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Evolution

Amyrel, a paralogous gene of the amylase gene family in *Drosophila melanogaster* and the *Sophophora* subgenus

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ABSTRACT **We describe a gene from *Drosophila melanogaster* related to the alpha-amylase gene *Amy*. This gene, which exists as a single copy, was named *Amyrel*. It is strikingly divergent from *Amy* because the amino acid divergence is 40%. The coding sequence is interrupted by a short intron at position 655, which is unusual in amylase genes. *Amyrel* has also been cloned in *Drosophila ananassae*, *Drosophila pseudoobscura*, and *Drosophila subobscura* and is likely to be present throughout the *Sophophora* subgenus, but, to our knowledge, it has not been detected outside. Unexpectedly, there is a strong conservation of 5′ and 3′ flanking regions between *Amyrel* genes from different species, which is not the case for *Amy* and which suggests that selection acts on these regions. In contrast to the *Amy* genes, *Amyrel* is transcribed in larvae of *D. melanogaster* but not in adults. However, the protein has not been detected yet. *Amyrel* evolves about twice as fast as *Amy* in the several species studied. We suggest that this gene could result from a duplication of *Amy* followed by accelerated and selected divergence toward a new adaptation.**

Acquisition of new biological functions is a main process of evolution. Because a new function involves new genes or new regulations of existing genes, a great deal has been focused on gene duplication events (1–3). Several processes may follow a gene duplication: concerted evolution that retains the similarity between duplicates, accelerated divergence toward putative new functions, or pseudogene formation. As molecular data have accumulated, it has been shown that many genes are members of multigene families having active or pseudogenic "companions" (3).

The amylase gene is an interesting model for studying evolution of multigene families. For over 30 years, it has been investigated widely in many organisms. Its enzymatic activity is revealed easily on electrophoresis gels (4). During the past decade, *Amy* genes were cloned and sequenced in a number of bacteria, fungi, plants, and animals. Alignments of AMY proteins have shown that, despite a high variability, a few blocks of amino acids were conserved (5, 6). In animals, many more amino acid stretches are conserved between species, and alignments remain easy.

Multicopy structures with various gene arrangements were found in various taxa: man and other Primates (7), rodents (8), and Crustacea (9). In Drosophilids, *Drosophila melanogaster* has two copies (10), *Drosophila pseudoobscura* has between one and three copies (11), *Drosophila eugracilis* and *Drosophila ficusphila* have two copies (12), and *Drosophila ananassae* has at least seven copies (13). It seems that multiplications (and loss?) of *Amy* genes have occurred independently in many animal lineages, raising the question of an adaptive advantage.

Focusing on amylase evolution in Drosophilids, we observed that some species harbored two types of genes: those with an intron at position 177, which is supposed to be ancestral (14), and those without an intron at this position. This was the case in *Drosophila takahashii*, *Drosophila lucipennis*, and in the *Drosophila obscura* group. Phylogenetic trees clearly showed that "intronless" genes of these species were excluded from the *Amy* tree but remained clustered together, suggesting that they were paralogs (14). Given the tree topology, we suspected that such a divergent gene might be present also in *D. melanogaster*. By using PCR primers specific to these genes, a fragment then was amplified from *D. melanogaster*. The present work describes the structure, chromosomal localization, and expression pattern of this gene, named *Amyrel* (for Amylase-related), in *D. melanogaster* and several species of the *Sophophora* subgenus and its evolutionary relationship with the classical *Amy* genes.

MATERIALS AND METHODS

The Canton-S strain of *D. melanogaster* was used. *D. subobscura* was from Montgenèvre, France, *D. pseudoobscura* was from Phoenix, Arizona, and *D. ananassae* was from Taï, Ivory Coast. DNA extraction and PCR conditions have been described (14). The primers used are listed in Table 1. Inverse PCR was performed by digesting genomic DNA with four-cutter restriction enzymes and religating the diluted cut DNA. The circularized DNA then was amplified with relevant primers. PCR products were cloned into the pGEM-T vector (Promega) and sequenced with an automated device, ABI 373 (Applied Biosystems).

Genomic clones were obtained for *D. melanogaster*, *Drosophila subobscura,* and *D. pseudoobscura*; minilibraries were obtained by cloning in pUC plasmid digestion fragments of required size (previously identified by Southern analysis). PCR fragments of *Amyrel* were used as probes for screening. Positive clones were treated for nested deletions and were sequenced. A genomic clone from *D. ananassae* had been obtained (J.-L.D.L., unpublished data).

