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EVIDENCE FOR AN INACTIVE TRANSPOSABLE *MARINER*-LIKE ELEMENT IN *ANOPHELES ALBIMANUS*

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ABSTRACT. Transposable elements may eventually be used as transformation vectors of anti-*Plasmodium* genes in natural populations of *Anopheles* mosquitoes. The *mariner* transposable element is widespread in most animal phyla and we wished to test for its presence in the important Latin American malaria vector, *Anopheles albimanus*. Degenerate primers were designed from the consensus of insect *mariner* elements and used in a polymerase chain reaction to amplify a fragment of the predicted size from *An. albimanus*. The fragment was cloned, sequenced, and determined to be a *mariner*-like element through multiple alignment with known insect *mariner* elements. Dot blot and Southern blot analyses showed only one or a few elements per haploid genome. The cloned fragment was used as a probe to isolate similar sequences from an *An. albimanus* genomic DNA library. A 659-base-pair clone was 57% similar at the DNA level with *mariner* elements from *Anopheles gambiae, Chrysoperla ptorapunda,* and *Haematobia irritans.* This low sequence similarity is comparable to that found among *mariner*-like elements in all insects. However, conserved amino acid motifs were not discovered nor was a single open reading frame found. These aspects suggest that the *An. albimanus mariner*-like element may represent an ancient transposition event but that the element is no longer active, the typical open reading frame having been disrupted through nucleotide insertions and deletions.

KEY WORDS Transposon, *mariner, Anopheles albimanus*

INTRODUCTION

The *mariner* transposable element was first identified as an insertion in the white gene of the fruit fly *Drosophila mauritiana* (Jacobson et al. 1986). This element is 1,286 base pairs (bp) long, with 28-bp short terminal inverted repeats and a single uninterrupted open reading frame of 1,038 bp. Transposable elements of the *mariner* family are now known to be widespread among most animal phyla, and 65 insect species representing 10 orders and 5 principal subfamilies have been shown to contain *mariner*-like elements (Robertson and Macleod 1993). Recently, *mariner*-like elements also were identified in *Anopheles gambiae* Giles, a major malaria vector in Africa (Robertson and Lampe 1995).

Transposable elements may eventually be used as transformation vectors of anti-*Plasmodium* genes in natural populations of *Anopheles* mosquitoes. The P transposable element has been used in germ line transformation of *Drosophila* (Rubin and Spradling 1981, 1982; Spradling and Rubin 1982) but has failed in the transformation of mosquitoes and many other insects (Morris et al. 1989). However, *mariner* transposable elements may also be good candidates and the suggestion has been made that a transposable element isolated from an insect species itself may prove to be the most suitable transformation vector for that species (Warren and Crampton 1994). *Anopheles albimanus* Wiedemann is an important vector of *Plasmodium vivax* malaria in Latin America. Identification of *mariner* elements in *An. albimanus* may eventually assist in the control of malaria in areas of Latin America where this species occurs. Here we demonstrate that a *mariner*-like element exists in *An. albimanus* but because no open reading frame could be found, that this element likely represents an ancient transposition event and that the current element is no longer active.

MATERIALS AND METHODS

Mosquito DNA extraction: Anopheles albimanus were captured in Chiapas, Mexico, and cultured in the laboratory for several generations. One hundred mosquitoes were ground in a mortar with liquid nitrogen and incubated at 50°C for 1.5 h in 4 ml of extraction buffer (100 mM Tris-HCl, pH 8.0, 50 mM NaCl, 50 mM ethylenediaminetetraacetic acid, 1% sodium dodecyl sulfate, and Proteinase K [Boehringer, Indianapolis, IN] 50 μg/ml). The mixture was extracted twice with 4 ml of phenol, and once with 4 ml of chloroform : isoamyl alcohol (24:1). The DNA was then precipitated by adding a one-tenth volume of 3 M sodium acetate (pH 5.2) and 2 volumes of cold absolute ethanol. After centrifugation the DNA was dissolved in 1 ml of 10 mM Tris-HCl plus 0.1 mM ethylenediaminetetraacetic acid (pH 7.6) and incubated with 10 μl RNAse (10 mg/ml) (Boehringer) at 37°C for 1 h, then extracted twice with phenol and chloroform. The DNA was ethanol precipitated as above and then washed with 70% ethanol, dried, and then dissolved in 10 mM Tris-HCl plus 0.1 mM ethylenediaminetetraacetic acid (pH 7.6). The DNA was quantified with an ultraviolet spectrophotometer.

