

gene has integrated into bacterial chromosome or it has expressed.

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Occurrence of a 'mariner' element in the silkworm *Bombyx mori*

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Occurrence of mariner element in *Bombyx mori* is reported here for the first time using degenerate primers in polymerase chain reaction (PCR) technique. The PCR product (Bm.MAR1) has been cloned and sequenced; it is 457 bp in length. DNA and peptide sequences of *Bombyx* mariner show a high degree of similarity with the mariner of the predatory mite, *Metaseiulus occidentalis* and low degree of similarity with the lepidopteran *Hyalophora cecropia*. Since no transcripts could be identified for the mariner of *B. mori* in northern hybridization, it appears to be a transcriptionally defective element.

SINCE McClintock¹ discovered mobile genetic element in maize, presence of such transposable elements has

been reported from a number of other eukaryotic genomes². A new transposable element 'mariner' has been identified and cloned from an unstable white mutant of *Drosophila mauritiana*, a sibling species of *D. melanogaster*³. The original mariner element is 1286 bp in length having 28 bp inverted repeats and an open reading frame (ORF) coding for a polypeptide of 345 amino acids. It is functional for germline transformation of *Drosophila*³. Lidholm *et al.*⁵ identified a mariner element in *Hyalophora cecropia* and showed about 40% DNA sequence identity with *Drosophila* mariner element. Using degenerate PCR primers, Robertson⁶ has shown the widespread occurrence of mariner elements in insects (17% among 400 species screened). Using the same PCR primers, Jeyaprakash and Hoy⁷ cloned a mariner element from a predatory mite *Metaseiulus occidentalis* and showed the presence of functional open reading frame. They constructed a transformation vector containing the mariner and made stable transformation of the predatory mite. Despite the fact that more than 400 species have been screened, the presence of mariner elements in the silkworm *Bombyx mori* has not been detected. This paper is the first report on the occurrence of mariner elements in *Bombyx mori*.

Bombyx mori strains (NB₄D₂, NB₁₈, KA, NB₇) were obtained from the Central Sericultural Research and Training Institute, Mysore. The genomic DNA isolation (from larval fatbody cells) was performed as described by Sambrook *et al.*⁸

The PCR was carried out using degenerate primers [MAR124F-5'-TGGGTNCCNCA YGARYT (17-mer) and MAR276R-5'-GGNGCNARRTCNGGNSWRTA (20-mer)⁶]. The conditions for PCR were: *Taq* polymerase buffer (1x), 2.5 mM MgCl₂, 150 μM dNTPs, 800 nM each primer, 0.25 units *Taq* polymerase, and 1-100 ng genomic DNA in a 25 μl total reaction volume⁹. For 35 cycles, amplification conditions were: denaturation at 94°C for 30 sec, annealing at 48°C for 30 sec and extension at 74°C for 1 min in Perkin-Elmer Thermol cycler. An aliquot from each reaction (10 μl) was electrophoresed on a 2% TBE gel. The amplified PCR product was purified by electro-elution and cloned into 'T' tailed plasmid. The clone was termed as pBm.MAR1.

The recombinant plasmid containing pBm.MAR1 was isolated and purified following standard methods⁸. The recombinant plasmid was transformed into *E. coli* strain DH5α; the plasmid DNA was isolated, purified and sequenced by conventional dideoxy method in an automated sequencer¹⁰. The sequence of *B. mori* mariner element was translated and compared with the translated sequence of mariner element of other species using the software 'GCG' (Genetic Computer Group, Wisconsin-Madison, USA)¹¹. Mariner sequences of other species were recovered from 'EMBL Gene bank' for comparison.

Using degenerate primers (MAR124F and MAR276R)



Figure 1. PCR amplification of mariner element from four different strains of *Bombyx mori* using degenerate primers (MAR 124F and MAR 276R). The samples in the lanes are: 1) λ marker; 2) No DNA control; 3) 100 ng, 4) 10 ng and 5) 1 ng of NB₄D₂ genomic DNA used for PCR; 6) 100 ng, 7) 10 ng and 8) 1 ng of NB₁₈ genomic DNA used for PCR; 9) 100 ng, 10) 10 ng and 11) 1 ng of KA genomic DNA used for PCR; 12) 100 ng, 13) 10 ng and 14) 1 ng of NB₇ genomic DNA used for PCR.

