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Expression of the GPDH-4 isozyme of *sn*-glycerol-3-phosphate dehydrogenase in three *Drosophila* species

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A fourth recently discovered isozyme of *sn*-glycerol-3-phosphate dehydrogenase (GPDH-4) in *Drosophila melanogaster* is shown to be a translational product of the *Gpdh* transcript which contains exons 1 through 7. This transcript was also found in two other *Drosophila* species, *D. busckii* and *D. virilis*. In contrast to *D. melanogaster* and *D. busckii*, the *Gpdh* transcript containing exons 1–6 is absent in *D. virilis* adults. The reason for this difference between *D. virilis* and the two other species is intriguing but remains elusive. We have ruled out the possibility that a replacement of an amino acid residue in exon 7 played any role in generating this interspecific variation.

Keywords: alternative splicing, *Drosophila*, evolution, GPDH, isozyme.

Introduction

In *Drosophila melanogaster* the enzyme *sn*-glycerol-3-phosphate dehydrogenase (GPDH, EC 1.1.1.8) is present in three isozymic forms. GPDH-1 is predominant in the adult flight muscle and involved in energy production for flight, whereas GPDH-3 is localized mainly in the fat body tissue, both in larvae and adult abdomens, and is postulated to serve a dual function regulating the cytoplasmic NAD^+/NADH ratio and providing *sn*-glycerol-3-phosphate as a precursor for lipid biosynthesis (Bewley & Miller, 1979). GPDH-2 is found in small amounts in those adult tissues where both GPDH-1 and GPDH-3 are expressed. As the functional GPDH molecule is a dimer (Collier *et al*., 1976) it is possible that GPDH-2 is a heterodimer composed of one GPDH-1 subunit and one GPDH-3 subunit (Wright & Shaw, 1969).

Gpdh transcripts are generated by alternative ways of processing the pre-mRNA (Cook *et al*., 1988). The *D. melanogaster Gpdh* gene has eight exons, a single promoter and multiple polyadenylation signals (Bewley *et al*., 1989; von Kalm *et al*., 1989). There are two alternate 3′ exons — exon 7 and exon 8. The transcript containing exons 1 through to 6 codes for GPDH-3 and the transcript containing exons 1–6, skipping exon 7 and including exon 8 encodes GPDH-1 (Bewley *et al*., 1989; von Kalm *et al*., 1989). The product of translation of the transcript containing exons 1–7 has not been identified, but it has been suggested that this transcript might be coding for a novel isozyme GPDH-4 (Wilanowski *et al*., 1995).

The amino acid sequence of GPDH has been obtained from a variety of organisms, and shown to be highly conserved over evolutionary time (Bewley *et al*., 1989). In fruit fly species this conservation extends to the nucleotide sequence (Kwiatowski *et al*., 1997) and isozyme patterns (Colgan, 1992). Despite this apparent congruity in the patterns of *Gpdh* variation, few data are available on the extent to which the regulation of the gene's expression is conserved.

For example, the GPDH electrophoretic phenotypes of *D. virilis* and *D. busckii* appear to be very similar to that of *D. melanogaster* (Fig. 1). However, Tominaga *et al*. (1992) were able to clone only two kinds of *Gpdh* cDNA from a *D. virilis* adult cDNA library — one contained exons 1–7 and the second exons 1–6 and 8. This result suggests that, unlike in *D. melanogaster*, the GPDH-3-encoding transcript

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might not be expressed in *D. virilis* adults. Recently, Wilanowski *et al*. (1995) have shown that in the *D. melanogaster* $Gpdh^{AKy33}$ allele the insertion of the *blood* retrotransposon into the noncoding part of exon 7 induces the production of a novel isozyme, GPDH-4, and causes an over-expression of the *Gpdh* transcript which contains exons 1–7. The comparison between *D. virilis* and *D. melanogaster* poses a number of questions regarding the evolution of *Gpdh* regulation. We have attempted to answer some of these questions by using P-element mediated transformation in *D. melanogaster* of a construct containing exons 1–7 and by investigating *Gpdh* expression in *D. virilis* and *D. busckii*.

