High Copy Number of Highly Similar *mariner*-Like Transposons in Planarian (Platyhelminthe): Evidence for a Trans-Phyla Horizontal Transfer

Jordi Garcia-Fernàndez, José Ramón Bayascas-Ramírez, Gemma Marfany, Ana Maria Muñoz-Mármol, Andreu Casali, Jaume Baguñà, and Emili Saló

Departament de Genètica, Facultat de Biologia, Universitat de Barcelona

Several DNA sequences similar to the *mariner* element were isolated and characterized in the platyhelminthe *Dugesia (Girardia) tigrina*. They were 1,288 bp long, flanked by two 32 bp-inverted repeats, and contained a single 339 amino acid open-reading frame (ORF) encoding the transposase. The number of copies of this element is approximately 8,000 per haploid genome, constituting a member of the middle-repetitive DNA of *Dugesia tigrina*. Sequence analysis of several elements showed a high percentage of conservation between the different copies. Most of them presented an intact ORF and the standard signals of actively expressed genes, which suggests that some of them are or have recently been functional transposons. The high degree of similarity shared with other *mariner* elements from some arthropods, together with the fact that this element is undetectable in other planarian species, strongly suggests a case of horizontal transfer between these two distant phyla.

Introduction

Mobile genetic elements (also called transposable elements or TEs) are widespread in all major phylogenetic groups (for a review, see Berg and Howe 1989; Kidwell 1992; McDonald 1993), and it is assumed that they represent a significant constituent of eukaryotic genomes. Questions about the origin and evolution of TEs themselves as well as their influence on the evolution of their hosts are still far from completely understood. The *mariner* transposable element is a small member of the Class II group of DNA elements (Plasterk 1993), which transpose directly from DNA to DNA. It represents one of the simplest forms of an autonomous DNA transposon as it consists of one gene without introns, flanked by two short inverted-repeat sequences (Hartl 1989). The encoded protein is presumably the transposase, which seems to be responsible for mediating transposition by excising, exchanging, and fusing DNAs in a coordinated manner.

Originally, the *mariner* transposon was described in *Drosophila mauritiana* (Jacobson et al. 1986) as the cause of somatic genetic instability of the mutant *peach* in the *white* locus. In contrast to the *Drosophila P* element, which is limited to transposing in germ line tissue (Rubin and Spradling 1982), it is functionally active in both germinal and somatic cell lines (Bryan et al. 1987). This element has also been found in organisms of the Arthropoda phylum other than *Drosophila* (Lidholm et al. 1991; Maruyama and Hartl 1991*a*; Robertson et al. 1992; Bigot et al. 1994). In a more exhaustive search for *mariner* elements in arthropods, performed by polymerase chain reaction (PCR), the distribution of *mariner* within this phylum has been shown to be widespread, albeit sporadic (Robertson 1993; Robertson and MacLeod 1993). Horizontal transfer or transmission of genetic material by means other than gametes has been put forward (Kidwell 1992, 1993) to account for both the presence of *mariner* elements in different representative members of arthropods, while being undetectable in many sibling species, and the high level of similarity shared by all the *mariner*-like sequences isolated, which is not consistent with the phylogenetic distances between the organisms bearing them.

Beyond arthropods, the presence of *mariner* elements has also been reported in nematodes (Sedensky et al. 1994). Our group detected a *mariner*-like sequence close to a planarian (phylum Plathyhelminthes) homeobox-containing gene and briefly reported its similarity to the arthropod elements (Garcia-Fernàndez et al. 1993*a*). Here, we report that the genome of the planarian *Dugesia tigrina* contains a high copy number of *mariner* elements. Genomic Southerns, PCR data, and sequence analysis of seven independently isolated planarian *mariners* indicates that they are highly conserved and that, probably, some of them are active transposons.

Key words: transposable element, planarian, *Dugesia tigrina*, *mariner*, *IS*630-*Tc*1 superfamily, horizontal transfer.

Address for correspondence and reprints: Dr. Jordi Garcia-Fernàndez, Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain. E-mail: jgarcia@porthos.bio.ub.es

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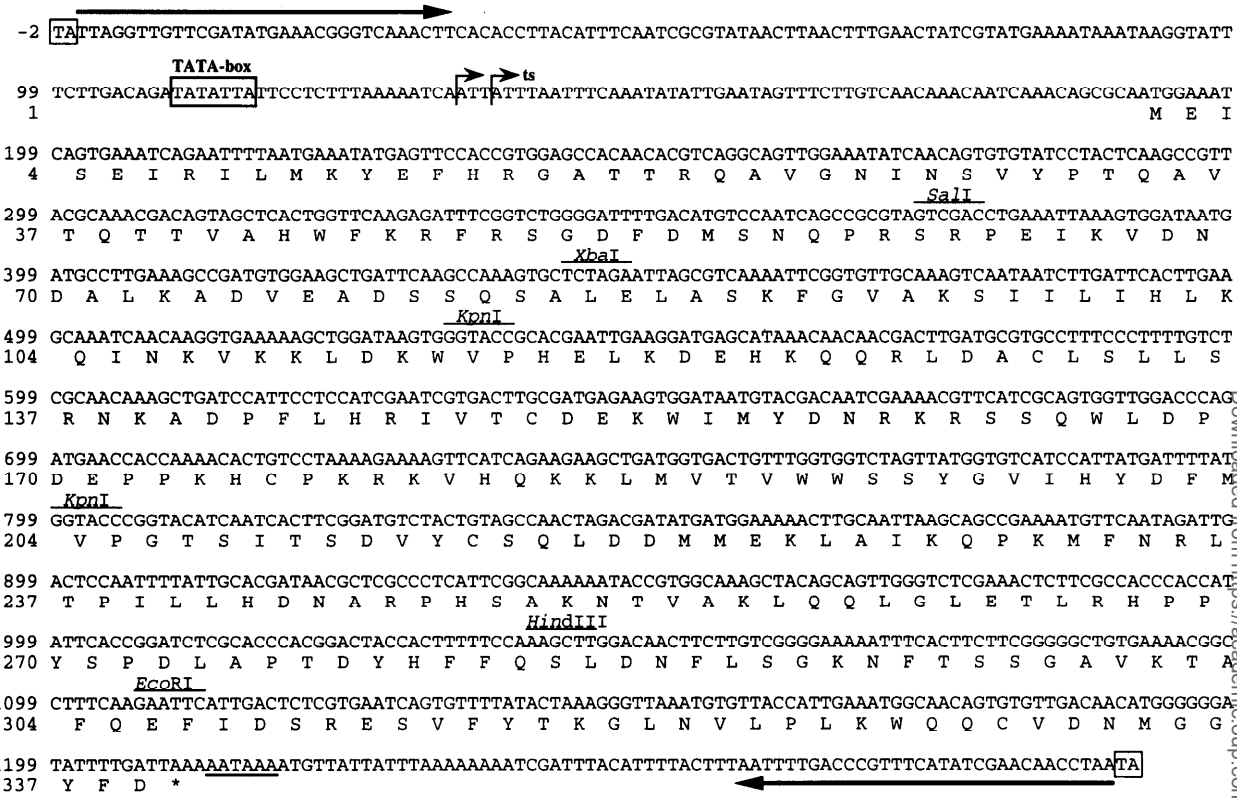

FIG. 1.—DNA sequence of the planarian *mariner*-1 element and the corresponding amino acid translation, encoding the transposase. The putative TATA box is boxed, and the polyadenylation signal is double underlined. The position of two putative transcription start points (ts) and the 32-bp terminal inverted repeats are indicated by arrows. The position of relevant restriction sites for the genomic Southern blot experiment (fig. 2) are also indicated, and the duplicated dinucleotide targets TA are boxed (the target sites are excluded from the nucleotide numeration).

