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Recent amplification of miniature inverted-repeat transposable elements in the vector mosquito *Culex pipiens*: characterization of the *Mimo* family

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Abstract

We describe a new family of repetitive elements, named *Mimo*, from the mosquito *Culex pipiens*. Structural characteristics of these elements fit well with those of miniature inverted-repeat transposable elements (MITEs), which are ubiquitous and highly abundant in plant genomes. The occurrence of *Mimo* in *C. pipiens* provides new evidence that MITEs are not restricted to plant genomes, but may be widespread in arthropods as well. The copy number of *Mimo* elements in *C. pipiens* (~1000 copies in a 540 Mb genome) supports the hypothesis that there is a positive correlation between genome size and the magnitude of MITE proliferation. In contrast to most MITE families described so far, members of the *Mimo* family share a high sequence conservation, which may reflect a recent amplification history in this species. In addition, we found that *Mimo* elements are a frequent nest for other MITE-like elements, suggesting that multiple and successive MITE transposition events have occurred very recently in the *C. pipiens* genome. Despite evidence for recent mobility of these MITEs, no element has been found to encode a protein; therefore, we do not know how they have transposed and have spread in the genome. However, some sequence similarities in terminal inverted-repeats suggest a possible filiation of some of these mosquito MITEs with *pogo*-like DNA transposons.

Keywords: Genome evolution; MITEs; *pogo*; Repetitive DNA; Transposons

1. Introduction

Recently, numerous families of short (100–500 bp) transposable elements with terminal inverted repeats have been found in high copy numbers in a wide range of plant genomes (Bureau and Wessler, 1992, 1994a; Bureau et al., 1996; Pozueta-Romero et al., 1996; Casacuberta et al., 1998; Song et al., 1998; Charrier et al., 1999). Based on common structural features, these elements have all been designed as MITEs (miniature-inverted-repeat transposable elements). Some mobile elements similar in structure to the plant MITEs have been described in fungi (Yeadon and Catcheside 1995) and in some vertebrates (Unsal and Morgan, 1995; Smit, 1996; Izsvák et al., 1999). In invertebrates, typical MITEs have been characterized from only two insect species: the yellow fever mosquito, *Aedes aegypti* (Tu, 1997), and the beetle, *Tenebrio molitor* (Braquart et al., 1999), whereas none have been described from the more extensively studied genome of *Drosophila melanogaster*. This distribution suggests that there might be a correlation between the level of MITE proliferation and the size and organization of the host genome; the larger it is, the higher the MITE copy number could be (Tu, 1997; Casacuberta et al., 1998; Braquart et al., 1999; Charrier et al., 1999).

To date, the mechanism of transposition of MITEs remains unknown. Several of their characteristics, including TIRs and short (2–3 bp) specific duplications generated upon insertion, suggest that MITEs may have spread by DNA transposition. This hypothesis is further

Abbreviations: bp, base pair; kb, kilobase; Mb, megabase; MITE, miniature inverted-repeat transposable element; PCR, polymerase chain reaction; SIR, subterminal inverted repeat; TIR, terminal inverted repeat.

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supported by the recent evidence that one plant MITE family has originated from a larger element encoding a transposase (Feschotte and Mouchès, 2000). However, so far, no excision event has been reported for a MITE. Furthermore, it is unlikely that DNA transposition, which by itself is a non-replicative process, is responsible for such a high level of proliferation in genomes (Izsvák et al., 1999; Feschotte and Mouchès, 2000).

Here, we report that the *C. pipiens* mosquito, an insect with a relatively large genome and a high amount of repetitive DNA, does contain MITEs. Structural analysis of one family, *Mimo*, indicates that it has a recent amplification origin and may have originated from a *pogo*-like DNA transposon, as previously demonstrated for a plant MITE family (Feschotte and Mouchès, 2000).

