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Numerical and structural chromosome variation in the swarm-founding wasp *Metapolybia decorata* Gribodo 1896 (Hymenoptera, Vespidae)

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Abstract The Neotropical Polistinae wasps are diverse in taxonomy, social behavior, and nesting founding characteristics. Although some species in this group have been used as models for studies on wasp's biology, they are poorly known in terms of cytogenetics. Here we reported an intraspecific numerical-structural chromosome variation in the swarm-founding wasp *Metapolybia decorata* from the Brazilian Atlantic Rainforest using conventional and molecular cytogenetic techniques. The observed structural chromosome change involved a telomeric fusion that resulted in a chromosome number range of 2n = 34–36. The origin and geographic distribution of the variant chromosome forms as well as their frequency and maintenance in the studied populations are discussed. In addition, we reported a novel and geographically restricted deletion in the fused chromosomes indicating that the species is undergoing a continued process of karyotype evolution leading to fused chromosome stabilization by elimination of inactive centromeric sequences. Evidence of differences in the telomeric sequences of this wasp was also found by in situ hybridization using the motif $(T_2AG_2)_7$ as probe.

Keywords rDNA · Fluorochromes · Fluorescent in situ hybridization · Telomeric fusion · Epiponini

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Introduction

Polistinae wasps have a cosmopolitan distribution and comprise 26 genera with approximately 900 species found mainly in tropical regions (Carpenter et al. 1996; Wilson 1971). Certain particular aspects of the biology of these insects, such as the haplodiploid sex determination system and different levels of social organization, which range from subsocial to eusocial, make these wasps appropriate model group for the study of population structure, genetics, and evolution.

Species in the tribe Epiponini are distributed in over 19 genera (Carpenter 2004) and are characterized as swarm-founding wasps that alternate polygyny and monogyny along the different phases of the colony life-cycle (West-Eberhard 1978; Queller et al. 1993). Another noteworthy characteristic of these wasps is the subtle morphological difference among castes. Interestingly, it was demonstrated that for some species the queen-worker dimorphism is accentuated in the later stages of the colony life cycle, whereas for others this distinction is seen throughout the colony life-cycle (Noll and Zucchi 2002; Baio et al. 2003).

Studies on *Metapolybia aztecoides* helped establish the model of cyclic oligogyny in epiponines (West-Eberhard 1978) and other *Metapolybia* species have been used as models for behavioral studies, such as those regarding regulation and organization of nest founding (Karsai and Wenzel 2000) and the factors related to morphological differentiation among castes (Baio et al. 2003).

Metapolybia comprises 16 species predominantly distributed in Central and South America (Richards 1978; Andena and Carpenter 2011). Their nests are built with a single sessile comb either directly on tree trunks or on walls. *Metapolybia decorata* Gribodo 1896 ranges between 7.0 and 10.0 mm in size and is dark colored with reddish brown

antennae and legs. This species has a wide distribution in South America and has been reported in Peru, Bolivia, Surinam, and Brazil (Richards 1978). Its nests range from 6 to 15 cm in diameter and are frequently found in the Atlantic Rainforest Central Corridor (hereon called ARCC).

Cytogenetic studies are still scarce for the Polistinae as a whole. In the Epiponini, studies using conventional staining methods were conducted on 15 species within 8 genera (Pompolo and Takahashi 1987, 1990a) and have shown an extensive interspecific variation in the haploid number ranging from n = 8 to n = 32 chromosomes. Interspecific haploid number variation found suggests that chromosome changes in the Epiponini have been frequent. In this study, using conventional and molecular cytogenetic methods, we investigate a complex numerical-structural chromosome variation in *M. decorata* likely resulting from telomeric fusion.

Materials and methods

Samples from 22 colonies of *M. decorata* analyzed in this study were collected in Igrapiúna [13°49′S, 39°08′W (one colony)], Itacaré [14°21′S, 39°10′W (one colony)], Ilhéus [14°47′S, 39°12′W (six colonies)], Una [15°17′S, 39°4′W (two colonies)], Camacan [15°25′S, 39°29′W (four colonies)], Arataca [15°22′S, 39°32′W (two colonies)] and Santa Teresinha [12° 44′S, 39°31′W (four colonies)] in the state of Bahia; and Linhares [19°25′S, 40°03′W (one colony)] and Colatina [19°31′S, 40°37′W (one colony)] in the state of Espírito Santo, Brazil. Adult voucher specimens were deposited at the entomological collection at the Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil.