For mRNA detection, flies were reared on axenic, sugar-free medium at 25°C, and several individuals were sampled at various time points from embryo to adult. The second- to third-instar molt was considered to occur 72 h after egg laying. For each time point, at least three individuals were assayed. Reverse transcription (RT)–PCR protocol was adapted from Huet *et al.* (15). RNAs were roughly extracted (16). Samples (1/100 of the extract) were treated with DNase/RNasin before RT-PCR. Negative controls were made on DNase-treated

Abbreviation: RT, reverse transcription.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. U69607 (*Amyrel D. melanogaster*), U53698 (*Amy35 D. ananassae*), U53477 (*Amy4N D. ananassae*), U53479 (*Amyrel D. ananassae*), U79724 (*Amyrel D. subobscura*), U80035 (*Amy D. subobscura*), andU82556 (*Amyrel D. pseudoobscura*)].

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Table 1. List of the primers used in this study

Primer name	Sequence (strand)	Position	Use
Amyrel1	GAGAACATCATTTCGGCGG (+)	196/214	(RT)PCR Amyrel
Amyrel2	TGGTCGAGACCCTTGAGGC (−)	521/539	(RT)PCR Amyrel
Rev1230	TTGCTGCCRTTRTCCCACC (−)	1273/1291	PCR Amy/Amyrel
Haerel279	TTCCAGGTGCAACAGTGCG (+)	493/511	inverse PCR Amyrel
Haerel855	CTCATCACCCGACTGGTGC (−)	1072/1090	inverse PCR Amyrel
Haerel1024	GAACGCCGTTCGGGATACG (+)	1241/1259	inverse PCR Amyrel
Haerel58	TCGTCCACTTGTTCGAGTG (+)	95/113	inverse PCR Amyrel
Haerel5	CCACCAATGGGGATTGTGC (−)	60/78	inverse PCR Amyrel
Intr1U	GTTCACCTCTTCGAGTGG (+)	94/111	RT-PCR Amy
Mel2	AGTCCAGCGAGGAGTAGGG (−)	427/445	RT-PCR Amy

Positions are numbered from the translation start. They were chosen with help of the program AMPLIFY 1.2 by Bill Engels. Some of them were designed to amplify *Amyrel* or *Amy* specifically; other primers were compatible with both classical and *Amyrel* genes. Primer Intr1U also matches to *Amyrel*.

samples without RT. To improve sensitivity, RT-PCR products were detected by Southern hybridizations with homologous radioactive probes. Individual organs from 96-h larvae (midgut, fat body, Malpighian tubules, and salivary glands) also were tested in the same conditions. *In situ* hybridizations on polytene chromosomes were prepared as described (13). The following software was used: the multisequence editor SEQAPP by Don Gilbert (Indiana University), CLUSTALW (17) (multiple alignment), KESTIM (18) (substitution rates), and MEGA (19) (codon usage).

RESULTS

The *Amyrel* Gene. In *D. melanogaster*, an internal part of *Amyrel* first was sequenced from a PCR product between primers Amyrel1 and Rev1230 and the surrounding regions by inverse PCR from −90 to 1699, relative to the translation start. Further data in flanking regions were obtained from genomic clones (two 4-kb-long *Pst*I fragments containing the left and right part of *Amyrel*, respectively). Using the full coding sequence as a probe, we performed Southern hybridizations that suggested that *Amyrel* is a single-copy gene (Fig. 1). Sequences from *D. subobscura*, *D. pseudoobscura* and *D. ananassae* were obtained readily from full-length genomic clones.

The coding sequence is 1482 bp long in both *D. melanogaster* and *D. ananassae*. In *D. pseudoobscura* and *D. subobscura*, an additional codon lies within the putative peptide signal. The gene is interrupted at position 655 (or 658) by a short intron (56 bp in *D. melanogaster* and *D. ananassae*, 60 bp in *D. subobscura,* and 68 bp in *D. pseudoobscura*), inserted between two codons. The intron position is quite unusual in amylase genes and seems not to be ancestral. The comparison between *Amyrel* and *Amy* (intron removed) shows high substitution rates per site and an overall divergence close to 40% in nucleotides and amino acids (see below). The length of *Amyrel* is equal to Drosophila *Amy*. Tree constructions based on either nucleic or protein data (Fig. 2 is from protein data) show that the divergence between *Amy* and *Amyrel* is higher than between any classical *Amy* genes within the *Drosophila* genus, but *Amyrel* remains inside the insect branch.

