Sequence Analysis of Active *mariner* Elements in Natural Populations of *Drosophila simulans*

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ABSTRACT

Active and inactive *mariner* elements from natural and laboratory populations of *Drosophila simulans* were isolated and sequenced in order to assess their nucleotide variability and to compare them with previously isolated *mariner* elements from the sibling species *Drosophila mauritiana* and *Drosophila sechellia*. The active elements of *D. simulans* are very similar among themselves (average 99.7% nucleotide identity), suggesting that the level of *mariner* expression in different natural populations is largely determined by position effects, dosage effects and perhaps other factors. Furthermore, the *D. simulans* elements exhibit nucleotide identities of 98% or greater when compared with *mariner* elements from the sibling species. Parsimony analysis of *mariner* elements places active elements from the three species into separate groups and suggests that *D. simulans* is the species from which *mariner* elements in *D. mauritiana* and *D. sechellia* are most likely derived. This result strongly suggests that the ancestral form of *mariner* among these species was an active element. The two inactive *mariner* elements sequenced from *D. simulans* are very similar to the inactive *peach* element from *D. mauritiana*. The similarity may result from introgression between *D. simulans* and *D. mauritiana* or from selective constraints imposed by regulatory effects of inactive elements.

THE transposable element *mariner* is highly variable in its expression from strain to strain and from one copy of the element to the next. One source of variation is that different copies of the element can have different nucleotide sequences (MARUYAMA, SCHOOR and HARTL 1991). The sequence variation is primarily in the form of nucleotide substitutions or small deletions or additions of one or two nucleotides, but larger deletions also occur (CAPY *et al.* 1991; MARUYAMA and HARTL 1991b). A second source of variation is that of position effects, in which *mariner* expression is modulated by flanking genomic sequences (MEDHORA, MARUYAMA and HARTL 1991). Other sources of variation in *mariner* expression include dosage effects of the target sequences, saturation effects at high levels of expression (GARZA *et al.* 1991), and possibly repression involving cytoplasmically transmitted factors (JACOBSON 1990). The level of *mariner* expression can be assayed in single individuals carrying the allele *white-peach* (w^{pch}), in which an inactive *mariner* element (denoted the *peach* element) is inserted in the promoter region of the *white* gene. In the presence of active *mariner* elements elsewhere in the genome, the *peach* element undergoes nearly precise excision during the development of pigment cells in the eye, and the result is the occurrence of mosaic animals with one or more pigmented facets in an otherwise peach-colored eye (BRYAN, JACOBSON and HARTL 1987; BRYAN, GARZA and HARTL 1990). Although this phenotypic assay monitors only *trans* activation of *peach* excision, the level of somatic excision of *peach* is usually highly correlated with germline excision and with *mariner* transposition (GARZA *et al.* 1991; MARUYAMA, SCHOOR and HARTL 1991).

Natural populations of *Drosophila simulans* are polymorphic for the presence of expressed *mariner* elements (CAPY *et al.* 1990). The expressed elements can be detected in testcrosses between males isolated from natural populations (or laboratory strains derived from them) with females homozygous for w^{pch} that carry no *mariner* elements other than *peach*. Among males that yield mosaic progeny, there is variation in the proportion of mosaics and in the level of mosaicism, which can range from one or a few pigmented facets per eye to multiple, overlapping, pigmented patches. Different natural populations can be classified according to the frequency of males that yield mosaic progeny as well as the proportion of mosaic progeny and the mean and variance in the level of mosaicism.

On the one hand, the expression of *mariner* in natural populations of *D. simulans* could represent a great diversity of *mariner* elements whose differing levels of expression result primarily from differences in nucleotide sequence. Alternatively, the active elements could all be members of a single subfamily of

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mariner elements that are highly homogeneous in nucleotide sequence. In this case, the differing levels of expression in natural populations would largely reflect position effects, dosage effects, possible repression, and perhaps other factors. To distinguish between these hypotheses, we have analyzed eight active *mariner* elements derived independently from five natural populations and one laboratory population of *D. simulans*. On the average, the active *mariner* elements were found to be 99.7% identical in nucleotide sequence. Hence, much of the variation in *mariner* expression in natural populations must reflect position effects, dosage effects, and perhaps other factors not directly related to the sequences of the active *mariner* elements themselves.