Chromosome preparations were obtained from cerebral ganglia of larvae, according to Imai et al. (1988). Chromosomes were stained with Giemsa (3 % stock solution in phosphate buffer pH 6.8) and C-banding following Sumner (1972), with modifications according to Pompolo and Takahashi (1990b). Fluorochrome staining (chromomycin A_3 [CMA_3]/4,6-diamidino-2-phenylindole [DAPI]) followed Schweizer (1976) with modifications proposed by Guerra and Souza (2002).

rDNA sites were located by fluorescent in situ hybridization (FISH) of *Arabidopsis thaliana* rDNA 45S probes labeled with cyanine 3 (Cy3) by nick translation (Invitrogen). Hybridizations were carried out according to Schwarzacher and Harrison-Heslop (1999) with 72 % of stringency. The slides were mounted in a Vectashield antifade solution (Vector Laboratories) containing DAPI.

To locate telomeric sequences, probes were generated by polymerase chain reaction (PCR) using primers $(TTAGG)_6$ and $(TAACC)_6$. PCR reactions followed Ijdo et al. (1991) with the modifications proposed by Sahara et al. (1999). The telomeric probe was labeled by Nick translation using BioNick Labeling System (Invitrogen). FISH procedures were performed with the following modifications: after the slides were washed, 100 µl of BSA 3 %/1xPBS were added to the slides, and the slides were covered with plastic coverslips and incubated in a humid chamber at 37 °C for 30 min. Then 150 µl of detection mix (0.07 % avidine conjugated with 3 % FITC/BSA) was added and the slides were incubated in a humid chamber at 37 °C for 1 h. The slides were then washed 3 times using 1xPBS for 2 min each.

Chromosomes stained with Giemsa, after C-banding and FISH were photographed using an Olympus BX51 epifluorescence photomicroscope equipped with a DP-72 digital camera (Olympus, Tokyo, Japan). Chromosomes stained with fluorochromes were observed using an epifluorescence microscope Leica DMRA2, and the images were captured using Leica IM50 software (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). On average 10 metaphases per specimen were analyzed. Karyotypes were constructed using Adobe Photoshop CS4 Extended version 11.0. Chromosomes were described according to Imai (1991) and arranged in decreasing size.

Results and discussion

The diploid chromosome number of *Metapolybia decorata* was found to vary from 2n = 34 to 2n = 36 and the haploid chromosome number from n = 17 to n = 18. These chromosome numbers are within the variation interval previously found in the tribe Epiponini (Pompolo and Takahashi 1987, 1990a). Individuals with 2n = 36 chromosomes, had 11 metacentric, one acrocentric, and six pseudoacrocentric chromosome pairs (A^M—with one of the arms entirely heterochromatic), $2k = 22M + 2A + 12A^M$ (Fig. 1). *Metapolybia* sp., studied by Pompolo and Takahashi (1990a) in the state of São Paulo, Brazil, showed n = 19 (2n = 38), with predominantly meta- and submetacentric chromosomes and complete absence of pseudoacrocentric chromosomes, showing a remarkable divergence from *M. decorata*.

The numerical chromosome variation found in *M. decorata* was a result of the presence of either one or two large metacentric chromosomes of the 4th chromosome pair in the specimens with 2n = 35 and 2n = 34, respectively (Fig. 1). The presence of a larger heterochromatic block in the pericentromeric region of these chromosomes was noted, as well a reduction in the number of pseudoacrocentrics when compared with specimens with 2n = 36. These results suggest that these large metacentric chromosomes in the 4th chromosome pair resulted from fusions involving the heterochromatic arms of two pseudoacrocentric chromosomes.