Materials and methods

Drosophila strains

Wild-type strains of *D. virilis* and *D. busckii* were provided by Allan R. Lohe. The *D. melanogaster* mutant allele $Gpdh^{AKy33}$ was isolated from a natural population at Cardwell, Queensland, Australia, in 1988 (Gibson *et al*., 1991). A *D. melanogaster* stock bearing a *Gpdh* null allele, $Gpdh^{nJH231}$, on a balancer chromosome *CyO* (Bewley & Lucchesi, 1977) was obtained from the Bowling Green Stock Centre. *Df (2L)*Gdh^{A} is a deficiency that spans the *Gpdh* locus (Lindsley & Grell, 1968). Flies heterozygous $Gpdh^{nJH231}/Df(2L)Gdh^{A}$ have no detectable GPDH activity and very poor viability.

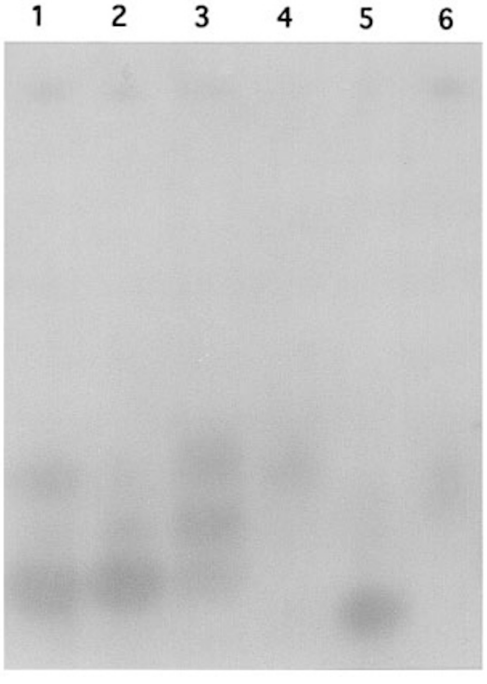

Fig. 1 Cellulose acetate GPDH electrophoretic phenotypes of *Drosophila virilis* and *D. busckii* in comparison with *D. melanogaster*. The cathode (+) is at the top of the gel; in pH 6.6 buffer the GPDH protein is negatively charged and migrates towards the anode. Lane 1, *D. melanogaster* $Gpdh^{S}$ (slow) allele whole adult fly. Lanes 2–4, *D. virilis*: 2, whole adult fly; 3, adult abdomen; 4, larva. Lanes 5–6, *D. busckii*: 5, whole adult fly; 6, larva.

Gpdh *gene constructs and transformation*

Two overlapping genomic clones which cover the *Gpdh* locus, *pDm59* and *pDm60*, were provided by the late Glenn C. Bewley (Cook *et al*., 1986). From these clones a construct, *pGpdh(1–7)*, was made to include exons 1–7, the promoter and the 3′ sequences necessary for polyadenylation (Bartoszewski, 1996). The region located downstream of the *Hin*dIII site in the noncoding part of exon 6 was replaced by a PCR-amplified fragment covering the entire exon 7. The fragment extends from 91 nucleotides upstream of exon 7 to 48 nucleotides upstream of exon 8. The splice sites between exons 6 and 7 are retained in the *pGpdh(1–7)* construct, but the entire exon 8 is removed (Fig. 2). The polyadenylation signals in exon 6 are located 3′ to the *Hin*dIII site so that they too are removed.

It is known that there is a competition between the reactions of splicing and cleavage-polyadenylation and if the competition from one pre-mRNA processing pathway is eliminated then the other pathway is exclusively followed by default (Peterson *et al*., 1994). In the *pGpdh(1–7)* construct effective polyadenylation can occur only in exon 7 as the only polyadenylation signals retained in this construct are those in exon 7. Consequently exon 7 is expected to be spliced to exon 6 as the splice sites in exons 6 and 7 are intact and the competing polyadenylation signals of exon 6 are removed. Thus the only mature *Gpdh* transcript that can be expressed from this construct is the one which contains exon 7 (Fig. 2). The *pGpdh(1–7)* construct is not able to produce GPDH-1 or GPDH-3.