The active *mariner* elements from *D. simulans*, plus two inactive elements, were compared with active elements of independent origin from laboratory strains of the sibling species *Drosophila mauritiana*. Parsimony analysis, including *mariner* sequences from *Drosophila sechellia* (CAPY *et al.* 1991) and inactive elements from *D. mauritiana* (MARUYAMA, SCHOOR and HARTL 1991), indicates that active elements are more closely related within species than between species. However, the two inactive *mariner* elements sequenced from *D. simulans* are more similar to some of the inactive elements from *D. mauritiana* than they are to the active elements in *D. simulans*. Possible reasons for these relationships are discussed.

MATERIALS AND METHODS

Natural populations and laboratory strains: Highly active *mariner* elements are denoted *Mos* (for *mosaic*). The *mariner* elements were isolated from the following natural populations of *D. simulans*: Agadir, Morocco (*Mos6a* and *Mos6b*); Bordeaux, France (*BordA*, an inactive element); Loua, Congo (*Mos* elements *L8* and *L14*); Mahé Island, Seychelles (*Mos* element *Sey2*); Madagascar (*Mos* element *MadB*); and Puerto Rico (*Mos* element *Pr1* and an inactive element *PrA*). Samples of the first three natural populations were maintained in the laboratory as isofemale lines and the others as mass cultures. The presence of active elements in these populations was previously reported by CAPY *et al.* (1990). An additional active *mariner* element (*Mos2*) was discovered in a laboratory strain of *D. simulans* carrying the *white* allele. Two new highly expressed *mariner* elements (*Mos3* and *Mos5*) were also found in the w^{pch} strain of *D. mauritiana* described by JACOBSON, MEDHORA and HARTL (1986). Laboratory strains of *D. simulans* used in isolating single active *mariner* elements from the natural populations were GB1, which carries the w^{pch} allele from *D. mauritiana* containing the inactive element *peach* but no other *mariner* elements (GARZA *et al.* 1991), and a laboratory strain carrying *y w* (*yellow* body and *white* eyes), which contains no *mariner* elements.

Isolation of *mariner* elements: Either of two mating schemes were used to identify active *mariner* elements. In the first, a single male from the natural population was crossed with two to three GB1 females; a single mosaic male was selected among the progeny and backcrossed to BG1, and this was repeated successively. After four generations of backcrosses, a single mosaic male was crossed with *GB1* females (cross A) and, after 4 days, crossed again with *y w* females (cross B). SOUTHERN (1975) blots were carried out with single *y w* F_1 males from cross B, using *mariner* as a probe. If several bands were detected, the backcrossing was continued for an additional generation using a single mosaic males from cross A mated with GB1 and remated with *y w*. This scheme was continued until the number of bands detected in the Southern blot was reduced to a single copy, and then genomic DNA of several *y w* F_1 males from cross B was extracted, the *mariner* element amplified by the polymerase chain reaction (PCR), and the amplified product sequenced. The activity of the sequenced *mariner* could be verified by the occurrence of mosaic male progeny in cross A. This procedure was used for the *Mos* elements *L8*, *L14*, *MadB*, *Sey2* and *Pr1*.

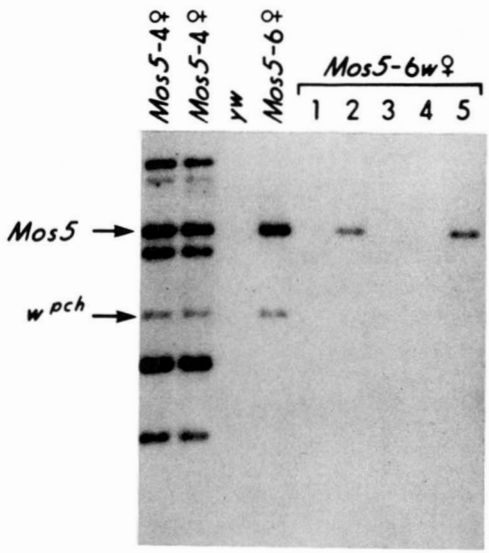

FIGURE 1.—Isolation of the active *mariner* element *Mos5* from *D. mauritiana*. The two lanes at the left contain genomic DNA from two single females in the fourth generation of backcrossing that carry *Mos5* along with several other copies of *mariner*. The *y w* strain used in the backcrosses is free of *mariner* elements. The lane labeled *Mos5–6* ♀ shows a mosaic w^{pch} female in generation 6 that contains only two copies of *mariner*, one of them the *peach* element and the other the active element *Mos5*. As expected, half of the *y w* female siblings carry only the active element *Mos5* (numbered lanes).

