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Evolution

Molecular evolution of inversions in *Drosophila pseudoobscura*: The amylase gene region

(chromosomal polymorphism/restriction fragment length polymorphism/inversion phylogeny/linked gene complex)

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ABSTRACT **The amylase region of the third chromosome of *Drosophila pseudoobscura* has been cloned and localized to cytological band 73A. It is contained within a series of highly polymorphic inversions and serves as a convenient tool for a molecular evolutionary analysis of the inverted gene arrangements. Amylase in *D. pseudoobscura* is a family of three genes, and some chromosomes have deletions for one or two of them. Two overlapping clones covering 26 kilobases were isolated and used as probes to survey DNA restriction map polymorphism among 28 lines, representing five of the major inversion types found in natural populations, as well as single chromosomes from the closely related species *Drosophila persimilis* and *Drosophila miranda*. Restriction-site differences are considerably greater among the various gene arrangements than among chromosomes with the same gene arrangement. Clustering the restriction map haplotypes yielded a dendrogram concordant with the phylogeny generated independently from cytogenetic considerations. The inversion polymorphism is estimated to be about 2 million years old.**

Inversions on the third chromosome of *Drosophila pseudoobscura* have served as a model genetic system for a long series of studies in population genetics and evolutionary biology. Th. Dobzhansky began work with these inversions, or gene arrangements, in the 1930s, and this system figured prominently in his seminal collaboration with Sewall Wright to measure the parameters of evolutionary theory. Changes in inversion frequency over space and time in nature were explained best by natural selection (1). Gene arrangements in experimental populations showed frequency changes due to selective differences that duplicated some, although not all, of the changes seen in nature (ref. 2; see also ref. 3).

Some 50 gene arrangements of the third chromosome are known from natural populations in western North America, ranging from British Columbia in the north to Mexico in the south (1, 4). There is, in addition, an isolated set of populations near Bogota, Colombia. On the assumption that each inversion arose only once, the breakpoints of the inversions have been used to construct a phylogeny of the gene arrangements (5). The importance of the inversions in population genetics stems from their ability to suppress recombination in heterozygotes for different gene arrangements, thus maintaining linked complexes of genes. The reduction in recombination in inversion heterozygotes is dramatic (6) but not complete, and over millions of generations even low levels of crossing over or gene conversion could lead to an appreciable exchange of genes between these inversions (7).

Prakash and Lewontin (8, 9) reported strong associations between particular allozymes at loci encoding soluble proteins and specific gene arrangements in *D. pseudoobscura* from several geographic sites, and they invoked selection as the cause of the associations. Nei and Li (10, 11) and Ishii and Charlesworth (12) studied mathematical models for drift, selection, and recombination on blocks of genes held inside inversions, however, and concluded to the contrary that associations between alleles and gene arrangements could persist without selection, as long as the inversions were of relatively recent origin.

The age of the inversions is thus an important consideration in deciding whether selection is involved in maintaining associations between alleles and inversions or whether the associations are simply the remnants of the complete association of each inversion with the alleles within it at the time of its formation. Dobzhansky and Sturtevant (5) suggested an origin of the inversions in or after the Pleistocene, about one million years ago, whereas Epling (13) argued from biogeography for an origin in the Miocene, more than 10 million years ago.

We present here an initial molecular analysis of the amylase (*Amy*) gene region, which is located within the inverted regions of most of the gene arrangements. We wish to know whether there is evidence of a single origin of each inversion, how old the gene arrangements are, whether the evolutionary relationship of the inversions inferred from restriction maps corroborates the independent cytogenetic phylogeny, and how effective the inversions have been in preserving linked gene complexes.

MATERIALS AND METHODS

Lines of *D. pseudoobscura* stocks homozygous for the third chromosome were constructed by using balancer stocks (15). Since balancer chromosomes were not available for *D. persimilis* and *Drosophila miranda*, stocks of these species were analyzed directly. Collection sites of the lines are given in Table 1. Salivary glands were dissected from third-instar larvae, 10 per line, and gene arrangements were diagnosed from squash preparations of the polytene chromosomes. The *Amy* genotype was determined for 10 individuals from each line by polyacrylamide gel electrophoresis (16).