Design of degenerate primers: The peptide se-

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Horn fly        TGTGACAATGG--ATGAAACATGGCTCCATCACTACAC-TCCTG--A---GTCC-AATCG-A---
G. lacewing     TGTGACAATGG--ATGAAACATGGCTCCATCACTACAC-TCCTG--A---GTCC-AAACG-A---
A. gambiae      TGTGACAATGG--ATGAAACATGGCTCCATCACTACAC-TCCTG--A---GTCC-AATCG-A---
Fruit fly       -GTTAC--TGGCGATGAAAAATGGATC----TTT----TTT--GTTA---GTCCTAAACGTAAA-
A. albimanus    -GTTACAATGG--ATGAGACGTGG-TG-AG-ACTGGGTGTCCAGCAAAACGTCC---TCGCATTT
consensus        ** **::***  **** *: *** *  :  ::*     *:: *  *   ****    ** *

Horn fly        CAG--TCGGCTGAGTGGACAGC-GACCGGTGAACCGTCTC-CG-AAGCGTGGA-AAGACTC---A
G. lacewing     CAG--TCGGCTGAGTGGACAGC-GACCGGTGAACCGTCTC-CG-AAGCGTGGA-AAGACTC---A
A. gambiae      CAG--TCGGCTCAGTGGACAGC-GAACGGTGAACCAGGTTCCG-AAGCGTGGA-AAGACTC---A
Fruit fly       AAG--TCA--TACGTTGATCCTGGAC-----AAC-------CGGCCACATCGA-CTG-CTCGACC
A. albimanus    AAGATTCG--TTTGTTG-TT---GACCCAT-TAC----TT-CGTTGGCTTT-ATATTACTCTGGA
consensus        **  **:  *  ** *      ** :  :  **    :  **   :* *  * :  :***   :

Horn fly        AAAGTCCA---CTG--GCAA-AGTA--ATGGCCTCTGTTT-TTTGG--GAT--GCGCATG--GAA
G. lacewing     AAAGTCCG---CTG--GCAA-AGTA--ATGGCCTCTGTTT-TTTTC--GAT--GCGCATG--GAA
A. gambiae      AAAGTCCG---CTG--GCAA-AGTA--ATGACCTCTGTTT-TTTGG--GAT--GCGAATG--GAA
Fruit fly       GAA-TCG----CTTTGGCAAGAAGACGATG--CTCTGTGT-TTGGTGGGATCAGAGCG-GT-G--
A. albimanus    AAAGTCCGTTTCCTTCGCCA-CTTAC-AT----T-TGTTCCTTTC----ATT-GCCCACGTTGCC
consensus       :**:**:    **   ** *   :*  **    * ***:  **:     **  *: :: *  *

Horn fly        AATAATTT---TTATC-G-A-TT-A--TCTT--C---AGA--AGG-GAAAAA-CC---A-TCAAT
G. lacewing     --TAATTT---TTATC-G-A-TT-A--TCTT--G---AGA--AGG-GAAAAA-CC---A-TCAAC
A. gambiae      --TAATTT---TTATC-G-A-TT-A--TCTT--G---AGA--AGG-GAAAAA-CC---A-TCACC
Fruit fly       --TCATTT--ACTAT--G-A-GC----TCTT--G---A-A--ACGCGGCGAAACGGTGAAT-A-C
A. albimanus    G-TAATTACAACAAACAGCAGTTCAGTTCTTATGGGCAGAGGATGCGCTTCGCCTGCCGTTTA-C
consensus         *:***      * : * * :: :  ****      *:*  * * *      *      * *

