Research Notes

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

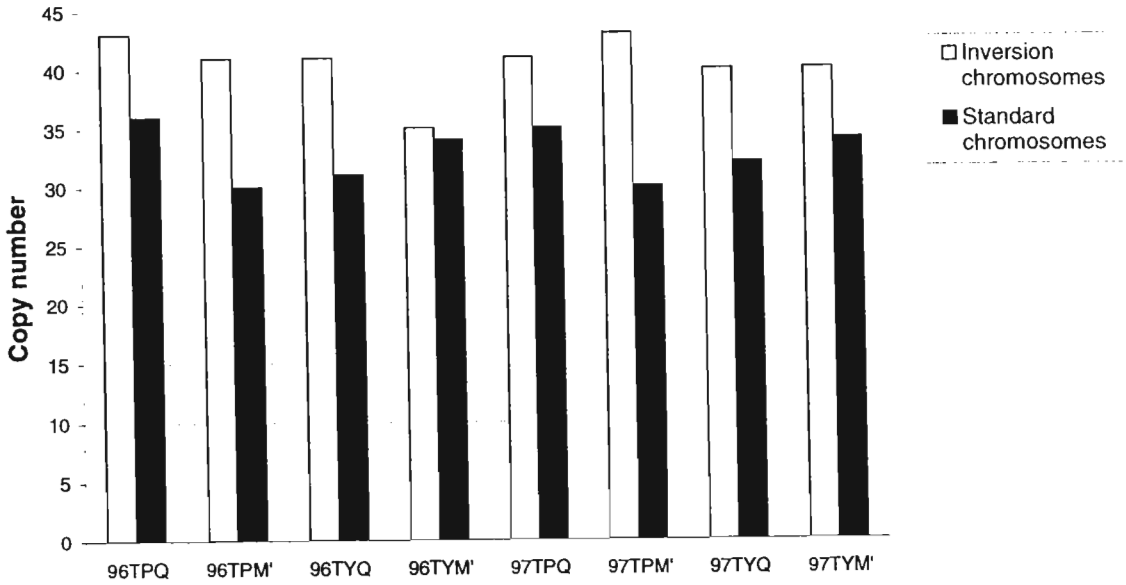

Figure 1. Overall comparison of copy numbers between five In(3L)P chromosome and standard 3L chromosomes.