D. pseudoobscura clones containing *Amy*-homologous sequences were isolated from a genomic library by plaque hybridization (17) using as a probe the plasmid pDm3.8 (18, 19) containing the *Drosophila melanogaster Amy* coding sequence. W. W. Doane (Arizona State University, Tempe) kindly provided pDm3.8. The library (a gift from C. H. Langley, National Institute of Environmental Health Sciences, Research Triangle Park, NC) was constructed from an *Mbo* I partial digest of *D. pseudoobscura* genomic DNA inserted into the *Bam*HI sites of EMBL4 (20). The strain of

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Abbreviations: AR, Arrowhead; CH, Chiricahua; KL, Klamath; SC, Santa Cruz; ST, standard; TL, Treeline.

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Table 1. Restriction site variation in the amylase region of *D. pseudoobscura*, *D. persimilis*, and *D. miranda*

			Amy 2								Amy 3										
Strain	GA	Amy	S (−1.7)	B (−1.5)	B (0.5)	H (1.7)	S (2.3)	X (2.8)	H (3.5)	H (4.3)	S (4.9)	B (5.1)	Z (6.6)	S (8.7)	E (9.6)	Z (12.8)	B (14.0)	E (14.4)	X (15.2)	B (15.3)	S (15.5)
1. Probe	ST	F	+	+		+	+		+		+	+	+				+		+	+	+
2. AH41	ST	F	+	+		+	+				+	+	+				+		+	+	+
3. AH155	ST	F	+	+		+	+				+	+	+				+		+	+	+
4. AH162	ST	F	+	+		+	+				+	+	+				+		+	+	+
5. AH38	ST	F	+	+		+	+		+		+	+	+				+		+	+	+
6. AH130	ST	F	+	+		+	+		+		+	+	+	+			+		+	+	+
7. AH69	ST	F	+	+		+	+		+		+	+	+				+		+	+	+
8. AH133	ST	F	+	+		+	+		+		+	+	+				+		+	+	+
9. M5	ST	F	+	+		+	+				+	+	+	+			+		+	+	+
10. M26	ST	F	+	+		+	+		+		+	+	+				+		+	+	+
11. M29	ST	F	+	+		+	+				+	+	+				+		+	+	+
12. BC420	AR	F	+	+		+	+				D	D	+				+		+	+	+
13. AH172	AR	F	+	+		+	+				D	D	+				+		+	+	+
14. BC86	CH	S	+	+	+	+	+			+	D	D	+			+	+				+
15. M18	CH	F	+	+	+	+	+			+	D	D	+				+				+
16. M19	CH	S	+	+		+	+			+	D	D	+				+				
17. M20	CH	F	+	+	+	+	+			+	D	D	+			+	+				+
18. M21	CH	F	+	+	+	+	+			+	D	D	+				+				+
19. BC16	SC	SS	+	+		+	+			+	D	D	+					+			+
20. BJ859	SC	F	+	+		+	+			+	D	D	+				+				+
21. BC93	TL	S	D	D				+			D	D			+	+					+
22. AH135	TL	SS	D	D							D	D				+		+			+
23. M22	TL	S	D	D							D	D			+	+					+
24. M23	TL	S	D	D							D	D				+	+	+			+
25. M24	TL	S	D	D							D	D				+	+	+			+
26. M25	TL	S	D	D							D	D				+	+	+			+
27. HI	TL	S	D	D		+		+			D	D				+		+			+
28. BOGER	TL	S	D	D		+					D	D				+	+	+			
29. PER	KL		+	+		+	+				+	+	+				+		+		+
30. MIR			+	+		+			+	+	D	D	+				+				