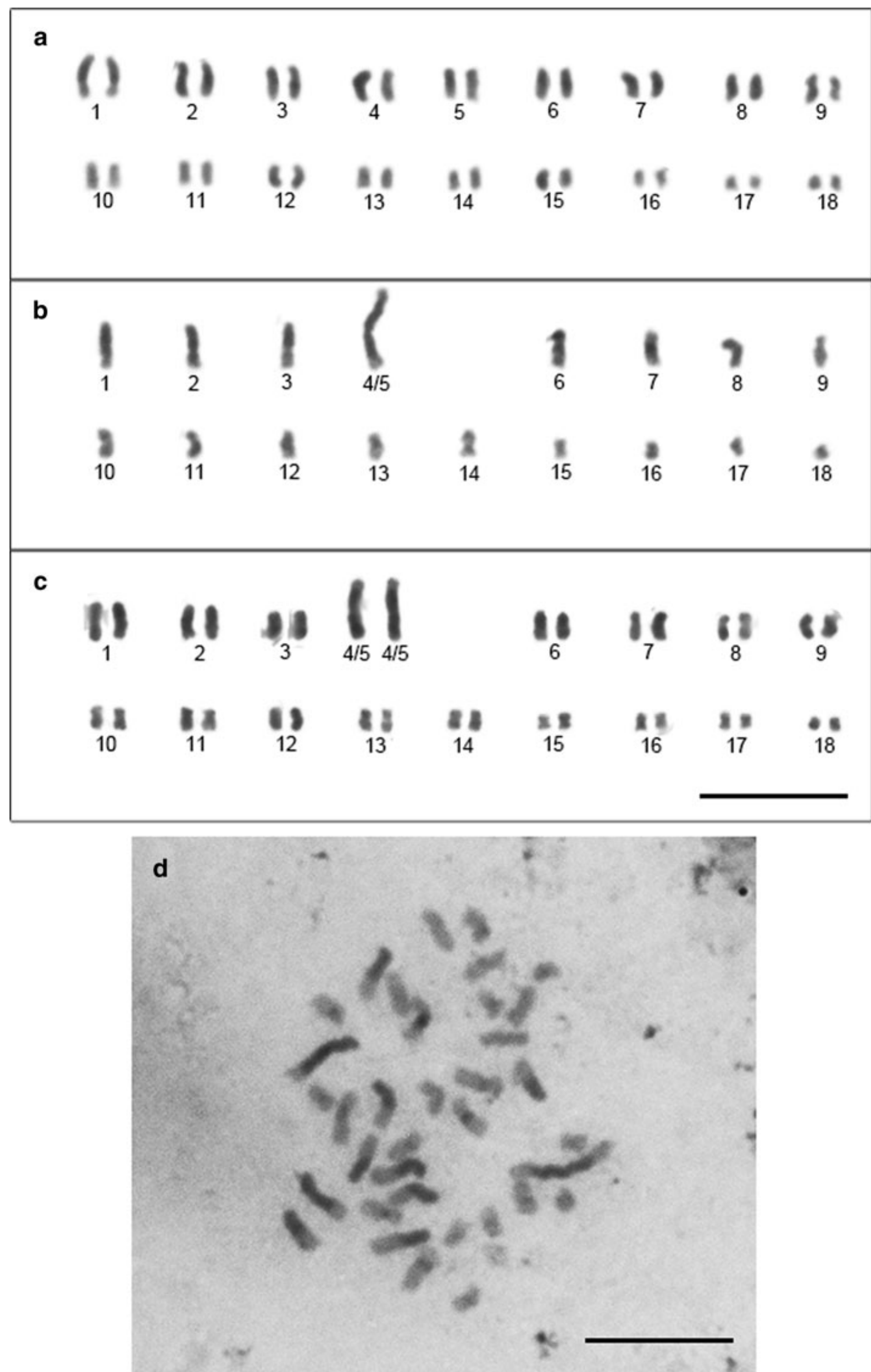

Fig. 1 Giemsa-stained karyotypes of *M. decorata*. **a** Female, 2n = 36; **b** male, n = 17 with one fused chromosome; **c** female, 2n = 34 with two fused chromosomes, and **d** C-banding in a metaphase of a female, 2n = 35. *Bar* represents 10 μm

