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Is Esterase-P Encoded by a Cryptic Pseudogene In *Drosophila melanogaster*?

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ABSTRACT

We have amplified and sequenced the gene encoding Esterase-P (Est-P) in 10 strains of *Drosophila melanogaster*. Three premature termination codons occur in the coding region of the gene in two strains. This observation, together with other indirect evidence, leads us to propose that Est-P may be a pseudogene in *D. melanogaster*. Est-P would be a "cryptic" pseudogene, in the sense that it retains intact the coding sequence (without stop codons and other alterations usually observed in pseudogenes) in most *D. melanogaster* strains. We conjecture that the β-esterase cluster may consist in other Drosophila species of functional and nonfunctional genes. We also conjecture that the rarity of detected pseudogenes in Drosophila may be due to the difficulty of discovering them, because most of them are cryptic.

Eukaryotic genomes contain, in addition to functional genes, sequences that are related to particular genes but exhibit changes that inactivate their expression. Such sequences have been designated "pseudogenes" (reviewed by Vanin 1985; Weiner et al. 1986; Wilde 1986). Since they were first described (Jaqu et al. 1977; Fedoroff and Brown 1978), pseudogenes have been identified in a variety of gene families. Some are unprocessed pseudogenes that have introns and are usually chromosomally linked to their functional counterparts, suggesting that they arise by tandem gene duplication, followed by divergence and inactivation. Processed pseudogenes lack introns and are believed to arise from reverse transcription of processed mRNA, followed by integration of the resulting DNA into a new chromosomal location in the genome. Most pseudogenes are presumably not expressed, although several are transcribed into RNA molecules (Fischer and Maniatis 1985; Sorge et al. 1990; Aubert et al. 1992; Currie and Sullivan 1994; Fürrab and Vanselow 1995), and processed pseudogenes that are translated into protein molecules have been discovered (McCarrey and Thomas 1987).

Pseudogenes along with their functional counterparts provide useful models for understanding gene evolutionary processes, since they allow the comparison of mode and rate of molecular changes in sequences with and without functional constraints (Li et al. 1981, 1985b; Miyata and Yasunaga 1981; Miyata and Hayashida 1981; Gojobori et al. 1982; Li 1982, 1983).

Only a few examples of pseudogenes are known in Drosophila. A transcriptionally active (unprocessed) alcohol dehydrogenase (*Adh*) pseudogene, closely linked to one or two functional counterparts, has been described in *D. mulleri* (Fischer and Maniatis 1985), *D. mojavensis* (Atkinson et al. 1988), *D. mettleri* (Yum et al. 1991), *D. hydei* (Menotti-Raymond et al. 1991), *D. buzzatii* (Schäfer 1992), *D. peninsulatini*, and *D. mercatorum* (Sullivan et al. 1994). In all these species, the reading frames of the *Adh* pseudogenes contain premature termination codons, resulting from one or more frameshifts, which along with indels prevent productive translation. Kylen et al. (1990) have found two unprocessed pseudogenes, included in the cecropin locus of *D. melanogaster*. Processed pseudogenes of alcohol dehydrogenase have been found in *D. yakuba* and *D. teissieri* (Jeffs and Ashburner 1991; Jeffs et al. 1994) and phosphoglyceromutase (*Pglum*) in *D. melanogaster* (Currie and Sullivan 1994). The *Adh* and *Pglum* processed pseudogenes lack introns and are located on different chromosomes (*Adh*) or different arms of chromosome (*Pglum*) from their functional counterparts. 

Collet et al. (1990) have detected a tandem duplication of the *Est-6* gene in *D. melanogaster*, denoted as *Est-P*. Both genes belong to the β-esterase gene cluster (Korochkin et al. 1987) and are located within subsection 69 A1-A3 on the left arm of polytene chromosome 3 (Procunier et al. 1991), with the same 5' to 3' orientation (*Est-P* is downstream from *Est-6*). Both genes comprise two exons, with length of 1387 and 248 bp, separated by a short intron, which is 51 and 56 bp long in *Est-6* and *Est-P*, respectively. The coding regions of the genes are separated by only 197 bp. The two genes show 64 and 60% similarity in the DNA and protein sequence, respectively (Collet et al. 1990).