2. Material and methods

2.1. Mosquito strains and genomic DNAs

The first *Mimo-Cp*1 copy was identified in a λ clone previously isolated from a genomic library of the Tem-R strain of *C. pipiens* (California, USA). All other copies are from the Ravenna strain of *C. pipiens* (Italy). For Southern experiments, we also used genomic DNAs from the following insects: *C. pipiens* Willow (California), *C. pipiens* Pro-R (California), *C. pipiens* cells (Taiwan), *C. hortensis* (collected in the field, South of France), *Aedes aegypti* Hanoï, *A. albopictus* Oahu 71, *A. triseriatus* cells (Trois Rivières, Canada), *Anopheles stephensi* (obtained from the MNHN Paris), *Drosophila melanogaster* (Canton), and *Musca domestica* (collected in the field, South of France).

2.2. Southern blot analysis

DNA was prepared from adult mosquitoes, as described previously (Mouchès et al., 1986). Aliquots of 10 μg of genomic DNA were digested to completion with *Eco*RI restriction endonuclease. Resulting fragments were separated on 1% agarose gels, transferred to a nylon membrane (Amersham) and hybridized at high stringency (65°C) with radiolabelled probes. Other procedures were as previously described (Mouchès et al., 1990). Probes were obtained by PCR amplification from plasmids carrying a copy of the element using a degenerate primer corresponding to the TIR (see below), gel-purified and labelled with α^{32}P-dCTP by random priming (Pharmacia).

2.3. Isolation of Mimo copies from a C. pipiens genomic library and estimation of copy number

A library was prepared by complete *Eco*RI digestion of genomic DNA from the Ravenna strain of *C. pipiens* and ligation into a λ-gt11 cloning vector (Stratagene). About 20 000 recombinant phages were plated and screened using a *Mimo-Cp*1 probe obtained by PCR amplification of the original *Mimo* element found in *CM-gag*4, a copy of the retroposon *CM-gag* (Bensaadi-Merchermek et al., 1997) (see Section 3.1). Prehybridization, hybridization and washing were carried out at 65°C, as previously described (Mouchès et al., 1990). After a first round of screening, a large number of positives were obtained. Several positive plaques were plugged in SM buffer and amplified, and each was used as a template for PCR amplification with universal primers for the arms of λ-gt11 vector. PCR parameters were the same as those described below, except that the annealing temperature was reduced to 54°C and the elongation time increased to 2 min 30 s. PCR products containing *Mimo* elements were identified by Southern hybridization with the *Mimo-Cp*1 probe, gel-purified and subcloned into pCR-TOPO plasmid vectors (Invitrogen).

Copy number for *Mimo* elements was estimated based on the ratio of positive phage plaques to the total number of plaques screened, taking into account the haploid genome size of *C. pipiens* of 540 Mb (Black and Rai, 1988) and an average genomic library insert size of 4 kb.

2.4. Additional Mimo copies isolated by PCR amplification

PCR reactions were performed according to standard procedures using a single oligonucleotide, 5′-CART-AGTTGTTCGGTAACTKG-3′, which is complementary to TIRs of the *Mimo-Cp*1 element. Cycling conditions were an initial 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 90 s at 55°C, 60 s at 72°C and a final 10 min elongation at 72°C. To assess *Mimo* length variation, additional PCR reactions were performed with increased elongation steps up to 2 min. PCR products were analysed on agarose gels and cloned directly into pCR-TOPO vectors (Invitrogen). Positive clones were identified by Southern hybridization with the *Mimo-Cp*1 probe.

2.5. Sequence analysis

Sequencing was done by the Eurogentec sequencing department with synthetic primers, using an ABI-377 automatic sequencer. Most of the sequence analysis was done using tools available at the Infobiogen WWW server (http://www.infobiogen.fr). Database searches

were performed with BLASTN (Altschul et al., 1990), using default parameters. Multiple sequence alignments were constructed by CLUSTAL W version 1.7 (Thompson et al., 1994) using default parameters. Pairwise alignments of nucleotide sequences were done using the ALIGN program (Myers and Miller, 1988) of the FASTA package. The ability of elements to form secondary structures was estimated by the DNA FOLD program (Zuker, 1994) available through the server http://mfold.wustl.edu/, and free energies were determined according to SantaLucia (1998). Sequences reported in this article appear in GenBank under the Accession Nos AF217611 to AF217616.

