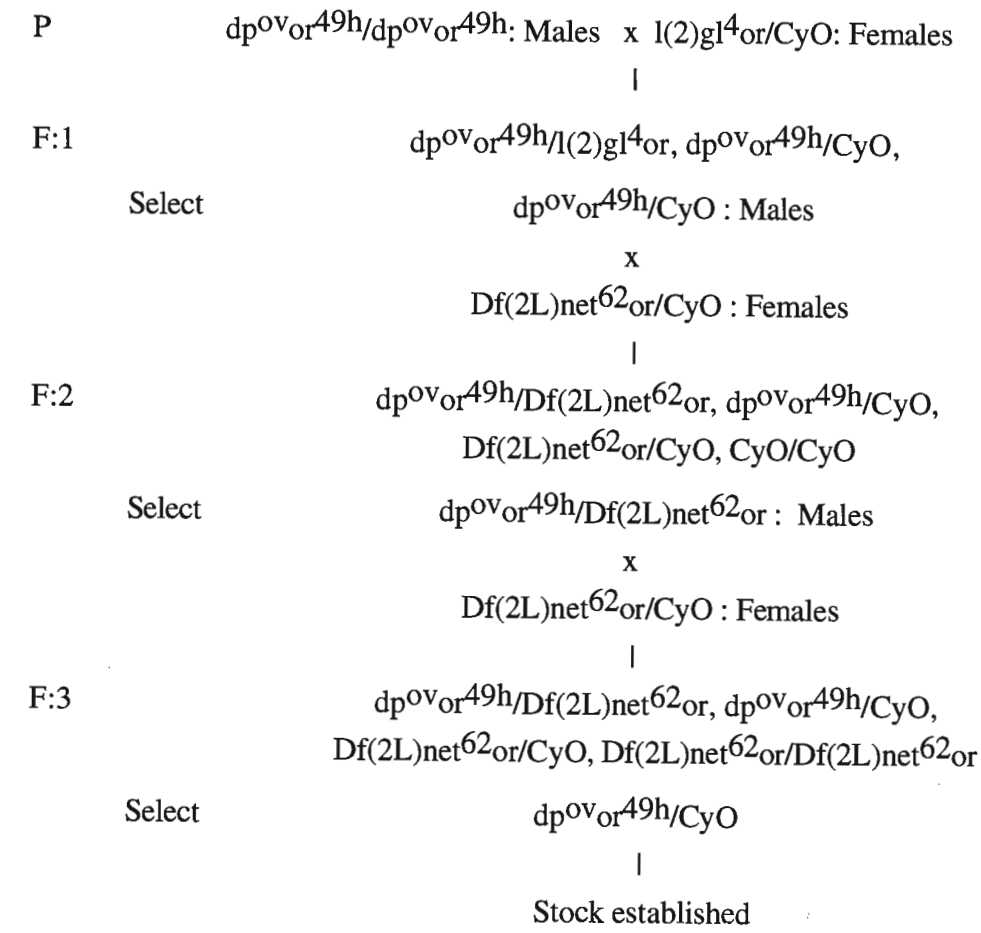

Figure 1. The scheme used for the isolation of the temperature-sensitive *l(2)gl* allele ($l(2)gl^{ts3}$). One of 2,500 chromosomes treated with EMS contained newly induced $l(2)gl^{ts3}$ allele that proved to be lethal at 29°C and viable at 22°C.

References: Gateff, E. and H.A. Schneiderman 1974, Wilhelm Roux's Arch. 176:23-65; Hanratty, W.P., 1984a, Wilhelm Roux's Arch. 193:90-97; Hanratty, W.P., 1984b, Wilhelm Roux's Arch. 193:98-107; Lewis, E.B., and F. Bacher 1968, Dros. Inf. Serv. 43:193; Perrimon, N., 1988, Dev. Biol. 127:392-407.

Derzhavets, Elena M., A.B. Korol, and E. Nevo. Institute of Evolution, University of Haifa, Mount Carmel, Haifa 31905, Israel. Increased male recombination rate in *D. melanogaster* correlated with population adaptation to stressful conditions.

Despite the broad interest in the problem of recombination evolution and abundance of theoretical models, it remains poorly studied experimentally and by observations in natural populations. Of the few studies that have been conducted, most have shown unequivocally that adaptation to stressful conditions or selection for quantitative traits can result in appreciable changes in the recombination system (reviewed in Korol *et al.*, 1994). Here we report the preliminary results on *D. melanogaster* male recombination from the opposing slopes of "Evolution Canyon", Lower Nahal Oren, Mount Carmel microsite in Israel). The South-facing slope (SF-slope) is warmer, drier, microclimatically more fluctuating, and less predictable than the North-facing (NF) slope (Nevo, 1995).

Materials and Methods: *Drosophila melanogaster* strains used: *ru cu ca*, a standard third-chromosome marker stock, classified as M-cytotype and carrying the following recessive mutations: *ru*, roughoid (3-0.0); *h*, hairy (3- 26.5); *th*, thread (3-43.2); *st*, scarlet (3-44.0); *cu*, curled (3- 50.0); *sr*, striped (3-62.0); e^s, ebony-sooty (3-70.7) and *ca*, claret

(3-100.7). See Lindsley and Zimm, 1992 for further details of all markers used. The stock was obtained from Bowling Green Stock Center, USA. Isofemale lines established earlier from inseminated females of the opposite slopes (Rashkovetsky *et al.*, unpublished; Derzhavets *et al.*, this issue) were used in this study.

Flies obtained from stations 2 and 6 of the SF- and NF-slopes, respectively, were reared on a standard medium at 25°C. In all tests three males were mated to three females in culture vials and transferred (three times) onto fresh medium. To measure the extent of male recombination on chromosome 3 the following crossing scheme was employed. Virgin *ru cu ca* females were mated to males of the tested lines, and F1 male progeny, heterozygous for markers, were crossed at 25°C to virgin females of strain *ru cu ca* to measure the frequency and distribution of recombination events. In order to evaluate possible effects of the cytoplasm and P-M system, the reciprocal hybrid males (which will be referred to as F1*) were also tested.

The presence of a recombinant genotype in a family of the test cross was taken to represent a single recombination event. If a male recombination event occurs pre-meiotically, clusters of individuals carrying the same recombinant genotype, or its reciprocal, can be found within a vial. Therefore, the occurrence of a particular recombinant type more than once in a family was treated as a cluster and taken to be derived from a single recombination event. In each experiment, recombinants were sought among the test cross progeny until 20 days after the parents had been introduced.

In a few cases the occurrence of mutations was also noted. Where necessary, putative recombinant offspring were subjected to progeny tests in order to confirm their genotypes.

Table 1. Frequency of male recombination on chromosome 3

	rucuca x line (F_1)			line x rucuca (F_1*)				Total		
Line	N	r	mrf(%)	N	r	mrf(%)	χ^2	N	r	mrf(%)
				South-facing slope, station 2, 3 isofemale lines						
2.23	3843	16	0.42	1258	5	0.40	0.03 ns	5101	21	0.41
2.25	3491	9	0.26	1436	3	0.21	0.00 ns	4927	12	0.24
2.9	3986	8	0.20	1581	4	0.25	0.00 ns	5567	12	0.22
χ^2	(df = 2)	3.33 ns			0.92 ns				4.07 ns	
Sum	11287	33	0.29	4263	12	0.28	0.00 ns	15550	45	0.29
				North-facing slope, station 6, 3 isofemale lines						
6.72	2096	1	0.05	1606	0	0	0.02 ns	3702	1	0.03
6.48	1941	3	0.15	1837	0	0	1.23 ns	3778	3	0.08
6.40	2155	1	0.05	1024	2	0.20	0.44 ns	3179	3	0.09
χ^2	(df = 2)	1.91 ns			6.73	(p < 5%)			1.35 ns	
Sum	6192	5	0.08	4467	2	0.04	0.11 ns	10659	7	0.07
				χ^2 (df = 1) heterogeneity (NF vs. SF) for mrf						
		7.31	(p < 1%)		6.23	(p < 2%)			14.88	(p < 0.1%)

N = the total progeny size; r = the number of recombinant events; F_1 and F_1* = direct and reciprocal hybrids; ns = the difference (heterogeneity) is not significant; χ^2 test was based on Kullback (1959) 'information statistics'.

Results and Discussion: Data presented in the Table clearly demonstrate an appreciable difference (four-fold) in the material established from flies of the opposite slopes with respect to male recombination frequency (mrf). Higher mrf value was associated with the SF-slope. No significant between-line variation was detected, beside some heterogeneity at the NF-slope in crosses 'tested line (female) × *ru cu ca* (male)'. The higher rate of recombination at the more stressful and unpredictable SF-slope corroborate the general expectation that stressful environment selects for enhanced recombination (Korol *et al.*, 1994).

One possible source of variation of recombination rates is cytoplasm (Thoday and Boam, 1956; Lawrence, 1963; Kruleva *et al.*, 1992). Besides, increased male recombination may be a manifestation and a diagnostic feature of hybrid dysgenesis (Kidwell *et al.*, 1977; Sved *et al.*, 1990). Our preliminary data indicate some tendency for different potential of P-M system in the laboratory isofemale lines established from flies of the SF- and NF-slopes of Lower Nahal Oren. Thus, to compare the opposite slopes with respect to male recombination we had to take these factors into account. This was accomplished by analyzing the direct and reciprocal hybrid males. As one could easily see from the Table, no differences in mrf between these crosses were found either for SF- or for NF-slopes. It is worth noting that beside the foregoing two putative factors (cytoplasmic effect and P-M status), the reciprocal hybrid males differed also for the X-chromosome. The absence of the reciprocal effect in the reported data allows us to conclude that all these factors were unimportant in the revealed difference between the slopes or that these factors worked in a compensating manner resulting in no difference between the reciprocal crosses.

Two additional points should be noted here: (i) The compared lines derived from nature from the opposite slopes of Lower Nahal Oren were maintained at standard laboratory conditions as isofemale lines for more than a year. Consequently, non-heritable factors should be excluded when explaining the observed four-fold difference in the rate of male recombination; (ii) The higher mrf at the SF-slope as compared to that at NF-slope was obtained on hybrid males from crosses of the compared lines to a common genotype (multiple marker stock). Thus, to be detectable under this experimental design, the genetic differences between the hybrids (differences in allelic states at 'rec-loci') should be dominant or, at least, non-recessive relative to corresponding alleles of the marker stock *ru cu ca*.

References: Kidwell, M.G., J.D. Kidwell and J.A. Sved 1977, Genetics 86: 813-833; Korol, A.B., I.A. Preygel and S.I. Preygel 1994, *Recombination Variability and Evolution.* Chapman and Hall, London; Kullback, S., 1959, *Information Theory and Statistics*. John Wiley, New York; Lawrence, M.J., 1963, Heredity 18: 27-46; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, Inc., San Diego; Kruleva, M.M., A.B. Korol, T.G. Dankov, V. Scorpan and I.A. Preygel 1992, Genome 34: 653-658; Nevo, E., 1995, Proc. R. Soc. Lond. B 262: 149-155; Sved, J.A., W.B. Eggleston and W.R. Engels 1990, Genetics 124: 333-339; Thoday, J.M., and T.B. Boam 1956, J. Genet. 53: 456-461.

Grasso, G., D. Bordne and K. White. Biology Department, Waltham, MA 02254. Deletions and lethals in the 66A region of *Drosophila.*

No chromosomal deletions are currently available that uncover 66A. We were interested in this region because a gene encoding phenalanine and tryptophan hydroxylase activities maps to 66A (Silva *et al.*, 1992; Neckemeyer and White, 1992). We generated deficiencies in the region in order to facilitate mutant isolation in the region.

Mutant recovery and initial analysis: Two genetic schemes were used to generate deletions in the 66A region. In both cases, gamma-rays were used to induce mutations. The standard radiation dose, from a ^{137}Cs source, was 40 Gy which was previously demonstrated to induce 0.5 lethal hits per chromosomal arm. The first scheme took advantage of a semi-dominant eye color mutation *Henna* (*Hn*) that has been mapped to 66A/B (Lindsley and Zimm, 1992). Two to four day old *Canton-S* males isogenized for third chromosome were irradiated and mated to *mdm^{VL476} red /TM6, Hn Ubx e* females. Males were discarded after 3 days. *Hn* mutants have darker brown eye color when homozygous or hemizygous. Thus flies with henna eyes in the F1 progeny reveal new mutation in the *Hn* gene or a deletion encompassing the *Hn* locus. Out of a total of 32,530 flies screened only two (HnD-1 and HnD-2) were phenotypically henna.

The second scheme relied on a transgene P[ry$^+$] insert (AS 463) kindly provided by A. Spradling that was mapped to 65D-66B region. To verify the cytological location of the P[ry$^+$] insert in AS436 stock a biotin-labeled probe containing P sequences was used in in situ hybridization on chromosomal squashes (Engels *et al.*, 1986); the insert was mapped to 65E7. Mutagenized *ry^{506}* P[ry$^+$]/*ry^{506}* P[ry$^+$] males were mated to *TM2, ry Ubx/MKRS, ry Sb* females. Progeny was screened for rosy eye color. The rosy flies could result from new mutations in the transposon borne *ry$^+$* gene or deletions of the P[ry$^+$] insert. Some of the deletions of the P[ry$^+$] element will have the adjacent region deleted as well. From a total of 75,000 chromosomes that were screened, 14 putative rosy mutations were isolated. It is possible that a minute locus, M(3)65F, localized between 65F10-66A (Moscoso del Prado and Ripoll, 1983) could have contributed to the low frequency of mutations observed in these two screens.

Table 1. Complementation analysis between the different deficiencies and available mutations in the region. The presence or absence of the DTPH gene was assayed by chromosomal in situ hybridization with a *Drosophila* TPH cDNA probe or by western immunoblot with an anti-DTPH antibody.

	65B pale	65C Df(3)Vn	65D Me	65E TM3Sb	66A TPH	66A Hn3Sb	66A pbl	66A M(3)
RM5-1	+	+	+	–	+	+	+	+
RM5-2	+	+	+	–	–	–	+	+
RM5-3	+	+	+	+	–	–	+	+
HnD-1	+	+	+	+	–	–	+	+
HnD-2	+	+	+	+	ND	–	–	+
RM1-13	+	+	–	+	+	+	ND	ND
RM2-2	+	+	+	–	+	+	ND	ND

Table 2. Complementation analysis among some of the generated deletions.

	RM5-1	RM5-2	RM5-3	HnD-1	HnD-2
RM5-1	–	–	–	+	+
RM5-2	+	–	–	–	+
RM5-3	+	–	–	–	+
HnD-1	+	–	–	–	+
HnD-2	+	+	+	+	–

Stocks were established for each new mutation. Both mutants recovered in scheme 1 were homozygous lethal. Of the 14 mutants recovered in scheme 2, six were homozygous lethal and eight were homozygous viable. These eight were tested for the presence of DTPH protein in immunoblots - all gave a positive signal. These eight lines were not further investigated.

Complementation analysis of the presumed deletions: Table 1 represents complementation analysis of the presumed deletions and some of the known mutations in the interval. RM1-13 failed to complement *Me* and thus was likely to be a small distal-most deletion. RM2-2 and RM5-1 were semi-lethal over the balancer *TM3, Sb e* which has a

breakpoint in 65E (Lindsley and Zimm, 1992) and thus are likely to be deletions that uncover a region in 65E. We were particularly interested in deletions that uncovered *Hn* and thus were likely to include 66A region. Of the 14 new *ry* mutations, two (RM5-2 and RM5-3) uncovered *Hn*. HnD-1 and HnD-2 both uncovered *Hn*, but only HnD-2 uncovered *pbl*. Table 2 represents inter se complementation tests with the five deletions RM5-1, RM5-2, RM5-3, HnD-1 and HnD-2. RM5-2, RM5-3, HnD-1 and HnD-2 were all embryonic lethal. The smaller RM5-1 deletion was larval lethal.

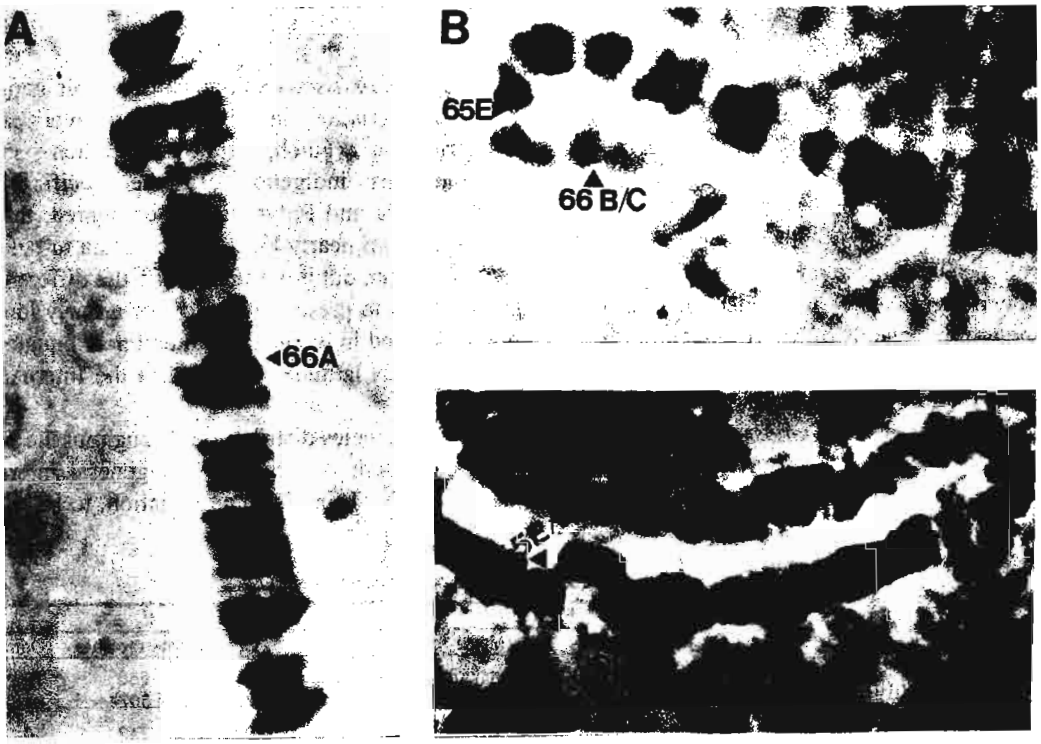

Figure 1: Microphotographs of third instar larvae salivary chromosome squashes of some of the deficiencies generated in 66A. **A**: HnD-1 in 66A6/9-66A21 **B**: RM5-2 in 65E-66B/C **C**: RM5-3 in 65E/F-66B/C.

Salivary chromosome analysis: Deletions HnD-1, RM5-3 and RM5-2 were analyzed by salivary chromosome analysis (Figure 1). As expected from the complementation analysis, both RM5-3 and RM5-2 were large deletions. Both RM5-2 and RM5-3 span the region 65E-F to 66B, but RM5-2 is poorly viable over the balancer chromosome *TM3, Sb e* that has a breakpoint in 65E, while RM5-3 survives better. In situ hybridization with probes for DTPH and DTH was undertaken to define deletions that were deleted for DTPH. Analyses of RM5-2/+ show that the DTH is still present and the DTPH is missing from the RM5-2 chromosome. HnD-1 is a small deficiency and was deleted for only a segment of 66A inclusive of the DTPH gene.

Generation of mutations within the RM5-2 deletion: To generate mutations that map within the deletion RM5-2, 1-3 day old homozygous *red e* males, isogenized for 3rd chromosome were starved for 6 hours and then fed 20 mM EMS dissolved in a 5% sucrose solution to which one drop of a red food colour was added (adapted from Lewis and Bacher, 1968). EMS treated males were then crossed to *TM3, Sb e/TM6B, Tb e* virgin females and removed after 3 days. From the F2, *Tb* (i.e., *red e**/TM6B, *Tb e*) male and virgin female progeny were selected and used to set up lines. Individual *red e**/TM6B, *Tb e* were selected and crossed to RM5-2/TM6B,*Tb e* and subsequentely to HnD-1/TM6, *Tb e.* Lines in which no F_2 progeny is Tb^+ (i.e., absence of */RM5-2) and lines in which few F_2 progeny is Tb^+ (i.e., semilethal) were established. A total of 8200 F2 lines were screened and 41 lethals were recovered. The frequency of lethal hits inside the DTPH deficiency is 1/200. Complementation analyses of these lethals shows that there are at least five complementation groups within the RM5-2 deficiency of which two map within the HnD-1 deficiency.

The deficiencies described above represent the only rearrangements so far available in the region around 66A and thus could be useful to analyze other genes located here. In general 66A and surroundings seem to be a cold-spot for P-element landing (Berg and Spradling, 1991) as well as for embryonic lethals (Jurgens *et al.*, 1985).

References: Berg, C.A., and A. Spradling 1991, Genetics 127:515-524; Engels, W.R., C.R. Preston, P. Thompson, and W.B. Eggleston 1986, BRL Focus 8:6-8; Lewis, E.B., and F. Bacher 1968, *Dros. Inf. Serv.* 43:193; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press Inc.; Moscoso del Prado, J., and P. Ripoll 1983, Genet. Res. 42:59-63; Neckameyer, W.S., and K. White 1992, JBC 267:4199-4206; Jurgens, G., E. Wieschaus, C. Nusslein-Volhard, and H. Kluding 1984, Roux's Archives of Developmental Biology 193:283-295; Silva, F.J., I. Bel, L.M. Botella, R.G.H. Cotton, and J. Ferre' 1992, Biochem J. 287:85-89.

Futch, D.G. San Diego State University, San Diego, California. Genetics of the pigment difference between Polynesian and Micronesian races of *D. ananassae*.

D. ananassae exhibits considerable geographically related variation in pigmentation over parts of its distribution (Futch, 1966). When samples of populations indigenous to the Pacific Islands of Micronesia and Polynesia are compared, the polytypic nature of the species becomes particularly apparent. Body color ranges from nearly black in Polynesia to pale yellow in Micronesia. Within each of these regions there is some inter-island variation, but it is small, while the difference in color in flies from the two regions is very distinct. These two populations seem to present evidence for a fairly long-standing isolation even though the islands of Micronesia and Polynesia are all located in the equatorial and near equatorial mid to eastern Pacific Ocean. Micronesia is mostly north of the Equator (to 20° N latitude) and west of the International Date Line (between 130° E and 180°).

Interestingly, populations of *ananassae* from less remote parts of the species distribution throughout the tropical and subtropical world; e.g., Hawaii, Asia, Africa, India, and Central and South America, are in general intermediate in pigmentation between the two extremes found in Micronesia and Polynesia. There is some variation, to be sure, so that, for example, pigmentation of American flies is slightly less than that of Hawaiian flies. These observations, along with some differences in the occurrence, distribution, and frequencies of certain paracentric inversions (Tomimura *et al.*, 1993), have led me to consider the species as being differentiated to the extent that there are three recognizable races, the light Micronesian race, the dark Polynesian race, and a widespread "cosmopolitan" race. Each of the former two probably owes its greater differentiation from the others to the occurrence of its populations on scattered and remote oceanic islands. Isolation between Micronesia and Polynesia is probably due to their locations on opposite sides of the Equator. According to accounts given by 18th and 19th century European explorers prevailing East to West ocean currents and winds seem to have produced a barrier to North-South maritime travel of Micronesian and Polynesian navigators (Sharp, 1964) so that there was little contact between these two groups of people in the past. This effect could also have served to prevent North-South Dispersal, or migration of *Drosophila* in this region. The selective significance of being light or dark in those two regions is not known.

Table 1. Observed distribution of F_2 phenotypes.

P_1 Cross			F_2			
Females	Males		Light	Intermediate	Dark	Total
Tonga(dark) x Majuro(light)		Females	0	688	79	767
		Males	78	507	61	646
Majuro(light) x Tonga(dark)		Females	99	742	0	841
		Males	106	579	78	763
C.D. at 5% = 3.19				C.D. at 1% = 4.64		

Table 2. Expected relative frequencies.

P_1			F_2		
Females	Males		Light	Intermediate	Dark
dark x	light	Females	0	0.875	0.125
		Males	0.125	0.750	0.125
light x	dark	Females	0.125	0.875	0
		Males	0.125	0.750	0.125

Table 3. Test of observed distribution to two-gene model.

P_1						
Females		Males	F_2	Chi square	Degree of freedom	p
dark	x	light	Females	3.394	1	0.10 > p > 0.05
			Males	5.9696	2	0.10 > p > 0.05
light	x	dark	Females	0.407	1	p > 0.50
			Males	4.429	2	0.50 > p > 0.10

From numerous crosses made between the dark Polynesian race of *ananassae* and its light sibling, *D. pallidosa*, from Samoa, I have seen that the presence of dark pigment was at least in part controlled by an X-linked gene or genes (Futch, 1966). This same asymmetric pattern of X-linked inheritance is also seen when reciprocal crosses are made between the dark Polynesian race and the light Micronesian races of *ananassae*. In such crosses if the P1 female is dark,

the F1 males are darker than if the P1 female is light. F1 females produced by either kind of cross have the same intermediate phenotype. Inheritance of this variation in pigmentation must be polygenic and allelic contributions seem to be additive.

To examine more closely the inheritance of this trait, crosses were made between two laboratory strains, one from each race, maintained by me at San Diego State. The strains chosen are the lightest and the darkest of the several that I have. The Micronesian race is represented by a strain from Majuro in the Marshall Islands and the Polynesian race by a strain from Tongatabu in the Tongan Islands. The F1 of reciprocal crosses between these two strains had the relatively uniform phenotype observed in the F1 of all crosses between dark and light flies. The females are intermediate and the males are lighter or darker depending on the race of the P1 female; dark females produce darker sons than light females.

When F1 hybrids are allowed to mate, F2 progenies are produced as shown in Table 1. The observed distributions of phenotypes seen in the F2 of both crosses fit a simple model in which two major genes, one X-linked and one autosomal, with alleles with additive effects determine the degree of pigmentation in these flies. Table 2 lists the relative frequencies expected for the phenotypes shown in Table 1 if this two gene model is correct. Table 3 summarizes a statistical analysis for these data using the Chi Square Test for Goodness of Fit of the observed frequency distributions to the distributions predicted by this model. None of the four F2 progenies produced by these crosses are significantly different from the predictions of the model. While the data seem to fit this simple two-gene model, there may be other genes with smaller effects that determine the amount of dark pigment characterizing these two races of *D. ananassae*. This might account for some of the difficulty encountered when trying to separate dark and intermediate F2. There is some variation in even the darkest of the pigmented flies.

References: Futch, D.G., 1966, Univ. Texas Publ. 6615: 79-120; Sharp, A., 1964, *Ancient Voyagers in Polynesia.* University of California Press, Berkeley and Los Angeles, pp. 150; Tomimura, Y., et al., 1993, in Drosophila ananassae, *Genetical and Biological Aspects* (Y.N. Tobari, ed.), Japan Scientific Societies Press, Tokyo, and Karger, Basel, 139-198.

Sidorenko, V.S. Institute of Biology and Pedology, Far Eastern Division of the Russian Academy of Science, Vladivostok - 22, Russia. Some unrecorded species of Drosophilidae from Viet Nam.

Up to the present, 14 species of the Drosophilidae (2 spp. of the genus *Leucophenga* Mik, 3 spp. of the genus *Dettopsomyia* Lamb, 3 spp. of the genus *Drosophila* Fallen, 4 spp. of the genus *Liodrosophila* Duda, 1 sp. of the genus *Scaptodrosophila* Duda, 1 sp. of the genus *Sphaerogastrella* Duda) have been known from Viet Nam (Okada, 1977). This report deals with 9 unrecorded species of Drosophilidae from Viet Nam collected by staff of the Laboratory of Insect Systematics of the Zoological Institute (St. Petersburg, Russia).

Subfamily Steganinae

Amiota (*Phortica*) *cardua* Okada, 1977; male, Viet Nam, Tam Dao, prov. Vinh Phu, 1000 m, 12.XI.1990.

Amiota (*Phortica*) *eugamma* Toda et Peng, 1990; 3 males, Viet Nam, prov. Vinh Phu, Tam Dao, 1000 m, 12.XI.1990.

Amiota (*Phortica*) *pseudotau* Toda et Peng, 1990; male, Viet Nam, prov. Vinh Phu, 1000 m, Tam Dao, 13.XI.1990.

Leucophenga (*Leucophenga*) *interrupta* Duda, 1924; 2 males, Viet Nam, pr. Ha Son Binh, Mai Chou, forest, 1, 2.XI.1990.

Leucophenga (*Leucophenga*) *ornata* Wheeler, 1959; 1 male, 1 female, Viet Nam, pr. Ha Son Binh, Mai Chou, forest, 31.X, 2.XI.1990.

Subfamily Drosophilinae

Zaprionus (*Anaprionus*) *bogoriensis* Mainx, 1958; 1 male, Viet Nam, pr. Ha Son Binh, Da Bac, Tuly, forest, 19.X.1990.

Zaprionus (*Anaprionus*) *grandis* (Kikkawa et Peng, 1938); 2 females, Viet Nam, pr. Hanoi, 70 km NW Hanoi, Ba Vi, 24.XI.1990.

Hypselothyrea (*Hypselothyrea*) *guttata* Duda, 1926; 10 males, 3 females, Viet Nam, pr. Ha Son Binh, Cao Phong, 31.X.1990; pr. Ha Son Binh, Mai Chou, forest, 1.XI.1990; pr. Vinh Phu, Tam Dao, 1000 m, forest, 13.XI.1990; pr. Hanoi, 70 km NW Hanoi, Ba Vi, 400 m, forest, 22.XI.1990.

Drosophila (*Dudaica*) *senilis* Duda, 1926; 1 female, Viet Nam, pr. Vinh Phu, Tam Dao, 1000 m, 16.XI.1990.

Acknowledgments: My hearty thanks are due to Prof. E.P. Nartshuk and Dr. S.A. Belokobylskij (St. Petersburg, Russia) for loaning me the very interesting material on Viet Namese Drosophilids. My special thanks to Prof. Masanori J. Toda (Sapporo, Japan) for his help in determination of some specimens.

References: Okada, T., 1977, A Catalog of the Diptera of the Oriental Region, III, Univ. Hawaii Press, Honolulu: 342-387.

De, A., and J.P. Gupta. Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi - 221005, India. Records of Drosophilid species from Bhutan.

In the past few decades, taxonomic studies of Drosophilidae have taken rapid strides in India, accumulating considerable data on native Indian species (Gupta, 1993). However, our knowledge regarding species inhabiting the adjoining countries still remains very meager and fragmentary.

Bhutan is one of these countries where such studies are yet to be initiated. It is a small country covering an area of about 47,000 sq km on the eastern Himalayan Mountain Range with China on the North and India on the South (Figure 1).

During our recent visit to this country, a cursory survey of the Drosophilid species was made at and around Phuntsholing in Bhutan. All together twenty species were collected, representing seven genera of the family Drosophilidae (Table 1). Among them, four species belonging to the genus *Mulgravea* and one species each of the genera *Lordiphosa* and *Nesiodrosophila* were detected as new species. Based on these results, it is inferred that the members of Drosophilidae are fairly distributed in this country and further surveying studies in this region may yield the occurrence of many more interesting species.

References: Gupta, J.P., 1993, Dros. Inf. Serv. 72: 87.

Figure 1. Position of Bhutan.

Table 1. Drosophilid species collected at and around Phuntsholing, Bhutan

Genus	Subgenus		Species	No. of flies collected: males	females
1. *Drosophila*	*Drosophila*		*nasuta*	24	66
	Sophophora		*melanogaster*	68	82
			ananassae	87	113
			bipectinata	3	2
			malerkotliana	43	21
			kikkawai	2	8
	Dudaica		*senilis*	3	4
2. *Lordiphosa*		**	*paraflabella*	3	2
3. *Microdrosophila*	*Oxystyloptera*		*paradistincta*	8	5
4. *Liodrosophila*			*trichaetopennis*	200	187
			fasciata	43	42
			ceylonica	11	8
5. *Nesiodrosophila*			*cirricauda*	8	17
		**	*neocirricauda*	14	6
6. *Hypselothyrea*	*Hypselothyrea*		*guttata*	1	1
7. *Mulgravea*			*ranipoolensis*	4	4
		**	*detriculata*	9	18
		**	*bhutanica*	5	—
		**	*peniglobosa*	14	10
		**	*spinisterna*	2	—
			Total number of flies collected		1148

** New species

Robertson, Hugh M. Department of Entomology, University of Illinois at Urbana-Champaign, 505 S. Goodwin, Urbana, IL 61801. Structure of the stable P element in *Drosophila melanogaster*.