+, Presence of a recognition site for a restriction enzyme; D, loss of a restriction site due to a 1.6-kb deletion in the coding region of *Amy2* or *Amy3*. Locations of genes *Amy2* and *Amy3*, the abbreviations for the endonuclease recognition sites, and their distances in kb from an arbitrary reference site are given. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal* I; X, *Xba* I; Z, *Xho* I. Not shown are the following restriction sites present at the indicated positions in all chromosomes studied: H at −9.0; B at −5.8; S at −5.6; X at −3.9; E at −3.4; X at −3.3; Z, the reference site, at 0; E at 2.9; X at 7.1; S at 9.7; X at 9.9; E at 10.0; Z at 12.3; H at 12.8; Z at 14.4; and B at 16.6. Also not shown are those restriction sites present in only one chromosome or those present in all but one. The following sites are present once only at the indicated positions: B at −9.7, in M22; S at −5.1 and S at −4.9, in the probe; S at 3.0 and S at 6.1, in MIR; H at 7.8, in BC16. The following sites are absent once only: E at 0.3, B at 3.4, and S at 3.8, all in MIR. The geographic location at which each strain was collected, the gene arrangement (GA) of the third chromosome in that strain, and the amylase allozyme (*Amy*) present are indicated. Locations of strains: Probe, McDonald Ranch, near Davis, CA; AH strains, Apple Hill, near Georgetown, CA; M strains, Mather, CA, near Yosemite Park; BC strains, British Columbia, Canada; BJ strains, Baja California, Mexico; HI, Hidalgo, Mexico; BOGER, Bogota, Colombia; PER (*D. persimilis*) and MIR (*D. miranda*), unknown locations. Amylase allozymes: F, fast; S, slow; SS, superslow.

D. pseudoobscura used for this library, a reference strain for electrophoresis from F. J. Ayala (University of California, Irvine), is homozygous for the standard gene arrangement.

Genomic DNA was prepared from individual lines (21). Restriction mapping was carried out by digestion of DNA with one or two restriction enzymes, followed by Southern blotting and hybridization to a labeled probe (22, 23).

Estimates of the nucleotide sequence divergence between pairs of chromosomes were calculated from the restriction maps (24). These distances were used to cluster the gene arrangement by the unweighted pair group method with arithmetic mean, yielding a dendrogram (24).

RESULTS

Cloning of the *Amy* Gene Region. The pDm3.8 plasmid contains a 3.8-kilobase (kb) genomic fragment from *D. melanogaster*, which includes the coding sequence for one of the duplicated *Amy* genes of that species (18, 19). By using this plasmid as a molecular probe, we isolated two phage clones (A1 and AC2) containing a contiguous 26-kb region of the *D. pseudoobscura* genome (Fig. 1). Hybridization of these clones to genomic Southern blots, to each other, and to pDm3.8 indicated three regions of similarity to the *Amy* structural gene of *D. melanogaster*. The three regions of similarity contain *Bam*HI and *Sal* I restriction sites (Fig. 1), as do the *Amy* genes of *D. melanogaster* (18, 19).

Evidence that the functional *Amy* gene of *D. pseudoobscura* was cloned comes from several results. Amylase allozyme variation maps to position 33.0 on the linkage map of the third chromosome, which roughly places *Amy* in sections 73 to 75 on the cytological map of the third chromosome (25). *In situ* hybridization (26) of biotin-labeled clones A1 and AC2 to polytene chromosomes of *D. pseudoobscura*, in fact, localized these clones to band 73A of the third chromosome. Hybridization with *D. melanogaster* clone pDm3.8 also localized this same site (27).

Further support that the A1/AC2 region contains the functional *Amy* structural gene comes from analysis of mRNA transcripts. Probing of a Northern blot containing poly(A) RNA from adults of *D. melanogaster* and *D. pseudoobscura* with clones A1 or AC2 indicated one major transcript of ≈1450 base pairs in both species. A transcript of this same size was detected by probing with pDm3.8, although in this case the *D. melanogaster* transcript hybridized more intensely, as expected.

