Characterization and chromosomal mapping of the DgmarMITE transposon in populations of Dichotomius (Luederwaldtinia) sericeus species complex (Coleoptera: Scarabaeidae)

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Abstract

Transposable elements are dispersed repetitive DNA sequences that can move within the genome and are related to genome and chromosome evolution, adaptation, and speciation. The aim of this study was to characterize and determine the chromosomal location and accumulation of a Mariner-like element in populations of four phylogenetically related species of the Dichotomius (Luederwaldtinia) sericeus complex. Mapping of the isolated element was performed by fluorescent in situ hybridization in different populations of analyzed species. Characterization of the isolated element revealed a degenerated transposon, named DgmarMITE. This transposon is 496-bp-long, AT rich (57%), and contains 24 bp terminal inverted repeats. In situ mapping revealed presence of this element only in two out of four species analyzed. DgmarMITE sites were located in heterochromatic and euchromatic regions and varied in location and number on the karyotypes of Dichotomius (L.) gilletti and D. (L.) guaribensis across different populations. These results demonstrate differential accumulation of the DgmarMITE in genomes of these species, which is probably due to the occurrence of ectopic recombination and cross-mobilization of the element mediated by the transposase of closely related or unrelated transposable elements.

Keywords: Mariner-like elements, cross-mobilization, chromosome evolution.

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Introduction

Mariner transposable elements are DNA transposons that exhibit broad diversity in their structure. Mariner elements are characterized by a size of about 1,300 bp, a single ORF (open reading frame) encoding a transposase, a conserved catalytic domain [DD(34)D] necessary for transposition, and two terminal inverted repeats (TIRs) of 28-30 bp flanked by a TA dinucleotide resulting from target site duplications (Robertson, 1995; Robertson and Lampe, 1995; Plasterk et al., 1999). During transposition, the encoded transposase recognizes the TIRs and catalyzes excision of the two DNA strands at the donor site and fusion of the element at another site in the genome (Lampe et al., 1996).

The Mariner elements are probably the most widely distributed family of transposable elements (TEs) in nature, being represented in diverse taxa, such as rotifers, fungi, plants and vertebrates. Their wide distribution in metazoan species, including arthropods (Robertson and Lampe, 1995; Wallau et al., 2014), is probably related to horizontal transfer events (Robertson 1995; Robertson and Lampe, 1995; Lampe et al., 2003) which, for example, account for the presence of the Mariner_Tbel and Mariner1_BT families in phylogenetically distant species such as insects and mammals (Oliveira et al., 2012). These elements have been found in a wide range of insects from different orders, including Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera and Coleoptera (Robertson and Lampe, 1995; Palacios-Gimenez et al., 2014).

The existence of nonfunctional Mariner elements is common, including a large number of inactive copies in different genomes (Lohe et al., 1995). Some of those inactive elements, the miniature inverted repeat transposable elements (MITEs), do not encode the enzyme necessary for their transposition and therefore require the transposase of other elements for their mobilization (Kidwell, 2005). The origin of these TEs is related to the internal degeneration of autonomous elements (Déprá et al., 2012). MITEs are distinguished from their autonomous counterparts by their high copy number, compact structure, short terminal in-
verted repeats, genomic preference, and DNA sequence identity (Feschotte et al., 2002; Feng, 2003).

Regarding the speciose order Coleoptera, Mariner elements have so far been described in only a few species belonging to the families Chrysomelidae, Buprestidae, Cerambycidae, Laemophloeidae, Meloidae, Scarabaeidae, Staphylinidae and Tenebrionidae (Robertson, 1993; Robertson and Macleod, 1993; Robertson et al., 2002; Lampe et al., 2003; Richards et al., 2008; Rivera-Vega and Mittapalli, 2010; Oliveira et al., 2013; Xavier et al., 2014). However, data from chromosome mapping of Mariner TEs in Coleoptera are limited to two species of Coprophanaeus, one of Diabrotica (Oliveira et al., 2013) and one Dichotomius (Xavier et al., 2014), all genera belonging to the family Scarabaeidae. Despite the small number of studies, TEs have been associated with important evolutionary processes in Scarabaeidae, such as chromosome rearrangements (Oliveira et al., 2013), dispersion of 18S rDNA sites (Cabral-de-Mello et al., 2011a,b), and dynamics of the repetitive DNA fraction that constitutes the constitutive heterochromatin (CH) in the genomes of Dichotomius species (Cabral-de-Mello et al., 2011c).