A particular insertion of a P[ry^+ Δ2-3] element generated by Laski *et al.* (1986) on the third chromosome at 99B has the unusual properties of producing enormous transposase activity, yet being itself almost completely stable (Robertson *et al.*, 1988). This element has proved to be useful as a stable source of transposase, particularly in mutagenesis and enhancer trapping experiments. I have examined the structure of this P insert and it provides a possible explanation for its stability.

The homozygous Δ2-3(99B) strain was first examined by Southern blotting with probes from the P transposable element and the *rosy* gene. This work led to the conclusion that the insert consists of two copies of the original construct, in head-to-head orientation, with approximately 360 bp missing from the junction of the 5' ends of the P elements (Figure 1). The exact nature of this deletion was determined by the cloning and sequencing of a PCR amplification product of approximately 350 bp generated with the primers 2223 and 3646 (kindly provided by William Engels) indicated in Figure 1. One of the P element sequences is deleted from position 1-19, while the other is deleted from position 1-348. The two 3' ends of this composite duplicated element were examined using inverse PCR with primers designed to the terminal *rosy* sequences and both are intact, however the eight base pairs flanking each end are not direct repeats of each other. Efforts to examine the original insertion site in wildtype genomic DNA using PCR with multiple primer sets designed from these flanking sequences failed because no amplification products could be obtained.

This structure of the Δ2-3(99B) P element provides an explanation for its stability in two regards. First, it does not have functional 5' ends but rather two 3' ends. Mullins *et al.* (1989) found that such constructs with symmetrical ends were not mobilized efficiently by P element transposase. Second, the duplicated Δ2-3(99B) structure yields a 20 kb composite element and large P elements are less efficiently mobilized. Together these properties are likely sufficient to explain its stability. The resultant composite element has one functional P transposase gene and two copies of the *rosy* gene. Extensive efforts to mutate this *rosy* marker using EMS failed (S.H. Clark and A. Chovnick, personal communication), which might best be explained if both of these copies are functional, requiring a major deletion or double mutation to eliminate both. Failure to amplify the original insertion site from wildtype genomic DNA indicates that a large region was deleted during formation of the composite element, perhaps in a single event between two nearby insertions of the original element.

References: Laski, F.A., D.C. Rio, and G.M. Rubin 1986, Cell 44: 7-19; Mullins, M.C., D.C. Rio, and G.M. Rubin 1989, Genes Dev. 3: 729-738; Robertson, H.M., C.R. Preston, R.W. Phillis, D.M. Johnson-Schlitz, W.K. Benz, and W.R. Engels 1988, Genetics 118: 461-470.

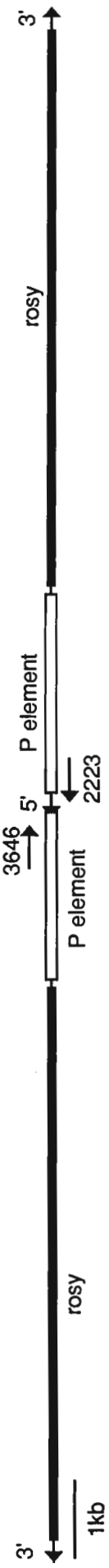

Figure 1. Schematic of the duplicated composite Δ2-3(99B) P element. The 20 kb element is drawn approximately to scale, with the P transposase gene sequences as an empty box, the *rosy* gene sequences as a solid box, and the remaining P sequences as a thin line. The two 3' ends of the P elements are shown as arrows, and the single truncated 5' end is indicated. The orientations and approximate positions of the primers used to amplify the junction fragment are shown.

Makunin, I.V., N.G. Kholodilov, and S.A. Shestopal. Institute of Cytology and Genetics, Novosibirsk 630090, Russia. Nucleotide sequences from the proximal part of the *Broad-Complex* locus of *D. melanogaster*.

The 963 bp *Hin*dIII/*Eco*RI genomic fragment from the proximal part of the *Broad-Complex* (*BR-C*) was sequenced (Figure 1 and 2). This sequence partially overlaps DM138D4T STS (Madueno *et al.*, 1995). It allows one to map contig N16 to 2B3-5 - 2B6 chromosome region.

The 1.2 kb cDNA clone named k9 was isolated from the third instar larvae cDNA library. The terminal sequences of the k9 clone were determined according to the method of Maxam-Gilbert. This clone is composed of two different parts: a sequence homologous to the *protein disulfide isomerase* (*pdi* or *dpdi*) gene (Makunin and Shestopal, 1995; McKay *et al.*, 1995) and a sequence from the 2B region. Nucleotide sequence comparison of the 2B part of k9 and 963 bp *Hin*dIII/*Eco*RI genomic fragment showed that these sequences share 106 bp (Figure 1 and 2). The k9 clone was used as a probe in Northern analysis of the Canton-S total RNAs. Bands corresponding both to *pdi* and *BR-C* transcripts were detected (Figure 3). Surprisingly, no homology was found between the k9 cDNA clone and the known *BR-C* transcript sequences (DiBello *et al.*, 1991). It seems that *BR-C* transcripts have an additional common sequence at the 3' end of the *BR-C* locus.

References: Belyaeva *et al.*, 1987, Chromosoma 95: 295-310; DiBello *et al.*, 1991, Genetics 129: 385-397; Madueno *et al.*, 1995, Genetics 139: 1631-1647; Makunin and Shestopal 1995, Dokl. Akad. Nauk (in Russian) 345: 561-563; McKay *et al.*, 1995, Insect Biochem. Mol. Biol. 25(5): 647-654.

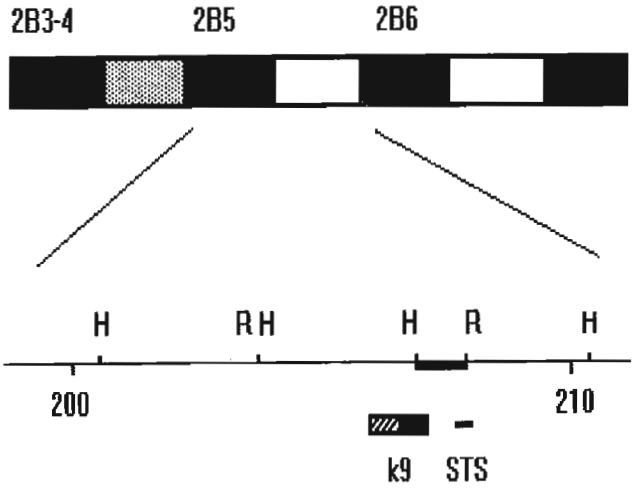

Figure 1. Cytological (above) and molecular (below) localization of 963 bp *Hin*dIII/*Eco*RI genomic fragment derived from 1479 phage (Belyaeva *et al.*, 1987), which is shown as a bold line. H - *Hin*dIII, R - *Eco*RI. In the K9 cDNA clone a dashed part corresponds to the *pdi* transcript, a black one to the sequence from the 2B region. STS - DM138D4T Sequence Tagged Site.

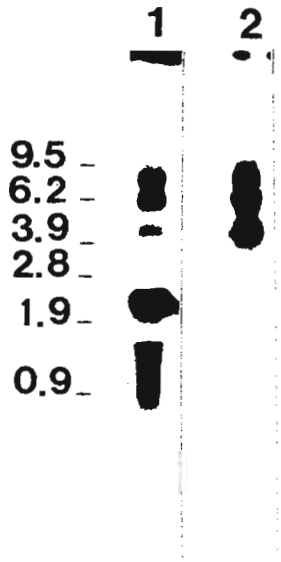

Figure 2. Comparison of sequences: genomic 963 bp *Hin*dIII/*Eco*RI fragment from 1479 phage (Belyaeva *et al.*, 1987) (1, X97997); part of k9 cDNA clone (2, X97998); DM138D4T STS (3, Z32387). Sites for *Hin*dIII and *Eco*RI were shown in small letters and subscribed above the sequence. The points stand for the same nucleotides.