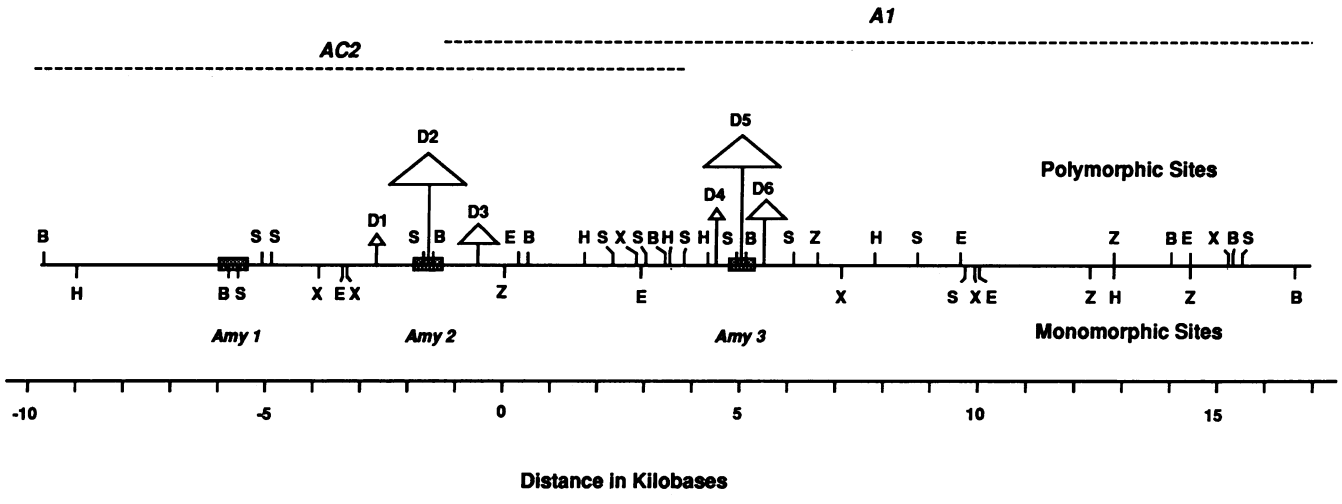

FIG. 1. Map of restriction site polymorphisms for the amylase gene region of *D. pseudoobscura*. The genomic regions contained in the two phage clones (AC2 and A1) are indicated above the restriction site map. Polymorphic and monomorphic restriction sites among *D. pseudoobscura*, *D. persimilis*, and *D. miranda* lines are indicated above and below the line, respectively, as follows: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal* I; X, *Xba* I; Z, *Xho* I. The scale is oriented with 0.0 at an *Xho* I site. The positions of the three genes *Amy1*, *Amy2*, and *Amy3* are stippled on the restriction map. Deletions relative to the ST map are shown as triangles above the map.

Confirmation that our clones contain a family of three *Amy* genes comes from sequencing and somatic transformation studies published elsewhere (28, 29). As depicted in Fig. 1, we have named these genes *Amy1*, *Amy2*, and *Amy3*. The three genes have been separately subcloned and sequenced, and their evolutionary relationships to each other and to the *Amy-p*[1] gene of *D. melanogaster* have been inferred (28). *Amy3* contains a stop codon and is thus a pseudogene. The three genes have been tested for expression by injection into *D. melanogaster* eggs homozygous for an *Amy* null allele (29). *Amy1* was expressed at a high level, *Amy2* was at a very low level, and *Amy3*, as expected, was not expressed at all. Our results presented below show that some gene arrangements carry deletions for *Amy3* and some, for both *Amy2* and *Amy3*, so it is clear that *Amy1* is the functional *Amy* gene.

Restriction Map Variation. By using our clones A1 and AC2 as molecular probes, we have determined the genomic restriction maps of the *Amy* region for 28 lines of *D. pseudoobscura*, one line of *D. persimilis*, and one line of *D. miranda*. The results are given in Fig. 1 and Table 1. Among the lines of *D. pseudoobscura* examined, 11 were homozygous for the standard (ST) gene arrangement, 2 were homozygous for Arrowhead (AR), 5 were homozygous for Chiricahua (CH), 2 were homozygous for Santa Cruz (SC), and 8 were homozygous for Treeline (TL). Our samples span the entire distribution of the species north to south, including British Columbia, California, Mexico, and Colombia. For *D. persimilis* we present the map for one Klamath (KL) chromosome.

Of the 44 restriction sites mapped within the 26 kb covered by our probes, 28 sites were variable. The one *D. persimilis* chromosome had no unique differences from the *D. pseudoobscura* chromosomes, whereas the *D. miranda* chromosome had 5 unique restriction-site differences from all of the other chromosomes.