P-element mediated transformation, with a wild-type *white* gene as marker, was used to obtain transformants. The *pGpdh(1–7)* construct was subcloned into the *pW8* vector (Klemenz *et al*., 1987) and transformed into *D. melanogaster* embryos by microinjection (Bartoszewski & Gibson, 1994); the recipient strain was homozygous for *white* eye colour. All the experimental procedures involved in making the construct and in transforming it into *D. melanogaster* have been described in detail by Bartoszewski (1996).

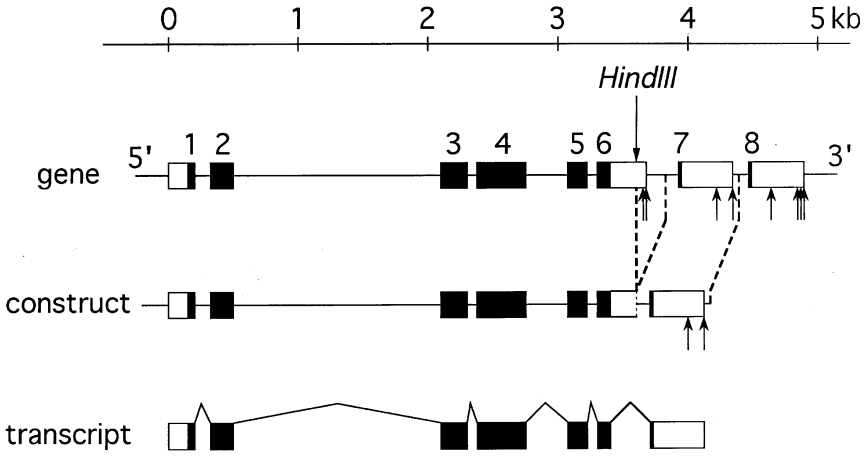

Fig. 2 The structure of the *Drosophila melanogaster Gpdh* gene (upper diagram) is shown according to Bewley *et al*. (1989) and von Kalm *et al*. (1989). Solid boxes indicate the coding regions, and open boxes denote the transcribed but untranslated regions. Arrows below the upper diagram show the locations of putative polyadenylation signals. In the construct *pGpdh(1–7)* the part of the cloned *Gpdh* gene located downstream of the *Hin*dIII restriction site in exon 6 was replaced with a PCR amplified fragment covering the entire exon 7 (middle). After this modification the only mature transcript (lower) that can be expressed is the one containing exon 7, because the only polyadenylation signals retained in the *pGpdh(1–7)* construct are located in exon 7.

GPDH electrophoresis

GPDH electrophoresis on cellulose acetate membranes with a buffer at pH 6.6 was performed according to Gibson *et al*. (1986).

Rapid amplification of cDNA ends (RACE) and identification of RACE products

The RACE analysis of the 3′ ends of *Gpdh* transcripts was carried out as previously described (Symonds *et al*., 1995). A primer specific to exon 5 of the *D. virilis Gpdh* gene, JG119 (5′-CGGGCAAGACAATCGAAGAT-3′) was based on the sequence published by Tominaga *et al*. (1992). For the study of *D. busckii* we used the JG11 primer (5′-AGAAGCTGCAGGGCCCACCCA-3′), which is specific to exon 5 of the *D. melanogaster Gpdh* gene. The RACE products obtained were subcloned into *pBluescript* as before (Wilanowski *et al*., 1995) and identified by sequencing with the ABI Prism dye terminator cycle sequencing reaction kit (Perkin-Elmer) according to the manufacturer's instructions.

Results

The *pGpdh(1–7)* construct was expressed in the *D. melanogaster* flies made heterozygous $Gpdh^{nJH231}/Df(2L)Gdh^{A}$ so that they lack endogenous GPDH activity (Bewley & Lucchesi, 1977). A number of separately produced transformant lines were obtained but each displayed identical GPDH electrophoretic phenotypes. The phenotype was compared with those of the $Gpdh^{F}$ and $Gpdh^{AKy33}$ alleles. As predicted, the transgenic flies express only one GPDH isozyme, which has the same electrophoretic mobility as GPDH-4 in $Gpdh^{AKy33}$ (Fig. 3). This result very strongly supports the hypothesis that this isozyme, named GPDH-4, is a product of translation of a *Gpdh* transcript containing exons 1 through 7 (Wilanowski *et al*., 1995).