Figure 3. Northern analysis of k9 cDNA expression. Total RNA from white prepupae was probed with the k9 clone (lane 1) or with 1 kb *Stu*I/*Bam*HI fragment from the dm527 clone (provided by G.M. Guild, see DiBello *et al.*, 1991) contained the core part of the *Broad-Complex* transcript (lane 2). The hybridization with the k9 clone reveals patterns of transcription similar but not equal to those of the core part of the *BR-C*. An additional signal on lane 1 corresponds to the protein disulfide isomerase transcript (McKay *et al.*, 1995). Size markers in kilobases are indicated to the left.

```
2     TTATGCGCATTAAACTTATTTTATTTGCCAACTGCTGCGCCTAACGTGCG      50
2     TTCTCTAAAGAGTCGGATTCCAGTAAGAAAAGATAAAGGTCCTACGGATC     100

2     GGCGGCAGATTAGATTGTATAAGATGTGTGTATTTTTTTTTTTTTTCGAG     150
                                                  HindIII
1                                                  aagctt
2     GGAGCCGTAATTGTAAATGTAGCTCATATATATAAGACTAGAGT......     200

1     AATTCGGGTCTAATGTTTAAAGTCGGCGGGGCACGGAGAAGACAAACGTG     250
2     .....AAA.........................................

1     CAGAAGCGAAACACAAGAGACAAACAAAATCAACCAACAAACAAAAGAAA     300
      ..................................A........A...

1     AACGTTATCAGGGATAAAATATCAAGTATGCAGTAGGAAAGATAATTTTT     350
1     TCTACTTATTCACGAATACGACGAATAAAGTAACAAAAATTAAAATCACT     400
1     TTTTTATATACACATACATATACACACGCGAGCGTATAAACGATAAATGT     450
1     GCATATGCGTATGCGCAAGCTAAGCGAAACAACTAAACTAATGTCTATGT     500
1     ATACATGTATATACTTTACACCATTTACCATATACAATTTATAAGCCGAT     550
1     ATGCCGATGATATACACATATACAATGTTAGTTTGGTGTATAGAAGACAG     600
1     ATGAGCTGTAAACGTTTTTGCAAATCAAGAAAATAACGTATTTAACAAAA     650
1     CCGCAAAGATAACTCAGTGATTTTTACCAGCGAGATACAAATGTTCTGAA     700
1     ATTAAATTAACTAACAGATAATAAGCGCCGACGAGCGAATCAGCAAATCG     750
1     AGTAGCTGCGCGGAGTATCAGTGCAGAATCATAAGTGATGGACCGCAGTT     800
1     AGCACTGCCCCAAATAGCGTCTGATCATGTTGCATGCAGTCTAGGACGGG     850
1     GCAGGCAAACATGCAACAACCACCAAGCCCAGACTGGAAGCGAAAAAAAA     900
1     AAAAAACAAAAAGAAAAAGAAAAACACCCACTAGGATCTGTGTTAAGAAA     950

1     ATGTTTAAGTCAAAAG-ACGAAGAATGTGTATCAATTCAATGTCTCAGGA    1000
3       ........N.....T..............................

1     ATTTCTTAAAATTTAACCAATTCACACCTTATACTATAGAACACATTGCA    1050
3     ......................T.........................

1     GGAACGTCTGGAAAGACAGACAGCTAACAACAAACTCTTCCAAACCAACA    1100
3     .................................................

1     CACCTCCAATGGTGCTGCATATCATCATCTCATCATCAAGTGCGAGTTTT    1150
3     .................................................

         EcoRI
1      CCgaattc
3     .........TTGTCAAAAAAAA
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Uysal, Handan[1], and Zafer Bahçeci[2]. [1]Atatürk University, Science and Art Faculty, Department of Biology, 25240 Erzurum, Turkey, and [2]Gazi University, Faculty of Education, Kirsehir, Turkey.
Effect of lead acetate and mercury chloride on offspring production in *Drosophila melanogaster*.

Previous researchers have investigated the effects of various stress conditions such as chemicals, temperature shocks (heat and cold), radiations, and anoxia on the development of Drosophila melanogaster. On the other hand, it was found that different salts affect the development of *D. melanogaster* (Islam *et al.*, 1986). In this study the effects of lead acetate and mercury chloride on the offspring production in *D. melanogaster* have been examined. The wild type *D. melanogaster* was used in this present investigation. The flies were reared on the cornmeal-agar food medium. Traces of propionsaure were added as mould inhibitor. The doses of lead acetate and mercury chloride used in this study per 100 ml of food medium were 0 (control), 0.05, 0.1, 0.5, 1.0, and 5.0%. The experiments were started with 7 pairs of flies, equally shared by virgin females and unmated males, per population jar (size 15 cm x 5 cm). The experiments were replicated three times for both chemicals. Counting of flies on the control and treated foods was made for three successive generations. Experiments were conducted at 25±1°C.

Table 1. Offspring production in *D. melanogaster* at different concentrations of lead acetate added to food.

	No. of flies at concentrations:					
generations	0.00%	0.05%	0.1%	0.5%	1.0%	5.0%
0 (parental)	14	14	14	14	14	14
1	295	119	115	99	84	75
2	310	151	143	140	141	97
3	327	241	225	183	153	143

Table 2. Offspring production of *D. melanogaster* at different concentrations of mercury chloride added to food.

	No. of flies at concentrations:					
generations	0.00%	0.05%	0.1%	0.5%	1.0%	5.0%
0 (parental)	14	14	14	14	14	14
1	347	337	311	229	224	96
2	364	342	316	229	224	168
3	382	345	328	233	239	186

The results are given in Tables 1 and 2. Both lead acetate and mercury chloride reduced the number of offspring of the fly ($P < 0.01$). It was found that with the increase of doses the offspring production decreased. Lead acetate produced a more drastic effect on *Drosophila* than did mercury chloride. This situation has been seen in Figures 1 and 2.

Islam *et al.* (1986) reared *D. melanogaster* on copper and ferrous sulfates added food medium and determined the decreasing of offspring production. Our results support the above findings. These results may be due to the following three reasons. Several heavy metals prevent the hatchability and so decrease the offspring production (Dhingra and Vijayakumar, 1987), larvae and pupae, highly sensitive to heavy metals, may decrease the offspring production yield (Pascoe *et al.*, 1989) and the blocking of spermatogenesis may also decrease the number of offspring (Marcos *et al.*, 1981).

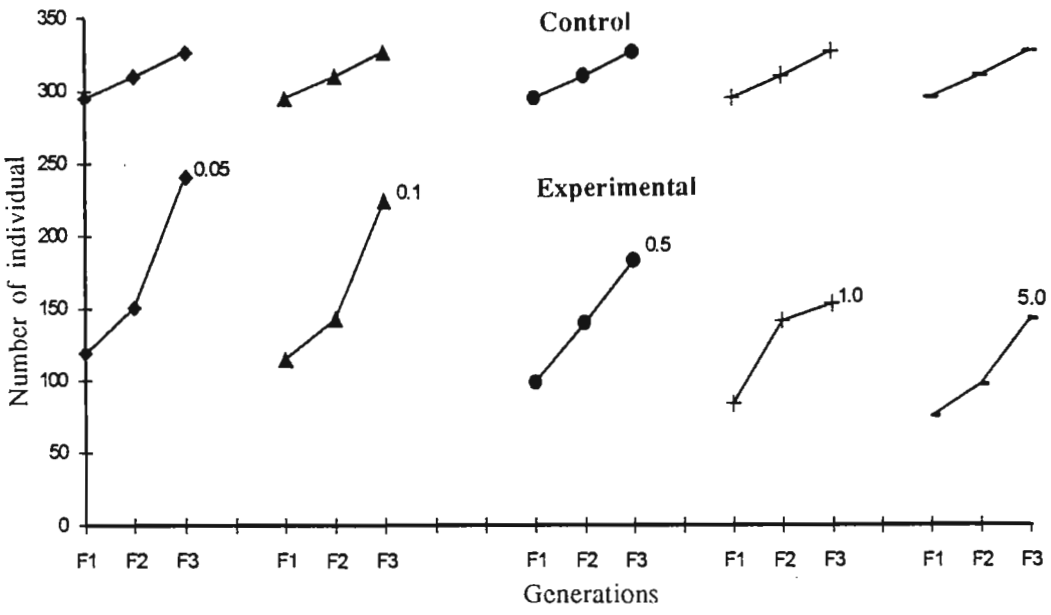

Figure 1. Effect of lead acetate on offspring production of *D. melanogaster*.

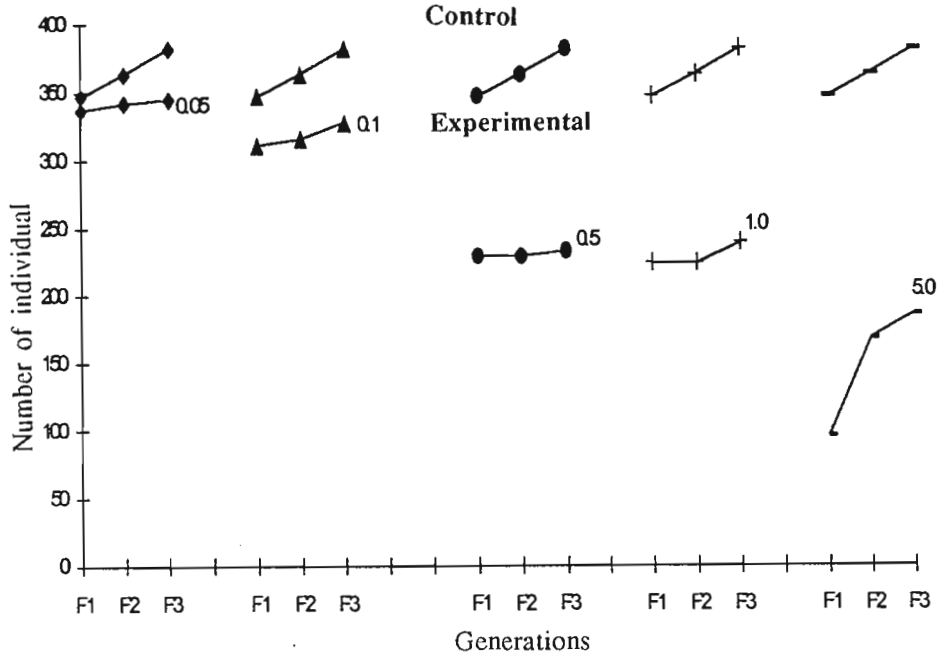

Figure 2. Effect of mercury chloride on offspring production of *D. melanogaster*.

In this study, it was also observed some morphological abnormalities, such as arrow, atrophy, folded on the wings and decreased body size (Uysal, 1994), in offspring after treatment of lead acetate and mercury chloride.

As a result, the lead acetate and mercury chloride in different doses have caused a decrease in the number of offspring produced and morphological abnormalities.

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Kosuda, Kazuhiko. Biological Laboratory, Faculty of Science, Josai University, Sakado, Saitama, 350-01, Japan. Viability of *Drosophila melanogaster* female flies carrying melanotic tumours.

There is a general agreement that the melanotic tumour development is in an aggregation of melanized haemocytes and it is under genetic control (Salt, 1970; Sparrow, 1978). Melanotic tumours generally appear in larval stages, especially in the third instar larvae shortly before pupation in most instances. They can be seen with unaided eyes as dense black bodies of irregular shape within the body cavity, usually floating in the haemocoel and present as single or multiple masses. In previous reports, Kosuda (1990, 1991) described a new strain, C-104, in *Drosophila melanogaster*, in which melanotic tumours can be detected under the microscope in the abdomen of female flies, exclusively in the vicinity of the spermathecae. They often become macroscopically visible as dark black lumps in the abdominal cavities when they are fully grown. Melanotic tumour formation was disclosed to increase with female ages and they do not develop in male flies (Kosuda, 1990). A gene responsible for this melanotic tumour formation was named *tu91k* and assigned to the second chromosome of *D. melanogaster* (Kosuda, 1992). In the present investigation, the effect of tumour formation on adult viability was studied.

Female flies of the C-104 strain were maintained for three weeks at 29°C, since this melanotic tumour develops in the aged female flies and the incidence of melanotic tumour formation was accelerated by the high rearing temperature (Kosuda, 1990). Female flies were maintained in vials with food and transferred every two or three days to fresh vials. At the time of transfer, dead flies were individually dissected and examined for the melanotic tumour formation under the microscope. They were classified into one of three classes according to the numbers of spermatheca attached or enveloped by the melanotic tumours. The incidence of melanotic tumour formation was also investigated for the living flies. If the melanotic tumour has a deleterious effect on the adult viability of its carriers, the incidence of melanotic tumour formation in the dead flies is expected to be higher than that in the living adults.

Table 1. Deleterious effect of melanotic tumour formation on female viability in the C-104 of *Drosophila melanogaster*. Ratios I and II are the proportion of tumour formation on the individual basis and the spermatheca basis, respectively. They are calculated by the following formulae: Ratio I = (A+B) / Total and Ratio II = (A+2B)/(2 x Total), respectively.

	No. of female flies					
	No. of spermatheca attached or encapsulated by melanotic tumours			Total	Ratio I	Ratio II
	0	1 (A)	2 (B)			
Alive	85 (63.9%)	29 (21.8%)	9 (6.7%)	133	.286	.177
Dead	112 (49.3%)	80 (35.2%)	35 (15.4%)	227	.507	.330

Table 1 gives the incidence of melanotic tumour development in the dead and living female flies at the age of three weeks after eclosion. The indices of Ratio I and II in the living flies are comparable to those in the previous report (Kosuda, 1990). The incidence of melanotic tumour formation in the dead flies, 0.507 per individual, is remarkably higher than that (0.286) in the living female flies. The difference is statistically highly significant, for chi-square values with one and two degrees of freedom in the contingency tables are 13.65 and 12.88, respectively. This fact implies that the mortality of female flies in which melanotic tumours develop is much higher than that of non-carriers of melanotic tumours. Since the aggregation and the subsequent cellular encapsulation by haemocytes accompanied by melanization in the C-104 strain are generally considered to be the typical common non-self recognition and self defense reactions in insects and other invertebrates (Nappi, 1973; Ho *et al.*, 1982; Chen and Lawrence, 1985; Collins *et al.*, 1985; Nayer *et al.*, 1989), self defense reaction and non-self recognition seem to be accomplished at the expense of the organisms.

References: Chen, C.C., and B.R. Lawrence 1985, Int. J. Parasitol. 70: 421-428; Collins, F.H., *et al.*, 1986, Science 234: 607-610; Ho, B.C., *et al.*, 1982, Parasitology 85: 567-575; Kosuda, K., 1990, Gerontology 36: 121-125; Kosuda, K., 1991, Dros. Inf. Serv. 70: 123-124; Kosuda, K., 1992, Genet. Sel. Evol. 24: 561-565; Nappi, A.J., *et al.*, 1989, Parasitology 66: 23-32; Nayer, J.K., *et al.*, 1989, J. Med. Entomol. 26: 159-166; Salt, G, 1970, *The Cellular Defense Reaction in Insects*. Cambridge University Press; Sparrow, J.C., 1978, In: *The Genetics and Biology of* Drosophila (Ashburner, M., and T.R.F. Wright, eds.), volume 2b, Academic Press, London, pp. 277-313.

Chatterjee, R.N. Department of Zoology, University of Calcutta, Calcutta, India. The 87A and 87C heat shock loci are induced unequally in a strain of X chromosomal aneuploid of *D. melanogaster*.

The heat shock genes of *D. melanogaster* provide a good experimental system for studying the coordinated control of gene expression. When *D. melanogaster* cells are shifted from 25°C to 37°C, the synthesis of heat shock RNA and protein is realised. In polytene tissues, the transcription of new RNAs is associated with the activation of puffs at specific sites on the polytene chromosomes (Ritossa, 1962; Ashburner and Bonner, 1979). Hochstrasser (1987) has noted that development temperature of the early stage of the larvae can cause the

unequal expression of the 87A and 87C loci of the polytene chromosomes of *D. melanogaster*. In this report, preliminary results on the effect of X chromosomal aneuploidy on the heat shock puffs 87A and 87C of the salivary gland chromosomes of *D. melanogaster* are presented.

For the present investigation, the male aneuploid, Dp(9A-11A) was constructed from X;Y translocation stocks of *D. melanogaster* by the method of Lindsley *et al.* (1972). The stocks used were: T(X;Y) 11A/Y; T(X;Y) 9A/FM7; C(1)RM:=Y and the Oregon R strain of *D. melanogaster*. By crossing T(X;Y)9A/FM7 females to T(X;Y)11A males, males aneuploid for the region 9A-11A were generated (Prasad *et al.*, 1981). The aneuploid males were viable and were recognised by their size and non-*Bar* phenotype. These males were selected and crossed to *y w f* attached-X virgin females for a permanent stock.

Salivary glands from third instar larvae (either from Oregon R strain or from aneuploid males) were dissected out in Drosophila Ringer at pH 7.2. One gland from the pair was incubated at 37°C (treated) for 30 min and the remaining on was incubated at 24°C (control) for the same period. Subsequently, they were labeled with ^{3}H-uridine (500 mCi/ml, Sp. activity 17,200 mCi/mM, obtained from BARC Bombay) for 10 min at the respective temperature. Cytological preparations were then made and processed for autoradiography.

Results show that, in contrast to the wild type, the heat shock induced incorporation of ^{3}H-uridine at 87A and 87C puffs was unequal in aneuploids (Figure 1). While the mean 87C/87A grain ratio in heat shocked wild type (Oregon R) glands was close to 1.0 (0.93 ± 0.07), it was 2.07 ± 0.11 in aneuploid males (Table 1). The activity of the 87A puff is comparable in both control and aneuploid males. However, 87C puff was significantly induced in aneuploid males. Thus, the higher 87C/87A grain ratio in Dp(9A-11A) males was due to a higher rate of ^{3}H-uridine uptake in the 87C puff. This was also evident from the puffing activity pattern of the 87C locus of the aneuploid males (see Figure 1).

Figure 1. Photomicrograph of a segmental aneuploid X chromosome in male, duplicated for the segments 9A-11A, showing ^{3}H-uridine labeling. Note the increased puffing activity and ^{3}H-uridine incorporation pattern on the 87C heat shock puff. Bar represents 10 μm.

Table 1. Analysis of 87A/87C activity in individuals with *Dp* (9A-11A).

Strains	No. nuclei observed	Mean no. of grain + S.E. 87A	87C	Mean 87C/87A grain ratio ± S.E.
Oregon R male	15	26.7 ± 1.7	25.2 ± 1.9	0.93 ± 0.07
Dp(9A-11A) male	12	23.5 ± 1.8	48.6 ± 2.1	2.07 ± 0.11*

* $P < 0.01$

The differential puffing activity of the 87A and 87C loci in the Dp(9A-11A) males is puzzling. It is possible that the X chromosomal aneuploidy may have some influence in regulation of 87A and 87C loci in heat shock condition. Earlier, differential regulation of 87A and 87C has actually been documented in salivary glands under certain extreme growth condition and in *in vitro* treatments (Hochstrasser, 1987).

Acknowledgments: This work was supported by CSIR research grant [37(850)/94/EMR-II] to RNC.

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Rim, N.R., and K.Y. Kwon. Chonbuk National University, Chonju 560-756, Korea. Relationship between gene frequencies and environmental variables in time series natural populations of *D. melanogaster*.

To the extent that a particular environmental factor affects the relative fitnesses of genotypes, correlation between that environmental factor and gene or inversion frequencies may be expected. Therefore, the present study was focused on what factors among the surrounding conditions we established cause seasonal difference in inversion frequencies from *Drosophila melanogaster* populations.

To analyze seasonal changes of gene frequencies in a Korean locality Chonju natural populations, samples were carried out monthly from August in 1982 to December in 1985, and made up 28 male collections and 27 female collections. To get chromosomes of sperm, every wild male was crossed to several virgin females homozygous for all standard chromosome sequences. Seven F1 larvae from each mating were selected and tested to analyze gene arrangements in haploid sets of sperm chromosomes produced by individual wild males. And to test egg chromosomes, a single larva from a little of each female fly inseminated in nature was used. For the behavior of gene frequencies with climatic surroundings, a back step multiple regression was employed.

Table 1. Environmental variables (mean and Cv) established in the present study. Cv = coefficient of variation.

Environmental variables	Abbreviations of variables	
	Mean	Cv
For 7 days, before sampling		
Average daily temperature		CMT/7
Daily minimum temperature	MLT/7	
Average wind speed	MWS/7	
Radiation on horizontal surface		CRH/7
For 14 days, before sampling		
Average daily temperature		CMT/14
Daily maximum temperature	MHT/14	
Average relative humidity	MHU/14	
Radiation on horizontal surface		CRH/14
For 30 days, before sampling		
Average daily temperature		CMT/30
For a month, 30 days between 90 days to 60 days before sampling		
Average daily temperature	MMT/90	CMT/90
Daily maximum temperature	MHT/90	CHT/90
Daily minimum temperature	MLT/90	
Daily temperature range	MTR/90	CTR/90
Average relative humidity	MHU/90	CHU/90
Radiation on horizontal surface	MRH/90	CRH/90

From 58 collections of both males and females, a total of 41 different inversions, seven cosmopolitan inversions and 34 endemic ones, were found to be the paracentric inversions only. Table 1 shows the environmental variables (environmental means and environmental variabilities) which were selected as coordinates through three steps of concerning procedures. Multiple regression analysis of gene frequencies are presented in Table 2 (environmental means, Mean) and Table 3 (environmental variabilities, Cv). Table 4 is coordinately synthesized together dependent criteria with the environmental means and the environmental variabilities.

Tables 2, 3, and 4 present that some of the environmental variables are affecting almost all dependent criteria, although there are subtle differences between male and female.

Most of the inverted sequences of genes, and the other sequences, were influenced by such conditions, especially as MMT/90, MHT/90, MHU/90, and MHT/14 of the environmental means and CMT/90, CMT/14, and CRH/90 of the environmental variabilities. Therefore, it seems that for *D. melanogaster*, gene frequencies are particularly more related to the environmental means that influenced two months ago from the point of sampling time than to the other variables. And among all of the environmental factors we established, the temperature-related variables exhibited a tendency effective much more to change the frequencies of inverted chromosome sequences. In Table 4, the environmental variables were particularly concentrated to *In(3)LP* and *In(3)RP* of inverted sequences and 3L and 3R in the standard sequences. We could note that there are negative correlations between the inverted sequences and the standard sequences in both 2nd and 3rd chromosomes. Although a few dependent variables correlated insignificantly to each of the environmental variables at the 5% level, most of the regression coefficients of the finally selected independent variables show significant influence to the dependent variables. Therefore, it is considered that the less effective variables are cooperatively contributed with each other as polyvariable effects.

References: Mulley, J.C., J.W. James, and J.S.F. Barker 1979, Biochem. Genet. 17: 105-126; Rim, N.R., B.S. Lee, and T.H. Lee 1988, Dros. Inf. Serv. 67: 67-69.

Table 2. Regression analyses of gene frequencies with environmental means (MEAN).

	Inverted sequence							Standard sequence						No. of inversions			
Variables	*2Lt*	*2RNS*	*3LP*	*3RC*	*3Rmo*	*3RP*	FI		*2L*	*3R*	*2nd*	*3rd*	0	1	2	3
Male																
MMT/90							-6.35**				8.59	[illegible]	6.38**	-10.13*	-9.54**	
MHT/90	86.99*	2.98**	0.79**	0.38*		0.71**	6.65**	-81.99*	-3.10**	-0.81**	-0.62**	-74.94*	-6.76*	-6.68**	10.65*	9.32**	112.61*
MLT/90	-93.54*	-3.01*						88.13*	3.13**			71.90*					121.45*
MTR/90	-22.68*							21.39*				17.74*			-1.25*		-29.73*
MHU/90		1.01**					1.15**		-1.02**			-1.16**	-1.04*	-1.15**		1.55**	
MRH/90	-0.38*	0.44*	-0.67**		0.78**	-0.21	-0.30**	0.43**	-0.41**	0.67**			0.51**	0.30**	-0.16	-0.49**	-0.27
MLT/7			0.43**	-0.50**		-0.63**				0.44**	0.62**		1.54*			-1.96**	-0.41*
MWS/7			0.40**				-0.33**			-0.40**			-0.41**	-0.33**	0.19	0.31*	
MHT/14		0.32*			-0.33*				-0.32*			-0.33	-1.20			1.85*	
MHU/14					0.24										0.19		
Full R^2	0.69	0.85	0.76	0.52	0.65	0.76	0.88	0.71	0.85	0.76	0.72	0.85	0.81	0.88	0.87	0.77	0.62
P	0.01	0.00	0.00	0.13	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Female																	
MMT/90			-13.56**	-13.66		-8.20*	-17.76**			13.67**	7.59*	13.42*	19.24**	17.35**	-16.33*	-10.08**	-19.77*
MHT/90	0.74**	0.55**	12.60**			8.08*	17.56**	-0.75**	-0.54**	-12.70**	-7.52*	-76.71*		-17.18**	16.47**	9.56**	
MLT/90				14.68	1.22**							68.29	-19.97**				20.40*
MTR/90				2.11*			-1.54*					18.34	-3.93**	1.51*	-2.03**		3.35*
MHU/90	-0.54**	0.32*	2.53**	0.72	-0.64*	1.38*	1.04*			-2.54**	-1.32*		-2.22**	-1.03*		2.04**	1.38*
MRH/90		0.30*	-0.78**	-0.26	0.36*	-0.25	-0.37**	0.54**	-0.32*	0.77**	0.21	0.27*	0.53**	0.37**	-0.21	-0.56**[1]	-0.49**
MLT/7			-2.03**		2.35*	-1.59*			-0.32*	1.98**	1.47*		1.45*			-1.74*	-1.86*
MWS/7			0.55**		-0.47*					-0.55**			-0.30*			0.43**	
MHT/14			2.51**		-2.45*	1.65*	0.33			-2.48**	-1.53	-0.22	-1.87**	-0.32		2.01*	2.70**
MHU/14					-0.89*										0.31*		
Full R^2	0.72	0.75	0.84	0.66	0.78	0.78	0.83	0.74	0.75	0.83	0.79	0.77	0.85	0.84	0.83	0.76	0.67
P	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02

FI: frequencies of inversion-carrying flies.
P: probability
* < 0.05
** < 0.01

Table 3. Regression analyses of gene frequencies with environmental variablilites (Cv: coefficient of variation).

	Inverted sequence							Standard sequence						No. of inversions			
Variables	*2Lt*	*2RNS*	*3LP*	*3RC*	*3Rmo*	*3RP*	FI	*2L*	*2R*	*3L*	*3R*	*2nd*	*3rd*	0	1	2	3
Male																	
CMT/90		0.65	-0.44*	-0.51**		-0.73**	-0.97**			0.45*	0.72**		0.73**	0.97**	-0.81**	-0.71**	1.59*
CHT/90	-0.61*	-1.35**			-0.32			0.59**	0.72**			1.19**					-2.15**
CTR/90		0.16							-0.16								
CHU/90																	
CRH/90			-0.40*	-0.32	0.46**	-0.26	-0.37*			0.39*	0.25		0.36*	0.37*		-0.33*	
CMT/7																	
CRH/7	0.26						0.28*							-0.29*	0.25*		
CMT/14		0.72*		0.56**	0.25	0.63**	0.33*		-0.84**		-0.65**		-0.51**	-0.33*	0.68*	0.44*	
CRH/14		-0.25*							0.23*								
CMT/30		-0.89**							0.99*						-0.73*		0.29
Full R^2	0.41	0.85	0.42	0.51	0.47	0.67	0.72	0.38	0.87	0.43	0.69	0.76	0.64	0.72	0.81	0.57	0.59
P	0.36	0.00	0.35	0.15	0.23	0.01	0.00	0.47	0.00	0.32	0.01	0.00	0.02	0.00	0.00	0.07	0.05
Female																	
CMT/90	-0.57**	1.03	-0.44*	-0.91**		-1.12**	-0.92**	0.68**	-1.06	0.44*	1.08**		0.94**	0.92**	-1.24**	-0.58*	-0.40*
CHT/90		-1.50*							1.51*			0.62**					
CTR/90					-0.34												
CHU/90															0.36*		
CRH/90	-0.39*	0.30*	-0.37	-0.45*	0.52**	-0.56**	-0.43**	0.51**	-0.32*	0.36	0.45**		0.45**	0.43**	-0.53**	-0.36*	-0.29
CMT/7												0.31*			-0.29		
CRH/7				0.35	-0.37*	0.43**	0.40**	-0.27			-0.36**		-0.35*	-0.39**	0.51**		
CMT/14				-0.94													-0.36*
CRH/14																	
CMT/30		0.43**		1.20*		0.44**			0.45**		-0.40**		-0.24		0.54*		
Full R^2	0.55	0.74	0.43	0.58	0.54	0.83	0.76	0.59	0.75	0.41	0.78	0.71	0.68	0.76	0.86	0.46	0.57
P	0.11	0.00	0.36	0.08	0.12	0.00	0.00	0.07	0.00	0.40	0.00	0.01	0.01	0.00	0.00	0.29	0.09

FI: frequencies of inversion-carrying flies.
P: probability
* < 0.05
** < 0.01

Table 4. Regression analyses of gene frequencies with coordinated environmental variables (MEAN and Cv).

	Inverted sequence							Standard sequence						No. of inversions			
Variables	*2Lt*	*2RNS*	*3LP*	*3RC*	*3Rmo*	*3RP*	FI	*2L*	*2R*	*3L*	*3R*	*2nd*	*3rd*	0	1	2	3
Male																	
MMT/90	-2.22		-6.46	-20.61**			-7.75**	5.99*		5.66		8.49**	7.57*	7.81**	-8.38*	-9.54*	-10.08*
MHT/90	2.88*	2.98**	6.49*	12.09**		3.38*	7.71**	-6.26**	-3.10**	-5.98	-0.62**	-8.47**	-7.55**	-7.97**	6.08**	9.32*	9.58*
MLT/90		-3.01**		8.36		-3.37*			3.13**						3.09		
MHU/90		1.01**	1.10	1.03*		1.06*	1.34**	-0.82*	-1.02**	-0.96		-1.30*	-1.31*	-1.35**	0.45*	1.55*	1.75*
MRH/90	-0.39*	0.44*	-0.74**		0.73**	-0.23	-0.37**	0.45**	-0.41**	0.73**			0.50**	0.37**	-0.16	-0.49**	-0.34*
MLT/7			-1.68**			-2.76**	-1.50*			1.59*	0.62**		2.63**	1.50*		-1.96**	-3.42**
MWS/7			0.465**			0.40*	0.43**	-0.26		-0.46**			-0.52**	-0.44**	0.33**	0.31*	0.57**
MHT/14		0.32*	1.59		-0.24	1.69*	1.44**		-0.33*	-1.44		-0.39*	-1.86*	-1.45*		1.85*	2.42*
CMT/7						-0.59							0.50				-1.00*
CRH/7						0.31	0.19						-0.24	-0.19	0.21*		0.43
Full R^2	0.64	0.83	0.78	0.53	0.64	0.79	0.88	0.67	0.83	0.77	0.75	0.82	0.84	0.88	0.90	0.77	0.66
P	0.02	0.00	0.00	0.11	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Female																	
MMT/90			-16.27**	-13.56	-4.54	-7.64*	-17.98**			16.41**	14.67*	10.08	19.98**	17.45**	-12.26*	-13.10**	-20.53*
MHT/90	0.74**	0.55*	15.01**	7.85		7.54*	13.37**	-0.75**	-0.54**	-15.13**	-10.55*	-5.92*	-15.93**	-13.01**	6.90*	12.30**	13.03*
MLT/90				6.07	5.75*		4.49				-4.11	-4.97	-3.32	-4.34	6.23*		7.07
MHU/90			2.95**	0.70	-1.18**	1.35**	1.65**			-2.98**	-1.32*		-2.51**	-1.63**		2.39**	1.38*
MRH/90	-0.54**	0.32*	-0.78**	-0.26	0.35*	-0.23	-0.41**	0.54**	-0.32*	0.76**	0.23	0.23	0.52**	0.40**	-0.23	-0.57**	-0.50**
MLT/7		0.30*	-3.44**			-2.00**	-1.63		-0.32*	3.43**	1.78*		2.65**	1.57		-2.66**	-1.90*
MWS/7			0.69**				0.24			-0.69**			-0.41**	-0.25		0.47**	
MHT/14			3.36**		-0.62*	2.06*	1.59			-3.35**	-1.90*		-2.65**	-1.53		2.31**	2.74**
CMT/7			-0.68*				-0.45			0.69*		0.26	0.49	0.43		-0.73*	
CRH/7			0.26*			0.22	0.24			-0.27*	-0.19		-0.25*	-0.23	0.26*		
Full R^2	0.71	0.72	0.90	0.68	0.63	0.83	0.85	0.74	0.73	0.89	0.82	0.74	0.89	0.85	0.81	0.82	0.71
P	0.01	0.01	0.00	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01

FI: frequencies of inversion-carrying flies.
P: probability
* < 0.05
** < 0.01

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Sensory projections from the peripheral labellar hairs of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae).

We have been interested in the anatomical and functional aspects of chemosensory systems of *Drosophila melanogaster* for the last several years (Nayak and Singh, 1983; Stocker and Singh, 1983; Stocker *et al.*, 1983; Venkatesh and Singh, 1984; Singh and Singh, 1984; Singh and Nayak, 1985).

In adult *Drosophila*, gustatory sensilla are mainly located on the proboscis, legs, anterior margin of the wing and in the pharynx (Wildermuth and Hadorn, 1965; Palka *et al.*, 1979; Nayak and Singh, 1983). Taste hairs are arranged on the labellar lobes of proboscis roughly in three rows: a peripheral and two medial rows (Falk *et al.*, 1976). Hairs in the medial rows contain 1 mechanosensory + 4 chemosensory neurons while hairs of the peripheral row contain 1 mechanosensory + 2 chemosensory neurons (Nayak and Singh, 1983). Golgi silver impregnation studies of the labellar sensilla of adult *Drosophila*, revealed mainly seven types of sensory fibres: type I coiled fibre, type II shrubby fibre, type III ipsilateral ventral fibre, type IV ipsilateral dorsal fibre, type V contralateral ventral fibre, type VI contralateral dorsal fibre and type VII central fibre, all projecting to the ventral and middle regions of the suboesophageal ganglion (SOG) (Nayak and Singh, 1985). These sensory terminals were characterized according to the morphology of arborizations and the region of volume of the SOG occupied by the arborizations.

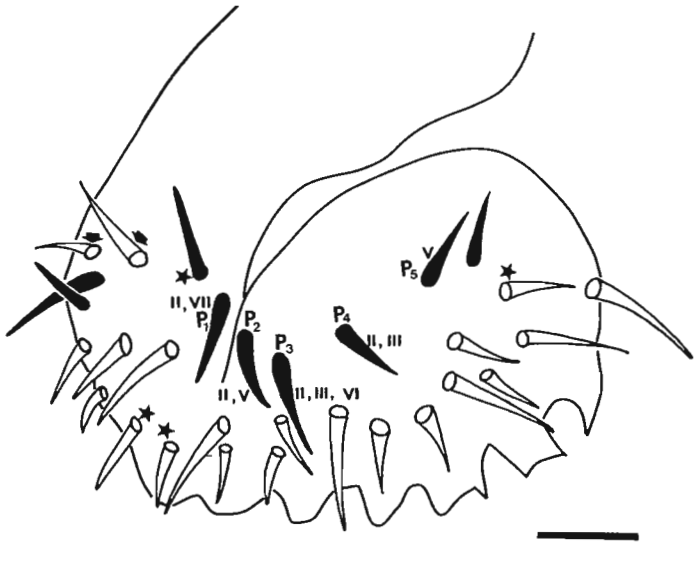

Figure 1. Diagram of the labellum, showing position and neuronal composition of various sensilla. Sagittal view dorsal on top. Unshaded = 4CS+1M; Solid filled = 2CS+1M; Marked stars = variable composition, either 2-4CS = 1M; marked arrow = composition not known. CS = chemosensory neuron/s; M = mechanosensory neuron. Scale = 25μm