The second procedure was sightly different but based on the same idea. A single male from a natural population, or from the *Mos*-containing *w* stock of *D. simulans*, was crossed to w^{pch} females. Mosaic males in the first generation (or spontaneous mosaic males found in the w^{pch} stock of *D. mauritiana*) were crossed with *y w* females of *D. simulans*. Successive backcrosses were carried out in each generation by mating a single mosaic female with *y w* males, and, after 4–6 days, the number of *mariner* copies in the female was estimated by Southern blotting. Because the female carries the w^{pch} allele, the blot always detects the inactive *peach* element plus one or more additional *mariner* elements. If more than one additional copy was found, the backcrossing was continued. When just one additional copy was detected, then the *y w* siblings of the female were sacrificed and genomic DNA extracted from individual flies; one aliquot was used to ascertain the presence of a single *mariner* element by Southern blotting, and another aliquot reserved for PCR amplification when flies containing a single *mariner* element were identified. This procedure was used for *Mos3*, *Mos 5*, *Mos 2*, *Mos6a* and *Mos6b*. Figure 1 shows a Southern

blot obtained during isolation of *Mos5* in generations 4, 6 and 7. As expected, half of the *y w* females arising from the last cross carried the *Mos5* element.

Two inactive *mariner* elements from *D. simulans* (*PrA* and *BordA*) were identified by chance in the SOUTHERN (1975) hybridizations. In these cases, no mosaic males were found in the progeny of the cross between a male and *GB1* females, but a single band hybridizing with *mariner* was observed in the diagnostic Southern blot of the male.

Southern hybridization: Preparation of genomic DNA, Southern blotting, and hybridization were carried out as described by MARUYAMA and HARTL (1991a). Genomic DNA was completely digested with the restriction endonucleases *Hin*dIII and *Bam*HI, which cleave in genomic DNA outside the *mariner* element. Hybridization was carried out with the probes pchIII (the complete *mariner* element), pchIV (approximately the 5′ half), or pchV (approximately the 3′ half), as described in (MARUYAMA and HARTL 1991a).

PCR and DNA sequencing: All *mariner* elements were amplified by PCR according to SAIKI *et al.* (1988). The primers correspond to the inverted repeats of the complete *mariner* element (that is, the 5′ end of each strand). The PCR products were purified using CL-6B columns as described in DUBOSE and HARTL (1990). Primers used for sequencing are given in CAPY *et al.* (1991). To reduce the likelihood of sequence differences resulting from incorporation errors during amplification, at least two independent PCR products were sequenced for each *mariner* element.

RESULTS

Sequence analysis: All elements, with one exception, proved to be 1286 base pairs (bp) in length. The exception was the inactive element *PrA* from Puerto Rico, which is 1285 bp. The best alignment between various *mariner* elements, including *Mos1* and *peach*, is obtained when single-nucleotide gaps are introduced (MARUYAMA and HARTL 1991a). With the sequences presented here, the gaps are introduced at position 1248 in *Mos3*, *peach*, *PrA* and *BordA*; at position 1253 in *PrA*; and at position 1254 in *Mos1*, *Mos5*, *Mos2*, *Mos6a*, *Mos6b*, *L8*, *L14*, *MadB*, *Sey2* and *Pr1*. With the gap in each sequence the consensus is 1287 bp.

The nucleotide differences among the sequenced elements are summarized in Table 1. The *peach* and *Mos1* sequences are from JACOBSON, MEDHORA and HARTL (1986) and from MEDHORA, MARUYAMA and HARTL (1991), respectively. The *Mos1* and *peach* elements are used for comparison of active and inactive forms of *mariner*.

In the consensus sequence of 1287 bp, 27 sites are polymorphic in the sense that at least one element shows a difference when compared to the others. Among the 27 polymorphic sites, 10 are unique to individual elements. Seven of the polymorphisms result in putative amino acid replacements in the long open reading frame.

