79

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

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

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

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

Molecular characterization of the insertion site in eight P-insertion lines from the Kiss Collection. <u>Liebl, Eric C.</u> Department of Biology, Denison University, Granville OH 43023.

As part of the genetics laboratory at Denison University my undergraduate students and I have both meiotically mapped the P{w+} inserts (data not shown) and molecularly characterized the insertion sites of eight P-insertion lines from the Kiss Collection (Torok et al., 1993) available through the Bloomington Stock Center. We carried out plasmid rescue of 3' flanking DNA after digesting genomic DNA with EcoRI (Bier et

al., 1989). This rescued 3' flanking DNA was sequenced using a primer that recognizes the Pelement's inverted repeat, yielding 600 – 900 nucleotides of sequence. These genomic sequences were used in BLAST searches during the week of 4-29-99 against the Berkeley Drosophila Genome Project database (www.fruitfly.org).

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

Two of these lines (P174, P1112) had significant matches to both genomic clones and cDNAs or ESTs in the database, leading us to

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

Bloomington Stock ^a	3' Flanking DNA matchb	
P539	AQ034143; bases	
I(2)k04203	503-764	
~~	(1.6e – 52)	
P996	AQ025938; bases	
I(2)k10609	122-183	
	(7.4e-3)	

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

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

Table 2. Results of BLAST searches.

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

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

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

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

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

d. Alleles of previously identified genes are indicated.

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

References: Bier, E., H. Vassin, S. Shepherd, K. Lee, K. McCall, S. Berbel, L. Ackerman, R. Carretto, T. Uemura, E. Grell, L.Y. Jan, and Y.N. Jan 1989, Genes and Development. 3: 1273-1287; Roch, F., F. Serras, F. Cifuentes, M. Corominas, B. Alsina, A. Lopez-Varea, R. Hernandez, D Guerra, S. Cavicchi, J. Maguna, and A. Garcia-Bellido 1998, Molec. Gen. Genet. 257(2): 103-112; Torok, T., G. Tick, M. Alvarado, and I. Kiss 1995, Genetics 135: 71-80.

Drosophila female receptivity to males with different sound parameters values.

Manfrin, Maura Helena ¹, Rosana Tidon-Sklorz ², and Fábio de Melo Sene³. ¹ Departamento de Biologia – Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo. Av. Bandeirantes 3900, 14040-901, Ribeirão Preto, SP, Brasil. FAX: 016 – 6336482. E-mail: mhimanfri@rgm.fmrp.usp.br, ² Departamento de Genética e Morfologia, Instituto de Ciências Biológicas, Universidade de Brasília, Brasil. E-mail: rotidon@unb.br, ³ Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo. 14040-901, Ribeirão Preto, SP, Brasil. E-mail: famesene@usp.br

Drosophila males from the various species usually have specific displays during courtship (Spieth, 1974; Ewing and Benet-Clark, 1968); these, when detected by the females should stimulate them until they are physiologically ready to mate. Generally, potentially receptive females wait at least one complete courtship sequence before adopting an acceptance behavior. The most conspicuous courtship behavior is some form of

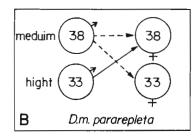
wing movement that produces a specific acoustic stimulus (Ewing and Bennet-Clark, 1968; Spieth, 1974; Ewing, 1979). The importance of this sound stimulus for conspecific identification and successful mating has been shown, using experimentally, different methodologies for each of the several Drosophila species studied (Manning, 1967; Bennet-Clark and Ewing, 1967; Spieth, 1974; Schilcher, 1976; Ewing, 1978; Kyriacou and Hall, 1982; Ikeda et al., 1981; Liimatainen et al., 1992). The interpulse interval (IPI) and the fundamental frequency (FF) are the sound stimulus parameters considered most appropriate for identification, as they vary among the species but are characteristic to each one (Bennet-Clark and Ewing, 1969; Chang and Miller, 1978; Tomaru and Oguma, 1994).

Drosophila mercatorum

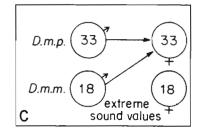
Drosophila mercatorum (Paterson and Wheller, 1942) belongs to the mercatorum subgroup of the repleta group. The populations of this species are divided into two subspecies, typical

INTRA SUBSPECIES

nedium 21 21 A Dm.mercatorum



INTER SUBSPECIES



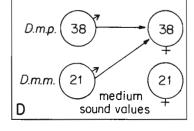


Figure 1. Graphical representation of *Drosophila mercatorum* female receptivity to males with different sound parameter values. A and B – crosses among lineages of the same subspecies with different values of sound parameters (Low, Medium and High) C and D – crosses among lineages of the two subspecies.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

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