Our subsequent studies using horseradish peroxidase (HRP) as a neuronal marker showed that an individual taste hair in the medial rows on the proboscis has types I, II, IV and VI neurons, which project to the distinct regions of the SOG (Shanbhag and Singh, 1989). Their functional roles could also be inferred from the selective uptake of HRP from solutions containing different solutes such as sucrose or sodium chloride. The putative roles assigned were type I coiled fibre corresponding to water sensing neuron, type II shrubby fibre as mechanosensory neuron, type IV ipsilateral dorsal fibre as sugar or low concentration of sodium chloride sensing neuron, and type VI contralateral dorsal fibre corresponding to potassium chloride or high concentration (> 0.5 M) of sodium chloride sensing neuron (Shanbhag and Singh, 1992). Similar selective uptake of neuronal marker cobalt (II) was subsequently reported for Type IV and type VI neurons which are likely to represent neurons eliciting attraction and repulsion responses, respectively, when the fly is subjected to the classical proboscis extension response (PER) test (Dethier, 1976).

Electrophysiology had earlier shown the presence of one neuron sensing sugar, another sensing water, and two salt sensing neurons in similar taste hairs (Siddiqi and Rodrigues, 1980; Zacharuk, 1980; Rodrigues and Siddiqi, 1981; Arora *et al.*, 1987). However, the peripheral taste hairs on the proboscis have received less attention. Therefore, we decided to study these peripheral hairs, in the hope that we may be able to identify types III, V and VII fibres, which were functionally not characterized until now. Cobalt-lysine solutions were used to label neurons in the undamaged peripheral hairs P_1-P_5 on the labellum (Figure 1). As expected, the fibre types III, V and VII were found in these hairs.

For these studies 3 to 5 day old *D. melanogaster* Canton S female flies were used. A microcapillary of glass filled with 370 mM cobaltous lysine containing 1% Triton X 100 was capped over undamaged tip of taste hair and kept overnight at 5°C (Yetman and Pollack, 1986). Subsequently, flies were processed and silver intensified according to

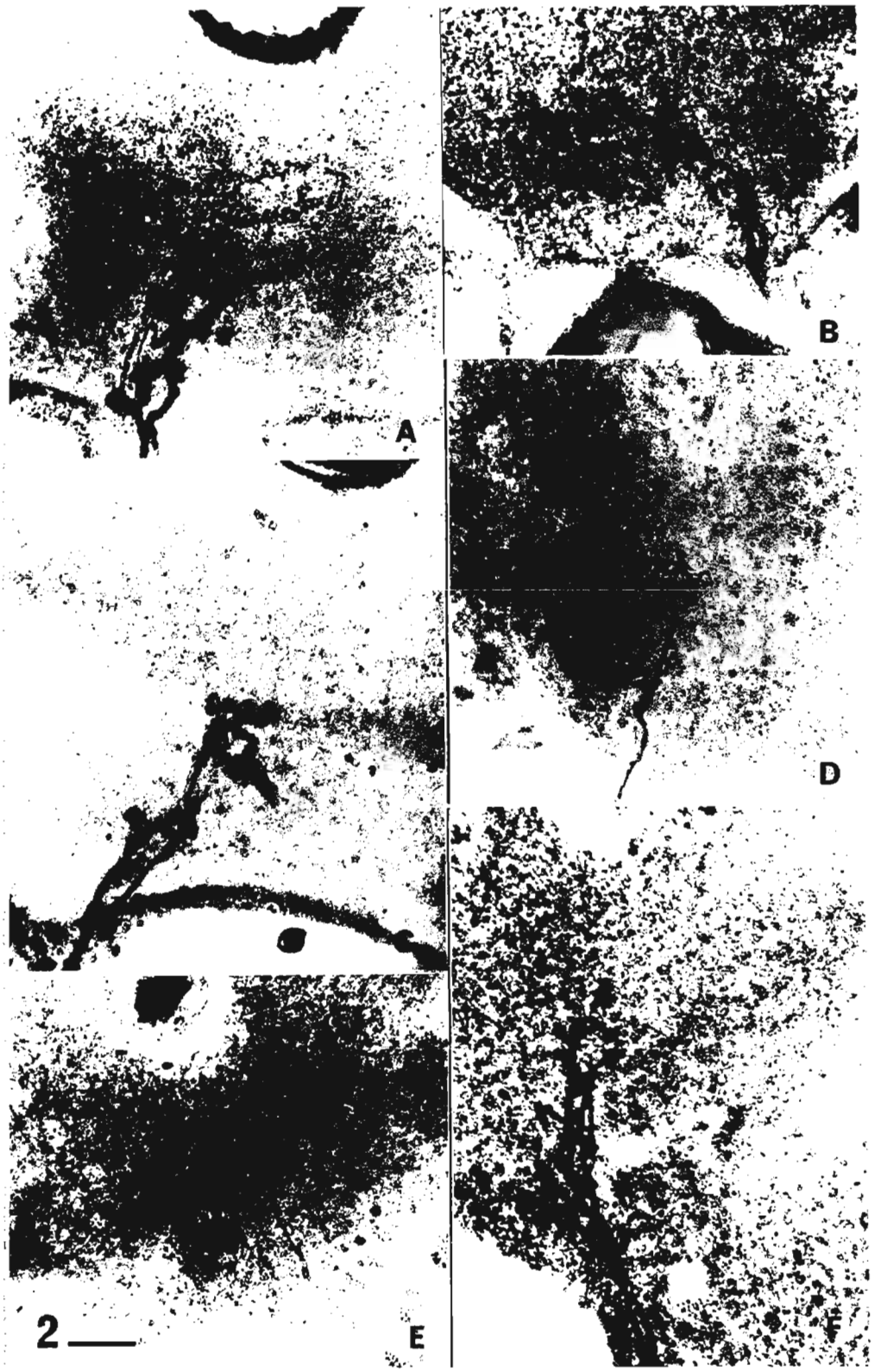

Figure 2. Photomicrograph of neuron labelled by Co(II) uptake present in individual labellar sensory hair of *D.melanogaster*. Dorsal on top. A, Hair P3 - type II, III and VI; B, Hair P2 - type II and V; C, Hair P1 - type II and VII; D, Hair P4 - type II and III; E, Hair P5 - type V; F, Hair P1 - II and IV (0.1M sucrose + cobalt II). Scale = 25μm.

(Bacon and Altman, 1977). The specimens were dehydrated in a graded series of ethanol embedded in Durcupan ACM (Fluka) through intermediate propylene oxide steps and polymerised at 60°C overnight (Nayak and Singh, 1983). Twenty µm thick sections were cut on a sliding microtome fitted with a steel knife, in frontal or in sagittal planes and mounted with Permount under glass cover slip. The success rate of such labelling was approximately 20% and approximately 100 successful preparations were used for analysis.

Photographs were taken on a Zeiss RS III photomicroscope. Diagrams of silver impregnated fibres were reconstructed on Leitz-Vario-Orthomat 2 photomicroscope with plan-apo objectives and a camera lucida attachment.

Sensory neurons on the proboscis project to the brain via labial nerve (Miller, 1950; Nayak and Singh, 1983). When 5 peripheral hairs P_1-P_5 were capped with cobaltous lysine and Triton X 100, most of the successful preparations showed two types of sensory fibres. These fibres occupied ventrolateral (VL) and ventromedial (VM) regions of SOG. When cobalt was mixed with 0.1M sucrose or 0.1M sodium chloride we find that the fibre projects to the same region. Most of the hairs on the labellum show one or two neurons stained by cobalt(II) uptake. Out of the two neurons stained, one is mostly mechanosensory. We have classified these fibres on the basis of their morphology and position of arborizations in the SOG according to the nomenclature used by Nayak and Singh (1983) and Nayak and Singh (1985). In the peripheral hairs we have seen that types III, V and VII are stained. These types of arborizations are specific for peripheral hairs only.

Peripheral hair P1 on the labellum: cobalt-lysine labels two types of fibres of P1 hair. One is thick and dark, resembling type II shrubby fibre and the other thin, faintly staining fibre projecting to the central most region of SOG is type VII fibre (Figures 2C, 3C).

Peripheral hair P2: Two types of fibres are labelled in this hair, the type II shrubby fibre and the type V contralateral ventral fibre (Figures 2B, 3B).

Peripheral hair P3: Two or three types of fibres are labelled in this hair. One is darkly staining type II fibre, while others are weakly staining types VI and III fibres (Figures 2A, 3A).

Peripheral hair P4: Two types of fibres are stained. One darkly stained being type II fibre and the other is faintly stained type III ipsilateral ventral fibre (Figure 2D).

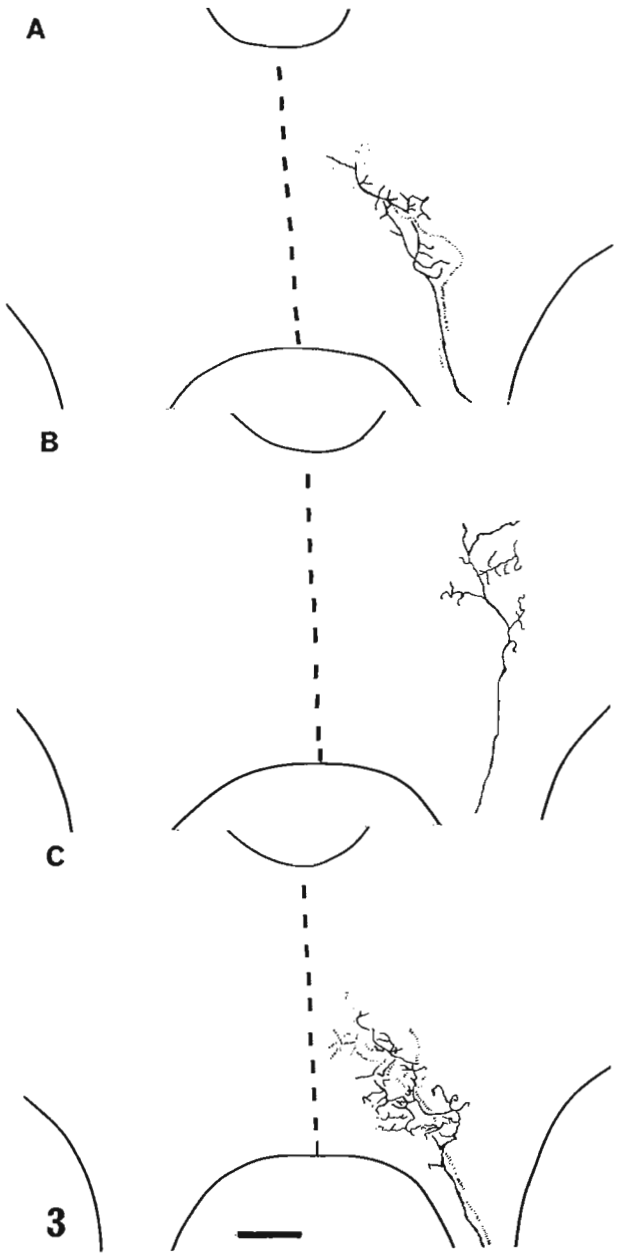

Figure 3. Diagrammatic reconstruction of cobaltous (II) stained neuron of labellar sensory hairs of *D. melanogaster*. Dorsal on top. A, Type III; B, Type V; C, Type VII. Scale = 50µm.

Peripheral hair P5: On cobalt staining, this hair shows only one type of arborizations and they belong to type V contralateral ventral fibre (Figure 1E).

We have also used cobaltous lysine mixed with 0.1M sucrose, 0.1 or 0.6M sodium chloride. With lower concentration of sodium chloride and sugar mixer, which elicits attraction response by the fly, as expected type IV fibre is visualized (Figure 2F). But at the higher concentration of sodium chloride, which evokes repulsion response in the fly, type III fibre was labelled with cobalt (II).

Gustatory hairs on the labellum of *D. melanogaster* in the two medial rows are morphologically quite distinct from those of the peripheral row. Medial row hairs have bifurcated tips with a hole on each tip. Dendrites innervate only one arm of the Y-shaped prong of the tip, the other arm is extension of the crescentic lumen filled with sensillar lymph (Nayak and Singh, 1983), whereas the peripheral hairs have a single shaft with a hole at the tip (Falk et al., 1976). In addition, the neuronal composition of medial and peripheral row sensilla is different, the former usually has 1 mechanosensory + 4 chemosensory neurons and the latter has 1 mechanosensory + 2 chemosensory neurons respectively, except a few variations (Falk et al., 1976; Nayak and Singh, 1983).

These experiments show that the peripheral hairs on the labellum have different set of neurons such as types III, V, and VII which are not present in the medial row sensilla. Only type II mechanosensory neuron and type IV attraction eliciting neuron are common to both categories.

Acknowledgments: We thank Dr. Shubha Shanbhag, Mrs. Kusum Singh, and M.P. Pitale for helpful suggestions.

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Wenk, María José, and Raúl Godoy-Herrera. Departamento de Biología Celular y Genética, Facultad de Medicina, Universidad de Chile, Casilla 70061, Santiago, Chile. Initial behaviors of *Drosophila gaucha* in the absence of food, water, and conspecifics.

Initial changes in the expression of the activity of an individual subjected to a new environment are called reactivity (O'Dell and Burnet, 1988). The magnitude of reactivity would reflect individual responses to new environments (Connolly, 1967). In the *Drosophila* genus, most studies on locomotor activity and reactivity have concentrated on the cosmopolitan species *Drosophila melanogaster*, while very little is known about the behaviors of other species of *Drosophila* in restricted environments (Ashburner, 1989). Investigations on initial behavioral responses of *Drosophila* adults in poor environments may contribute to understanding how behavior helps animals to adjust to changing environments thus preventing extinction (Hoffmann and Parsons, 1991). Which are the immediate behaviors of individuals of an endemic species of *Drosophila* when transferred to poor living conditions? To explore this problem initial behaviors of *Drosophila gaucha* flies in environments with limited social and physical ecological resources were measured. These consisted of: i) Petri dishes without food, water, and conspecifics, and ii) bottles with food and water but without conspecifics.

D. gaucha Jaeger and Salzano is a neotropical, endemic species belonging to the *mesophragmatica* group (Brncic, 1957), which lives in Southern Brazil and Central-West Argentina. A stock of flies collected in Capoes, Brazil, was used. Flies were taken 8 days after post-hatching, thus ensuring virginity of males and females (Koref-Santibañez and del Solar, 1961). After being collected, 30 flies of both sexes were deposited in bottles with food. Forty eight hours before starting the experiments they were sexed, and thereafter the sexes were kept separately at 18°C. When the flies were 12 days old individual males were gently introduced into an empty Petri dish; after 5 sec allowed for settling down their behavior was recorded every 3 sec for up to 26 sec. The same procedure was followed for females. Both sexes were tested in parallel at the same time of the day.

In a further experiment, individual males and females were placed in half-pint bottles with about 30 ml of food (Burdick, 1954), and their behavior was also observed every 3 sec for a period of 26 sec. Again, before observing their behaviors the flies were allowed to settle down for 5 sec.

Once the behavior of a fly was recorded, the Petri dish was washed and dried before introducing a new individual. The bottles with food were also used once per fly and then discarded. One hundred and forty five flies of each sex were measured in the Petri dishes and 60 in the bottles with food.

Individual registers were performed for each of the flies tested by recording their positions in the Petri dish every 3 sec. The same procedure was followed for the flies introduced into bottles. The behaviors recorded are listed in Table 1. Locomotion means translation from one place to another; it was estimated by comparing the position of the fly in each observational interval with respect to its previously recorded position. Turning means rotation in space. A turning activity was recorded when a fly changed its direction with respect to the previous position recorded. An event of jumping was registered when a fly jumped from one wall to another or from one place to other on the same wall. Sometimes the flies fell over on their back shaking legs for a few sec. However, they rapidly recovered and went on moving exploring the environment. Some flies shook their abdomen without falling over; this type of movement reminds one of seizure-like movements. Flies were also observed to clean their legs, wings and antennas with their forelegs (grooming or preening; Connolly, 1967).

The behaviors observed in the Petri dishes and in bottles were recorded as a sequence of discrete events of activity. In Table 1 the behaviors are expressed as a percentage of the total number of behavioral events recorded during 26 sec. In the Petri dishes the total number of behavioral events were as follows: i) males = 1127 ($\bar{x} \pm$ S.E = 7.77 $\pm$ 0.44); females = 1120 ($\bar{x} \pm$ S.E. = 7.72 $\pm$ 0.85); in the bottles: i) males = 491 ($\bar{x} \pm$ S.E. = 8.18 $\pm$ 0.23), females = 496 ($\bar{x} \pm$ S.E. = 8.30 $\pm$ 0.72). These means of activity are not significant (t test, df = 203). Thus, in the Petri dishes and bottles males and females show a very similar number of events of activity.

Table 1 also shows that in the Petri dishes grooming is the most frequent behavior in both sexes, while the less frequent is jumping. In the bottles, locomotion and grooming are frequent in both sexes, while the less frequent behavior is falling over. Some of the females exhibit seizure-like movements in both environments; these are not observed in males. Sometimes the flies did not move (standing).

Table 1. Percentages ± S.E. per individual of initial behaviors exhibited by virgin males and females of *D. gaucha* during 26 sec. Individual flies were observed in isolation in a Petri dish without food and conspecifics, and in a bottle with fold but without other flies. Number of flies were: (i) Petri dishes = 145 per sex, (ii) bottles = 60 per sex.

	Percentage per individual			
	Males		Females	
Behavior	Petri dishes	Bottles	Petri dishes	Bottles
Locomotion	32.30 ± 2.11	37.48 ± 2.30	37.91 ± 1.82	35.08 ± 2.75
Turning	4.44 ±1.32	4.89 ± 2.43	8.94 ± 1.23	3.43 ± 1.73
Jumping	1.60 ± 0.37	7.74 ± 1.39	2.74 ± 1.05	11.90 ± 2.05
Falling over	3.11 ± 1.78	2.44 ± 0.44	4.11 ± 0.92	2.42 ± 1.01
Grooming	54.94 ± 1.21	30.94 ± 3.24	51.01 ± 1.63	38.10 ± 3.72
Standing	3.82 ±1.18	3.67 ± 0.73	9.45 ±1.48	4.23 ± 1.05
Seizure-like movements	—	—	2.85 ± 0.13	4.84 ± 0.91

The frequency of each behavior depends on the sex and the environment in which the flies are observed. In the Petri dishes the amount of locomotion of *D. gaucha* females is greater than that of males (t test = 2.01, $p < 0.05$, df = 288); turning behavior is also more frequent in the females than in the males (t test = 2.50, $p < 0.05$, df = 288); females stand more than do males (t test = 2.97, $p < 0.05$, df = 288). However, the percentage of jumping, falling over and grooming is similar in both sexes (t test, df = 288).

In a bottle with food, both sexes of *D. gaucha* show similar behaviors, with the exception of the percentage of grooming which is higher in females (t test = 3.18, $p < 0.05$, df = 188).

In the males, the percentages of locomotion, turning, falling over and standing in the Petri dishes are similar to those calculated for the bottles (t test = 1.66, 0.16, 0.37, and 0.45, respectively, df = 203). Jumping increases in bottles, while grooming decreases (Table 1; t test = 4.27, and 6.88, respectively, df = 203). *D. gaucha* females show similar locomotion and falling over in the Petri dishes and bottles (t test = 0.49, and 1.24, df = 203). However, jumping and seizures increase in the bottles (t test = 3.95, and 2.16, respectively, $p < 0.05$, df = 203), while turning, grooming and standing behaviors decrease (t test = 2.60, 3.18, and 2.16, respectively, $p < 0.05$, df = 203).

In poor environments, the number of initial behavioral events of *D. gaucha* is the same for males and females, but the frequency of a particular behavior depends on the sex of the flies. For example, only females exhibited seizure.

In comparison with the Petri dishes, in the bottles males and females of *D. gaucha* increased frequencies of jumping; the females also increased seizures (spontaneous activity). An increase in jumping could be in connection with space available which is greater in the bottles than in the Petri dishes. Jumping behavior could merely reflect aborted attempts of flying. On the other hand, as a by-product, seizure and grooming movements could produce slight air currents

inside the Petri dishes and bottles helping to spread female pheromones. In the sibling species *D. pavani* these substances seem to attract conspecifics of both sexes (Godoy-Herrera and Fenner, 1984). Table 1 also suggests that poor living conditions seem to affect female initial behaviors more deeply than male ones. It is important to keep in mind that the bottles offer more food and space for the flies than do the Petri dishes. Thus behavioral changes observed in the bottles could be produced by food, a greater space or a combination of these two factors.

Initial behaviors of *D. gaucha* adult flies in a restricted environment may be divided into two behavioral systems: i) those involved in exploring the environment (locomotion, turning and standing), and ii) those that we presume correspond to spontaneous activity (jumping, falling over, grooming and seizure). It is thought that these behaviors may be produced by an intrinsic instability of the central nervous system (Burnet *et al.*, 1974). Alternatively, they may reflect the central excitatory state of the brain of *D. gaucha* flies (Hay, 1985).

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David, J.R., B. Moreteau, P. Gibert, J.P. Morin, and G. Pétavy. CNRS - UPR 9034, Laboratoire Populations, Génétique et Evolution, 91198 Gif-sur-Yvette Cedex, France. Drosophilids from an arid Afrotropical country, the Republic of the Niger: faunistics and phenotypic plasticity.

With a few exceptions (*e.g.*, cactophilic desert species) most drosophilids are heat and desiccation sensitive. The highest species diversity is found in rainforests of various continents. How the number of species decreases when the climate becomes more arid and sometimes warmer is poorly documented. African countries of the Sahel region, south of Sahara desert, offer a convenient opportunity to investigate this problem, and we present here an ecological analysis from Niamey, the capital of the Republic of Niger.

The present survey was done in July 1995, *i.e.* at the beginning of the rainy season, and several rains occurred during the collection period. Maximum daily temperature was 37-38°C in sunny days, but less in rainy days (31-32°C). During the night, the temperature decreased to about 25°C but could be lower (22°C) in case of rain. Meteorological records indicate for July an average maximum of 34°C and an average minimum of 24°C. The daily average is therefore less than 30°C, close to 29°C. The period of the present survey was clearly in agreement with usual average climatic conditions in July.

Drosophila collections were made either by using attractive banana traps or by sweeping with a net over fallen resources, mostly mangoes and citrus. A species living in gombo flowers (*Hibiscus esculentus*) was collected by aspiration. Different habitats were prospected ranging from artificial, human constructions (a brewery and a grocery in Niamey) to city gardens and more natural sites like orchards. Two orchards were located along the Niger river and were quite humid. Orchards and gardens were covered with trees providing shadow and cool microclimatic conditions. Although adult flies were abundant in all these places, few larval breeding sites were identified.

Faunistic observations: Collection data are given in Table 1. Altogether 12 species have been found, for a total of 2606 individuals. Three species were clearly dominant, representing more than 92% of the community. *D. melanogaster*, the well-known cosmopolitan species, was present in all habitats (except flowers) and as usual, much more abundant in buildings and domestic habitats. The two *Zaprionus* are also widespread species for which evidence exists of recent human introduction in remote oceanic islands such as Seychelles, Mauritius or Canary islands. Moreover *Z. indianus* also exists in India from where it was first described. *Zaprionus* individuals were not found in the brewery, presumably because of the reluctance of these flies to enter human constructions (David, 1979). In the grocery, the repellent effect of the building on *Zaprionus* was also clear, as compared with the abundance of *D. melanogaster*. *ScaptoDrosophila latifasciaeformis* was quite abundant in garden and orchards. This species is probably native to tropical Africa. *D. yakuba*, belonging to the *D. melanogaster* subgroup, is widespread in tropical Africa but was quite rare in Niamey. It was presumably already found from Niger republic, but misidentified as *D. teissieri* (Tsacas *et al.*, 1981). *D. malerkotliana* is an Asiatic species which has been introduced in many tropical countries including Africa and America.

ScaptoDrosophila aterrima is a small flower breeding species, widespread in Africa and for which some evidence exists of human transportation (Tsacas *et al.*, 1988). The last five species were represented by 16 individuals. *Leucophenga dudai* was identified by Dr. L. Tsacas. The four others are typical Afrotropical flies which are abundant in more humid countries (Tsacas *et al.*, 1981).

Table 1. Faunistic and ecological observations (number of flies) in different habitats from Niamey and suburbs. Marked species are known to be transported by man. *Z.: Zaprionus, D.: Drosophila, S.: Scaptodrosophila, L.: Leucophenga.*

Species	Niamey City				Suburbs				Total
	brewery (Braniger)	grocery	garden 1	garden 2	orchard 1	orchard 2	orchard 3	gombo flowers	
Z. indianus *		12	242	43	273	73	455		1098
Z. tuberculatus *		35	94	16	474	7	62		688
D. melanogaster *	170	306	48	92	23	4	17		660
S. latifasciaeformis *			39	18	27	18	19		121
D. yakuba			4		24		3		31
D. malerkotliana *		2	6	3	4		2		17
S. aterrima *								15	15
D. greeni				1	1		3		5
S. mokonfim					4		1		5
Z. ghesquierei					2		1		3
L. dudai							2		2
D. fima					1				1

Table 2. Results of a morphological analysis of size characters of wild living flies. n: number of individuals; m ± s.e.: mean (in mm x 100) ± standard error; CV: coefficient of variation; W/T: wing/thorax ratio.

Species		n	Wing length		Thorax length		W/T ratio		correlation
			M	CV	M	CV	M	CV	
D. melanogaster	♀	110	222.44 ± 1.31	6.18	93.18 ± 0.66	7.43	2.391 ± 0.008	3.32	0.90
	♂	100	192.64 ± 1.19	6.15	79.83 ± 0.62	7.71	2.417 ± 0.008	3.18	0.92
Z. indianus	♀	100	254.96 ± 2.00	7.86	115.86 ± 1.06	9.19	2.204 ± 0.006	2.63	0.97
	♂	100	244.52 ± 2.13	8.72	110.76 ± 1.14	10.26	2.212 ± 0.006	2.77	0.97
Z. tuberculatus	♀	110	258.00 ± 1.83	7.44	117.42 ± 0.99	8.87	2.201 ± 0.006	2.73	0.96
	♂	100	242.32 ± 1.65	6.79	110.36 ± 0.95	8.64	2.200 ± 0.006	2.78	0.97

Up to now, only five drosophilids were known from Niger (Tsacas *et al.*, 1981). All these species were collected again in the present survey, and seven more have been discovered. We may conclude that the drosophilid fauna of the Republic of the Niger is depauperate, as expected from the arid climate. By comparison, 138 species have been found in Ivory Coast, a more humid country south of the Republic of Niger. Species in Niamey are expected to have developed some adaptive processes for tolerating heat and desiccation.

Phenotypic variability of the 3 most abundant species: Wild living *Drosophila* are known to exhibit a high level of phenotypic variability (David *et al.*, 1980; Coyne and Beecham, 1987; Moreteau *et al.*, 1995), and such variations are mostly due to plasticity, *i.e.* to an heterogeneity of larval feeding conditions and also to variations in developmental temperature. Samples of at least 100 flies of each sex of the three dominant species were studied. In all cases, wing and thorax length were measured. Moreover, for *D. melanogaster*, thoracic and female abdomen pigmentations were also analysed. Flies were collected in different habitats. ANOVA (not shown) failed to evidence any significant effect of the habitats, and thus the data were pooled into a single sample. For the thorax, the average pigmentation of the thoracic trident (see David *et al.*, 1985) was 0.10 ± 0.03 in females and 0.15 ± 0.02 in males. In other words, very few individuals exhibited a visible trident, as could be expected from a population living in a warm and arid climate (David *et al.*, 1985). For the abdomen pigmentation of females, on the other hand, the average pigmentation of the last three segments was 10.14 ± 0.38. This value appears to be higher than in European population living in a temperate environment (David *et al.*, 1990; Moreteau *et al.*, 1995). The difference is especially striking if we consider that flies in Niamey developed at a high temperature, close to 28-29°C. This result apparently does not match the adaptive hypothesis according to which lighter flies are selected in warmer climates (David *et al.*, 1990) and further investigations are needed.

Average values of size traits are given in Table 2. A first general observation concerns the high level of between individual variability, as shown by the coefficients of variation, always superior to 6% and sometimes reaching 10%. This confirms the heterogeneity of developmental conditions in nature. There is some evidence that *Zaprionus* individuals

could be even more variable than *D. melanogaster*. Also, in all cases, variability was higher for the thorax than for wing length. Finally the wing/thorax ratio is much less variable than the traits, and this is due to the positive correlation between them (Table 2).

D. melanogaster values were lower than those observed in natural European populations (Moreteau *et al.*, 1995) and this presumably has two origins: first the developmental temperature was higher, but also the genetic size is less, according to well-known latitudinal clines (Capy *et al.*, 1993). *Zaprionus* were never investigated so that we lack comparative data. Length values were higher than in *D. melanogaster*, an indication of a bigger genetic size. On the other hand, the wing/thorax ratios were similar but much less than in *D. melanogaster*. A last observation concerns the sexual dimorphism, expressed by the female/male ratio for wing or thorax length. Average values are given below:

	Wing length	Thorax length
D. melanogaster	1.156	1.167
Z. indianus	1.040	1.046
Z. tuberculatus	1.064	1.064

In *D. melanogaster*, the dimorphism is quite high and the values found in nature are similar to those found in laboratory experiments (David *et al.*, 1994). In *Zaprionus*, on the other hand, the dimorphism is much less, *i.e.* male are almost as big as females. Important differences are found between *D. melanogaster* and *Zaprionus* concerning the wing/thorax ratio, and the sex dimorphism. This illustrates different evolutionary strategies in the Drosophilid family and a need for more extensive comparative studies.

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Hasson, Esteban, Juan C. Vilardi, Antonio Fontdevila. [1]G.I.B.E, Depto. Cs. Biológicas, Fac. Cs. Exactas y Naturales, Universidad de Buenos Aires. 1428 Buenos Aires, Argentina. [2]Depto. Genética y Microbiología, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona. Spain. Long term variation of inversion frequencies in a natural population of *Drosophila buzzatii* from Argentina.

Introduction: Evidence for the adaptive basis of *Drosophila* inversion polymorphims in natural populations by means of direct methods (sensu Endler, 1986) are scarce. Recently, we have demonstrated the adaptive significance of the chromosomal polymorphism in the Arroyo Escobar population (Argentina) of the cactophilic species *Drosophila buzzatii* by means of selection components analysis (SCA) (Hasson *et al.*, 1991). This method involves the detection of natural selection through the changes of inversion frequencies between successive stages during one generation by sampling as many life cycle stages as possible. However, an underlying assumption of the method employed, for the detection of larval and adult (longevity) viabilities, is that the population is stable and no significant changes in inversion frequencies occur in successive generations at the same life stage (see Ruiz *et al.*, 1986).In this paper, we report the results of a long term study of the inversion polymorphism in a *D. buzzatii* natural population. **Materials and Methods**: The population analyzed (Arroyo Escobar) is situated approximately 30 km North of Buenos Aires (Argentina) (see Hasson *et al.*, 1991 for a detailed description of the population). In this locality *D. buzzatii* breeds and feeds on the rotting cladodes of *Opuntia vulgaris* (Hasson *et al.*, 1992). This site has been regularly sampled from December 1979 to March 1989 (Table 1). Inversion frequencies were estimated in November 1986 and March 1989 from the offspring of wild males collected with banana baits and individually crossed to females of a laboratory homokaryotypic stock. For the remaining samples inversion frequencies were estimated by the cytological analysis of one larva from the offspring of each wild female collected. Simultaneous samples have shown non-significant differences between estimates of inversion frequencies obtained by these two methods (Hasson *et al.*, 1991).

