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Conservation of a highly repeated DNA family of *Aedes albopictus* among mosquito genomes (Diptera: Culicidae)

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Summary. The genomic organization and chromosomal localization of a cloned 0.79-kb highly repeated DNA fragment, H-115, isolated from *Aedes albopictus* has been examined. The cloned fragment is a part of a larger unit of 1.86 kb that is tandemly repeated in the *Ae. albopictus* genome. The H-115 family of sequences are located at the intercalary position on chromosome 1 in *Ae. albopictus*. Similar patterns of in situ and Southern blot hybridization results are obtained in *Ae. aegypti*, *Ae. seatoi*, *Ae. flavopictus*, *Ae. polynesiensis*, *Ae. alcasidi*, and *Ae. katherinensis*. The H-115 sequences are widely conserved in Culicidae and are found in *Haemagogus equinus*, *Tripteroides bambusa*, and *Anopheles quadrimaculatus* by hybridization under high stringency conditions. The H-115 sequences are also tandemly repeated in *Hg. equinus* with a monomer unit of 1.86 kb and in *Tp. bambusa* with a slightly diverged monomer unit of 1.90 kb. In *Anopheles quadrimaculatus*, the H-115 sequences are dispersed throughout the genome. Partial sequence analysis shows that the H-115 insert is 62% AT and contains two perfect inverted repeats and numerous perfect direct repeats. The occurrence of inverted repeats with potential to form intrastrand palindromic structure suggests that the H-115 family of sequences may be involved in chromatin condensation.

Key words: Repetitive DNA – Genomic organization – Chromosomal localization – DNA sequence – Mosquitoes

Introduction

Repetitive DNA sequences comprise a large proportion of eukaryotic genomes (Singer 1982; Bouchard 1982). There may be hundreds or thousands of families of repetitive DNA sequences in a genome, varying in sequence length from two to several thousand base pairs (Singer 1982; Bouchard 1982). These sequences may exist as long tandemly repeated arrays or may be dispersed throughout the genome (Singer 1982; Bouchard 1982). The variation in the amount of repetitive DNA sequences has been shown to be associated with changes in nuclear DNA content (Flavell et al. 1974; Black and Rai 1988). Moreover, much of the evolutionary history of eukaryotic genomes reflects the turnover of repetitive sequences, and speciation may be a consequence of such turnover (Rose and Doolittle 1983).

The mosquito family Culicidae is classified into three subfamilies, Toxorhynchitinae, Culicinae and Anophelinae, and contains approximately 3,100 described species. Among them, the diploid chromosome complement of about 200 species and the 1C-DNA content of 38 species are known to date (Jost and Mamelli 1972; Rao and Rai 1987a, b, 1990). A nearly eightfold difference in nuclear DNA content is associated with the same numbers of three pairs of chromosomes in all but one species of mosquitoes so far examined (Rao and Rai 1987a, b; Rao and Rai 1990). Of these three subfamilies, only species of Anophelinae display sex-chromosome dimorphism (White 1980). Reassociation DNA kinetic studies in mosquitoes have shown that the variation in nuclear DNA content is due mainly to differences in the amount of repetitive sequences (Black and Rai 1988). A linear relationship has been found between the 1C-DNA content and the proportion of genome represented by repetitive DNA sequences that make up 20–84% of mosquito genomes (Black and Rai 1988). Therefore, mosquitoes can be used as excellent models to study the role of repetitive sequences in molecular mechanisms of speciation. The first step in understanding the role of repetitive

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sequences in speciation is to examine the genomic and chromosomal organization of repetitive sequences. However, not much is known about the genomic and chromosomal organization of repetitive DNA sequences in mosquitoes (Redfern 1981; Gale and Crampton 1987; Black and Rai 1988).

In this communication we report on the genomic organization and chromosomal localization of a cloned 0.79-kb highly repeated DNA fragment, H-115, in the Asian tiger mosquito, *Aedes albopictus*. This clone was isolated from a library of cloned DNA fragments generated by ligation of *Eco*RI fragments from the Oahu (Hawaii) strain of *Ae. albopictus* at the *Eco*RI site of the plasmid pUC 12 (McLain et al. 1987). Furthermore, we have also examined the genomic organization and chromosomal localization of H-115 DNA in six other *Aedes* species. To determine as to how widespread the sequences homologous to H-115 DNA are in other genera of the mosquito family Culicidae, we also hybridized DNAs isolated from *Haemagogus equinus*, *Tripteroides bambusa* (both from the subfamily Culicinae) and *Anopheles quadrimaculatus* (subfamily Anophelinae) with H-115 DNA. A partial nucleotide sequence of the H-115 insert is also presented.