Nucleotide Divergence and Codon Usage in *Amy* and *Amyrel*. Table 2 shows that the divergence between *Amy* and *Amyrel* is similar in the four species with *K*a values around 0.37, suggesting that *Amyrel* has undergone similar selective constraints at the protein level in the different taxa. An important divergence from *Amy* also may have occurred in their common ancestor. In contrast, the synonymous rates are different between species and are correlated negatively to the C content in the third position and to the codon bias, as often reported (20–22). It is known that, in Drosophila, *Amy* is highly biased for its codon usage, especially in *D. melanogaster* (23) and *D. pseudoobscura* (24). In these two species, 88% of the *Amy* codons end with G or C, the latter being most preferred. It seems that *Amyrel* genes are a bit less constrained because of a lower codon bias. In *D. ananassae*, in which several classical but divergent *Amy* genes exist [*Amy35* and *Amy4N* in our study (J.-L.D.L., unpublished data)], the codon bias for *Amy* is lower than in *D. melanogaster or D. pseudoobscura* and similar to that of *Amyrel* (Table 2). However, comparisons between *Amy* and *Amyrel* indicate that for most synonymous groups, the same codons are preferred in the two genes for the species studied. This finding may reflect the general C-ending preference reported in the *Sophophora* subgenus (21). But, of interest, the high bias toward codon TTC (Phenylalanine), which is a typical trait in all Drosophila *Amy* genes known to date, is common to both types of genes. Tables available for codon usage in pooled Drosophila genes (25) or *Xdh* or *Adh* do not show such a bias. On the other hand, the low usage of GGG (glycine) or TTA (leucine) in *Amy* and *Amyrel* is in accordance with the Drosophila general usage.

The divergence between the *Amyrel* genes is higher than between the *Amy* genes (Table 3). *Amyrel* seems to be a fast

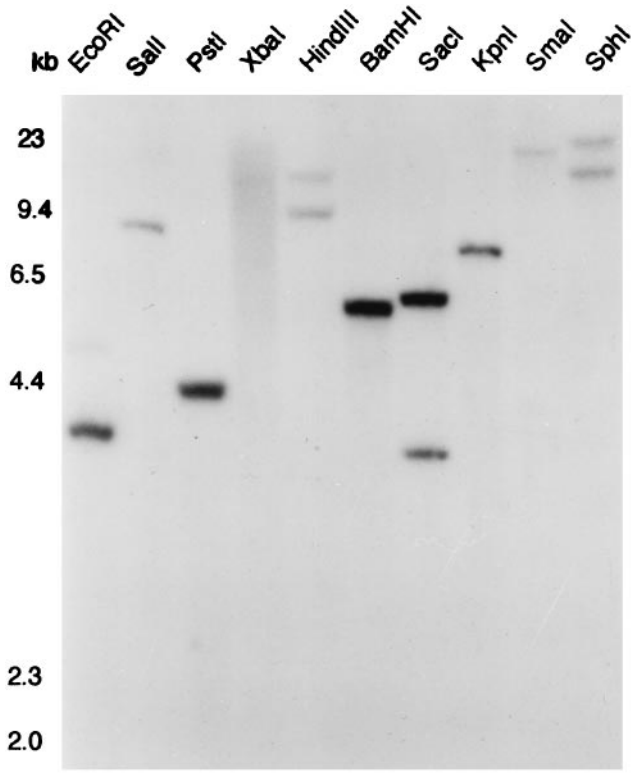

FIG. 1. Southern blot of genomic DNA from *D. melanogaster* probed with the entire *Amyrel* gene (^{32}P-labeled). To eliminate signal from *Amy*, washing was stringent (63°C, 0.25 × standard saline citrate, 1%SDS). The size and number of fragments suggest that *Amyrel* is single copy. For *Pst*I, the band is double (see text).