Polytene chromosome slides were obtained following the technique described by Fontdevila *et al.* (1981). Data were analyzed by means of regression analysis of inversion frequencies as a function of time (months). As the number of chromosomes analyzed in each sample varied widely, standardized inversion frequency deviations (Christiansen *et al.*, 1976) were used in the regression analysis. For each sample and arrangement these deviations were estimated as: $(p_i - p_o) / \sqrt{(2N_i / p_o (1 - p_o))}$, where p_i is the frequency of one arrangement in sample i and $(1- p_i)$ is the frequency of the other arrangements, p_o and $(1 - p_o)$ are the frequencies in the total of all samples and $2N_i$ is the number of chromosomes analyzed in each sample.

Results and Discussion: The chromosomal arrangements in the population sampled and their frequencies estimated, during a ten-year period, are listed in Table 1. From the initial record on December 1979 (Fontdevila *et al.*, 1981) the frequency of 2st (= standard) decreased from 0.3 to about 0.1. The frequency variation of each second chromosome arrangement was studied by means of regressions on time (in months) (Figure 1). The regression coefficient for 2st was negative and highly significant ($F_{1,6} = 13.86$, $p = 0.0098$) indicating that its frequency has been decreasing ($y = 5.855 - 0.083x$, where y is the standardized frequency deviation at time x) during the period considered (Figure 1). Conversely, the regression for 2j was positive and highly significant ($F_{1,6} = 25.39$, $p = 0.0024$) ($y = -5.052 + 0.069x$). The frequency of arrangement $2jz^3$ only showed non-significant ($F_{1,6} = 0.12$, $p = 0.74$) small random changes. Arrangements $2jq^7$ and 4s remained at negligible frequencies. These temporal patterns of variation for 2st and 2j may be considered as indicative of directional selection.

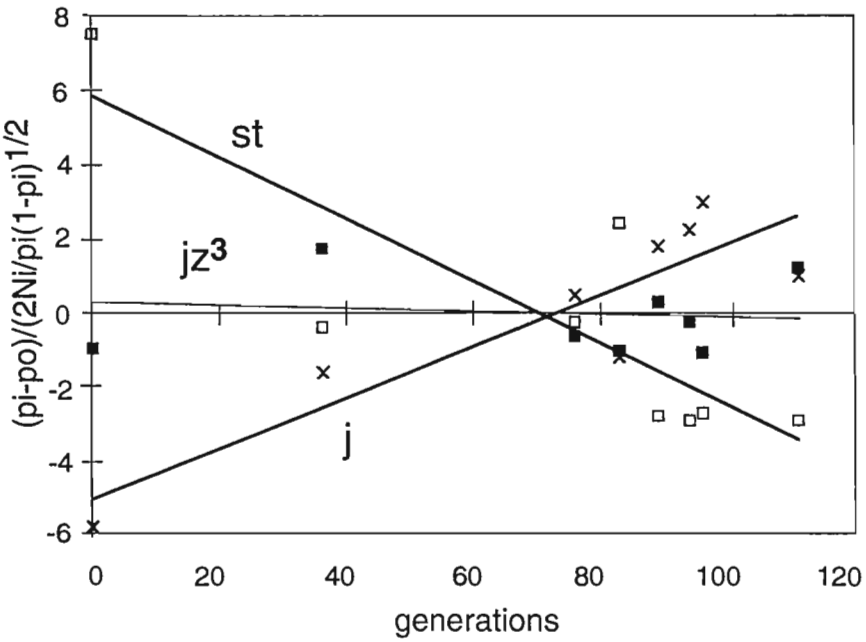

Figure 1. Plot of standardized deviations $(p_i - p_o) / \sqrt{(2N_i / p_o (1 - p_o))}$ of second chromosome inversion frequencies in function of time (months) (see text for explanation). st, open squares; j, crosses; jz^3, filled squares.

In our previous work we have shown the occurrence of endocyclic selection in the population of Arroyo Escobar (Hasson *et al.*, 1991). In the sample of third instar larvae the frequency of 2st was significantly lower than in the sample of immature adults emerged from the natural substrates and the reverse picture was observed for arrangement 2j, indicating that pupal viability is higher for 2st carriers. In addition, it was also shown that females carrying the 2j arrangement have a decreased fecundity. Finally, the significant decrease of the frequency of 2st observed when the samples of immature and mature adults were compared, led to the conclusion that it impairs longevity. However, the estimation of this latter fitness component is based on the assumption that inversion frequencies do not change between successive generations (Ruiz *et al.*, 1986).

According to the present results, temporal stability can be accepted for $2jz^3$, which showed a significant longevity effect in our selection component analysis (Hasson *et al.*, 1991). On the other hand, the longevity effect detected against 2st might be an overestimation. Yet, the frequency change per generation for 2st, when the regression coefficient is calculated with non-transformed frequencies, is more than one order of magnitude lower than the change between the mature and immature adult stages and consequently such overestimation may be considered minimal.

Those antagonistic effects observed by Hasson *et al.* (1991) seem to be occurring simultaneously with the temporal pattern reported in the present paper. A possible explanation for the long term trends is that the antagonistic effects are not completely balanced, so that 2j is favoured at the expense of 2st. Recent collections in South American

natural populations revealed macrogeographic patterns of variation in the inversion polymorphism of *D. buzzatii* (Hasson *et al.*, 1995). The observation of negative latitudinal clinal variation for 2st suggests that its reduced frequency in this Southern population could result, at least partially, as a response to the different selective pressures accompanying the environmental changes. Host shifts have been proposed to account for the differences detected by SCA between two natural populations (Hasson *et al.*, 1991), providing an alternative explanation for the temporal pattern observed in Arroyo Escobar. A significant effect of different host plant tissues (cladodes *vs* fruits) on the relative fitness of each inversion was demonstrated in laboratory populations derived from flies collected in Arroyo Escobar (Ruiz and Fontdevila, 1985). The frequency of 2st increased in populations fed with fruits, while 2j was favoured at the expense of 2st when the trophic resource were *Opuntia* cladodes. In this context, it is suggestive that in Arroyo Escobar, larvae are only found in *O. vulgaris* rotting cladodes and not in fruits.

Table 1. Inversion frequencies in the *D. buzzatii* natural population of Arroyo Escobar from December 1979 to March 1989. (N = number of chromosomes analyzed).

		Chromosome 2				Chromosome 4	
Sample	N	st	j	jz[a]	jq[7]	st	s
Dec '79	392	0.310	0.385	0.296	0.015	1	0
Dec '82	48	0.146	0.416	0.416	0.021	1	0
Apr '86	64	0.156	0.562	0.265	0.017	0.984	0.016
Nov '86	731	0.201	0.510	0.284	0.005	0.989	0.011
May '87	408	0.116	0.576	0.308	0	1	0
Oct '87	620	0.124	0.576	0.297	0.003	0.983	0.017
Dec '87	128	0.078	0.664	0.258	0	1	0
Mar '89	343	0.109	0.558	0.332	0	1	0

(1) Data from Fontdevila *et al.*, 1982
(2) Data from Ruiz and Fontdevila unpublished.

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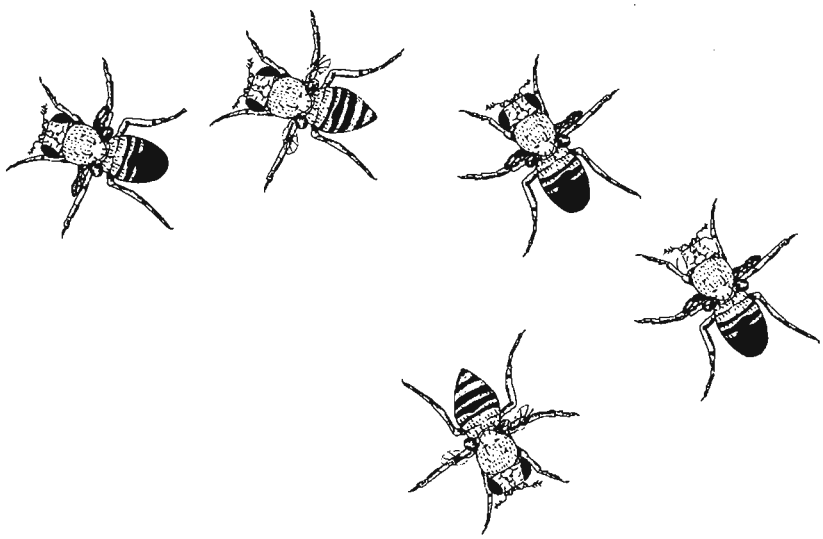

Levy, Estrella, Pedro Fernández Iriarte, Juan J. Fanara, and Esteban Hasson. GIBE, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Ciudad Universitaria (1428), Buenos Aires, Argentina, e-mail: emlevy@BIOUBA.UBA.AR fax: (541) 782- 0620.
Oviposition preference of *Drosophila buzzatii* on different cactus species.

Introduction: In the genus *Drosophila*, the preference for different oviposition sites would be important for niche separation between species (Carson, 1971; Shorrocks, 1975). *Drosophila buzzatii* is a cactophilic species of the *repleta* group that feeds and breeds on the necrotic tissues of several cactus species (Hasson *et al.*, 1992). Fields studies have revealed that *D. buzzatii* emerges mainly from the necrotic cladodes of several *Opuntia* species, e.g. *O. vulgaris, O. quimilo, O. sulphurea*, etc. (Hasson *et al.*, 1992; Fanara, 1995).

In addition, it was shown that *D. buzzatii* can utilize columnar cacti such as *Trichocereus terschekii* and *Cereus validus*, which are the main breeding resources of its sibling *D. koepferae* (Hasson *et al.*, 1992; Fanara, 1995).This emergence pattern can be the result of either differential attraction and/or oviposition preference (Fanara *et al.*, 1995). In the Argentinian northwestern population of Quilmes (Tucumán Province) *D. buzzatii* and *D. koepferae* coexist and utilize the necrotic tissues of *O. sulphurea* (Os) and *T. terschekii* (Tt). Fanara (1995) find no evidence of differential attraction of both species to the host plants.

In the present work, we report a study of oviposition preference in *D. buzzatii* under laboratory controlled conditions.

Materials and Methods: Fly strains: the flies utilized in this study correspond to the fourth laboratory generation of a stock originated from 100 isofemale lines of *D. buzzatii* collected in the natural population of Quilmes in early Autumn 1992 (see Hasson *et al.*, 1995 for geographic information). A glass population cage (100 x 40 x 40 cm) was set up with fifty 7 day-old virgin females and males. Eight small petri dishes (diameter 2.5cm) containing homogenates prepared with rotting tissues of *O. sulphurea* and *T. terschekii*, four of each, were offered to the flies. Flies were kept inside the cage during three days. Every 12 hours the petri dishes were removed from the cage for egg-counting and replaced with fresh cactus medium. The experiment was conducted at 25°C and a photoperiod of 12 h. The number of eggs was recorded in the 48 petri dishes and the number of flies visiting both cactus species was recorded periodically.

Results and Discussion: An average of 22.4 (43.75%) and 28.8 (56.25%) fly-visits were observed on Tt and Os, respectively. The number of fly-visits to each cacti is plotted against time (sample) in Figure 1. The analysis of variance testing for differential attraction to the cactus species showed that the average number of fly-visits to both cactus were not significantly different ($F_{1,11} = 1.29$, $p = 0.28$). A total of 6137 eggs were counted, 2379 (38.76%) in Os and 3758 (61.24%) in Tt. The number of eggs laid on each cactus species on a per sample basis is shown in Figure 2. The

significance of the difference in the number of eggs laid on each cactus was tested by means of an ANOVA, with cactus and sample as the main effects. The number of eggs counted on Tt was significantly larger than on Os ($F_{1,36} = 8.14$, $p = 0.007$). Samples were also shown to be significantly heterogeneous ($F_{5,36} = 9.4$, $p < 0.0001$), and the cactus x sample interaction was also significant ($F_{5,36} = 6.3$, $p = 0.0003$). The regression test of number of eggs on time for both cactus species did not reveal any significant trend ($F_{1,4} = 3.8$, $p = 0.12$ in Os and $F_{1,4} = 0.18$, $p = 0.69$ in Tt).

From Figure 2 it can be deduced that females remated during the experiment as it is suggested by the increase in the number of eggs observed after the first oviposition (sample 3 of Os. and 4 of Tt.). However, the patterns observed in Os and Tt were quite different. On one hand, after mating females laid the eggs preferentially of Os (compare the number of eggs in sample 1 in Figure 2). After, this first "choice" females laid more eggs on Tt (sample 2 in Figure 2), and this pattern was repeated in the next samples.

How can we interpret this pattern? One possible explanation might be that flies mate preferentially on Os and after the first oviposition they move on to Tt. Another possibility might be the existence of variation for oviposition behavior in the population of flies employed. Unfortunately, the experimental design does not allow us to discriminate between both explanations.

Further analysis of the present results gave more certainties about *D. buzzatii* oviposition behavior. In Figure 3 the ratio between variance and average of the number of eggs is plotted against time (samples). All samples but one, Tt sample 4, showed a ratio larger than one, indicating that oviposition was contagious. Fanara (1995) have shown that larval viabilities of *D. buzzatii* on Os and Tt are not significantly different. If the present results are extrapolated to natural conditions, the oviposition preference of *D. buzzatii* females for Tt and the absence of differences in larval viability on Os and Tt, observed by Fanara (1995), it is difficult to explain the emergence pattern. One likely explanation is that the sibling species *D. koepferae* might represent a possible competitor, during oviposition and larval life. The results of Fanara (1995) showing different oviposition strategies for *D. buzzatii* and *D. koepferae*, and that the latter is more viable than *D. buzzatii* in Tt, point to that direction. In fact, Krebs and Barker (1993) have shown this kind of interspecific interaction for the pair *D. buzzatii* - *D.*

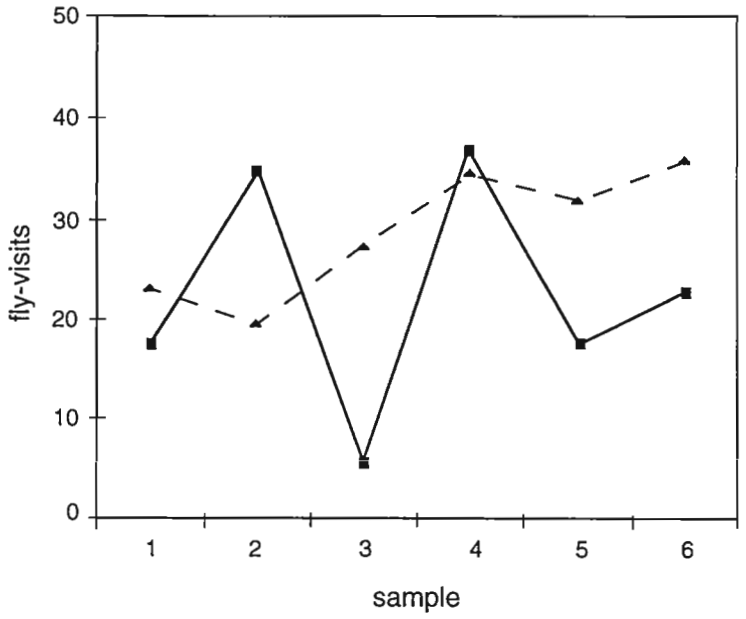

Figure 1. Total number of flies visiting both cactus species per sample. Squares, *T. terschekii*; Triangles, *O. sulphurea.*

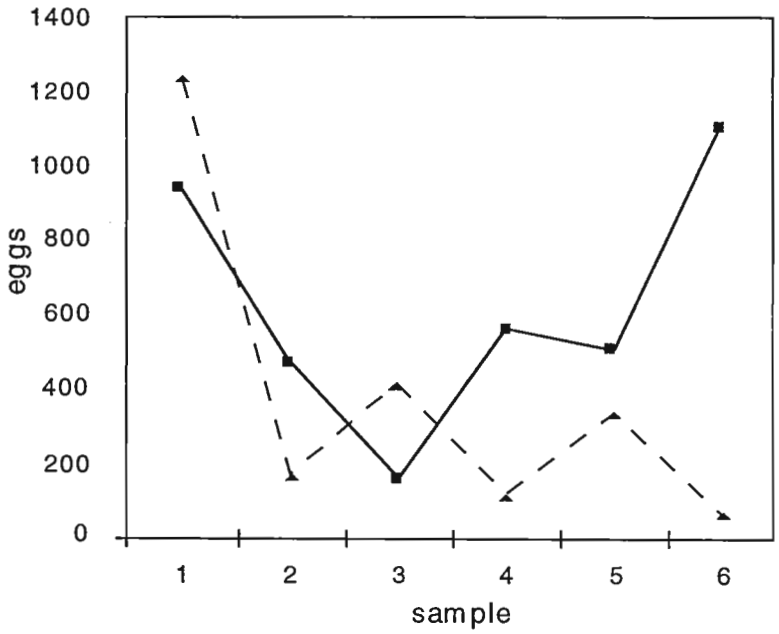

Figure 2. Counts of eggs layed on each cactus species. Squares, *T. terschekii*; Triangles, *O. sulphurea.*

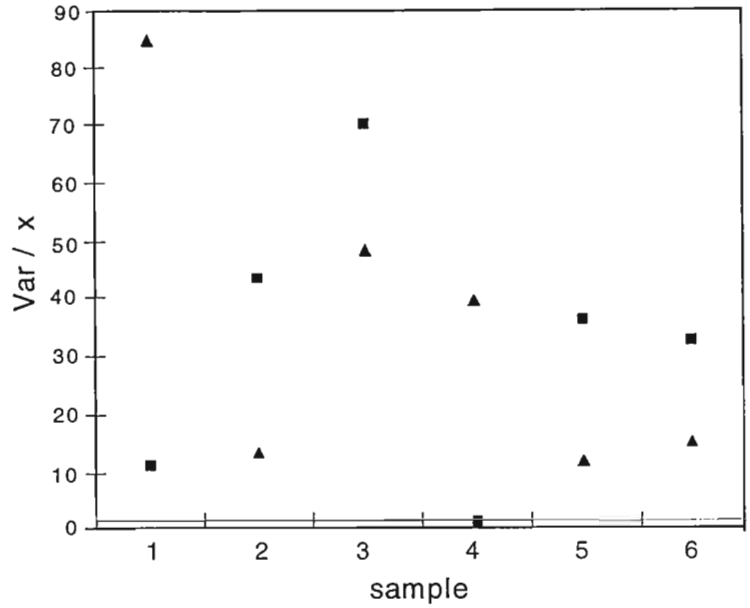

Figure 3: Variance/ mean ratio distribution for each cactus and sample. Squares, *T. terschekii*; Triangles, *O. sulphurea.*

aldrichi. While the viability of preadults of *D. buzzatii* was not affected by the presence of increasing numbers of *D. aldrichi* larvae, the reverse was not true revealing a significant interspecific effect. Whether this effect is present in the pair *D. buzzatii* - *D. koepferae* is not known, but experiments testing this hypothesis are under way.

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Pandey, M.B., and D.S. Misra. Department of Entomology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. Larval activity of *Drosophila ananassae* for pupation under simulated field condition.

The different biological activities for feeding, shelter, oviposition and pupation, etc., of any organism is mostly governed by the behavioural preference. Realising this fact, scientists are making all efforts to understand the behavioural activities of economic insects/pests so as to assist in the maintenance of natural balance. *Drosophila ananassae*, commonly known as vinegar fly, works as fermentator and decomposer of ripe fruits. Accordingly, it affect the keeping quality of fruits in nature. But how this fly discharges its biological activities have been the major concern of the scientists under laboratory conditions and not under natural conditions.

Therefore, an attempt has been made to understand the larval behaviour of *D. ananassae* pertaining to the selection of pupation sites under simulated natural condition.

It is quite difficult to understand the larval behaviour of the test organism, i.e., *D. ananassae*, which was obtained from the Mysore University, Mysore, and was maintained under the laboratory of Insect-Pest Management laboratory of the Department, to the greatest precision under natural conditions unless the condition is simulated. Hence, the field simulated condition was created by filling the petriplates (3 cm high x 10 cm in diameter) to a depth of 1 cm dried pulverised sterile soil. Thereafter, it was randomly planted with 20-25 pieces of Doob grass (*Cynodon dactylon*). One grape, sliced in two equal halves, each having one cavity equal to the size of its seed, was placed approximately in the centre of the grass planted petriplates. Each half was seeded with five 4-day-old larvae (10 larvae/petriplate) and were maintained under

Table 1. Pupation preference of *Drosophila ananassae* larvae in relation to micro-habitats.

Microhabitat (M)	Period			
	First	Second	Third	Average
On the outer surface of grape (M_1)	16	16	17	16.33
Inside the grape (M_2)	10	15	18	14.33
On or inside soil (M_3)	67	61	57	61.66
On the grass (M_4)	02	04	06	04.00
Total number of pupae	95	96	98	96.32

cover lid. There were ten replicates per treatment and repeated three times in a fortnight. Every plate possessed soil, grass and sliced grape fruit as microhabitat for pupation, though the sliced fruit resulted in two microhabitats, one being the cavity and another the outer surface of the fruit These microhabitats were designated as follows:

M_1 - Inside the fruit] on-fruit population
M_2 - On the skin of fruit]
M_3 - On or in the soil] off-fruit population
M_4 - On the grass]

After pupation, the number of pupae observed in each microhabitat was counted separately.

Tables 1 and 2 show the results of the pupation behaviour of *D. ananassae* larvae in relation to microhabitats. It is evident from both the tables that the larvae prefer to undergo pupation mostly in soil than on grass or on/in fruits. A total of 57-67 larvae averaging 61.66 larvae pupated on or inside the soil. The least preferred pupation site was grass. This constituted a total of 63-69% off-fruit pupation and 26-35% on-fruit pupation.

Further, the pupation on fruit surface or inside fruit remained at par among themselves and significantly lowest when compared with the pupation observed in soil. It is interesting to note that most of the larvae (95% - 98%) underwent pupation in spite of microhabitational hindrances.

Table 2. Percentage of on-fruit and off-fruit pupation in *Drosophila ananassae*.

Period	% on-fruit ($M_1 + M_2$) = S*	% off-fruit ($M_3 + M_4$) = R*	% Total Survivorship
First	26	69	95
Second	31	65	96
Third	35	63	98

* S = Sitters, R = Rovers

The cause of pupation on/in soil than on/in fruit seems to be largely governed by the moisture of the pupation site. In general, larvae during wandering phase, in search of site for pupation, go away from the moisture source, hence are negatively correlated (Pandey and Singh, 1993). Earlier laboratory trials, too, have confirmed the similar behaviour and the same behaviour is also exhibited by the larvae under simulated natural condition (Sokolowski *et al.*, 1986). Further, the flies emerging from such pupae are said to be rover, hence those pupating on/in fruit automatically fell under the sitter category. Accordingly, it is inferred that some proportion of flies, those pupating in/on fruit, always remain near the food source, hence assist in its further progeny than the rovers which are more exposed to natural calamities or vice versa.

Acknowledgments: Authors are thankful to CSIR, New Delhi, for providing financial assistance as SRF to Manju Bala Pandey, and to Dr. H.A. Ranganathan, Mysore University, for supplying stock of *D. ananassae*.

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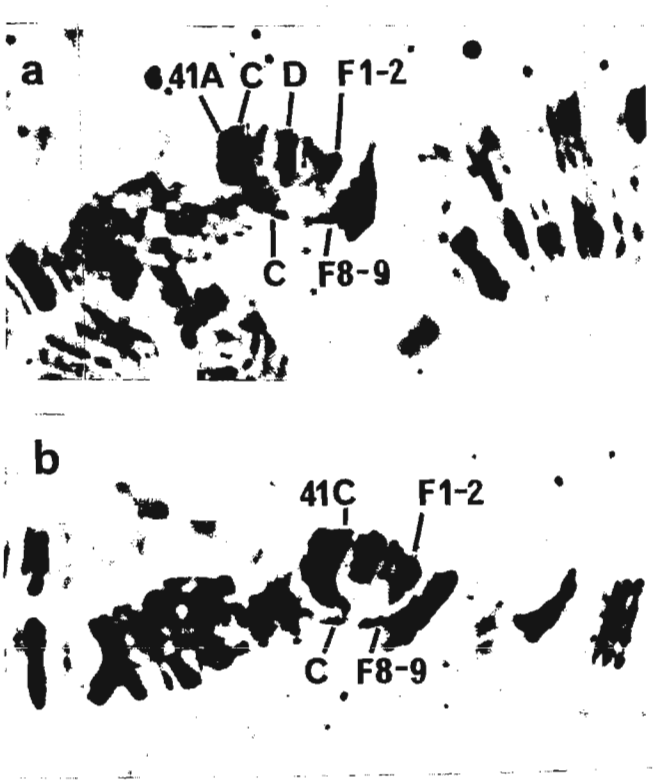
Koryakov, D.E.,[1] and I.F. Zhimulev.[1, 2] [1]Department of Cytology and Genetics, Novosibirsk State University, [2]Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, 630090 Novosibirsk, Russia. Mapping of deficiencies and loci in the chromosome 2R eu-heterochromatin junction point in *Drosophila melanogaster.*

The break points of three deficiencies in the eu-heterochromatin junction point of chromosome 2R have been mapped. *Df(2R)MS24* removes chromosome region between 41C and 42A1-2 and *Df(2R)MS2*^{10} removes heterochromatin of the chromosome 2R from the centromere to 41E1-2 (Koryakov *et al.*, 1996). *Df(2R)MS2*^{8} removes a part of banded region of the chromosome 2R between 41C and 41F8-9. In homologue with the deficiency there are only 41AC bands connected with 41F8-9 (Figure 1a, b).

Df(2R)MS2^{10} according to Morgan *et al.* (1939) uncovers *stw* and *ap*, *Df(2R)MS2*^{4} uncovers both *stw* and *ap* as well as *MS2*^{8} uncovers *stw* only. The *stw* and *ap* loci originally were mapped by Schultz in 41B-C (see Lindsley and Zimm, 1992). According to Dimitri (1991), deficiencies *MS2*^{4} and *MS2*^{8} also uncover *l(2)41e* and *l(2)41Ah* loci.

Using our new cytological data on mapping of these deficiencies, *stw* can be located between 41E1-2 and 41F8-9 (between distal break point of *MS2*^{10} and distal beak point of *MS2*^{8}) and *ap* between 41F8-9 and 42A1-2 (between distal break point of *MS2*^{8} and distal break point of *MS2*^{4}), as well as *l(2)41Ae* and *l(2)41Ah* would be placed between 41C and 41E (between proximal break point of *MS2*^{8} or *MS2*^{4} and distal break point of *MS2*^{10}).

Figure 1 (next page). Basement of 2R chromosome with the heterozygote deficiency *Df(2R)MS2*^{8}. Normal homologues are above in both cases. In rearranged homologue region 41C is connected with 41F8-9 band.



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Derzhavets, Elena M., E. Nevo, and A.B. Korol. Institute of Evolution, University of Haifa, Mount Carmel, Haifa. 31905, Israel. Potential for the P-M hybrid dysgenesis in differentiated *D. melanogaster* populations at 'Evolution Canyon', Lower Nahal Oren, Mount Carmel.

Analysis of the genetic basis of adaptation in nature may be especially successful if comparisons could be done between populations inhabiting contrasting conditions, *e.g.* mild versus stressful (Parsons, 1993). Such an opportunity is provided by a unique natural model, the "Evolution Canyon" microsite at Lower Nahal Oren, Mount Carmel (Haifa, Israel) (Nevo, 1995). The opposing slopes, separated by only 200 m (bottom) and 500 m (top), represent dramatic biotic contrasts and divergence due to the higher (up to 300%) radiation on the South-facing slope (SF-slope) as compared with the North-facing slope (NF-slope). The SF-slope is warmer, drier, microclimatically more fluctuating, and less predictable than the NF-slope. For regular collections we have at each slope three stations of increasing altitudes plus one station at the valley bottom between the slopes, in total seven stations.

A series of isofemale lines of *D. melanogaster* have been established and tested for temperature preferences of females with respect to egg laying activity (Rashkovetsky *et al.*, unpublished). The estimates obtained in this study show a strong differentiation between the slopes with respect to temperature adaptation. This is not a trivial fact, because of the small distance between the slopes. Thus, selection differences should be very strong to overcome the migration effect. Here we report the results of evaluation of potential for P-M hybrid dysgenesis as characterized by gonadal sterility of F1 hybrid females in diagnostic crosses.

Material and Methods: Wild type inseminated females were collected in June-July 1994 from the two opposite slopes of Lower Nahal Oren, at stations 2 (SF-midslope) and 6 (NF-midslope) (6 females from each station). The resulting isofemale lines were kept under standard laboratory conditions by mass cultures until gonadal dysgenesis (GD) sterility was evaluated in November-December 1995. The following laboratory stocks, kindly provided by the Bowling Green Stock Center, USA, were used as standards in diagnostic crosses: Harwich, a wild-type laboratory P strain, and Canton S, a wild-type laboratory M strain. Standard diagnostic tests were used for measuring GD sterility potential

(Kidwell, 1983): cross A: Canton S females x males of the tested strain (for evaluation of P-activity); and cross A*: females of the tested strain x Harwich males (for testing P-susceptibility).

For each cross, three males and three females were mated in vials and immediately placed at 29°C. The vials were kept for a week at this temperature and then the parents were discarded. The F1 flies emerging by days 11-14 were transferred to vials with fresh medium at 25°C. After flies had matured for two to four additional days, F1 females were screened for gonadal sterility by dissecting to detect rudimentary ovaries. A score was used only in calculations if ten or more F1 females were produced in the cross in the vial. About 50 females were taken at random for dissection. An ovary was scored as nondysgenic if even one ovariole was developed (Schaeffer *et al.*, 1979). The females with two dysgenic ovaries were classified as sterile. The frequency of gonadal sterility was calculated by dividing the number of dysgenic females by the total number of scored females. The strains were identified according to Kidwell's criteria (Kidwell *et al.*, 1983).

F1 females resulted from the cross Canton S female x Harwich males were used as a control. In addition, the intrastrain sterility of each isofemale line was tested. In these intrastrain crosses, both sexes were scored for GD. All control crosses were done concurrently with the experimental crosses.

Results and Discussion: The mean frequency of GD sterility is presented in the Table for each of the isofemale lines from the opposite slopes. A low level of P-activity, as manifested by the frequency of dysgenetic ovaries in cross A, was found: 0 to 3.3% for the SF-facing slope (station 2), and 0 to 5.4% for the NF-facing slope (station 6). Tests for heterogeneity showed no significant differences either within or between slopes. Thus, the heterogeneity within SF-slope was $\chi^2 = 3.78$, df = 5 ($P > 0.5$) whereas for NF-slope $\chi^2 = 11.08$, df = 5 ($P = 0.05$). Student's t-test for arcsin(sqrGD)-transformed frequencies showed no inter-slope differences: $t = 0.43$, df = 10 ($P > 0.3$).

Table 1. Gonadal dysgenesis sterility in isofemale lines from the opposite slopes of Lower Nahal Oren.

Line	Diagnositic crosses		Intrastrain crosses	
	cross A	cross A*	Female	Male
		Station 2 (South-facing slope)		
2.2.I	1.22 ± 1.21	0	8.33 ± 3.57	0
2.19	2.08 ± 2.06	1.61 ± 1.60	4.00 ± 2.77	3.45 ± 2.40
2.23	3.39 ± 2.36	5.08 ± 2.86	1.67 ± 1.65	0
2.29	0	12.79 ± 3.60	7.27 ± 3.50	0
2.25	1.37 ± 1.36	22.62 ± 4.56	11.54 ± 4.43	0
2.9	0	28.07 ± 5.95	17.31 ± 5.25	6.00 ± 3.36
χ^2 (df = 5)	3.78 ns	40.74	($p << 0.001$)	
		Station 6 (North-facing slope)		
6.1.3	1.59 ± 1.57	0	1.79 ± 1.77	0
6.72	0	0	0	0
6.48	0	0	1.72 ± 1.71	0
6.2.F	0	2.04 ± 2.02	15.91 ± 5.51	0
6.40	5.45 ± 3.06	2.94 ± 2.05	10.00 ± 3.57	1.79 ± 1.77
6.44	0	70.18 ± 6.06	8.93 ± 3.81	0
χ^2 (df = 5)	11.08 ns	212.2***	($p << 0.001$)	
**t (df = 10)	0.63 ns	0.43 ns		

Mean sterility GD(%) ± SEGD is given for each line; ns = non-significant difference or heterogeneity; ** Student's test for arcsin (sqrGD) - transformed rates. *** Between-line heterogeneity for station 6 becomes non-significant when the line 6.44 is excluded: χ^2 (df = 4) = 4.79, $p>0.3$: χ^2 test was based on Kullback (1959) 'information statistics'.

Different results were obtained when testing for P-susceptibility (ovarian dysgenesis in cross A*). Here, the involved lines from the SF-slope manifest an approximately even distribution within the range of 0-28%. According to the accepted criteria based on sterility estimates in crosses A and A* (Kidwell, 1986), the first three lines from station 2 should be classified as Q (GD<10%), and the reminder three as weak M'-lines (GD > 10%).

A somewhat different distribution was displayed by the GD-values for lines of the NF-slope. With an exception of one line, # 6.44, all others showed a low level of GD (from 0 to 2.9%) and could, therefore, be identified as Q-lines. Because of high P-susceptibility (GD = 70.2%), line # 6.44 should be classified as a strong M'. In order to exclude the effect of other (non-dysgenetic) factors of sterility we have conducted for this line an additional diagnostic cross: females P (Harwich) x males 6.44. No sterile females were found in this test conforming to the dysgenetic nature of the foregoing result in the A* cross.

The obtained results, based on the accepted classification criteria (Kidwell *et al.*, 1983), indicate different patterns of gonadal sterility in the material derived from the opposite slopes: approximately equal presentation of Q and M' lines associated with the SF-slope, but a predominating tendency to Q-status at NF-slope. It is known that after 5 to 15-20 generations of cultivation in laboratory conditions newly established isofemale lines approach stabilization of the P-M status (Kidwell *et al.*, 1988; Periquet *et al.*, 1989). This steady state does not necessarily reflect the real situation of the respective natural population. Therefore, further tests with freshly caught flies are needed. They will allow us to get a more reliable estimation of the effect of the contrasting natural conditions on the potential of the P-M system of hybrid dysgenesis as a function of differential ecological stress on a microscale.

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Ronsseray, and M.H. Hamelin 1989, Heredity 63: 47-58; Schaeffer, R.E., M.G. Kidwell, and A. Fausto-Sterling 1979, Genetics 92: 1141-1152.

Koryakov, D.E.[1] and I.F. Zhimulev.[1, 2] [1]Department of Cytology and Genetics, Novosibirsk State University, 630090 Novosibirsk, Russia. [2]Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, 630090 Novosibirsk, Russia. FAX: (3832) 35 65 58 or (3832) 35 67 45, E-mail: zhimulev@ benpc.bionet.nsk.su. Distribution of chromosome rearrangement break points along the polytene chromosomes of *D. melanogaster*.

