Evolution of the Transposable Element *mariner* in *Drosophila* Species

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ABSTRACT

The distribution of the transposable element *mariner* was examined in the genus *Drosophila*. Among the eight species comprising the *melanogaster* species subgroup, the element is present in *D. mauritiana*, *D. simulans*, *D. sechellia*, *D. yakuba* and *D. teissieri*, but it is absent in *D. melanogaster*, *D. erecta* and *D. orena*. Multiple copies of *mariner* were sequenced from each species in which the element occurs. The inferred phylogeny of the elements and the pattern of divergence were examined in order to evaluate whether horizontal transfer among species or stochastic loss could better account for the discontinuous distribution of the element among the species. The data suggest that the element was present in the ancestral species before the *melanogaster* subgroup diverged and was lost in the lineage leading to *D. melanogaster* and the lineage leading to *D. erecta* and *D. orena*. This inference is consistent with the finding that *mariner* also occurs in members of several other species subgroups within the overall *melanogaster* species group, Within the *melanogaster* species subgroup, the average divergence of *mariner* copies between species was lower than the coding region of the *alcohol dehydrogenase* (*Adh*) gene. However, the divergence of *mariner* elements within species was as great as that observed for *Adh*. We conclude that the relative sequence homogeneity of *mariner* elements within species is more likely a result of rapid amplification of a few ancestral elements than of concerted evolution. The *mariner* element may also have had unequal mutation rates in different lineages.

TRANSPOSABLE elements comprise a class of multigene families whose members are capable of moving from one chromosomal location to another. Although subject to extensive genetic and molecular study, transposable elements are little understood from the standpoint of evolutionary history. Transposable elements can rapidly increase or decrease in copy number without apparent phenotypic effects, which distinguishes them from conventional single-copy nuclear genes. A second distinction is that at least some transposable elements can undergo horizontal transfer between different species. For these reasons and others, tranposable elements are subject to somewhat different evolutionary forces than conventional single-copy genes.

Phylogenetic studies indicate that many transposable elements are distributed in discontinuous fashion among related taxa (DOWSETT and YOUNG 1982; MARTIN, WIERNASZ and SCHEDL 1983; STACEY *et al.* 1986). When a particular species lacks a transposable element that is found in closely related species, the finding is generally attributed to stochastic loss of the element in the lineage of the particular species. Conversely, when a species contains a transposable element not found in near relatives, the presence of the element is generally attributed to novel acquisition of the element by interspecific transfer from a more distantly related taxon. The occurrence of interspecific transfer is illustrated by the *P* transposable element in Drosophila. The *P* element appears to have spread in *Drosophila melanogaster* only in the past few decades, since it is prevalent in contemporary natural populations but completely absent in old laboratory strains and in species closely related to *D. melanogaster* (KIDWELL 1983). However, homologues of the *P* element are prevalent in more distantly related species comprising the *willistoni* and *saltans* species groups (DANIELS *et al.* 1984; DANIELS and STRAUSBAUGH 1986; LANSMAN *et al.* 1987) [see ENGELS (1989) for review]. The discovery of a functional *P* element in *D. willistoni* that is virtually identical in nucleotide sequence with the *D. melanogaster* element strongly argues that the element was transferred recently into the *D. melanogaster* genome from one of these species (DANIELS *et al.* 1990). On the other hand, many transposable elements reveal a more complex pattern in their species distribution. For example, the transposable element designated *I* is also thought to have spread recently in populations of *D. melanogaster*, similar to the situation with *P*. However, inactive elements homologous to *I* are found in sibling species of *D. melanogaster*, and it is not clear whether rapid spread of the *I* element was initiated by interspecific transfer or whether some process allowed the activation of previously inactive elements already in the genome (BUCHETON *et al.* 1986) [see FINNEGAN (1989) for review].

In addition to issues concerning the distribution

and abundance of transposable elements, much remains to be learned about forces governing the extent of nucleotide sequence variation between different copies of transposable elements within and among species. Although it has been suggested that dispersed, moderately repetitive sequences might undergo rapid concerted evolution as do tandemly repeated sequences (ARNHEIM 1983), there is no strong evidence for this view [see DOVER (1988) for review]. For the rDNA gene family in humans, it has been shown that concerted evolution of rDNA sequences within the same chromosome is much more rapid than among rDNA sequences in nonhomologous chromosomes (SEPARACK, SLATKIN and ARNHEIM 1988). In the case of transposable elements, if sequences within a family are virtually identical, then this could result either from concerted evolution by specific genetic exchange mechanisms, such as gene conversion, or from recent amplification of a few ancestral copies of the element.

We report here a systematic study of the evolution of the transposable element *mariner* in the *melanogaster* species subgroup of Drosophila. The autonomous *mariner* element is 1286 nucleotides in length, includes a single, long open reading frame encoding a putative protein of 345 amino acids (presumed to be a transposase necessary for transposition), and has terminal inverted repeats of 28 base pairs (JACOBSON, MEDHORA and HARTL 1986). Eight species are assigned to the *melanogaster* species subgroup, which radiated in the Afro-tropical region. Two alternative trees have been proposed for the species phylogeny, with three distinct species complexes recognized within the subgroup (Figure 1, modified from LACHAISE *et al.* 1988). Based on genetic distance data derived from allozymes and nucleotide sequences of the *Adh* genes, the three complexes are thought to have split 6–15 million years ago (BODMER and ASHBURNER 1984; LACHAISE *et al.* 1988). The species *D. erecta* and *D. orena* are restricted to a small part of Africa, whereas *D. teissieri* and *D. yakuba* are widespread on the African mainland. These four species are reproductively completely isolated from one another, and they do not hybridize even in the laboratory. In the *melanogaster* species complex, *D. sechellia* and *D. mauritiana* are insular species (restricted to the Seychelles and Mauritius, respectively), while *D. simulans* and *D. melanogaster* have spread worldwide. The three most closely related species are *D. simulans*, *D. sechellia* and *D. mauritiana*, and they produce fertile female offspring when mated with each other (LACHAISE *et al.* 1988). Although the *mariner* element was first identified in *D. mauritiana*, homologous sequences are also present in *D. simulans*, *D. sechellia*, *D. yakuba* and *D. teissieri*. However, the species distribution of *mariner* is discontinuous in that it is not naturally found in *D. melanogaster*, *D. erecta* or *D. orena*.