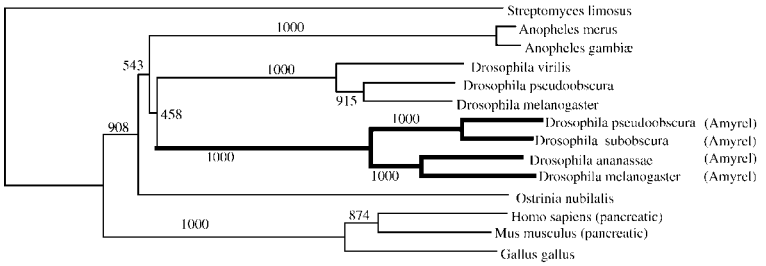

FIG. 2. Distance tree from an alignment of the proteins by CLUSTALW (Neighbor joining method) showing the position of *Amyrel* relative to *Amy*. The *Amyrel* branch is the bold line. The tree was rooted with a Streptomycete (6). Numbers along branches are bootstrap values (1,000 replicates). [GenBank accession numbers: *S. limosus*, M18244; *A. merus* (mosquito), U01210; *A. gambiae*, L04753; *D. virilis*, U02029; *D. pseudoobscura*, X76240; *D. melanogaster*, X04569; *O. nubilalis* (moth), U04223; *H. sapiens*, P04746; *M. musculus*, P00688; and *G. gallus*, U63411.]

evolving gene if compared with *Amy* and also with *Adh*, *Adh-dup*, or *Xdh*, for instance. *K*a values between *D. pseudoobscura* and *D. melanogaster* are 0.07 ± 0.008, 0.05 ± 0.008, 0.04 ± 0.008, and 0.07 ± 0.004 for these four genes, respectively. The *K*a value for *Amyrel* is 0.13 ± 0.012. The higher synonymous rates (Ks) in *Amyrel* would be correlated to the lower codon bias of this gene compared with *Amy*, which is known for its low synonymous rate (like *Adh*; see ref. 21). Also, the high ratios Ks/Ka are indicative of protein-coding capacity (26).

The AMYREL Protein. The conceptual product AMYREL is a protein of 493 aa (55.3 kDa) in *D. melanogaster* that differs by 42% from the classical AMY protein. The divergence is higher within the first 30 residues, which makes the N-terminal part difficult to align, except for the putative cleavage site for the peptide signal (23) (Fig. 3). Several amino acid stretches highly conserved in animal or even bacterial alpha-amylases (5, 6) are present in AMYREL. Cysteine residues that are involved in disulfide bridges (6) are conserved particularly (asterisks in Fig. 3), suggesting that AMYREL may be an alpha-amylase. However, the AMYREL proteins from the four species studied have lost the conserved motif GHGA (positions 325–328), and, in ten positions (arrows in Fig. 3), they share identical residues different from the residues conserved in animal alpha-amylases. These residues might be involved in a specific function of AMYREL. Two additional cysteines also are found in AMYREL at positions 442 and 465 of the alignment, which might create a new disulfide bridge and, hence, a different tertiary structure.

Flanking Regions. We have sequenced the 3′ flanking regions of *Amyrel* in *D. melanogaster*, *D. ananassae*, and *D. subobscura* and the 5′ regions in these species and in *D. pseudoobscura*. The high level of interspecific conservation of long stretches of noncoding sequences (Fig. 4) is a striking and unexpected result, given the situation in *Amy*, in which only a few short motifs have been found to be conserved upstream to the gene (27). Within the 3′ region, a polyadenylation site, AATAAA, is usually found in *Amy* genes. In *Amyrel*, this motif is included in an almost perfectly conserved stretch of 60–70 bp. Significant homologies between downstream sequences remain visible for several hundreds of base pairs (not shown on Fig. 4): AACTGGASTTAGTCTAACA (241–260); TGCCWCGACAACGASA (263–278); CAGCTGRCACWCTGT (391–406); CTGCAAATAGAARAAGKTSGCATT (423–446); and TGAAATTGTAGTTGGSTGYTCTGG (449–472). The 3′ region in *D. melanogaster* corresponds from positions 260 to 594 to sequence-tagged site Dm0827 (28), that maps at the same locus.

In the 5′ flanking regions, three long motifs have been preserved during evolution (Fig. 4): (*i*) −183 to −169; (*ii*) −129 to −84; and (*iii*) −80 to −69. The latter has been recognized by Magoulas *et al*. (29) to be involved in amylase regulation. But, except for this case, our search in databases for similar motifs was not conclusive. The putative TATA box was found in the four sequences, but no obvious CAAT box has been detected so far.