In the most recent synopsis of localization of chromosome rearrangements in *D. melanogaster* polytene chromosomes (Mukhina *et al.,* 1981) it was shown that the chromosome regions demonstrating high frequencies of break points usually display such traits as late replication, underrepresentation of DNA in polytene chromosomes and ectopic pairing. Regions showing these traits are usually called intercalary heterochromatin (Hannah, 1951; Mukhina *et al.,* 1981; Zhimulev *et al.,* 1982).

Since the last publication numerous data on chromosome rearrangements have been published in recent years. In this note the locations of the rearrangements induced in *D. melanogaster* at random or those found in populations are plotted on the polytene chromosome map. New chromosome rearrangements are listed in the Table 1.

Table 1. List of chromosomal rearrangements in the polytene chromosomes of *Drosophila melanogaster.*

Rearrangements	*Df*	*Dp*	*In*	*T* and *Tp*	Various rearrangements including those with not correct localization of one of the breaks	Breaks produced in the first generation	References
I. Spontaneously arisen			22				Oshima *et al.*, 1964
			2				Grossman, 1967
			23				Rim *et al.*, 1986
			6				Santos *et al.*, 1991
			20				Koryakov *et al.*, 1994
		1	13				Inoue, 1988
			30				Zacharopoulou and Pelecanos, 1980
			10				Roca *et al.*, 1982
			58				Paik, 1979
			19				Inoue and Watanabe, 1979
			15				Das and Singh, 1991
Total (I)		**1**	**212**				
II. Induced by mobile elements			103	4			Yamaguchi and Mukai, 1974
	3	12	23	3			Yannopoulos and Zacharopoulou, 1980
							Berg *et al.*, 1980
			14				Engels and Preston, 1984
			31				Lim, 1988
			8				Ising and Block, 1981
				69			
Total(II)	**3**	**12**	**179**	**76**			
III. Induced by artificial mutagens				5		69	Hilliker, 1985
			11				Reuter *et al.*, 1985
			38	29	13		Omelianchuk *et al.*, 1991
			18	35			Volkova and Buzykanova, 1991
			10	167			Trusis and Hilliker, 1984; Hilliker and Trusis-Coulter, 1987; Hilliker *et al.*, 1991
				77			Yamamoto, 1987
						77	Demakova *et al.*, 1994
Total(III)			**77**	**313**	**13**	**146**	
Total (I-III)	**4**	**12**	**468**	**389**	**13**	**146**	

To prepare histograms both new rearrangements and those listed in Mukhina *et al.* (1981) were used. Data in Figure 1E show that the most active in chromosome rearrangement formation are the following regions: 2B, 2F, 3C, 5E, 7BC, 11A, 12E, 17C and 20th. Data for the autosomes are not so detailed as for the X, and nevertheless in numerous regions chromosome rearrangements occur more often than in others. These are: 22A, 24D, 25A, 27A, 29F, 30AB, 31B, 33B, 34A, 34CD, 35AB, 35D, 36D and 40 regions in the 2L (Figure 2D); 41, 42A, 42B, 47A, 48C, 50A, 50C, 51D, 53D, 55EF, 56D, 56F, 57AB, 57F, 59D, 60A, 60D and 60F in the 2R (Figure 3D); 61C, 62A, 64C, 64D, 64E, 66A, 66BC, 67DE, 70C, 75C and 80AC in the 3L (Figure 4D); 84B, 84D, 87B, 87D, 87E, 90A, 96A, 96F, 98B, 98C, 98F and 100F in the 3R (Figure 5D).

Data in Table 2 show that as a rule chromosome rearrangements peaks are located in chromosome regions with delayed completion of replication.

Table 2. Correspondence of chromosome rearrangement and late replication sites in polytene chromosomes.

	Degree of late replication*							
Chromosomes	strong		moderate		weak		none	
X	3C	12E	7BC				2B	5E
	11A	20	17C				2F	
2L	25A	35D	22A				27A	34A
	33B	36D	24D				29F	34CD
	35AB	40	30AB				31B	
2R	41		50A	59D	42B	57AB	42A	57F
	50C		53D		47A	60F	48C	60A
	56F		56D		51D		55EF	60D
3L	64C	75C	64E		64D		61C	66BC
	57DE	80AC	70C		66A		62A	
3R			84D		90A		84B	98F
			87E		96A		87B	100F
			98C		98B		96F	

* according to Zhimulev et al., 1982.

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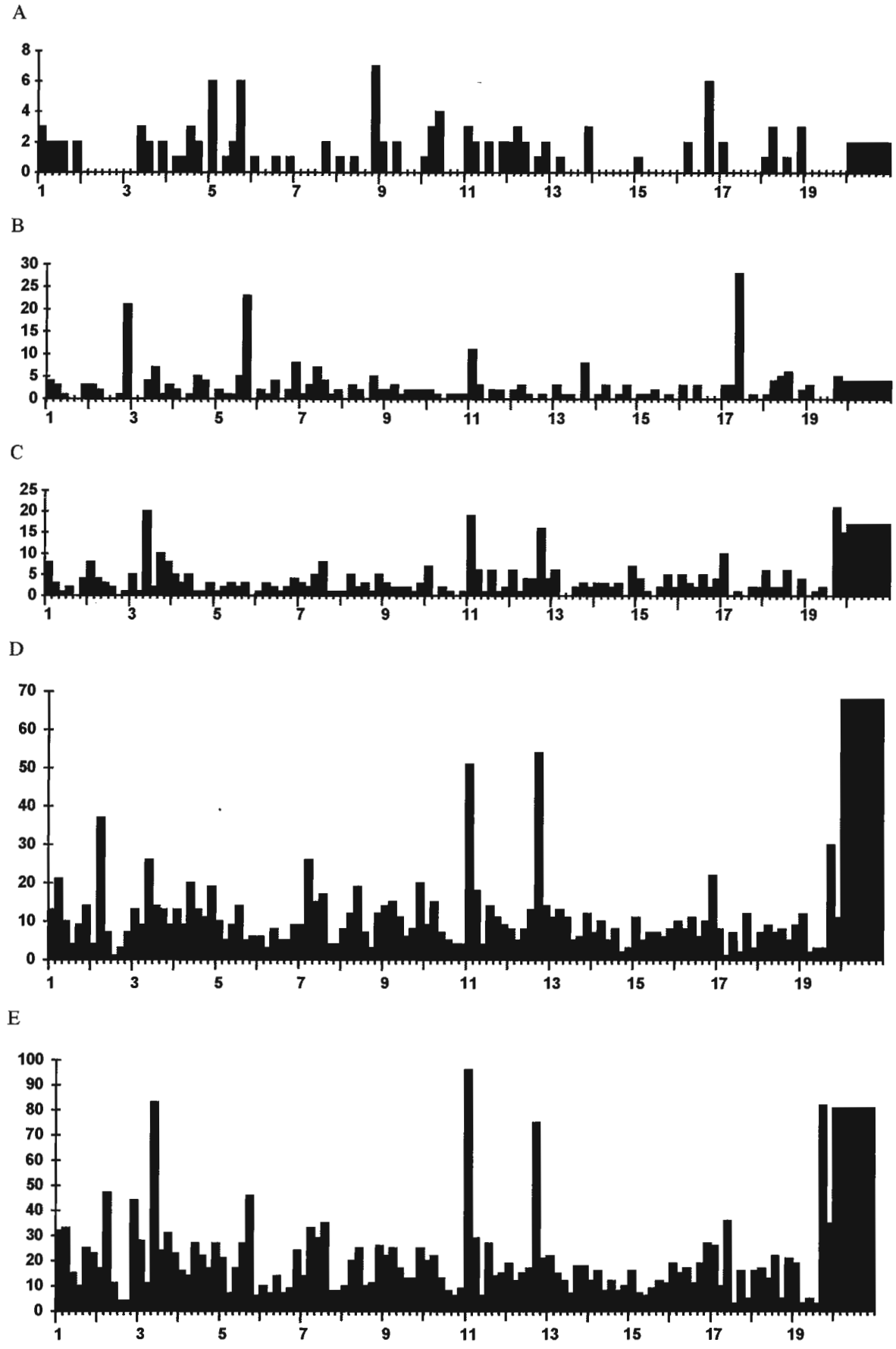
Figures 1-6. Distribution of chromosome rearrangement breaks along the X chromosome (Figure 1), 2L chromosome (Figure 2), 2R chromosome (Figure 3), 3L chromosome (Figure 4), 3R chromosome (Figure 5) and fourth chromosome (Figure 6). Abscissa:chromosome regions according to Bridges' revised maps. Ordinate:number of breaks in the letter subdivision of the map.

Figure 1 (next page). A - inversions spontaneous, B - inversions induced, C - translocations and transpositions, D - sum of the data of Kaufmann and Prokofyeva-Belgovskaya and Khvostova (see Mukhina *et al.,* 1981 for references), E - sum of A - D plus deficiencies.


A
8
6
4
2
0
1 3 5 7 9 11 13 15 17 19
B
30
25
20
15
10
5
0
1 3 5 7 9 11 13 15 17 19
C
25
20
15
10
5
0
1 3 5 7 9 11 13 15 17 19
D
70
60
50
40
30
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10
0
1 3 5 7 9 11 13 15 17 19
E
100
90
80
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40
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0
1 3 5 7 9 11 13 15 17 19

Figure 2. A - inversions induced, B - inversions spontaneous, C - translocations and transpositions, D - sum of A - C plus deficiencies.

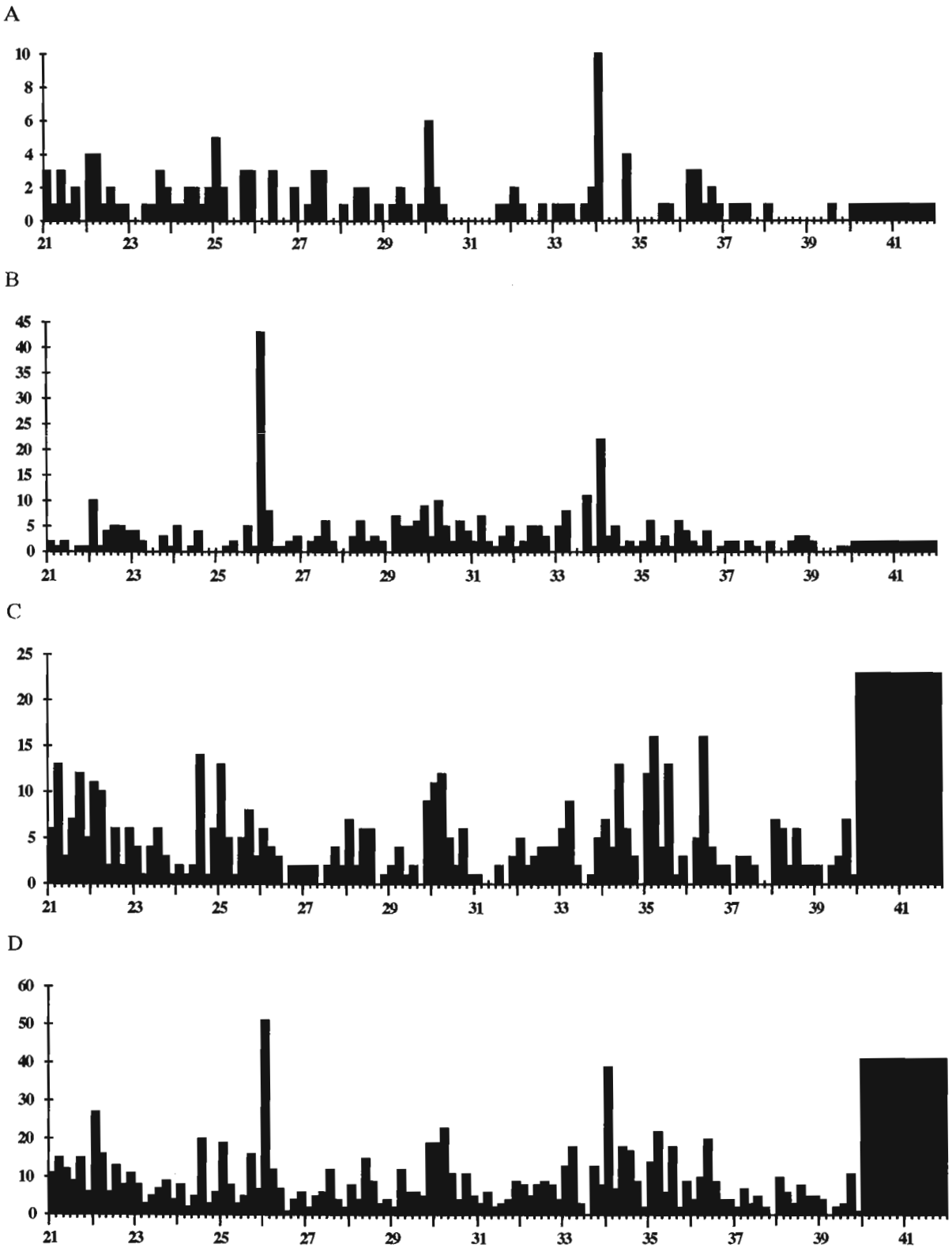


Figure 3. A - inversions spontaneous, B - inversions induced, C - translocations and transpositions, D - sum of A - C plus deficiencies.

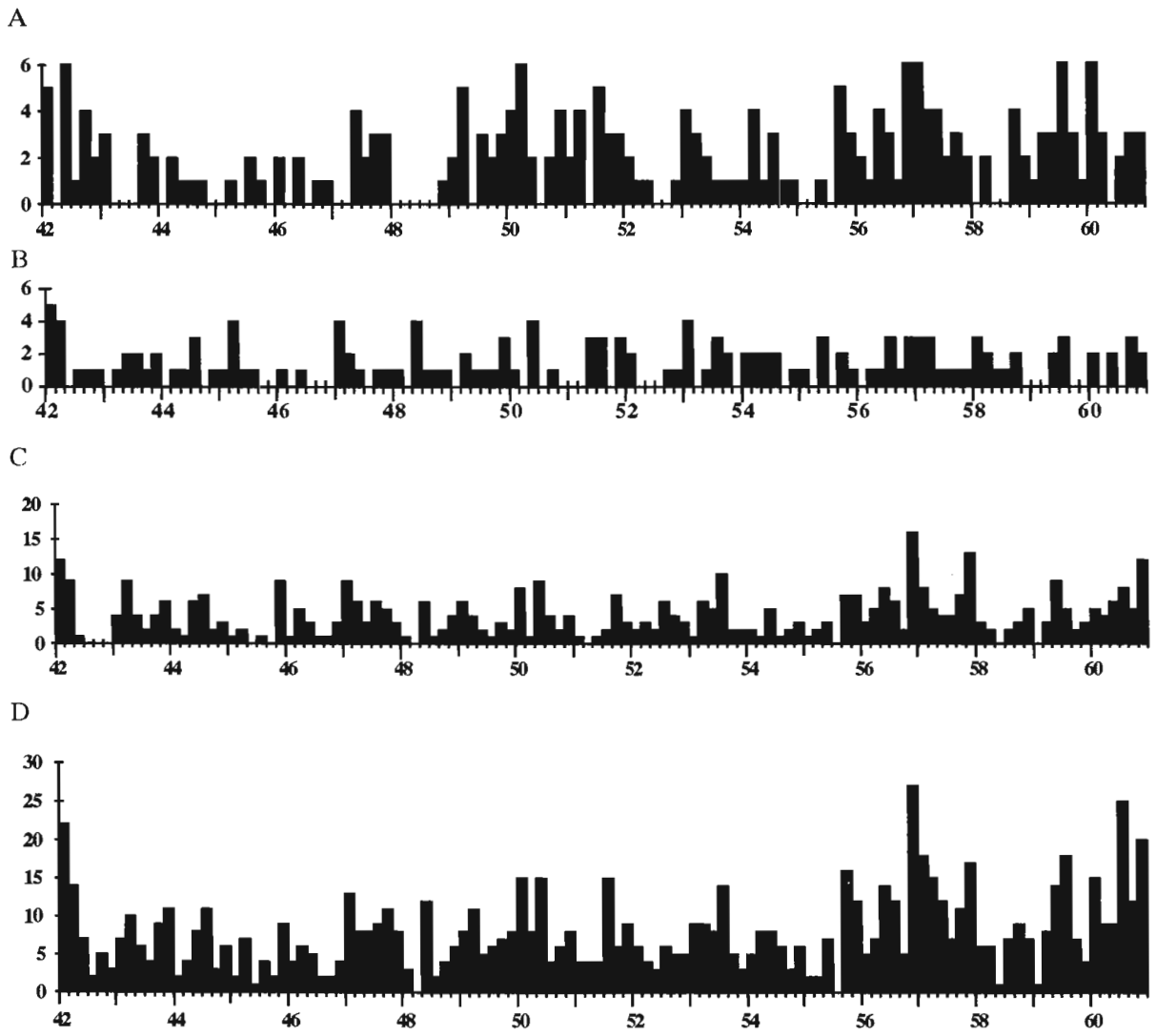


Figure 4. A - inversions spontaneous, B - inversions induced, C - translocations and transpositions, D - sum of A - C plus deficiencies.

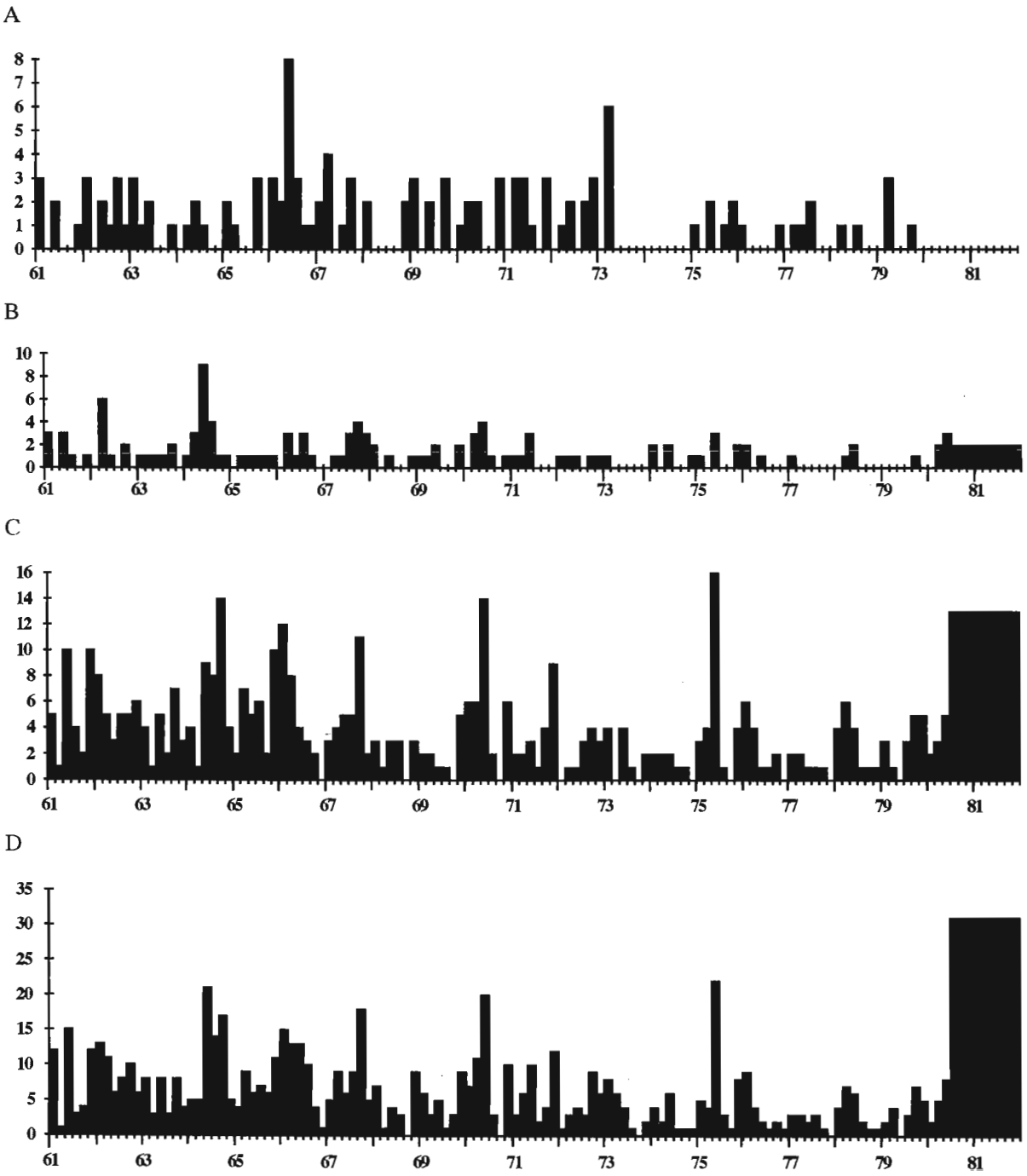


Figure 5. A - inversions induced, B - inversions spontaneous, C - translocations and transpositions, D - sum of A - C plus deficiencies.

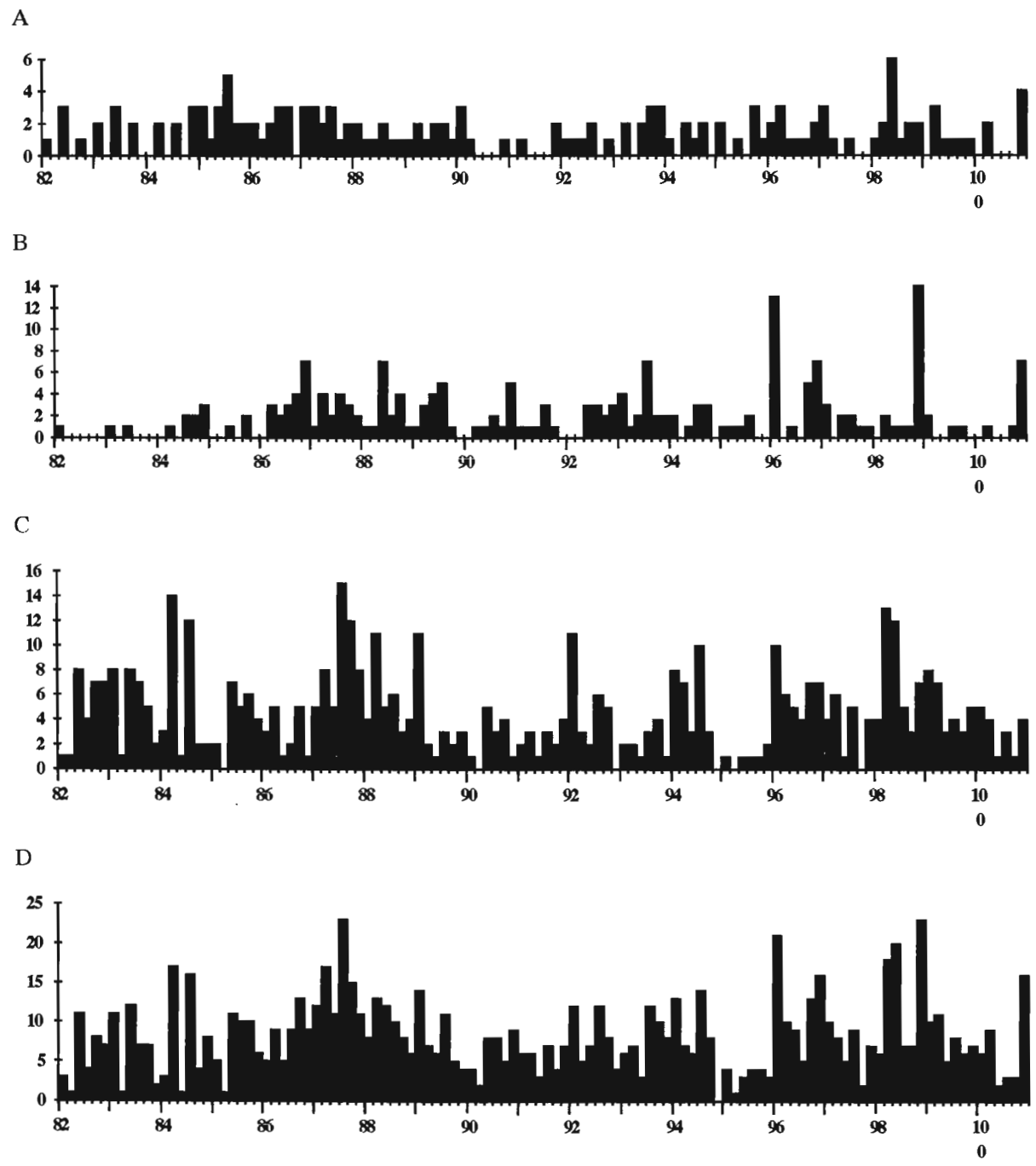
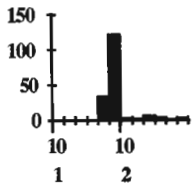


Figure 6. sum of all breaks.



Zhimulev, I.F., V.F. Semeshin, G.H. Umbetova, I.E. Vlassova, and E.S. Belyaeva. Institute of Cytology and Genetics, Novosibirsk 630090, Russia. Transcription in the 100B3/4-5 interband of *Drosophila melanogaster* polytene chromosomes.

For many years the problem of functional organization of polytene chromosome interbands remains intriguing. The interbands are decondensed and under EM look like very small puffs but, nevertheless, their transcriptional activity has not been convincingly proven.

The technique of localization of antibodies against DNA/RNA molecular hybrids on polytene chromosomes (Rudkin and Stollar, 1977; Alcover *et al.*, 1982; Buesen *et al.*, 1982; Diez and Barettino 1984; Vlassova *et al.*, 1985; Lezzi *et al.*, 1989) gives two very important advantages: i) antibodies bind DNA/RNA molecular hybrids formed after DNA denaturation and renaturation with RNA synthesized *in situ* in the same chromosome region. Therefore fluorescence of the chromosome region corresponds to a site of real RNA synthesis. ii) resolution of localisation of the RNA-synthesizing region is noticeably higher than usual autoradiography can give.

Interband analysis under light microscope is difficult because they have rather small sizes. Very often in polytene chromosome regions showing decondensation of chromatin and looking like interbands, numerous microbands can be found on Bridges revised maps or on EM maps. Therefore, only those interbands are real which do not contain even smallest bands which can puff and show some transcriptional activity. Another problem is related with resolution of cytological methods. If on one or both sides of transcriptionally inactive interband two active or partially active bands are situated, the tracks of their activity can lay over an interband. As a consequence, such interband will show some transcriptional activity.

For the analysis of transcriptional activity, interbands which are situated between a pair of big densely stained bands, not showing transcriptional activity, are most suitable. No microbands should be located within such interbands. In previous studies it was shown that such real interbands are not numerous in *Drosophila melanogaster* polytene chromosomes (Semeshin *et al.*, 1979). In this paper the real interband was mapped under EM and their transcriptional activity was analyzed using DNA/RNA antibodies localization.

Third instar larvae from PS1 to PS10 and 0, 4-6 hours prepupae of Batumi-L wild strain or gt^{E6}/gt^{13z} were used. Aging of larvae (PS - Puff Stages, see Ashburner, 1975) was performed by morphology of the salivary gland duct (Zhimulev *et al.*, 1981), the age of prepupae was scored in hours after puparium formation.

EM techniques were described in detail by Semeshin *et al.* (1979). Preparations for immuofluorescent analysis were made as described earlier (Vlassova *et al.*, 1985). In each case, on the average eight photographs of a chromosome region have been analysed.

The 100B according to preliminary data contains real interband (Semeshin *et al.*, 1979). In the region 100B (Figure 1) at high magnification of EM four bands can be easily seen at any PS: three thick ones, 100B1-2, 100B4-5, and 100B8 which are singlets but not doublets as it was shown in light microscope maps (Lindsley and Zimm, 1992). Fourth band, 100B3, is very thin and lies very closely to 100B1-2. Similar banding pattern in the 100B region was found by Heino *et al.* (1985). Broad interband is clearly seen between 100B3 and 100B4-5 (Figure 1).

By immunofluorescent localization of DNA/RNA hybrids it has been shown previously that the 100B3/100B4-5 interband of chromosome 3R is transcriptionally active in Oh *gt* prepupae (Vlassova *et al.*, 1985). These data are well reproduced on the polytene chromosomes of Batumi-L strain (Figure 2b, b'). Moreover, at PS8-9 and PS14-16 the fluorescence of this region is enhanced, as seen from comparison of Figures a, a', c, c'. The enhancement of the transcription in the interband is likely to be due to activation of the 100B3 band adjoining the 100B1-2 dense band.

The 100B3/100B4-5 interband is active during most part of studied period of development. So, data fit very well with the proposal by Zhimulev and Belyaeva (1975) that interbands are constantly transcriptionally active. All previous cytological data support this idea as well. By means of Electron Microscopic autoradiography, incorporation of ^{3}H-uridine was shown in 100B3/100B4-5 interband (Semeshin *et al.*, 1979). Antibodies against RNA-polymerase II were shown to be located in the interbands of *Drosophila* and *Chironomus* polytene chromosomes (Jamrich *et al.*, 1977a,b). Location of the RNA-polymerase II coincides with ^{3}H-uridine incorporation which is especially very well seen on stretched chromosomes (Sass and Bautz, 1982).

RNP granules which are the products of locus activity are located in any chromosome regions showing any extent of chromosome decompaction (see for review, Zhimulev, 1994). Their location in interbands has been demonstrated by many authors (Perov and Chentsov, 1971; Alonso and Berendes, 1975; Perov *et al.*, 1975; Skaer, 1977, 1978, Mott *et al.*, 1980; Mott and Hill, 1986).

On the other hand, cloning and sequencing the *D. melanogaster* interband DNA in the 61C region has shown two very short overlapping open reading frames, 354 and 555 bp which may witness for their genetic activity (Demakov *et al.*, 1993). But frequencies of codon usage (Ashburner, 1989) within these frames are statistically different from those

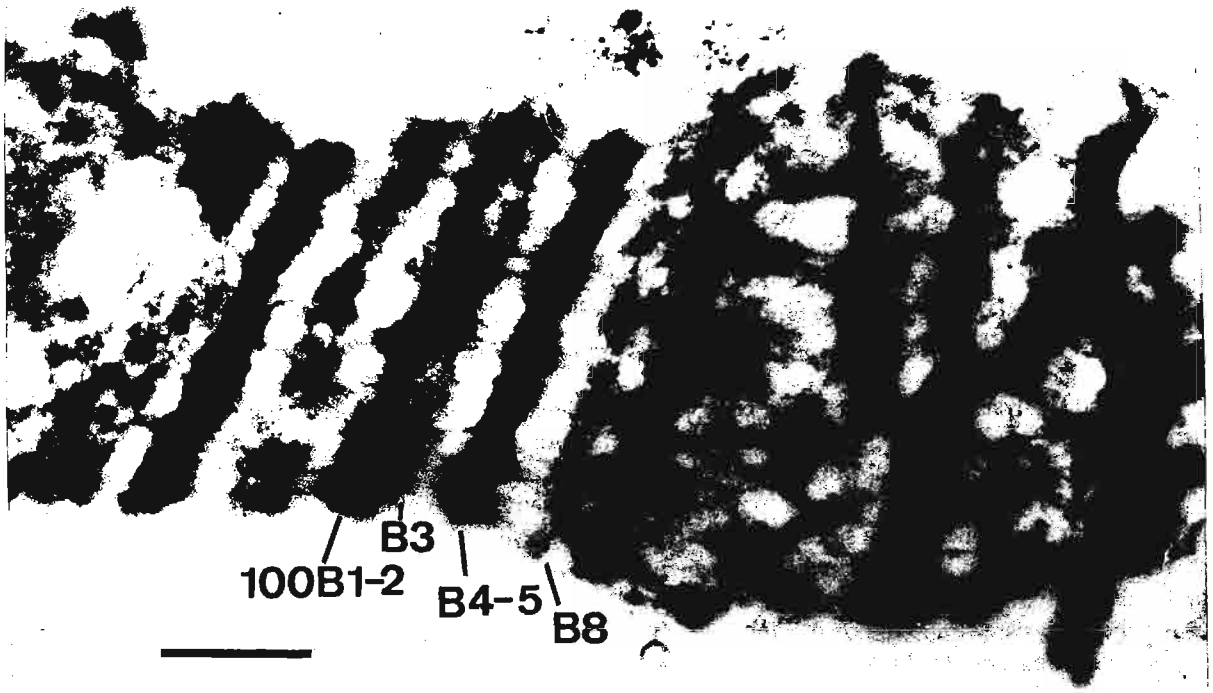


Figure 1. Electron microscopical mapping of the 3R chromosome region 100B at Puff Stage 1 - PS. Bar corresponds to 1 μm.

which are usually found in *Drosophila* proteins. A short open reading frame 420 bp long was found in the 3C5-6/3C7 interband (Rykowski *et al.*, 1988; Ramos *et al.*, 1989) In EM analysis of *Drosophila* stocks transformed with foreign DNA, it has been shown that if transposon insertion occurs into interbands, new small bands can be found in the site of insertion. In 11 stocks from 15 studied, the insertions of transposed DNA take place into interbands and all these stocks are viable as homozygotes (Semeshin *et al.*, 1989, 1994 and in preparation). Therefore, at least these 11 interbands do not carry functions essential for survival. These findings make difficult interpretations of interbands as gene loci coding for proteins despite on their transcriptional activity, and the problem of interband organisation becomes now more enigmatic.

Acknowledgments: This work was supported by a grant from the Genetic Programme of Russian Federation and Russian Foundation of Fundamental Research (RFFI).

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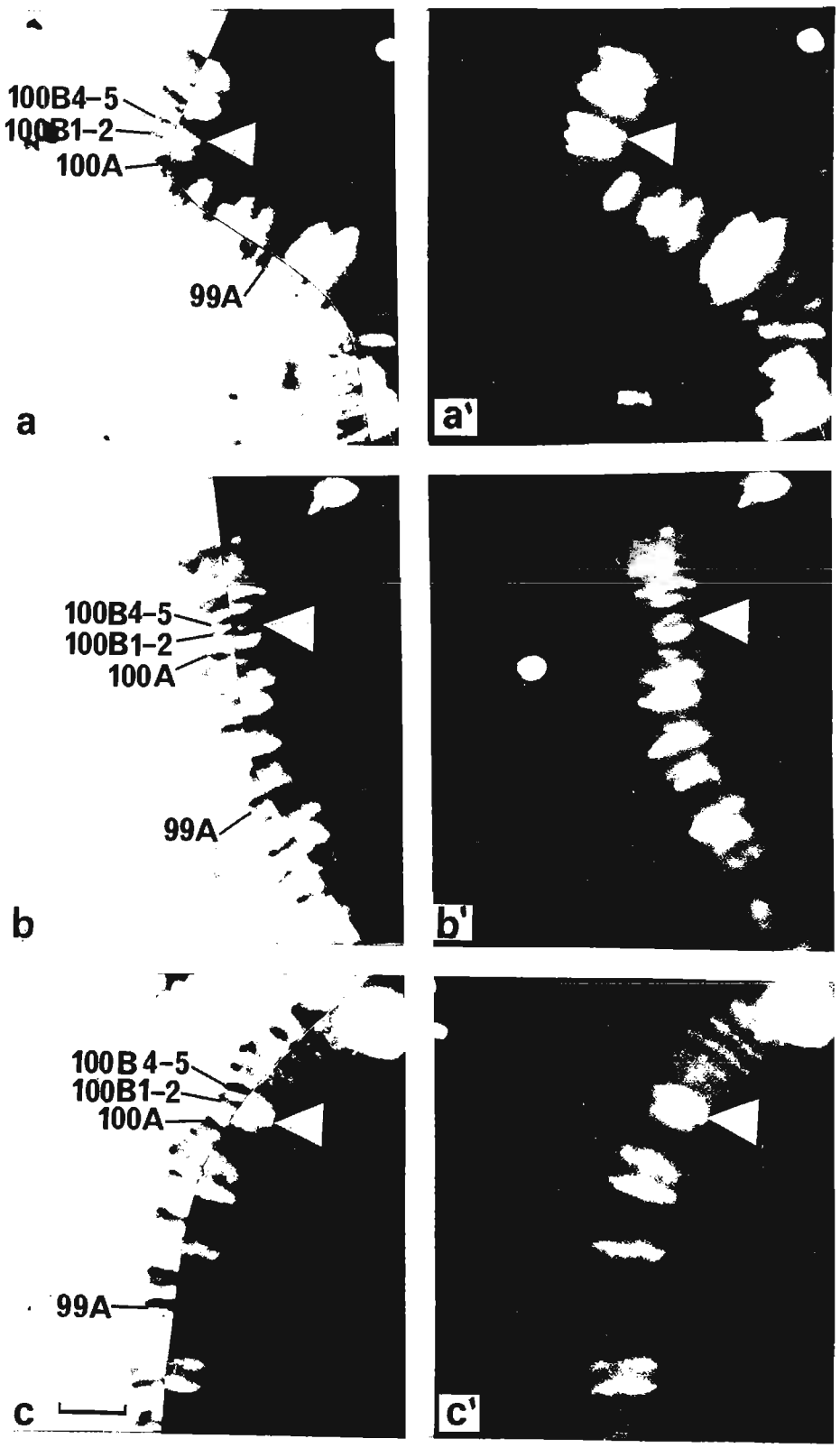

Figure 2. DNA/RNA hybrid immunofluorescence in the interband 100B3/100B4-5 of the 3R chromosome (Batumi-L wild type) during normal development: a, a' - PS8-9, b, b' - PS10-11, c,c' - PS14-16. Arrowhead points to the interband fluorescence. Bar corresponds to 3 μm.

Ivannikov, Andrey V., and Ilya K. Zakharov. Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Division, Novosibirsk, 630090, Russia. Tel (3832) 35 61 36; FAX (3832) 35 65 58; e. mail: zakharov@cgi.nsk.su. Differences in mutations rates between males with small and large body sizes from natural populations of *Drosophila melanogaster.*

Body size in *Drosophila* varies widely. It has been reported that numerous morphological and biometrical traits of the adult phenotype such as body weight, size of wings, legs, thorax and other organs are strongly dependent on the conditions of larval development in the majority of insects, including *Drosophila*. The dependence is based on complex modifications and interrelations of growing organs and enfolding endocrine processes determining metamorphosis (for review, see David, *et al.*, 1983). Of the various environmental factors decreasing body size pertinent here are high temperature during larval development, crowding, and food deficiency. The effects of these factors alone or combined on body size can be readily reproduced in experiments and in models (Powell and Taylor, 1979). However, when concerned with natural *Drosophila* populations, it is difficult, if at all feasible, to disclose the major factors causing morphometric differences between flies. The underlying causes are, indeed, complex ranging from external to intrinsically genetic.

A vantage point of view may be offered. By distinguishing ecological from physiological and genetic factors affecting development, on the one hand, and by estimating spontaneous mutation rate in natural populations of *Drosophila melanogaster*, on the other hand, there appear possibilities of bridging a gap between *Drosophila* ecophysiology and genetics and linking certain environmental factors which affect unfavorably development and also most probably act as mutagens and/or regulators of the mutation process.

Our aim here is to determine and compare spontaneous mutation rates in *Drosophila melanogaster* males from natural Altai populations differing in the complex character of body size.

Collection sites and formation of groups according to size. *Drosophila melanogaster* males were taken from four populations of fruit processing factories in Altai, south of West Siberia, at the towns Zmeinogorsk, Biisk, Gorno-Altaisk and settlement Pospelikha which are located at the northern boundary of their distribution area of this species. The flies were caught during the last part of September, 1992, by the end of their reproduction season in West Siberia. Males were first visually divided into groups according to body size: large, small and intermediate. The mean weight of each group of males was estimated as 0.26-0.53 mg for small, 0.78-0.79 mg for intermediate, and 0.74-0.90 mg for large body sizes.

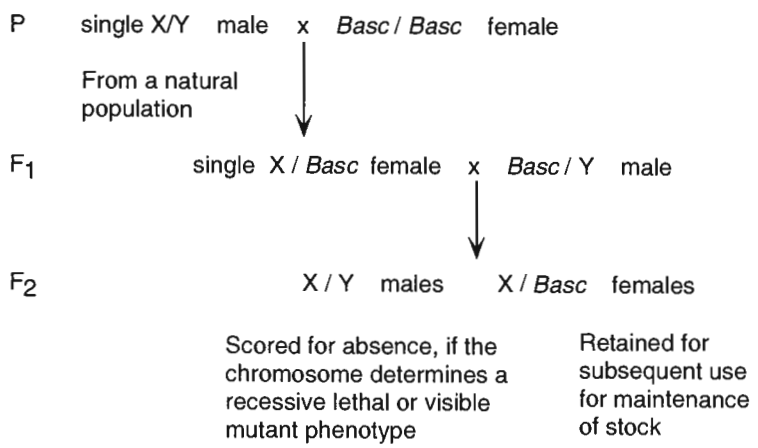

Figure 1. Breeding program.

Estimation of mutability rate. Muller's standard method was used to estimate mutability rate (Grigliatti, 1986). It is based on determination of the arisal frequencies of sex-linked recessive lethal and visible mutations. We used the standard Basc chromosome to detect sex-linked recessive lethal and visible mutations. Basc / Y males and homozygous females are viable and fertile. The Basc chromosome suppresses crossing over in the X. The frequency of mutations arising in the X chromosome was determined according to the scheme in Figure 1. The data for the frequencies of the *de novo* arising of sex-linked recessive lethal and visible mutations are presented in Table 1. The four populations were homogeneous with respect to the arisal frequencies of the analysed mutation, and pooling of the results for each population appeared justified. Of the 7 arisen mutations in 4,770 examined gametes, 6 were identified in males with small, 1 in those with intermediate, and none in those with large body sizes. The revealed differences between mutability of small and large males were significant (P = 0.005, according to the test for independence in a 2x2 contingency table). It should be noted that, when males with intermediate body weight were pooled with those of small or large body weights, the above differences remained consistently significant.

The higher mutability rate in males with small than large body size from natural populations of *Drosophila melanogaster* from Altai may not be a ubiquitous phenomenon. However, it should be stipulated that the revealed difference is a fact meriting consideration. Among the causes underlying these differences may be implied the dependence of mutation rates in different populations on seasons of the year, temperature, larval density, physical properties and chemical composition of food and other variations in environmental conditions of development (see review by Woodruff, *et al.*, 1983).

The question can be raised, what if the higher mutability of the chromosome sample from males with small body weight compared with those with large body weight from wild populations may not be only a response to natural selections? If not, then, perhaps, the revealed differences may be explained by a parallelism of the effects of environmental conditions which, by effecting unfavorable larval development decrease body size in adults, on the one hand, and, by acting alone or together, destabilize the genome manifested as increase in mutation rate, on the other hand.

Table 1. Frequencies of the *de novo* arising lethal and visible recessive mutations in the X chromosome gametes of males with different body sizes in *Drosophila melanogaster* populations from Altai, September 1992.

	Collection sites									
Body size	Pospellkha		Zmeinogorsk		Biisk		Gorno-Altaisk		Total	
groups	N	n	N	n	N	n	N	n	N	n
Small	683	4	—	—	455	1	403	1	1541	6
Intermediate	—	—	536	1	518	0	—	—	1054	1
Large	553	0	617	0	297	0	708	0	2175	0

N = sample size; n = number of arisen mutations.

It is known that body size in flies is closely related to population density: smaller adults develop from larvae grown in overcrowded conditions. It is reasonably expected that the fertility of the smaller would be reduced compared to the larger flies. Furthermore, increase in mutability contributes to higher mortality.

Thus, the prevalence of small over large body sizes in terms of mutability, which we observed in *Drosophila melanogaster*, is most probably not an artifact. If it is a real event, mutability effect would be consistent with an important principle of the organization of living systems, subjugation to common interests in an ecosystem with regulatory consequences restricting population size at high densities.

The results we obtained may be relevant to the monitoring of the spontaneous mutation process in wild *Drosophila* populations. Measurements of mutation rate are performed, as a rule, from a population as a whole. According to our present data it is expedient to take into account a set of factors, which can affect the life history of populations such as the time course of changes in size, sites and methods of collection of samples of tested individuals, among others.

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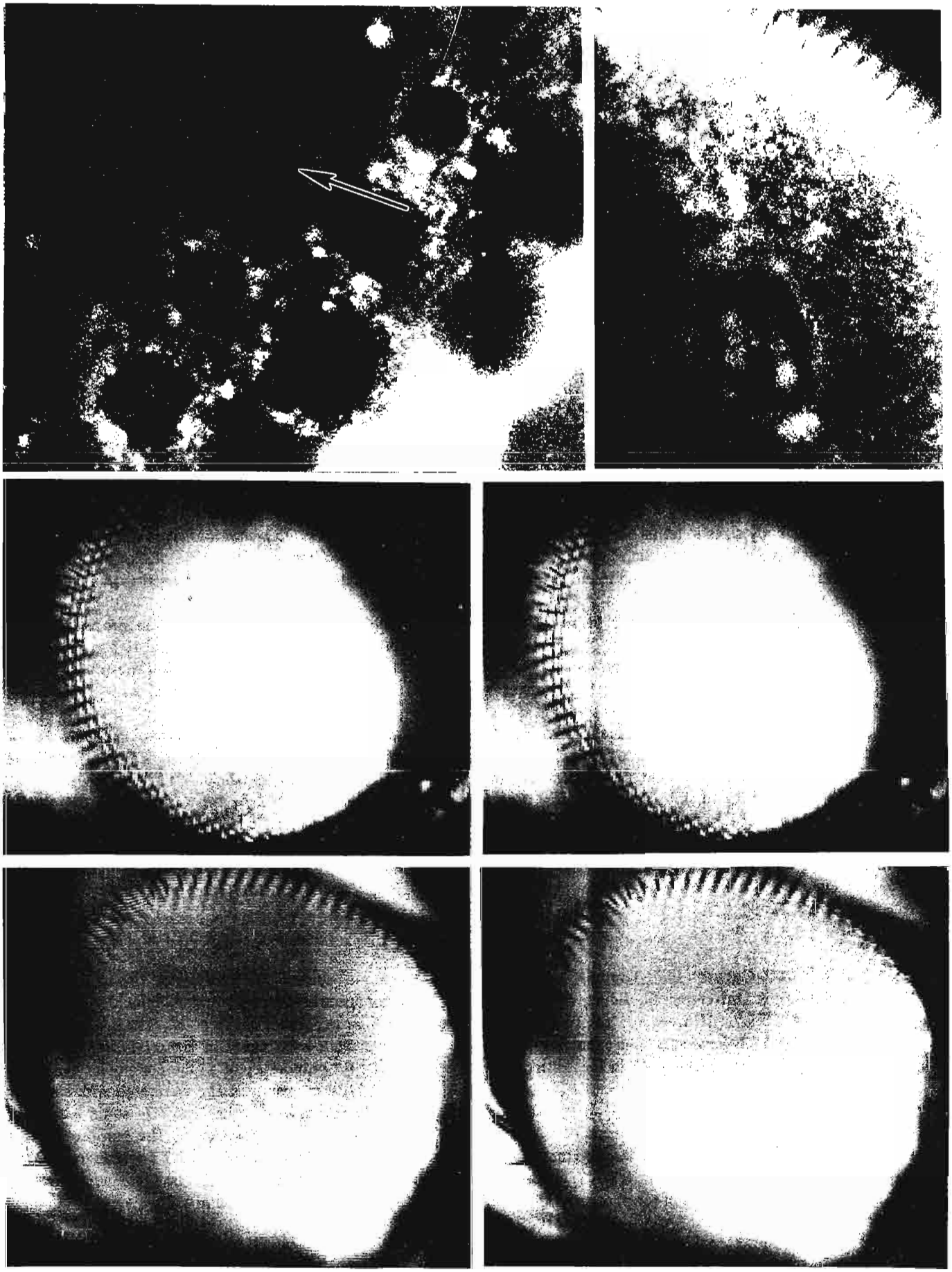
Hunnius, D.J.[1], J.P. Carulli[2], C.F. Thomas[3] and W.S. Stark.[1] [1]Saint Louis University, St. Louis, MO USA. [2]Waltham, MA USA. [3]University of Wisconsin, Madison, WI USA. Microscopy and genetics of a *Drosophila virilis* mutant lacking R7 in the compound eye.

In studies of *Drosophila* opsin phylogeny, receptor spectral sensitivity and compound eye structure (Carulli *et al.*, 1994; Stark *et al.*, 1993; Stark *et al.*, 1994), a white-eyed strain of *Drosophila virilis* which lacks the R7 photoreceptor in the compound eye (here called R7-) was examined and compared with a white-eyed R7+ strain. The white-eyed R7- strain was from the Bowling Green Stock Center. The purposes of this study and report are four-fold: (1) to describe the optical microscopy which made the presence of R7 a visible trait, (2) to determine the mode of inheritance of R7-, (3) to reestablish the white-eyed R7+ strain lost since the first studies, and (4) to present confocal microscopy of the photoreceptor autofluorescence made possible by our new white-eyed R7+ strain.



The deep pseudopupil was viewed as described in many of this laboratory's studies since the introduction of the well-known sevenless mutant (Harris *et al.*, 1976). The conversion of R1-6 rhodopsin to metarhodopsin in white eyes could be viewed and photographed to enhance the decision of presence or absence of R7 (Stark *and* Johnson, 1980). The accompanying photographs show white-eyed R7+ (top row) and R7- (second row) eyes without (left) *vs.* with (right) blue light induced rhodopsin to metarhodopsin conversion.

To determine the mode of inheritance of R7- in *Drosophila virilis*, as well as to determine whether this mutation is X-linked or autosomal, a series of crosses were performed using an X-linked white-eyed mutation to mark the X chromosome. White-eyed, R7- males were crossed with virgin red-eyed, R7+ females. This cross yielded no R7- flies, suggesting that the mutation is recessive. Virgin F1 females from this cross were backcrossed to white-eyed R7- males, and the female progeny of this cross included roughly equal numbers of white-eyed R7-, white-eyed R7+, red-eyed R7- and red-eyed R7+ individuals. This suggests that the R7- mutant is autosomal rather than X-linked. Had the mutation been X-linked, an excess of white-eyed R7+ females would have been expected from this cross. A number of mutations of *Drosophila melanogaster* result in an R7- phenotype, including sevenless, bride of sevenless, son of sevenless, etc. The genetic studies may help to identify evolutionary homology between the R7- mutation in *Drosophila virilis* and one of the known R7- mutations in *Drosophila melanogaster*.

Repeated selections and single pair crosses using the deep pseudopupil optics on etherized flies were necessary to re-establish a white-eyed R7+ strain. Our immediate motivation for having a white-eyed *Drosophila virilis* stock on hand was to study R7 autofluorescence and multiciplicity in the laser scanning confocal microscope. *Drosophila melanogaster* photoreceptor autofluorescence (Stark *et al.*, 1977) was recently examined in the confocal microscope (Lee *et al.*, 1996). Importantly, white eyes were found to be optimal for receptor visualization since fluorescence of eye color pigments obscured receptor imaging. Using *Drosophila virilis*, we replicate the following conclusions from the Lee *et al.* (1996) study: (1) R1-6 and R7 were clearly visible in the deep pseudopupil using fluorescein optics (third row, left); and (2) using immersion oil to optically neutralize the cornea, some R7s appeared dim (arrow) while most appeared bright (third row, right). Focusing through depth (a "z-series"), possible because of the low depth of field (optical sectioning) in the confocal microscope, verified that the dim R7s were not a plane of focus artifact. Similarity of R7 receptor multiplicity in *Drosophila virilis* and *Drosophila melanogaster* is of interest because these species diverged approximately 60 million years ago (Carulli *et al.*, 1994).

Acknowledgments: Supported by NIH grant EY07192 to WSS and DRR-570 at the Integrative Microscopy Resource (IMR) at the University of Wisconsin.

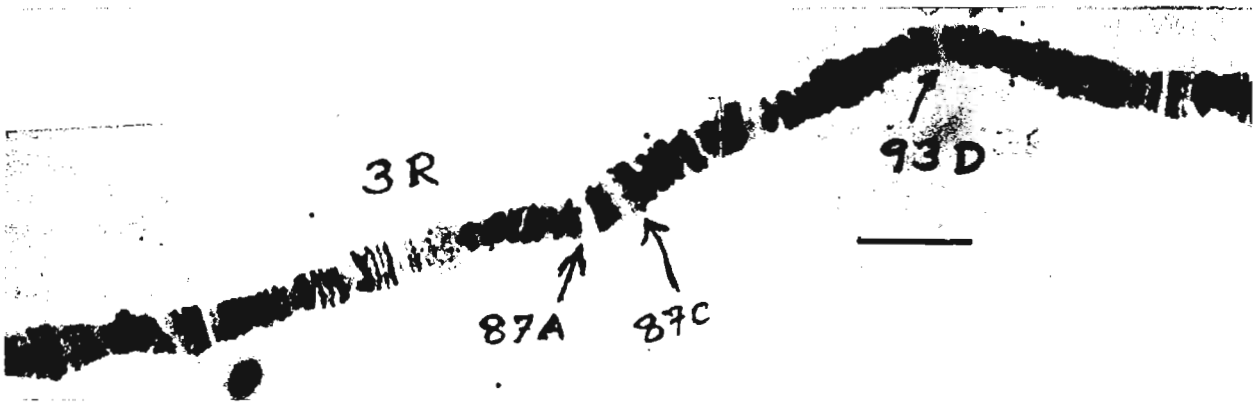
References: Carulli, J.P., *et al.*, 1994, J. Mol. Evol. 38:250-262; Harris, W.A., *et al.*, 1976, J. Physiol. (Lond.) 256:415-439; Lee, R.D., *et al.*, 1996, Micros. Res. Tech. in press; Stark, W.S., *et al.*, 1993, Dros. Inf. Serv. 72:164-167; Stark, W.S., *et al.*, 1994, Dros. Inf. Serv. 75:102-103; Stark, W.S., *et al.*, 1977, J. Comp. Physiol. 121:289-305; Stark, W.S., and M.A. Johnson 1980, J. Comp. Physiol. 140:275-286.

Ghosh, P., and A.S. Mukherjee. Genetics Research Unit, Department of Zoology, University of Calcutta, India. Induction of a new round of DNA replication in some major heat shock puffs in the 3R arm of polytene chromosomes of *Drosophila melanogaster* at 37°C.

In response to heat shock, *Drosophila* salivary gland tissues react by the strong activation of a limited number of specific genes (Ritossa, 1962, 1964; Morimoto, 1993; Ashburner, 1969, 1970). It is generally manifested by the appearance of a set of proteins, the heat shock proteins or hsps (Lindquist, 1986; Tissieres, 1974), which is presumed to ensure survival under stressful conditions by involvement in damage protection or damage repair due to their action as molecular chaperons (Morimoto, 1990, 1993). The Hsps are also proposed to protect cells depleted in ATP from rapid necrotic death by inhibiting the aggregation of cytoskeletal proteins (Kabakov and Gabai, 1994). However, the overwhelming multiplicity of the stress inducers have presented a challenge to the search for a common stress signal transduction pathway. Despite the fact that a large amount of work has been done on the molecular biological aspects, not much headway has been done toward the replicative organization of these novel ubiquitous genes. No significant works have been done on DNA replication pattern in *Drosophila* polytene chromosomes after thermal stress. For this the present investigation has been undertaken to know the replication status of the heat shock genes at thermal stress. In our present investigation it has been revealed that a few specific chromosomal sites (87A, 87C, 93D, of the right arm of the third chromosome) of *Drosophila melanogaster* puffed out upon a thermal stress of 37°C, which remain almost unpuffed in normal physiological condition. This result corroborates the earlier findings of Ritossa (1962). ^{3}H-thymidine autoradiography of salivary gland nuclei after thermal shock (37°C) reveals that the physical stress evokes a new round of DNA replication at these heat shock puffs at late "S" phase where normal replication cycle is going to be terminated.

Figure 1a. Photomicrographs of ^{3}H thymidine autoradiogram of polytene chromosome of *Drosophila melanogaster* salivary gland nuclei after heat shock treatment. The right arm of the third chromosome is presented here.

Figure 1b. Photomicrograph of polytene chromosome of *Drosophila melanogaster* salivary gland nucleus in normal physiological condition. The right arm of the third chromosome is presented here. scale = 10 μm.



Such type of novel replication (secondary initiation or re-initiation) was already described in *Drosophila hydei* polytene tissues by Chatterjee (1982) in an experimental situation where salivary glands of *Drosophila* were pretreated with an antibiotic puromycin. We have also noticed the inducing effect of the same drug on polytene chromosomes in *Drosophila melanogaster* (Ghosh, 1996). In the case of heat shock treated nuclei, all the three heat shock puff sites, the 3H-TdR induced silver grains were considerably higher in comparison to other developmental puffs (50CD selected as internal control) and the same puff (50CD) in control conditions (25°C) (Table 2; Figure 1a, b).

The transcriptional profile of the heat shocked polytene nuclei after ^{3}H-uridine autoradiography reveals that these heat shock puffs have been shown to be competent to transcribe RNA actively, which corroborates the earlier findings of Ritossa (1964).