Three RACE product bands were obtained from *D. virilis*: two both from adults and larvae, with one

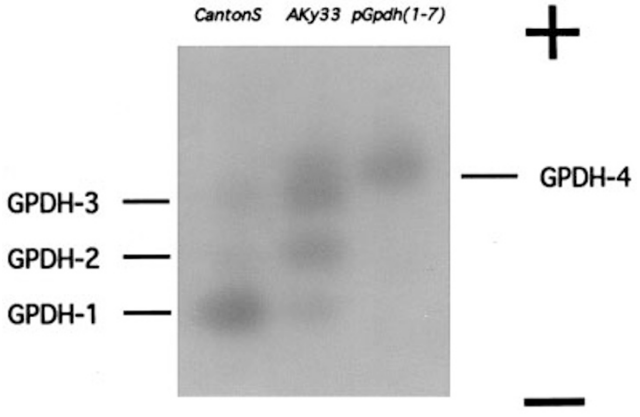

Fig. 3 Electrophoresis on a cellulose acetate membrane of GPDH from adult *Drosophila melanogaster* transformed with the *pGpdh(1–7)* construct. Samples from the left: the *Canton S* stock which carries the $Gpdh^{F}$ (fast) allele, the $Gpdh^{AKy33}$ allele, heterozygotes $Gpdh^{nJH231}/Df(2L)Gdh^{A}$ transformed with *pGpdh(1–7)*.

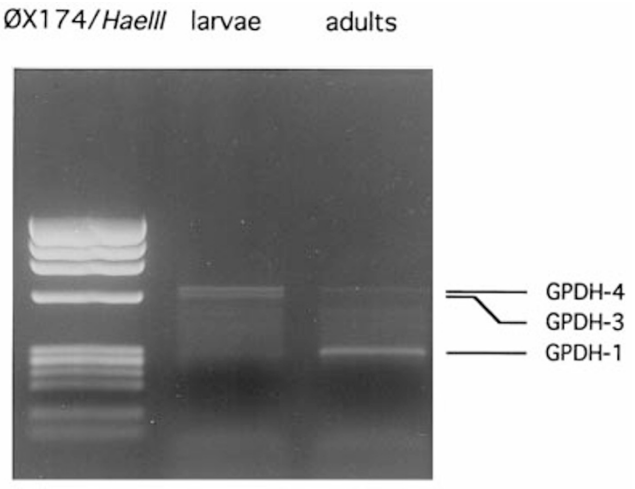

Fig. 4 Two percentage agarose gel showing the results of the RACE experiment on *Drosophila virilis*. Samples from the left: size standard, RACE products amplified from larvae and adult flies. The intensities of the bands are related not only to the abundance of the corresponding transcripts but to the sizes of the RACE products as well.

band common to both life stages (Fig. 4). All the fragments visible in Fig. 4 have been cloned and sequenced. The band expressed in larvae and adults was found to represent a transcript containing exon 7, that is, the equivalent of the GPDH-4-encoding transcript in *D. melanogaster*. These data also show that the transcript containing exons 1–6 — the homologue of the GPDH-3-encoding transcript — is present in *D. virilis* but only in larvae, whereas the equivalent of the *D. melanogaster* transcript coding for GPDH-1 is unique to the adult stage of *D. virilis*.

In *D. busckii*, initial RACE experiments gave several products (Fig. 5). Upon sequencing, only two of them — indicated in the figure — were found to correspond to *Gpdh* transcripts; other bands resulted from nonspecific amplification. For example, GenBank searching showed that the $\approx$1 kb RACE fragment represented a transcript encoding acetyl-coenzyme A carboxylase (EC 6.4.1.2). The sensitivity of the RACE method was low probably because of the use of the JG11 primer (Symonds *et al*., 1995), which was based on the *D. melanogaster Gpdh* sequence (Bewley *et al*., 1989; von Kalm *et al*., 1989) and had two mismatches in comparison with the *D. busckii Gpdh* sequence (R. Spencer Wells, personal communication).