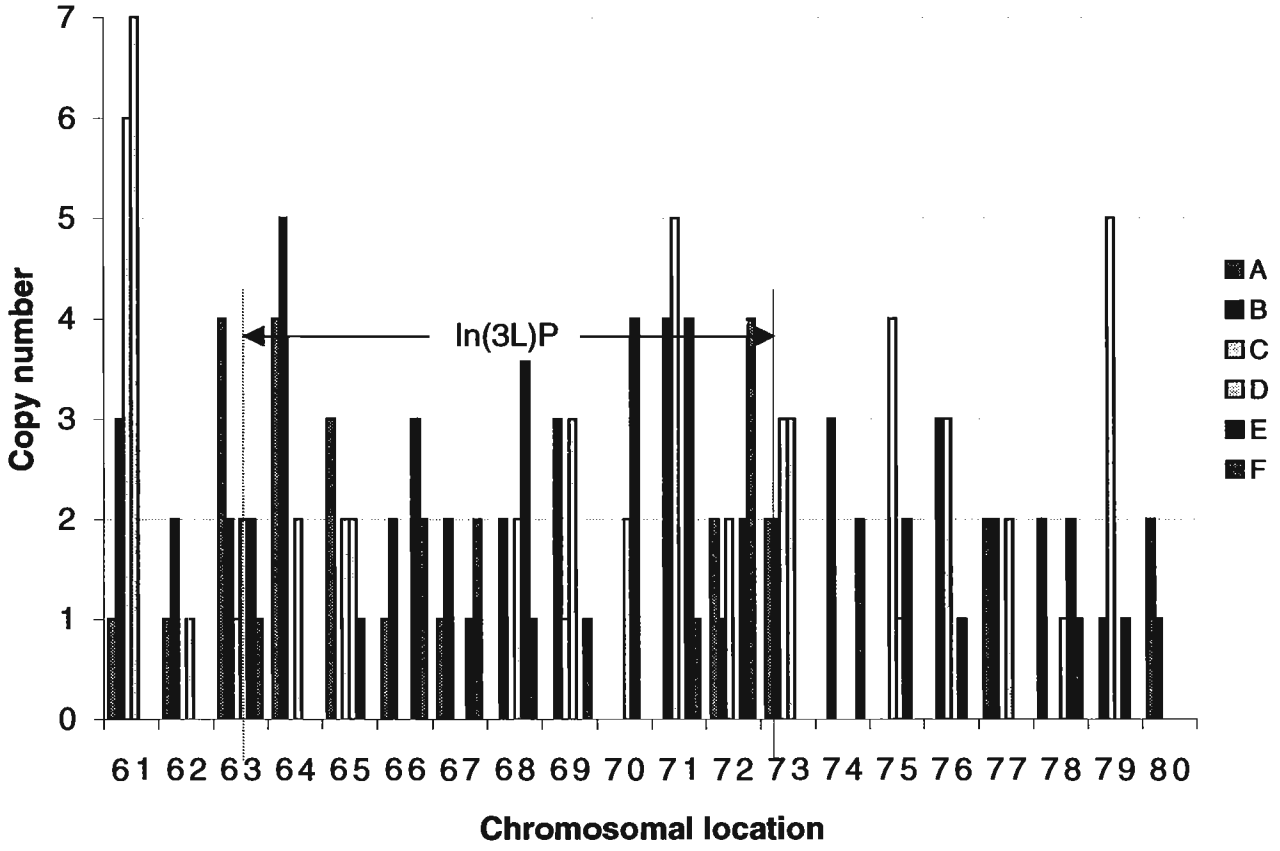

Figure 2. In(3L)P Q types 96,97.

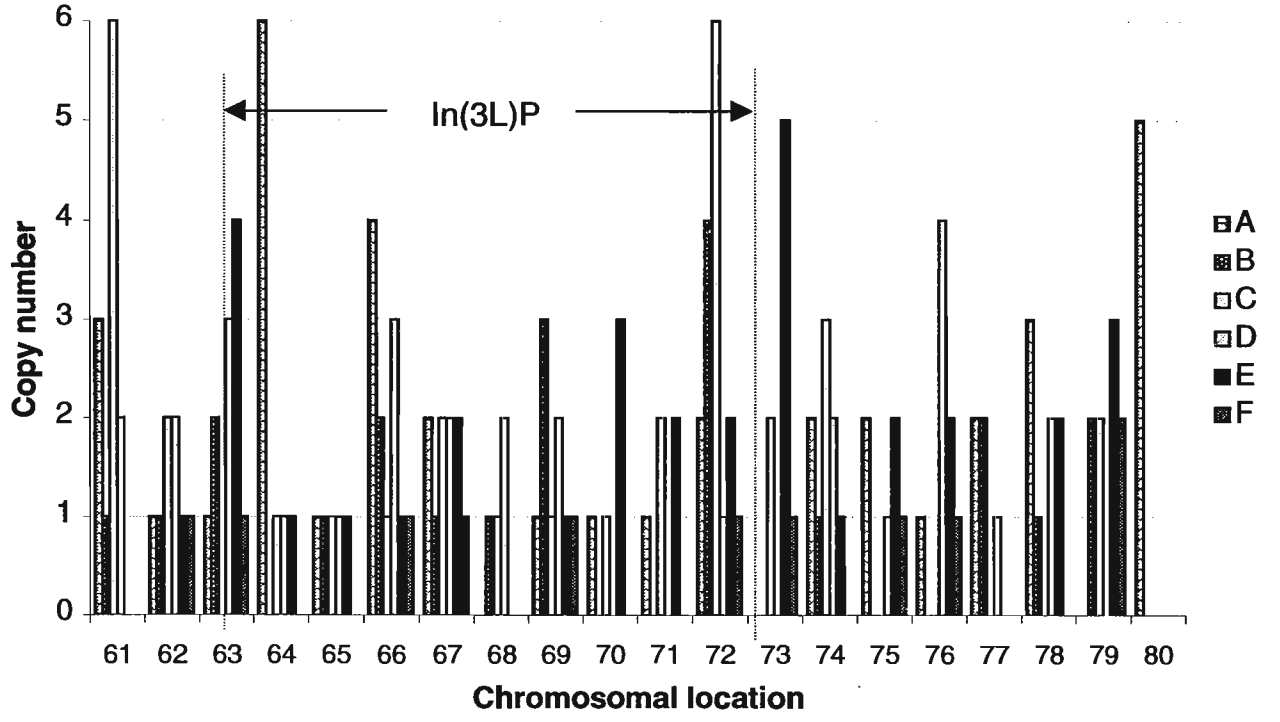

Figure 3. In(3L)P M' types 96,97.

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

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

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

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

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

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

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

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

References: Bridges, C.B., 1935, J. Heredity, 26:60-64; Lefevre, G., 1976, In: *The Genetics and Biology of* Drosophila, vol.1. Academic Press, London, pp.31-66; Kidwell, M.G., 1983, Proc. Natl. Acad. Sci. USA 80:1655-1659; Kidwell, M.G., 1986, In: Drosophila: *A Practical Approach* (D.B. Roberts, ed.), IRL Press, Washington DC, pp. 70; Kim, D.I., 1994, Dros. Inf. Serv. 75:157-159; Sniegowski, P.D., and B. Charlesworth, 1994, Genetics 137:815-827; Koryakov, D.E., and I.R. Zhimulev, 1996, Dros. Inf. Serv. 77:126-132.

Sexual dimorphism apparent in size-related wing asymmetry.

Norry, Fabian M.*, Marcelo Cortese, and Esteban Hasson. Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina. *Correspondence. Telephone: (0054-1) 782-0566. FAX: (0054-1) 782-0620. E-mail: fnorry@bg.fcen.uba.ar or fmnorry@cbiol.uba.ar.

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

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

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