Material and Methods

Species

Ten Platyhelminthes species belonging to different families were used in this study. *Dugesia tigrina* (1), *Dugesia gonocephala* (2), *Dugesia (Schmidtea) mediterranea* (3), *Dugesia polychroa* (4), *Polycelis felina* (5) *Geoplana* sp. (6), and *Monocelis lineata* (7) belong to the order Seriata; *Dalyellia viridis* (8) is from the order Neorhabdocoela; *Discocelis tigrina* (9) belongs to the order Polycladida; and *Macrostomum tuba* (10) is from the order Macrostomida. Finally, *Prostoma ehilardi* (11) is a representative of the phylum Nemertina. Animals were collected as follows: species 1, 2, 3, 5, 9, 10, and 11 were collected near Barcelona (Spain); species 4 comes from Germany; species 6 from Uruguay; species 7 from Italy; and species 8 from Madrid (Spain). Two-week-starved organisms were used in all experiments. Genomic DNAs were obtained as previously described (Garcia-Fernàndez et al. 1993*b*).

Library Screening

Approximately 100,000 recombinant clones from a genomic library of *Dugesia tigrina* were screened for sequences homologous to a partial *mariner* element found upstream of *Dth-2* gene (Garcia-Fernàndez et al. 1993*b*). The DNA probe extended from nt 444 to nt 1010 of figure 1 and contained only a coding sequence. Hybridization with the ^{32}P-random primed labeled probe was carried out on nitrocellulose filters (Schleicher & Schuell) at high-stringency conditions (Sambrook et al. 1989).

Restriction Analysis and Sequencing

Seven positive clones were randomly selected, characterized by restriction mapping, subcloned into pBluescript +SK (Stratagene) using standard techniques (Sambrook et al. 1989), and sequenced. Sequencing was carried out using T7 DNA polymerase (Pharmacia), with further subcloning and use of specific internal primers. Nucleotide sequence data were analyzed with the Sequence Analysis Software Package of the Genetics Computer Group of the University of Wisconsin (Deveraux et al. 1984).

Southern and Northern Blotting

Five μg of total genomic DNA of *Dugesia tigrina* was single-digested with either *Eco*RI, *Hin*dIII, *Bam*HI,

*Pst*I, *Xho*I, *Sma*I, *Pvu*II or *Kpn*I, and double-digested with the following enzyme combinations: *Xba*I/*Hin*dIII, *Sal*I/*Eco*RI, and *Xba*I/*Eco*RI. The same quantity of DNA from the planarian *D. mediterranea* was digested with either *Eco*RI or *Bam*HI. DNAs were separated in a 0.8% agarose gel and transferred to nylon membranes (Amersham) by capillary blotting. Total cellular RNA was isolated from intact and regenerating planarians, by the guanidinium thiocyanate method (Chirgwin et al. 1979). Hybridization was carried out at high-stringency conditions as previously described (Garcia-Fernàndez et al. 1991), with the same probe used in the library screening.

PCR Amplification of Several Plathyhelminthes Genomic DNA

PCR was performed on 100 ng of genomic DNA from each tested species. The reaction (50 µl) was made in Promega buffer including 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.4 mM of primer (5′ TTAGGTTGTT-CGATATGAAACG 3′, positions 1–22 of the inverted repeat) and 1.5 units of Taq polymerase. PCRs were carried out in a Perkin-Elmer programmable thermal controller for 30 cycles. Cycling conditions were 94°C for 1 min, 58°C for 1.5 min, and 72°C for 1 min. After the PCR, a fraction of the reaction was run in a 1% agarose gel, transferred to nylon membranes, and hybridized with the same probe and in the same conditions used in the library screening.

Results

Detection of a *mariner* Element

While analyzing the genomic structure of the planarian homeobox gene *Dth-2* (Garcia-Fernàndez et al. 1993*b*), the restriction maps of different lambda clones did not match completely outside the gene region. In the clone λS5 we found, 1 kb upstream of the transcription start of *Dth-2* and in the opposite orientation to this gene, an unusually long open-reading frame (ORF) of 263 amino acids. A computer search indicated a high level of similarity with the transposase coded by the *Drosophila mauritiana mariner* element (Jacobson et al. 1986; Maruyama and Hartl 1991*b*). Then, we hybridized the phages encompassing the same region with a probe derived from this ORF. Only some subsets of the phages showed a hybridization signal, indicating the polymorphic presence of this element in that genomic region.

Copy Number and Genomic Distribution of *mariners* in *Dugesia tigrina*

In order to isolate a full-length *mariner* element, we screened a genomic library of *D. tigrina* at high-stringency conditions, with a probe containing the open-reading frame of the *mariner* element. Approximately 7,000 positives were found in the screening of 100,000 independent lambda plaques, and nine randomly selected positives were further analyzed (λM1–9). Their restriction map showed no similarities, indicating that they corresponded to different genomic regions. Assuming that *mariner* copies are dispersed in the genome and taking into account the haploid size of the genome of *D. tigrina* ($1{,}4 \times 10^9$ bp, E.S. and J.B., unpublished data), the number of positives obtained in the screening of a genomic library with the *mariner* probe indicated that *D. tigrina* contained approximately 8,000 copies per haploid genome of *mariner*-like sequences, approximately 0.7% of its genome.

One of the clones (λM2) was further characterized, and the complete *mariner* element was sequenced. Figure 1 shows the sequence and protein translation of this element, named planarian *mariner*-1.

