Evolutionary Conservation of the Chromosomal Configuration and Regulation of Amylase Genes among Eight Species of the *Drosophila melanogaster* Species Subgroup


*Department of Biology, University of Ottawa; and †Laboratoire de Genetique et Biologie Evolutive, C.N.R.S.

Nuclear DNA was extracted from each of the eight species comprising the *Drosophila melanogaster* species subgroup. Southern hybridization of this DNA by using a molecular probe specific for the alpha-amylase coding region showed that the duplicated structure of the amylase locus, first found in *D. melanogaster*, is conserved among all species of the *melanogaster* subgroup. Evidence is also presented for the concerted evolution of the duplicated genes within each species. In addition, it is shown that the glucose repression of amylase gene expression, which has been extensively studied in *D. melanogaster*, is not confined to this species but occurs in all eight members of the species subgroup. Thus, both the duplicated gene structure and the glucose repression of *Drosophila* amylase gene activity are stable over extended periods of evolutionary time.

Introduction

Within the genus *Drosophila*, the *melanogaster* species subgroup includes eight species. *Drosophila melanogaster* and *D. simulans* are cosmopolitan, and *D. yakuba*, *D. teissieri*, *D. erecta*, *D. orena*, *D. mauritiana*, and *D. sechellia* are endemic to the Neotropical region, the last two having been found only in the islands of Mauritius and the Seychelles, respectively (see Lemeunier et al. 1986 for a review). On the basis of their male genitalia (Tsacas and Bocquet 1976) and of their polytene chromosome structures (Lemeunier and Ashburner 1976), these species were split into two complexes. More recent analyses (Cariou 1987) suggest the existence of three complexes rather than two. The first of these complexes contains *D. orena* and *D. erecta*, the second comprises *D. yakuba* and *D. teissieri*; and the third is represented by *D. melanogaster*, *D. simulans*, *D. mauritiana*, and *D. sechellia*. Several estimates of the divergence times between these species have been made (see Cariou 1987 for a review). Both molecular (Ashburner et al. 1984) and biogeographical (Lachaise et al. 1988) data can be used to infer an initial split between the *erecta-orena* complex and the other six species ∼15 Myr ago (Mya), followed by the divergence between the two remaining complexes as recently as 2.5–3.0 Mya.

The alpha-amylase (E.C.3.2.1.1.) locus in *D. melanogaster* has been extensively studied (see Doane et al. 1987; Hickey and Benkel 1987 for reviews). Amylase enzymes
within this species are encoded by two closely linked genes located ~ 4 kb apart (Gemmill et al. 1985; Levy et al. 1985; Boer and Hickey 1986; Benkel et al. 1987). Although natural populations of D. melanogaster are highly polymorphic for amylase allozyme phenotypes, including both single-banded and multiple-banded enzyme phenotypes (Hickey 1979; Singh et al. 1982), all strains examined at the DNA level within this species have a duplication of the amylase gene (Gemmill et al. 1986; S. Abukashawa, B. F. Benkel, and D. A. Hickey, unpublished data). The major purpose of the present study was to determine whether this duplication, which appears ubiquitous within D. melanogaster, also extends to the other sibling species within the melanogaster subgroup. Recent results on enzyme polymorphisms (Ogoubi et al. 1987) indicate that the duplication must be present in at least several of the other sibling species.

It is also interesting that amylase genes within D. melanogaster are glucose repressible (Hickey and Benkel 1982; Benkel and Hickey 1986a, 1986b). Therefore, the second aim of the present study was to test whether the repression occurred within the other members of the melanogaster species subgroup.

Material and Methods

The following Drosophila stocks were used in the present study: Drosophila melanogaster (Canton-S and Oregon-R, two commonly used laboratory strains); D. simulans (Antanarivo, a strain collected in Madagascar in 1984); D. mauritiana (Les Galets, collected in 1985); D. sechellia (strain 228, collected in the Seychelles Islands, 1981); D. yakuba (strain Tai 12, collected in the Ivory Coast); D. teissieri (strain 201-5, collected in Brazzaville in 1977); D. erecta (strain 220-S, collected in the Ivory Coast, 1980); D. orena (the only known strain of this species was collected in 1975). The two strains of D. melanogaster were obtained from the Mid-America Stock Center (Bowling Green, Ohio) and all other strains were from the collection of the Laboratoire de Biologie et Genetique Evolutives (CNRS, Gif-sur-Yvette, France). All stocks were maintained on commercially prepared medium (Carolina Biological Supply Co.).