Materials and methods

Species examined

All mosquito species examined in this study were obtained from laboratory populations maintained in the Vector Biology Laboratory, Department of Biological Sciences, University of Notre Dame. Table 1 provides a list of species and strains with their sources and dates of colonization in the laboratory.

Table 1. Sources and dates of colonization of species and strains used in this study

Species	Strain, source and date of colonization
Aedes albopictus	Oahu, Hawaii, L. Rosen, 1971; Savannah, USA, J.D. Miller, 1988; Koh Samuii, Thailand, D. Gould, 1970
Ae. seatoi	Bangkok, Thailand, D. Gould, 1972
Ae. flavopictus	Nagasaki, Japan, Y. Eshita, 1981
Ae. Aegypti	Rockefeller Institute, USA, D.W. Jenkins, 1959; Houston, USA, D. Sprenger, 1986
Ae. polynesiensis	Suva, Fiji, D. Gubler, 1982
Ae. alcasidi	Taiwan, J. Lien, 1981
Ae. katherinensis	Australia, G. Davis and P. Whelan, 1982
Haemagogus equinus	Panama, J.L. Peterson, 1979
Tripteroides bambusa	Kyusu Island, Japan, W. Hawley, 1987
Anopheles quadrimaculatus	Savannah, USA, H. Schoof, 1971

DNA blot hybridization

High-molecular-weight DNA was extracted from female pupae using the procedure described by Blin and Stafford (1976). Restriction enzyme digestions were performed under the conditions recommended by the supplier (Promega USA). The digested DNA samples were resolved on 0.8% agarose submarine gel in TBE buffer with ethidium bromide (0.5 µg/ml) at 1.5 V/cm. DNA fragments in the gel were transferred to a nylon filter as described in Sambrook et al. (1989). The filter was prehybridized for 3–4 h at 40 °C with shaking in a solution containing 5 × SSC, 1% SDS, 10% dextran sulphate, 50% formamide, 100 µg/ml calf-thymus DNA, and 1 × Denhardt's solution. The hybridization was carried out overnight at the same temperature following the addition of H-115 DNA labelled with P^{32}-dCTP (3,000 Ci/mmole) according to the nick-translation procedure of Sambrook et al. (1989). The filter was washed with shaking in 1 × wash buffer (0.3 *M* NaCl, 0.06 *M* Tris, pH 8.0, 0.002 *M* EDTA, pH 8.0) (2 × 10 min) at room temperature, 1 × wash buffer plus 1% SDS (2 × 30 min) at 60 °C, and finally in 0.1 × wash buffer (2 × 30 min) at room temperature. The filter was exposed to a Kodak XOMAT X-ray film at −70 °C with an intensifying screen for 1–3 days.

In situ hybridization

Chromosome squash preparations were made from pupal testes and neural cells of colchicine-treated fourth instar larvae as described in Rao and Rai (1987b). In situ hybridization was carried out following the procedure of Kumar and Rai (1990).

DNA sequencing

The 0.79-kb insert was excised from the plasmid pUC 12 and was recloned in the pBluescript SK + II phagemid (Stratagene USA). The single-stranded DNA was isolated (Sambrook et al. 1989) and sequenced by the dideoxy chain-termination method (Sanger et al. 1977) with a T7 DNA polymerase kit (Promega USA) and S^{35}-dATP (1,000 Ci/mmole). The band compression and stops were resolved using deaza-dGTP and Taq DNA polymerase (Promega USA). Nucleotide sequences were analyzed using the MacVector program (IBI USA).