Based on the restriction map, we designed a genomic Southern blot experiment, in order to investigate the size distribution and the sequence conservation of *mariners* in the *D. tigrina* genome. Total genomic DNA was digested with restriction enzymes that did not have recognition sites in planarian *mariner*-1 (*Sma*I, *Xho*I, *Pst*I, *Bam*HI, and *Pvu*II); enzymes that had one restriction site (*Eco*RI and *Hin*dIII); one enzyme that presented two internal sites (*Kpn*I); and combinations of two enzymes that had one restriction site each within *mariner* (*Xba*I plus *Hin*dIII, *Xba*I plus *Eco*RI, and *Sal*I plus *Eco*RI). The probe used (from nt 44 to nt 1010, containing only coding region) revealed a multiple-band pattern, typical of repetitive DNA dispersed in the genome, in those digestions with enzymes that did not present any internal site (fig. 2). Most of the hybridization corresponded to high molecular weight bands: this could be due to the enzymes used, which are rare-cutter in the A/T rich genome of planarian (Garcia-Fernàndez et al. 1991). However, when digesting with either *Eco*RI or *Hin*dIII, whose target sites were not so rare in the *D. tigrina* genome, the hybridization signal presented a well-defined inferior limit (fig. 2). This inferior limit precisely matched the distance from the internal restriction sites to the 5′ end of *mariner*-1 (1,100 bp for *Eco*RI and 1,040 bp for *Hin*dIII). The smear along these lines strongly suggested a random distribution of *mariner* copies rather than distribution in tandem repeats. Even in short exposure times, no discrete bands were detected (data not shown). Digestions with enzyme combinations that presented internal sites gave an interesting result: a strong, discrete

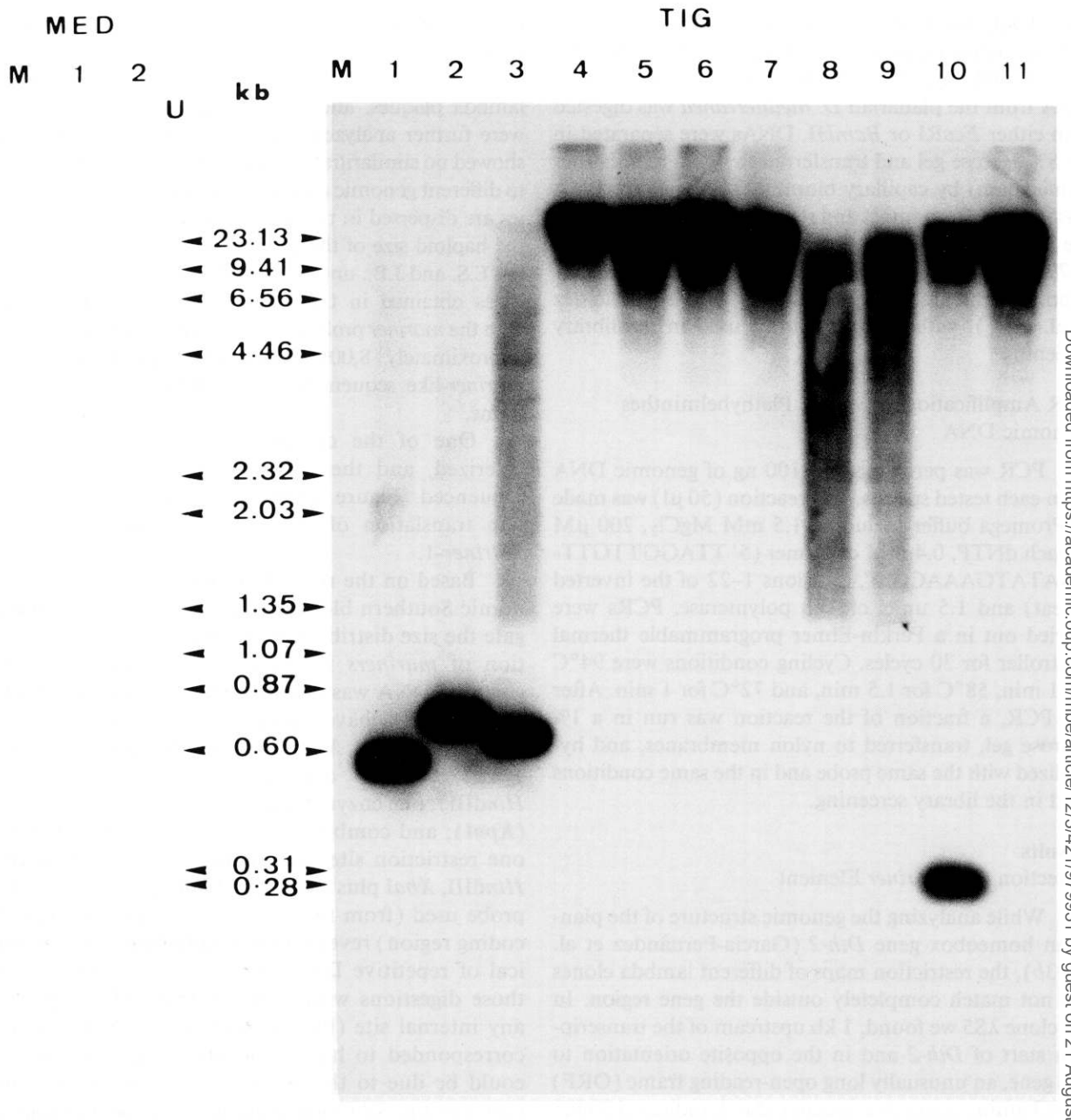

FIG. 2.—Genomic Southern blots of *Dugesia (S) mediterranea* (MED) and *Dugesia (G) tigrina* (TIG). Approximately 5 μg of genomic DNA was digested by the corresponding enzyme(s), electrophoresed on a 0.8% agarose/TBE gel, transferred to a nylon membrane (Amersham), and probed at high-stringency conditions with a ^{32}P-labeled *mariner*-1 (from nucleotides 440–1010; fig. 1). *M*, molecular weight marker. *Dugesia mediterranea* DNA was digested with *Bam*HI (1) or *Eco*RI (2), and *D. tigrina* DNA was digested with *Xba*I/*Hin*dIII (1); *Sal*I/*Eco*RI (2); *Xba*I/*Eco*RI (3); *Sma*I (4); *Xho*I (5); *Pst*I (6); *Bam*HI (7); *Eco*RI (8); *Hin*dIII (9); *Kpn*I (10); and *Pvu*II (11).

band of the expected sizes for *mariner*-1 were detected (547 bp for *Xba*I/*Hin*dIII, 663 bp for *Xba*I/*Eco*RI, 733 bp for *Sal*I/*Eco*RI, and 268 bp for *Kpn*I; fig. 2). It is noticeable that in the latter, a light smear is evident, due to the fact that the probe used hybridized with fragments of *mariner* not included in between the two *Kpn*I sites.

High Sequence Similarity in *Dugesia tigrina mariners*

We sequenced seven different *mariner* elements of *D. tigrina* (named *mariner*-1,-2,-3,-5,-7,-8 and -9) plus the partial isolated clone near *Dth-2* (*mariner*-0, including nt 1–1,010). All these copies were extremely similar at the nucleotide level and flanked by two almost perfect inverted repeats of 32 bp. Five of them contained a single

ORF coding for 339 amino acids, and 5′ and 3′ untranslated regions of 158 bp and 45 bp, respectively (excluding the inverted repeats). *Mariner*-2 presented a nucleotide deletion at position 1197 that enlarges the ORF through the genomic region, and *mariner*-7 shows another single nucleotide deletion at position 455 that produces a frameshift and thus a truncated transposase (stop codon at position 486–488). Figure 3 depicts the differences among the planarian *mariners* sequenced. On the average, all elements were more than 99% similar at the nucleotide level, no differences being observed in the inverted repeats, while a single substitution was observed in the 5′ and 3′ untranslated regions in *mariner*-9 and *mariner*-3, respectively (fig. 3). Most of the substitutions were scattered in the coding region.