It has been suggested that an increase in chromosome arm length by tandem growth of the heterochromatin would increase the risk of reciprocal translocations due to unspecific heterochromatin interactions. Centric fusion is a mechanism through which the excess of heterochromatin from pseudoacrocentric chromosomes can be eliminated (Imai et al. 1986, 1988). Although this type of rearrangement is relatively uncommon, it has been observed in ants of the *Myrmecia pilosula* species complex (Imai et al. 1994) and in wasps in the genus *Vespula* (Hoshiba et al. 1989). Scher and Pompolo (2003) also suggested the occurrence of Robertsonian rearrangements (centric fission and fusion) as a possible cause of karyotype variation in *Trypoxylon nitidum*. The presence of a large heterochromatic block in the

interstitial portion of the fused chromosomes of *M. decorata* likely corresponds to remaining heterochromatic parts of the fused pseudoacrocentrics.

CMA_3 and DAPI staining showed brightly conspicuous heterochromatin blocks (Figs. 2, 3a). The 4th and 5th chromosome pairs in the 2n = 36 karyotype (Figs. 2a, 3a) showed a very similar length and CMA_3/DAPI staining when compared the fused chromosomes arms (Fig. 2b, c) indicating their likely involvement in chromosomal fusion event. The centromere of most chromosomes, including the pseudoacrocentrics, was positive for CMA_3 staining (Figs. 2, 3a). Moreover, the fused chromosomes showed two additional centromere-like CMA_3 positive markings in the middle of both chromosome arms (Fig. 2b, c, e). This feature also varied, revealing two types of fused chromosomes. The first type, herein named Fs1, showed three CMA_3^+/$DAPI^-$ bands, one centromeric and two interstitial. The second type, Fs2, lacks an interstitial band in one of the arms, which is shorter (Figs. 2c, 3b). Type Fs2 must then be considered a submetacentric chromosome when compared with its pair Fs1, which is a typical metacentric.

The CMA_3^+/$DAPI^-$ interstitial bands, typical of centromeres in the studied species, localized in the middle of chromosome arms in Fs1 and on the long arm in chromosome Fs2, likely represent sequences from inactive centromeres of the original chromosomes involved in the fusion. Considering this hypothesis, the fusion process might have been followed by centromeric inactivations that stabilized the fused chromosome and maintained the present primary constriction. Although the molecular mechanism of centromere inactivation has not been fully established, it is thought to involve epigenetic modification