Results

Genomic organization

The basic genomic organization of the H-115 clone was examined by Southern blot hybridization. To determine whether the cloned 0.79-kb *Eco*RI repetitive DNA fragment was from dispersed or tandemly repeated sequences, genomic DNA from the Oahu strain of *Ae. albopictus* was digested separately with *Eco*RI, *Bam*HI, *Bgl*I, *Xho*I, *Pvu*II, and *Pst*I. The digests were electrophoresed in an agarose gel, blotted to a nylon filter and probed with P^{32}-labelled H-115 DNA. If the cloned fragment was a part of tandemly repeated sequences, a single or multiple arithmetic ladder of bands would be observed. The *Eco*RI pattern showed a band 1.86 kb in length (Fig. 1, lane 1), indicating that the cloned DNA fragment belonged to tandemly repeated sequences. This also indicated that one *Eco*RI site was present in each 1.86-kb monomer unit. Hereafter we would refer to these se-

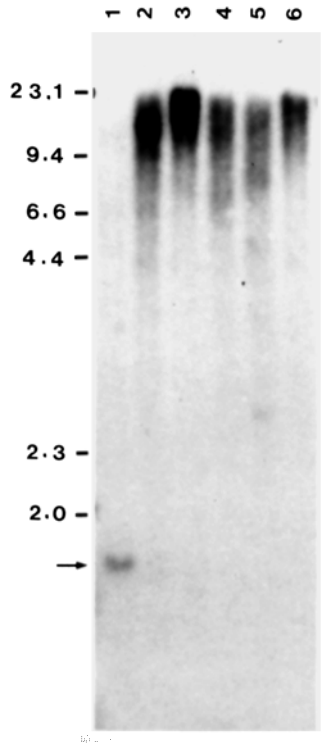

Fig. 1. Southern blot hybridization analysis of the *Aedes albopictus* DNA. The DNA samples (1 μg) were digested with *Eco*RI (*lane 1*), *Bam*HI (*lane 2*), *Bgl*I (*lane 3*), *Xho*I (*lane 4*), *Pvu*II (*lane 5*), and *Pst*I (*lane 6*), and the blot was probed with H-115 DNA. Molecular weight markers are indicated at the *left* in kb

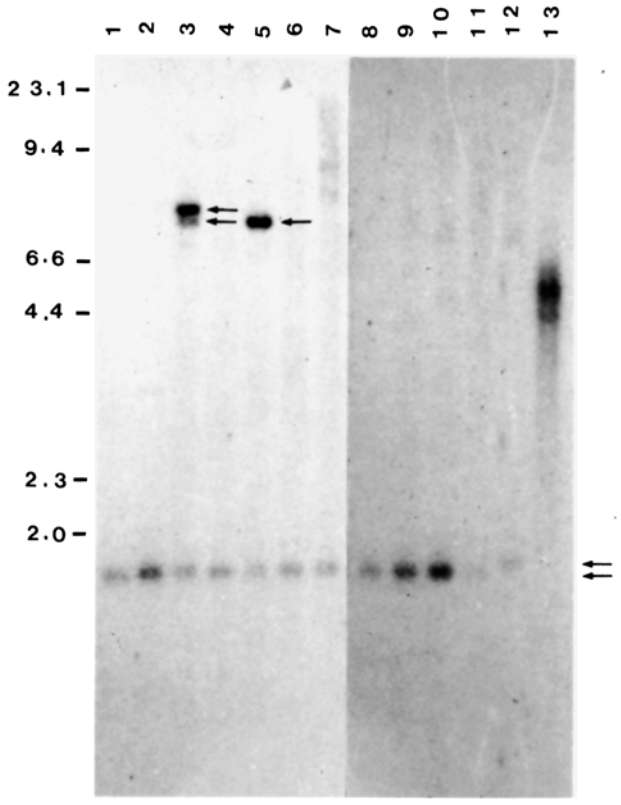

Fig. 2. Southern blot hybridization of *Eco*RI-digested DNA samples from different species to H-115 DNA. *Lanes 1–13* correspond to *Ae. aegypti* (Rockefeller), *Ae. aegypti* (Houston), *Ae. albopictus* (Oahu), *Ae. albopictus* (Savannah), *Ae. albopictus* (Koh Samuii), *Ae. flavopictus*, *Ae. seatoi*, *Ae. polynesiensis*, *Ae. alcasidi*, *Ae. katherinensis*, *Haemagogus equinus*, *Tripteroides bambusa*, and *Anopheles quadrimaculatus*. Each lane contains 1 μg DNA. Molecular weight markers are indicated at the *left* in kb

