Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics, 2013
Vol. 66, No. 1, 59–64, http://dx.doi.org/10.1080/00087114.2013.780443

Cytogenetic studies of five taxa of the tribe Attini (Formicidae: Myrmicinae)

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Cytogenetic studies have been carried out on more than 750 ant taxa and are an important tool in evolutionary, taxonomic and phylogenetic studies. However, less than 10% of the species reported in the tribe Attini have been studied. The aim of the present study was to describe the chromosomes of five attine ants collected in Viçosa, Minas Gerais state, Brazil, at present unknown. The ant karyotypes reported are: *Sericomyrmex* sp. (2n = 50, 44m + 6sm, n = 25, 22m + 3sm); *Trachymyrmex relictus* (2n = 20m); *Trachymyrmex* sp. (2n = 22, 18m + 4sm); *Apterostigma madidiense* (n = 23, 7m + 10sm + 5st + 1a) and *Apterostigma steigeri* (2n = 22, 20m + 2sm). C-banding showed that heterochromatin was present in the centromeric region of all chromosomes of *T. relictus*. Future cytogenetic studies on members of the tribe Attini will provide important information to discuss chromosome evolution in this ant group.

Keywords: ant; *Apterostigma*; chromosomes; heterocromatin; *Sericomyrmex*; *Trachymyrmex*

Introduction

The tribe Attini is a monophyletic group of ants that arose about 50 million years ago (Chapela et al. 1994; Schultz and Meier 1995; Mueller et al. 1998; Schultz and Brady 2008; Mehdiabadi and Schultz 2009) and have a symbiotic relationship with the fungus they cultivate for food (Weber 1966). This tribe is exclusive to the New World and is mainly found in the Neotropical region (Mayhé-Nunes and Jaffé 1998). These ants are grouped in 15 genera with 297 described species (Brandão et al. 2011).

Most of the natural and ecological history data refer to the "higher attine" (Mayhé-Nunes and Jaffé 1998; Leal and Oliveira 2000; Schultz and Brady 2008; Mehdiabadi and Schultz 2009; Brandão et al. 2011), including the genera *Atta*, *Acromyrmex*, *Trachymyrmex* and *Sericomyrmex*. The largest amount of data is on leaf-cutter ants (*Acromyrmex* and *Atta*) because of their associated agricultural losses. Data of other groups of ants remain obscure, with insufficient scientific information (Lattke 1999). The difficulty of studying, and especially collecting, "lower attine" nests, is mainly due to the fact that the nests are inconspicuous, small and sometimes located in chambers deep in the soil (Mackay et al. 2004; Hernández-Marín et al. 2005; Rabeling et al. 2007, 2009).

The *Trachymyrmex* and *Sericomyrmex* genera can present large colonies with some thousands of workers with increased individual size, unlike the "lower attine" nests which normally present some tens of workers. *Trachymyrmex* is probably the most derived of the monomorphic Attini, because some species present polymorphisms in size among workers (Brandão and Mayhé-Nunes 2007). It is therefore a key group to understand the phylogenetic relationships of the "higher attine", because it is considered to be the putative sister group of the leaf-cutter ants (Brandão and Mayhé-Nunes 2007; Schultz and Brady 2008).

Cytogenetic studies of the family Formicidae have already been carried out on more than 750 ant taxa (Lorite and Palomeque 2010) and are an important tool in evolutionary, taxonomic and phylogenetic ant studies (Imai et al. 1994; Mariano 2004; Delabie et al. 2008; Lorite and Palomeque 2010; Aguiar et al. 2010; Mariano et al. 2012). There is great variation in the chromosome number reported in ants, from 2n = 2 in *Myrmecia croslandi* Taylor, 1991 (Crosland and Crozier 1986) to 2n = 120 in *Dinoponera lucida* Emery, 1901 (Mariano et al. 2008).

Less than 10% of the total number of species of the tribe Attini have been cytogenetically studied (Goñi et al. 1983; Santos-Colares et al. 1997; Murakami et al. 1998; Barros et al. 2010, 2011). The tribe Attini shows variation in the diploid number of chromosomes, ranging from 2n = 8 in *Mycocepurus* sp. (Murakami et al. 1998) and *Mycocepurus goeldii* Forel, 1893 (Barros et al.

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2010) to 2n = 54 in *Mycetarotes paralellus* Emery, 1906 (Barros et al. 2011).