Cytogenetic studies have been carried out in only 18 of the 165 described Dichotomius species, including molecular cytogenetics studies in 15 species (Cabral-de-Mello et al., 2008, 2011a,b; Silva et al., 2009; Korasaki et al., 2012; Xavier et al., 2014). This genus presents groups of closely related species (Sarmiento-Garcés and Amat-Garcia, 2009), including Dichotomius (Luederwaldtinia) sericeus complex (Coleoptera: Scarabaeidae). This complex was recently taxonomically revised by Valois et al. (2017), raising the number of species from five to eight. More specifically, D. sericeus var. aterrimus (Luederwaldt, 1929) was synonymized with D. sericeus and four new taxa, D. guaribensis, D. gilletti, D. iannuzziae, and D. catimbau have been described.

Species of the genus Dichotomius present the derived karyotype 2n = 18, Xyp, with meta-submetacentric chromosome morphology and presence of a large metacentric pair (Silva et al., 2009; Cabral-de-Mello et al., 2011a). The constitutive heterochromatin, located in pericentric regions of all autosomes, show similar patterns of highly and moderately repeated DNAs (C0t-1 DNA fraction) distribution in the six analyzed species (Cabral-de-Mello et al., 2011c). Furthermore, the 45S rDNA is predominantly located in the distal region of the third autosome pair, whereas the SS rDNA and H3 histone were co-located in the proximal region of the second pair in 14 analyzed species (Cabral-de-Mello et al., 2011a,b).

The aim of this study was to access whether distinct populations present differential patterns of location and accumulation of Mariner-like elements. Therefore, we characterized and mapped Dgmar/MITE sequences in chromosomes of phylogenetically related species of the Dichotomius (Luederwaldtinia) sericeus complex belonging to different populations.

Material and Methods

Specimens sampling

All species investigated herein belong to Dichotomius (L.) sericeus complex. Dichotomius (Luederwaldtinia) gilletti and D. (L.) iannuzziae were collected in Aldeia (7°53'48" S, 35°10'47"W) and Igarassu (7°48'37"S; 34°57'25"W), remnants of the Atlantic Rain Forest in the state of Pernambuco, Brazil. Additionally, individuals of D. (L.) schifleri and D. (L.) guaribensis were collected in Maracaípe (8°31'26"S 35°1'31"W), Pernambuco. D. (L.) guaribensis was also collected in REBIO Guaribas (6°42'41"S 35°11'17"W), Paraíba, Brazil. The individuals were collected using pitfall traps, in compliance with IBAMA/SISBIO guidelines (Permanent license No. 16278-1 for the collection of zoological material, authorization No. 41761-4 for collection in a Federal Conservation Unit for scientific purposes, and the license No. 50438-1, specific for D. (L.) schifleri). The specimens were identified by the taxonomist Dr. Fernando Silva, from the Universidade Federal do Pará, in Brazil.

DNA extraction and isolation of the transposable element

DNA samples of the four species of Dichotomius mentioned above were obtained from the pronotum tissue. Genomic DNA was extracted according to the protocol described by Sambrook and Russell (2001). Mariner elements were amplified by PCR using the MOS_N679 primer from Drosophila (5’GCCCATATGTCGAGTTTCGTCGAC). The volume of each PCR assay was 25 μL containing 1 ng genomic DNA, 1X PCR buffer, 5 mM MgCl2, 0.2 mM dNTP (Invitrogen), 1 pmol primer, and 1 U Taq polymerase (Invitrogen). The PCR conditions were 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 49 °C for 30 s and 72 °C for 1.20 s, and a final extension step at 72 °C for 5 min.