Species differences: The average number of nucleotide differences between active and inactive elements in *D. simulans* and *D. mauritiana* are given in Table 2. Active elements in both species are very similar, and the average number of nucleotide differences between them is 6.5. Likewise, the average number of differences between inactive elements in the two species is 1.5. On the other hand, the level of *mariner* polymorphism between the species is significantly greater than that within either species. (The Student's *t* test yields $P < 0.01$ in the comparison with *D. simulans*, and $P < 0.01$ in the comparison with *D. mauritiana*). Statistical significance is also obtained when the comparisons are based on all known *mariner* sequences in the two species. This result implies that the active *mariner* elements in *D. simulans* are more closely related to each other than to those in *D. mauritiana*. Indeed, when all published sequences of active *mariner* elements are compared, certain species-specific combinations of sites can be identified, as shown in Figure 2. A single difference at position 375 distinguishes between active elements from *D. simulans* and *D. mauritiana*, four sites distinguish between *D. simulans* and *D. sechellia*, and six sites distinguish between *D. mauritiana* and *D. sechellia*.

An analogous comparison among inactive elements is not possible because of the small number of available sequences. However, comparison of the mean number of nucleotide differences between active and inactive elements is also highly significant ($P < 0.01$).

Active *vs.* inactive *mariner* elements: Nucleotide identities between *mariner* elements are generally high, ranging upward from 98.9% between the active *Mos* element *L8* and the inactive elements *peach*, *PrA* or *BordA*. The distributions of identity percentages within and between active and inactive elements are shown in Figure 3. The distributions are clearly distinct, and the proportion of the distributions that overlap is very low (about 2.5% of the total area). The mean values of the distributions are significantly different ($99.6 \pm 0.02\%$ *vs.* $99.1 \pm 0.02\%$, $P < 0.01$), suggesting that at least some of the differences between active and inactive elements are independent of species of origin.

One consistent difference between active and inactive elements is a base substitution T → A in position 1203, which causes a Phe → Leu amino acid change that may have a significant affect on the activity of the putative transposase (MARUYAMA, SCHOOR and HARTL 1991). The present results are consistent with this hypothesis. In particular, both inactive elements obtained from *D. simulans* have an A at position 1203, while all the active elements from both *D. simulans* and *D. mauritiana* have a T at this position. Four other nucleotide substitutions could also contribute to the loss of activity of the inactive elements in *D. simulans*: T → C at position 64, A → T at position 154, C → A at position 305, and A → G at position 662, all of which lead to amino acid replacements (Ser → Pro, Thr → Ser, Thr → Lys, and Asn → Ser, respectively).

TABLE 1

Nucleotide differences among active and inactive *mariner* elements from *D. mauritiana* and *D. simulans*

Species	Origin	Element	Nucleotide sequence	Activity
D. mauritiana	*white-peach* strain	*Mos1*	TTGGCGA TGCAGATAACCAGGGCT TT-	Active
D. mauritiana	*white-peach* strain	*Mos3*	TTGGCAA TGCAGATCACTAGGCCT -TA	Active
D. mauritiana	*white-peach* strain	*Mos5*	TTGGCGA TGCAGATAACCAGGGCT TT-	Active
D. mauritiana	*white-peach* strain	*peach*	CTGGCAT TGAAGATCGGCAGGGCA -AA	Inactive
D. simulans	*white* strain	*Mos2*	TTGGCAA TGCAAATCACCAAGGCT TT-	Active
D. simulans	Agadir (Morocco)	*Mos6a*	TGAGCAA TGCAAATCACCAAGGCT TT-	Active
D. simulans	Agadir (Morocco)	*Mos6b*	TGAGCAA TGCAAATCACCAAGGCT TT-	Active
D. simulans	Loua (Congo)	*L8*	TGGTCAA TGCAAGTCACCAATGCT TT-	Active
D. simulans	Loua (Congo)	*L14*	TGGTCAA TGCAAGCCACCGGGGCT TT-	Active
D. simulans	Madagascar	*MadB*	TGGTTAA TGCAAATCACCAAGGAT TT-	Active
D. simulans	Seychelles	*Sey2*	TGGGCGA TGCAAATCACCAAGGCT TT-	Active
D. simulans	Puerto-Rico	*Pr1*	TTAGCAA TGCTAATCACCAAGGCT TT-	Active
D. simulans	Puerto-Rico	*PrA*	CTGGCAT GGAAGATCGGCAGGGCA --A	Inactive
D. simulans	Bordeaux (France)	*BordA*	CTGGAGT TAAAGATCGCCAGGGCA -AA	Inactive
			* ** ** * *	
			6811111 22333456688889911 111	
			4712235 26067243602994602 222	
			27924 14525516203896020 455	
			93 834	

Sequences of *peach* and *Mos1* are from JACOBSON, MEDHORA and HARTL (1986) and MEDHORA, MARUYAMA and HARTL (1991). Numbers beneath the sequences are nucleotide positions in *mariner*, and the asterisks denote nonsynonymous changes in the putative coding region.