From the results, it can be concluded that the thermal stress may invoke an unconventional replication (secondary initiation) in the heat shock puff sites of *Drosophila melanogaster*.

Table 1. Frequency of ^{3}H-thymidine labelling over the three heat shock puff sites (87A, 87C, 93D) and on 50 CD (developmental puff) of *Drosophila melanogaster* polytene chromosome in control and after heat shock treatment.

Puff sites	Early pattern				Late pattern			
	Control		Heat Shock		Control		Heat Shock	
	♂	♀	♂	♀	♂	♀	♂	♀
87A	10	12	4	5	14	15	80	82
87C	11	14	4	6	12	16	82	86
93D	50	52	6	8	10	10	60	62
50 CD	60	62	4	6	6	8	20	22

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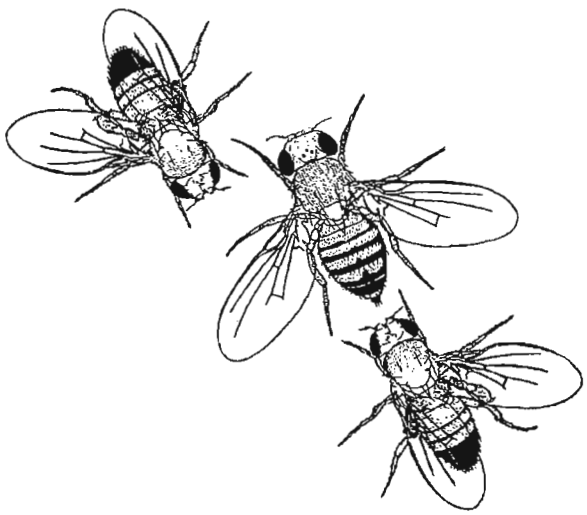
References: Ashburner, M., and J.J. Bonner 1979, Cell 17: 241-254; Ashburner, M., 1970,

Table 2. Data on the ^{3}H-thymidine incorporation into the heat inducible puff sites (87A, 87C, 93D and 50 CD) in the polytene chromosomes of the two sexes in control and heat shocked salivary gland nuclei of *Drosophila melanogaster.*

Heat induced puff sites	Males		Females	
	Control	Treated	Control	Treated
87A	4.0 ± 0.2	12.8 ±1.38	4.2 ± 0.3	13.6 ± 0.75
87C	4.2 ± 0.3	13.9 ± 0.2	4.4 ± 0.1	14.2 ± 0.2
93D	4.1 ± 0.4	9.5 ± 0.3	4.6 ± 0.1	10.2 ± 0.2
Developmental puff (Non heat inducible) site				
50CD	4.0 ± 0.2	5.0 ± 0.2	4.8 ± 0.2	5.4 ± 0.3

The mean grain number was counted in each case by 20 observation of labelled (^{3}H-thymidine) nuclei at advanced (late) 'S' phase.

Chromosoma 31: 356-376; Chatterjee, C., 1983, PhD Thesis; Ghosh, P., 1995, PhD Thesis; Kabakov, A.E., and V.L. Gabai 1994, Cell Biol. 4: 193-195; Lindquist, S., 1986, Ann. Rev. Biochem. 55: 1151-1191; Morimoto, R.I., A. Tissieres, and C. Georgopoulos 1990, New York: Cold Spring Harbor Laboratory Press; Morimoto, R.I., 1993, Science 259: 1409-1410; Ritossa, F.M., 1962, Experientia 18: 571-573; Ritossa, F.M., 1964, Exp. Cell Res. 36: 515-523; Tissieres, A., H.K. Mitchell, and U.M. Tracy 1974, J. Mol. Biol. 84: 389-398.



Guide to Authors

Drosophila Information Service prints short research and technique articles, descriptions of new mutations, stock lists, directory information, and other material of general interest to *Drosophila* researchers. The current publication schedule for regular issues is annually in late summer/early fall. To meet this target date, the deadline for submission of materials is typically 30 April. Later submissions can occasionally be accommodated by contacting the editor by email or telephone. Special issues will also be prepared on an irregular schedule.

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

Submission: Submissions are accepted at any time, but the deadline for the annual issue will be about 30 April or until the issue is full. To help minimize editorial costs, proofs will not be sent to authors unless there is some question that needs to be clarified or they are specifically requested by the authors at the time of submission. The editor reserves the right to make minor grammatical and stylistic changes if necessary to conform to DIS format.

Manuscripts should be submitted in duplicate. If possible, a 3.5" or 5.25" diskette should also be sent with the manuscript in Microsoft Word, ASCII, WordPerfect or other common word-processing format. This improves the speed and accuracy of preparing manuscripts and is always greatly appreciated.

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

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

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

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

Drosophila Information Newsletter (DIN)

We all owe a debt of gratitude to the excellent work done by Carl Thummel (Department of Human Genetics, Eccles Institute, University of Utah) and Kathy Matthews (Department of Biology, Indiana University) in developing the electronic forum for exchanging information. DIN has stopped at volume 20, and its editors encourage everyone to begin making use of the bionet.drosophila newsgroup in its place.

You can participate in bionet.drosophila using newsreader software on your local system, by an email subscription, or through the archive in FlyBase/News/bionet.drosophila. If you want an email subscription, send a message to biosci-server@net.bio.net with the following line in the body of the message:

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Mutation Notes - Drosophila melanogaster

Report of Michael Bender.
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New lethal loci in the 42AB region of the *Drosophila melanogaster* 2nd chromosome.

Twenty mutations lethal over *Df(2R)20B* (42A10-12 to 42B1, R. Kreber and B. Ganetzky, personal communication) were isolated in an F2 EMS screen for new *EcR* alleles. Five of these proved to map to the *EcR* gene and are described elsewhere (Bender *et al.,* manuscript in preparation). Each of the remaining mutants was tested for complementation to all other lethals in the group and could be placed in one of three new complementation groups. One member of each of the four groups within *Df(2R)20B* was complementation tested to other 42A region deficiencies (provided by R. Kreber and B. Ganetzky) to generate the map shown in Figure 1. Deletion mapping showed that *EcR* is the most proximal group within *Df(2R)20B*. The proximal to distal order of groups 6G1, 5CC1 and 4LL1 is not known. It is likely that we are near saturation for lethal loci within this deficiency because we have isolated multiple alleles in all but one of the complementation groups described here.

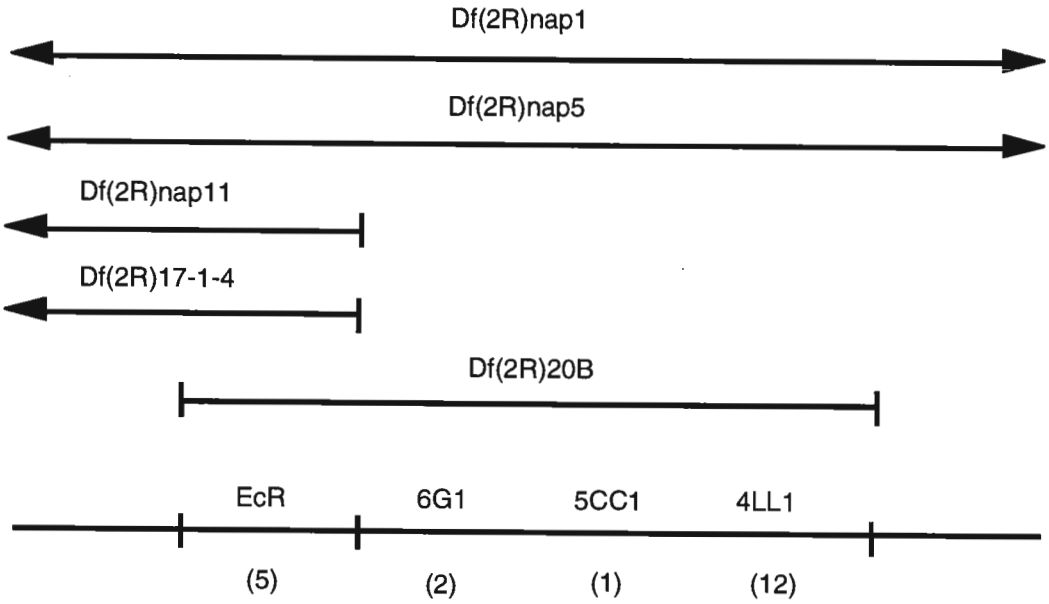


Figure 1. Deletion mapping of lethal mutations in 42AB. Four lethal complementation groups are shown at bottom. The number of alleles in each group is shown in parentheses. The extent of chromosomal deletions is shown by bars at top.

Acknowledgements: I thank R. Kreber and B. Ganetzky for providing 42A deficiencies prior to publication, D.S. Hogness, in whose laboratory this work was initiated, and the University of Georgia Research Foundation for support of this work.

Report of Zimm, G.G., and D.L. Lindsley. Department of Biology, University of California, San Diego, La Jolla, CA 92093-0322. *Upturned* revisited with description of a new allele.

Among the progeny of phenotypically wild-type males treated with ethyl methanesulfonate, one fly with curly wings was recovered; the phenotype was subsequently inherited as a dominant mutation that segregated with chromosome 2; crossing over with *al b c px sp* was normal, and no straight-winged recombinants with *c* (2-75.5) were recovered among 161 testcross progeny. This result places the new mutation in the vicinity of *Upturned* (*U*). The allele

U^1 is associated with a pericentric inversion with breakpoints at 40F and 53A according to Bridges and Li (Morgan, Bridges, and Schultz, 1935). The genetic map position originally given for *U* was 70± based on the mapping of a putative second allele, U^{H20}, isolated by Tanaka (1937); more recently Ashburner has revised this position to 2-[76], based on the 53A breakpoint of *In(2LR)*U^1 (FlyBase, 1994). *In(2LR)* U^1 is homozygous lethal, and U^{H20}, now lost, was homozygous viable. The new mutation is also homozygous lethal, but it survives in heterozygous combination with U^1. If the new mutation is an allele of *U*, then at least one of the above two is not a lethal allele. To clarify this ambiguity, we generated an X-ray-induced revertant of U^1 and found that it is lethal in combination with the new mutant; thus we conclude that the new mutation is a lethal allele of *U*, whereas U^1 is a viable allele with a lethal mutation elsewhere on the chromosome; we designate the new allele U^2. U^1/U^2, U^1/Cy , and U^2 */Cy* flies show an additive effect of the curly-wing phenotype.

We have been unsuccessful in finding a deficiency that uncovers the lethality of U^2 . The fact that neither four deficiencies for *c* at 52D1-7 (Davis and MacIntyre, 1988) nor two for *Khc* at 52F5-53A1 (Saxton, pers. comm.; FlyBase, 1994) extend far enough to the right to include 53A, coupled with our inability to establish stocks of nearly all X ray-induced revertants of either U^1 or U^2, implies the existence of haplo-insufficiency that is coincident with or closely linked to *Upturned.* No duplications are available to cover the 53A1 breakpoint of *In(2LR)*U^1.

The viability of U^1 over *Df(2L)C'*, a deficiency for the 2L heterochromatin (Hilliker and Holm, 1975), indicates that the lethal mutation in *In(2LR)*U^1 is not associated with the breakpoint at 40F. Furthermore, we were unable to cover the lethality of U^1 in male homozygotes with *Dp(2;Y)G*, a duplication that includes the 40F salivary region.

In our hands U^1 (but not U^2) exhibits reduced penetrance, with its expression depending on genetic background and in general being less reliable in males than in females. In the original description (Bridges, 1935), U^1 was said to cause, in addition to upwardly curled wings, crossed posterior scutellar bristles, darker than normal body color with dark waxy wings, and eyes mottled with light flecks. Of these phenotypes only upturned wings, crossed posterior scutellars, and darker than normal body color are currently observed in U^1 and U^2 heterozygotes and U^1/U^2 heteroalleles. Since U^1 is associated with a pericentric inversion with one break in proximal 2L heterochromatin, we speculated that perhaps U^1 was originally recovered in combination with a *lt*-bearing homologue and that the eye mottling was variegation associated with removal of the *lt* locus from its normal heterochromatic to a distal euchromatic environment in *In(2LR)*U^1; U^1/lt flies exhibit variegated eyes in addition to *upturned* wings, crossed postscutellar bristles, and darker body color.

References: Bridges, 1935, Dros. Inf. Serv. 3: 5-19; Bridges, 1937, Dros. Inf. Serv. 7: 5-17; Davis and MacIntyre, 1988, Genetics 120: 755-66; FlyBase, 1994, Nucleic Acids Res. 22: 3456-58; Hilliker and Holm, 1975, Genetics 81: 905-21; Morgan *et al.,* 1935-36 , Year Book - Carnegie Inst. Washington 35: 289-97; Saxton, pers. comm.; Tanaka, 1937, Dros. Inf. Serv. 8: 11.

Report by Ruplekha Sur, Sudipa Basu, Saswati Ghosh, and A.S. Mukherjee.
Genetics Research Unit, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700019, India.

Mutations in *Drosophila* induced by P-element mediated mutagenesis.

P-lac W was mobilized from the X chromosome to autosomes in several independent lines, following the technique of Bier *et al.* (1989). These lines were scored for P(lwB) mediated insertional mutations with easily detectable phenotypic expressions. Four such interesting mutations were observed, which have not yet been genetically mapped.

1. *ecl*: *eclipse* (Figure 1)
   location: 2- (not located)
   origin: P-element induced
   discoverer: R. Sur, 1995
   phenotype: All heterozygous males and females with anterior half of the eye orange and posterior half of the eye white. All homozygous flies with anterior half of the eye red and posterior half of the eye white. Not somatic variegation. The ratio of males and females is 1:1 and all the flies are completely viable and fertile. Penetrance 100%. RK1.

2. *air*: *aircraft* (Figure 2)
   location: 2- (not located)
   origin: P-element induced

discoverer: R. Sur and S. Basu, 1995

phenotype: Homozygous flies with wings held out at an angle of 45-60° to longitudinal axis of the body. The flies are completely viable and fertile but are unable to fly. Penetrance 100%. RK1.

1

2

Figures 1 and 2.

3. *fs(2)R: female sterile (2) of Ruplekha*

location: 2- (unmapped

origin: P-element induced

discoverer: R. Sur, 1995

phenotype: Homozygous females are sterile and exhibit low viability in comparison with heterozygous females. Homozygous females are morphologically normal but with extremely low fecundity. Eggs laid by homozygous females

sometimes survive up to a maximum of 23 hours after which they die and turn brown in colour; no larva is observed. Homozygous males, generated by crossing heterozygous males and females, and heterozygous males and females are completely viable and fertile, while homozygous females are sterile. The genitalia and ovary of homozygous females appear to be normal. Viability and sterility of homozygous females are not affected by a change of temperature between 18°C and 25°C. RK2.

4. *afl^{ts}*: *autosomal female lethal*
location: 2- (unmapped)
origin: P-element induced
discoverer: R. Sur, S. Basu, and S. Ghosh, 1995
phenotype: Most homozygous flies die as embryos or larvae, with rare escapers. Escapers are semi-sterile. Lethality increases in crowded condition. *afl^{ts}* mutants are temperature sensitive; viability of homozygotes increases with a decrease in temperature. At 25°C viability of females is 0.19% and that of males is 0.74%. At 22°C the viability of females is 1.09% and that of males is 2.04%. At 18°C the viabilities of females and males are 2.7% and 6.3%, respectively. The viability of homozygous females is always lower than that of homozygous males (Figure 3). The escapers are morphologically normal.

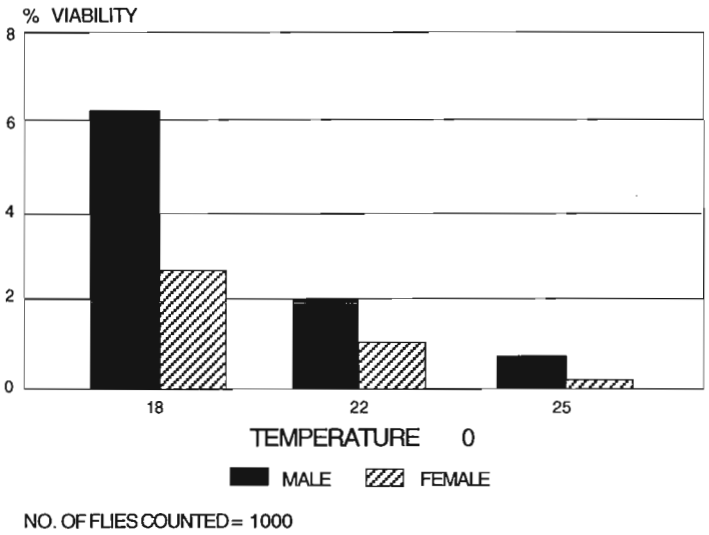


Figure 3. Histogram showing percent viability of afl^{ts}/ afl^{ts} at different temperatures.

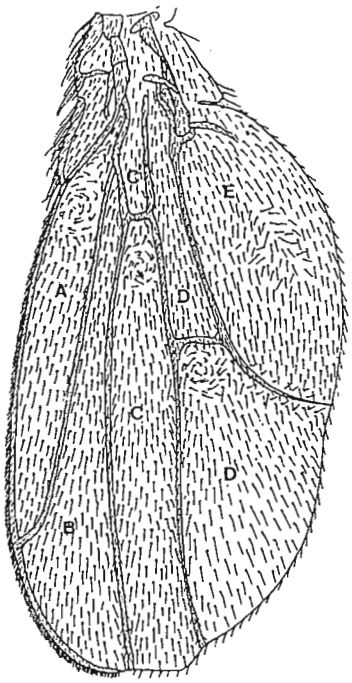
References: Bier, E., H. Vaessin, S. Shepherd, K. Lee, K. McCall, S. Barbel, L. Ackerman, R. Caretto, U. Tadashi, E. Grell, L.Y. Jan, and Y.N. Jan 1989, Genes and Develop. 8: 1273-1287.

Report of Samuel Moyer.
Department of Biology, Burlington County College, Pemberton, NJ.
flightless gene on the second chromosome

fll2: flightless-2nd
location: 2-100.5
origin: Spontaneous
discoverer: Moyer, 1974
phenotype: Unable to sustain wing beat, can jump once every few seconds if stimulated. Normal wings and use for courtship and preening; nearly normal running. Better fitness than vestigial wings after F2 cultures. Needs further investigation of muscles and biochemistry. Preliminary study found no gross muscle abnormalities; muscles receive signal and action potential for a short, fast burst. Might involve structural proteins of flight muscle (Mogami and Hotta, 1981, Mole. Gen. Genet. 183: 409).

Report of R. E. Pacella Norman and N. van Schaik.
Department of Genetics, University of Witwatersrand, Johannesburg, South Africa.
paisley

An irregular whorling in the pattern of wing hairs was found to be present in the Oregon R Resistant (ORR) strain [derived from an Oregon R line by Dapkus and Merrell (1977) through recurrent selection for resistance to DDT]. Adler (1992) suggests that an alteration in the orientation of the trichomes may be due to mutations in genes that disrupt the



polarity of the wing hairs. Whorling occurs spontaneously on wings of practically all ORR flies and varies with temperature with higher expression at 18°C than at 25-29°C. Whorling occurs more often on wings of female flies than male flies. The whorling is either a spiralling arrangement of the trichomes (as seen in the proximal part of the marginal cell, and in restricted fields of the first, second, and third posterior cells) or a disarrangement of the wing hairs along veins L4 and L5 (Figure 1). An increase in the number of cells with two hairs (two hair spots) was observed on these wing blades. the gene responsible for this whorling effect was characterized and named paisley (*ply*). It was shown to be recessive and located around position 89.6-90 on chromosome 2.

ply: paisley
origin: spontaneous in Oregon R-R flies selected for resistance to DDT
location: 2-89.6-90

References: Adler, P.N. 1992, BioEssays 14: 735-741; Dapkus, J., and D.J. Merrell 1977, Genetics 87: 685-697.

Figure 1. Diagramatic representation of wing blade of *Drosophila melanogaster* showing the different sectors where whorling of the trichomes most often occurs.

Mutation Notes - Other Species

Report of R. Banerjee and B.N. Singh.
Department of Zoology, Banaras Hindu University, Varanasi, India.

A spontaneous mutation in *Drosophila bipectinata.*

Studies on population and behavior genetics of *Drosophila bipectinata* have been initiated in our laboratory (Singh and Chatterjee, 1991; Singh and Pandey, 1991; Singh and Das, 1991; Das and Singh, 1992; Srivastava and Singh, 1993a, b; Singh and Sisodia, 1995, 1996; Sisodia and Singh, 1996a, b; Singh and Banerjee, 1995; Banerjee and Singh, 1995). Two spontaneous mutations in *D. bipectinata* (*se* eye color - autosomal recessive and *ct* wing sex-linked recessive) were detected in laboratory stocks raised from flies collected from different localities which have already been described (Singh *et al.*, 1995a). In the *se* mutant stock, several flies with bilateral outgrowths on the thorax were detected which appeared to be unique phenotypic change in *D. bipectinata* caused due to mutation, but its exact nature could not be understood from the results of different crosses (Singh *et al.*, 1995b).

We detected a spontaneous mutation (black body color) in F_1 progeny resulting from the cross involving Trivandrum and Kottayam stocks of *D. bipectinata.* Several flies showing black body color in the F_1 generation of this cross were found and a separate laboratory stock of this new mutation could be established which is being maintained in our laboratory. The mutant stock was crossed reciprocally with a wild stock and in the F_1 all the flies of both crosses were normal in body color. In both the crosses, nearly a 3:1 ratio between normal and mutant flies was found in the F_2 generation, which indicates that black body color in *D. bipectinata* is a recessive autosomal mutation.

Acknowledgments: Financial support from the UGC, New Delhi, in the form of a research project to BNS and a JRF to RB is gratefully acknowledged.

References: Banerjee, R., and B.N. Singh 1995, Proc. Zool. Soc. (Calcutta) 47: 125-133; Das, A., and B.N. Singh 1992, Korean J. Genet. 14: 173-178; Singh, B.N., and R. Banerjee 1995, Cytobios, in press; Singh, B.N., and S. Chatterjee 1991, Genome 34: 849-852; Singh, B.N., and A. Das 1991, Biol. Zent bl. 110: 157-162; Singh, B.N., and

M.B. Pandey 1991, Ind. J. Exp. Biol. 29: 926-929; Singh, B.N., and S. Sisodia 1995, Biol. Zent bl. 114: 95-101; Singh, B.N., and S. Sisodia 1996, Biol. Zent bl. 115: in press; Singh, B.N., S. Sisodia and R. Banerjee 1995a, Dros. Inf. Serv. 76: 83-84; Singh, B.N., S. Sisodia, and R. Banerjee 1995b, Current Science 69: 771-772; Sisodia, S., and B.N. Singh 1996a, Braz. J. Genet., in press; Sisodia, S., and B.N. Singh 1996b, Zool. Studies 35: in press; Srivastava, T., and B.N. Singh 1993a, Ind. J. Exp. Biol. 31: 460-462; Srivastava, T., and B.N. Singh 1993b, Evol. Biol. 7: 193-205.

Report of F. Mestres.

Dept. de Genètica, Facultat de Biologia, Universitat de Barcelona, Spain.

Spontaneous *yellow* mutation in *Drosophila subobscura.*

Yellow mutant flies spontaneously arose in a stock of *D. subobscura* kept in the laboratory for nine years. This stock has a lethal O chromosome obtained in the natural population of Gilroy (California) maintained with the balancer chromosome

The *yellow* mutation is recessive, located in the A (sexual) chromosome and has been previously described in *D. subobscura* (Krimbas, 1993).

References: Krimbas, C.B., 1993, Drosophila subobscura. *Biology, Genetics, and Inversion Polymorphism.* Verlag Dr. Kovac, Hamburg.

Report of M. Pascual and F. Mestres.

Dept. de Genètica, Facultat de Biologia, Universitat de Barcelona, Spain.

List of mutants of *Drosophila subobscura.*

Va/Ba: O chromosome balanced lethal strain (Sperlich *et al.*, 1977). It carries the dominant mutants *Varicose* (irregular thickening at the junctions of the wing and short irregular side branches to the veins; lethal in homozygous condition) and *Bare* (the number of macrochaetes and rarely microchaetes is variably reduced but bristle sockets are present; lethal in homozygous condition). More details of this strain are presented in Mestres *et al.* (1990) and some particularities are described in Mestres and Busquets (1991) and Orengo and Mestres (1993).

ch cu: Homokaryotypic strain (O_{3+4}, E_{st}, J_{st}, U_{st} and A_{st}). This strain carries the recessive mutants *cherry* (bright red eye color) and *curled* (wings curled concave upwards) both located on the O chromosome (Mestres *et al.*, 1995).

af: Mutation characterized by the fusion of the first two proximal articles of the first pair of legs. It is recessive and located in the U chromosome. Other abnormalities are associated with this mutant: interruption of some longitudinal or transverse veins, twisted legs, reduction in the number of legs and extra articles (Mestres, 1985). The mutant strain is homokaryotypic: O_{3+4}, U_{st}, E_{st}, J_1 and A_{st} (Mestres and Pegueroles, 1987).

sL4L5: Veins L4 and L5 don't reach wing margin due to this recessive mutation (Mestres, 1993). Most of the homozygous flies for this mutant present wings upward being perpendicular to the longitudinal body axis. The mutation is located in the O chromosome.

scc: Recessive mutation producing curved sex combs. The proximal sex comb is twisted and curved 45 degrees and the number of teeth is seldom reduced. The trait presents variable expressivity. Few individuals were detected with both sex combs affected. The mutation is located in the A (sexual) chromosome (Pascual and Mestres, 1995).

References: Sperlich, D., H. Feuerbach-Mravlag, P. Lange, A. Michaelidis, A. Pentzos-Daponte 1977, Genetics 86:835-848; Mestres, F., G. Pegueroles, A. Prevosti and L. Serra 1990, Evolution 44:1823-1836; Mestres, F. and M.D. Busquets 1991, Dros. Inf. Serv. 70:145-146; Orengo, D.J. and F. Mestres 1993, Rev. Bras. Genet. 16:471-475; Mestres, F., J. Balanyà, C. Segarra, A. Prevosti and L. Serra 1992, Evolution 46:1564-1568; Mestres, F., L. Serra and F.J. Ayala 1995, Genetics 140:1297-1305; Mestres, F. 1985, Dros. Inf. Serv. 61:214-215; Mestres, F. and G. Pegueroles 1987, Dros. Inf. Serv. 66:190-191; Mestres, F. 1993, Dros. Inf. Serv. 72:70-71; Pascual, M. and F. Mestres 1995, Dros. Inf. Serv. 76:82-83.

Technique Notes

Weisser, Wolfgang W., and Gebreselassi Asefa. Department of Biology, Imperial College at Silwood Park, Ascot, Berks. SL5 7PY, U.K. The effects of propionic acid on fly behaviour and a medium for long-term experiments with *Drosophila* and *Drosophila*-parasitoids.

Introduction: Long-term experiments with *Drosophila* often encounter problems with fungal infections. To control moulds growing on culture media, Nipagin (Methyl-paraben or p-Hydroxy-benzoic acid methyl ester) is commonly added as a broad-spectrum fungicide. However, in our long-term experiments, Nipagin proved inefficient against infection by *Aspergillus niger* (van Tiegh). The fungus overgrows the culture medium, thereby killing all fly offspring. Ashburner and Thompson (1978) recommend adding Propionic Acid (PA) to the medium in doses of 12-40 mM undissociated acid. However, in our experiment, PA in doses of 13 mM and 8 mM cleared fungal infections but led to a drastic decline in the densities of *Drosophila ananassae* (Dol.) and *Leptopilina victoriae* (Nordlander) (Hymenoptera: Eucoilidae). We therefore started a series of experiments to optimise the use of PA with respect to mould prevention and fly performance.

Material and Methods: *D. ananassae* and *L. victoriae* were originally collected in the Philippines and subsequently reared in half-pint milk bottles at 25°C, RH 70%, L:D 12:12. Kalmus medium was prepared as food for the flies as follows: 20 g Agar, 35 g dry baker's yeast, 50 g sugar and 9 g Kalmus-mixture (5 parts tartaric acid, 2 parts ammonium-sulphate, 0.5 parts magnesium-sulphate-7-hydrate, and 1.5 parts potassium-dihydrogen-orthophosphate) are added to 1 litre water and boiled for 2 minutes. 1 g Nipagin is added to the medium after cooling. In our long-term experiments we use 60 ml plastic containers (diameter 3.7 cm, height 5.5 cm) as 'patches' for fly and parasitoid reproduction (Weisser *et al.* unpubl.). To minimise fly numbers emerging from single patches and to prevent desiccation during the 5 week period during which patches remain in an arena, each patch contains 8 g of Kalmus medium on top of 35 g agar (20 g in 1l water + 1 g Nipagin). 1 g yeast paste (mixture of 20 g dried baker's yeast (ALLINSON, Maidenhead, UK, in 45 ml water) is placed on top of the Kalmus layer.

In experiment A, PA was added to Agar (pH = 6.4) and Kalmus (pH = 2.4) in the following concentrations of undissociated acid: (A1) no PA (control); (A2) 0 ml/litre in Agar, 0.1 ml/litre in Kalmus (= 1.3 mM undissociated PA); (A3) 10 ml/l (4 mM), 0 ml/l Kalmus; (A4) 10 ml/l, 0.1ml/l; (A5) 20 ml/l (8.1 mM), 0.1 ml/l; (A6) 20 ml/l, 0.25 ml/l (3.3 mM); (A7) 20 ml/l, 0.5 ml/l (6.7 mM). Experiment B followed from the results of exp. A. Undissociated PA was added to Agar and Kalmus as follows: (B1) no PA (control); (B2) 5 ml/litre in Agar (2 mM), 0.1 ml/litre in Kalmus; (B3) 5 ml/l, 0.25 ml/l; (B4) 10 ml/l, 0.1 ml/l; (A5) 10 ml/l, 0.25 ml/l; (B6) 15 ml/l (6mM), 0.1 ml/l; (B7) 15 ml/l, 0.25 ml/l. In both experiments, 6 containers were prepared for each treatment, three of which were inoculated with *A. niger*. The remaining pots (21 for 7 treatments) were positioned at random in a fly population cage (containing ca. 300 (A) or 500 (B) flies) free of *A. niger* infection. The number of flies sitting on the medium or the rim of the containers were counted 4 times over the next 3 days. After 1 week the containers were removed from the arena. All flies emerging until 15 days after the start of the experiment were scored. Data was transformed for analysis if appropriate. Numbers in brackets [ ] refer to standard deviation.

Results: Table 1 shows results of experiment A. While the mean number of flies sitting on the rim is the same for all treatments ($F_{6,20} = 0.89$, $p = 0.34$), fewer flies landed on the medium in treatments with higher concentrations of PA ($F_{6,20} = 11.8$, $p < 0.001$). The average number of flies emerging varied between treatments ($F_{6,20} = 6.1$, $p < 0.05$). In the control (A1), *A. niger* overgrew the medium in all 3 replicates within five days. After 10 days, one replicate of treatment (A2) was covered completely by fungus; the others two replicates were 50% covered. No growth of fungus was observed in treatments (A3)-(A7).

In experiment B, there were no difference in the number of flies sitting on the rim between treatments ($F_{6,20} = 2.2$, $p = 0.1$). The control (B1) attracted more flies onto the medium than any other treatment ($F_{6,20} = 15.7$, $p < 0.001$; Tukey test). Emergence data were (B1) 307 [44.2], (B2) 109.3 [78.8], (B3) 56.7 [24.1], (B4) 77.7 [41.5], (B5) 35 [18.5], (B6) 31.3 [34.2], (B7) 24 [11.8]. Means differed significantly ($F_{6,20} = 11.0$, $p < 0.001$), with (B1) having a higher mean than all other treatments (Tukey test). The control (B1) was covered by *A. niger* within 5 days. After 10 days, limited growth was observed in replicates of treatment (B2) but in no other treatment.

Discussion: One effect of PA on *D. ananassae* is that it detracts flies from oviposition, in particular at higher concentrations. Because of the high pH of Agar, concentrations of around 10 mM resulted in a strong vinegar smell, and very few eggs were laid by females. A further effect of PA was to cause the mixture of live baker's yeast with Kalmus to exude water, resulting in difficulties for flies walking and ovipositing onto the medium. In culture bottles with 13 mM PA in Kalmus, the entire medium liquefied after one week, resulting in drowning of flies. On the other hand, the

Table 1. Obervational data (means over 4 observations and 3 replicates) and production of flies in experiment A. Means in rows that share same letters differ significantly at 5% level (Tukey test)

Experiment A	A1	A2	A3	A4	A5	A6	A7
Flies on Rim	4.9	7.5	5.2	7.8	6.8	3.2	6.4