Two common variants in sequence length were found (Table 1 and Fig. 1). All eight of the TL chromosomes carry D2, a 1.6-kb deletion (relative to the ST chromosome of the probe) that removes the *Bam*HI and *Sal* I restriction sites marking the coding region of *Amy2*. A second deletion, D5, again 1.6 kb long relative to the probe, occurs in all the chromosomes we studied except for the ST chromosomes in *D. pseudoobscura* and KL, the one-step derivative of ST in *D. persimilis*. Four other deletions (relative to the probe) were discovered. Both of the AR chromosomes contain D1, a 0.3-kb deletion between the *Xba* I site at position −3.3 and the *Sal* I site at position −1.7, and AR strain BC420 carries D4, a second 0.3-kb deletion between *Hin*dIII at position 4.3 and *Sal* I at position 4.9. The *D. miranda* chromosome has D3, a 0.6-kb deletion between *Bam*HI at position −1.5 and *Xho* I at position 0, as well as D6, a 0.8-kb deletion between *Bam*HI at position 5.1 and *Sal* I at position 6.1.

The data in Table 1 show a striking pattern: different chromosomes with the same gene arrangement show strong similarities to each other, whereas chromosomes with different gene arrangements are considerably different. The TL chromosomes are particularly instructive because they were collected over a range of many thousands of kilometers, from Canada in the north to Mexico and Colombia in the south. This pattern is reflected in the dendrogram obtained by the unweighted pair group method with arithmetic mean, shown in Fig. 2, which is based on the phenetic similarity of the restriction maps. Other methods of phylogenetic reconstruction yielded similar results.

Polymorphic variants—restriction sites, deletions, and allozymes—were tested in pairwise combinations for linkage disequilibrium, using Fisher's exact test. In the 28 *D. pseudoobscura* lines, there were 18 sites at which the rarer variant was present at least twice, and 60 of the 153 pairwise combinations of these sites, or 39.2%, were statistically significant at the 5% level. The 28 lines came from 7 localities, however, and differences between localities could account for part of the linkage disequilibrium. Consequently, we tested the 11 strains from Mather, CA, separately. There were 11 polymorphic sites, and 22 of the 55 pairwise comparisons of them, or 40.0%, were statistically significant. Thus the strong linkage disequilibrium of sites in the 26-kb *Amy* region does not appear to be the result of combining genetically differentiated populations but represents substantial linkage disequilibrium among the gene arrangements. By contrast, there were only 5 significant pairwise comparisons of polymorphic sites out of a total of 153, or 3%, in the 32-kb alcohol dehydrogenase (*Adh*) region (30). The fourth chromosome has no polymorphism for inversions, so the *Adh* region serves as a control for the effects of inversions on linkage disequilibrium on the third chromosome.

DISCUSSION

The *Amy* region has an unexpected complexity in *D. pseudoobscura*. Earlier breeding studies gave no indication of