The minimum interaction theory proposed by Imai et al. (1994) assumes that chromosome fission played an important role in ant karyotype evolution, resulting in an increase in the number of chromosomes and a decreased chromosome size. Other chromosome rearrangements have also been reported to occur in ants, including inversions, translocations, chromosome fusions and supernumerary chromosomes (reviewed in Lorite and Palomeque 2010).

The aim of the present study was to contribute to ant cytogenetics from the tribe Attini, with special reference to the genera *Apterostigma*, *Trachymyrmex* and *Sericomyrmex*.

Materials and methods

Cytogenetic studies were carried out on *Sericomyrmex* sp., *Trachymyrmex relictus* Borgmeier, 1934, *Trachymyrmex* sp., *Apterostigma steigeri* Santschi, 1911 and *Apterostigma madidiense* Weber, 1938. Ants were collected in Viçosa, Minas Gerais State, Brazil (20°41′20″ S–20°49′35″ S; 42°49′36″ W–42°54′27″W) from November 2008 to October 2009 (Table 1).

The colonies were kept in an incubation chamber (BOD) at 25°C at the Laboratório de Citogenética de Insetos, Universidade Federal de Viçosa. Metaphases were obtained according to Imai et al. (1988) using individual cerebral ganglia and testis from larvae (after meconium defecation). At least 10 metaphases were analyzed per individual, using conventional coloration (Giemsa 4%) and photographed using an Olympus® BX 60 microscope attached to a Q Color 3 Olympus® image capture system. Due to sample availability, C-banding technique was performed only on the chromosomes of *T. relictus*. This technique is used to identify heterochromatin and was applied according to Sumner (1972) with the times modified as follows: 4 min in HCl 0.2 N at room temperature, rinsed with distilled water at room temperature, 11 min and 30 s in $(BaOH)_2$ 5% at 60°C, 30 s in HCl 0.2 N at room temperature, rinsed with distilled water at room temperature, 12 min in 2 × saline sodium citrate (2XSSC) solution pH 7 at 60°C, rinsed in distilled water at room temperature and stained with Giemsa (8%) for 30 min.

The karyotype of each species was set by pairing homologous chromosomes (in females), then arranging chromosomes by decreasing size and classifying by chromosome arm ratio as per Levan et al. (1964): metacentric (m, r = 1–1.7); submetacentric (sm, r = 1.7–3); subtelocentric (st, r = 3–7) and acrocentric (a, r > 7). The chromosomes were arranged in karyotypes with the Corel Photopaint X3 ® and Image Pro Plus® software.

Adult specimens were identified by Dr. Jacques H. C. Delabie and deposited in the ant collection at the Laboratório de Mirmecologia do Centro de Pesquisas do Cacau (CPDC/Brazil), record number #5570. *Sericomyrmex* sp. and *Trachymyrmex* sp. workers were deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Results and discussion

The diploid number ranged from 2n = 20 to 2n = 50, in *T. relictus* and *Sericomyrmex* sp., respectively (Table 1).

Genus Sericomyrmex

The chromosome number observed for *Sericomyrmex* sp. was 2n = 50 (Figure 1a) and n = 25 (Figure 1b). The genus *Sericomyrmex* has 22 described species (Brandão et al. 2011) but only one has been reported cytogenetically: *Sericomyrmex amabilis* Wheeler, 1925 from Barro Colorado, Panamá, which also presented 2*n* = 50 chromosomes (Murakami et al. 1998). Although the samples we analyzed were not identified at the species level, the population of *S. amabilis* studied by Murakami et al. (1998) may represent a different species because it was not previously reported in South America.

Sericomyrmex sp. and *S. amabilis* are separated by more than 5000 km and show the same chromosome number and similar morphology. Murakami et al. (1998) considered all the chromosomes to be meta- or submetacentric. In our sample the larger chromosomes were very similar, but the morphology of the smaller chromosomes could not be precisely compared. *Sericomyrmex* sp. presented 22 pairs of metacentric chromosomes and three pairs of submetacentric chromosomes.

Genus Trachymyrmex

The chromosomes of *T. relictus* (Figure 2a–c) and *Trachymyrmex* sp. were studied (Figure 2d). All

Table 1. Studied species, sampling site, sample size (number of colonies – individuals), chromosome number: diploid (2*n*) and/or haploid (*n*), and karyotypic formulae following Levan et al. (1964).