3. Results

3.1. Mimo, a family of highly reiterated elements in the genome of C. pipiens

The first *Mimo* element was discovered during the analysis of a genomic clone from the Tem-R strain of *C. pipiens*, which contains a copy of the retroposon *CM-gag* (Bensaadi-Merchermek et al., 1997). Sequence alignment of this copy with the canonical *CM-gag*1 element (Accession No. AF030588) shows that it is interrupted by a 348 bp insertion at position 1678 (Fig. 1). The presence of almost perfect TIRs and flanking 2 bp direct repeats suggests that this insertion may be a transposable element. A computer-assisted search in current DNA databases using this insertion sequence as a query failed to detect any similarity with previously described sequences. However, it shares a strong structural similarity with the newly described class of short transposons called MITEs (see Section 3.2). We have named this insertion sequence *Mimo-Cp*1 for MITE mosquito.

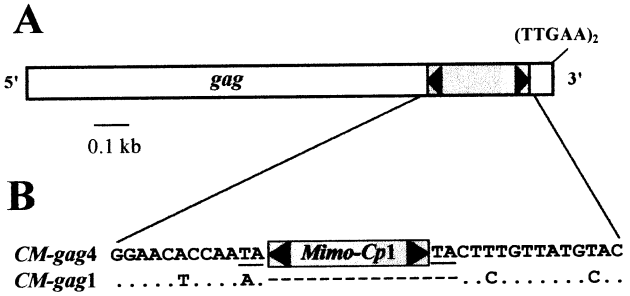

Fig. 1. Insertion of *Mimo-Cp*1 in a *CM-gag* retroposon copy. (A) Schematic structure of the *CM-gag*4 copy. The single ORF (encoding a putative *gag* protein) is represented by a hatched box. TTGAA tandem repeats define the 3′ end of *CM-gag* elements (Bensaadi-Merchermek et al., 1997). *Mimo-Cp*1 element is boxed in grey with black triangles for TIRs. (B) Partial sequence alignment of copies 1 and 4 of *CM-gag*. the putative target site duplication is underlined. Alignment is performed from position 1667 to 1690 of the published sequence of *CM-gag*1, GenBank No. AF030588 (Bensaadi-Merchermek et al., 1997).

*Mimo-Cp*1 was used as a probe to investigate the presence of related sequences in several dipterous species. Southern hybridizations with various genomic DNA digests reveal a long continuous smear for all tested strains of *C. pipiens* (data not shown). This demonstrates that *Mimo-Cp*1 belongs to a family of highly reiterated elements in this species. No hybridization signal was detected in other insects, including *Aedes* and *Anopheles* mosquito species. All *C. pipiens* strains contain about the same copy number of *Mimo* elements, but some variations in the banding pattern suggest that several locations are polymorphic between strains. The copy number of the *Mimo* MITEs in the *C. pipiens* genome was estimated by screening a genomic library from the Ravenna strain with *Mimo-Cp*1 as a probe. Based on the ratio of positive plaques to the total number of plaques screened and assuming a haploid genome size for *C. pipiens* of 540 Mb (Black and Rai, 1988), the copy number of *Mimo* elements is $\sim$1000 per haploid genome. This is probably an underestimation since several *Mimo* copies can be present in the same phage clone.

3.2. Mimo is a novel family of MITEs with a high sequence conservation

The variability of *Mimo* family was analysed by characterization of several copies. Using *Mimo-Cp*1 as a probe, we screened a genomic library from the Ravenna strain of *C. pipiens*. Two positive phage clones were randomly chosen and further characterized. Each genomic clone contained a *Mimo* copy with both structural and sequence similarity to the original *Mimo-Cp*1 element (Fig. 2).