In order to infer if there was any evolutionary trend of the *mariner* sequences, we analyzed the number and distribution of the nucleotide substitutions in the coding region. As this number is very low between every two single sequences, we have considered all of them as a group. According to *mariner*-1 sequence, there were 26.5% synonymous and 73.5% nonsynonymous positions. We found a total of 7 synonymous versus 20 nonsynonymous substitutions (including 1-bp deletions and a nucleotide substitution in the stop codon as single nonsynonymous events). Hence, these sequences appeared to be evolving neutrally as the nucleotide substitutions distributed randomly through synonymous and nonsynonymous positions ($\chi^2 = 0.007$; $P > 0.95$).

Although there is no definite proof that these *mariner* copies are functional, several features of their sequence suggest that, at least some of them, are or have been recently transcribed (see fig. 1). First, in most elements analyzed, the ORF is continuous, with neither stop codons nor deletions nor frameshifts, which have usually been observed in *mariners* of other organisms (Lidholm et al. 1991; Maruyama and Hartl 1991*b*; Robertson 1993; Robertson and MacLeod 1993; Bigot et al. 1994; Sedensky et al. 1994). Second, many of the characteristic signals of transcribed genes are found in planarian *mariners*. In all of them, the 5′ untranslated region contains a possible TATA box that agrees with the *consensus* (Breathnach and Chambon 1981), and a sequence very similar to that of other planarian transcription start sites (Garcia-Fernàndez et al. 1993*b*) is found at an appropriate distance from the TATA box. Furthermore, the region surrounding the codon start ATG is in agreement with the requirements of the leader sequences and translation start points (Hultmark et al. 1986). With respect to the 3′ untranslated region, the stop codon is followed by a canonical polyadenylation signal. Finally, the codon usage analysis of the presumptive ORF shows a clear bias toward the use of A or T in silent positions (60% of silent third-codon positions were A or T), a percentage very similar to other functional planarian (Garcia-Fernàndez et al. 1993*b*) and other lower invertebrate genes (Fisher and Bode 1989).

The terminal inverted repeats were 32 bp in length, with only one mismatch between the left and right repeats. The insertion of all planarian *mariners* analyzed seems to have produced a duplication of the dinucleotide TA, characteristic of the *IS*630-*Tc*1-*mariner* insertions

	188	333	353	371	406	455	480	619	632	642	713	717	868	878	914	925	960	995	1026	1033	1057	1067	1099	1105	1156	1166	1197	1251
mar-0	G	T	C	G	A	T	C	T	G	A	C	T	C	C	C	A	T	C										
		Phe	Leu	Gly	Leu	Ser	Thr	Phe	Val	Asp	His	Phe	Ala	Pro	His	Ala	Leu	Pro										
mar-1	G	T	A	A	G	T	T	C	G	A	C	G	A	C	C	T	T	C	A	T	G	A	C	A	G	T	G	T
		Phe	Met	Ser	Leu	Ser	Ile	Phe	Val	Asp	His	Cys	Ala	Pro	His	Ala	Leu	Pro	Tyr	Phe	Leu	Asn	Ala	Gln	Val	Leu	Gly	
mar-2	G	T	C	G	G	T	C	T	A	A	C	G	A	C	C	T	T	A	G	T	G	A	T	A	A	T	-	T
		Phe	Leu	Gly	Leu	Ser	Thr	Phe	Met	Asp	His	Cys	Ala	Pro	His	Ala	Leu	Thr	Cys	Phe	Leu	Asn	Ala	Gln	Val	Leu		
mar-3	G	T	C	G	G	T	C	T	G	A	C	G	A	C	C	T	T	A	G	T	G	A	T	A	G	T	G	C
		Phe	Leu	Gly	Leu	Ser	Thr	Phe	Val	Asp	His	Cys	Ala	Pro	His	Ala	Leu	Thr	Cys	Phe	Leu	Asn	Ala	Gln	Val	Leu	Gly	
mar-5	G	T	C	G	G	T	C	T	G	G	C	G	A	C	C	T	T	C	A	T	C	T	C	A	G	T	G	T
		Phe	Leu	Gly	Leu	Ser	Thr	Phe	Val	Gly	His	Cys	Ala	Pro	His	Ala	Leu	Pro	Tyr	Phe	Phe	Tyr	Ala	Gln	Val	Leu	Gly	
mar-7	G	T	C	G	G	-	C	T	G	A	T	G	C	C	C	T	T	C	A	T	G	A	C	T	G	C	G	T
		Phe	Leu	Gly	Leu		Thr	Phe	Val	Asp	Tyr	Cys	Ala	Pro	His	Ala	Leu	Pro	Tyr	Phe	Leu	Asn	Ala	His	Val	Ser	Gly	
mar-8	G	T	C	G	G	T	C	T	G	A	C	G	A	G	T	T	G	C	A	C	G	A	C	A	G	T	G	T
		Phe	Leu	Gly	Leu	Ser	Thr	Phe	Val	Asp	His	Cys	Ala	Ala	Tyr	Ala	Arg	Pro	Tyr	Phe	Leu	Asn	Ala	Gln	Val	Leu	Gly	
mar-9	A	A	C	G	G	T	C	C	G	A	C	G	A	C	C	T	T	C	A	T	G	A	C	A	G	T	G	T
		Tyr	Leu	Gly	Leu	Ser	Thr	Phe	Val	Asp	His	Cys	Ala	Pro	His	Ala	Leu	Pro	Tyr	Phe	Leu	Asn	Ala	Gln	Val	Leu	Gly	

FIG. 3.—Alignment of eight planarian *mariner* sequences. Only sites differing among the sequences of *mariner*-1 are shown. The numbers at the top indicate the positions of the variable sites in the *mariner* sequence. Deletions are indicated by dashes. Numeration is according to fig. 1. *Mariner*-0 sequence is 1,010 nucleotides long, while *mariner*-7 has a nucleotide deletion at position 455 that produces a stop codon at positions 486–488 and is here considered as a frameshift. In *mariner*-2 the terminal stop codon is skipped by a deletion of nt 1197. Planarian *mariner* sequences have been deposited in the GenBank/EMBL databases under the accession numbers X71979, X80776, X80777, and X80893–80897.