PCR products were separated by electrophoresis on 1% agarose gel. A band of approximately 500 bp obtained from D. (L.) gilletti (Supplementary Figure S1) was isolated from the gel using the Zymoclean™ Gel DNA Recovery Kit (Sinapse) according to the protocol of the manufacturer.

Cloning and sequencing

The isolated DNA fragment was cloned using the pGEM-T Easy Vector (Promega) according to manufacturer’s instructions. The insert was isolated by PCR using the M13 primer (M13F 5’-GTAAAACGACGGCCAGT 3’, M13R 5’-CAGGAAACACGATATGAC) (Zhang et al., 2001).

The PCR reagents were the same as described above. The PCR conditions were 95 °C for 3 min, followed
by 30 cycles at 95 °C for 30 s, 55 °C for 1 min and 72 °C for 2 min, and a final extension step at 72 °C for 5 min. For sequencing, the M13 PCR product was purified with ExoSAP-IT (Affymetrix/USB) and sequenced in an ABI3730XL automated sequencer (Applied Biosystems) by Macrogen Inc.

Editing and analysis of the transposable element

The chromatograms of forward and reverse strands of the M13 PCR product were analyzed with the Pregap4 software of the Staden package (Bonfield et al., 1995) in order to generate consensus sequences. Only bases with a Phred value of 20 or higher were considered in this analysis. Vector sequences were removed using the VecScreen tool. The consensus sequence used as a query sequence in GenBank and RepBase Censor for correct identification and classification of the elements. In addition, the presence of ORFs was investigated using the ORFfinder tool.

Chromosome preparations, C-banding and fluorescent in situ hybridization (FISH)

Cytological preparations of our four target species were obtained by the classical testicular follicles squashing technique in 50% acetic acid. Two male individuals of each species were analyzed. C-banding was performed on D. (L.) gilletti and D. (L.) guaribensis karyotypes following Sumner (1972). FISH was performed according to the protocol of Pinkel et al. (1986), with modifications as proposed by Cabral-de-Mello et al. (2010). The probe of the transposable element was labeled with dUTP-digoxigenin (Roche) and detected with anti-digoxigenin-rhodamine (Roche).

Photodocumentation

Hybridization images were captured with a Leica DM 2500 epifluorescence microscope. Brightness and contrast of the images were optimized using the Photoshop CS5 program.

Results

The presence of fragments amplified by the MOS_N679 primer of Mariner elements (Figure S1) and hybridization signals of the DgmarMITE were observed in only two out of the four analyzed species, namely Dichotomius (L.) gilletti and D. (L.) guaribensis. The element isolated from D. (L.) gilletti was 496 bp-long, rich in AT (57%), and had perfect TIRs of 24 bp. The consensus sequence used as a query sequence in GenBank and RepBase revealed 100% similarity with TIRs of the AfMar2 Mariner-like element of the grasshopper Abracris flavolineata (Figure 1) (accession number: KJ829354.1). The sequence between TIRs had no similarities to previously described elements. In addition, the largest identified ORF contained only 30 amino acids and showed no similarity to any transposase.

The species Dichotomius (L.) guaribensis, D. (L.) gilletti, D. (L.) iannuzziae and D. (L.) schifferi presented similar karyotypes with 2n = 18, and meta-submetaacentric chromosomal morphology. However, distinct sexual determination systems were observed: D. (L.) gilletti, and D. (L.) iannuzziae had a Xyp system, whereas D. (L.) schifferi and D. (L.) guaribensis presented a Xy1 sex bivalent configuration (Figure 2). C-banding revealed pericentromeric constitutive heterochromatin in all autosomes, and additionally, along the entire length of the seventh bivalent and X chromosome of Dichotomius (L.) gilletti and D. (L.) guaribensis (Figure 2a,b).