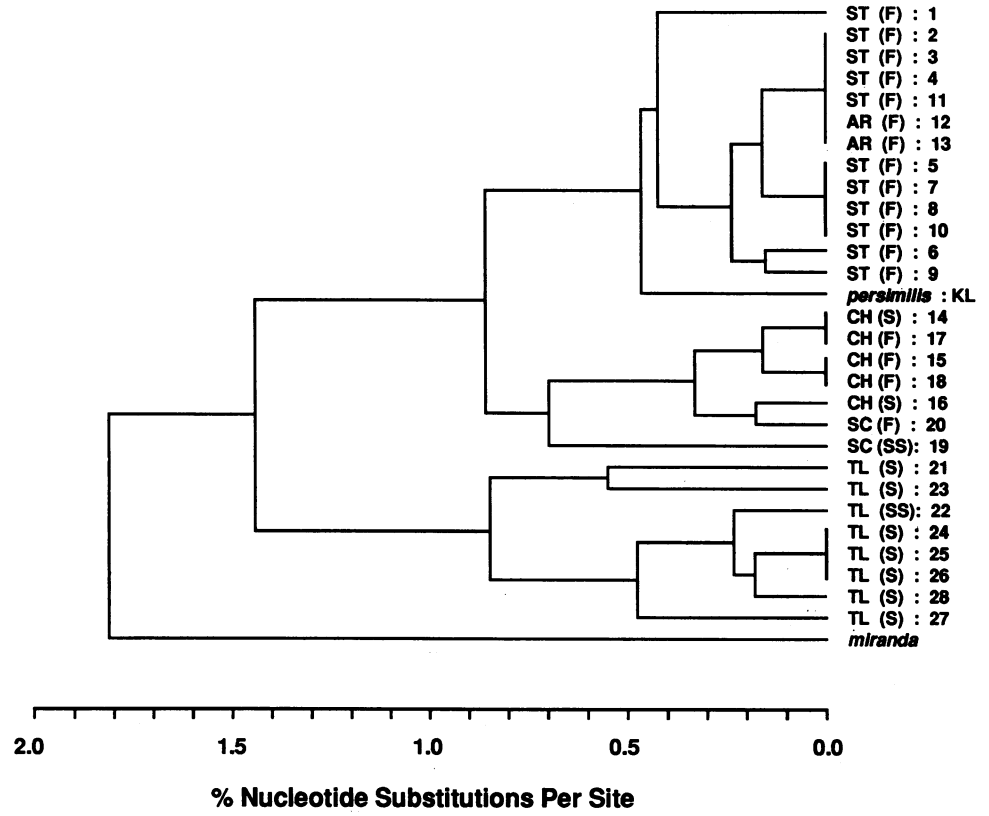

FIG. 2. Dendrogram of restriction map haplotypes for the *Amy* gene region of *D. pseudoobscura*, *D. persimilis*, and *D. miranda* obtained by the unweighted pair group method with arithmetic mean. Each chromosome is identified by gene arrangement, *Amy* allozyme, and strain number, and explanations of the abbreviations and geographic origins of the strains are given in Table 1. Note that the same haplotype was found in several chromosomes. The scale of distances measures half the nucleotide sequence divergence between sequences.

multiple genes (8, 31, 32) and, indeed, only *Amy1* seems to be expressed at a level that makes it a functional gene (29).

Nucleotide sequence diversity, essentially equivalent to per nucleotide heterozygosity, is very high for *Amy* in *D. pseudoobscura*. Among the 28 *D. pseudoobscura* chromosomes that were studied, mean heterozygosity is 1.81% with some lines identical to one another and some showing as much as 4.4% sequence divergence. This level is comparable to that found in the *Adh* and *Xdh* regions in this same species and supports the view that *D. pseudoobscura* harbors significantly more DNA sequence polymorphism than *D. melanogaster* (30, 33, 34). For example, mean heterozygosity within ST (n = 11), CH (n = 5), and TL (n = 8) gene arrangements ranges from 0.4 to 1.0%. Heterozygosity per nucleotide for the comparable gene regions of *D. melanogaster* (including *Amy*) is only 0.4%, ranging from 0.2 to 1.0% (34). Thus, the level of nucleotide polymorphism found within *D. melanogaster* as a species is approximately equivalent to that found within a single gene arrangement in *D. pseudoobscura*.

Sequence length variants that were repetitive and might be transposable elements were not detected in the *Amy* region of *D. pseudoobscura*. Similar results were obtained for the *Adh* region of *D. pseudoobscura* (23, 30) but stand in sharp contrast to the results of similar surveys of variation in virtually all gene regions that have been examined in *D. melanogaster*, including *Amy*. In *D. melanogaster*, chromosomes sampled from natural populations have large insertions, many of which have been shown to be transposable element insertions at frequencies of 0.002–0.018 per kb (average 0.004) per line surveyed (34). There is, however, surprising variation for the number of *Amy* structural genes in *D. pseudoobscura*.

The phylogenetic tree of gene arrangements depicted in Fig. 3 is an abbreviated version of one of the most carefully documented phylogenies in evolutionary cytogenetics. The construction of this tree is based on an assumption of parsimony, that each step occurred by a single unique inversion. However, it has been shown for *D. melanogaster* that transposable elements can repeatedly induce breaks at the same, or nearly the same, location, giving rise to the same inversion several times (35). Such a polyphyletic origin would mean that sequences contained within copies of a particular inversion, particularly those from widely separated localities, need not be monophyletic and that a phylogeny for those sequences might not be concordant with the cytogenetic phylogeny of Fig. 3. Neither of these possible consequences of polyphyly was observed in our study.