quences as the H-115 family. A smeared hybridization pattern in the top area of the gel was observed with other restriction enzymes (Fig. 1, lanes 2–6), indicating that the recognition sites for these enzymes were rare or completely lacking in the H-115 family of sequences. To determine the presence and organization of H-115 sequences in other species of mosquitoes, genomic DNA samples from all species and strains were digested with *Eco*RI, and the blot was probed with P^{32}-labelled H-115 DNA. Hybridization under high stringency conditions with the H-115 clone to *Eco*RI-digested DNA from all species detected positive signals (Fig. 2). The Southern profile showed a single band 1.86 kb in length in all species except *Tp. bambusa* and *An. quadrimaculatus*. A slightly larger fragment with a length of 1.90 kb was seen in *Tp. bambusa* (Fig. 2, lane 12). In *An. quadrimaculatus* the probe showed a dispersed hybridization (Fig. 2, lane 13). Two bands of 7.44 and 7.08 kb in the Oahu strain (Fig. 2, lane 3) and a band of 7.08 kb in the Koh Samuii strain (Fig. 2, lane 5) of *Ae. albopictus* were also observed. It might be noted that the genomic DNA samples from the Oahu strain in Figs. 1 (lane 1) and 2 (lane 3) were from the same DNA preparation. This indicated that two bands of 7.44 (a tetramer of the 1.86-kb fragment) and 7.08 kb resulted from partial digestion. When the genomic DNA from the Koh Samuii strain was redigested with *Eco*RI and the blot was probed with ^{32}p-labelled H-115 DNA, only 1.86-kb band was observed, indicating that the 7.08-kb band in this strain was also the result of partial digestion (data not shown).

Chromosomal localization

To determine the chromosome location of H-115 DNA, H^3-labelled H-115 DNA was hybridized in situ to chromosome preparations from the Oahu strain of *Ae. albopictus*. Autoradiographic silver grains were located at the intercalary position of the smallest pair of chromosomes (chromosome 1) (Fig. 3a, b). In situ hybridization of H^3-labelled H-115 DNA to meiotic chromosomes from the testes of pupae showed that H-115 DNA was located on both homologues of chromosome 1 (Fig. 3c). In interphase nuclei, a single cluster of silver grains was observed (Fig. 3d). Similar patterns of in situ hybridization results were also observed for the Savannah and Koh Samuii strains of *Ae. albopictus* (data not shown), *Ae. aegypti*,

