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SHORT NOTE

Identification of *mariner* elements from house flies (*Musca domestica*) and German cockroaches (*Blattella germanica*)

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Abstract

Full-length *mariner* elements were isolated and sequenced from house flies (*Musca domestica*) and German cockroaches (*Blattella germanica*). The amino acid sequence of the house fly *mariner* element (accession number: AF373028) showed 99.5% identity with *Mos1* and *peach* elements, whereas the German cockroach *mariner* element (accession number: AF355143) showed 98.8% and 99.8% identity, respectively. Sequence analysis revealed that the *mariner* elements in house flies and German cockroaches differed from the active *Mos1 mariner* element by seven and 15 nucleotides, respectively. Four essential nucleotide substitutions at positions 64, 154, 305, and 1203, which have been proposed to contribute to the loss of activity of the inactive elements, were detected in the German cockroach *mariner* element. In contrast, although the *mariner* element in house flies contained substitutions at positions 64, 154, and 305, it retained T at position 1203, identical to active *mariner* elements. Mariner is present in approximately eight copies in the German cockroach genome.

Keywords: *Musca domestica*, *Blattella germanica*, *mariner*, transposable element.

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Introduction

The *mariner* transposable element was first discovered spontaneously in the *white peach* mutant of *Drosophila mauritiana*. The element was described as 1286 bp in length with 28 bp inverted terminal repeats (Jacobson *et al*., 1986). The majority of naturally occurring *mariner*-like elements (MLEs) are inactive due to multiple in-frame terminations, deletions or insertions, and frame-shift mutations that disrupt the open reading frame (ORF). Some non-autonomous elements, such as the *peach* element, *Ma331*, *Ma310,* and *Ma311* from *D. mauritiana*, have ORF with only simple nucleotide substitutions that produce a protein but cannot support transposition (Hartl *et al*., 1997). Only a few characterized *mariner* elements, such as *Mos1* (also referred to as *Dmmar1* (*Drosophila mauritiana mariner1*), Robertson & Asplund, 1996), *Mos5* from *D. mauritiana*, and *Himar1* from *Haematobia irritans* (Bryan *et al*., 1987; Robertson & Lampe, 1995a,b) have been demonstrated to be autonomous.

MLEs have an exceptionally wide distribution among different distantly related insects, including species of different orders (Robertson & Lampe, 1995a). Horizontal transmission has been proposed as an explanation for the widespread distribution of MLEs (Lohe *et al*., 1995). Successful transposition of *mariner* transposable element in *D. melanogaster* (Lidholm *et al*., 1993), Australian sheep blow flies (Coates *et al*., 1997), and yellow fever mosquitoes (Coates *et al*., 1998) indicates that *mariner* transposition is independent of host specific factors (Lampe *et al*., 1996). The wide host range and its independence from host specific factors have made *mariner* a promising candidate for germ line transformation, thus attracting great interest for potential applications such as the genetic manipulation of target genes or transferring foreign DNA sequences into the genome of insect species, especially those of economic and medical importance.

The house fly, *Musca domestica*, and German cockroach, *Blattella germanica*, are serious insect pests. In addition to their economic importance, they pose significant health

hazards and act as mechanical vectors and reservoirs of both human and animal pathogens. Resistance to insecticides is now an immense practical problem challenging the management of these insect pests. For global understanding of insecticide resistance development, investigation of gene expression and function in response to insecticides has become an important research area. The long-term goal of our research is to develop a transposon-based transformation system to transfer exogenous DNA into the genome of insects in order to study gene expression and function in the resistance of economically and medically important insects. Using the *peach* and *Mos1* transposable elements as transformation vectors, we investigated the presence of endogenous *peach* and *Mos*-related elements in the genome of the house fly and German cockroach, as the endogenous copies of transposable elements in the genome are thought to interfere with the activity of the exogenous transposase (Coates *et al*., 1995; Atkinson *et al*., 1993). This study is the first to report the presence of entire endogenous *mariner* transposable elements containing undisturbed open reading frames of 1035 nucleotides in both house flies and German cockroaches. The nucleotide sequences, structure characteristics, copy numbers, and possible activities of the *mariner* elements are presented in this paper.

Results and discussion

The mariner *elements and sequence analysis*

Four genomic DNA clones (F2R1, F4R5, F3R4, and F2R3) from three house fly strains and three clones (F2R1, F4R5, and F3R4) from two German cockroach strains covering the full-length of *mariner* elements were isolated (Fig. 1), using an overlapping PCR amplification strategy with different primer pairs. Sequence analysis revealed that the *mariner* elements in all the insects were 1286 bp in length. Each element contained an undisrupted open reading frame of 1035 nucleotides (172–1206), encoding a putative protein of 345 amino acid residues. This study is the first to indicate that there are entire endogenous *mariner* transposable elements in both house flies and German cockroaches that encode transposases.