Furthermore, we used PCR amplification of genomic DNA with a degenerate primer for the TIRs to assess length heterogeneity among the *Mimo* copies. Numerous PCR fragments, ranging from approximately 300 to 400 bp, were obtained from Ravenna genomic DNA and subsequently cloned. Twelve of them were randomly chosen for further characterization. All clones strongly hybridize with the *Mimo-Cp*1 probe under high-stringency conditions, which further indicates that there is a high sequence homogeneity between *Mimo* elements. Ten of the 12 copies are also homogeneous in size, ranging from 310 to 380 bp. The shortest of these clones (*Mimo-Cp*6, 311 bp) contains several large deletions in the central region of the element (Fig. 2) but a similar substitution rate compared to other *Mimo* copies (data not shown). Two PCR clones were significantly longer, with 520 and 707 bp, respectively. Sequence analysis for these two clones reveals that they contain 'regular' *Mimo* copies (*Mimo-Cp*4 and *Mimo-Cp*5 in Fig. 2) interrupted by other mobile elements (see Section 3.3). The pairwise

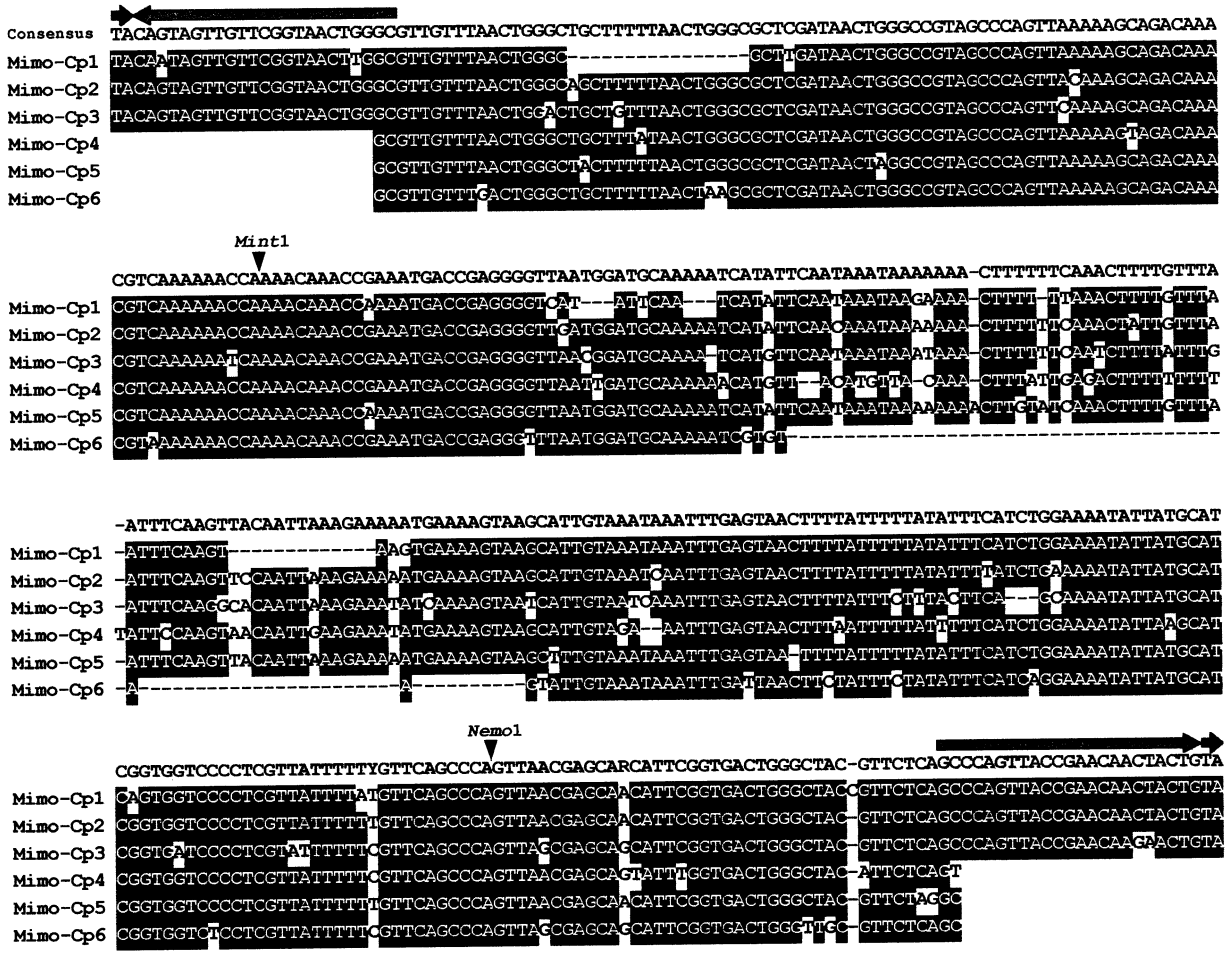

Fig. 2. Multiple sequence alignment of the Mimo family of elements. Mimo sequences were aligned using the CLUSTAL W program (Thompson et al., 1994) with default parameters. *Mimo-Cp*1, *Cp*2 and *Cp*3 were isolated from *C. pipiens* genomic libraries, so we align the entire *Mimo* sequence for these copies with their TIRs (grey arrows) and TA flanking direct repeats (black arrows). *Mimo-Cp*4, *Cp*5 and *Cp*6 were isolated by PCR using a degenerated TIR sequence as a primer, so only internal sequences are aligned. Conserved nucleotides (>50%) are indicated by white letters on a black background. Based on a simple majority rule, a consensus sequence was determined. *Mint*1 and *Nemo*1 insertion sequences are found into *Mimo-Cp*4 and *Mimo-Cp*5, respectively. They were extracted for clarity, but their positions are indicated above the consensus sequence.