Species	Locality	Colonies – individuals	2*n* (*n*)	Karyotypic formula 2*n* (*n*)
Sericomyrmex sp.	Viçosa, MG	1 – 10	50	44m + 6sm
			(25)	2m + 3sm
Trachymyrmex relictus Borgmeier, 1934	Viçosa, MG	5 – 21	20	20m
			(10)	(10m)
Trachymyrmex sp.	Viçosa, MG	1 – 5	22	18m + 4sm
Apterostigma steigeri Santschi, 1911	Viçosa, MG	2 – 6	22	20m + 2sm
Apterostigma madidiense Weber, 1938	Viçosa, MG	1 – 4	(23)	(7m + 10sm + 5st + 1a)

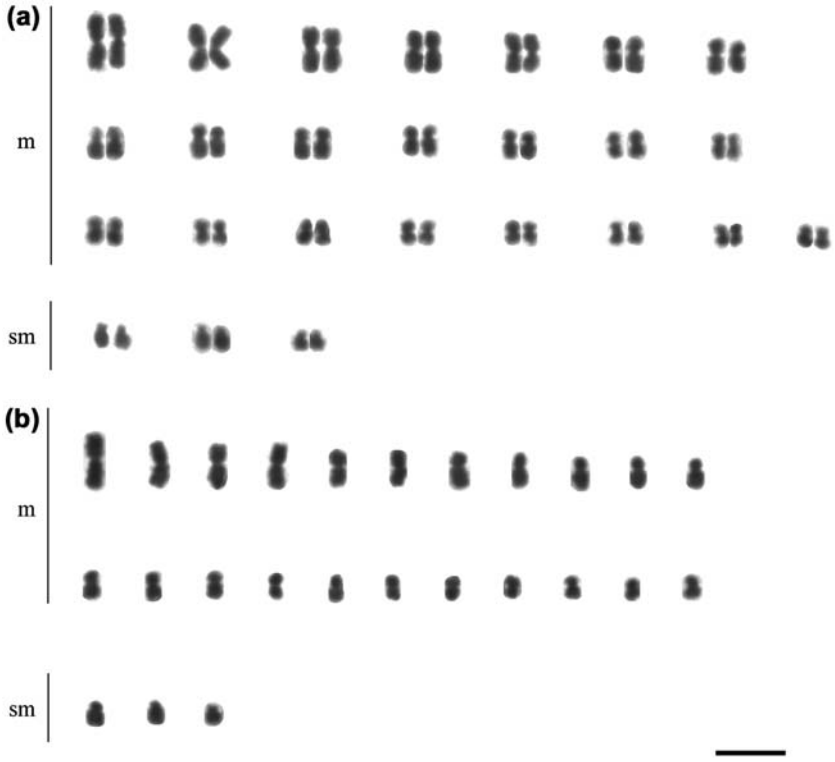

Figure 1. Karyotypes of *Sericomyrmex* sp.: (a) female (2n = 50); (b) male (*n* = 25). Scale bar = 5 μm.

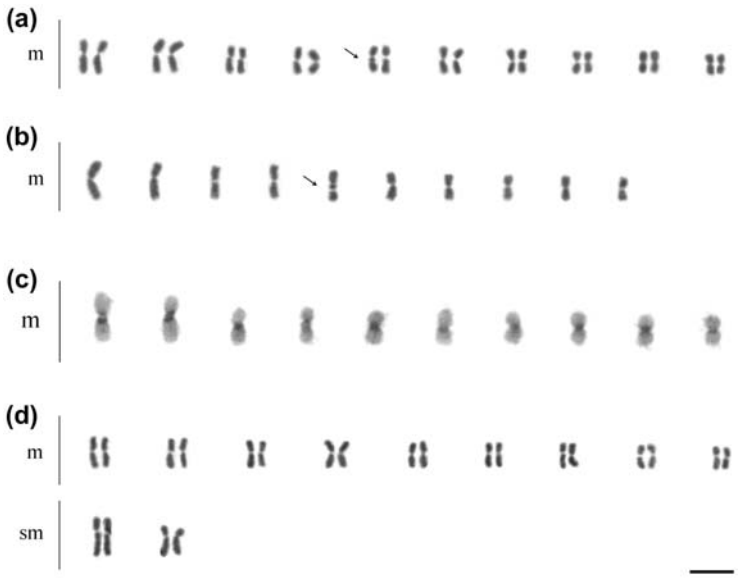

Figure 2. Karyotypes of *Trachymyrmex* species: (a) female of *T. relictus* (2*n* = 20); (b) male of *T. relictus* (*n* = 10); (c) C-banding of a male of *T. relictus*; (d) female of *Trachymyrmex* sp. (2*n* = 22). Arrows indicate secondary constriction on the fifth pair of chromosomes suggesting that this pair may carry the nucleolus organizer regions (NORs) Scale bar = 5 μm.