Nevertheless, the RACE analysis of *D. busckii* indicated that the homologue of the GPDH-1-encoding transcript was unique to the adult stage and the one coding for GPDH-3 was present both in adults and larvae. This experiment failed to detect a transcript containing exon 7 in *D. busckii*. To test further whether this transcript was present in *D. busckii*, we used a more direct approach. A *D. melanogaster* exon-7-specific primer, JG99 (5′-GACGGCATGATGGACGTATC-3′), was used with JG11 to PCR amplify only the fragment unique to the transcript containing exons 1–7. By this method we detected this transcript both in adults and larvae of *D. busckii* (data not shown). Our findings are summarized in Table 1.

Discussion

The GPDH electrophoretic phenotypes of *D. virilis* and *D. busckii* appear to resemble that of *D. melanogaster* (Fig. 1). However, the RACE analyses (Figs 4 and 5) showed that although the pattern of *Gpdh* transcription is similar in *D. busckii* and *D. melanogaster*, it differs from that in *D. virilis*. In *D. melanogaster*, both from adult and larval cDNA samples, three RACE products are obtained that correspond to the transcripts encoding GPDH-1, GPDH-3 and GPDH-4, respectively (Symonds *et al*.,

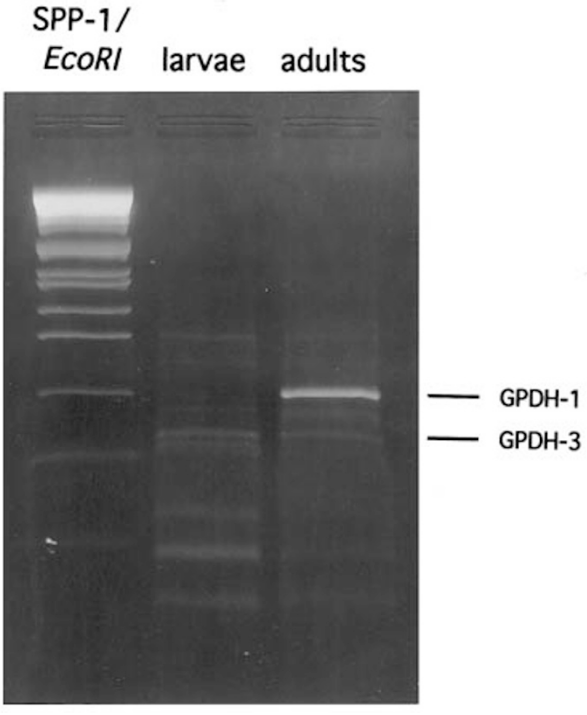

Fig. 5 The results of the RACE experiment on *Drosophila busckii*. Lanes from the left: size standard, RACE products obtained from larvae and adult flies. The bands confirmed by sequencing to represent *Gpdh* transcripts are indicated; other bands visible in this figure arose from nonspecific amplification.

Table 1 Comparison of developmental patterns of expression of GPDH isozymes in three *Drosophila* species

Isozyme	RNA structure	*D. melanogaster*	*D. virilis*	*D. busckii*
GPDH-1	Exons 1–6 + 8	Adults	Adults	Adults
GPDH-2	None (heterodimer)	Adults	Adults	Adults
GPDH-3	Exons 1–6	Adults + larvae	Larvae	Adults + larvae
GPDH-4	Exons 1–6 + 7	Only in $Gpdh^{AKy33}$	Adults + larvae	Adults + larvae