Horn fly        AGTG-A-----CT--A-TTATAT-G---GCGTTATTGGAGCG-TTTGA--AGGT-CGAAATC-GC
G. lacewing     AGTG-A-----CT----TTATAT-G---GCGTTATTGGAGCG-TTTGA--AGGT-CGAAATC-GC
A. gambiae      AGTG-A-----CT--A-TTATAT-G---GCGTTATTGGAGCG-TTTGA--AGGT-CGAAATC-GC
Fruit fly       GGC--AC---GCT--A-CCA-ACA----AC--AATTG-ATCAATTTGA--ACCGTGCGCTTCAGA
A. albimanus    TG-GCACCTTCCTCCACTTA-ATAGTTTGCTTAATTG-A-C--TTCCACCAAG--CGAA--CAGC
consensus        * : *     **  : ::* *: :   :* : **** * *  **  *  * :  ::::  * *:

Horn fly        GGCAAAATGGCCCCA-TATG-A-AG--AAGAAAAAAGT--GT--TG-TTCCA--CCAAGAC----
G. lacewing     GGCAAAACGGCCCCA-TATG-A-AG--AAGAAAAAAGT--GT--TG-TTCCA--CCAAGAC----
A. gambiae      GGCAAAACGGCCCCA-TATG-A-AG--AAGAAAAAAGT--GT--TG-TTCCA--CCAAGAC----
Fruit fly       G--AAAACGACCGGAATATCAA-AA--AAGACAACACAGGGTCATTTTTCT---CCATGAC----
A. albimanus    AT-AAAACGATTGG--TACGCACCGCAAATAAAATTCTT-GT--TGATTCCATTCCATGACCTGG
consensus          **** *       ** : *  :  ** *:**   :  **  *: ***::  *** ***

Horn fly        AACGCA---TCGTGC---CAC---AAGTC-ATTGAGAACAATGGCA-AAAAT-TCATGAATTGGG
G. lacewing     AACGCA---CCGTGC---CAC---AAGTC-ATTGAGAACGATGGCA-AAAAT-TCATGAATTGGG
A. gambiae      AACGCA---CCGTGC---CAC---AAGTC-ATTGAGAACGATGGCA-AAAAT-TCATGAATTGGG
Fruit fly       AACGCT---CCAT-CACATACGGCAAGAGCGGTTCGCGACACGTTGGAAACACTCA---ATTGGG
A. albimanus    AATGCAAAAACAAGCAGCCAGGAAAAAAA-ATAGACCA-GATGGAGGAAAG-CTCAT--CTTGCA
consensus       ** **:    *  :*   :*    **    :: ::  :  *:*:   ***   ***:   ***

Horn fly        TTTCCAATTGCTTC----C-CCACC-CAACGTATTCTCCA-GATCTGGCCCCCAGCG-ACTT---
G. lacewing     CTTCGAATTGCTTC----C-CCACC-CACCGTATTCTCCA-GATCTGGCCCCCAGCG-ACTT---
A. gambiae      CTTCGAATTGCTTC----C-CCACC-CACCGTATTCTCCA-GATCTGGCTTCCAGCG-ACTT---
Fruit fly       -----AAGTGCTTC----C-GCATG-CGGCTTACTCACCA-GACCTGGCCCC-ATCCGA-TTACC
A. albimanus    AGA--AATTGCTTCAAAACTCCATTTCGGCGAA-ACGCGTTGTTTTG-CT---A-C--A-TT---
consensus            **:******    * :**   *  *: *  * *   * : ** *    * *  * **

Horn fly        ---T-TTCTTGTTC--T----CA-G-AC---CTCAAAA--G----G-ATGCT--CGCA-G-G-GA
G. lacewing     ---T-TTCTTGTTC--T----CA-G-AC---CTCAAAA--G----G-ATGCT--CGCA-G-G-GA
A. gambiae      ---T-TTCTTGTTC--T----CA-G-AC---CTCAAAA--G----G-ATGCT--CGCA-G-G-GA
Fruit fly       ACCTATTC-GCTTCGATGGGACACGCACTCGCTGAGCAGCGCTTCG-ATTCTTACGAAAGTGTGA
A. albimanus    -C-TAT-CTTTTTC--T----C--G--C-----GAA-AG-G-T--GCAGCCT---GC---TGCGA
consensus          * * *:: ***  *    *  *  *      *: *  *    * *  **   *:    * **