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                 1                                 34
Pmar1            TTAGGTTGTTCGATATGAAAC--GGGTCAAACTT
Hcmle            TTAGGTCCTTACATATGAAATTAGCGT--TTTGT
Mos-1            CCAGGTGTACAAGTAGGGAATGTCGGT--T----
Derec            TTAGTTTGGCAAATATCTCCCTTCCGCCTTTTTG
Cplor            TTAGGTTGGCTGATAAGTCCC--CGGTC-----T
CeZK370          TTAGGTTGGTCAAAAAGTCTTTG----CAAAATT
consensus        TTAGGT       ATA G         GT
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FIG. 4.—Alignment of the left inverted repeat of *mariner*-like sequences from *Dugesia (G) tigrina* (Pmar-1), *Hyalophora cecropia* moth (Hcmle; Lidholm et al. 1991), *Drosophila mauritania* Mos-1 (Medhora et al. 1991), *Drosophila erecta* (Derec, GenBank accession number U08094), lacewing *Crisoperla plorabunda* (Cplor, Robertson et al. 1992), and *Caenorhabditis elegans* ZK370 (GenBank accession numbers M98552 and L18807). A consensus sequence between all these elements is also shown.

(Jacobson et al. 1986; Doak et al. 1994). Although the inverted-repeat length varies in different families of *mariner* elements from 27 bp to 38 bp, a central core of similarity is observed in the elements isolated up to now (fig. 4), even when comparing distantly related members.

The putative transposase coded by planarian *mariners* is 339 amino acids long. There are no reports on the structure of *mariner* transposases, although a clear similarity to *Tc*-1 transposase has been pointed out (Henikoff and Henikoff 1992; Doak et al. 1994; Robertson 1995), defining some conserved amino acid boxes. Several features of the sequence can be noted: it is very rich in charged amino acids (24%, 11% basic residues and 13% acidic residues), and some usually rare residues are relatively abundant in *D. tigrina mariners* (7 residues of Trp, 10 of Tyr, and 16 of Phe). The conserved amino acids observed in the superfamily *Tc*1-*mariner* may be key residues for the function of the transposase, and they are mostly conserved in planarian *mariners* (fig. 5). It is worth mentioning the conservation of the D(34)D motif, which is similar to the known relevant D(35)E motif of bacterial transposases and retrovirus-retrotransposon integrases (Doak et al. 1994), and the presence of the Phe residue 356, which has been shown to be critical for


```
                       1
Planarian mariner-1    ......MEISEIRILMKYEFHRGATTRQAVGNINSVYPTQAVTQTTVAHWFKRFRSGDFDMSNQPRS.RPEIKVDNDALKADVEADS...SQSALELASK
H.cecropia MLE         ......MANMKYRYIYEYEFYRGTSAAETARRINNVYGAGAAKESKVRFWFQRFRSGIFDLQNQPR.GRPEIKVENEEIKA#V*ADP...SQSTSEIAAG
D.mauritiana Mos-1     .MSSFVPNKEQTRTVLIFCFHLKKTAAESHRMLVEAFGEQVPTVKTCERWFQRFKSGDFDVDDKEH.GKPPKRYEDAELQALLDEDD...AQTQKQLAEQ
D.erecta               ....MEFTNAEIRAILKFSFVKGKSARETFREINGVLGDGTLSLRTAEEWFRRFRAGENDTMDKPAGGRPVTTNTDQIMEYI...EL.DRHVASRDIAQE
C.plorabunda cons      ......MEKKEFRVLIKYCFLKGKNTVEAKTWLDNEFPDSAPGKSTIIDWYAKFKRGEMSTEDGERSGRPKEVVTDENIKKIHKMILNDRKMKLIEIAEA
C.elegans cons         .MTIIKLERRDVRLLLLYEFRLGHSAMEAERNICGAMGEGALSYNTAKSWFQKFKNGDFSLEEIERSGRPVELNEEDLVKLVEEEPR....LSLREMEEK
C.elegans ZK370        MTENLLAERHALRGVFLYEFPQSCNCNEARRNMCVVLGKSSVTYNTMKFWFEKFTKKNYDLDDKPR*DRSRLDIDEDISRALEDDSR....ATSRELSAT
consensus                     E    R    YEF  G     EA R       G       T    WF   F   G FD      R GRP                        E A
                                   *         *                                                  **

                       101
Planarian mariner-1    FGVAKSIILIHLKQINKVKKL.DKWVPHELKDEHKQQRLDACLSLLSRNKADP..FLHRIVTCDEKWIMYDNRKRSSQ...WLDPDEPPKHCPKRKVHQK
H.cecropia MLE         FGVSDKTVLIYLKQIGKVKKL.E*WVPHELSESNLQTRVDCCVTLLNRHNNEG..ILNRIITCDEKWILYDNRKRSSQ...WLNPGDPAKSCPKRKLTQK
D.mauritiana Mos-1     LEVSQQAVSNRLREMGKIQKV.GRWVPHELNERQMERRKNTCEILLSRYKRKS..FLHRIVTGDEKWIFFVSPKRKKS...YVDPGQPATSTARPNRFGK
D.erecta               MGVSHQTILNHLQKAGYK.KKLDVWVPHDLTQKNLLDRINACDMLLKRNELDP..FLKRMVTGDEKWITYDNIKRKRS...WSKAGESSQTVAKPGLTAR
C.plorabunda cons      LKISKERVGHIIHQYLDMRKLCAKWVPRELTFDQKQQRVDDSERCLQLLTRNTPEFLRRYVTMDETWLHHYTPESKRQSAEWTATGEPSPKRGKTQKSAG
C.elegans cons         LECCHSTIARHLGRLGFTSKL.GTWVPHELSASQKLTRVNVCTQLLTFRRKFD..WLNNLVTGDEKWVLYVNHSRKRQ...WLPIGEKGIPTPKPDLHPK
C.elegans ZK370        LKHPQRIIINHLQKTGKIEKF.GQLVPHKLSDSQKNCFV#LSLSLLTRKRTTD..WVKDIITGNDKWVLYVSHTRKKE...WVPVEETATPDLK*ELHGK
C.cerasi cons                                      NDAQKERRLEACLSLLSRNKTEP..FLNRIVTCDEKWIMYDNRKRSSQ...WLDRAEPPKHCSKRGIHQK
consensus              L          L    GK  KL   WVPHEL   QK  R D C  LL R         L RIVT DEKW  Y N  R  Q   W   GE     K    K
                                  *                     *  *                      **

                       201
Planarian mariner-1    KLMVTVWWSSYGVIHYDFMVPGTSITSDVYCSQLDD.MMEKLAIKQPKMFNR.LTPILLHDNARPHSAKNTVAKLQQLGLETLRHPPYSPDLAPTDYHFF
H.cecropia MLE         KLLVSVWWTSAGVVHYSFLKSGQTITADIYCQQLQT.MKEELAAKQPRLVNR.SRPLLLHDNARPHTAQQTTTKLDELQLECLRHPPYSPDLAPIDYHFF
D.mauritiana Mos-1     KTMLCVWWDQSGVIYYELLKPGETVNTARYQQQLIN.LNRALQRKRPEYQKRQHRVIFLHDNAPSHTARAVRDTLETLNWEVLPHAAYSPDLAPSDYHLF
D.erecta               KVLLCVWWDWKGIIHNELLPYGQTLNSTTYCQQLDR#LKQAIDQKRPELANRK.GVVFHQDNARPHTSLMTRQKLRELGWEVLSHPPYSPDIAPSDYHLF
C.plorabunda cons      KVMASVFWDAHGIIFIDYLEKGKTINSDYYMALLER.LKVEIAAKRPHMKK..KKVLFHQDNAPCHKSLRTMAKIHELGFELLPHPPYSPDLAPSDFFLF
C.elegans cons         KIMICVWWGVQGPVHWELLPTNKTITADYYCAQLDR.VAEKTNGKYEK.......LYFLHDNARPHVAKKTFQKLQDLGWTVLPHPPYSPDLAPTDYHLF
C.elegans ZK370        KVLLSIGRDSKGVISRELLPDFATINAGLYCI*LEK.VVHAHRLHRPRGSK....LLLLHDNARPHTTFKTRQKLQTVGIQILSYPSYSPGLAPTDYHLF
C.cerasi cons          KLMVTVWWSASSVIHHSFMEPGTSITAVVYCKQLDD.MMRNLAVKQVRLVNR.SAPILLHDNARPHTAQMTVAKLQQLELETFPHPP
consensus              K M  VWW    G I     L  G TI   D YC QL          K P           LHDNARPH A   T  KL  LG E L HPPYSPDLAP DYHLF
                             **                 *   *                      **   *        *        *  *  **** *     *

                       300
Planarian mariner-1    QSLDNFLSGKNFTSSGAVKTAFQEFIDSRESVFYTKGLNVLPLKWQQCVDNMGGYFD*
H.cecropia MLE         RNLDNFLHGKKFNSYSVVQTAFKEFIDRRPHAFFNKGINELPVRWQKCINNNGAYFD#
D.mauritiana Mos-1     ASMGHALAEQRFDSYESVKKWLDEWFAAKDDEFYWRGIHKLPERWEKCVASDGKYFE*
D.erecta               LSMANALGGVKLNSKEACEKWLSEFFANKEGGFYEGGIMKLPSRWKQIIEQNGAYLN*
C.plorabunda cons      SDLKRMLAGKKFGCNEEVIAETEAYFEAKPKEYYQNGIKKLEGRYNRCIALEGNYVE*
C.elegans cons         LSLSDYMRDKQFDDEEHLKTELSTFFSSRSPDFFSRGIMMLPSKWQQVVDTNGEYLCE*
C.elegans ZK370        RSLQNHLAGQKFHDRKVVETGLDDFFAS*SQEF*AEGTVQLPLCWQEVIGINGKYITH*
consensus                L   L GK F     V       FF       F   GI  LP  WQ C      G Y  *
                         *                                  *               *
```