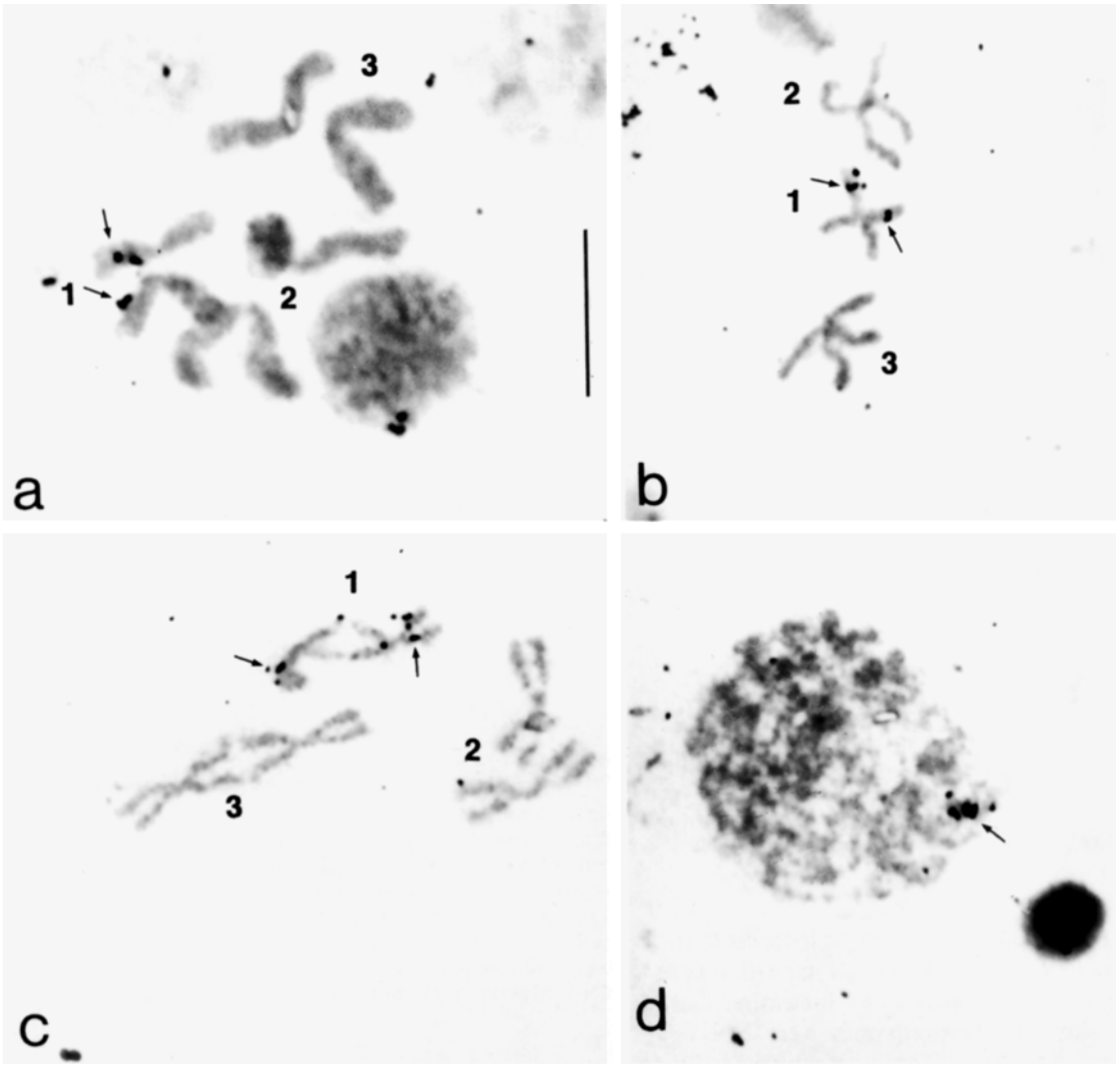

Fig. 3a–d. Chromosomal localization of H-115 DNA in *Aedes albopictus* (Oahu strain): **a** mitotic metaphase plate, **b** meiotic metaphase-II, **c** two cells from the same spermatocyte at metaphase-II, **d** meiotic interphase nucleus. *Arrow* points to sites of hybridization. Exposure time 4 months. *Bar* = 10 μm

Ae. seatoi, *Ae. flavopictus*, *Ae. polynesiensis*, *Ae. alcasidi* and *Ae. katherinensis* (Fig. 4).

DNA sequence

The nucleotide sequence of only 284 bp of the H-115 insert was determined reading from the 5′ end because of frequent stops and T clustering that could not be solved completely using deaza-dGTP and Taq DNA polymerase (Fig. 5). The sequence was 62% AT and consisted of two perfect 10-bp inverted repeats and numerous perfect direct repeats 4–11 bp in length. The two perfect 10-bp inverted repeats have the potential to form an intrastrand palindromic structure with a loop (Fig. 6). An extensive search of the GenBank database revealed that the 284-bp sequence shared very little homology with previously published sequences.

Discussion

We have demonstrated that the H-115 family of repetitive DNA sequences isolated from the *Ae. albopictus* genome are widely conserved in Culicidae and are found in the nine species examined belonging to two subfamilies. The Southern hybridization analysis indicates a tandem genomic organization of the H-115 family of sequences in the different species of mosquito examined except in *An. quadrimaculatus*. To our knowledge, the H-115 family is the third tandemly repeated sequence (satellite