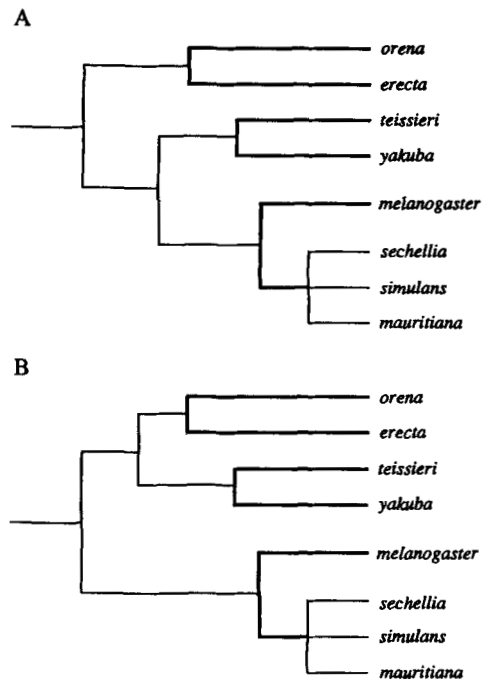

FIGURE 1.—Two alternative phylogenetic trees of the *melanogaster* species subgroup. Unequivocal relationships are indicated by thick lines and equivocal relationships by thin lines (redrawn from LACHAISE *et al.* 1988).

Two hypotheses may be proposed to explain the discontinuous distribution of *mariner* in the *melanogaster* species subgroup. One model is that *mariner* was present in the common ancestor before the eight contemporary species diverged but was lost in the lineages leading to *D. orena*, *D. erecta* and *D. melanogaster*. If this model is correct, the gene phylogeny of *mariner* sequences among the species is expected to be congruent with the phylogeny of the species, and sequences homologous to *mariner* might be found in other species related to the *melanogaster* species subgroup. The other model for the distribution of *mariner* assumes that one or more recent horizontal transfers introduced the element into the five species possessing it some time after the divergence of the subgroup began. In this case the phylogeny of *mariner* sequences would have no necessary correlation with the phylogeny of the species, and the *mariner* sequences should all be very similar to each other. Although the models are not necessarily mutually exclusive, the weight of the evidence may be determined by comparing the phylogeny of *mariner* sequences with the phylogeny of the species that contain them. These comparisons require knowledge of *mariner* sequence variation, both within and between the species in the subgroup.

We have carried out Southern blot hybridizations on representative species of the major *Drosophila* species groups in the genus *Drosophila* in order to detect

the presence of *mariner* homologues. The genus *Drosophila* consists of two major subgenera. The subgenus *Sophophora* contains seven species groups, one of which is the *melanogaster* species group. Although further subdivision of the species groups into subgroups is difficult, the *melanogaster* species group is conventionally divided into 10 to 11 subgroups, including the *melanogaster* subgroup (ASHBURNER 1989; THROCKMORTON 1975; LEMEUNIER *et al.* 1986). We have found that sequences homologous to *mariner* are prevalent within the *melanogaster* species group, and we have cloned and sequenced multiple copies of the element from various members of the *melanogaster* species subgroup within this species group. Judged from these sequences, the phylogeny of the *mariner* elements is congruent with the phylogeny of the species within the subgroup. The rate of sequence evolution of *mariner* appears to be somewhat slower than that of the single-copy nuclear gene, alcohol dehydrogenase (*Adh*). Furthermore, *mariner* elements from closely related species can share nucleotide polymorphisms, implying that concerted evolution has had little effect on *mariner* evolution over the time scale of evolution of these species.

MATERIALS AND METHODS

Fly stocks: Isofemale lines of species in the *melanogaster* species subgroup were obtained from the following individuals: *D. simulans*, courtesy J. R. DAVID and R. S. SINGH; *D. melanogaster* and *D. sechellia*, courtesy of J. R. DAVID; and *D. yakuba*, *D. teissieri*, *D. erecta* and *D. orena*, courtesy of M. SOLIGNAC. DNA from various Hawaiian *Drosophila* species was kindly provided by R. DESALLE. All other species were obtained from the National Drosophila Species Resource Center, Bowling Green State University, Bowling Green, Ohio. All species examined for the presence of the sequences homologous to the *mariner* element are listed in Table 1.

DNA probes: Four overlapping regions covering *mariner* were subcloned into pUC18 to use as probes, as shown in Figure 2 (see also JACOBSON, MEDHORA and HARTL 1986). Plasmid DNA was prepared by CsCl density gradient centrifugation, and probe DNA was isolated by a modification of the glass-powder method of VOGELSTEIN and GILLESPIE (1979).

DNA blotting experiments: Genomic DNA for Southern blot hybridization experiments was prepared as described in LIS, SIMON and SUTTON (1983). Restriction digestion, gel electrophoresis, and transfer to filters were performed according to MANIATIS, FRITSCH and SAMBROOK (1982). Southern blots were carried out essentially as in SOUTHERN (1975). Probes were labeled with ^{32}P to high specific activity by the method of FEINBERG and VOGELSTEIN (1983, 1984). Filter hybridizations were performed at 65° for 12–16 hr in a solution of 1% Sarcosyl, 100 mg/ml salmon sperm DNA, 0.5 M NaCl, 0.1 M Na_2HPO_4, 5 mM Na_2EDTA, pH 7.0. Filters were washed at room temperature, first with 1% Sarcosyl, 1 mM Tris (pH 7.0), and then three times with 1 mM Tris (pH 8.0).

Cloning and sequencing: Genomic DNA from each species was prepared according to KUNER *et al.* (1985). DNA was partially digested with *Sau*3A and size-fractionated by gel electrophoresis. DNA fragments of 13–22 kilobases were isolated from the gel and ligated into the *Bam*HI site of the lambda vector EMBL3. Recombinant lambda phage were packaged *in vitro* with Gigapack (Stratagene, La Jolla, California), and the resulting libraries were screened with the *Ssp*I-*Nhe*I fragment of *mariner* (Figure 2). To avoid sequencing the same *mariner* element twice, the genomic position of each clone was examined by preparing DNA from each positive lambda clone (HELMS *et al.* 1985), digesting the DNA with one or more endonucleases having no restriction sites within the *mariner* element, and separating the frag-