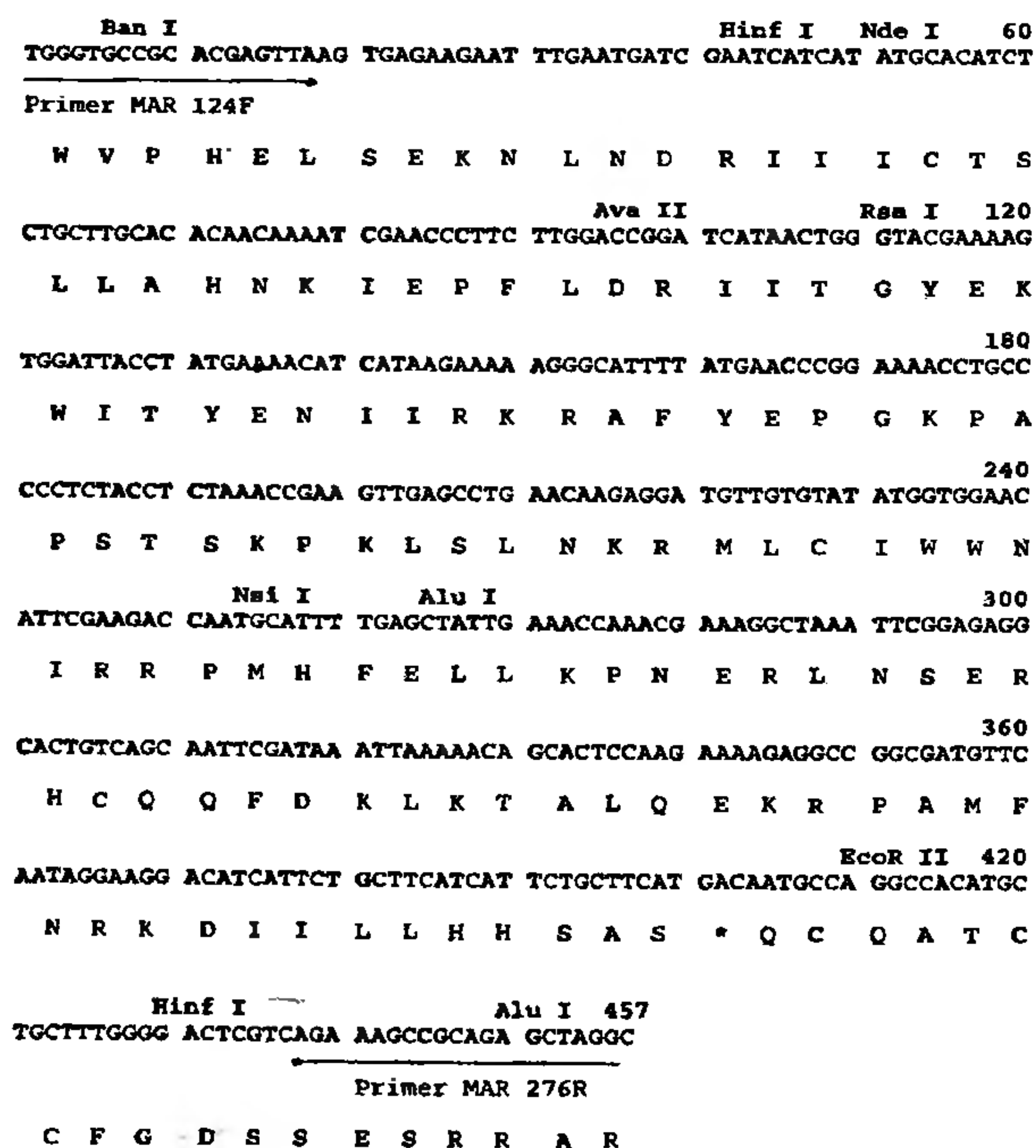


Figure 2. The PCR amplified *B. mori* mariner (Bm.MAR1) sequence was cloned and sequenced. The restriction map and amino acid sequence of Bm.MAR1 are shown.

PCR amplification of genomic DNA from *B. mori* strains resulted in the production of a single fragment approximately 450 bp. The PCR products obtained in all the four strains were identical in size (Figure 1). The PCR product was equal in size to the mariner product amplified in *D. mauritiana* using the same primers⁶. The PCR product from *B. mori* represents internal sequence of the mariner and the purified product was cloned. Six clones were obtained from the PCR product amplified from *B. mori* strain NB₇ and all the clones showed identical size on agarose gel fractionation. One of the clones was completely sequenced and it contained 457 base pairs (Figure 2). The possible existence of an ORF was tested in all the three frames. There were a number of stop codons in the second and third frames, while there was one stop codon in the first reading frame at 399 bp. The 457 bp pBm.MAR1 was compared with PCR-amplified clones (using the same primers) of mariner from *Hyalophora cecropia*, *Anopheles gambiae*, *Haematobia irritans*, *Metaseiulus occidentalis* (using 'GAP' software of GCG) and the data is presented in Table 1. The mariner sequences of the above species were translated into amino acid sequences and aligned using Clustal V multiple sequence alignment program of GCG software (Figure 3). Peptide similarity and identity of the mariner from these species were compared. *M.*