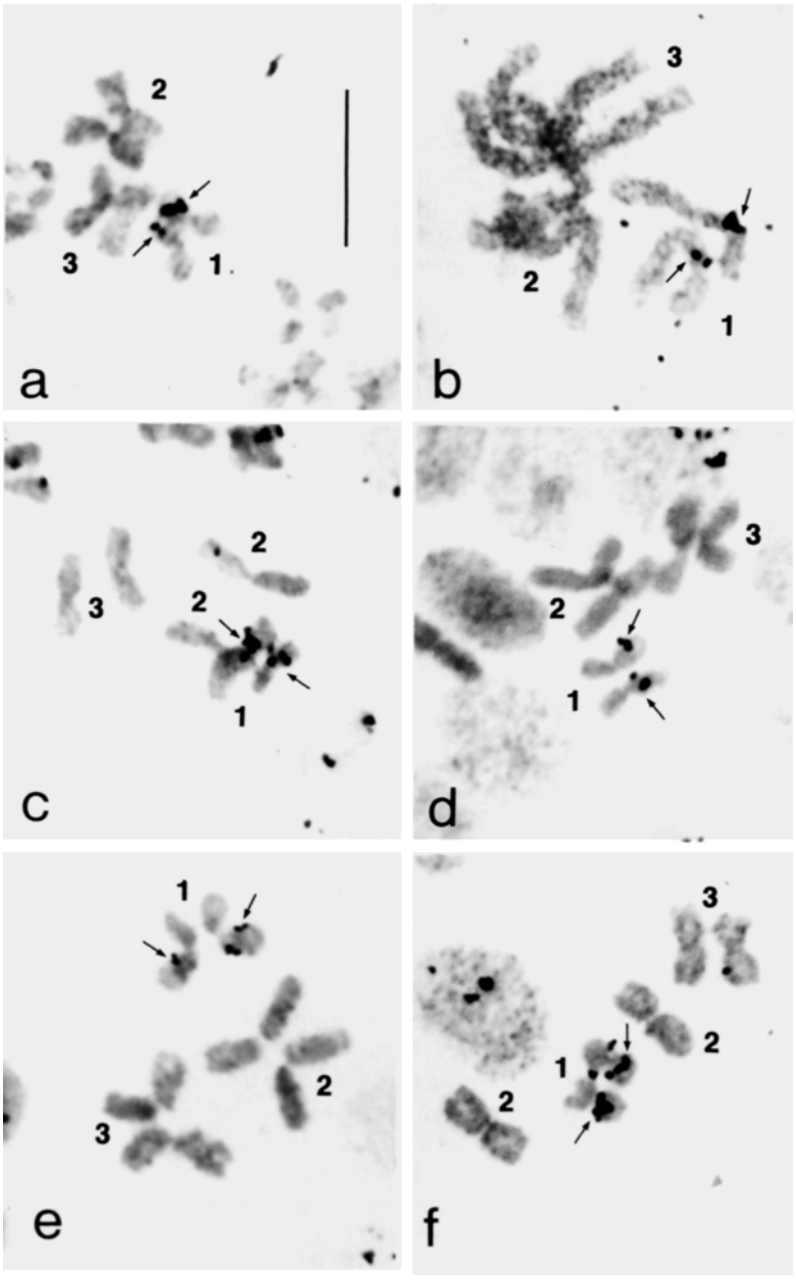

Fig. 4. Chromosomal localization of H-115 DNA on mitotic chromosomes of *a Aedes aegypti* (Rockefeller), *b Ae. seatoi*, *c Ae. flavopictus*, *d Ae. polynesiensis*, *e Ae. alcasidi*, and *f Ae. katherinensis*. Exposure time 7 months. *Bar* = 10 μm

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AATTCATCTG  GAAATTTCTT  CAAGAAGGAC  TCCTCCTAGA  ACTCTTTCTG  AGATTCCTCC  AGGAACTTCG  70
                        ----------> 1                                  ==========> 2

TGAAGGATTG  ATTTTACCAG  GAACTCCTTC  AAGTATTTCT  CTTGGAGTTC  CTCGAGGGAT  TTTTTCTGGA  140
                        ==========> 3

ATTTCTTGAC  AGATTATATC  AGGAACTTCT  TCAGTGATTT  TAACAGGAGT  TTTTTCAAGT  ATTTAAAGAG  210
                        ==========> 2                                  ==========> 3

GAAGTGCTAC  AGGAGTCCTT  CAGATTTTTT  TCCTGGATTT  TTCAGCATTT  CTTCTGAATT  GCTGGAGCGT  280
            ----------> 1

TCTC
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Fig. 5. A partial nucleotide sequence of the H-115 insert reading from the 5′ end. The *closed arrows* mark the position and the direction of inverted repeats; the *open arrows* mark the position and direction of direct repeats. Each pair of inverted repeats and direct repeats is numbered with the same *arabic numerals*; direct repeats of 10 or more base pairs are only *marked*