In Drosophila species, the β-esterase gene cluster includes two or (only in *D. pseudoobscura*) three known closely linked genes (Oakeshott et al. 1993, 1995), which have the same direction of transcription and similar exon/intron structure (Yenikolopov et al. 1983, 1992–1994; Currie and Sullivan 1994; Furst and Vanselow 1995). The genes in *D. yakuba* and *D. teissieri* have been designated Est-6 and Est-10, respectively. It is likely that the *Est-6* pseudogene and the *Est-P* pseudogene arose from the *Est-6* gene by reverse transcription and integration of the resulting DNA into the genome.
We propose that whereas the productive translation of the gene in those strains. GATAATG TAGGGTGAAGATTCA

AIA D. melanogaster strains (see reviews in Richmond et al. 1993, 1995). EST-6 plays an important role in the reproducti

bly coding for the products of the two genes. They have, accordingly, proposed distinct physiological functions for the products of the two genes.

We have amplified and sequenced EST-P in 10 strains of D. melanogaster and found three premature termination codons in two of the 10 strains, which should prevent productive translation of the gene in those strains. We propose that EST-P may be a pseudogene in D. melanogaster, which is not essential although it retains a potentially functional coding sequence in a majority of the D. melanogaster strains we have studied.

MATERIALS AND METHODS

Drosophila strains: The D. melanogaster strains were derived from wild flies collected by F. J. Ayala (October 1991) in El Rio Vineyard, Lockeford, California. The strains were made fully homozygous for the third chromosome by means of crosses with balancer stocks, as described by Seager and Ayala (1982). The strains were named in accordance with the superoxide dismutase electrophoretic alleles they carry, Fast (F) or Slow (S), as follows: 255S, 510S, 521S, 94F, 174F, 357F, 377F, 521F, 581F, 968F.

PCR primers, amplification, and sequencing: Total genomic DNA was extracted using the procedure described by Palumbi et al. (1991). The EST-6 and EST-P sequences, previously published by Collet et al. (1990), were used for designing PCR and sequence primers. The amplified fragments (2.5 kb long) encompassed part of EST-6 exon II, the intergenic region between EST-6 and EST-P, the whole EST-P gene, and 360 bp of the 3’-untranslated region. The two primers used for the PCR amplification reactions were as follows: 5’-GATAAAGTGGTAGGAAGATCC-3’ (forward primer), and 5’-CATTGAGCTGCGAATGTC-3’ (reverse primer).

All PCR reactions were carried out, as described by Kwa-
indicating that these alterations are not a cloning artefact. In both strains, the termination codons are located within exon I of Est-P (see Figures 1 and 2). The inferred polypeptide sequences are significantly shorter than the complete one (355 and 461 amino acids, instead of the 544 amino acids in the complete sequence). The missing polypeptide segments include two cysteine residues (positions 517 and 538), the catalytic histidine (position 464), and one (in the 510s strain), or three (in the 357F strain) potential N-linked glycosylation sites (positions 420, 456, 509), which are important for maintaining esterase function.

Figure 4 shows a phylogenetic tree of \( \beta \)-esterase coding sequences in Drosophila. In \( D. \) pseudoobscura and \( D. \) buzzatii the \( \beta \)-esterase genes cluster together, suggesting that they may be evolving in accordance with the concerted pattern, which is common for duplicated genes (Arneheim 1983; Hickey et al. 1991; Shibata and Yamazaki 1995), or that the gene duplications are not very ancient. The two \( \beta \)-esterase genes from \( D. \) melanogaster show, on the contrary, much greater divergence.

The rate of synonymous substitutions is likely to be the same in the products of gene duplication, whether functional or not. The rate of nonsynonymous substitutions, however, is likely to be greater in a pseudogene than in a functional gene. Table 1 compares synonymous as well as nonsynonymous substitutions between the Est-6 genes of \( D. \) melanogaster, \( D. \) simulans and \( D. \) mauritiana, as well as between the \( D. \) melanogaster Est-P and the Est-6 gene of all three species. The relative rates of synonymous \((K_s)\) and nonsynonymous \((K_a)\) substitutions are examined by means of the \( \gamma \) parameter (Satta 1993), which is simply the ratio \( K_s/K_a\). This ratio is about twice as large between Est-\( P \) and Est-6 genes, as between the Est-6 genes \((t = 4.59, P < 0.01)\), indicating that the rate of nonsynonymous substitutions is greater in Est-\( P \), as expected if it were a pseudogene.