The amino acid sequences of the house fly *mariner* element (accession number: AF373028) showed 99.5% identity with both the *Mos1* and *peach* elements; the German cockroach *mariner* element (accession number: AF355143) showed 98.8% and 99.8% identity with the *Mos1* and *peach* elements, respectively. Both house fly and German cockroach *mariner* elements contained an acidic signature of three aspartic acid residues D,D(34)D (Fig. 2), which were thought to be a binding domain for a divalent cation necessary for catalysis (Hartl *et al*., 1997). Sequence analysis revealed that the house fly *mariner* element differed from *Mos1* and *peach* in seven and six nucleotide positions, respectively, distributed throughout its length (Fig. 3), while the German cockroach *mariner* element had 15 and two nucleotide substitutions compared with *Mos1* and *peach*, respectively. *Mariner* elements from both house flies and German cockroaches shared four nucleotide differences in the open reading frame at positions 305 (C to A), 636 (A to C), 662 (A to G), and 800 (C to G) (Figs 2 and 3), resulting in Thr to Lys, a synonymous change, Asn to Ser, and Pro to Arg amino acid changes, respectively, compared with *Mos1* (Medhora *et al*., 1991). In addition, a substitution of T to A at position 1203, resulting in a Phe to Leu substitution, was detected in the German cockroach *mariner* element. All five substitutions are identical to the inactive *peach* element.

Activity vs. inactivity

Among several changes between active and inactive *mariner* elements, the Phe to Leu substitution at position 1203 is consistent between active and inactive *mariner* elements in both *D. mauritiana* and *D. simulans*. The amino acid change from Phe to Leu may have a significant negative effect on the activity of the transposase (Maruyama *et al*., 1991; Capy *et al*., 1992a). In addition, three other nucleotide substitutions, T to C at position 64, A to T at position 154, and C to A at position 305, resulting in a Thr to Lys substitution, have been suggested to contribute to the loss of activity of the inactive elements (Capy *et al*., 1992a). A weakly active element, *MB1*, isolated from *D. simulans* (Maruyama *et al*., 1991) contains only two mutations at positions 1203 and 1248 compared to active elements *Mos1* and *Ma351* isolated from a non-mosaic w^{pch} strain of *D. Mauritiana* (Jacobson *et al*., 1986; Maruyama *et al*., 1991), further indicating the importance of the substitution at position 1203 for the *mariner* activities. Position 1248 is downstream of the putative coding region and is polymorphic in the active elements, thus any change at this position is unlikely to affect the

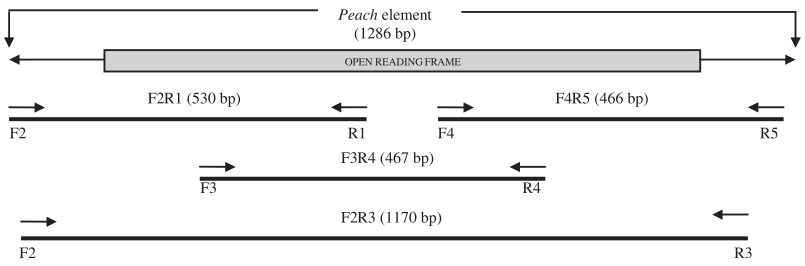

Figure 1. Graphic representation of the PCR amplification of *mariner* elements from house flies and German cockroaches. Primer positions corresponding to the *peach* element (M14653) and the sizes of the PCR fragments are indicated.

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Mos1      1   MSSFVPNKEQTRTVLIFCFHLKKTAAESHRMLVEAFGEQVPTVKTCERWFQRFKSGDFDV  60
peach     1   MSSFVPNKEQTRTVLIFCFHLKKTAAESHRMLVEAFGEQVPTVKKCERWFQRFKSGDFDV  60
HF        1   MSSFVPNKEQTRTVLIFCFHLKKTAAESHRMLVEAFGEQVPTVKKCERWFQRFKSGDFDV  60
GC        1   MSSFVPNKEQTRTVLIFCFHLKKTAAESHRMLVEAFGEQVPTVKKCERWFQRFKSGDFDV  60