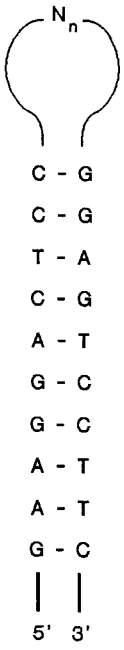

Fig. 6. Possible intrastrand palindromic structure generated by two 10-bp inverted repeats

DNA) described in mosquitoes, the other two being the satellite DNAs of *Anopheles stephensi* (Redfern 1981) and *Anopheles melas* (Gale and Crampton 1987). In the absence of any cross-hybridization or sequence data it is impossible to know of any clear relationships between these three tandemly repeated sequences. However, comparison of the in situ and Southern hybridization results of the H-115 DNA of *Ae. albopictus* with those of the satellite DNAs of *An. stephensi* and *An. melas* indicates that any relationship among these DNA sequences is highly unlikely. Unlike the situation in mosquitoes, tandemly repeated sequences have been extensively characterized in other insects such as *Drosophila*, *Sarcophaga*, the wasps, and the grasshoppers (see John and Miklos 1979; Arnold and Shaw 1985; Bigot et al. 1990; Hershfield and Swift 1990).

The Southern hybridization experiment in the Oahu strain of *Ae. albopictus* demonstrates that, in addition to the 1.86-kb band and its tetramer 7.44-kb band, there is a band of 7.08 kb that is not a multiple of the 1.86-kb fragment. A similar finding has been described in rye, *Secale cereale*, by Sandery et al. (1990): in addition to the 1.1-kb band and its multiple, the 2.2-kb band, there are higher molecular size bands that are not multiples of the 1.1-kb band. An insertion event by unrelated sequences into the original array of 1.1-kb repeats was postulated to explain this phenomenon in rye (Sandery et al. 1990). The presence of a 7.08-kb band in *Ae. albopictus* could also be explained by the insertion of an unrelated sequence in the H-115 family. No band of the size of the cloned fragment was observed in the Southern blot of *Eco*RI-digested *Ae. albopictus* DNA, indicating that the cloned 0.79-kb fragment is a part of the 1.86-kb monomer.

It has been proposed that the mosquito family Culicidae evolved from the *Mochlonyx*-like ancestor with $2n=8$ of the nematocera family Chaoboridae (Rao and Rai 1987b). Rao and Rai (1987b) proposed that a single line arose from the *Mochlonyx*-like ancestor, which then split into two: one that led to the mosquito subfamily Anophelinae through the *Chagasia*-like ancestor with $2n=8$; the other split into two separate lines leading to other two subfamilies, Culicinae and Toxorhynchitinae. The hybridization of H-115 DNA to the *An. quadrimaculatus* DNA indicates that the sequences homologous to H-115 DNA were present before the subfamilies Culicinae and Anophelinae diverged from each other.

The subfamily Culicinae is divided into ten tribes including Aedini and Sabethini; the tribe Aedini is classified into nine genera including *Aedes* and *Haemagogus*. The tribe Sabethini is divided into nine genera including *Tripteroides* (see Knight and Stone 1977). Based on this classification, a taxonomic correlation exists between the genomic organization of the H-115 family and the taxonomic grouping of the species. For example, all species from the tribe Aedini have a monomer unit of 1.86 kb, whereas *Tp. bambusa* of the tribe Sabethini has a different 1.90-kb monomer unit. The presence of a constant 1.86-kb monomer unit in all species belonging to the tribe Aedini suggests that there may be some selective constraint on the size of such units. These findings also suggest that the genus *Haemagogus* is evolutionarily more closely related to the genus *Aedes* than is the genus *Tripteroides* and corroborate their taxonomic groupings. *An. quadrimaculatus* of the subfamily Anophelinae does not possess any of these monomers, and the H-115 family of sequences are dispersed in its genome. Since *An. quadrimaculatus* is the only species analyzed from the subfamily Anophelinae, we do not know if the dispersed organization of the H-115 family is also present in other species of this subfamily. At present, we also do not know as to how the H-115 family of sequences are organized in the ancestor of the mosquito family Culicidae.