sequence identity between *Mimo* copies is high, ranging from 74.3 to 95.8%.

A consensus sequence (Fig. 2) was calculated from the six *Mimo* copies (i.e., three elements obtained from genomic clones and three copies isolated by PCR amplification). *Mimo* elements are A+T-rich (~60%) and have no coding capacity. They possess 23 bp TIRs and additional short subterminal inverted repeats, so they could form stable secondary structures (ΔG values in the range of −53.5 to −68.6 kcal/mol). Analysis of the borders of the three *Mimo* copies isolated from the genomic libraries reveals that they are flanked by TA duplications (Fig. 2). This indicates that *Mimo* elements may insert preferentially at TA sites in the *C. pipiens* genome and that the target sequence is probably duplicated upon insertion. Since these features fit well with those defining the plant MITE families (Wessler et al., 1995), we conclude that *Mimo* elements belong to a novel family of highly conserved MITEs.

3.3. Some Mimo elements contain other MITE-like elements

Two of the 12 *Mimo* copies randomly chosen among PCR clones obtained from *C. pipiens* genomic DNA are interrupted by other putative transposable elements. Insertions occurred at two distinct positions in these copies (indicated in Fig. 2) but, interestingly, both have been inserted in *Mimo* elements after a CCA site. Comparison of *Mimo-Cp*4 and *Mimo-Cp*5 with other *Mimo* elements (Fig. 3) suggests that both insertion events were followed by a duplication of the dinucleotide CA. Southern experiments using each of these insertion sequences as a probe against *C. pipiens* genomic DNA show that these elements are highly reiterated in its genome (data not shown).

The first of these elements, named *Mint*1 (miniature nested transposon), was recognized as a 141 bp insertion sequence in *Mimo-Cp*4. Unlike classical MITEs, *Mint*1