Table 2 gives the \( \gamma \) parameter for the 10 Est-\( P \) alleles as well as for the Est-6 alleles of the same 10 strains (unpublished data of E. S. Balakirev and F. J. Ayala). Estimates of \( \gamma \) are potentially subject to large error whenever the number of substitutions between two alleles is smaller than 10 (including both synonymous and nonsynonymous sites) (Satta 1993) as it is the case for these Est alleles. We have overcome this problem by counting the total numbers of substitutions between all 10 alleles of each gene, and calculating \( K_s \) and \( K_a \) for the total values. Table 2 shows that \( \gamma \) is greater for Est-\( P \) than for Est-6, reflecting that the rate of nonsynonymous substitutions is faster in Est-\( P \) (\( K_a \) is about double). This is what is expected if Est-\( P \) is a pseudogene.

Table 3 gives the \( \gamma \) values for pairwise comparisons between three esterase genes of \( D. \) pseudoobscura. The data suggest that Est-5A is a pseudogene. If we combine the data of Tables 1–3, the value of \( \gamma \) is significantly greater for the comparisons involving the presumptive pseudogenes Est-\( P \) or Est-5A \((t = 5.5, P < 0.005)\).

DISCUSSION

Collet et al. (1990) have argued that Est-\( P \) is a functional gene in \( D. \) melanogaster, based on several lines of evidence: transcriptional activity, no premature termination codons, intact splicing sites, presence of initiation and termination codons, significantly less replacement-site than silent-site differences in the coding regions of both Est-6 and Est-\( P \). Our results suggest rather that Est-\( P \) is probably not a functional gene, at least not an essential one.

Two of the 10 \( D. \) melanogaster strains studied have premature termination codons. These strains are fully homozygous for the third chromosome, yet viable, although the termination codons will prevent productive translation. Translation of the two deficient genes would yield polypeptides that lack essential amino acids and are significantly shorter than the complete sequences (355 and 461 amino acids instead of 544 amino acids).
acids in the complete sequence). As noted above, the deleted polypeptide fragments include two cysteine residues, the catalytic histidine, and one (for 510S strain), or three (for 357F strain) potential N-linked glycosylation sites, all of which are important for maintaining esterase function. The catalytic histidine and the cysteine sites are strictly conserved in all Drosophila's β-esterase sequences already published (data not shown). The catalytic histidine is one of three key residues in the esterase molecule that directly effect the esterase reaction (CARTER and WELLS 1988; MYERS et al. 1988; SCHRAG et al. 1991; SUSSMAN et al. 1991). The cysteine residues are involved in the formation of disulfide bridges, which support the three-dimensional molecular structure, and are crucial for molecular conformational stability. It is known that the active sites of other serine hydrolases with similar catalytic mechanisms are stabilized by disulfide bridges (MYERS et al. 1988). Est-6 is a glycoprotein (MANE et al. 1983) and contains four potential N-linked glycosylation sites, three (strain 357F) or two (strain 510S) of which are lost in Est-P sequences. We can assume that protein molecules lacking those sites are not functionally efficient.

The fact that two perfectly viable strains lack functional Est-P genes suggests that Est-P is not an essential gene and may be a pseudogene. There are also indirect sources of evidence that support the hypothesis that Est-P is a pseudogene.

I. The Est-6 promoter has four regulatory elements and the most important one, which controls gene expression in the ejaculatory duct, is located in the region from −511 to −1132 bp, upstream from the initiation codon (LUDWIG et al. 1993). The intergenic region between Est-6 and Est-P is only 197 bp, and thus there is not enough sequence to account for an Est-P promoter, if we extrapolate from Est-6 that the Est-P active promoter should be longer than 197 bp. Some significant part of the promoter was lost during or after duplication. This event destroyed normal gene regulation in one of the duplicated products and determined which