TABLE 1

Distribution of sequences hybridizing with *mariner*

Subgenus *Sophophora*	Group *willistoni*
Group *melanogaster*	− *capricorni*
Subgroup *melanogaster*	− *nebulosa*
− *erecta*	− *paulistorum*
++ *mauritiana*	Group *saltans*
− *melanogaster*	− *neocordata*
− *orena*	− *saltans*
++ *sechellia*	− *sturtevanti*
++ *simulans*	Subgenus *Drosophila*
++ *teissieri*	Group *virilis*
++ *yakuba*	− *littoralis*
Subgroup *takahashii*	− *lummei*
− *lutescens*	− *montana*
− *paralutea*	− *virilis*
− *prostipennis*	Group *robusta*
− *pseudotakahashii*	− *lacertosa*
− *takahashii*	− *melanica*
Subgroup *eugracilis*	− *robusta*
− *eugracilis*	− *sordidula*
Subgroup *suzukii*	Group *repleta*
+ *lucipennis*	− *arizonensis*
+ *mimetica*	− *buzzatii*
+ *pulchrella*	− *eohydei*
+ *rajasekari*	− *mojavensis*
Subgroup *montium*	− *mercatorum*
− *auraria*	− *mulleri*
+ *barbarae*	− *neohydei*
+ *bicornuta*	− *paranaensis*
− *birchii*	− *repleta*
+ *diplacantha*	Group *immigrans*
− *jambulina*	− *albomicans*
+ *kikkawai*	− *immigrans*
+ *nikananu*	− *nasuta*
+ *pennae*	Group *funebris*
+ *quadraria*	− *funebris*
++ *seguyi*	Hawaiian *Drosophila*
+ *serrata*	− *adiastola*
++ *tsacasi*	− *grimshawi*
− *vulcana*	− *heteroneura*
Subgroup *ananassae*	− *mimica*
+ *ananassae*	− *neutralis*
+ *malerkotliana*	− *punalua*
Group *obscura*	Subgenus *Dorsilopha*
− *affinis*	− *buschii*
− *algonquin*	
− *bifasciata*	Genus *Zaprionus*
− *miranda*	− *inermis*
− *persimilis*	++ *tuberculatus*
− *pseudoobscura*	

Species examined for the presence of sequences that hybridize with mariner. The ++ and + signs indicate the relative abundance of hybridizing sequences; − indicates no detectable hybridization signal.

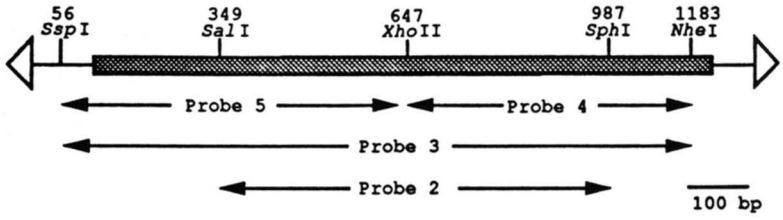

FIGURE 2.—Restriction map of *mariner*, showing the regions covered by different probes.

ments on agarose gels alongside genomic DNA digested with the same restriction enzymes. After transfer to filters and hybridization with *mariner* as probe, lambda clones representing independent *mariner* elements were selected by identifying those distinct fragments that comigrated with genomic DNA fragments also hybridizing with the probe. The DNA fragment containing *mariner* from each lambda clone was subcloned into M13mp18 and M13mp19 and sequenced by the dideoxy chain termination method of SANGER, NICKLEN and COULSON (1977) using Sequenase (United States Biochemical).

Sequences of *Adh* from *D. teissieri* and *D. yakuba* were kindly provided by P. JEFFS and M. ASHBURNER.

RESULTS

Distribution of *mariner* in the *D. melanogaster* species subgroup: In order to estimate the number of copies of *mariner* in the genome, DNA digested with various combinations of restriction enzymes that do not cleave within the element was transferred to nylon membranes and probed with an internal *Ssp*I-*Nhe*I *mariner* fragment that includes most of the element (probe 3 in Figure 2). To detect the presence of deleted elements or restriction-site polymorphisms, the same filters were also hybridized with two distinct internal fragments, an *Ssp*I-*Xho*II fragment that includes approximately the 5′ half of the element (probe 5), and an *Xho*II-*Nhe*I fragment that includes approximately the 3′ half of the element (probe 4).

Figures 3 and 4 show that the *mariner* element is distributed discontinuously among species in the *melanogaster* species subgroup. For example, sequences hybridizing with *mariner* are found in the three closely related species, *D. mauritiana*, *D. simulans* and *D. sechellia*, but not in their sibling species, *D. melanogaster*. Hybridizing sequences are also found in *D. yakuba* and *D. teissieri*, but no detectable hybridization is observed in *D. erecta* or *D. orena*. Among the species containing the element, a striking difference is found in the copy number and the pattern of intraspecific variation. *D. mauritiana* has the largest number of bands hybridizing with *mariner*, but its sibling species *D. simulans* and *D. sechellia* have the lowest number of bands. Furthermore, *D. sechellia* does not show any evidence for intraspecific variation in the copy number or genomic positions of the elements.

Figure 4 also illustrates intraspecific variation in the copy number and genomic positions of *mariner* elements in strains of *D. simulans* from different regions of the world. The number of bands hybridizing with

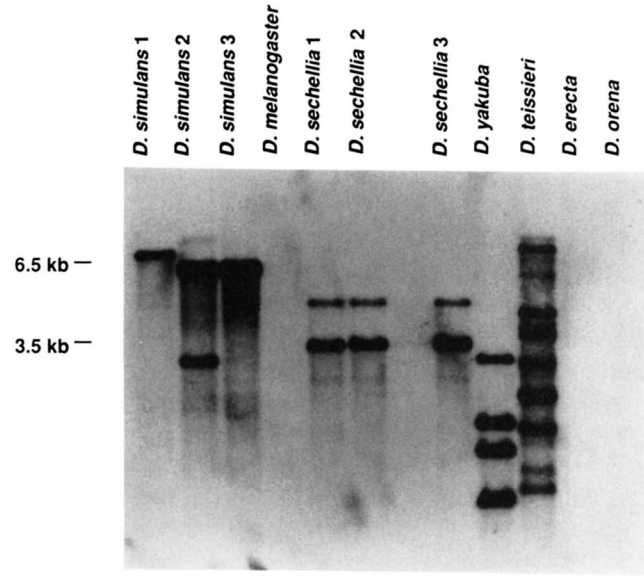

FIGURE 3.—Southern blot hybridization of species in the *melanogaster* species subgroup. Each lane contains DNA from about 10 flies digested with *Hin*dIII and *Bam*HI. The filter was hybridized with the *Sal*I-*Sph*I fragment (probe 2). Hybridization with the *Ssp*I-*Nhe*I fragment (probe 3) revealed no additional hybridizing bands.

mariner in the *D. simulans* lines is generally small (occasionally none), but lines from Afro-tropical regions show the highest number of bands. However, the variation in genomic position is not very great, and a few hybridizing bands of the same size are present in the majority of the strains representing regions as far apart as Africa and North America (Figure 4). For example, a 6.6 kb *Eco*RI-*Bam*HI band is observed in strains from Capetown, Congo, and the United States. Thus, it appears that *mariner* has a low copy number in most strains of *D. simulans*, and some genomic sites with insertions appear to be nearly fixed.