Horn fly        AAAAAATTGGCT-GCAATGAAGAGGTGATCGCCGAA-A--CTGAGGCCTATTTTGAGGCCGAA--
G. lacewing     AAAAATTTGGCT-GCAATGAAGAGGTGATCGCCGAA-A--CTGAGGCCTATTTTGAGGCAAAACC
A. gambiae      CAAAATTTGACT-GAAATGAAGAGGTGGTCGCCGAA-A--CTGAGGCCTATTTTGAGGCAAAACC
Fruit fly       AAAAA--TGGCTCG-A-TGAAT-GGT--TCGCCGCAAAAGACGATGAGTTCTACT-GGC------
A. albimanus    C------TGTCT-G-AAGGAAGA-TT--TC-CCGAACAA-CCGA-CACT-GTCGTGGGC--ACTC
consensus             ** ** * *: ***::  *  ** ***:* *  : **   :*  *    ***  :
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Fig. 1. The alignment of *mariner* elements with the AaMc1 sequence from *Anopheles albimanus*. *, conserved nucleotide; :, conserved nucleotides excluding the fruit fly; –, gap inserted to optimize the alignment. The alignment was performed by CLUSTAL W (1.7), Houston, TX.

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Horn fly        ---GGAGTACTACC-AAA-ATG-GT-A-T-TAAAAAATTGGAAGGTCGTTATAATCGTTGTATCA
G. lacewing     GAAGGAGTACTACC-AAA-ATG-GT-A-T-CAAAAAATTGGAAGGTCGTTATAATCGTTGTATCG
A. gambiae      GAAGGAGTACTACC-AAA-AAG-GT-A-T-CAAAAAATTGGAAGGTCGTTATAATCGCTGTATCG
Fruit fly       GT-GGA--A-T-CC-ACA-AA---T-TGCCCGAGAGATGGGAA-------A-AAT-G-TGTAGCT
A. albimanus    CTTGG----CTA---AAGGACGAGTCAGTCCCC--GA--GAAAGGT-GT-A-AATAG--GT----
consensus          **    :*:   *:  * : :* : :       *  * **::: :: * *** *  **

Horn fly        CTCTTGAAAGG-AACTATCTTGA-ATAA-TAAAAA-CTTATTTT-GACAAAAAAAAATGGGTTT-T
G. lacewing     CTCTTGAA-GGGAACTATGTTGA-ATAA-TAAAAA-CGAATTTTC-ACAAAAAAA-TGTGTTT-T
A. gambiae      CTCTTGAA-GGGAACTATGTTGA-ATAA-TAAAAA-CGAATTT-CGACAAAAAAA-TGTGTTT-T
Fruit fly       AGC--GAC-GGCAAATACTTAGA-ATAAATGATTTTTTCTTTTTCCACAAAATTTAAC-GTGT-T
A. albimanus    CTCT-----GGGA-CTAGCG-GAGAGGT-TGG----CG-AGTG-CCTCAGTC---GTC--TCAGT
consensus       ::*:     ** * :**    ** *    *      :  : *     **       :   *   *

Horn fly        TCTTTG-TTAG--AC----CGGGGACTTATCAGCCAAC-CTGTT-A-CTGAGC
G. lacewing     TCTTTG-TTAG--AC----CGGGGACTTATCAGCCAAC-CTGTT-ATCTT---
A. gambiae      TCTTTG-TTAG--AC----CGGGGACTTATCAGCCAAC-CTGTT-A--TAA--
Fruit fly       T--TTGATTAAAAAAAAAACG---ACATTTCA--T-AC-TTGTACACCTGA--
A. albimanus    TC--TG---AC-CA-----CGAGCAGTTCTG-GCC-ACGCTGG--A--TCGA-
consensus       *:  **   *   *     ** : * :* *  ::: ** :**   *  *
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Fig. 1. Continued.