TABLE 2

Mean number of nucleotide differences between *mariner* elements in *D. simulans* and *D. mauritiana*

	Active elements		Inactive elements	
	D. mauritiana $n = 3$	*D. simulans* $n = 8$	*D. mauritiana* $n = 1$	*D. simulans* $n = 2$
Active elements				
D. mauritiana	2.7 ± 1.3	6.5 ± 0.3	9.0 ± 0.0	9.0 ± 0.0
D. simulans		4.1 ± 0.4	11.6 ± 0.6	11.6 ± 0.4
Inactive elements				
D. mauritiana				1.5 ± 0.5
D. simulans				3.0 ± 0.0

Sequence differences among the elements are given in Table 1.

Comparison among all known *mariner* sequences in the two species also suggests that positions 64, 154 and 305 may contribute to reduction in activity. On the other hand, the substitution at position 662 is less likely to be implicated in the reduction in activity, because two inactive elements from *D. mauritiana* (*Ma310* and *Ma311*) are identical to *Mos1* at this position (MARUYAMA, SCHOOR and HARTL 1991).

Phylogeny of mariner elements: Figure 4 presents a phylogeny inferred from all published *mariner* sequences along with those of the present work. Only *mariner* sequences in the three species belonging to the *simulans* complex were considered (*D. simulans*, *D. mauritiana* and *D. sechellia*). The identity matrix used for phylogenetic inference included 23 *mariner* sequences with 49 polymorphic sites. Using the program PAUP to obtain unrooted phylogenetic trees by maximum parsimony (SWOFFORD 1989), the length of the shortest tree was 51 with a consistency index of 0.843, and 48 trees were retained. The differences between the trees were inconsequential for present purposes and concerned only the grouping of the active elements to the right of node II.

The phylogenetic analysis indicates that all of the active *mariner* elements are grouped together. Two elements, *MB1* from *D. simulans* and *Ma341* from *D. mauritiana*, that show low activity when introduced into the genome of *D. melanogaster* are also close together (MARUYAMA, SCHOOR and HARTL 1991). All the other elements from *D. simulans* exhibit high activity as judged by giving at least 17% mosaic males in the progeny of crosses with GB1. The elements *Mos1* and *Ma351* from *D. mauritiana* have differing activity levels, but even the less active element *Ma351* has an activity much greater than that of *MB1* and *Ma341* (MARUYAMA, SCHOOR and HARTL 1991). The activity of the *mariner* elements from *D. sechellia* is uncertain, and conflicting results have been reported for the proportion of mosaic flies (1% *vs.* 25%) in interspecific crosses with *D. simulans* or *D. mauritiana* (COYNE 1989; CAPY *et al.* 1991).

Figure 4 also indicates that the active *mariner* elements from *D. simulans* are more closely related to each other than to those of *D. mauriatiana*. Moreover, the full-length *mariner* elements from *D. sechellia* are more closely related to those of *D. simulans* than to those of *D.mauritiana* (CAPY *et al.* 1991). Finally, the two inactive elements from *D. simulans* (*PrA* and *BordA*) are very similar to the inactive element *peach* in *D. mauritiana*.

Examination of the nucleotide substitutions along the branches of the tree in Figure 4 shows that the differences between nodes I and II (C → T at position 64, T → A at position 154, and A → C at position