Mos1      61  DDKEHGKPPKRYEDAELQALLDEDDAQTQKQLAEQLEVSQQAVSNRLREMGKIQKVGRWV 120
peach     61  DDKEHGKPPKRYEDAELQALLDEDDAQTQKQLAEQLEVSQQAVSNRLREMGKIQKVGRWV 120
HF        61  DDKEHGKPPKRYEDAELQALLDEDDAQTQKQLAEQLEVSQQAVSNRLREMGKIQKVGRWV 120
GC        61  DDKEHGKPPKRYEDAELQALLDEDDAQTQKQLAEQLEVSQQAVSNRLREMGKIQKVGRWV 120

Mos1      121 PHELNERQMERRKNTCEILLSRYKRKSFLHRIVTGDEKWIFFVNPKRKKSYVDPGQPATS 180
Peach     121 PHELNERQMERRKNTCEILLSRYKRKSFLHRIVTGDEKWIFFVSPKRKKSYVDPGQPATS 180
HF        121 PHELNERQMERRKNTCEILLSRYKRKSFLHRIVTGDEKWIFFVSPKRKKSYVDPGQPATS 180
GC        121 PHELNERQMERRKNTCEILLSRYKRKSFLHRIVTGDEKWIFFVSPKRKKSYVDPGQPATS 180

Mos1      181 TARPNRFGKKTMLCVWWDQSGVIYYELLKPGETVNTARYQQQLINLNRALQRKRPEYQKR  40
Peach     181 TARPNRFGKKTMLCVWWDQSGVIYYELLKRGETVNTARYQQQLINLNRALQRKRPEYQKR  40
HF        181 TARPNRFGKKTMLCVWWDQSGVIYYELLKRGETVNTARYQQQLINLNRALQRKRPEYQKR  40
GC        181 TARPNRFGKKTMLCVWWDQSGVIYYELLKRGETVNTARYQQQLINLNRALQRKRPEYQKR  40

Mos1      241 QHRVIFLHDNAPSHTARAVRDTLETLNWEVLPHAAYSPDLAPSDYHLFASMGHALAEQRF 300
Peach     241 QHRVIFLHDNAPSHTARAVRDTLETLNWEVLPHAAYSPDLAPSDYHLFASMGHALAEQRF 300
HF        241 QHRVIFLHDNAPSHTARAVRDTLETLNWEVLPHAAYSPDLAPSDYHLFASMGHALAEQRF 300
GC        241 QHRVIFLHDNAPSHTARAVRDTLETLNWEVLPHAAYSPDLAPSDYHLFASMGHALAEQRF 300

Mos1      301 DSYESVKKWLDEWFAAKDDEFYWRGIHKLPERWEKCVASDGKYFE 345
Peach     301 DSYESVKKWLDEWFAAKDDEFYWRGIHKLPERWEKCVASDGKYLE 345
HF        301 DSYESVKKWLDEWFAAKDDEFYWRGIHKLPERWEKCVASDGKYFE 345
GC        301 DSYESVKKWLDEWFAAKDDEFYWRGIHKLPERWEKCVASDGKYLE 345
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Figure 2. Alignment of the amino acid sequences of *mariner* elements, *Mos1* (X78906), *peach* (M14653), *HF* (from house flies, AF373028), and *GC* (from German cockroaches, AF355143). The different amino acid residues among the *mariner* elements are in bold and the acidic signature of the three aspartic acid residues D,D(34)D is underlined.