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B.mori      SEKNLNDRIIII---CTSLLAHNKIEPFLDRIITGYEKWITYENIIRKRA---FYEPGKPAPSTSKPKLSLNKRMLCIWW
H.cecropia  SESNLQTRVDC---YVTLNLRHNNERKMYDNRKGSLOWL-----NPGDPAKSCPKR*LTQKKLLVSVLV
M.occidentalis SERQKEVRLTV---CRELLSRYKNKSFLYRIITSDEKWIYYDNPGRKRS---WVSPGEPAEKSVRRNRFGKKTMLCVWW
D.mauritiana  NERQMERRKNT---CEILLSRYKRKSFHLRIVTGDEKWIFFVNPKRKKS---YVDPGQPATSTARPNRFGKKTMLCVWW
A.gambiae    TFDQKHQRVDDSERCLQLLTRNTPE-FLRRYVTMDQTLHHTPEPESNRKSAQWTATDEPAPKRGKTQKSAGKVIASVFW
H.irritans   TFDQKQQRVDDSERCLQLLTRNTPE-FFRRYVTMDQTLHHTPEFDQQAETATGEPSPKRGKTQKSAGKVMASVFW
      . . R          LL .          W.          . P.          . K          .

B.mori      NIRRPMHFELL-KPNERLNSERHCQQFDKLTALQEKRPAMFNRKDIILLHHSAS*QCQATCCFGDS
H.cecropia  D*RRCHSLQ-LSKMWPNDYSRYLLSATASHEGRTSC*TSEIGQSL*VTAASRQRKTTHC*TNNH*VK
M.occidentalis DQRGVIYHELL-KPGETVDTARYQQQLIDLNRVAVKEKRPNWDQVRN-ASF CSTTTLSVTPRSRRRKL
D.mauritiana  DQSGVIYYELL-KPGETVNTARYQQQLINLNRALQRKRPEYQKRQHRVIFLHDNAPSHTARAVRDTL
A.gambiae    DAHGIIIFIDYLEKGTINSDDYIMALSERFKVEIAA-KRPHMKKKK--VLFHQDNAPCHKSLRTMAKI
H.irritans   NAHGIIIFIDYLEKEKTINSDDYIMALL*RLKVEIAA-KWPHMKKKKVLFDQ--DNAPCHKSVRTMAKI
      . .          L K          K..          DNAP
    
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Figure 3. Alignment of translations of mariner PCR fragments from *D. mauritiana*, *H. cecropia*, *A. gambiae*, *H. irritans*, *M. occidentalis* and *B. mori*. They were translated using 'GCG' software; the '-' symbol indicates frameshift to maintain an aligned reading frame and the asterisk indicates stop codon. The translations were aligned using Clustal V multiple sequence alignment program with final manual alignment.

Table 1. Comparison of Bm.MAR1 sequence with mariner elements of other species. The mariner sequences were retrieved from 'EMBL' data bank and compared using 'GAP' program of 'GCG' software

Species	DNA sequence identity (%)	Peptide similarity (%)	Peptide identity (%)
<i>D. mauritiana</i>	57	52	39
<i>H. cecropia</i>	47	44	25
<i>A. gambiae</i>	39	46	24
<i>H. irritans</i>	41	48	27
<i>M. occidentalis</i>	49	57	41

occidentalis showed maximum peptide similarity (57%) with *B. mori* mariner sequence, and minimum similarity was noted with *H. cecropia* (44%). In peptide identity, the maximum similarity was observed with *M. occidentalis* and minimum with *A. gambiae*. Though *B. mori* and *H. cecropia* belong to the same order Lepidoptera, their mariner elements are diversified. The mariner element of *B. mori* appears to be close to *M. occidentalis* or *D. mauritiana*.

The northern blot of total RNA from *B. mori* was probed with Bm.MAR1. No detectable signal was obtained, indicating the absence of mariner specific mRNA population. Since there was a break in the coding sequence of *B. mori* mariner, it was found to be a defective mariner. Possible use of the mariner for the construction of vector for a stable germline transformation of *B. mori* needs to be confirmed only after cloning and sequencing of a number of mariner elements from more silkworm strains (both mulberry and non-mulberry

silkworms). This is the first report on *B. mori* mariner and cloning of full length mariner from other strains is under progress.

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