Intraspecific variation in the distribution of *mariner* was also studied by Southern blot experiments using multiple isofemale lines from each species: 12 lines of *D. mauritiana*, five lines of *D. sechellia*, six lines of *D. yakuba*, seven lines of *D. teissieri* and two lines of *D. erecta*. For *D. orena* only one strain was used because all the extant lines are derived from one collection in 1975 from Cameroon (LACHAISE *et al.* 1988). For the cosmopolitan species *D. simulans* and *D. melanogaster*, more than 15 isofemale lines representing different regions of the world were screened for the presence of *mariner*. Southern blot hybridization with distinct internal fragments of *mariner* does not reveal any

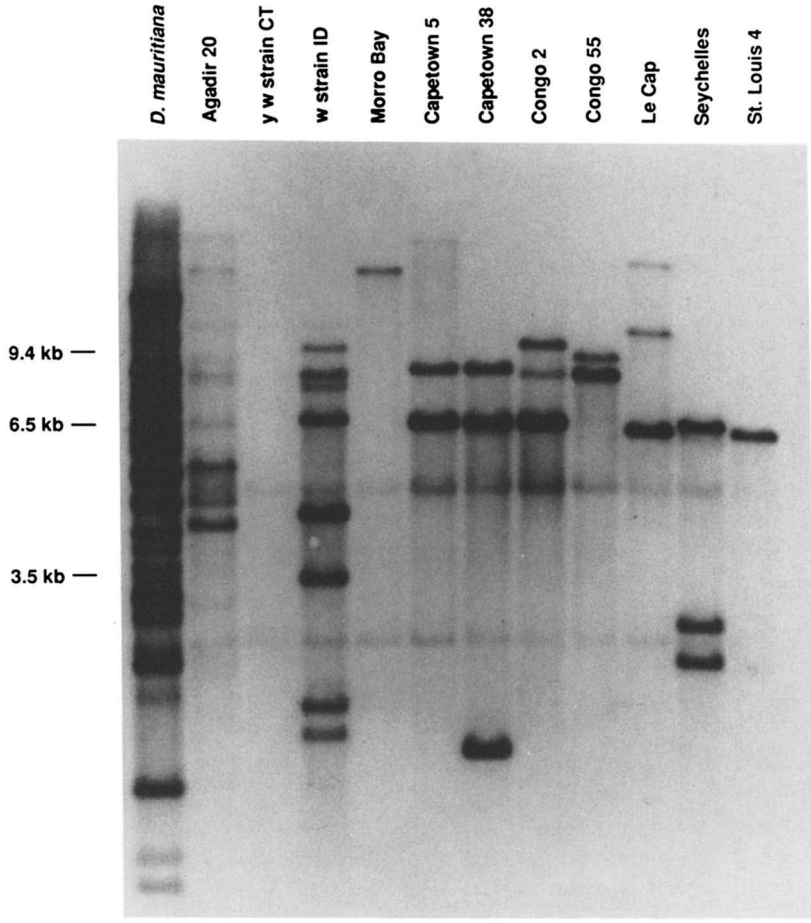

FIGURE 4.—Southern blot hybridization of *D. simulans* lines. The leftmost lane contains *D. mauritiana* as a control. The *y w* strain is a laboratory strain maintained for many years at the California Institute of Technology. The *w* strain is a laboratory strain obtained from the Indiana University Stock Center. All other strains were isolated recently from natural populations. Each lane contains DNA from about 10 flies digested with *Eco*RI and *Bam*HI. The filter was hybridized with the *Ssp*I-*Nhe*I fragment (probe 3). The Morro Bay strain shows two hybridizing bands in other blots (data not shown). Blots using DNA from single flies show that the heavy bands are shared by all the individuals in the strain, whereas the weak bands are present only in some individuals (data not shown).

detectable difference in hybridization pattern in *D. mauritiana*, indicating that there are no major deletions or restriction site polymorphisms among the elements (Figure 5). Each of the *D. mauritiana* strains examined also shows a unique pattern of hybridization, suggesting that at least some of the elements in the genome are active (data not shown). The present observations confirm and extend the results of JACOBSON, MEDHORA and HARTL (1986), who reported that *D. mauritiana* has 20–30 copies of *mariner* per genome, great variation in the genomic positions of the elements, and few deleted copies.

All five isolates of *D. sechellia* examined show the same pattern of hybridization as the three strains shown in Figure 3. When the entire *mariner* element is used as probe, two bands are observed, but only one hybridizes with the *Xho*II-*Nhe*I fragment from the 3′ end (compare Figure 5, A with B). This result suggests that there are two copies of *mariner* at fixed locations in the genome, and that one copy has a deletion of most of the 3′ half of the element.

The sibling species *D. yakuba* and *D. teissieri* are widespread in Africa (LACHAISE *et al.* 1988). Variation in the distribution of *mariner* within each species was studied using strains collected from areas spanning the continent. Strains of *D. yakuba* from different parts of Africa show from 4–8 hybridizing bands. Two examples are illustrated in Figure 5. Unlike *D. sechellia*, hybridization with distinct internal fragments of *mariner* does not detect any large deletions in the elements (compare Figure 5, A with B). The presence of a moderate number of distinct bands suggests the presence of at least some active *mariner* elements in the *D. yakuba* genome.

In *D. teissieri*, hybridization with two distinct internal *mariner* fragments shows a strikingly different pattern. In all strains examined, the *Ssp*I-*Xho*II fragment from the 5′ end hybridizes to over 15 genomic bands (Figure 5A), while the *Xho*II-*Nhe*I fragment from the 3′ end hybridizes only to a few bands (Figure 5B). This result indicates that the majority of *mariner* copies in this species have a deletion of most of the 3′ half of the gene. The sequence of the deleted elements is shown below.