Chromosomal Localization. A PCR fragment of the *Amyrel* coding sequence was used as a probe for *D. subobscura*. For *D. melanogaster* and *D. ananassae*, genomic adjacent fragments were used instead. Fig. 5 shows the chromosomal labeling of the biotinylated probes. In *D. melanogaster*, *Amyrel* maps at 53D1–3, (*Amy* is at 54A1B1; ref. 30 and our data). In *D. subobscura*, *Amyrel* and *Amy* map at 74A and 55D, respectively, at the two opposite tips of the acrocentric chromosome E. In *D. ananassae*, *Amyrel* is at position 76C on the 3L arm (see ref. 31 for cytogenetical nomenclature), distant from a cluster of classical genes (81C; ref. 13).

***Amyrel* Expression in *D. melanogaster*.** RT-PCR experiments revealed that *Amyrel* is transcribed at the second and third larval instars with a maximum in young and middle third-instar larvae (Fig. 6). In contrast to *Amy*, no transcript was detected in adults. Tissue activity was found in the midgut and perhaps in the fat body (not shown). More refined experiments will be necessary to study tissue expression precisely.

DISCUSSION

Gene amplification techniques are powerful tools to detect duplicated copies of genes. *Amyrel* was discovered in the course of a PCR study on classical genes (14). Its high divergence with *Amy* may explain why it had not been characterized earlier.

Table 2. Intraspecific comparison between *Amy* and *Amyrel* in the four species studied

	melanogaster	*pseudoobscura*	*subobscura*	*ananassae* Amy35	*ananassae* Amy4N
Overall nucleotide divergence (%)	43.3	41.1	37*	41.5	43.7
Overall aminoacid divergence (%)	41.8	43.3	40*	40.6	41.2
Ka Amyrel/Amy	0.37	0.36	0.31*	0.37	0.36
Ks Amyrel/Amy	1.32	0.96	0.85*	1.08	1.37
Overall GC richness, Amyrel/Amy (%)	55.5/62.8	59.6/62.8	61.5/61*	57.4/57.7	57.4/54.4
%C 3rd position, Amyrel/Amy	36.7/62.0	46.2/63.1	51.1/52.4*	43.6/50.0	43.6/42.4
%G+C 3rd position, Amyrel/Amy	67.1/87.6	79.2/87.6	81.3/80.3*	74.1/73.8	74.1/63.4

Asterisks indicate that the sequence available is incomplete for *Amy* in *D. subobscura*. Percentages of C and G+C at the third codon position were calculated excluding codons ATG (Met) and TGG (Trp). These values are indicative of the level of codon bias (22).

Table 3. Interspecific substitution rates per sites (*Amyrel*/*Amy*)

	melanogaster	*subobscura*	*pseudoobscura*	*ananassae Amy35*	*ananassae Amy4N*
melanogaster	—	0.92/0.52*	1.00/0.38	0.89/0.63	0.89/1.00
subobscura	0.13/0.06*	—	0.41/0.22*	0.83/0.63*	0.83/1.32*
pseudoobscura	0.13/0.07	0.05/0.04*	—	1.05/0.53	1.05/0.84
ananassae Amy35	0.08/0.04	0.13/0.06*	0.13/0.07	—	—/1.06
ananassae Amy4N	0.08/0.04	0.13/0.07*	0.13/0.07	—/0.04	—

Ks (synonymous) are above the diagonals; Ka (non-synonymous) are below. Both types of classical *Amy* genes of *D. ananassae* have been compared. The asterisks indicate that computing has been done by using the partial sequence of *D. subobscura* and the corresponding sequences of the other species.

Indeed, it is most likely that *Amyrel* had been cloned first by Gemmill *et al.* (30) along with *Amy* in *D. melanogaster*. The actual chromosomal localization of *Amyrel* is the same as that of their clone λ Dm32. However, Gemmill *et al*. found no expression by Northern blotting and considered this copy as a pseudogene. Brown *et al.* (24) observed an *in situ* hybridization signal in *D. pseudoobscura* additionally to the *Amy* locus and also suspected a pseudogene.