1995; Wilanowski *et al*., 1995). The most significant difference in *D. virilis* is the lack of the GPDH-3-encoding transcript in adults. The transcript coding for GPDH-4, however, was found in *D. virilis* adults (Fig. 4) which suggests that in this species the least anodal isozymic band might be GPDH-4. We base this hypothesis on a comparison between the GPDH electrophoretic phenotype (Fig. 1) and the outcome of the RACE analysis (Fig. 4). In *D. virilis* the most anodal GPDH staining band and the GPDH-1-encoding transcript are both unique to adults, consequently we argue that this band represents the GPDH-1 isozyme. Thus it is reasonable to assume that the least anodal GPDH staining band in *D. virilis* adults is encoded by the only other *Gpdh* transcript expressed at this stage of development and hence it is the homologue of the GPDH-4 isozyme in *D. melanogaster*. The middle band in *D. virilis* might then be a heterodimer composed of one GPDH-1 subunit and one GPDH-4 subunit. Similarly, the middle band in *D. melanogaster* and *D. busckii* adults might be a mixture of heterodimers made of one GPDH-1 subunit and one GPDH-3 or GPDH-4 subunit (Wilanowski *et al*., 1995).

These findings prompt a question about the basis of the variation in *Gpdh* expression in the *Drosophila* genus. A comparison of *Gpdh* sequences obtained from *D. melanogaster* (Bewley *et al*., 1989; von Kalm *et al*., 1989) and *D. virilis* (Tominaga *et al*., 1992) revealed that the splice sites in exons 6 and 7 are perfectly conserved but the sequences adjacent to polyadenylation signals in these exons are very different in *D. melanogaster* and *D. virilis*. This result provides new support to the hypothesis originally formulated by Cook *et al*. (1988) that the polyadenylation site selection is the regulated event in *Gpdh* expression.

The change of isozymic patterns could have been triggered by a mutation in exon 7 which is translated only in GPDH-4 (Bewley *et al*., 1989; von Kalm *et al*., 1989). There is one amino acid difference between *D. virilis* and *D. melanogaster* in this exon. It is located at residue 353, which is phenylalanine in *D. virilis* but serine in *D. melanogaster* (Tominaga *et al*., 1992). *Drosophila busckii* is reputed to be the only other *Drosophila* species that has the same mutation in exon 7 as *D. virilis* (Wells, 1996). However, in *D. busckii* the transcripts coding for GPDH-3 and GPDH-4 are expressed both in adults and larvae, which is different from *D. virilis* (Table 1). It remains possible that some other mutation in the GPDH protein is associated with the shift in the GPDH isozymic pattern. There are seven amino acid replacements in *D. virilis* in comparison with the *D. melanogaster* $Gpdh^{S}$ allele (Tominaga *et al*., 1992). Unfortunately, as many as three of them are unique to *D. virilis* (Wells 1996) so this supposition is very difficult to test.

The data overall emphasize that seemingly similar phenotypes can be produced by different regulatory patterns in different species. Further studies on the expression of the *Gpdh* transcripts in various species of Diptera should shed light on this important evolutionary phenomenon.

Acknowledgements

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References

BARTOSZEWSKI, S. 1996. *Analysis of Regulation of Expression of the* sn-*Glycerol-3-phosphate Dehydrogenase (*Gpdh*) Gene of* Drosophila melanogaster *by P-element Transformation*. Ph.D. Thesis, Australian National University.

BARTOSZEWSKI, S. AND GIBSON, J. B. 1994. Injecting un-dechorionated eggs of *Drosophila melanogaster* under ethanol. *Drosoph. Inf. Serv.*, **75**, 205–206.

BEWLEY, G. C. AND LUCCHESI, J. C. 1977. Origin of α-glycerophosphate dehydrogenase isozymes in *Drosophila melanogaster* and their functional relationship in the α-glycerophosphate cycle. *Biochem. Genet.*, **15**, 235–251.

BEWLEY, G. C. AND MILLER, S. 1979. Origin and differentiation of the soluble α-glycerolphosphate dehydrogenase isozymes in *Drosophila melanogaster*. *Isozymes: Curr. Top. Biol. Med. Res.*, **3**, 23–52.

BEWLEY, G. C., COOK, J. L., KUSAKABE, S., MUKAI, T., RIGBY, D. L. AND CHAMBERS, G. K. 1989. Sequence, structure and evolution of the gene coding for *sn*-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster*. *Nucl. Acids Res.*, **17**, 8553–8567.