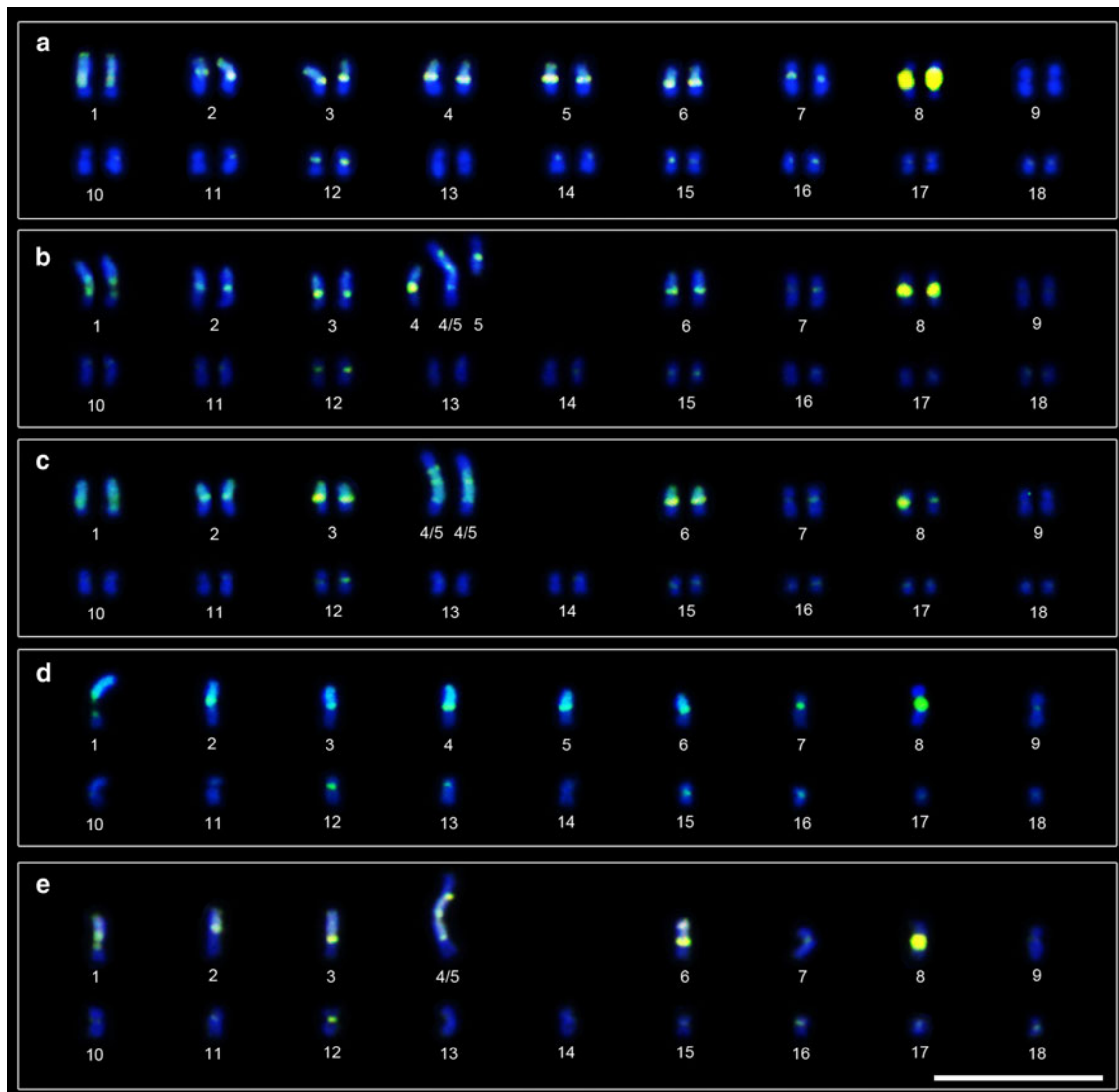

Fig. 2 Fluorochrome stained karyotypes of *M. decorata.* **a** Female, 2n = 36; **b** female, 2n = 35 with one fused chromosome; **c** female, 2n = 34 with two fused chromosomes; **d** male, n = 18, and **e** male, n = 17 with one fused chromosome. *Bar* represents 10 μm

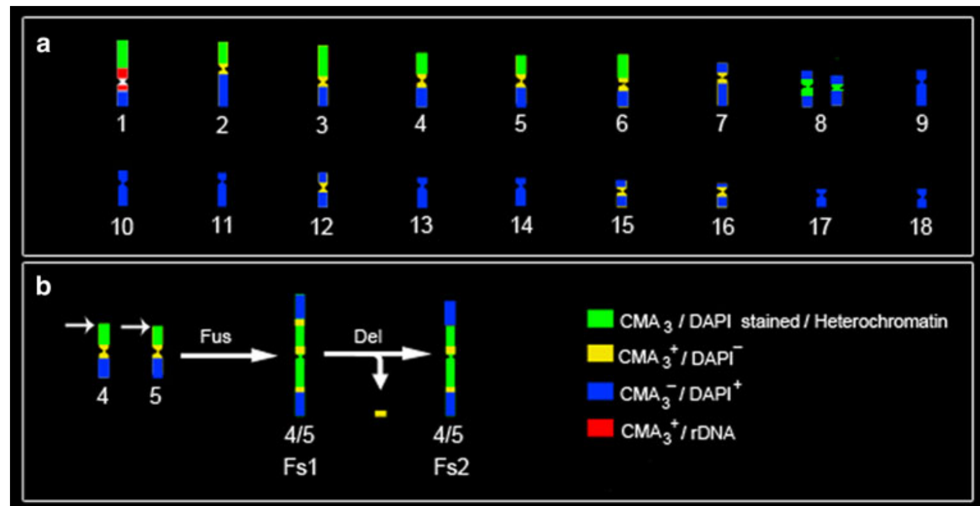

Fig. 3 **a** Ideogram showing the CMA_3/DAPI staining, heterochromatin and rDNA sites. **b** Diagram showing *M. decorata* putative chromosomal fusion event and the resulting two types of fused chromosomes. Fus = chromosome fusion and Del = deletion event

factors that alter heterochromatin structure (Stimpson and Sullivan 2010). A similar mechanism of fusion and alteration of the functional centromere was described in ants for the *Myrmecia pilosula* species complex (Imai and Taylor 1989). This is the first report of this type of rearrangement via fusion and alteration of the functional centromere in the subfamily Polistinae.