Mapping of DgmarMITE probes on the karyotype of D. (L.) gilletti revealed signals in all chromosomes, except for pairs five and seven of the Igarassu population (Figure 3a), and pair five of the Aldeia population (Figure 3b). Overall, DgmarMITE sequences were predominantly located in euchromatic regions in individuals from both populations, except in the Igarassu population, for which signals were detected at heterochromatic regions of chromosome pairs six and eight (Figure 3a). Similarly, in Aldeia population, DgmarMITE was restricted to the heterochromatic region of pair two (Figure 3b). In addition, five heteromorphic pairs were observed in Aldeia individuals (Figure 3b).

Mapping of DgmarMITE probes on the karyotype of D. (L.) guaribensis revealed their location in heterochromatic regions of all autosomes and of the X chromosome in both populations (Figure 3c,d). Additional signal was observed on the Y chromosome of the Guaribus population (Figure 3c). Furthermore, DgmarMITE sites were observed in euchromatic regions of all autosomes, except pair eight, in specimens from Maracaípe (Figure 3d). Overall,

### Table

| Transposable element | TIR 5’ | Internal sequence (bp) | TIR 3’ |
|----------------------|--------|------------------------|--------|
| DgmarMITE            | GCCATATGTCGAGTTTGTGCCAGG | 448 | TGTCAGCGAAACTGCACATATGCC |
| AfMarMITE            | GCCATATGTCGAGTTTGTGCCAGG | 422 | TGTCAGCGAAACTGCACATATGCC |

Figure 1 - Alignment of terminal inverted repeats (TIRs) of the elements DgmarMITE and AfMar2.
stronger FISH signals were observed in the karyotypes of individuals from Guaribas when compared to specimens from Maracaípe (Figure 3c,d).

Discussion

The karyotype observed in *D. (L.) guaribensis* (*2n* = 18) is considered derived from the ancestral number reported for the family Scarabaeidae (*2n* = 20), but conserved in most species of *Dichotomius*. The configuration of the sexual bivalent (*X_Y*), which has been reported so far only in *D. schiffleri*, also differs from the ancestral Scarabeidae *X_Y* (Cabral-de-Mello et al., 2008, 2011a; Silva et al., 2009; Xavier et al., 2014). The derived karyotypes of *D. gilletti* and *D. iannuzziae* observed in this study are similar to those described by Cabral-de Mello et al. (2011a) prior to the taxonomic revision by Valois et al. (2017). In Cabral-de Mello et al. (2011a), these species were referred to as *D. sericeus* and *D. laevicollis*, respectively. The presence of constitutive heterochromatic blocks in pericentromeric regions of all autosomes, as observed in *D. (L.) gilletti* and *D. (L.) guaribensis*, is a common feature in the genus *Dichotomius* (Cabral-de-Mello et al., 2011c), and has also been reported for the other two species investigated herein: *D. (L.) iannuzziae* (Cabral-de-Mello et al., 2011c) and *D. (L.) schiffleri* (Xavier et al., 2014).

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**Figure 2** - C-banding in metaphase I of *Dichotomius (L.) gilletti* (a) and metaphase I of *D. (L.) guaribensis* (b). Bar = 5 μm.

**Figure 3** - Fluorescent in situ hybridization of the element DgmarMITE in meiotic cells of two individuals of *Dichotomius (Luederwaldtina) gilletti* (a, b) and *D. (L.) guaribensis* (c, d). (a) Metaphase I of an individual from Igarassu population; (b) diplotene of specimen from Aldeia population; (c) metaphase I of individual from Guaribas population; (d) metaphase II of specimen from Maracaípe population. Note the localization and the size of the signals between populations. Bar = 5 μm.
DgmarMITE, the TE characterized here, presented features shared by all MITEs such as a large copy number, which was observable with FISH resolution, lack of transposase coding, AT richness, and conservation of TIR structure (Kuang et al., 2009). In most cases, sequence similarity between a MITE and its closest element is restricted to the TIRs (Feschotte and Pritham, 2007). The similarity between the TIRs of DgmarMITE and the AfiMar2 element of A. flavolineata indicates that the former belongs to the Mariner family.