COLGAN, D. J. 1992. Glycerol-3-phosphate dehydrogenase isozyme variation in insects. *Biol. J. Linn. Soc.*, **47**, 37–47.

COLLIER, G. E., SULLIVAN, D. T. AND MACINTYRE, R. J. 1976. Purification of α-glycerophosphate dehydrogenase from *Drosophila melanogaster*. *Biochim. Biophys. Acta*, **429**, 316–323.

COOK, J. L., SHAFFER, J. B., BEWLEY, G. C., MACINTYRE, R. J. AND WRIGHT, D. A. 1986. Isolation of a genomic clone for *Drosophila sn*-glycerol-3-phosphate dehydrogenase using synthetic oligonucleotides. *J. Biol. Chem.*, **261**, 11751–11755.

COOK, J. L., BEWLEY, G. C. AND SHAFFER, J. B. 1988. *Drosophila sn*-glycerol-3-phosphate dehydrogenase isozymes are generated by alternate pathways of RNA processing resulting in different carboxyl-terminal amino acid sequences. *J. Biol. Chem.*, **263**, 10858–10864.

GIBSON, J. B., WILKS, A. V., CAO, A. AND FREETH, A. L. 1986. Dominance for *sn*-glycerol-3-phosphate dehydrogenase activity in *Drosophila melanogaster*: evidence for differential allelic expression mediated via a *trans*-acting effect. *Heredity*, **56**, 227–235.

GIBSON, J. B., CAO, A., SYMONDS, J. AND REED, D. 1991. Low activity *sn*-glycerol-3-phosphate dehydrogenase variants in natural populations of *Drosophila melanogaster*. *Heredity*, **66**, 75–82.

KLEMENZ, R., WEBER, U. AND GEHRING, W. J. 1987. The *white* gene as a marker in a new P-element vector for gene transfer in *Drosophila*. *Nucl. Acids Res.*, **15**, 3947–3959.

KWIATOWSKI, J., KRAWCZYK, M., JAWORSKI, M., SKARECKY, D. AND AYALA, F. J. 1997. Erratic evolution of glycerol-3-phosphate dehydrogenase in *Drosophila*, *Chymomyza*, and *Ceratitis*. *J. Mol. Evol.*, **44**, 9–22.

LINDSLEY, D. L. AND GRELL, E. H. 1968. Genetic variations of *Drosophila melanogaster*. *Publ. Carnegie Inst. Wash.*, *Number 627*.

PETERSON, M. L., BRYMAN, M. B., PEITER, M. AND COWAN, C. 1994. Exon size affects competition between splicing and cleavage-polyadenylation in the immunoglobulin μ gene. *Mol. Cell. Biol.*, **14**, 77–86.

SYMONDS, J. E., GIBSON, J. B., WILKS, A. V. AND WILANOWSKI, T. M. 1995. Molecular analysis of a *Drosophila melanogaster sn*-glycerol-3-phosphate dehydrogenase allozyme variant that has cold labile activity. *Insect Biochem. Mol. Biol.*, **25**, 789–798.

TOMINAGA, H., SHIBA, T. AND NARISE, S. 1992. Structure of *Drosophila virilis* glycerol-3-phosphate dehydrogenase gene and a comparison with the *Drosophila melanogaster* gene. *Biochim. Biophys. Acta*, **1131**, 233–238.

VON KALM, L., WEAVER, J., DEMARCO, J., MACINTYRE, R. J. AND SULLIVAN, D. T. 1989. Structural characterization of the α-glycerol-3-phosphate dehydrogenase-encoding gene of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 5020–5024.

WELLS, R. S. 1996. Nucleotide variation at the *Gpdh* locus in the genus *Drosophila*. *Genetics*, **143**, 375–384.

WILANOWSKI, T. M., GIBSON, J. B. AND SYMONDS, J. E. 1995. Retrotransposon insertion induces an isozyme of *sn*-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 12065–12069.

WRIGHT, D. A. AND SHAW, C. R. 1969. Genetics and ontogeny of α-glycerophosphate dehydrogenase isozymes in *Drosophila melanogaster*. *Biochem.. Genet.*, **3**, 343–353.