Drosophila genomic DNA was isolated using a modification of the procedure described by Davis and Davidson (1984). In brief, several hundred flies or larvae (~1 g wet weight) were dounce homogenized, and a nuclear pellet was prepared by low-speed centrifugation. Nuclei were lysed by treatment with proteinase K and sarkosyl. Nuclear DNA was purified by digestion with DNase-free RNase and proteinase K followed by phenol extraction and ethanol precipitation. DNA from each strain (10 µg) was digested with a variety of restriction enzymes according to the manufacturers’ instructions. The digested DNA was electrophoresed and blotted onto nylon membranes (Biotrans; ICN Biomedicals) by the method of Southern (1975). These membranes were hybridized to a D. melanogaster amylase cDNA 32P-labeled probe (Benkel et al. 1987) overnight at 65 °C in 6 × saline sodium citrate (SSC), 5 × Denhardt’s solution, 0.5% sodium dodecyl sulfate (SDS) (w/v), 10 mM ethylene diaminotetraacetate (EDTA), and 100 mg salmon-sperm DNA/ml. Filters were washed three times for 30 min in 5 mM sodium phosphate pH 6.5, 1 mM EDTA, and 0.2% SDS (w/v) at 65 °C. Under these hybridization conditions, only those sequences with >80% sequence identity would be expected to bind the probe.

Glucose repressibility of amylase expression was assayed by the method described by Benkel and Hickey (1986a). Larvae of each strain were subjected to two dietary treatments, one of which contained 10% glucose while the other treatment lacked glucose. Third-instar larvae were harvested, and larval homogenates were electrophoresed and stained for amylase activity as described by Hickey (1981).
Fig. 1.—Southern analysis of genomic DNA. Panel A, DNA digested with BamHI; panel B, DNA digested with HindIII; panel C, DNA double-digested with BamHI and Sall. Samples are as follows: lane 1, Drosophila melanogaster (Canton-S); lane 2, D. simulans; lane 3, D. mauritiana; lane 4, D. sechellia; lane 5, D. yakuba; lane 6, D. orena; lane 7, D. erecta. Probe and conditions used are described in the Material and Methods section. Fragment sizes are in base pairs.

Results

The Southern hybridizations shown in figure 1 indicate that the amylase coding sequences from all members of the melanogaster species subgroup are quite similar. This conclusion is based on strong hybridization bands under conditions of high stringency. This similarity is to be expected based on the phylogenetic relatedness of these
Amylase Genes in *Drosophila melanogaster* of the Subgroup 563

![Diagram of molecular structure of the amylase region](image)

**Fig. 2.—** Molecular structure of the amylase region. A simplified map of the amylase region in *Drosophila melanogaster* is shown (top); coding regions are indicated by boxes; *BamHI* and *SalI* restriction sites which occur in all eight species are shown without parentheses; the *BamHI* sites that are lacking in both amylase coding regions of *D. teissieri* and *D. erecta* are shown in parentheses. The crosshatched bars indicate regions that hybridize to the probe used. A scale, in kilobase pairs, indicating the fragment sizes is also shown (bottom).

species. In addition to the cross-hybridization, there is extensive similarity in the restriction patterns of the amylase loci in all eight sibling species. This is especially true for enzymes, such as *SalI* and *BamHI*, that cut within the coding region (for detailed restriction maps of the amylase region, see Gemmill et al. 1985, 1986; Benkel et al. 1987). Figure 1A shows that five species have identical *BamHI* patterns. Of the remaining three species *Drosophila orena* (fig. 1A, lane 6) has two bands of intermediate size (1.3 kbp and 4.5 kbp, respectively) in addition to the 700-bp and 6.0-kbp fragments that compose the common pattern, suggesting the presence of either a pseudogene or a restriction polymorphism for an extra *BamHI* site in *D. orena*. In contrast, *D. erecta* (fig. 1A, lane 7) and *D. teissieri* (data not shown) have a single larger *BamHI* fragment of ~7.4 kbp and are lacking the smaller *BamHI* fragment of ~700 bp. When restriction enzymes that do not have recognition sites within the coding region are used, such as *HindIII* (fig. 1B), a much higher level of interspecific variation is observed in digests with *EcoRI* (data not shown), which also cuts only in flanking sequences.

To explore the basis for the difference between the common *BamHI* pattern and that found in *D. erecta* and *D. teissieri* (fig. 1A), we doubly digested DNA samples with *BamHI* and *SalI*. It is known that the coding sequence of *D. melanogaster* amylase contains a single *SalI* site, which lies between two *BamHI* sites (Boer and Hickey 1986; Gemmill et al. 1986; Benkel et al. 1987). Thus, double digestion of *D. melanogaster* DNA with *SalI* and *BamHI* results in a pattern consisting of a 200-bp, a 500-bp, and a 6.0-kbp band, when hybridized with the amylase coding-region probe. The results of the double digests are shown in figure 1C. Although *D. erecta* lacks the small (700-bp) *BamHI* fragment that is characteristic of *D. melanogaster* (fig. 1A), it does show the 500-bp *BamHI*-*SalI* fragment that is also found in *D. melanogaster* (fig. 1C). We interpret these patterns as meaning that the upstream *BamHI* site is absent from both of the duplicated coding sequences in *D. erecta* and *D. teissieri*. The inferred restriction map is outlined in figure 2. We confirmed this interpretation by performing single *SalI* digests (data not shown). In this case all eight species give a single strong hybridization band of ~6.4 kb in length.