quences of *mariner* elements from *An. gambiae,* the horn fly *Haematobia irritans,* and the green lacewing *Chrysoperla ptorapunda* were aligned with GeneWorks (Intelligenetics Inc., Mountain View, CA). A variety of conserved regions were identified and potential primer sites for polymerase chain reaction (PCR) were analyzed using Oligo (4.03, National Bioscience, Cascade, CO). Potential primers were compared with *mariner* nucleotide sequences from other insects to identify variable sites. The upper primer was 5′-GTN ACN ATG GAY GAR AMN TGG-3′, which corresponds with amino acids VTMDET(K)W, whereas the lower primer was 5′-GGN GCR TTR TCN TGR TGR AA-3′, corresponding to amino acids FHQ(H)DNAP. Oligonucleotides were synthesized by Operon Technologies Inc, Alameda, CA.

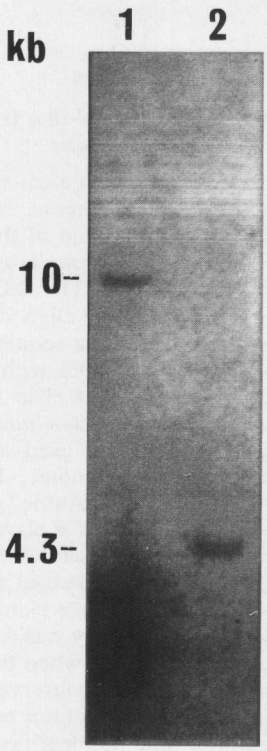

Fig. 2. Southern blot. Ten micrograms of *Anopheles albimanus* genomic DNA was digested with *Hin*dIII (lane 1) or *Eco*RI (lane 2) and hybridized under stringent conditions with the *mariner*-like LK fragment labeled with [α-^{32}P] deoxycytidine triphosphate.

Degenerate primer PCR: Polymerase chain reaction was conducted in 50 μl reaction volume with 60 ng of *An. albimanus* genomic DNA, 10 pmole of each primer, 200 μM of each deoxynucleoside triphosphate, and 1 unit of *Taq* DNA polymerase (Boehringer) in 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1 mg/ml gelatin. The thermal cycling program consisted of initial denaturation at 95°C for 5 min, followed by 10 cycles of 94°C (denaturation) for 1 min, 36°C (annealing) for 1 min, and 72°C (extension) for 1 min, followed by 25 cycles with the same conditions but with an annealing temperature of 52°C.

Cloning and sequencing of the PCR product: The PCR product (10 μl) was treated with *Pfu* DNA polymerase (2.5 units) at 72°C for 30 min and ligated into the *Eco*RV linearized pBluescript SK(−). The ligation was performed in 20 μl reaction volume with 1 unit of T4 DNA ligase (Boehringer) at 15°C for 8 h and the plasmid was transformed into competent XL1-blue cells (Sambrook et al. 1989). The insert : vector ratio was 200:1. Recombinant colonies containing the appropriate insert size were identified by PCR. A portion of the colony was suspended in 100 μl of water, heated for 15 min at 95°C, centrifuged for 30 sec, and 10 μl of the supernatant was used as template for PCR in a 20 μl reaction volume using the original PCR conditions and primers. Plasmid DNA from posi-