Our studies include two gene arrangements, ST and TL, for which more than five chromosomes were analyzed. The ST chromosomes came from three populations in California and the TL chromosomes came from five widely separated locations spanning the entire north–south range of the species. In every case the ST chromosomes carry three *Amy* genes and

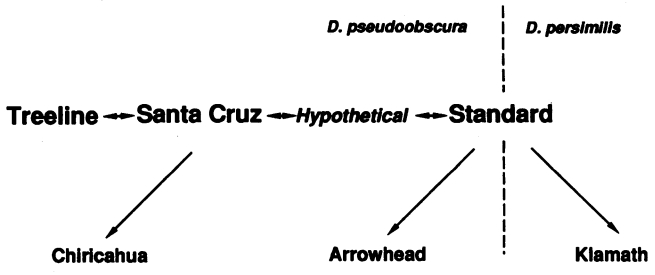

FIG. 3. Cytogenetic phylogeny of gene arrangements of *D. pseudoobscura* and *D. persimilis* examined in the present study.

a distinctive profile of restriction sites that cause them to cluster together in the dendrogram of Fig. 2. Likewise, the TL chromosomes from all of our populations, including the isolate at Bogota, Colombia, contain only one *Amy* gene and have similar patterns of restriction sites that cause them to cluster in our dendrogram. It should be noted that the dendrogram was constructed without information on the duplicate coding regions and that the evidence from the duplications and restriction site polymorphisms is fully concordant. Thus, our data are consistent with a single origin of each inversion, followed by multiplication and spread to other geographic regions.

The dendrogram based on restriction site polymorphism (Fig. 2) fits the cytogenetic phylogeny of *D. pseudoobscura* and *D. persimilis* gene arrangements very well (refer to Fig. 3). The ST chromosome and its single-step derivatives AR and KL form one cluster, SC and its single-step derivative CH form another, and TL forms a third. These clusters represent the three main families of inversions (1) in the gene arrangement phylogeny of *D. pseudoobscura*. The molecular data thus provide a striking independent corroboration of the evolutionary relationships of the inversions. The KL chromosome of *D. persimilis* clusters with the ST chromosomes of *D. pseudoobscura*. This relationship is consistent with our studies of the *Adh* locus in these same species, which showed *D. persimilis* clustering with *D. pseudoobscura* strains at a level of divergence less than that which separates TL from the other inversions. *D. miranda* shows the greatest divergence from the *D. pseudoobscura* and *D. persimilis* strains in the *Amy* region, at a level nearly the same as that reported for *Adh* (23, 30).

The time at which the *D. pseudoobscura* gene arrangements diverged may be estimated if we assume a constant rate of substitution and if we can calibrate this molecular clock with respect to absolute time (24). From studies of DNA·DNA hybridization, a substitution rate of at least 1.7-nucleotide substitutions per million years since last common ancestor has been estimated for single-copy nuclear DNA in species of the *D. melanogaster* subgroup (14). By using this rate, we estimate that *D. miranda* diverged from *D. pseudoobscura* 2.1 million years ago, whereas *D. persimilis* separated more recently, perhaps only 0.5 million years ago. The sequence divergence among the gene arrangements suggests that the TL chromosomes diverged from the SC and ST families of inversions about 1.7 million years ago, or at least 5 million generations ago, and that the ST and SC families diverged about 1.0 million years, or 3 million generations, ago. These estimates of divergence time for the inversion polymorphism in *D. pseudoobscura* indicate that the inversion system is fairly old, although not quite as old as Epling's estimate of >10 million years, based on biogeography (13). *D. pseudoobscura* has probably been polymorphic for the gene arrangements for at least 5 million generations.

Given the apparent age of the inversion system, there appears to have been ample time for recombination to diminish the associations between restriction map variants and inversions. Nevertheless, significant linkage disequilibrium between restriction sites was observed across the entire 26-kb region. The linkage disequilibrium in the *Amy* region stands in sharp contrast to similar measurements in the *Adh* region (23), which is located on a chromosome lacking inversion polymorphism. The inversions are evidently effective in maintaining blocks of genes in the face of the homogenizing effect of recombination.

Ishii and Charlesworth (12) have summarized the rates of gene exchange between inverted and noninverted chromosomes in several species of *Drosophila*, including *D. pseudoobscura*, and they concluded that a rate of gene conversion on the order of 10^{-4} per generation is typical for a locus near the middle of a simple inversion. They further estimated that associations generated by chance when an inversion occurs would decay after $\approx 10^4$ generations unless impeded by selection. Our data on TL are instructive in this regard. TL chromosomes from locations as far apart as Canada and Colombia share a distinctive profile of restriction sites, and the dendrogram in Fig. 2 suggests that TL diverged from the ST and SC families of inversions 5×10^6 generations ago. Our data support the concept of linked gene complexes within the inversions, held together for considerable spans of time. It is not clear how these complexes are maintained, but natural selection is very likely involved.