Another polymorphism of CMA_3^+/DAPI$^-$ bands in this species was observed in the 8th chromosome pair as two strong signals, a larger and a smaller one, present in the interstitial region of the homologous chromosomes (Figs. 2c, 3a). As it has been already reported for other hymenopterans (Araújo et al. 2000; Rocha et al. 2002), chromosome segments strongly stained by CMA_3, therefore rich in GC, mostly frequently contain ribosomal genes responsible for the formation of the nucleolus organizer regions (NORs). However, FISH analysis of *M. decorata*, showed the presence of ribosomal genes only in proximal region of the heterochromatic arm of the pair one (Figs. 3a, 4).

FISH analysis with the insect telomeric probe $(T_2AG_2)_7$, did not hybridize to the chromosomes of *M. decorata*. This probe previously hybridized to the terminal regions in chromosomes of other hymenopterans such as in 33 ant species (Okazaki et al. 1993; Meyne et al. 1995; Sahara et al. 1999; Lorite et al. 2002) and in the honeybee *Apis mellifera* (Sahara et al. 1999). The absence of the motif $(T_2AG_2)_7$ has been reported for several insects such as in Coleoptera (Okazaki et al. 1993; Sahara et al. 1999; Frydrychová and Marec 2002), Hemiptera (Okazaki et al. 1993; Sahara et al. 1999; Grozeva et al. 2011), Odonata, Ephemeroptera, Dermaptera, Mecoptera (Frydrychová et al. 2004), and Diptera (Okazaki et al. 1993; Sahara et al. 1999). This absence of hybridization to the telomere of *M. decorata* suggests an alteration in the telomeric motif in this wasp species, which must be further investigated.

Telomeric fusion events described so far mostly refer to rearrangements involving chromosomes with acro/telocentric morphology (Slijepcevic 1998). In these cases, the chromosome break points are localized in regions near the centromere, which generally leads to the loss of one of the primary constrictions or less frequently to the formation of dicentric chromosomes. In this study, however, besides the fact that the break and fusion points are distant from the centromeres, it is noteworthy that the heterochromatic arms

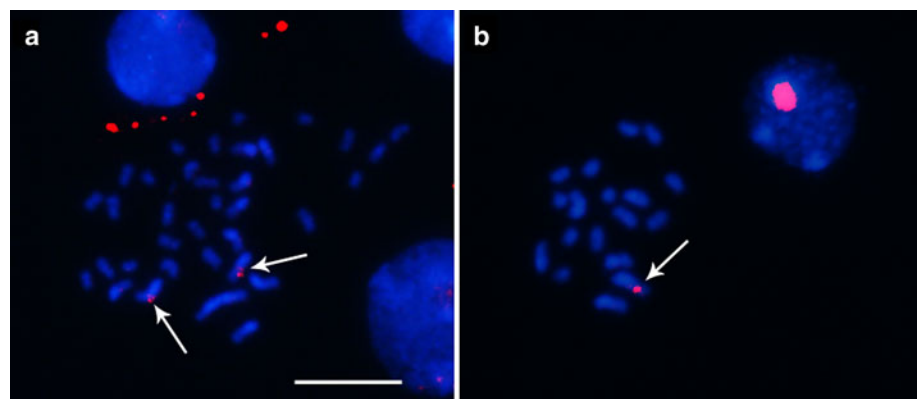

Fig. 4 Fluorescent in situ hybridization using rDNA probe in *M. decorata*. **a** Female, 2n = 35; **b** male, n = 18. *Arrows* indicate rDNA sites. *Bar* represents 10 μm

are involved in the fusion rearrangement. According to Imai (1991), these heterochromatic blocks formed by the accumulation of terminal heterochromatin may contain, among other repetitive sequences, inactive centromeres and telomeres. Following this hypothesis, it is likely that the break occurred in a region adjacent to an inactive centromere in the heterochromatic arm in one of the pseudoacrocentric chromosomes involved in the fusion process. The subsequent activation of this centromeric region would confer a metacentric morphology to the resulting fused chromosomes, as observed here.