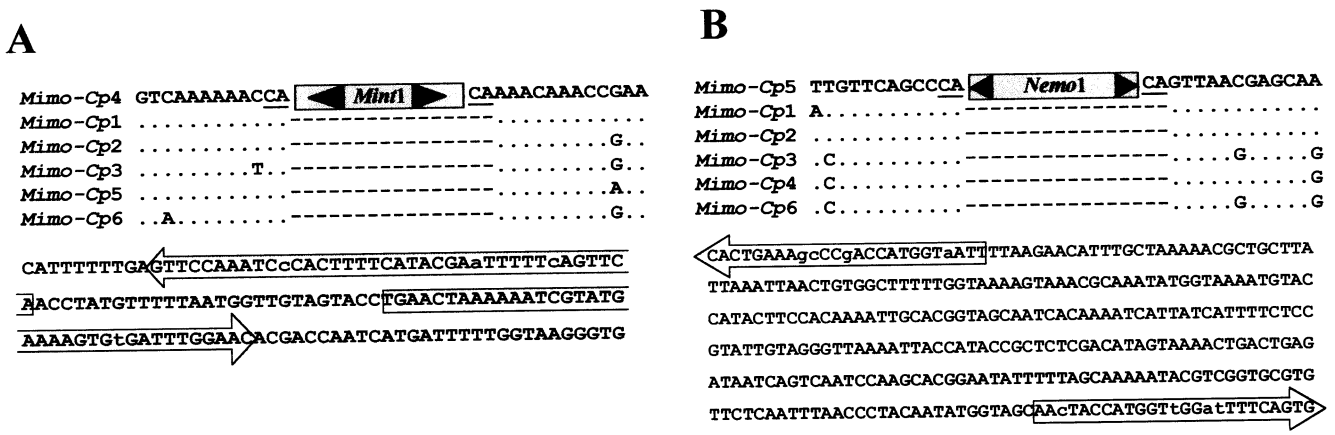

Fig. 3. MITE-like elements nested in *Mimo* elements. (**A**) Polymorphism corresponding to *Mint*1 insertion into *Mimo-Cp*4 and sequence of *Mint*1 MITE-like element. Subterminal inverted-repeats are boxed. (**B**) Polymorphism corresponding to *Nemo*1 insertion into *Mimo-Cp*5 and sequence of *Nemo*1 MITE-like element. TIRs are boxed. In each multiple alignment, dots denote identity, and dashed lines indicate gaps corresponding to the element insertion and one copy of the presumed target site. Putative target site duplications are underlined.

do not have recognizable TIRs but possesses nearly perfect (2 mismatches out of 37 bp) subterminal inverted-repeats (SIRs) so that it can be folded into a stem-loop structure (Fig. 3A). The absence of TIRs has recently been reported for two miniature transposon-like elements of the rice genome, *Pop* and *Crackle* (Song et al., 1998), that also have SIRs and the potential to form secondary structures. Although we assume that additional *Mint* sequences are needed to confirm this unusual structure, *Mint*1 probably belongs to this new type of MITEs.

*Mimo-Cp*5 is interrupted by another putative transposon that we have named *Nemo*1 (for nested mosquito MITE). This element is 324 bp long and contains imperfect 25 bp TIRs (Fig. 3B). Like *Mimo* and *Mint*1, *Nemo*1 is A+T-rich and does not appear to encode a protein. Because it has several SIR motifs, it could presumably form stable secondary structures ($\Delta G = -53.3$ kcal/mol). Based on these features, it is likely that *Nemo*1 is also a member of a new MITE family.

Mimo, *Mint* and *Nemo* elements have no significant sequence identity to each other or to any other known transposable elements. However, some striking similarities, in both size and sequence, exist between TIRs and target site duplications of *Mimo* and *Nemo* and those of several MITEs and Class II transposons (Fig. 4). This indicates a possible filiation of these *C. pipiens* MITEs with DNA transposons (see Section 4.4).

Element	TSD	TIRs	Organism	Type	Size,bp
Mimo	TA	CAGTAGTTGTTCGGTAACTGGGC	*C. pipiens*	MITEs	324
Pogo	TA	CAGTA-TAATTCGCTTACTGCATCGA	*D. melanogaster*	PLE	2121
MER(II)	TA	CAGTNGTCCCTCGNTATCCGCGGG	*H. sapiens*	MITEs	488
Tigger2	TA	CAGTTGACCCTTGAACAACaCGGG	*H. sapiens*	PLE	2417
Tigger1	TA	CAGGCATACCTCGTTTTATT-GCG	*H. sapiens*	PLE	2708
Nemo1	CA	CACTGAAAgCCCgACCATGGTAATT	*C. pipiens*	MITEs	346
Wujin	TA	CAGTGAAACCTCCATGA-GTTCGA	*A. aegypti*	MITEs	185
Lemi1/Emi	TA	CAGTAAAACCTCTATAA-ATTAAT	*A. thaliana*	PLE/MITEs	2114/524