Distribution in other *Drosophila* species: Species from other *Drosophila* species groups were also probed with *mariner*. Some representative examples are shown in Figure 6, and the results concerning presence (+ or ++, according to relative abundance) or absence (−) of hybridizing fragments are summarized in Table 1. In addition to the *melanogaster* species subgroup, *mariner* homologues are found in several

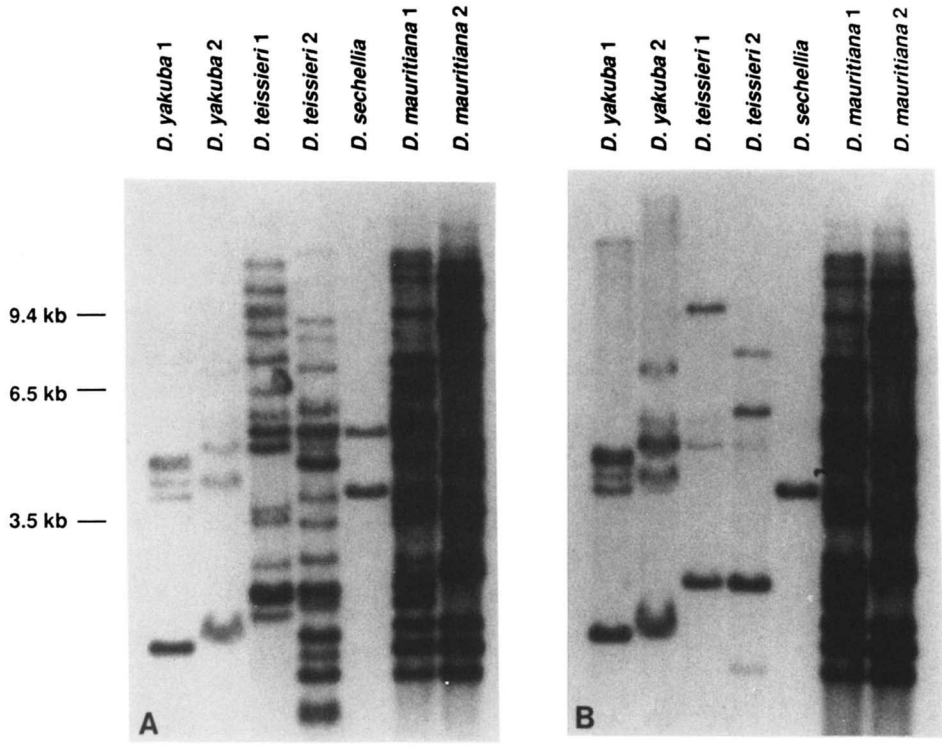

FIGURE 5.—Southern blot hybridization showing large deletions in *mariner* elements in some species of the *melanogaster* species subgroup. DNA was digested with *Eco*RI and *Bam*HI, except for *D. sechellia* DNA, which was digested with *Bam*HI and *Hin*dIII. (A) Hybridization with the *Ssp*I-*Xho*II fragment (probe 5). (B) Hybridization with the *Xho*II--*Nhe*I fragment (probe 4).

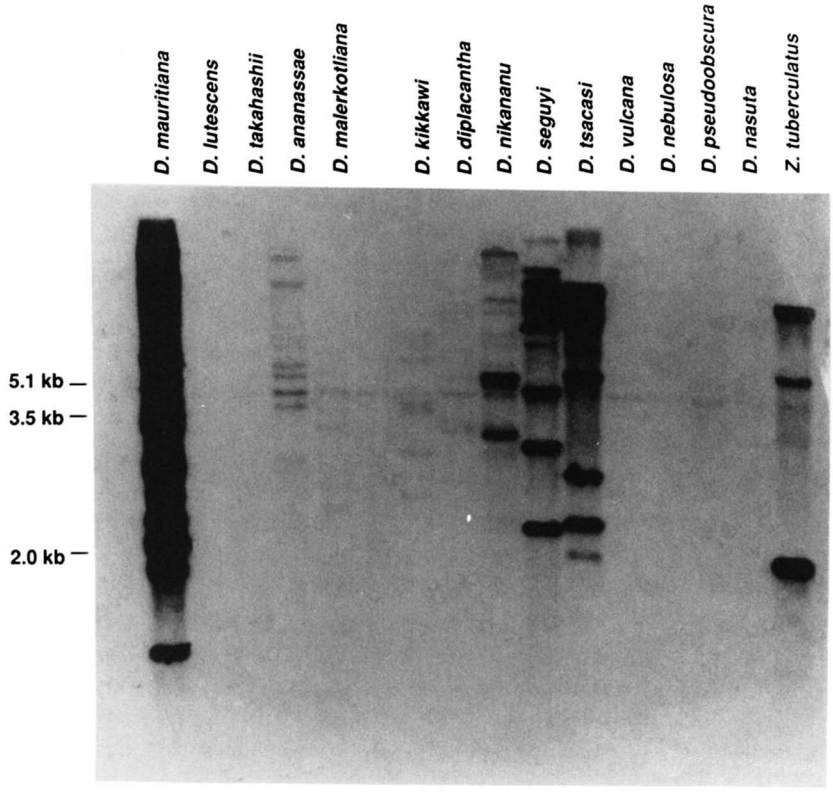

FIGURE 6.—Southern blot hybridization of species of representative species groups. See also Table 1. DNA was digested with *Eco*RI and *Bam*HI, and the filter was probed with the *Xho*II-*Nhe*I fragment (probe 4). In the *ananassae* subgroup, *D. ananassae* and *D. malerkotliana* do not show any hybridization when probed with the *Ssp*I-*Xho*II fragment (probe 5). Some species of the *montium* subgroup (*D. kikkawai*, *D. diplacantha*, *D. nikananu*, *D. seguyi*, *D. tsacasi* and *D. vulcana*) show an abundance of restriction fragments that hybridize with the *mariner* probe. Sequences homologous to *mariner* have not been found in the *takahashii* (*D. lutescens*, *D. takahashii*) or *eugracilis* subgroups, which are generally considered to be most closely related to the *melanogaster* subgroup (LEMEUNIER *et al.* 1986). In species outside the *melanogaster* species group (Table 1), we have observed no sequences that hybridize with *mariner*. However, notable exceptions are species within the genus *Zaprionus*. When the hybridization is carried out at low stringency, homologous sequences are detected in some species of the *immigrans* species group (data not shown). In the blot, lane 6 is intentionally left blank.

other subgroups in the *melanogaster* species group, namely, the *montium*, *suzukii* and *ananassae* subgroups. However, presence of homologous elements has not been detected in the *takahashii* and *eugracilis* subgroups, which are generally considered to be more closely related to the *melanogaster* subgroup (LEMEUNIER *et al.* 1986). In species outside the *melanogaster* species group (Table 1), we have observed no *mariner* homologues, with the notable exception of species within the genus *Zaprionus*. When the hybridization is carried out at lower stringency, however, homologous sequences are detected in some species of the *immigrans* species group, which the genus *Zaprionus* is related to (data not shown).