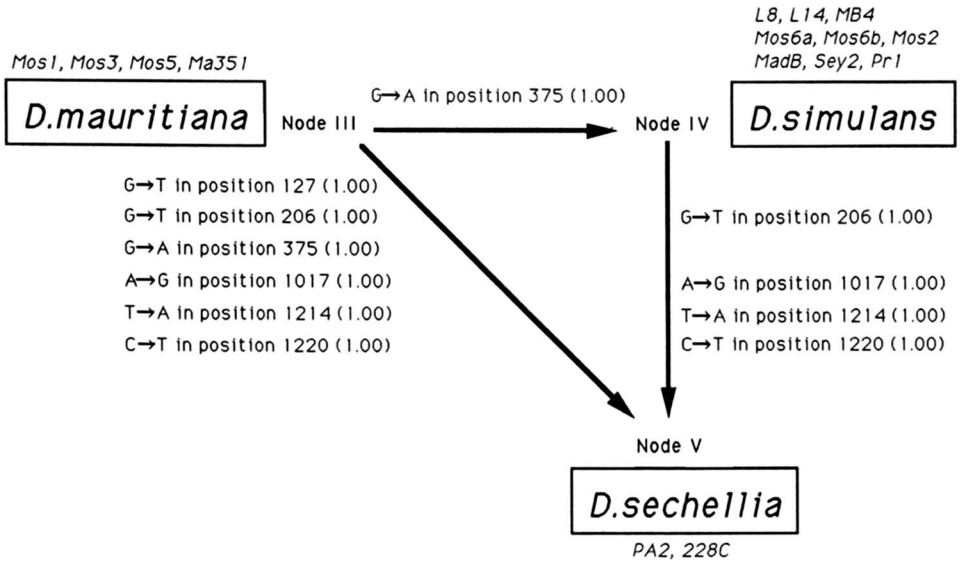

FIGURE 2.—Nucleotide substitutions that are consistent between the sibling species. The positions of the sites are numbered as in JACOBSON, MEDHORA and HARTL (1986). The values in parentheses are the consistency indexes, and the nodes III, IV and V correspond to the nodes in Figure 4.

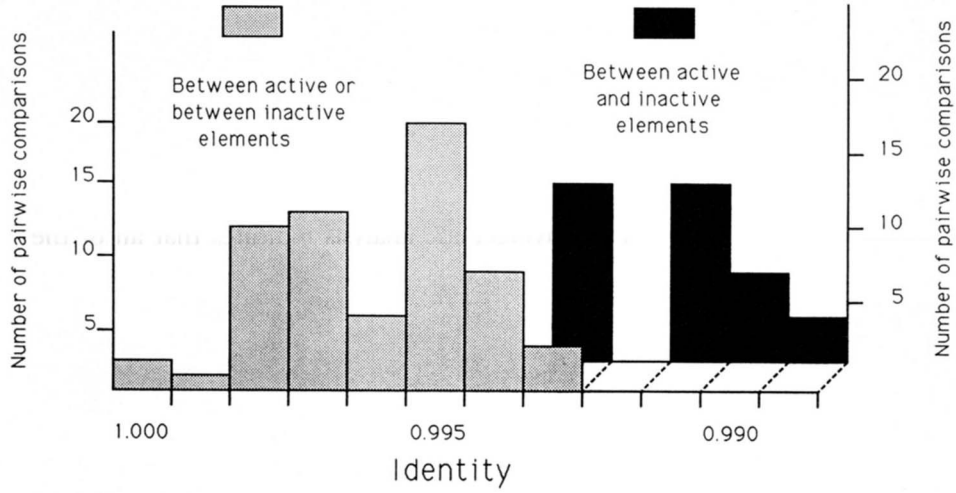

FIGURE 3.—Distribution of nucleotide identity scores between pairs of active or pairs of inactive *mariner* elements (gray), and between active elements compared with inactive elements (black). The overlap between the distributions is about 2.5% of the total area.

305) correspond to the transition between inactive and active elements, and the differences between nodes II and III (A → T at position 1203 and A → T at position 1253) correspond to the transition between elements with low activity and more highly active elements. Hence, the activity of these *mariner* elements can, in part at least, be predicted largely from sequence alone. The only discrepancy is the position of the element *Ma331* from *D. mauritiana*, which is grouped with *Mos1* and *Mos5* but which is inactive when introduced into the genome of *D. melanogaster* (MARUYAMA, SCHOOR and HARTL 1991). On the other hand, *Ma331* may simply represent an independently derived lineage of inactive elements; there are nine nucleotide differences between *Ma331* and *Mos1*, including three amino acid replacements and a single nucleotide deletion causing a frameshift five codons from the carboxyl end of the putative transposase.

DISCUSSION

Sequence similarity among active elements in *D. simulans*: Seven active *mariner* elements derived from five geographically diverse natural populations of *D. simulans* were sequenced and compared with an active element from a laboratory population of *D. simulans*. All active elements were very similar in nucleotide sequence, averaging 99.7% nucleotide identity. What small differences exist are not correlated with the level of *mariner* expression, which suggests that variation in *mariner* expression in natural populations (CAPY *et al.* 1990) is strongly influenced by position effects, dosage effects, and perhaps other factors beyond the *mariner* sequence itself.