Fig. 4. Homologies in TIRs and target site duplications between some of the mosquito MITEs and DNA transposons of the *pogo* family. Alignment of the TIRs sequences was constructed by eye. *Mimo*, *Nemo*1, *Wujin* (Tu, 1997) and *MERs*(II) (Smit and Riggs, 1996) can all be associated with a *pogo*-like Class II element (PLE). According to TIR similarities, *Mimo* is closer to *pogo*, *Nemo* and *Wujin* are closer to *Lemi*1, and *MERs*(II) are closer to *Tiggers*. For each group, conserved bases are highlighted by white letters on a black background. TIRs, target site duplication (TSD) sequences and length of *Wujin* and *Lemi*1/*Emigrant* and *Mimo* families are deduced from their consensus sequences (Tu, 1997; Casacuberta et al., 1998; Feschotte and Mouchès, 2000 and this work). Since only one copy of *Nemo*1 is available, and since it possesses imperfect TIRs, we have aligned the 5′ TIR of *Nemo*1, but we have indicated mismatches in lower-case letters. *MER*(II) represents a general consensus based on a simple majority rule that we have determined for the second group of human *MERs*, i.e., *MER28*, *MER8*, *MER2*, *MER44*, *MER46*, *MER6* and *MER7* (Smit and Riggs, 1996). N indicates a highly variable nucleotide. Other information on human transposons is from Smit and Riggs (1996), and data on *pogo* elements are from Tudor et al. (1992).

4. Discussion

4.1. Occurrence of the Mimo family of MITEs in the C. pipiens genome

We have shown that *Mimo* is a family of repetitive elements from the mosquito *C. pipiens* with a high structural and sequence homogeneity. *Mimo* sequences do not share a sequence similarity with any transposable element described to date, but they do possess all the characteristics that defined the novel class of transposons called MITEs (Wessler et al., 1995). These include a small and homogeneous size, the presence of TIRs and the absence of coding capacity. Moreover, *Mimo* elements can be potentially folded into stable secondary structures, another frequent characteristic of MITEs. It is noteworthy that, to date, very few MITE families have been described in animals, while it is argued that MITEs are the most prevalent type of transposon associated with flowering plant genes (Wessler, 1998). To our knowledge, *C. pipiens* is only the third invertebrate species for which a highly reiterated MITE family is described. This provides new evidence that MITEs are not restricted to plant genomes, but occurred in arthropods also.

We estimate the copy number of *Mimo* elements to be $\sim$1000 per haploid genome. This is a lower copy number than those generally reported for other MITE families. For example, the *Tourist* family is present at 10^4 copies in the maize genome (Bureau and Wessler, 1992), and MITE families described from the yellow fever mosquito, *Aedes aegyti*, are reiterated at $\sim$2000 copies per genome (Tu, 1997). The DEC element is also present at a high copy number in the beetle *Tenebrio molitor* ($\sim$3500 copies, Braquart et al., 1999). This has led some authors to propose that the proliferation of MITEs may be associated with large and complex genomes, in both plant and animal kingdoms (Bureau and Wessler, 1992; Tu, 1997; Braquart et al., 1999; Charrier et al., 1999). In this case, the copy number of *Mimo* elements is in the range of those from the yellow fever mosquito, since the genome of *C. pipiens* is approximately 1.5-fold smaller than those of *A. aegypti* (540 against 810 Mb). Studies using reassociation kinetics have shown that *Aedes* and *Culex*, both members of the Culicines tribe, possess complex genomes with a short-interspersion pattern of repetitive elements (Black and Rai, 1988). It was also demonstrated that there has been a general increase in genome size during the evolution of Culicines mosquitoes, with all classes of repetitive DNA increasing linearly in amount with total genome size (Black and Rai, 1988). Our results are consistent with this previous work, showing that the genome of *C. pipiens*, like those of *A. aegypti*, does contain MITEs but in lower amounts. This difference could reflect a higher level of proliferation of MITEs, and probably other classes of transposable elements, in *Aedes* than in *Culex* and/or differing rates of 'junk' DNA elimination between these mosquito species, as recently shown between *Drosophila* fruit-fly and *Laupala* crickets (Petrov et al., 2000).