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                                                 1 1 1
                         1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 4 6 7 8 9 2 3 3
       1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 1 4 7 4 3 7 6 7 2 6
Mos1   CCAGGTGTACAAGTAGGGAATGTCGGTTCAAGTATGGGT
Ma351  CCAGGTGTACAAGTAGGGAATGTCGGTTCAAGTATGGAT
pch    CCAGGTGTACAAGTAGGGAATGTCGGTTCAAGCATGGAT
MB1    CCAGGTGTACAAGTAGGGAATGTCGGTTCAAGTATGGAT
MB4    --AGGTGTACAAGTATGGAATGTCGGTTCAAGTAGGTAT
Yak2   CCAGGTGTACAAGTATGGAATGTCGGTTCAACTTTTTCC
Yak3   CCAGGTGTACAAGTATGGAATGTCGGTTCAACTTTGTCC
Teis32 ------------------------------TGCTTTGTCC
                   *               * *  ***

       ATAATCGAGACGACAAGGAGTCAAGTGCACTTTGT-TGA
       ATAATCGAGACGACCAGTAGTCAAGTGCACTTTGT-TGA
       ATTATAGAGACGACCGGGAGTGAAGTGCACAT-GAATGA
       ATAATCGAGACGACCAGGAGTCAAGTGCACAT-GAATGA
       ATAATCGAAACGGCCAGGAGTCAAATTCACTATGT----
       TCAGACGAAACGACCAAGAGCCCTAAGTGTTTTAT-TGG
       TCAGACACAAAAACCAAGAACCCAATGTGCTTTAT-TGG
       TCAGACGAAGCGATCAAGGGCCCAATGTGCTTTAT-TGG
       ** **   *       *   *  *  ** *  *     *
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FIGURE 7.—Alignment of eight *mariner* sequences. Only sites differing among the sequences are shown. The numbers at the top indicate the positions of the variable sites in the *mariner* sequence. Deletions are indicated by minus signs. Phylogenetically informative sites are indicated by asterisks. The best alignment is obtained by a deletion of T at position 1248, and addition of A at position 1254, in the sequences of *pch* and *MB1*. Thus, the numbering of nucleotides is through 1287 instead of 1286. The open reading frame runs from 172 through 1209.

DNA sequence analysis: The aligned nucleotide sequences from four species of the *melanogaster* species subgroup are presented in Figure 7. For *D. mauritiana*, sequences of three *mariner* copies are shown. The copy of the element denoted *peach* (*pch*) was previously cloned from the *white* locus (JACOBSON, MEDHORA and HARTL 1986), and the *Mos1* sequence is a highly active *mariner* element that causes a high rate of excision of the *peach* element from its insertion point in the *white* locus (MEDHORA, MACPEEK and HARTL 1988; MEDHORA, MARUYAMA and HARTL 1991). The sequence denoted *Ma351* was obtained from a clone chosen at random from a w^{pch} library (JACOBSON, MEDHORA and HARTL 1986). Figure 7 indicates that all three elements from *D. mauritiana* are 1286 nucleotides in length. An additional five copies of *mariner* isolated from *D. mauritiana* also show no heterogeneity in length (data not shown).

For *D. simulans* and *D. yakuba*, two copies of *mariner* from each species were sequenced. In the Morro Bay strain of *D. simulans*, one copy (designated *MB1*) was full-length (1286 base pairs), while the other (designated *MB4*) had a few base pairs deleted at the ends of the terminal inverted repeats. The two *D. yakuba* copies (*Yak2* and *Yak3*) were both full-length, and there were no chain-terminating base substitutions observed in the open reading frame of the sequence, consistent with the Southern blot experiments suggesting that these elements are, at least potentially, active.

For *D. teissieri*, which was found to possess a number of deleted copies, the genomic library was screened first using the entire *mariner* element as a probe, and positive clones were then hybridized separately with

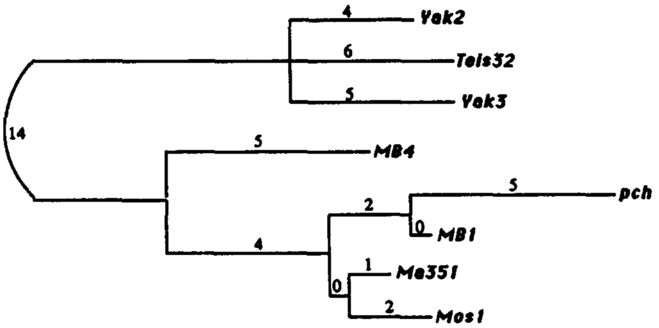

FIGURE 8.—Maximum parsimony tree of eight *mariner* sequences, using PAUP (SWOFFORD 1985). Deletions at the ends of *MB4* and *Teis32* were treated as unique single characters. The compensating insertion/deletion at positions 1248 and 1254 was treated as a single character. The number of mutational steps between the nodes are indicated along the branches.

the two distinct internal fragments. Among 14 clones that showed hybridization with the entire *mariner* fragment, only three hybridized with both internal fragments, whereas 11 hybridized with the *Ssp*I-*Xho*II fragment but not with the *Xho*II-*Nhe*I fragment. Thus, the ratio of intact copies to the total number of elements is 3:14, which is roughly consistent with the results of the Southern blot experiments. One of the three apparently complete *mariner* copies (*Teis32*) was chosen for further study. As seen in Figure 7, while the element possesses an intact open reading frame, it is missing a portion of the 5′ terminal inverted repeat. We also studied the sequences of three elements randomly chosen from the 11 clones containing *mariner* elements with a deletion in the 3′ half of the gene. All three share an identical internal deletion that spans nucleotide positions 544–1260, which includes the putative stop codon of the open reading frame and the putative polyadenylation site (JACOBSON, MEDHORA and HARTL 1986).

Phylogeny of *mariner* elements: A phylogenetic tree was constructed from the eight *mariner* sequences in Figure 7. All of the phylogenetically informative sites were consistent with each other, and therefore maximum parsimony analysis yields only one tree with no inconsistencies (Figure 8). In Figure 8, the numbers represent mutational steps separating any two nodes. Although these numbers are small, it is clear that the *mariner* sequences from *D. yakuba* and *D. teissieri* form one phylogenetic group and those from *D. mauritiana* and *D. simulans* form another. Within *D. mauritiana* and *D. simulans*, however, the *mariner* sequences do not form clusters according to the species classification. That is, *MB1* from *D. simulans* is grouped with the *mariner* sequences from *D. mauritiana*, while *MB4* is off by itself. The simplest explanation of these shared polymorphisms is that they arose prior to speciation, which would indicate that *mariner* elements have not undergone rapid concerted evolution within these genomes. These species are closely related, and

	Mos1	Ma351	pch	MB1	MB4	Yak2	Yak3	Tei32
Mos1	0.002	0.007	0.003	0.008	0.019	0.019	0.019	
Ma351	0.006	0.002	0.007	0.019	0.019	0.019		
pch	0.004	0.012	0.023	0.024	0.023			
MB1	0.008	0.019	0.020	0.019				
MB4	0.017	0.018	0.018					
Yak2	0.007	0.007						
Yak3	0.008							
Tei32								

FIGURE 9.—Pairwise divergence among the eight *mariner* sequences. The divergence was calculated as the number of nucleotides differing between any two sequences divided by the total number of nucleotides in *mariner*.

they are known to share polymorphisms in the *Adh* gene that arose prior to speciation (COYNE and KREITMAN 1986; STEPHENS and NEI 1985).