Based on the frequency of presence/absence of fused chromosomes in different geographic locations (Table 1), we can classify this variation as a chromosome polymorphism. These data indicate that the meiotic segregation of these chromosomes apparently has not led to the production of gametes with unbalanced chromosomes and consequent fertility loss. This allows us to suggest that this chromosome mutation has no negative impact on the fitness of the carriers. In fact, considering its high frequency in the population, it is either neutral or may increase fitness.

The significantly higher frequency of individuals with fused chromosomes in the sampled populations, also evidenced within individual colonies, could be explained by the species' reproductive behavior. The founding females (queens) present in the initial stages of the colony would be closely related. In the colonies analyzed, the primordial female progenitors (mother queens), or even a single female, therefore, carrying fused chromosomes would have passed them on to their descendants. By exerting reproductive control, such queens would be responsible for maintaining this chromosome mutation within colonies and consequently in the population, which could explain the high frequency observed. This hypothesis seems to be in accordance with the reproductive control behavior of the queens over the workers suggested by some authors (West-Eberhard 1978; Noll and Zucchi 2002, Hastings et al. 1998). As well, this is in accordance with the observation that colonies of species within the Epiponini, despite their characteristic poligyny, show a high level of genetic homogeneity (Queller et al. 1988, 1993; Hastings et al. 1998).

The chromosome Fs1 showed a wide distribution, being reported in colonies along the ARCC, from the northern region of the state of Espírito Santo (Linhares) to southern Bahia (Igrapiúna). Serra da Jibóia (Santa Teresinha), more to the north of the studied area is an Atlantic Rainforest fragment surrounded by Caatinga (Queiroz et al. 1996), which is isolated from the ARCC, where there were no reports of this chromosome fusion (Fig. 5; Table 1). It is possible that these populations in Serra da Jibóia are isolated from the other populations where the polymorphism is present. It is likely that the fusion may have originated in

Table 1 Number of individuals sampled and chromosome numbers observed in the different colonies of *M. decorata* analyzed

Locality (Colony number)	Females 2n = 34	Females 2n = 35	Females 2n = 36	Males n = 17	Males n = 18
Santa Teresinha, BA (1)	–	–	4	–	3
Santa Teresinha, BA (2)	–	–	6	–	1
Santa Teresinha, BA (3)	–	–	–	–	2
Santa Teresinha, BA (4)	–	–	4	–	–
Ilhéus, BA (1)	1	4	–	1	–
Ilhéus, BA (2)	–	1	–	–	4
Ilhéus, BA (3)	–	2	1	–	1
Ilhéus, BA (4)	–	3	9	–	–
Ilhéus, BA (5)	–	2	10	–	–
Ilhéus, BA (6)	–	–	–	6	–
Una, BA (1)	–	3	–	–	–
Una, BA (2)	–	1	–	–	1
Camacan, BA (1)	–	–	2	–	–
Camacan, BA (2)	–	4	–	–	–
Camacan, BA (3)	–	2	2	–	–
Camacan, BA (4)	–	1	3	–	–
Arataca, BA (1)	2	6	–	2	–
Arataca, BA(2)	–	1	1	–	–
Itacaré, BA (1)	1	2	–	3	–
Igrapiúna, BA (1)	–	1	2	2	–
Linhares, ES (1)	–	3	5	–	–
Colatina, ES (1)	–	–	4	–	1
Total (22)	4	36	53	14	13
%	3.33	30	44.16	11.66	10.83

populations in the central and southern areas of the ARCC after the isolation of these populations. In contrast the fused chromosome Fs2 was reported only in Arataca (Fig. 5). The restricted distribution of chromosome Fs2 is consistent with the hypothesis of a more recent origin. The loss of a CMA_3^+/$DAPI^-$ band and the consequent shortening of the chromosome arm indicate the deletion of one of the inactivated centromeric regions (Fig. 3b) and that the species is undergoing a continued process of karyotype evolution leading to fused chromosome stabilization by elimination of inactive centromeric sequences.