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>The rate of synonymous and nonsynonymous substitutions, and the γ parameter for intra- and interspecific comparisons between Est-P and Est-6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comparison</th>
<th>$K_s$</th>
<th>$K_a$</th>
<th>γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>mel Est-P vs. mel Est-6</td>
<td>0.241</td>
<td>0.572</td>
<td>0.422</td>
</tr>
<tr>
<td>mel Est-P vs. sim Est-6</td>
<td>0.237</td>
<td>0.576</td>
<td>0.411</td>
</tr>
<tr>
<td>mel Est-P vs. mau Est-6</td>
<td>0.234</td>
<td>0.580</td>
<td>0.409</td>
</tr>
<tr>
<td>mel Est-P vs. sim Est-6</td>
<td>0.029</td>
<td>0.109</td>
<td>0.203</td>
</tr>
<tr>
<td>mel Est-6 vs. mau Est-6</td>
<td>0.025</td>
<td>0.097</td>
<td>0.259</td>
</tr>
<tr>
<td>sim Est-6 vs. mau Est-6</td>
<td>0.009</td>
<td>0.041</td>
<td>0.212</td>
</tr>
</tbody>
</table>

The species abbreviations are as follows: mel, D. melanogaster; sim, D. simulans; mau, D. mauritiana. $K_s$, rate of synonymous; $K_a$, rate of nonsynonymous; rate of γ = $K_a / K_s$. 
of the two Est-6 or Est-P remained an essential gene. Termination codons may then have appeared later by chance, since there were no functional constraints that would strongly select against them. Pseudogenes have been detected that have no obvious defects (such as frame-shift mutations and/or premature termination codons) and retain high sequence similarity to their functional counterparts, but lack promoter elements. The deletion of promoter parts completely inactivates the expression of these genes (SIBBALD and BLENCOE 1990; MATTERS and GOODENOUGH 1992). A possible escape of the argument we have just advanced is to assume that a segment of the Est-6 gene may have been highjacked into becoming the promoter of Est-P (see COLLET et al. 1990). This seems unlikely but is, of course, impossible.

2. Members of a multigene family often share an evolutionary process called concerted or coincidental evolution (ARNHEIM 1983). Several molecular mechanisms and theoretical models have been proposed to explain patterns of concerted evolution (NAGYŁAKI 1984; OHTA 1984). Both nonreciprocal (gene conversion) and reciprocal (unequal sister chromatid exchange) recombination of DNA among members of gene families contribute toward their concerted evolution, so that the members of the family retain much greater similarity than expected in terms of the time since their independent origin. The phylogenetic tree of the Drosophila \( \beta \)-esterase genes suggest that a gene duplication may have occurred previous to the divergence of the subgenera Sophophora (which includes \( D. \) melanogaster and \( D. \) pseudoobscura) and Drosophila (\( D. \) buzzatii). If so, concerted evolution might account for the greater similarity between the two (or three) paralogous genes of each species than between the orthologous genes from different species. This is the situation observed in \( D. \) pseudoobscura and \( D. \) buzzatii, whereas the divergence between the two paralogous genes is much greater in \( D. \) melanogaster (Figure 4). This greater divergence could, however, be accounted for if Est-P would have acquired a new function. We submit, however, based on the overall of evidence, that it is rather the loss of function of Est-P that accounts for its extensive divergence from Est-6.

3. COLLET et al. (1990) detected Est-P transcripts in late larvac and adults of each sex. However HEALY et al. (1991) failed to find the Est-P protein product among 22 esterases in \( D. \) melanogaster, assayed by polyacrylamide gel electrophoresis and isoelectric focusing. As we have mentioned above, some pseudogenes can be transcriptionally active. Thus, the presence of Est-P transcripts is not definitive proof of functionality, while the absence of the protein product in a functional organism is indirect evidence of lack of (at least, essential) function.

4. In functional genes most nonsynonymous substitutions are deleterious and are eliminated from a population by negative selection. As a consequence, in functional genes the rate of nonsynonymous substitutions is generally significantly lower than the rate of synonymous substitutions. Pseudogenes are not subject to functional constraints, and thus synonymous and nonsynonymous substitutions rates are expected to be the same, and the expected ratio of the rates, \( \gamma = K_n/K_s \), should gradually become one (KIMURA 1983). However, the \( \gamma \) values of a pseudogene and a paralogous functional gene may be fairly similar, if the duplication occurred recently, or if the pseudogene became nonfunctional recently. The possibility of gene conversion between the pseudogene and its functional counterpart also has to be taken into account, since it will contribute to conserve the similarity of the \( \gamma \) values.