Based on morphological, allozyme, and geological histories, Pashley et al. (1985) suggested that the *Ae. albopictus* and the *Ae. scutellaris* subgroups of *Aedes* diverged from one another about ten million years ago. The three species examined from the *Ae. albopictus* subgroup (*Ae. albopictus*, *Ae. seatoi* and *Ae. flavopictus*) and from the *Ae. scutellaris* subgroup (*Ae. polynesiensis*, *Ae. alcasidi* and *Ae. katherinensis*) have the H-115 family of sequences on chromosome 1. The site of the H-115 family on chromosome 1 has thus been conserved for approximately ten million years.

The chromosome complement of all species of Culicinae consists of three pairs of homomorphic chromosomes: a pair of small chromosomes (chromosome 1), a

pair of large chromosomes (chromosome 2), and a pair of intermediate size chromosomes (chromosome 3) (Rai 1963, 1966; White 1980; Rao and Rai 1987b). The sex is determined by a pair of alleles for which the females are homozygous and the males are heterozygous (Gilchrist and Haldane 1947). In species where the correlation between the linkage groups and chromosomes has been made the sex-determining alleles are found to be located on chromosome 1 (McDonald and Rai 1970; Baker et al. 1971; Dennhöfer 1972). Thus, in the males one chromosome 1 is male determining and the other is female determining, while both chromosome 1 are female determining in the females. C-banding analysis has shown a centromeric block of heterochromatin in each of three pairs of chromosomes in all species of Culicinae so far examined (Motara and Rai 1978; Rao and Rai 1987b). In addition to centromeric blocks of heterochromatin, an intercalary band of heterochromatin is also found on the female-determining chromosome 1 in a few species, namely, *Ae. aegypti*, *Ae. albopictus*, *Ae. polynesiensis*, and *Ae. alcasidi* (Motara and Rai 1978; Rao and Rai 1987b). The intercalary band of heterochromatin is absent in *Ae. katherinensis* (Rao and Rai 1987b). Since the H-115 family of sequences are present at the intercalary position of chromosome 1 in species with and without the intercalary band of heterochromatin and also on both male- and female-determining chromosomes, it is unlikely that the H-115 family is located in the intercalary band of heterochromatin. In situ hybridization studies have previously shown that tandemly repeated DNA sequences are usually, but not exclusively, located in heterochromatic blocks of chromosomes (see John and Miklos 1979). The H-115 family is one of the few examples, such as tandemly repeated DNAs of *Vicia faba* and *Triturus vulgaris meridionalis*, which are not located in heterochromatin (see John and Miklos 1979; Timmis et al. 1975; Vignali et al. 1991).

The nucleotide sequence analysis indicates that there are many internal homologous sequences (direct repeats) distributed throughout the 284-bp length of the H-115 insert sequenced. However, no internal tandem repetition of direct repeats was observed. It appears that during the long period of amplification and maintenance of sequences, an internal tandem subrepeat structure might have been lost by sequence divergence through many base substitutions, deletions, and additions.

The role of repetitive DNA sequences is highly speculative. The association of tandemly repeated sequences with centromeric and telomeric heterochromatin (John and Miklos 1979) has led to suggestions that they have a role in chromosomal pairing (Rose and Doolittle 1983). On the other hand, Orgel and Crick (1980) suggested that repetitive sequences are parasitic elements and are concerned solely with their survival in the genome without fulfilling any meaningful function. Based on the sequence analysis of the satellite DNAs of parasitic wasps, Bigot et al. (1990) have suggested that inverted repeats with the potential to form intrastrand palindromic structures may play a role in the mode of chromatin condensation. The presence of two 10-bp inverted repeats with the potential to form an intrastrand palindromic structure with dyad symmetry in the H-115 family indicates the same function for this family.

In conclusion, the present study has shown that the H-115 family of repetitive DNA sequences isolated from *Ae. albopictus* are found in nine species of mosquitoes belonging to four genera and two subfamilies under high stringency conditions of hybridization. Further study is needed to know if the H-115-related sequences are also present in other genera of the three subfamilies of mosquitoes. We suggest that in situ hybridization of the H-115 clone in combination with other probes on the chromosomes of mosquito species will be useful for constructing the chromosome phylogeny of mosquitoes on which virtually no work has been done.

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