FIG. 5.—Alignment of *Dugesia (G) tigrina mariner*-1 transposase with available sequences of complete or reconstructed *mariner* transposases from other organisms. Conserved residues with the *mariner* consensus sequence (Robertson 1995) are highlighted in bold. Residues also shared by the *Tc*1-*mariner* superfamily (Doak et al. 1994; Robertson 1995) are indicated below by a bold asterisk. The glutamic acid residues of the D34D motif are indicated by arrowheads. An asterisk (*) indicates a stop codon in the aligned reading frame, and the pound symbol (#) indicates a frameshift to maintain an aligned reading frame. MLE sequence references are as follows: *Hyalophora cecropia* moth (Lidholm et al. 1991), *Drosophila mauritania* Mos1 (Medhora et al. 1991), lacewing *Crisoperla plorabunda* (Robertson et al. 1992), the partial sequence of the ant *Chromatogaster cerasi* (Robertson and MacLeod 1993), *Drosophila erecta* (GenBank accession number U08094), *Caenorhabditis elegans* cons (first reported by Sedensky et al. 1994; the consensus sequence of three elements according to Robertson (1995) is used here, GenBank accession numbers X77804 and U10438), and *C. elegans* ZK370 (GenBank accession numbers M98552 and L18807).

the *mariner* activity in *Drosophila* (Maruyama and Hartl 1991*a*).

Alignment of the transposase of planarian *mariner*-1 with the transposases or reconstructed ORFs from elements of *Drosophila,* moth, lacewing, and nematode shows a similarity of 36.2%, 56.5%, 31%, and 38%, respectively (fig. 5). In addition, comparison with the subfamilies of *mariner* transposases recently described, based on a fragment coding for 148 amino acids (amino acids 131–286 in the numeration of planarian *mariner*-1) amplified by PCR in a wide range of arthropods, mainly insects (Robertson and MacLeod 1993), clearly shows that the planarian *mariners* cluster with the *cecropia* subfamily, with a maximum of 73% similarity in this region with an element from the ant *Chromatogaster cerasi* (Robertson and MacLeod 1993).

Expression of *mariner* Elements during Planarian Regeneration

As planarian *mariner* elements presented most of the sequence motifs required for functional active genes, we studied their expression by Northern blot (fig. 6). *Mariner* expression is ubiquitous, showing a smear along the lane, with some stronger bands of high molecular weight.

The observed smeary pattern of expression suggests a hitchhiking effect, due to *mariners* being cotranscribed together with some genes in which they are inserted nearby, more than a specific expression of the elements. Some of the detected bands could be merely the result of "white-out" by saturation of the membrane by rRNA; this may well be the case of the signals located just above and below of the 18S RNA. However, since in planarians and other lower invertebrates the 28S RNA is cleaved in the process of the obtention of RNA (Farlenga and Gamble 1987), no saturation effect could be claimed for large RNA sizes. Thus, the highest stronger bands would represent highly expressed genes or a local enrichment in transcripts of a particular range of sizes.

Nevertheless, no transcripts of the expected size for an autonomous expression of *mariner* (about 1,200 nt) are detected. This is somewhat surprising, as most of the analyzed elements presented signals of functional genes. A "white-out" effect is difficult to put forward in this case, as the 18S RNA in planarians has a size of approximately 2,000 nt (Riutort et al. 1992). In addition, the actin probe detected without problems a band of a similar size (1,200 nt) to that expected for *mariner.* On the other hand, if only a small fraction of the *Dugesia tigrina mariner* elements are fully autonomous and functional, the low level of transcription would be difficult to detect in a Northern blot or may be even masked by the smear due to hitchhiked expression.