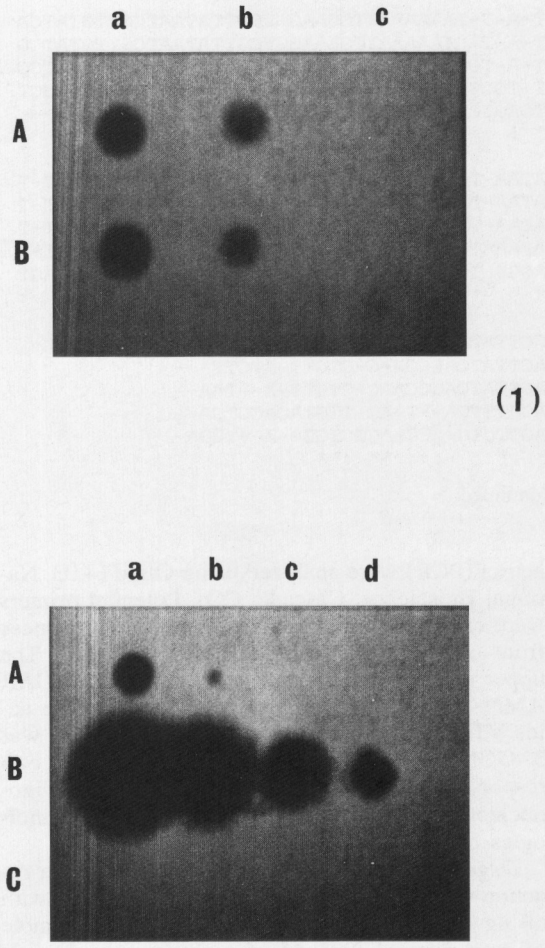

Fig. 3. Dot blot. The DNAs were spotted onto a nylon membrane and hybridized with the *mariner*-like LK fragment labeled with [α-^{32}P] deoxycytidine triphosphate. (1) Comparison of (A) *Anopheles gambiae* with (B) *Anopheles albimanus.* The a, b, and c columns contain 6.5 μg, 1.3 μg, and 0.13 μg of DNA, respectively. (2) Estimation of copy number in (A) *An. albimanus,* (B) the LK fragment, or (C) *Escherichia coli.* a, b, c, and d are 5×10^8, 5×10^7, 5×10^6, and 5×10^5 molecules, respectively.

tive clones was obtained with mini-preps (Sambrook et al. 1989), purified on Plasmid Select-250 Spin Columns (5 Prime->3 Prime, Inc., Boulder, CO). The insert was sequenced with T3 and T7 primers end-labeled with ^{32}P and *f*mol™ Sequencing Kit (Promega, Madison, WI) according to manufacturer instructions. The sequence ladders were separated by 5% acrylamide-urea gel (Sambrook et al. 1989).

Isolation of a mariner*-like gene from genomic DNA library:* The cloned PCR product (LK fragment) was labeled with ^{32}P and used as a probe to screen an *An. albimanus* genomic DNA library. The library contained *An. albimanus* genomic DNA digested with *Sau*3AI and inserted into lambda DASH (Stratagene, Foster, CA) with an average insert size of 15 kilobases (kb). Colony hybridization was performed at 42°C in the presence of 50% formamide (Sambrook et al. 1989). The orientation of the *mariner*-like fragment in the insert of the positive clone was analyzed by long PCR using the Expand™ kit (Boehringer) with primers designed from the identified fragment and universal T7 or T3 primers, which flanked the vector multiple clone sites. The PCR product was sequenced directly with primer walking strategy.

Dot blot and Southern blot hybridizations: The DNA was dotted onto nylon filters. This included the LK fragment as a positive control and to examine relative copy number, *An. gambiae,* and *Escherichia coli* as a negative control. Identical amounts of DNA were spotted onto the filter and then hybridized with the ^{32}P-labeled LK fragment (Liu et al. 1993). The hybridization signals were scanned by a densitometer and the copy numbers were estimated by comparison of the signal intensity. To analyze the genomic distribution of the elements, 10 μg of genomic DNA of *An. albimanus* was digested and used in Southern blot and hybridized with the cloned LK fragment labeled with ^{32}P under high-stringency conditions (Southern et al. 1975).

RESULTS

Identification of a *mariner*-like fragment in *An. albimanus*

The expected ~300-bp fragment was amplified from *An. albimanus, An. gambiae,* and the honey bee *Apis mellifera.* But no band of the appropriate size appeared in *E. coli, Salmonella typhi, Amoeba* spp., or with human DNA. The 282-bp-long sequence of *An. albimanus* was aligned with 4 other insect *mariner* sequences. The sequence similarity at the nucleotide level was 50% with *An. gambiae* and 41% with *D. mauritiana.* The similarity between *An. gambiae* and *D. mauritiana* was 43%.

The 282-bp fragment was used as a probe to screen an *An. albimanus* genomic DNA library. Four clones termed AaMc1, AaMc2, AaMc3, and AaMc4 were isolated. Long PCR showed that these had a 12.5-kb or a 8.4-kb insert. A 659-bp region of the 12.5-kb clone was sequenced and compared with the 4 other insect *mariner* elements (Fig. 1). The overall sequence similarity was 41%. However, sequence similarity was 57% when the *Drosophila* sequence was excluded. No conserved region was obvious at the amino acid level nor could a continuous open reading frame be identified.