The presence of DgmarMITE in the taxonomically similar species D. (L.) gilletti and D. (L.) guaribensis [considering the genus revision of Valois et al. (2017)] and therefore possibly phylogenetically closer, suggests an origin of this element in the common ancestor of these species. An alternative hypothesis is that this element may have originated independently in the species D. (L.) gilletti and D. (L.) guaribensis by horizontal transfer events, which are frequently observed for Mariner-like elements (Robertson, 1995; Robertson and Lampe, 1995), including MITEs, as described previously for the Stowaway element in the plant family Pooidaeae (Minaya et al., 2013). The DgmarMITE origin may be recent or not however, since older TEs accumulate preferentially in heterochromatic regions (Juna-kovic et al., 1998), as has been previously proposed for other TEs in Scarabaeidae (Oliveira et al., 2013). Genome colonization of this MITE possibly occurred earlier in D. (L.) guaribensis than in D. (L.) gilletti. In D. (L.) gilletti, the preferential location in euchromatic regions, the presence of heteromorphic pairs in the population of Aldeia, and absence of the signal in one or two chromosome pairs, suggest that this element originated most recently.

In addition to D. (L.) gilletti, predominantly euchromatic signals have been reported for TEs in D. (L.) schiffleri (Xavier et al., 2014), for the grasshopper species Eyprepocnemis plorans (Montiel et al., 2012) and A. flavolineata (Palacios-Gimenez et al., 2014). The occurrence of DgmarMITE in euchromatic regions can influence gene expression and/or gene and chromosome mutations (Kidwell and Lish, 2000; Feschotte and Pritham, 2007). However, it is also possible that the element is inserted in pseudogenes or even other dispersed repetitive sequences in euchromatin, as proposed for Mariner family elements of E. plorans (Montiel et al., 2012). On the other hand, the presence of MITEs in heterochromatic regions, as observed for DgmarMITE in D. (L.) guaribensis, is not common, since these TEs are preferentially associated with genes (Lu et al., 2011). However, heterochromatic enrichment of these elements has been described in other organisms, such as in the insect Anopheles gambiae (Quesneville et al., 2006) and in the plants Oryza sativa (Lu et al., 2011) and Arabidopsis thaliana (Guo et al., 2017).

Mapping of DgmarMITE in D. (L.) gilletti and D. (L.) guaribensis showed variation in the location and number of sites between species and populations. These findings suggest that this non-autonomous element may be cross-mobilized to different regions of host genomes using the transposase of either an older or a newly emerged transposon, in this latter case DgmarMITE accumulation occurs by a process known as “snowball effect” (Feschotte et al., 2005). The transposase used by DgmarMITE could belong to a closely related TE as observed between the inactive peach element and the transposase of Mariner-like Mos1 in Drosophila melanogaster (Garza et al., 1991), or to a phylogenetically distant TE, as observed between an element of the Stowaway family and Osmar transposase, an autonomous Mariner-like element in the rice genome (Feschotte et al., 2003; Yang et al., 2009).

With respect to copy-number variation in heterochromatic regions of D. (L.) guaribensis chromosomes, an increase in DgmarMITE copy number in the Guaribas population probably results from transposition-independent events, including ectopic recombination and concerted evolution. The latter has been observed for highly repetitive DNA sequences such as Mariner elements found in the heterochromatin of Drosophila erecta (Lohe et al., 1995). An alternative hypothesis to explain this variation is that this element is undergoing a reverse process with quantitative and random copy loss in the genomes of individuals from Maracaípe population. In this scenario, DgmarMITE would be undergoing senescence, the last stage of the transposable element “life cycle”, as described by Kidwell and Lisch (2000).

Mapping of DgmarMITE in species of the Dichotomius (Luederwaldtinia) sericeus complex contributed to increase our knowledge about the location and distribution of TEs in dung beetle genomes. This analysis also revealed the accumulation of DgmarMITE in the karyotype of two species. Plausible mechanisms underlying such accumulation include the occurrence of cross-mobilization and/or ectopic recombination in heterochromatic regions. However, we cannot completely rule out the possible involvement of other molecular mechanisms discussed here. Therefore, further characterization and chromosome mapping should be extended to other species within this complex of species.

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