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1. Dobzhansky, Th. (1944) *Carnegie Inst. Washington Publ.* **554**, 47–144.
2. Wright, S. & Dobzhansky, Th. (1946) *Genetics* **31**, 125–156.
3. Anderson, W. W. (1989) *Genome* **31**, 239–245.
4. Olvera, O., Powell, J. R., de la Rosa, M. E., Salceda, V. M., Gaso, M. I., Guzman, J., Anderson, W. W. & Levine, L. (1979) *Evolution* **33**, 381–395.
5. Dobzhansky, Th. & Sturtevant, A. H. (1938) *Genetics* **23**, 38–64.
6. Dobzhansky, Th. & Epling, C. (1948) *Proc. Natl. Acad. Sci. USA* **34**, 137–141.
7. Chovnick, A. (1973) *Genetics* **75**, 123–131.
8. Prakash, S. & Lewontin, R. C. (1968) *Proc. Natl. Acad. Sci. USA* **59**, 398–405.
9. Prakash, S. & Lewontin, R. C. (1971) *Genetics* **69**, 405–408.
10. Nei, M. & Li, W. H. (1977) *Genet. Res.* **25**, 31–43.
11. Nei, M. & Li, W. H. (1980) *Genet. Res.* **35**, 65–83.
12. Ishii, K. & Charlesworth, B. (1977) *Genet. Res.* **30**, 93–106.
13. Epling, C. (1944) *Carnegie Inst. Washington Publ.* **554**, 145–183.
14. Caccone, A., Amato, G. D. & Powell, J. R. (1988) *Genetics* **118**, 671–683.
15. Pavlovsky, O. & Dobzhansky, Th. (1966) *Genetics* **53**, 843–854.
16. Yardley, D. G., Anderson, W. W. & Schaffer, H. E. (1977) *Genetics* **87**, 357–369.
17. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
18. Levy, J. N., Gemmill, R. M. & Doane, W. W. (1985) *Genetics* **110**, 313–324.
19. Gemmill, R. M., Levy, J. N. & Doane, W. W. (1985) *Genetics* **110**, 299–312.
20. Frischauf, A., Lehrach, H., Puustka, A. & Murray, N. (1983) *J. Mol. Biol.* **170**, 827–842.
21. Bingham, P. M., Levis, R. & Rubin, G. M. (1981) *Cell* **25**, 693–704.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Schaeffer, S. W. & Aquadro, C. F. (1987) *Genetics* **117**, 61–73.
24. Nei, M. (1987) *Molecular Evolutionary Genetics* (Columbia, Univ. Press, New York).
25. Yardley, D. G. (1974) *Drosoph. Inf. Serv.* **51**, 25.
26. Montgomery, E., Charlesworth, B. & Langley, C. H. (1983) *Genet. Res.* **49**, 31–41.
27. Doane, W. W., Gemmill, R. M., Schwartz, P. E., Hawley, S. & Norman, R. A. (1987) in *Isozymes*, eds. Rattazzi, M. C., Scandalios, J. G. & Whitt, G. S. (Liss, New York), Vol. 14, pp. 229–266.
28. Brown, C. J., Aquadro, C. F. & Anderson, W. W. (1990) *Genetics* **126**, 131–138.
29. Hawley, S. A., Norman, R. A., Brown, C. J., Doane, W. W., Anderson, W. W. & Hickey, D. A. (1990) *Genome* **33**, 501–508.
30. Schaeffer, S. W., Aquadro, C. F. & Anderson, W. W. (1987) *Mol. Biol. Evol.* **4**, 254–265.
31. Prakash, S., Lewontin, R. C. & Hubby, J. L. (1969) *Genetics* **61**, 841–858.
32. Powell, J. R. (1979) *Genetics* **92**, 613–622.
33. Riley, M. A., Hallas, M. E. & Lewontin, R. C. (1989) *Genetics* **123**, 359–369.
34. Aquadro, C. F. (1990) *UCLA Symp. Mol. Cell. Biol.* **122**, 179–189.
35. Engels, W. R. (1983) *Annu. Rev. Genet.* **17**, 315–344.