Here, we have shown that *Amyrel* has a full-length coding sequence and is transcribed in larvae. This divergent gene has remarkable features, making it a special case in the amylase family of Drosophila. The intron–exon structure is very original and does not correspond to an ancestral state because the intron site is not shared in other genomic sequences available (insects or vertebrates). Indeed, this intron is spliced correctly, as revealed by RT-PCR using primers that surround the intron (data not shown). Other data, such as significant codon bias and high Ks/Ka ratios, also are in favor of an active gene. However, AMYREL has not been detected by the usual technique for amylase electrophoresis. Given the number of charged residues, AMYREL should migrate faster than the classical AMY1 allele.

Another unexpected and interesting result is the high interspecific conservation of noncoding flanking regions. *D. melanogaster*, *D. ananassae*, and *D. subobscura* are not closely related, and the divergence time between these species (20–35 million years) is too long to allow conservation without selection. The pattern of highly conserved blocks distributed along a much less conserved sequence suggests a functional role for these motifs. In addition, further sequencing of these regions indicates that *Amyrel* might be surrounded very closely by other putative coding genes (J.-L.D.L., unpublished results), which could have an influence on the conservation of the flanking sequences.

The chromosomal localizations of *Amyrel* genes compared with *Amy* are variable; whereas both genes are in rather close regions in *D. melanogaster* (53D vs. 54A), they can be very distant in other species. Chromosomal rearrangements are likely responsible for this variation, but it is difficult to know which situation is ancestral because of the uncertain interspecific correspondences between chromosomal arms. However, the large distances between *Amyrel* and *Amy* in each species (even in *D. melanogaster*) suggest that they have evolved without any contact (unequal crossover or gene conversion). Moreover, such events, common in classical *Amy* genes that often are arranged tandem (24, 32), are avoided by the single-copy structure of *Amyrel*.

Until now, we have not found *Amyrel* outside the *Sophophora* subgenus, but it has been detected by PCR in *Drosophila willistoni*, which is considered the most divergent member of this subgenus. The confirmation that *Amyrel* is restricted to the *Sophophora* subgenus would indicate that the gene has undergone a very fast evolution, with a high rate of nonsynonymous substitutions compared with *Amy*: 40% amino acid substitutions in <60 million years [according to the estimated divergence time between *D. melanogaster* and *Dro-*

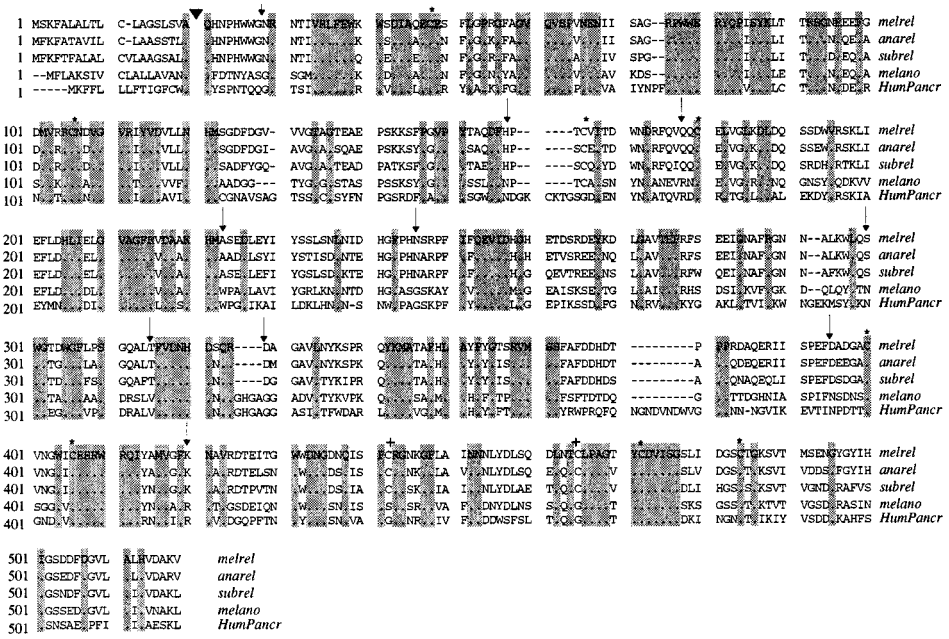

FIG. 3. Putative AMYREL proteins of *D. melanogaster* (melrel), *D. ananassae* (anarel), and *D. subobscura* (subrel) and AMY proteins from *D. melanogaster* (melano) and pancreatic amylase of man (HumPancr) were aligned by CLUSTALW and edited with SEQAPP. Shading indicates invariant residues. Vertical arrows show positions where AMYREL sequences share common residues that are different from conserved residues of animal AMY proteins. Asterisks mark the positions of cysteines involved in disulfide bridges in the pig and putatively in other animal AMY proteins. Plus signs mark additional cysteines in AMYREL.