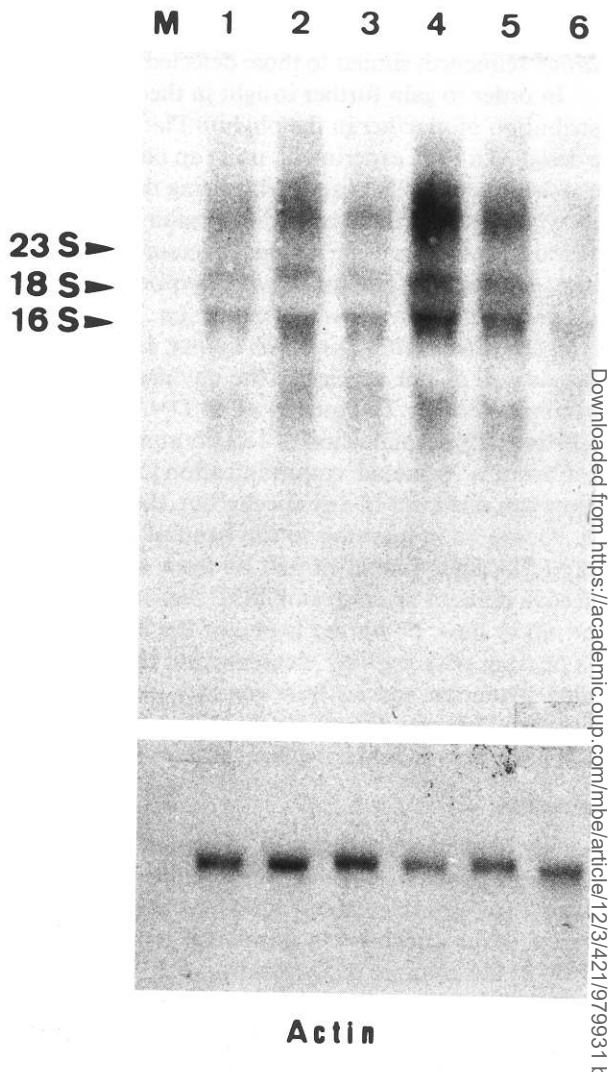

FIG. 6.—Northern blot analysis of total RNA, 10 μg per lane, from intact planarians (lane 1) and animals in different stages of regeneration (lane 2, 1h; lane 3, 12 h; lane 4, 3 d; lane 5, 5d; lane 6, 7 d; where h is hour(s) and d is day(s) of regeneration). The blot was hybridized with the same probe as in fig. 2. The blot was stripped and rehybridized with a 5C actin probe (Fyrberg et al. 1981) as a control of the RNA levels. *Escherichia coli* ribosomal RNAs were used as size markers.

Finally, the observed temporal quantitative changes during regeneration may reflect a general increase in transcription activity during planarian regeneration (Collet 1990).

mariner Distribution in Plathyhelminthe Species

The presence of *mariner*-like sequences in the genome of a close species *Dugesia mediterranea* was tested by Southern blot hybridization (fig. 2). Interestingly, no hybridization signal was observed, even with longer exposures or less stringent hybridization conditions (data

not shown), which suggests that this species did not bear *mariner* sequences similar to those detected in *D. tigrina.*

In order to gain further insight in the phylogenetic distribution of *mariner* in the phylum Platyhelminthes, we designed a PCR experiment, using an oligonucleotide from the inverted repeat and hybridizing the PCR products with a probe from the coding region. Several Platyhelminthe species were tested: *Dugesia tigrina, Dugesia gonocephala, Dugesia mediterranea, Dugesia polychroa, Polycelis felina, Geoplana s.p, Monocelis lineata, Dalyellia viridis, Discocelis tigrina, Macrostomum tuba,* as well as one species of the phylum Nemertina: *Prostoma ehilardi.* The quality of all DNAs was previously tested by amplification of 18S ribosomal sequences (S. Carranza, personal communication). No amplification was observed in any species but *Dugesia tigrina* (fig. 7), where in addition to the band of the expected size (1,288 bp), a band of 650 bp from a presumably defective element was also amplified. This result suggests that, up to now, *D. tigrina* is one of the few species in this phylum with *mariner* elements and that if any analyzed planarian species bore *mariner*-like sequences, they should be quite different to those found in *D. tigrina,* or, at least, they would have divergent inverted repeats.

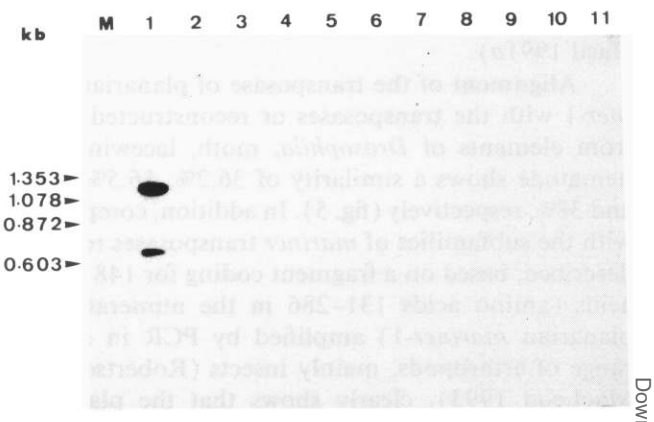

FIG. 7.—Phylogenetic distribution of *mariner*-like sequences in Platyhelminthes. A Southern blot from amplification PCR products of genomic DNAs using inverted repeat-derived primer was hybridized with the same probe as in fig. 2. Ten different species of Platyhelminthes and one Nemertina were tested. Lane 1, *Dugesia tigrina;* 2, *Dugesia gonocephala;* 3, *Dugesia mediterranea;* 4, *Dugesia polychroa;* 5, *Polycelis felina;* 6, *Geoplana sp.;* 7, *Monocelis lineata;* 8, *Dalyellia viridis;* 9, *Discocelis tigrina;* 10, *Macrostomum tuba;* and the Nemertine 11, *Prostoma ehilardi.*

Discussion

We described the isolation of several *mariner* elements in planarian, showing that *mariner* is present in a high copy number, about 8,000 copies per haploid genome, thus constituting a middle repetitive DNA family of the genome of *Dugesia tigrina.* These results were remarkable per se, as in other organisms *mariner* sequences are present in much lower numbers, and most of them are not full ORFs but degenerated copies with in-frame stop codons and nucleotide deletions (Maruyama and Hartl 1991*b;* Robertson and MacLeod 1993; Bigot et al. 1994; Sedensky et al. 1994; but see Capy et al. 1990, 1992; Maruyama and Hartl 1991*a;* Maruyama et al. 1991, for references on naturally occurring active elements in *Drosophila*).

Therefore, some interesting biological and evolutionary questions were posed: Were these sequences conserved? How were they distributed in the genome? Had they ever been or were they still functional? How did they spread so much in the *D. tigrina* genome? Were they also present in other Platyhelminthe species? If the answer to the last question is negative, how did this element appear in *D. tigrina?* In order to shed some light on these questions, we performed a genomic Southern blot with different restriction enzymes, a Northern blot with RNA of different regenerative stages, isolated and sequenced some independent *mariner*-containing clones, and analyzed the presence of *mariner*-like sequences in other Platyhelminthe species by PCR or Southern blot.

The originally described *mariner* element, together with four new sequenced clones, share the same basic structure: a full ORF of 339 aa, which codes for the presumptive transposase, flanked by two short-inverted repeats of 32 bp, and a duplicated dinucleotide TA in the integration site. The comparison of the inverted-repeat sequences showed a high conservation between the right and left repeats of the same or different elements. When this comparison was extended to the *mariner* elements from other organisms, some regions appear to be conserved, which thus suggests that they may be of importance for the function or recognition of the repeats.

