Duplication of the *Gpdh* gene in the *Drosophila virilis* species group.

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D. virilis species group is composed of twelve taxa and divided into the *virilis* and *montana* phylads. In order to examine the phylogenetic relationship of the *virilis* species group, we have determined the entire nucleotide sequence of the *Gpdh* gene. In the course of the study, we found the duplicated Gpdh genes in some of the species in the montana phylad. This paper reports that the duplicated Gpdh genes were confirmed by Southern blot hybridization and both the copies of the duplicated genes are transcribed.

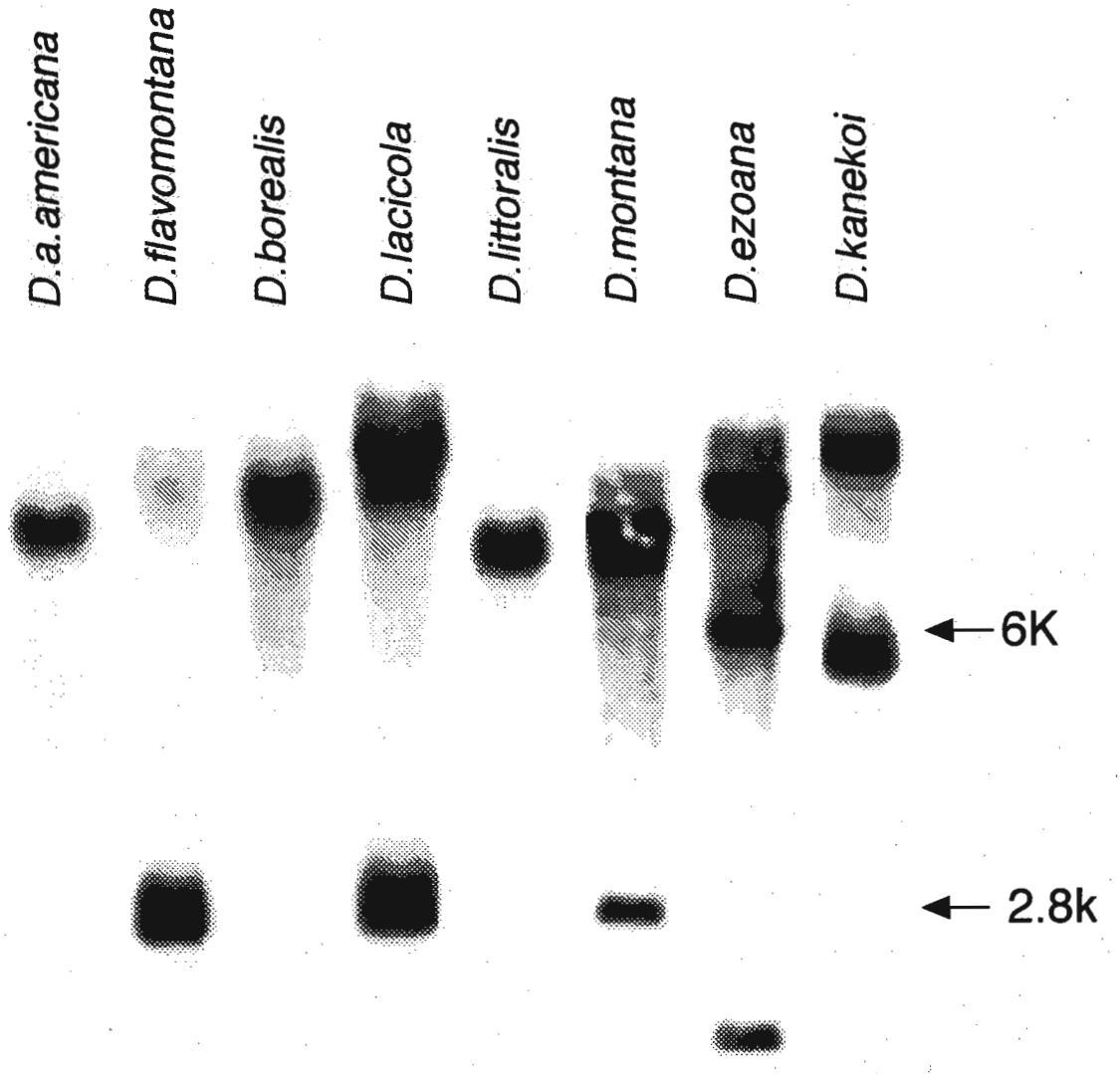

Figure 1. Southern blot analysis of *Sal*I-digested genomic DNA from the *montana* phylad species of the *D. virilis* group. *D. a. americana* is shown as a representative of the *virilis* phylad species.