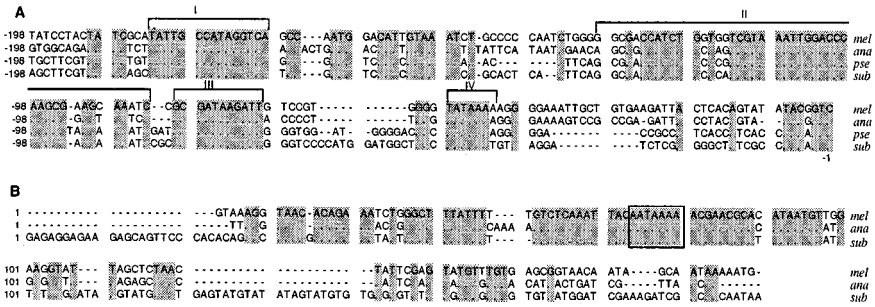

FIG. 4. (*A*) Alignment of 5′ regions of *Amyrel* in *D. melanogaster* (mel), *D. ananassae* (ana), *D. pseudoobscura* (pse), and *D. subobscura* (sub). Shading indicates invariant nucleotides. Long conserved sequences are numbered *i*, *ii*, and *iii* (see text); *iv* is the putative TATA box. CLUSTALW alignment was corrected manually. (*B*) Alignment of the 3′ regions (except *D. pseudoobscura*, which was not available). The putative polyadenylation site is boxed.

sophila virilis (33)]. Initially relaxed constraints on the new copy followed by positive selection may have allowed these changes. A recent intron insertion would have accompanied the differentiation of *Amyrel*. We also may notice that the rate of evolution of *Amyrel* among the four species studied so far seems to be about twice as fast as *Amy* (also suggested by Fig. 2). Data from other species will help in estimating the evolutionary dynamics of this new gene. It would be of interest to know whether the substitution rates have varied during evolution or among lineages. A recent study on a duplication of the *Adh* gene in the *Drosophila repleta* group (26) shows parallel results. In this group, a duplicated gene, *Adh*-Ψ, physically close to *Adh* formerly had been reported to be a pseudogene, but, like *Amyrel*, it shows a full-length ORF (with recruitment of codons from the upstream region) and exhibits a significant codon bias and a high Ks/Ka ratio. Also, despite its physical vicinity with *Adh*, which could facilitate gene conversion, *Adh*-Ψ evolves faster, and it has been shown that the substitution rate was higher in the past than recently. The same phenomenon might have occurred in *Amyrel*.

Duplications of *Amy* frequently have been found in the *Sophophora* subgenus but are not documented in other subgenera (12). As far as we know, the duplications were not followed by strong differentiation. However, *Amy* encodes a major digestive enzyme and may be subject to adaptation; a case of accelerated divergence in the *Amy* gene was observed in *Drosophila erecta* (34) and was attributed to adaptation to new resources. Similarly, the fast divergence of *Amyrel* and its

