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

Borie, N., C. Loevenbruck, and C. Biémont. UMR 5558 "Biométrie, Génétique et Biologie des Populations", Université Lyon1. -69622 Villeurbanne- France, borie@biomserv.univ-lyon1.fr, Telá: (33) 4 72 43 29 16, Faxá: (33) 4 78 89 27 19.

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

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

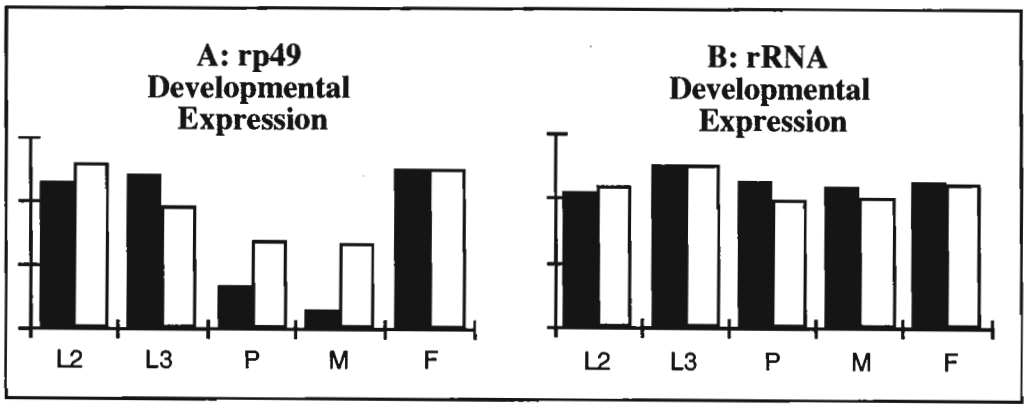

Figure 1: Expression patterns of rp49 (A) and rRNA (B) genes for different developmental stages: second instar larvae (L2), third instar larvae (L3), pupae (P), adult males (M) and females (F). Data correspond to mean values. (■) *D. melanogaster*, (□) *D. simulans*.

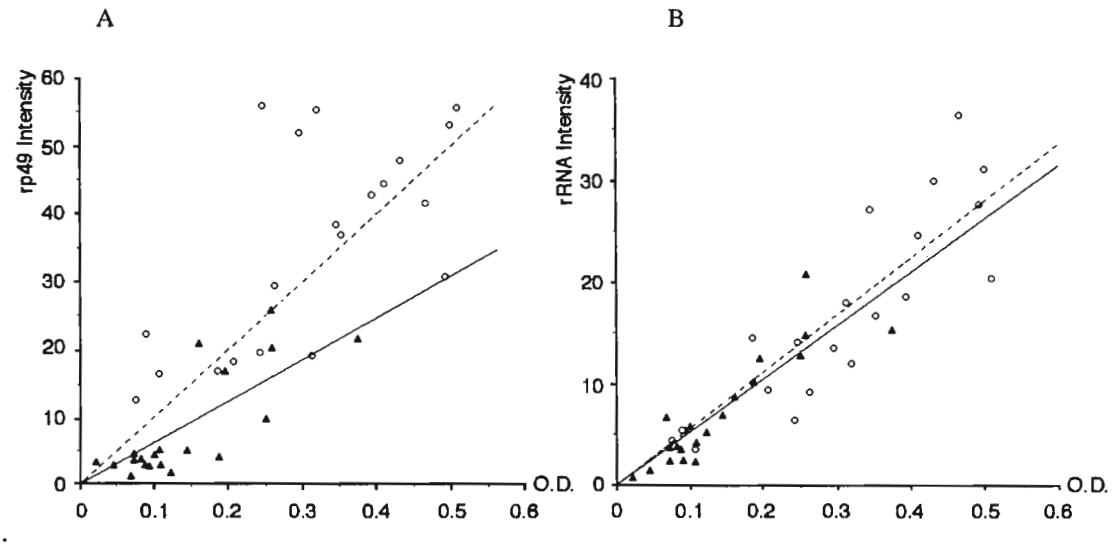

Figure 2: Relationship between rp49 (A) and rRNA (B) spot intensity and optical density (O.D.) for males (▲) and females (O). Data from the two species are pooled.

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

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

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

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

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

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

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

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

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

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

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