The results of the glucose repression test are shown in figure 3. For each species, larvae were grown both in the presence of added dietary glucose (repressing conditions) and in the absence of added glucose (derepressing conditions). Third-instar larvae were harvested, and aliquots having equal protein content were loaded onto electrophoretic gels. One such gel, stained to visualize the amylase activity, is shown in figure 3. *Drosophila sechellia* was not used in these experiments; this species has relatively
low levels of amylase activity under all dietary conditions and proved difficult to
maintain in the absence of dietary glucose. For the other seven species (labeled A–G
in fig. 3) the lane marked with a minus sign (−) is a larval sample from the derepressing
conditions while the lane marked with a plus sign (+) represents glucose-treated larvae.
In all cases, the enzymatic activity was greatly diminished by the addition of glucose
to the larval diet. We already know (Benkel and Hickey 1986a) that the degree of
repression in the Oregon-R strain of D. melanogaster (sample F in fig. 3) is more than
100-fold. By comparing lane pair F with the other samples in figure 3, we conclude
that the degree of glucose repression in all seven sibling species is in the range of 10
100-fold.

Discussion

The occurrence of gene families is relatively common among the higher eukaryotes
(see Maeda and Smithies 1986 for a recent review). The duplicated alpha-amylase
genes in Drosophila melanogaster (Gemmill et al. 1985; Benkel et al. 1987) may be
considered as a very small gene family with two members. The duplicated amylase
genesis are not tandemly arranged, however; rather, they are divergently transcribed
(Boer and Hickey 1986). Such an arrangement is expected to be evolutionarily more
stable than a tandem duplication, since there is not the same potential for generating
variations in gene copy number through unequal crossing-over. This expectation of
structural stability is borne out by the results presented here.

Because of the close linkage between the duplicated amylase genes (Gemmill et
al. 1985, 1986), and because of the similarity in their coding sequences (Boer and
Hickey 1986), there is the possibility of intrachromosomal paralogous recombination
between the two coding regions. Such recombination would result in an inversion of the upstream intergenic region. There is direct evidence for such recombination events at the amylase locus in *D. melanogaster* (Langley et al. 1988; Hickey et al., submitted; P. E. Schwartz and W. W. Doane, personal communication).

Gene conversion between linked inverted repeat sequences has also been reported for the major heat-shock genes in *Drosophila* (Leigh Brown and Ish-Horowicz 1981) and for the chorion genes of *Bombus mori* (Iatrou et al. 1984). Leigh Brown and Ish-Horowicz (1981) proposed a model for gene conversion that predicts an association between intrachromosomal recombination and gene conversion. This type of model was recently subjected to experimental verification in yeast (Willis and Klein 1987). They found that ~50% of intrachromosomal crossovers, which occurred between inverted repeat sequences, were associated with gene conversion. They also reported that the majority of the interactions between repeats are conversions that are resolved as noncrossovers. If the same is true of *Drosophila* sequences, it would mean that the observed frequency of crossovers is indicative of a much higher frequency of gene conversion.

Gene conversion would explain the coding sequence conservation between the two amylase genes observed in *D. melanogaster* (Boer and Hickey 1986). The hypothesis of concerted evolution is also supported by our observation (see figs. 1, 2) that restriction-site differences between species (such as the absence of a BamHI site) are shared by both genes within a species (e.g., *D. erecta*). Without invoking concerted evolution it is difficult to reconcile the observation that the duplication predated the speciation events (and is consequently not very recent) with the observation of little sequence divergence between the duplicated gene copies. Walsh (1987) has explored the evolutionary parameters that affect the balance between sequence divergence due to accumulated mutations and sequence conservation due to gene conversion.

Apart from the *Drosophila* amylase genes, we know of no other instances of glucose repression of gene expression among the invertebrates (see Benkel and Hickey 1986a). It is, however, an important aspect in the regulation of amylase gene expression in *D. melanogaster*, where it acts at the level of mRNA abundance (Benkel and Hickey 1987). Despite the fact that there have not been other reports of this phenomenon, it is unlikely that it occurs at only a single locus within a single eukaryotic species, namely, the alpha-amylase locus of *D. melanogaster*. There is indeed evidence that *D. melanogaster* genes other than amylase are subject to glucose repression (Benkel et al. 1987). In the present paper we have shown that the repression of alpha-amylase extends throughout the *D. melanogaster* subgroup. We also have unpublished evidence that glucose repression of amylase occurs in *D. pseudoobscura* and in the lepidopteran *Ostrinia nubilalis*.

**Acknowledgments**

This work was supported by an Operating Grant from NSERC Canada to D.A.H. and by a Postdoctoral Fellowship from C.N.R.S. France to V.P.

**LITERATURE CITED**


WALTER M. FITCH, reviewing editor

Received November 12, 1987; revision received March 15, 1988