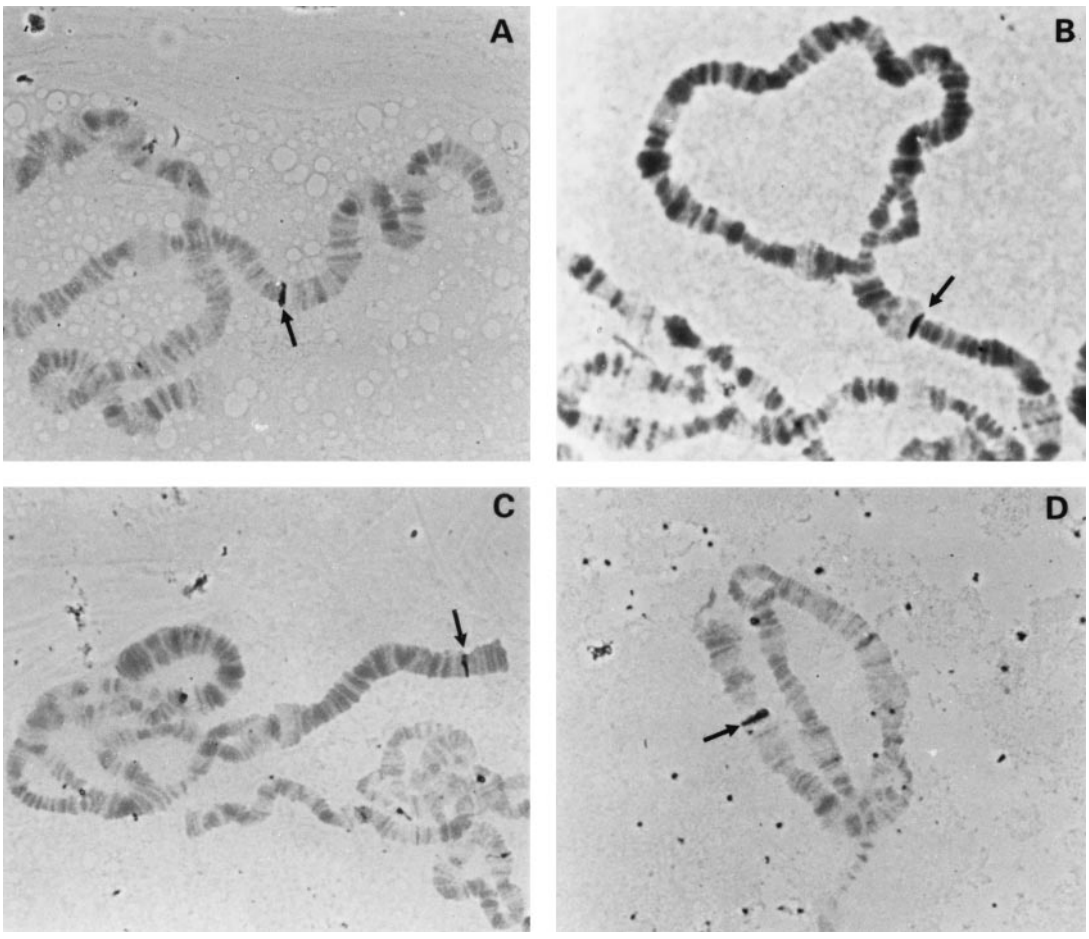

FIG. 5. *In situ* hybridizations and chromosomal localizations of *Amyrel* genes. (*A*) *D. melanogaster*. (*B*) *D. ananassae*. (*C*) *D. subobscura*. (*D*) *Amy* localization of *D. subobscura*.

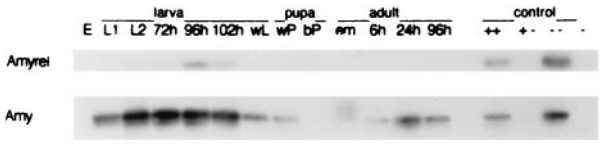

FIG. 6. RT-PCR analysis of *Amyrel* and *Amy* in *D. melanogaster*. The primers used are indicated in Table 1. Each time point was repeated on several individuals, one of which was representative and was selected for both genes. The sizes of amplified cDNAs are 344 bp for *Amyrel* and 352 bp for *Amy*. (E, embryo; L1, larva, first instar; L2, larva, second instar; 72h, L2/L3 molt time at 25°C; wL, wandering larva; w*p*, white pupa; b*p*, black pupa; em, emerging adult; ++, control (96-h larva) treated with DNase and RTase; +−, control treated with DNase and without RTase; − −, control with no DNase and no RTase; −, negative control.)

maintenance as an active gene also suggest an adaptation toward a new function or substrate. Further evidence for a new function is suggested by the amino acid differences between *Amyrel* and *Amy* in a number of usually conserved positions. In *Adh*-Ψ of the *D. repleta* group, substitutions in conserved regions of the protein also have been considered to be adaptive to new function (26). Other *Adh*-related genes of Drosophila, *FBP2* (35) and *jingwei* (36), were reported to have turned to novel functions, thus increasing genome potentialities. The esterase multigene family is another well known example of diversifying multiplication (37). In the amylase family, although regulatory differences are known (38, 39), *Amyrel* is a new example of such a structure and sequence divergence. For instance, the mouse salivary and pancreatic proteins are only 15% divergent. Biochemical studies will help in understanding the evolutionary meaning of this duplication and divergence as a paradigm of physiological adaptation through gene duplication.

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