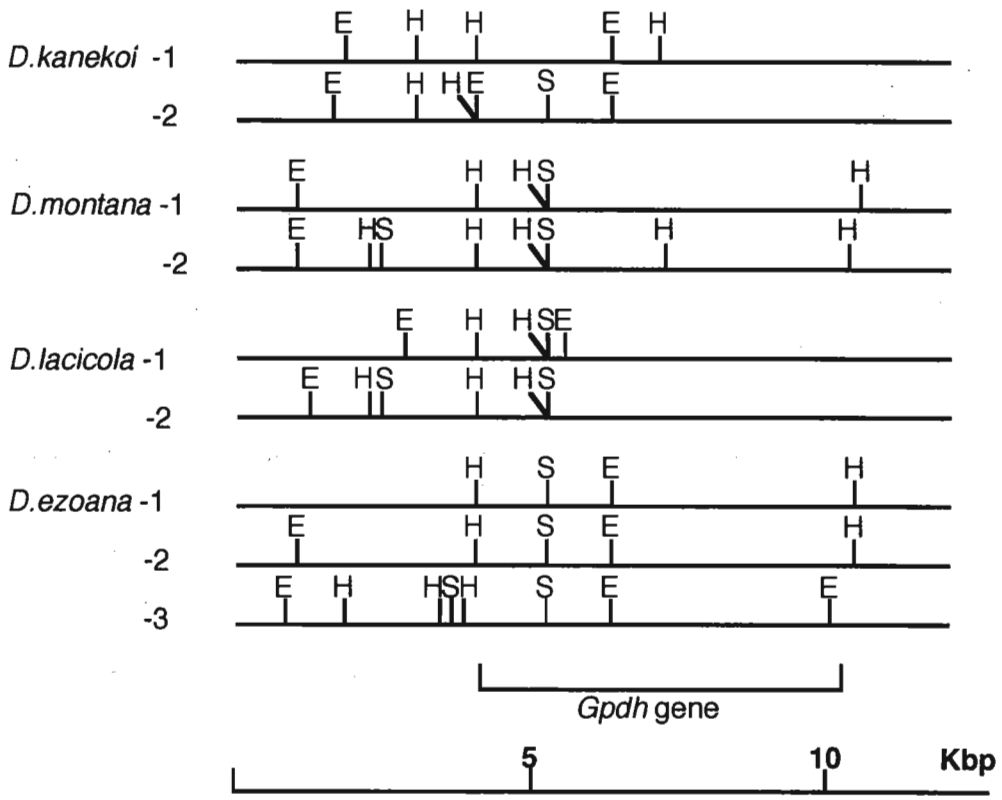

Figure 2. Restriction maps of the regions including the *Gpdh* gene obtained from different clones in lambda libraries of four species. Restriction enzyme abbreviations: E = *Eco*RI, H = *Hin*dIII, S = *Sal*I. The region of the Gpdh gene is shown at the bottom.

D. ezoana, D. montana, D. lacicola, D. littoralis, D. borealis, and *D. flavomontana* in the *montana* phylad were obtained from the National *Drosophila* Species Resource Center, Bowling Green State University, Bowling Green, Ohio. *D. kanekoi* was supplied by Dr. H. Watabe, Hokkaido University of Education.

Southern blot analysis was as follows: genomic DNAs of adult flies of each species prepared as previously described (Tominaga *et al.*, 1992) were digested with several restriction enzymes and the fragments separated by agarose gel electrophoresis were transferred onto nitocellulose membrane. A probe was designed on the basis of the *D. virilis Gpdh* gene sequence (Tominaga *et al.*, 1992): the probe is a sequence of 1342 bp containing exon 1 and 2. Southern blot analysis of total genomic DNA from the species belonging to the *montana* phylad revealed duplicated genes in some species (Figure 1); that is, *D. lacicola, D. montana*, and *D. kanekoi* showed a duplication, and *D. ezoana*, a triplication. *D. flavomontana, D. borealis*, and *D. littoralis* had a single copy as did the members of the *virilis* phylad. Similar results were also obtained when the Southern blot analysis was performed with some other restriction enzymes (data not shown). Since a single lambda dash clone never did contain any duplicated copies, these genes in the genome would be located at least 20 kb apart from each other.

Figure 2 shows the restriction maps of the regions including the *Gpdh* locus in these clones. It is clear from Figures 1 and 2 that the 2.8-K bands of *D. montana and D. lacicola* correspond to *Sal*I-*Sal*I fragments of *D. montana*-2 and *D. lacicola*-2, respectively. 6-K bands of *D. kanekoi* and *D. ezoana* are derived from D. *kanekoi*-2 and *D. ezoana*-1 or -2, and the small fragment band comes from *D. ezoana*-3.