Figure 9 summarizes the pairwise divergences (percent of nucleotides that differ) among *mariner* sequences from the four species. There is an unexpectedly low rate of divergence between *D. mauritiana-D.simulans* and *D. yakuba-D. teissieri* lineages. The table indicates that the average divergence of *mariner* between these two species clusters is less than 2%. A similar comparison for the coding region of the *Adh* gene gave 5% divergence (P. JEFFS and M. ASHBURNER, personal communication). On the other hand, the average divergence of *mariner* elements within species is about 1% and is roughly compatible with the intraspecific variation in the *Adh* gene of *D. melanogaster* (KREITMAN 1983). Including additional *mariner* sequences from *D. mauritiana* indicates that random *mariner* sequences from this species may differ by as much as 1.1% (data not shown), but this does not affect the comparison between species.

DISCUSSION

Two hypotheses may be proposed to account for the discontinuous distribution of the transposable element *mariner* in the *melanogaster* species subgroup of the genus *Drosophila*. According to the stochastic loss hypothesis, *mariner* was present in the ancestral group and was subsequently lost from some of the species during their evolution. On the other hand, according to the recent invasion hypothesis, *mariner* was recently transferred from an outside source into some species of the *melanogaster* species subgroup subsequent to its divergence. Several features of our results do not support the recent invasion hypothesis. First, *mariner* is not limited to the *melanogaster* species subgroup. Second, the *mariner* gene phylogeny is congruent with the species subgroup phylogeny. Third, a detailed sequence analysis of *mariner* copies shows that the pattern of divergence between species is not compatible with the pattern expected based on recent interspecific transfer. These findings are elaborated below.

In the *melanogaster* species group, *mariner* homologs are found in some other subgroups as well as the *melanogaster* species subgroup, namely the *montium*, *suzukii*, and *ananassae* subgroups, although the element is not observed in the subgroups *takahashii* and *eugracilis*. Furthermore, the *montium*, *suzuki* and *ananassae* subgroups are mainly Oriental in distribution, whereas the *melanogaster* species subgroup is Afrotropical (LEMEUNIER *et al.* 1986). Thus, the distribution of *mariner* is not limited to one subgroup or to one geographic area, as any simple form of the recent invasion hypothesis would predict.

Maximum parsimony analysis of the *mariner* sequences from the *melanogaster* species subgroup gives only one phylogenetic tree with no inconsistencies (Figure 8). When superimposed on the two alternative species trees (shown in Figure 1), the *mariner* phylogeny is consistent with either 1A or 1B. (The absence of *mariner* in both *D. erecta* and *D. orena* prevents discrimination.) Assuming that *mariner* was present before the diversification of the *melanogaster* species subgroup, both trees require that *mariner* was lost at least twice in lineages leading to *D. melanogaster* and *D. orena-D. erecta*. The possibility that a species could lose a transposable element is supported by the distribution of *mariner* in *D. simulans*, in which some strains lack the element entirely.

Assuming that *mariner* was present in the ancestral group before speciation, we might expect that *mariner* sequences would be as divergent as any single-copy gene between the species. However, the *mariner* sequences among the species have not diverged to the same extent as the *Adh* gene from the same species. We compared the rates of divergence between the coding region of the *Adh* gene and the entire sequence of the *mariner* gene, since it is believed that most of the 1286 nucleotide sequence of *mariner* encodes a transposase protein (JACOBSON, MEDHORA and HARTL 1986). If the sequences were under similar selective pressure, we should see a somewhat greater rate of divergence in *mariner*, since a small amount of noncoding region is included in the *mariner* sequence. The divergence in the 771-nucleotide coding region of *Adh* is 5% between *D. mauritiana* and *D. yakuba*, and this is roughly consistent with other data on the genetic distance between the two species. However, the average divergence of *mariner* sequences is approximately 2%, roughly half that of the *Adh* gene. Comparison between *D. mauritiana* and *D. teissieri* gives a similar result. Thus, it seems that *mariner* has diverged more slowly than the coding region of the *Adh* gene, which itself has diverged at a relatively slow rate (SHARP and LI 1989).

Although the other evidence argues against the recent invasion hypothesis, the slow rate of *mariner* evolution requires reconsideration of the possibility

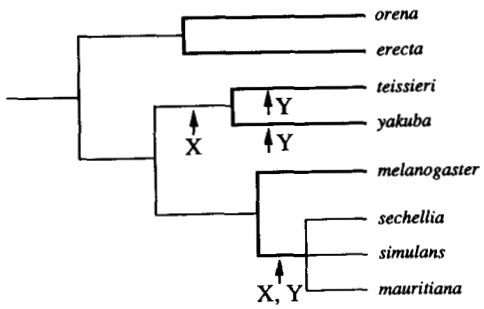

FIGURE 10.—Two scenarios of *mariner* invasion after the divergence of the *melanogaster* species subgroup. In scenario X, *mariner* invaded before *D. teissieri* and *D. yakuba* split, whereas in scenario Y, the invasion occurred after the species had split.

that *mariner* may have entered the *melanogaster* species subgroup after the species diverged. This would give the *mariner* elements a more recent common ancestor than the common ancestor of the *Adh* gene, and could explain the discrepancy in the rates of sequence evolution. One possibility is that *mariner* independently entered each of the ancestral species of the *yakuba* and *melanogaster* species complexes before the divergence of *D. yakuba* and *D. teissieri* (scenario X in Figure 10). This model would predict that *mariner* and *Adh* should be equally divergent between *D. yakuba* and *D. teissieri*, but this is not the case. In these species *mariner* sequences are 0.8% divergent but *Adh* sequences are more than 2% divergent. An alternative possibility is that *mariner* invaded the *melanogaster* species subgroup several times after the speciation of *D. yakuba* and *D. teissieri* (scenario Y in Figure 10). Scenario Y requires at least three independent invasions, into *D. yakuba*, *D. teissieri* and the species ancestral to *D. simulans*, *D. mauritiana* and *D. sechellia*. Although this model might explain why *mariner* is less divergent between species than the *Adh* gene, it creates another difficulty. For example, if we accept that interspecific transfer of *mariner* was frequent enough to allow three independent invasion events in the *melanogaster* subgroup, then we have to explain why *mariner* has not invaded other species of *Drosophila* and why the phylogeny of *mariner* is congruent with the species subgroup phylogeny.