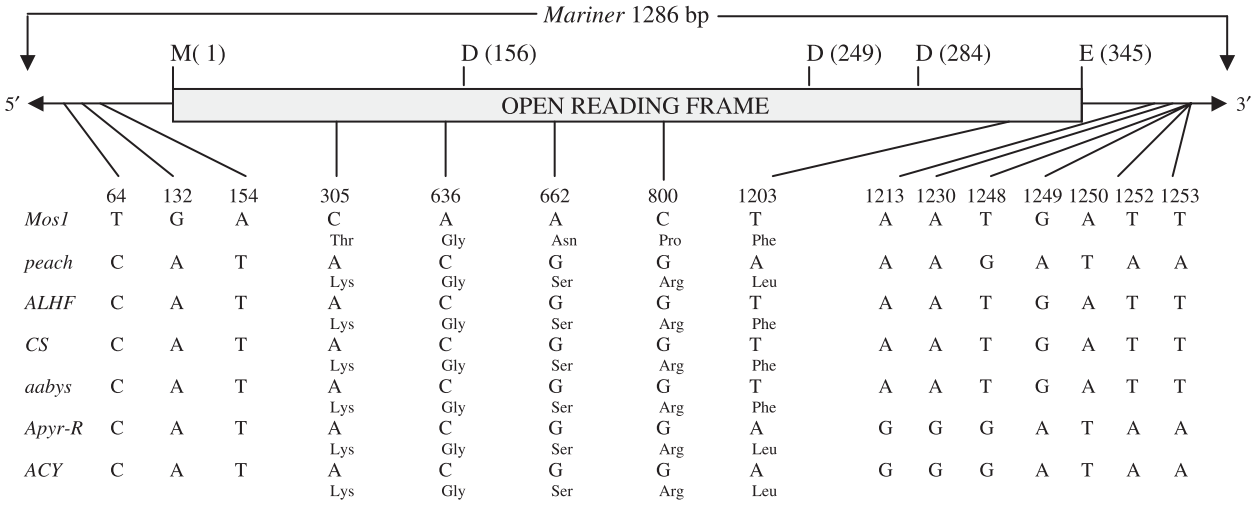

Figure 3. Schematic representation of sequence differences among *mariner* elements, *Mos1* (X78906), *peach* (M14653), and those in ALHF, CS and aabys house flies (AF373028), and in Apyr-R and ACY German cockroaches (AF355143).

activity. Although an A to G substitution at position 662, resulting in an Asn to Ser amino acid change, was present in inactive elements, it has been considered less likely to contribute to a reduction in activity as the two inactive elements from *D. mauritiana*, *Ma310* and *Ma311*, are identical to *Mos1* at this position (Maruyama *et al.*, 1991; Capy *et al.*, 1992a). Positions 132 and 636 have been examined and are thought to have little, if any, effect on activity (Maruyama *et al.*, 1991). Accordingly, four consistent nucleotide differences at positions 64, 154, 305 and 1203 in the inactive elements and one consistent difference at position 1203 in the weakly active elements have been distinguished from active elements (Capy *et al.*, 1992a). The *mariner* element in German cockroaches contained substitutions at positions 64, 154, 305, and 1203 similar to those in inactive elements, indicating that the element from German cockroaches might also be inactive. However, further functional analysis is required. In contrast, although the *mariner* element in house

flies contained substitutions at positions 64, 154, and 305, it retained T at position 1203, identical to active *mariner* elements, suggesting that the *mariner* element in house flies could be potentially active or weakly active. However, once again its functionality as a transposon requires further investigation.

The copy number and divergence of mariner *elements*

To estimate the number of copies of mariner elements in the house fly and German cockroach genomes, genomic DNA isolated from house flies and German cockroaches was subjected to Southern blot analysis with the probe prepared from F2R1 mariner fragment (Fig. 4). Digestion with *Hin*dIII and *Eco*RI resulted in six and eight bands, respectively, in German cockroaches. Considering that *Hin*dIII and *Eco*RI do not cut inside of the mariner element, we proposed that multiple copies, or at least eight copies, of the *mariner* element existed in German cockroaches, which was similar as the results of our dot blot analysis (data not shown). This result may further indicate that the mariner in the German cockroach is a non-autonomous element incapable of moving from one location to another. The German cockroach had a similar copy number of the *mariner* elements in its genome to those in the *Drosophila* genome, i.e. 0–10 copies in *D. simulans*, 20–30 in *D. mauritiana*, and three in *D. ananassae* (Capy *et al.*, 1992b; Robertson & Lampe, 1995b). Highly variable copy numbers in insects have been detected, from two copies in *D. sechellia* Capy *et al.* (1992a) to 17 000 copies in the horn fly, *Haematobia irritans* (Robertson & Lampe, 1995b). In contrast, digestion with *Hin*dIII and *Eco*RI resulted in smear in house flies, indicating that there are non-identical locations of the *mariner* elements in the genome of the individual house flies. This further suggests that the *mariner* element in house flies could be potentially active. Non-functional *mariner*-like elements containing multiple stop codons have been reported in house flies (Yoshiyama *et al.*, 2000). When taken together with the previous results, the results reported here suggest that potentially functional and non-functional *mariner* elements exist in the house fly genome. Multiple *mariner* elements with varying numbers of copies in the same genome are a common phenomenon (Lampe *et al.*, 2001), which can be attributed to horizontal transmission and genetic mutation drift in the molecular evolution of mariners (Robertson & Lampe, 1995b; Hartl *et al.*, 1997; Lampe *et al.*, 2001).

It has been demonstrated that divergent *mariner* elements or, to be precise, elements with minor changes in inverted terminal repeats and amino acid sequences do not interact (Lampe *et al.*, 2001). Thus, this new information on the sequences, divergence, and activities of *mariner* elements in house flies and German cockroaches has practical implications for selecting appropriate transposable elements as genetic tools for germ line transformation of house flies and/or German cockroaches.