Comparisons among the sibling species: *D. simulans*, *D. mauritiana* and *D. sechellia* are three sibling species so closely related that their polytene chromosomes are homosequential (LEMEUNIER and ASHBURNER 1984), and fertile interspecific hybrids can be obtained (COYNE and KREITMAN 1986; DAVID *et al.* 1974, 1976; LACHAISE *et al.* 1986, 1988). *D. simulans* is cosmopolitan with an area of origin mainly in East Africa (LACHAISE *et al.* 1986), while *D. mauritiana* and *D. sechellia* are endemic to Mauritius Island and the Seychelles Archipelago, respectively. *D. simulans* is

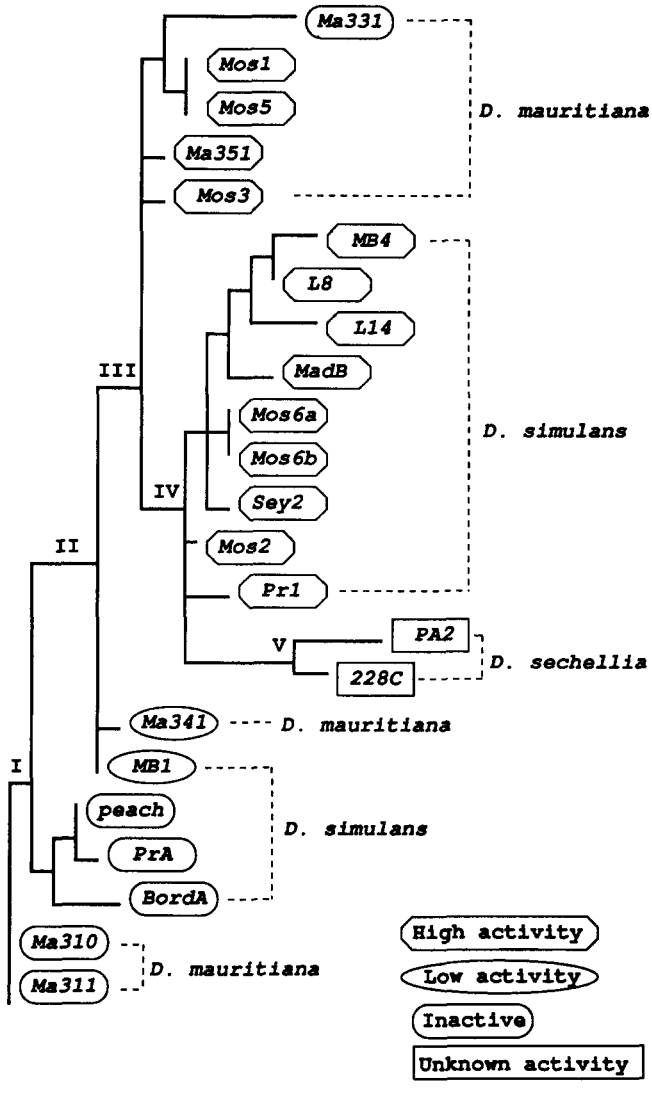

FIGURE 4.—Maximum parsimony tree of *mariner* sequences based on PAUP (SWOFFORD 1989), unrooted. The elements *Ma310*, *Ma311*, *Ma331*, *Ma351*, *Mos1* and *peach* are from *D. mauritiana* (JACOBSON, MEDHORA and HARTL 1986; MARUYAMA, SCHOOR and HARTL 1991; MEDHORA, MARUYAMA and HARTL 1991); *MB1* and *MB4* are from *D. simulans* (MARUYAMA, SCHOOR and HARTL 1991); and *PA2* and *228C* are from *D. sechellia* (CAPY *et al.* 1991). The length of the tree is 51 steps, and the consistency index is 0.843.

also present in the Seychelles but not on Mauritius Island (DAVID *et al.* 1989).

The most parsimonious phylogenetic tree of *mariner* sequences is obtained when the *D. mauritiana* and *D. sechellia* sequences are derived from *D. simulans*. A similar pattern is suggested by data on mitochondrial DNA (SOLIGNAC and MONNEROT 1986). The sequences of active *mariner* elements in *D. simulans* and *D. mauritiana* are also more closely related to each other than to those in *D. sechellia*, which is also in agreement with the mitochondrial DNA data (SOLIGNAC and MONNEROT 1986; SOLIGNAC, MONNEROT and MOUNOLOU 1986). On the other hand, the analysis of other characters suggests other patterns, and almost every possible relation between the three species is supported by some data (COYNE 1983; COYNE and KREITMAN 1986; CARIOU 1987; LACHAISE *et al.* 1988; CARIOU *et al.* 1990). Nevertheless, the bulk of the data support the view that both *D. mauritiana* and *D. sechellia* are derived from *D. simulans* and that the two latter species diverged at about the same time.