Our results revealed a marked chromosome variation in populations of *M. decorata* along the ARCC. The chromosome variation patterns found, as well as their

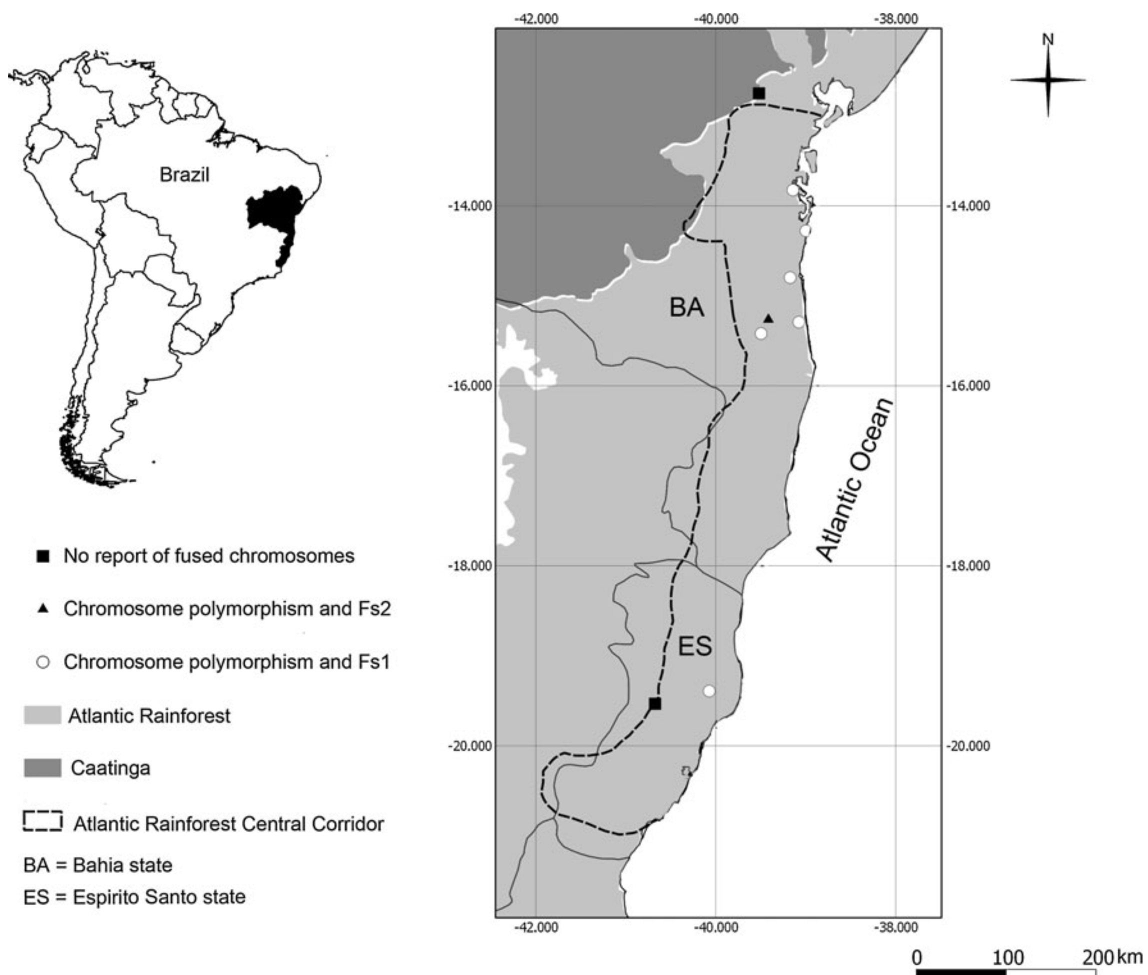

Fig. 5 Map showing collection sites with the distribution of chromosomal polymorphism and the two types of fused chromosomes

geographic distribution, can result from evolutionary and biogeographic processes on this species. Therefore, genetic analyses regarding the level of genetic differentiation among populations of this species along the Atlantic Rainforest should be revealing. Further analyses to test the presence of the insect motif $(T_2AG_2)_7$ and other motifs will be necessary to reveal the telomeric motif present in *M. decorata* as well as in other Epiponini.

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