Genomic distribution

Anopheles albimanus genomic DNA (10 μg) was digested with *Hin*dIII and *Eco*RI, which do not ex-

ist in *mariner* elements. Digested fragments were transferred to a nylon filter and ^{32}P-labeled *An. albimanus mariner*-like element was used in the hybridization. One 4.3-kb band appeared in the autoradiograph from the *Eco*RI-digested DNA, whereas a 10-kb, a 3-kb, and a 2-kb band appeared in the autoradiograph from the *Hin*dIII-digested DNA (Fig. 2).

Copy numbers

The intensity of hybridization of the 1st spot of *An. albimanus* genomic DNA (6.5 μg = 6.5×10^6 pg = 2.17×10^7 haploid genomes assuming ~0.3 pg/haploid genome) was most similar in intensity to the 4th spot (5×10^5 molecules) of the LK fragment annealed to itself (Fig. 3). This suggests that there are ~ 5×10^5 copies/2.17×10^7 haploid genomes = 0.023 copies/genome or no more than 1–2 copies of LK/*An. albimanus* genome.

DISCUSSION

We demonstrated the existence of a *mariner*-like element in *An. albimanus*. However, the dot blot and the *Eco*RI and *Hin*dIII digestion suggest only one or a few copies of the element. Nucleotide sequence similarity was relatively low (37–56%) with respect to other insect *mariner*-like elements but well within the range of other interspecific comparisons in insects. In addition, we found no open reading frame and no obvious regions of amino acid conservation with other *mariner* elements. Among 65 insect species, the amino acid similarity ranges from 23 to 45% (Holly and Langer 1992). This may indicate that the *mariner*-like element in *An. albimanus* represents an ancient transposition and that the element is no longer active. Nucleotide insertions and deletions may have caused shifts in the open reading frame.

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REFERENCES CITED

Holly, B. S. and P. J. Langer. 1992. The protein sequence predicted from a *Leishmania guyanensis* gp63 major surface glycoprotein gene is divergent as compared with other *Leishmania* species. Mol. Biol. Parasitol. 52: 141–144.

Jacobson, J. W., M. M. Medhora and D. L. Hartl. 1986. Molecular structure of a somatically unstable transposable element in *Drosophila*. Proc. Natl. Acad. Sci. USA 83:8684–8688.

Liu, K. Y., H. G. Zheng, B. C. Huang, B. Wang and Y. L. Cheng. 1993. The establishment of genomic DNA libraries for the human malaria parasite *Plasmodium falciparum*. Chung Kuo Chi Sheng Chung Hsueh Yu Chi Sheng Chung Ping Tsa Chih 11:116–119.

Morris, A. C., P. Eggleston and J. M. Crampton. 1989. Genetic transformation of the mosquito *Aedes aegypti* by microinjection of DNA. Med. Vet. Entomol. 3:1–7.

Robertson, H. M. and D. J. Lampe. 1995. Recent horizontal transfer of a *mariner* transposable element among and between Diptera and Neuroptera. Mol. Biol. Evol. 12(5):850–862.

Robertson, H. M. and E. G. Macleod. 1993. Five major subfamilies of *mariner* transposable elements in insects, including the Mediterranean fruit fly and related arthropods. Insect Mol. Biol. 2:125–139.

Rubin, G. M. and A. C. Spradling. 1981. Vectors for P element mediated gene transfer in *Drosophila*. Nucleic Acids Res. 11:6341–6351.

Rubin, G. M. and A. C. Spradling. 1982. Genetic transformation of *Drosophila* with transposable element vectors. Science 218:348–353.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–508.

Spradling, A. C. and G. M. Rubin. 1982. Transposition of cloned P elements into *Drosophila* germ line chromosomes. Science 218:341–347.

Warren, A. M. and J. M. Crampton. 1994. *Mariner*: its prospects as a DNA vector for the genetic manipulation of medically important insects. Parasitol. Today 2:58–63.