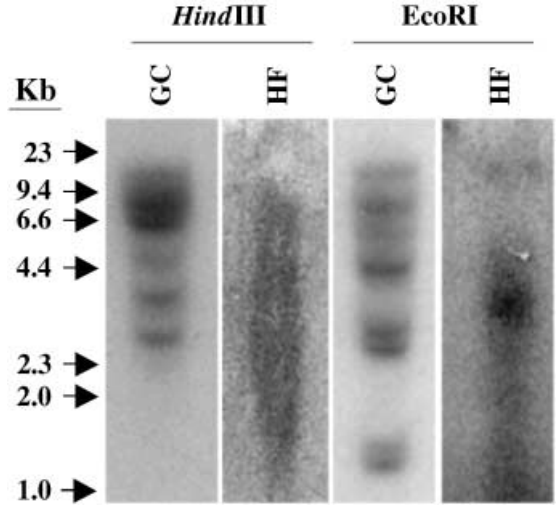

Figure 4. Southern blot analysis of the *mariner* transposable element in the genome of house flies and German cockroaches. Gnomic DNAs from house flies (HF, 40 μg) and German cockroaches (GC, 15 μg) were digested with *Hin*dIII and *Eco*RI. The membranes were hybridized with ^{32}P-labelled *mariner* F2R1 fragment.

Experimental procedures

Insects

Three strains of house flies and two strains of German cockroaches were used in this study: aabys and CS, insecticide-susceptible house fly strains obtained from Dr J. G. Scott (Cornell University); ALHF, an insecticide resistant house fly strain collected from Alabama in 1998; ACY (American Cyanamid Co., Clifton, NY), a susceptible German cockroach strain; and Apyr-R, a field strain collected from Alabama in 1999.

Genomic DNA preparation

Genomic DNA was extracted from adult flies and/or cockroaches. Insects were collected on dry ice and homogenized in an ice-cold homogenization buffer (10 mM Tris-HCl (pH 7.5), 60 mM NaCl and 10 mM EDTA; pH 8.0). SDS and proteinase K (Sigma Chemical Co.) were added to final concentrations of 1% and 200 μg/ml, respectively. The samples were extracted three times with equal volumes of phenol: chloroform (1 : 1) and once with an equal volume of chloroform. RNase (50 μg/ml) was added to the aqueous phase and the samples were incubated at 37 °C for 1 h, followed by another 2 h of incubation at 50 °C with the addition of proteinase K (100 μg/ml). The mixture was again extracted with phenol: chloroform. The aqueous phase was collected and NaCl was added to a final concentration of 0.1 M. DNA was precipitated with 100% ethanol.

Amplification, cloning and sequencing of the mariner *fragment*

To amplify the mariner fragment, the polymerase chain reaction (PCR) was performed with different primer pairs F2 (5′-CCAGGTGTACAAGTAGGGAATGTCGG-3′)/R1 (5′-CTCATGTGGCACCCATCTAC-3′), F3 (5′-GACGTCGACGACAAAGAGCAC-3′)/R4 (5′-ACCGTTTCGCCGGGTTTCAAG-3′), F4 (5′-CACGCTACCAACAACAATTG-3′)/R5 (5′-TCAGGTGTACAAGTATGAAATGTCG-3′), and F2/R3

(5′-CCATCTCTCGGGCAATTTGTG-3′) corresponding to sequences of the *peach* element (accession number M14653) described by Jacobson *et al*. (1986) (Fig. 1). PCR products were analysed by 0.8% agarose gel electrophoresis, purified from agarose gel using QIAquick Gel Extraction Kit (Qiagen), and cloned into PCR™ 2.1 Original TA cloning vector (Invitrogen). Sequences of PCR fragments from TA clones were verified by automated sequencing (Research Instrumentation Facilities, Auburn University). Sequence analyses of the *mariner* fragments from each strain were repeated at least three times with different preparations of genomic DNA, and three TA clones from each replication were verified by sequencing. In addition, the PCR products from three different preparations of genomic DNA for each strain were directly sequenced.

Southern blot analysis

Genomic DNAs of German cockroaches (15 μg) and house flies (40 μg) were digested to completion with *Eco*RI and *Hin*dIII in appropriate buffer. Samples were precipitated with ethanol and resuspended in TE buffer prior to electrophoresis on 0.8% agarose gels in 0.5× TBE. DNA was transferred to Nytran membranes (Schleicher and Schuell, Keene, NH, USA) as described by Sambrook *et al*. (1989). The membranes were then hybridized with ^{32}P-labelled *mariner* F2R1 fragment using QuickHyb solution (Stratagene, La Jolla, CA, USA). All Southern blot analyses were repeated three times with different preparations of DNA samples.

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