4.2. Evidence for a recent amplification history of Mimo

The first member of the *Mimo* family was identified as an insertion sequence in a copy of another repetitive element, the *CM-gag* retroposon. This insertion event, as well as a putative target site duplication, provides evidence for past mobility of this *Mimo* copy. Furthermore, the *CM-gag* retroposons are highly conserved, indicating a recent amplification of these retroelements (Bensaadi-Merchermek et al., 1997). The *CM-gag*4 copy also shares a high sequence similarity with other *CM-gag* elements (from 86 to 90%), which suggests a very recent insertion event of *Mimo-Cp*1 in *CM-gag*4. In addition, we show that *Mimo* elements isolated from *C. pipiens* are highly conserved in both terminal and internal sequences, even between two distinct geographical isolates and also when secondary insertions of mobile elements have occurred within them. This is in contrast with most MITE families described earlier. Generally, members of the same family share a good conservation in structure and in terminal sequences but are generally more divergent in internal sequences, with a sequence variation up to 30% being frequent between two members of the same family (Bureau and Wessler, 1994a,b; Pozueta-Romero et al., 1996; Rio et al., 1996; Smit and Riggs, 1996; Charrier et al., 1999; Izsvák et al., 1999). We conclude, therefore, that amplification of *Mimo* elements is relatively recent in the *C. pipiens* genome. It is possible that some *Mimo* elements may still be able to transpose.

4.3. MITEs may be a nest for other MITEs

Among the 12 *Mimo* copies randomly isolated by PCR using the TIR as a primer, we report that two copies contain nested transposable elements, named *Mint*1 and *Nemo*1. Although we acknowledge that this approach is not quantitative, the ratio of secondary insertions in *Mimo* copies (16.6%) could be higher because the PCR cycling conditions that we used (1 min extension) have probably enhanced amplification of the shorter *Mimo* elements rather than larger copies. Nested MITEs in other MITEs were also reported in *Sorghum bicolor* where *Stowaway-Sb*1 was discovered as an insertion in *Tourist-Sb*5 (Bureau and Wessler, 1994a) and in *A. aegypti* with the *Wujin-Aa*1 element being found in the *Wukong-Aa*5 copy (Tu, 1997). Thus, it is possible that MITEs could be a preferential insertion site for other MITEs. Nested insertions in *Mimo* elements further support that recent and multiple transposition

bursts of various MITE-like elements occurred in the genome of *C. pipiens*.

4.4. *Mimo and Nemo elements could have originated from pogo-like transposons*

The transposition mechanism of MITEs has not yet been elucidated. Due to several structural similarities with DNA transposons as well as some sequence similarity in TIRs, it has been suggested that MITEs could transpose via a DNA intermediate by using transposase activity encoded elsewhere in the genome by another element (Bureau and Wessler, 1994a; Unsal and Morgan, 1995; Smit and Riggs, 1996; Tu, 1997). This hypothesis is further supported by the recent discovery that some MITEs described from the *Arabidopsis* and human genome are deleted forms of larger *pogo*-like DNA transposons that potentially encode a transposase (Smit and Riggs, 1996; Feschotte and Mouchès, 2000). This also suggests that a similar mechanism for the origin of MITEs exists in distinct eukaryote genomes. In this regard, it is striking that *Mimo* and *Nemo*1 as well as *Wujin*, a MITE family from the *A. aegypti* genome (Tu, 1997), display significant TIRs similarities with *pogo*-like transposons (Fig. 4). In addition, insertion of these elements is probably followed by a 2 bp target site duplication that appears to be specific (TA) for *Mimo* and *Wujin* elements. This feature is reminiscent of the TA target site preference of *pogo*-like elements and other members of the Tc1/*mariner* superfamily (van Luenen et al., 1994; Hartl et al., 1997; Plasterk et al., 1999). It is therefore tempting to speculate that *Mimo* and *Nemo*1, from *C. pipiens*, as well as the *Wujin* family from *A. aegypti*, are *pogo*-like transposon derivatives. In this case, *pogo*-like elements may have resided, at least at an ancient time, in their genomes. We now have to investigate the presence of such elements in these mosquito genomes to extend the DNA transposon origin of MITEs to these families.

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