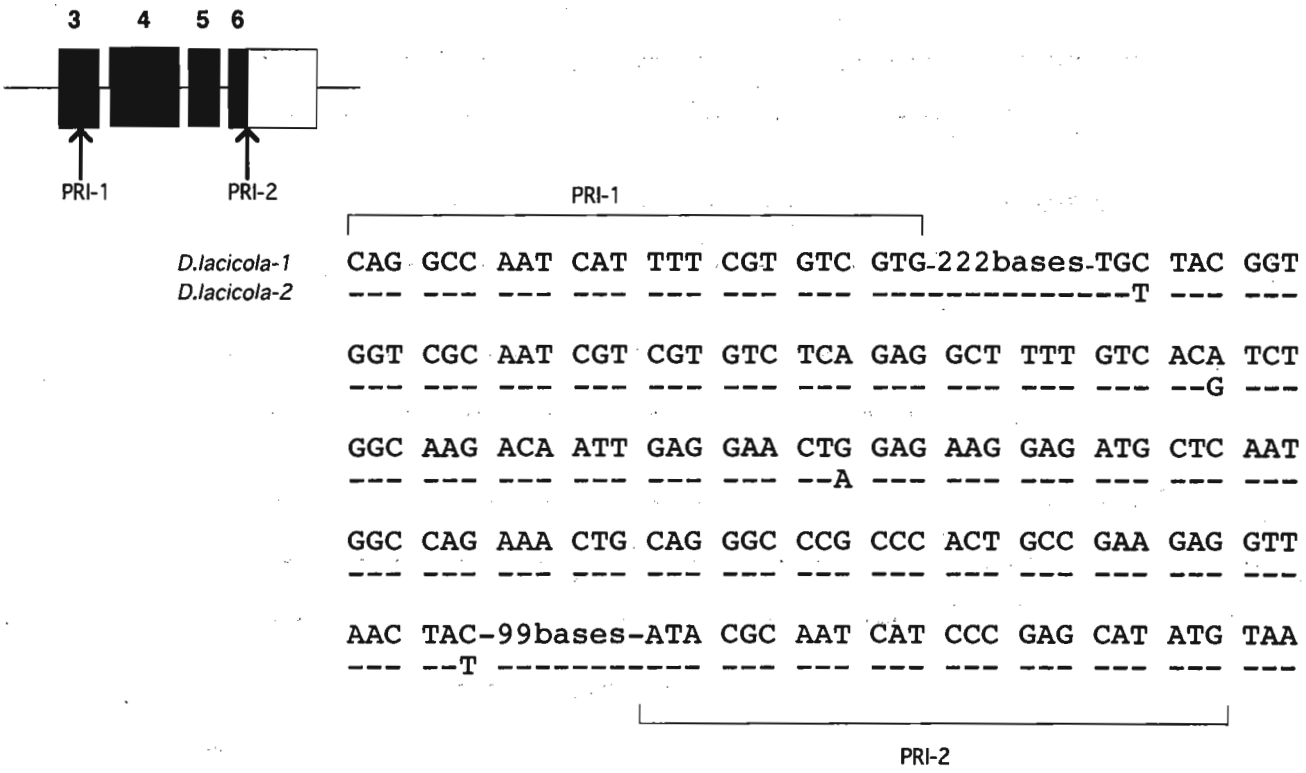

Figure 3. Comparison of nucleotide sequences between the two cDNAs from *D. lacicola*. cDNAs were amplified by RT-PCR from adult mRNA by use of two primers, PRI-1 and PRI-2, indicated in the *Gpdh* gene region at the top (Filled boxes show exons and open box, 3' non-translated). Dashes denote identical bases. 222 bases downstream of PRI-1 primer and 99 bases upstream of PRI-2 are abbreviated, for the two sequences are all identical to each other.

In order to test whether both copies of the duplicated gene are transcribed, cDNA from *D. lacicola* adult mRNA was amplified by means of RT-PCR using primers PRI-1 (nt number 5176-5199 in D. lacicola-1, 5'-CAGGCCAATCATTTTCGTGTCGTG-3') and PRI-2 (nt number 5786-5809 in D. lacicola-1, 5'-CATATGCTCGGGATGATTGCGTAT-3') (Figure 3). PCR was carried out for 25 cycles under the following conditions: denaturation at 94°C for 40 sec, annealing at 60°C for 40 sec, and extention at 72°C for 2 min. The 501-bp RT-PCR products were cloned into the PCR-script and sequenced by the dideoxy chain termination method (Sanger *et al.*, 1977). As shown in Figure 3, two different sequences of cDNA were obtained from *D. lacicola* adult flies. Four nucleotide substitutions were observed among the 501 bp, but no amino acid changes occurred. This means that both of the duplicated *Gpdh* genes are transcribed.

Gene duplication is now known to occur frequently in eukaryotes. Molecular evidence for gene duplication was recently reported at *Gpdh* (Koga *et al.*, 1988) locus in *D. melanogaster*. Compared with the *Gpdh* locus in *D. melanogaster* in which the duplication is tandem and incomplete, the duplicated genes in some species of the *montana* phylad are complete; that is, 5' flanking, 1-8 exons, introns, and 3' flanking are included. They are located more than 20 kb apart from each other and seem to be functional.

References: Koga, A., S. Kusakabe, F. Tajima, K. Harada, G.C. Bewley, and T. Mukai 1988, Proc. Jap. Acad. 64: 9-12; Sanger, F., S. Nicklen, and A.R. Coulson 1977, Proc. Natl. Acad. Sci., USA 74: 5463-5467; Tominaga, H., T. Shiba, and S. Narise 1992, Biochim. Biophys. Acta 1131: 233-238.