Be that as it may, if we observe that \( \gamma \) is greater between a putative pseudogene and its functional counterpart than between two corresponding functional genes, this will be evidence favoring the interpretation that the putative pseudogene is indeed a pseudogene. This will be the case whether the comparison is made between species or between alleles of the same species. Both tests, as summarized in Tables 1 and 2, favor the hypothesis that Est-P is a pseudogene.

If we assume that the rate of nonsynonymous substitutions is constant, the \( K_s \) values in Table 1 conform to the accepted phylogeny of the \( D. \) melanogaster group species, in which \( D. \) simulans and \( D. \) mauritiana are sister species, and \( D. \) melanogaster is sister to the cluster of the other two (e.g., POWELL and DESALLE 1995, Figure 4). The average \( K_s \) for the three Est-P:Est-6 comparisons in Table 1 is 0.576, or 5.6 times the average \( K_s \) between \( D. \) melanogaster and the other two species. If we assume that the \( D. \) melanogaster and \( ( \text{simulans + mauritiana} \) lineages diverged 2.5 million years ago (POWELL and DESALLE

---

**TABLE 2**

<table>
<thead>
<tr>
<th>Gene</th>
<th>( N_s )</th>
<th>( N_t )</th>
<th>( K_s )</th>
<th>( K_t )</th>
<th>( \gamma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est-P</td>
<td>34</td>
<td>22</td>
<td>0.029</td>
<td>0.047</td>
<td>0.624</td>
</tr>
<tr>
<td>Est-6</td>
<td>17</td>
<td>28</td>
<td>0.015</td>
<td>0.060</td>
<td>0.250</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>( K_s )</th>
<th>( K_t )</th>
<th>( \gamma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est-5A:Est-5B</td>
<td>0.133</td>
<td>0.504</td>
<td>0.438</td>
</tr>
<tr>
<td>Est-5A:Est-5C</td>
<td>0.161</td>
<td>0.397</td>
<td>0.406</td>
</tr>
<tr>
<td>Est-5B:Est-5C</td>
<td>0.092</td>
<td>0.311</td>
<td>0.288</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.
1995, Table IV), the Est-P:Est-6 duplication would have occurred 14 million years ago. This inference must, however, be received with considerable caution for reasons mentioned above, particularly the possibility of gene conversion between Est-P and Est-6. If we accept the duplication date of 14 million years ago, it follows that the phylogeny based on nucleotide distances (Figures 4) is in error (introduced by different rates of nucleotide evolution in different genes), since the divergence between the obscura and melanogaster lineages occurred about 40 million years ago (see, e.g., Table IV in Powell and Desalle 1995).

An Est-6 null allele has been found in the carnation eye color (car, X-linked, recessive) laboratory stock of D. melanogaster [sc ec ev et v z/FM, y\textsuperscript{3} sc b dm B 1] (Johnson et al. 1966). If null alleles were common for Est-6, we might propose that this gene also is a pseudogene. However, no Est-6 null alleles have been observed in natural populations (Voelker et al. 1980; Langley et al. 1981; Oakeshott et al. 1989). Moreover, Gilbert and Richmond (1982) have shown that Est-6 null-allele males have significantly lower reproductive fitness than typical (wild-type) Est-6 males. Saad et al. (1994) have, moreover, found strong association between the activity level of EST-6 and the reproductive fitness in D. melanogaster wild-derived lines. These results (see extensive discussion in Oakeshott et al. 1993, 1995) strongly suggest that Est-6 in D. melanogaster is engaged in adaptively significant physiological function and therefore cannot be a pseudogene. The null allele observed in a laboratory stock (Johnson et al. 1966) may represent a spontaneous null mutation that would have very low probability to survive in nature. It may be likely that this spontaneous null mutation arose as a consequence of hybrid dysgenesis (Sved 1979; Prudhommeau and Proust 1990; Georgiev and Yelagin 1992), which increases mutation rates in specific crosses that involve strains long cultured in the laboratory. In addition to the Est-6 null allele, Johnson et al. (1966) found another null allele for a separate esterase locus, Est-C in the same D. melanogaster stock. An abundance of null alleles certainly may occur in hybrid dysgenesis crosses. Because of the crosses used in our study to generate homozygous strains, the question arises whether our Est-P “null” alleles may have arisen by hybrid dysgenesis. This hypothesis seems unlikely, given that no other null alleles have been found in the numerous El Rio strains of D. melanogaster assayed in our laboratory for Sod, Xdh, Gpdh and other genes, even though all strains were made homozygous by the same extraction and maintenance procedures (Hudson et al. 1994; F. J. Ayala and collaborators, unpublished results).