When comparing the different planarian *mariners,* the sequence similarity is very high; this sequence conservation is confirmed by Southern blot analyses. Moreover, our results showed that *mariner* elements are not tandemly arrayed but rather dispersed in the genome. The number of detected nucleotide substitutions was very low, and they mostly produced amino acid changes. Interestingly, five of the seven elements analyzed showed a complete ORF. Thus, planarian *mariner* are the only nonarthropod elements reported with intact ORFs and signals of functional expressed genes, although these features do not guarantee that a particular copy is functional (Maruyama et al. 1991; Medhora et al. 1991). But these results, together with the high number of *mariner*-like sequences in the *D. tigrina* genome, the polymorphic presence of one *mariner* element in the 5′ region of the planarian *Dth-2* gene, and the at least hitchhiking expression of *mariners,* suggest that probably some of them are still or have recently been active transposons

in this organism. However, not all the *mariner* sequences should be functionally active neither under selective constraint. A reduced number of fully active transposons could be responsible for the maintenance and spread of the element, while the *mariner* sequences as an average could be evolving neutrally, rapidly acquiring mutations and becoming a stable repetitive DNA in the *D. tigrina* genome.

The amino acid similarity between complete *mariner* sequences from planarians and other organisms was variable and ranged from 28.4% to 56.5%. However, some parts of the coding sequences were more conserved than others, which indicates that they are important regions for the transposition function. Some authors reported that *mariner* belonged to a superfamily of transposons, together with *Tc*1, *Tes*1, and bacterial *IS* elements (Doak et al. 1994; Robertson 1995). All of them share conserved residues or boxes (fig. 5), one of them being the D(34)D motif, which is also conserved in the *mariner* sequences isolated in *D. tigrina.*

Another interesting issue is how the *mariner* elements have spread so much in the *D. tigrina* genome, while in most organisms they are present in low numbers (except for *Hyalophora cecropia,* where there are about 1,000 copies per genome). The *D. tigrina* used in our analysis is a planarian that reproduces asexually by fission. Planarians are well known for their ability to regenerate completely the body after excision, due to the totipotency of particular somatic stem cells, called neoblasts (Baguñà 1981). These are the only cells capable of reproducing, differentiating, and replacing the old differentiated cells, as well as giving rise to germ cells.

The *mariner* element is one of the few transposons which is active in both somatic and germ cells. In asexual forms they may colonize one or more totipotent neoblasts and then expand, as these cells divide and differentiate, into differentiated somatic cells. *Dugesia tigrina* is native to North America, where it is widely distributed, forming a polytypic species with sexual, asexual, or alternate sexual/asexual forms (Kenk 1976). Since sexual *D. tigrina* from North America also bears *mariner* elements (H. Robertson, personal communication), the most parsimonious hypothesis for the presence of current *mariner* in the asexual European forms would be that they were previously introduced in the sexual ancestors. Screening for *mariner* elements in other *D. tigrina* populations could give interesting data as to the origin and evolution of these elements and of their hosts.

The number of *mariner* elements in the *D. tigrina* genome, the highest so far reported for any species, remains to be clarified. It can be speculated that a factor similar to *Mos* of *Drosophila mauritiana* (Jacobson et al. 1986; Medhora et al. 1988) could exist in *D. tigrina. Mos* is a special *mariner* element that is capable of inducing not only its own transposition but also that of other *mariner* elements. It has been proposed as a possible evolutionary mechanism for a rapid increase and decrease in the number of *mariner* copies in the genome (Lidholm et al. 1991). A reasonable guess of the number of functional genes in *D. tigrina* is between 5,000 and 15,000. If this is so, an important fraction of these genes may have a *mariner* copy nearby. Such situation would represent an unbearable load to any genome, so it is worth considering whether it gives asexual *D. tigrina* any selective advantage. Conversely, asexual organisms cannot get rid of their *mariner* or other transposons by purifying selection through the germ line. Then, a complex equilibrium can be envisaged between rates of transposition (possibly accumulated by the high rate of neoblast proliferation) and excision (due mainly to recombinational events) to result in the actual copy number.

The presence and distribution of *mariner*-like sequences was also tested in the genome of another close planarian species, *Dugesia mediterranea* by Southern blot and hybridization, and in the genomes of different Platyhelminthes by PCR. No similar *mariner* sequences have been amplified in the analyzed species, although the possibility of sequence divergence in the inverted repeat region precluding the amplification of *mariner*-like sequences in other organisms cannot be discarded on the basis of our PCR results. Moreover, the Southern blot of the close species *D. mediterranea* showed clearly that no similar sequences could be detected even using low-stringency hybridization conditions. These results indicated that if present in other Platyhelminthes, the *mariner* elements would not be very similar to those of *D. tigrina* and that the distribution of this element in the phylum is irregular. The most extreme hypothesis is that only *D. tigrina* has been "infiltrated" by *mariner.*

Robertson and McLeod (1993) proposed the existence of several subfamilies within the *mariner* elements, according to sequence similarity. The planarian *mariner* element locates in the cecropia subfamily, closer to a partial *mariner* element from the ant *Chromatogaster cerasi.* Five of the subfamilies presented arthropod hosts, the *Caenorhabditis elegans* elements being the more divergent subfamily. Interestingly, the arthropod *mariner* sequence tree did not agree with the phylogenetic positions of the hosts bearing them (Maruyama and Hartl 1991*b;* Robertson 1993). This result, together with the undetectability of *mariner*-like sequences in sibling species, strongly supported the feasibility of horizontal transfer between organisms. A similar argument would apply to the results obtained with Platyhel-

minthes. It could be argued that *mariner* is a very ancient transposon which spread very early, in the precursor of most of the actual phyla. Some of the lines could have lost the transposon while others allowed its continuity. However, for this hypothesis to be accepted, some results remain to be understood: the sporadic distribution between sibling species, which have diverged recently in evolutionary time, and the great similarity shared by elements which are in very different phyla, which is in contrast to the divergence that some *mariner* elements present in more closely related species. Horizontal transmission of *mariner,* at least between some of the organisms, seems a more plausible explanation to account for the present distribution and sequence similarity among different hosts. Therefore, the presence of *mariner* in *D. tigrina* is one of the first examples in which trans-phyla horizontal transfer, possibly from an arthropod lineage to this Platyhelminthe species, must be invoked.

A further implication is the fact that this transferred transposon may be active in many different organisms (even from different phyla), which thus opens the possibility to develop a new genetic tool: an integrative system of heterologous DNA. In fact, a *mariner*-derived vector has recently been used to transform successfully *Drosophila melanogaster* germ line (Lidholm et al. 1993). Although the mode of operation and requirements of this transposon are not well known, as the knowledge of the host organisms able to foster *mariner* multiplication expands, it should be possible to develop a more general integrative vector. Such a general vector will certainly be of great interest for using on those organisms where transgenesis is hampered because no means for DNA transfer has yet been developed. However, if *Tc*1-*mariner* vectors have the capability of infiltrating the genome of most animals, the effects of possible accidental horizontal or vertical transfer will have to be considered very seriously.

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