	(3.5)	(4.9)	(3.2)	(3.8)	(6.7)	(3.4)	(2.6)
Flies on Medium	15.8^{a}	16.0^{b}	5.5^{c}	10.5^{d}	$4.0^{a,b}$	2.8^{a-c}	0.3^{a-d}
	(10.5)	(8.8)	(7.0)	(6.2)	(4.9)	(3.8)	(0.9)
Flies emerging	58.7^{a}	101.3^{b}	83.0^{c}	80.3^{d}	53.3^{e}	66.3^{f}	13.3^{b-df}
	(20.1)	(18.2)	(48.7)	(19.5)	(17.0)	21.2)	(7.8)

aggressive growth of *A. niger* makes treatment necessary. PA also diffused from Agar into the Kalmus layer of the experimental containers which explains the effectiveness of treatment (A3) against fungal growth. The difference in the number of flies emerging is therefore primarily a result of fewer visits by flies. We further tested for effects on parasitoids by using containers prepared as in experiment (B) for rearing *L. victoriae*. Parasitoids emerged from all treatments. For our long-term experiments we decided to use 5 ml/l PA for Agar and 0.1 ml/l for Kalmus. These experiments have now (March 1996) been running for 6 months with no obvious effect on flies or parasitoids. We also tested for fitness effects of PA by comparing egg-to-adult survival of flies in pots with PA at these concentrations to survival in pots without PA. In the range of 100-300 eggs (no larval competition, unpubl. data), there was no significant difference between treatments (mean survival 50.4% [12.1] (PA), 56.8% [8.1] (no PA), U = 115, p = 0.0575, n = 30).

Acknowledgments: This study was supported by NERC. Thanks to PM Reader for critical comments.

References: Ashburner M., and J.N. Thompson, Jr 1978, in *The Genetics and Biology of* Drosophila, Vol 2a, pp 2-109, Academic Press, London.

Schmidt, R., and G. Bächli. Zoological Institut, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland. Improved rearing conditions for larvae of *Musca domestica* L.

The wheat bran medium usually used to grow larvae of *Musca domestica* in the laboratory (Dübendorfer, 1971) is suitable for large populations. Since many techniques in develop-mental biology, e.g. transplantation of pole cells, X-ray treatment to induce mitotic recombination, or single pair crosses often reveal only small numbers of larvae, no or only very few adults were obtained from such experiments. Therefore, it was desirable to find a rearing technique with improved larval survival.

In nature, *M. domestica* is found in stables where the larvae develop on manure (West, 1951; Zimin, 1951). Therefore, we compared the suitability of porcine faeces for housefly rearing to that of wheat bran medium. Fresh faeces were obtained from pigs fed with AFL Mini Pig (EBERLE NAFAG AG, Gossau, Switzerland) containing wheat bran, straw, barley, soja extract, powdered hey, sugar-beet, powdered fish, molasse, wheat and corn. The faeces of these pigs are essentially odourless. Wheat bran medium is composed of 480 g wheat bran, 60 g wheat flower, 40 g milk powder, 20 g brewer's yeast, 900 ml of water and 20 ml ethanol containing 0.67 g Nipagin and 1.33 g Nipasol as fungicides. Freshly hatched first instar larvae were put on either wheat bran medium or on porcine faeces and were reared at 23° C and 65% relative humidity at a 12 hrs dark/light cycle. Table 1 shows the average number of adults obtained from larvae grown on the two different media.

Table 1. Average survivor rate of larvae grown on different media.

rearing medium	number of first instar larvae placed on medium	number of adults
wheat bran medium	773	40 (5.2%)
porcine faeces	704	244 (34.7%)

If less than 30 larvae were put on wheat bran medium, only about 3% (n=200) of the larvae survived to adulthood, whereas on pig's faeces about 20% (n = 77) adult flies were obtained. This shows that porcine faeces are about six times more efficient for housefly rearing than wheat bran medium, and that they are particularily suitable for the development of small populations of larvae.

References: Dübendorfer, A., 1971, Roux' Arch. Dev. Biol. 168: 142-168; West, L. S., 1951, *The Housefly, Its Natural History, Medical Importance*, New York; Zimin, L. S., 1951, Fauna USSR 18: No 4.

Resler, A.S., and H.H. Fukui. Department of Ecology, Evolution, and Behavior, University of Minnesota, 100 Ecology, 1987 Upper Buford Circle, St. Paul, MN 55108. Duration of DNA stability using the Engles' extraction method.

Fly DNA preparation for PCR can be simply accomplished using the Engles' method for extraction (Engels *et al.*, 1990). Here we quantify the shelf life of DNA using this method.

Fly stocks were supplied by Dr. Luckinbill of Wayne State University (Luckinbill *et al.*, 1984). Ten *Drosophila* from LB stock (lanes 1-5, 11-15) and ten LD2 (lanes 6-10, 16-20) underwent Engles' DNA preparation method. Single flies were macerated in a 0.5 ml tube for 5-10 seconds with a pipette tip containing 100 µl of a squishing buffer. The squishing buffer contained 10 mM Tris-Cl [pH 8.2], 1 mM EDTA, 25 mM NaCl, and 200 µg/ml Proteinase K. The Proteinase K was from a frozen stock, and 100 µl of the squishing buffer was added to each tube. The tubes were incubated at 37°C for 24 hours. The Proteinase K was inactivated by heating the tubes to 95°C for 2 minutes. The extracts were then centrifuged for 20 seconds and diluted with 100 µl of nanopure distilled water (Engels *et al.*, 1990).

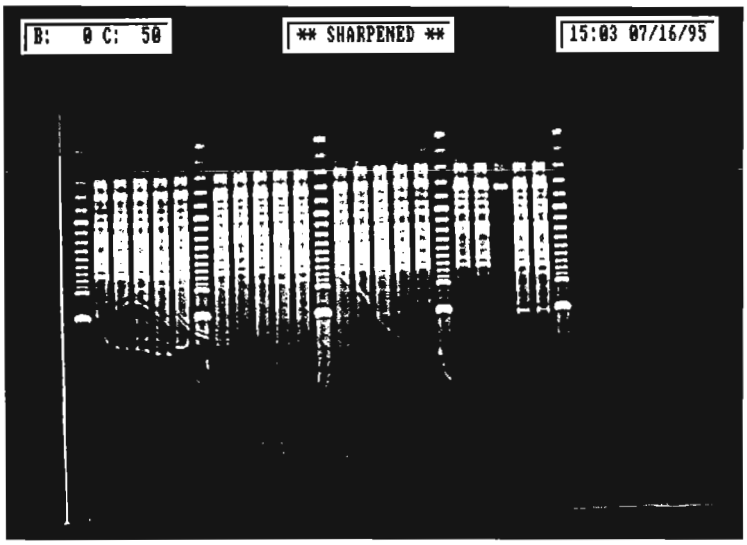


Figure 1. Week 1.

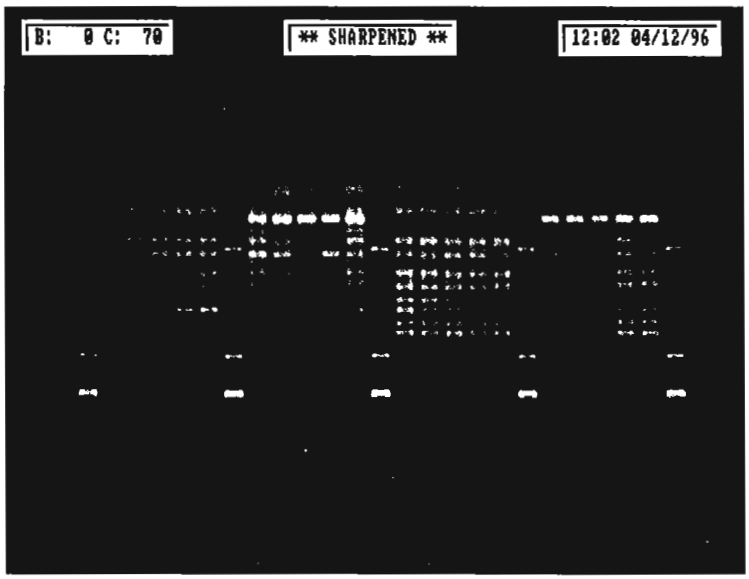


Figure 2. Week 38.

The preparation was stored at 4°C, and PCR was done weekly for 18 weeks and monthly thereafter. Amplification was carried out in a total of 25 µl with 10 mM Tris-HCl [pH 8.3], 50 mM KCl, 5.0 mM $MgCl_2$, 400 µM dNTPs, 0.7 µM of primer (10-mer Operon Technologies, Inc.), 20 units of AmpliTaq DNA polymerase, Stoffel Fragment (Perkin Elmer), and approximately 10 ng of DNA template. The DNA template was centrifuged for 20 seconds before addition into the reaction mixture. Prior to amplification, 28 µl Nujol mineral oil (Sigma) was overlaid on each reaction mixture. DNA amplification suggested by Burt *et al.* (1994) was performed with modification for a Hybaid Omnigene Thermal Cycler. After two initial cycles at 94°C / 36°C / 72°C for 3 min / 3 min / 3 min, the reaction mix was subjected to 40 cycles consisting of 94°C / 36°C / 72°C for 0.5 min / 0.5 min / 1 min. The amplification cycles were followed by a 7 minute final extension at 72°C. The products were resolved by electrophoresis for 3 hours (100 V) in gels composed of 1.4% agarose (Owl Scientific, Inc.) in 1× TAE buffer (0.04M Tris-acetate and 0.001M EDTA). DNA fragments were visualized and photographed under UV light after ethidium bromide staining.

We also compared the use of two different microcentrifuge tubes containing the DNA extracts. The two types were a 0.5 ml cap top microcentrifuge reaction tubes (USA/Scientific Plastics #1405-4400), lanes 1-10, and a 0.5 ml cryovial screw cap tube (Sarstedt #72-730-005), lanes 11-20.

We have found that DNA preparation using the Engles' method has produced consistent PCR-RAPD banding

patterns for at least 38 weeks. We found no difference in functional duration of the DNA between the two tube types due to evaporation or other factors.

Acknowledgments: We thank Jim Curtsinger for comments. This work was supported by National Institutes of Health grants AG 0871 and AG11722 to JWC.

References: Burt, A., D. A. Carter, T. J. White, and J. W. Taylor 1994, Molecular Ecology 3: 523-525; Engels, W. R., D. M. Johnson-Schlitz, W. B. Eggleston, and J. Sved 1990, Cell 62: 515-525; Luckinbill, L. S., R. Arking, M. S. Clare, W. C. Cirocco, and S. A. Buck 1984, Evolution 38(5): 996-1003.

Pacella Norman[1], R.E., N. van Schaik[1], and U. Graf[2]. [1]Department of Genetics, University of the Witwatersrand, Johannesburg, South Africa, and [2]Institute of Toxicology, ETH and University of Zurich, Schwerzenbach, Switzerland. New "High Bioactivation" cross for the SMART wing assay.

When screening toxic chemicals for genotoxicity in short-term tests, it becomes desirable to test them at sub-toxic exposure levels. The problem is that at these non-toxic levels, genotoxic effects will only be detected if the test system is optimally sensitive. The *Drosophila* somatic mutation and recombination wing spot test (SMART) is a convenient and well-validated short-term *in vivo* genotoxicity screening test. In 1989 Frölich and Würgler improved the detection capacity of the SMART assay by constructing high bioactivation (HB) tester strains carrying chromosomes 1 and 2 from the DDT resistant Oregon R (OR-R) strain (Dapkus and Merrell, 1977) conferring the chromosome substitution strains with a high constitutive level of cytochrome P450 enzymes active in bioactivation (Hällström and Bianck, 1985). The gene responsible for high constitutive expression of cytochrome P450 enzymes characteristic of insecticide resistant strains Hikone R and OR-R is presumed to be the same gene responsible for DDT resistance known as the *Rst(2)DDT* (Tsukamoto, 1958) (or *RI*) gene located at position 2-65 (Kikkawa, 1961).

A drawback of the high bioactivation lines is the presence of an irregular whorling of the trichomes on wing blades which interferes with scoring the wing for mutant spots (Graf *et al.*, 1991). The gene responsible for this whorling effect was characterized and named paisley (*ply*). It was shown to be recessive and located around position 90 on chromosome 2 and is thus linked to the *RI* gene responsible for high bioactivation. Graf and van Schaik established and evaluated an improved HB (IHB) cross producing OR-R heterozygous individuals (Graf *et al.*, 1991; Graf and van Schaik, 1992). These have a normal, undisturbed wing hair pattern while exhibiting P450-dependent bioactivation capacity equal to or even slightly higher than that of the original HB cross. The disadvantage of using the hybrid larvae of the IHB cross is that they are heterozygous for *RI* and ply genes. Several events can eliminate the wild type paisley gene (ply^+) resulting in the expression of whorling in certain individuals. Furthermore, the *RI* gene may not be completely dominant and hence *RI* homozygotes may be more sensitive to the effects of promutagens than *RI* heterozygotes. Tests of DDT resistance and results of biochemical assays, as well as the findings of Graf and van Schaik (1992), seem to indicate that the original *ORR*; *mwh* stock does not carry the *RI* gene on chromosome 2, although it may carry resistance alleles on the first chromosome. Hence, progeny from the original HB cross may also be heterozygous for the *RI* gene. New HB strains were constructed that are homozygous for the *RI* gene but whorl-free. This was done by separating the *ply* gene from the *RI* gene. The newly-constructed SMART HB stocks *ORR*; flr^3 and *ORR*; *mwh* were shown to have higher bioactivation capacity and improved sensitivity for the detection of borderline promutagens that the standard cross and IHB cross. AFG2 and sterigmatocystin, which were only detected positive at the high concentrations in the other crosses, were positive at sub-toxic exposure levels in the new HB cross. It was interesting to note that the animal carcinogen, zearalenone, was negative at all concentrations in the SMART standard cross but was detected positive at the two higher concentrations by the new HB cross, demonstrating the improved sensitivity of the new HB cross (Pacella, 1993).

Biochemical assays provided direct evidence that both the newly-constructed stocks *ORR*; flr^3 and *ORR*; *mwh* overexpressed P450 cytochrome-dependent activities. The pNA demethylase activity in the new HB tester strains and original *ORR*; flr^3 strain was similar to that in the OR-R strain (from which they were constructed) and 9-10 fold higher than in the standard and original *ORR*; *mwh* tester strains. The pNA demethylase activity in third instar larvae from the new HB cross was also 5-fold higher than in larval microsomes from the standard cross.

The main advantage of the new HB cross is to combine the high bioactivation capacity of RI homozygous individuals with the ease of scoring the wings using the same criteria as for the standard cross. This cross has, however, been validated with a small number of chemical mutagens and it should, therefore, be used in parallel with the standard cross which has been validated with over 330 chemicals so far. Another drawback of using this cross is the higher spontaneous mutation rate of the HB crosses compared with the standard cross. Promutagens previously reported

negative in Drosophila should now be retested using the sensitive new HB cross to characterize further the properties of this new SMART cross.

References: Pacella, R.E., 1993 Thesis, University of the Witwatersrand, Johannesburg, South Africa; Dapkus, D., and D.J. Merrell 1977, Genetics 87: 685-697; Hällström, I., and A. Blanck 1985, Chem.-Biol. Interact. 56: 173-184; Kikkawa, H., 1961, Annu. Rep. Sci. Works Fac. Sci. Osaka Univ. 9: 1-20; Tsukamoto, M., 1958, Dros. Inf. Serv. 32: 87; Frölich, A., and F.E. Würgler 1989, Mutation Res. 216: 179-187; Graf, U., N. van Schaik, and R.E. Pacella 1991, Dros. Inf. Serv. 70: 247-248; Graf, U., and N. van Schaik 1992, Mutation Res. 271: 59-67.

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Other Announcements

Conference Programs

In an effort to provide as diverse a source of information on *Drosophila* genetic researach as possible, Drosophila Information Service will print programs for research conferences whenever space allows. The editor invites conference organizers or participants to submit copies of meeting programs and a brief description of the theme, location, and time the conference was held. DIS will endeavor to publish the names of authors, the affiliation of the senior author or corresponding presenter, and the title of the talks or posters. This can then be used by readers of DIS to locate individuals pursuing problems of common interest, locate possible postdoctoral researchers, and find sources of materials or information that may not have yet been published in other journals. Even if space does not permit publishing all proceedings, we hope that a listing of regional and national conferences will be useful.

37th Annual *Drosophila* Research Conference
27 April to 1 May 1996, San Diego, California, U.S.A.

The 37th Annual *Drosophila* Research Conference was held at the Town & Country Hotel, San Diego, California, and the 1996 Program Chairs were Sanford I. Bernstein (San Diego State University), Gary H. Karpen (The Salk Institute), and James W. Posakony (University of California, San Diego). The conference was sponsored by the Genetics Society of America, 9650 Rockville Pike, Bethesda, Maryland 20814-3998. Large numbers of slide presentations and posters were presented. The plenary session lectures are listed here, grouped by topic as in the conference program.

Baker, Bruce (Department of Biological Sciences, Stanford University, Stanford, CA 94305). Sex in the 90's: Old acquaintances and new beginnings.

Rose, Michael (Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717). Evolution of aging.

Bienz, Mariann (Laboratory of Molecular Biology, Medical Research Council, Hills Road, Cambridge CB2 2QH, England). Endeoderm induction: Signals and their nuclear targets.

Orr-Weaver, Terry (Department of Biology, Whitehead Institute, MIT, Nine Cambridge Center, Cambridge, MA 02142). Knowing when to let go: Sister-chromatid cohesion.

Wasserman, Steve (Department of Biochemistry, University of Texas SW Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235). Signal transduction in embryonic dorsal-ventral patterning.

Schubiger, Gerold (Department of Zoology, University of Washington, Seattle, WA 98195). Cell determination-transdetermination: Who cares?

Geyer, Pam (Department of Biochemistry, University of Iowa, Bowen Science Bldg., Iowa City, IA 52242). Effects of the suppressor of Hairy Wing insulator protein on gene expression.

McKeown, Michael (Department of MBLV, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037-1099). Nuclear receptor interactions in growth and differentiation.

Sehgal, Amita (Neurosciences, University of Pennsylvannia Medical School, 233 Stemmler Hall, Philadelphia, PA 19104). Role of the timeless gene in the circadian clock.

McKearin, Dennis (Department of Biochemistry, University of Texas SW Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235). Regulation of germ cell differentiation.

Heberlein, Ulrike (Gallo Center, Neurology Department, University of California, San Francisco, Bldg., 1, Rm. 101, SFGH, San Francisco, CA 94110). Morphogenesis of the *Drosophila* retina: How hedgehog pushes the furrow.

Taylor, Barbara (Department of Zoology, Oregon State University, 3029 Cordley Hall, Corvallis, OR 97331). Sex, flies, and videotape: Genetic regulation of sexual differentiation in the CNS.

Doe, Chris (Department of Cell and Structural Biology, University of Illinois, HHMI, 505 South Goodwin Avenue, Urbana, IL 61801). Asymmetric determinants and the specification of CNS cell fates.

Steward, Ruth (Department of Molecular Biology and Biochemistry, Waksman Institute, Rutgers University, P.O.Box 759, Piscataway, NJ, 08855). Nuclear targeting of dorsal protein.

SPECIAL KEYNOTE ADDRESS:

Wieschaus, Eric (Nobel Laureate; Department of Molecular Biology, Princeton University, Washington Road, Princeton, NJ 08542). Embryonic transcription and the control of development pathways.

WORKSHOPS:

Molecular Biology of Ecdysone Response
Chair: Linda L. Restifo (ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721).

Evolution and Development
Chair: Susan Brown (Division of Biology, Kansas State University, Manhattan, KS 66506).

Immunity
Chair: Deborah Kimbrell (Department of Biology, University of Houston, Houston, TX 77204).

RNA Processing
Chair: Stephen Mount (Department of Zoology, University of Maryland, College Park, MD 20742).

Stem Cells and Asymmetric Division During Development
Co-Chairs: Suma Datta (Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843).
HaiFan Lin (Department of Cell Biology, Duke University Medical School, Durham, NC 27710).

Aging and Stress
Chair: John Tower (Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089).

Muscle
Chair: Eric Fyrberg (Department of Biology, The Johns Hopkins University, Baltimore, MD 21218).

Laboratory Stock Lists on FlyBase - Your Contribution is Needed

The FlyBase Consortium*

FlyBase contains descriptions of more than 42,000 *Drosophila melanogaster* alleles and aberrations. The U.S. and European stock centers combined maintain just over 10,000 stocks. Which of the 'uncollected' mutations are lost, and which are held in individual laboratory collections? Which stock center stocks are unique and which widely held? Researchers and stock center curators face these questions daily, and the answers can be difficult to find. Your help is needed to make a more comprehensive list of extant mutations available to the fly community.
We urge you to join your colleagues Michael Ashburner, Adelaide Carpenter, Antonio Garcia-Bellido, Christiane Nusslein-Volhard, Gunter Reuter, and Bill Saxton in helping to make location of alleles and aberrations not held in stock centers easier by contributing your stock list to FlyBase.

At present, laboratory stock lists are in FlyBase as flat files, searchable from the **Stocks** or **Searches** sections. When laboratory stocks are fully incorporated into the relational FlyBase tables users of FlyBase will also have access to laboratory stock data from within gene and aberration reports. To reduce the chance of inappropriate stock requests being directed to laboratories, reports of alleles and aberrations held in laboratory collections will be automatically suppressed in these sections of FlyBase if the mutation is available from a stock center.

The format of the stock list you provide to FlyBase needs to conform to a standard style, but the content is up to you. Lab lists can include stock numbers, full genotypes, cytology, etc. or they can be simple lists of individual alleles and aberrations available from your laboratory. Certain fields or whole records can be flagged such that they do not appear in the public version of your list, or you can remove these from the list you send to FlyBase.

Contact Kathy Matthews by e-mail (matthewk@indiana.edu) or phone (812-855-5782) to contribute your stock list to FlyBase.

How to prepare your stock list for FlyBase

To allow computerized parsing of a stock list the format must follow certain rules. In general, genotypes should be written according to the rules of *Drosophila* nomenclature. An explanation of *Drosophila* nomenclature can be found in the **Documents** section of FlyBase. The parsing process allows flexibility in some areas, and requires strict adherence to the rules in others. These are the major requirements:

1. **Use formatted superscripts or square brackets for allele symbols.** FlyBase indicates superscripts in plain text by enclosing them in square brackets, e.g., Sb[1]. This format can be converted back and forth between superscripts and brackets as needed. If you use a common word processing or spreadsheet program and have encoded superscripts, we can do the conversion to brackets after we receive your list. If you use other paired symbols, such as Sb<1> exclusively for superscripts, you can change them to [and] with a simple find and replace. If you use unpaired symbols, such as Sb~1, replace your symbol with [and FlyBase will add] (using software we've written for the purpose).

2. **All alleles must include an explicit allele symbol**. In the past, the allele designation 1, which is given to the first identified mutant allele of a gene, was implied by the absence of a superscript on a gene symbol. For example, the symbol for the first mutant allele of the gene *cinnabar* was written *cn*, rather than cn^1. This approach allows for significant ambiguity since superscripts are sometimes omitted from genotypes unintentionally (for example, many *white* alleles shown as *w* are not w^1). All alleles in genotypes to be parsed by FlyBase must carry an explicit allele designation. Use your judgement and knowledge of the stock in deciding whether the allele should be shown as 1, meaning it is highly likely to be allele 1, as 1?, meaning it is probably allele 1 but with less certainty, or *, meaning it is a mutant allele of the indicated gene, but which one is anyone's guess.

3. **Use standard syntax.** More flexibility is allowed in syntax than in other genotype components, but uniformity helps. These are the basics. A semicolon (;) followed by a space separates nonhomologous chromosomes. A solidus (/) separates homologues (spaces surrounding the / are optional, but be consistent within your list). Alleles are listed in the wild-type left-to-right order and are separated by a space. Commas are used to separate aberrations from alleles and/or to indicate a

break in the left-to-right order of the items listed. Aberrations precede alleles (transposon insertions may be treated as either aberrations or alleles in this context). A colon (:) is used to separate alleles on heterologous components of translocations and transpositions, or heterozygous alleles on compound chromosomes. Aberration and allele symbols are written only once when homozygous. The X and Y chromosomes are treated as homologues when writing genotypes. Some examples:

ru^1 h^1 st^1 ry^{506} e^1
y^1; pr^1 ord^1/CyO
Df(3L)Pc, ri^1 p^p/TM3, Sb^1 Ser^1
C(4)RM-P2, ci^1 ey^R; gvl^1 sv^n
y^* w^* P{lacW}3-76a

For a more thorough treatment of genotype syntax see the FlyBase nomenclature document.

4. **Use valid gene-allele, aberration and transposon symbols, or recognized synonyms.** FlyBase can help you with this task. The biggest change for many of you will be in transposon nomenclature. The short format is *Element Symbol{Construct Name}Insertion Identifier*, for example *P{PZ}Kr^{00895}*. See the Transposons section of the FlyBase nomenclature document for a complete description of transposon nomenclature. Even if you aren't sure what should go inside and/or to the right of the braces it will help the process if you use P{} (or H{} for hobo, M{} for mariner, etc.) so the parser recognizes these as transposons.

Once your list is in parsable format FlyBase will use software to try to match each component of each stock genotype to an aberration, transposon, or allele in FlyBase. Exact matches result in a link to the appropriate FlyBase object. Matches based on synonymy will be shown to you for approval (mismatches can occur when a symbol is a synonym for more than one gene). A list of nonmatches will be provided to you for resolution. Some nonmatches will be easily resolved typos, others will take some back and forth between you and FlyBase. If your stock list includes unpublished alleles, aberrations, and transposons these will need to be added to FlyBase via a personal communication from you to FlyBase describing these new mutations.

The end result will be a stock list of genotypes using valid FlyBase symbols for all components and these will be fully linked to alleles, aberrations, and transposons in FlyBase. We will return to you a file that contains both your original genotype and the translated version containing only valid symbols.

5. **Structure your stock list to be machine readable**. The FlyBase genotype-parsing software uses files in comma-separated-values (csv) format. Commas separate fields and quotation marks surround (or delimit) data in a field. Spreadsheet programs such as FilemakerPro can produce this format automatically from your data tables. In most cases FlyBase can produce a csv file for you if you use a common word processing program to maintain your stock list, but more care in the formatting of each stock record is required with a word processor. Use a separator that does not appear in your data, such as a tab, between fields, and include all fields, in the same order, in every record, even when fields are blank. Use a hard return at the end, and only at the end, of each record. For example, the structure of your stock records might look like this (with tabs and hard returns shown as code):

Stock Number[left tab]Genotype[left tab]Source[left tab]Comments[HRt]

Records with empty Source and Comments fields, respectively, would look like this:

22[left tab]Df(2R)abc/CyO[left tab][left tab]DEB-induced, 1996[HRt]
35[left tab]y^1 cv^1 v^1 f^1 car^1[left tab]Bloomington[left tab][left tab][HRt]

*The current members of the FlyBase Consortium are: W. Gelbart, W. Rindone, J. Chillemi, Russo, M. Crosby, B. Matthews, D. Emmert, and M. Mahoney, Harvard University, Cambridge, MA, USA, M. Ashburner, R. Drysdale, A. de Grey, E. Whitfield, and G. Millburn, University of Cambridge, Cambridge, UK, T. Kaufman, K. Matthews, D. Gilbert, and V. Strelets, Indiana University, Bloomington, IN, USA and C. Tolstoshev, NCBI, Bethesda, MD, USA.

E-Mail Addresses for U.S. Researchers

The following list of e-mail addresses was provided by FlyBase and is intended to provide a directory for quick reference. It only includes *Drosophila* researchers in the United States. Privacy regulations in some other countries prohibit publication of foreign e-mail addresses, but they may be available directly from FlyBase. The FlyBase directory also includes full mailing addresses, telephone, and FAX numbers, where available. Since e-mail addresses can change, we encourage you to go to FlyBase if you discover one of these listings is no longer active. In addition, if you find an error in your own listing, this might remind you to update your FlyBase directory listing if you have not already done so. Printing addresses can be ambiguous, since the number "zero" and the letter "oh" look similar when printing is done in capitals, and comparable problems arise when using lower case. Here, I have printed the number **"one"** in **bold**, to distinguish it from the letter "el".

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