The original loss of function of Est-P in D. melanogaster may have happened owing to the deletion of part of the promoter region when the Est-6 duplication occurred. After that, due to lack of functional constraints, null mutations, leading to stop codons would be tolerated and persist in some individuals. A relative abundance of premature stop codons is likely to arise only as a result of frameshift mutation. Nevertheless, stop codons are likely to be rare even in pseudogenes or nonessential genes. First, we note that the rate at which a null mutation becomes fixed in a population is highly dependent on population size and mutation rate (Li 1980, 1983). In a very large population, a null mutation may never become fixed (Fisher 1935; Nei and Roychoudhury 1973; Li 1980, 1983). Moreover, Modiano et al. (1981) have shown that the pattern of codon usage in human globin genes is such that codons that can mutate by single nucleotide substitution to a termination codon are never used whenever the corresponding amino acid is specified also by other triplets. Thus, the number of opportunities to mutate to an untranslatable codon is reduced to the minimum compatible with the amino acid composition of a gene. They propose that living organisms have adopted mechanisms that considerably reduce mutations with especially severe consequences (Modiano et al. 1981). The combination of these two factors (i.e., fixation rate of null mutations and pattern of codon usage) may explain why the Est-P gene lacks premature stop codons in most individuals, despite the fact that the Est-6/Est-P duplication event is probably as old as the divergence of the repleta and melanogaster group (Collet et al. 1990), which has been estimated to have occurred 44 million years ago by some authors (Kwiatowski et al. 1994), but 60–80 million years ago by others (e.g., Throckmorton 1975). We may consider Est-P as a “cryptic” pseudogene, because only two strains out of 10 studied strains exhibit stop codons. In all other strains the coding sequence of Est-P is intact, without premature stop codons or other alterations typically observed in pseudogenes.

In spite of extensive molecular genetic studies, few pseudogenes have been found in Drosophila (see Introduction). This might be because many of them are cryptic, for similar reasons as we have just pointed out for Est-P in D. melanogaster. Particularly, the β-esterase gene cluster in other Drosophila species may also consist of functional and nonfunctional genes. The results displayed in Table 3, for pairwise comparisons between the β-esterase genes of D. pseudoobscura, suggest that Est-5A may be a pseudogene. Negative evidence supporting this conjecture is that translational products have not been identified for this gene (Brady et al. 1990). The similarity of K\textsubscript{s} values in Table 3 suggests that the two gene duplications giving rise to Est-5A, Est-5B, and Est-5C in D. pseudoobscura may have occurred in rapid succession, with the Est-5A:Est-5B being the most recent. If we assume that K\textsubscript{s} is constant between D. melanogaster and D. pseudoobscura, the β-esterase duplications would have occurred ~8 million years ago (assuming again 2.5 million years for the divergence between D. melanogaster and D. simulans). The same reservations pointed out above, particularly the possibility of gene conversion, indicate that the inferred time of divergence between the D. pseudoobscura β-esterase
genes should be received with much caution. Indeed, Brady et al. (1992; see also Oakeshott et al. 1995) have proposed that a gene duplication occurred before the divergence of D. melanogaster and D. pseudoobscura, so that D. melanogaster Est-P and D. pseudoobscura Est-5A are orthologous to each other, whereas D. melanogaster Est-6 is orthologous to D. pseudoobscura Est-5B (and Est-5C).

Pseudogenes are named using the designation of the functional counterpart with additional prefix “ψ”. We propose that the Est-P pseudogene in D. melanogaster be designated as ψEst-6. To prevent confusion in the Drosophila ß-esterase nomenclature, we prefer to maintain the suffix “-6” in the pseudogene abbreviation, indicating its likely origin as a duplicated product of the gene designated Est-6 in D. melanogaster.

We thank all members of our laboratory, particularly Francisco Rodriguez-Trelles, Alberto Garcia Saez, Stephen Rich, Andrei Tatarenkov, Jan Kwiatowski, Kevin Bailey, Victor Dehllipins, Carlos Machado, Heather Carstens and Douglas Skarcey for encouragement, help, and discussion. This work is supported by National Institutes of Health Grant GM-42997 to F.J.A.

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