We therefore prefer the hypothesis that *mariner* was present in the ancestral species prior to the radiation of the *melanogaster* species subgroup, and that the element was lost in the lineages leading to *D. melanogaster* and *D. orena-D. erecta*. Acceptance of this model implies that *mariner* has evolved rather slowly in the *melanogaster* species subgroup. Although genes under different selective pressures are known to have different rates of nucleotide substitution, the paucity of substitutions, even at the presumed silent sites in *mariner*, remains difficult to explain. On the other hand, the data on intraspecific variation have revealed many nucleotide polymorphisms within *mariner* elements in *D. mauritiana*, as evidenced by the fact that two random copies of *mariner* from this species may differ by as much as 1.1%.

The relatively high rate of *mariner* polymorphism within *D. mauritiana*, as contrasted with the relatively low rate of divergence among species, suggests the possibility that *mariner* elements may have different mutation rates in different lineages. Another example of possible differential mutation rates was reported in a region near the *glue* gene cluster in *D. melanogaster* (MARTIN and MEYEROWITZ 1986), in which noncoding sequences in salivary region 68C adjacent to the gene cluster are significantly more conserved than the nearby coding regions. The hypothesis of differential mutation rates might be particularly apt for transposable elements. Some quiescent transposable elements in Drosophila are located in heterochromatic regions where gene expression appears to be low (BUCHETON *et al.*1986; DANIELS *et al.* 1984; MIKLOS *et al.* 1988; SIMONELIG *et al.* 1988). In species where the elements undergo active transposition they are usually found in euchromatic regions. In *D. mauritiana*, where *mariner* reaches the highest copy number among the sibling species, the elements are located primarily in euchromatic regions (data not shown), and some copies have been shown to be highly active (BRYAN and HARTL 1988; MEDHORA, MARUYAMA and HARTL 1991). In *D. simulans*, however, *mariner* is located in both heterochomatic and euchromatic regions (data not shown). If genes in heterochromatin have reduced mutation rates, then it is possible that *mariner* could have long resided in heterochromatin and only recently become active in *D. mauritiana*, resulting in a higher mutation rate in this species.

The slow rate of evolution and shared polymorphisms betwen closely related species are often explained by introgression between species. Although the introgression of mtDNA is speculated to have occurred from the population of *D. simulans* in Madagascar to that of *D. mauritiana* endemic to Mauritius (SOLIGNAC and MONNEROT 1986), there is no evidence for introgression of any nuclear genes in natural populations. In the case of the *Adh* gene, the *simulans* alleles most closely related to *mauritiana* alleles are not limited to populations in the Afro-tropical region where the two species coexist, suggesting that the shared polymorphisms are due to retention of polymorphisms that predated speciation and not due to introgression (COYNE and KREITMAN 1986; STEPHENS and NEI 1985). Two observations support the same conclusion for *mariner*. First, the Morro Bay strain that we studied was collected in California, geographically very distant from Mauritius, where *D. mauritiana* is endemic. Second, active copies of *mariner*, which constitute a subset of all *mariner* sequences (K. MARUYAMA, unpublished data), are found in *D. simulans* populations worldwide as well as in *D. mauritiana*,

indicating that active *mariner* elements existed in the common ancestor of the two species (CAPY *et al.* 1990). A similar observation holds for the pair of sibling species *D. yakuba* and *D. teissieri* (unpublished data, but see Figure 8). Although their mtDNA shows striking similarity, these two species show almost complete reproductive isolation, and there is no evidence for nuclear introgression (MONNEROT, SOLIGNAC and WOLSTENHOLME 1990). Furthermore, species of the melanogaster species complex (*D. mauritiana* and *D. simulans*) and the yakuba complex (*D. yakuba* and *D. teissieri*) do not hybridize either under laboratory conditions or in nature. Thus, the relatively slow rate of sequence evolution of *mariner* in the *melanogaster* subgroup and the shared polymorphisms between *D. mauritiana* and *D. simulans* are not likely to result from introgression.

The retention of ancestral polymorphisms suggests that the *mariner* family of elements has not undergone rapid sequence homogenization. Although it has been suggested that transposable elements might undergo rapid concerted evolution or homogenization (HEY 1989), the concept is difficult to apply to dispersed genes that can change rapidly in copy number. The shared polymorphisms and lack of species-specific nucleotide changes between closely related species suggests the absence of concerted evolution of *mariner* transposable elements. Indeed, the relative homogeneity of *mariner* sequences within species may simply reflect rapid amplification of the elements from a few ancestral sequences and the coalescence process (KINGMAN 1980), without any involvement of particular homogenizing mechanism relevant to multigene families.

We have shown that each species in the *melanogaster* species subgroup is characterized by a unique *mariner* distribution. The copy number varies greatly. For example, *D. mauritiana* has about 30 copies of *mariner*, *D. teissieri* has about 15, and the element is absent in *D. melanogaster*, *D. orena*, and *D. erecta*. Variation in copy number within *D. simulans* is wide, and some strains lack the element. The species also differ in the *mariner* elements themselves. For example, most of the copies of *mariner* in *D. teissieri* have a deletion of approximately the 3′ half of the element (nucleotides 544–1260), whereas deleted copies appear to be rare in *D. mauritiana*. The contrast observed with *mariner* between *D. mauritiana* and *D. teissieri* is observed within *D. melanogaster* with the transposable *P* element system, in which some strains have accumulated copies of *P* with a characteristic deletion (designated *KP* elements), whereas other strains carry full-length and heterogeneous copies (BLACK *et al.* 1987).

One might explain the differences in copy number and molecular characteristics of *mariner* in different species by assuming that each species has a distinct equilibrium state. However, given the wide range of variation, it is possible that none of the species are at equilibrium, and that the different states merely represent different stages in the evolution of a transposable element. We have provided evidence suggesting that *mariner* was lost at least twice in the *melanogaster* subgroup. The absence of *mariner* in the *takahashii* and *eugracilis* subgroups, considered to be the closest relatives of the *melanogaster* subgroup, also suggests that the element was lost completely in the lineages leading to these subgroups. Theoretical work suggests that, if the transposition process depends on copy number, then the transposable element system will go extinct in the genome unless autonomous copies have some advantage in terms of frequency of transposition over nonautonomous copies (KAPLAN, DARDEN and LANGLEY 1985). This is a rather pessimistic model from the standpoint of a transposable element, and if it is accepted, then rare instances of horizontal transfer to new species would provide a necessary mechanism for an element to remain extant over long periods of evolutionary time. Indeed, it seems very likely that some kind of horizontal transfer of *mariner* between species must be invoked to account for the presence of the element in the genus *Zaprionus*.

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