At present, *D. simulans* and *D. mauritiana* are generalist species, and the effective sizes of their populations are probably greater than that of *D. sechellia*, which is specialized to the fruit of *Morinda citrifolia*, which is highly toxic for *D. simulans* (R'KHA, CAPY and DAVID 1991). CARIOU *et al.* (1990) have suggested that the effective size of natural populations of *D. sechellia* may be about 10 times smaller than those of the sibling species, which is corroborated by the level of enzymatic and mtDNA polymorphisms (SOLIGNAC and MONNEROT 1986; CARIOU 1987; CARIOU *et al.* 1990). Therefore, the different ecological status and effective sizes of the three species may contribute to differences in the number of copies of *mariner* in their genomes.

Evolution of active *mariner* elements: We have shown previously that two copies of *mariner* can be detected in *D. sechellia* (CAPY *et al.* 1991). One copy is a potentially active full-length element, while the other contains three deletions including one that eliminates much of the 3′ end (CAPY *et al.* 1991). In the phylogeny in Figure 4, the position of the two full-length *D. sechellia* elements *PA2* and *228C* suggests that they are active, and this suggestion is supported directly by interspecific crosses between *D. sechellia* and strains of *D. simulans* and *D. mauritiana* containing the w^{pch} allele.

Our results demonstrate that active and inactive *mariner* elements in *D. simulans* are virtually identical to those in *D. mauritiana*, with nucleotide identities ranging from 99.3% to 99.9%. However, when the active elements are considered by themselves, three distinct clusters corresponding to the three species can be distinguished (Figure 4), implying the existence of some species specificity. This result strongly suggests that one or more closely related active elements were present in the ancestor of the three species and that the species-specific characteristics were acquired after their separation. This conclusion agrees with the hypothesis of MARUYAMA, SCHOOR and HARTL (1991), who proposed that the ancestral form of *mariner* among these species was an active element and that the inactive elements are derived. In this respect *mariner* is comparable to other transposable elements in *Drosophila*, such as the *P* element (reviewed in ENGELS 1989), since inactive *P* elements in *D. melanogaster* seem to have arisen after the invasion of the genome by active elements (KIDWELL 1986, 1989; BLACK *et al.* 1987; MISRA and RIO 1990).

Origin of inactive elements: Assuming that inactive

elements are derived from active elements, and that the rate of evolution of inactive elements should be greater than that of active elements, one would expect a greater divergence between inactive *mariner* elements than between active elements. However, the results show that the average nucleotide identity between inactive elements (99.8%) is of the same magnitude as the average nucleotide identity between active elements (99.7%).

One hypothesis to explain the similarity among inactive elements in *D. simulans* and *D. maurtinana* is that there are actually some selective constraints, perhaps because inactive *mariner* elements have regulatory effects of some sort. Several distinct types of regulatory effects of inactive *P* elements are well documented (SIMMONS and BUCHOLZ 1985; BLACK *et al.* 1987; MISRA and RIO 1990). A second hypothesis, not mutually exclusive, is that of recent introgression of one genome into the other. Such an introgression would have had to predate worldwide expansion of *D. simulans*, since the two inactive *D. simulans* elements come from geographically distant populations in Puerto Rico and Bordeaux. Introgression from *D. simulans* into *D. mauritiana* has been suggested by the analysis of mitochondrial DNA (SOLIGNAC and MONNEROT 1986). Three cytoplasmic races can be distinguished in *D. simulans* (SiI, SiII and SiIII), two in *D. mauritiana* (MaI and MaII), and the similarity between the restriction maps of SiIII and MaI strongly suggests introgression from *D. simulans* of Madagascar or Réunion into *D. mauritiana*. It has been suggested that the island of Mauritius was colonized at least two times by *D. simulans*, the first resulting in the formation of *D. mauritiana* and the second leading to introgression in which part of the *D. simulans* genome was absorbed (SOLIGNAC and MONNEROT 1986; AUBERT and SOLIGNAC 1990).

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