chromosomes of *T. relictus* (2n = 20) were metacentrics, like most chromosomes of *Trachymyrmex* sp. (2*n* = 22), with the exception of two pairs of submetacentric chromosomes. A secondary constriction on the fifth pair of chromosomes was observed in the female (Figure 2a) and male (Figure 2b) chromosomes of *T. relictus*, suggesting that this pair may carry the nucleolus organizing regions (NORs).

Fifty-two species of the *Trachymyrmex* genus have been described (Brandão et al. 2011). Murakami et al.

(1998) reported the chromosomes of three taxa from Barro Colorado, Panama: *Trachymyrmex* sp. 1 (2n = 12 chromosomes), *Trachymyrmex* sp. 2 (2n = 18 chromosomes) and *Trachymyrmex septentrionalis* (McCook, 1881) (2n = 20 chromosomes). The karyotype of *T. relictus* (Figure 2a–c) shown in the present study is similar to the one describe by Murakami et al. (1998) for *T. septentrionalis*.

C-banding indicated the presence of heterochromatin blocks restricted to the centromeric region in all *T. rectilus* chromosomes (Figure 2c). Murakami et al. (1998) described interstitial heterochromatin blocks in four pair of chromosomes, in addition to the centromeric blocks in all chromosomes of *Trachymyrmex* sp. 1 (2n = 12). The authors suggested the possibility that chromosome fusion had occurred in this genus.

Trachymyrmex species show variation in the diploid number (2n = 12, 18, 20, 22). However, chromosomes with tiny arms, small chromosomes and/or pseudoacrocentric chromosomes were not observed in the present study, nor previously reported in this genus (Murakami et al. 1998). This would indicate that chromosomal rearrangement events of the centric fission type may have occurred according to the minimum interaction theory, but until now *Trachymyrmex* data are insufficient to support such an assumption. Future cytogenetic studies involving the chromosome heterochromatin analysis will be necessary to evaluate chromosome rearrangements involved in the karyotype evolution of this group of ants.

Genus Apterostigma

Two species were studied cytogenetically – *A. steigeri* and *A. madidiense*, with a diploid number of 2n = 22 (Figure 3a) and a haploid number of n = 23 (Figure 3b) respectively (Table 1). Secondary constriction was observed in at least one of the homologous of the largest metacentric pair of *A. steigeri*, indicating that this pair may carry the NORs. Until now no secondary constriction was reported in the genus *Apterostigma* (Fadini and Pompolo 1996; Murakami et al. 1998).

Forty-six species of the genus *Apterostigma* have been described (Fernández 2003; Agosti and Johnson 2005), but only four taxa have been studied cytogenetically: *Apterostigma mayri* Forel, 1893 (2*n* = 24, Murakami et al. 1998) and *Apterostigma* sp. from Barro Colorado, Panamá (2n = 24, Murakami et al. 1998); *Apterostigma* sp. from Minas Gerais, Brazil (2n = 20, Fadini and Pompolo 1996) and *Apterostigma* sp. from Montagne des Singes, French Guiana (2n = 32, Mariano et al. 2011).

Apterostigma madidiense presented n = 23 chromosomes in males, the highest chromosome number reported in the genus *Apterostigma*.

Tsutsui et al. (2008) raised the possibility of genome duplication in the *Apterostigma* genus, because studies indicate that *Apterostigma dentigerum* Wheeler, 1925 has about twice the DNA content of other species in the tribe Attini including *Atta cephalotes* (Linnaeus, 1758), *Atta colombica* Guérin-Méneville, 1844 and *S. amabilis*. However, since there are no reports of cytogenetic studies on A. dentigerum, a hypothesis in relation to possible chromosomal change in this species should await data on chromosome number and morphology.

The alternative hypothesis of centric fission seems more probable than the polyploidy hypothesis. This is based on the fact that the consequences of centric fission rearrangements will increase the number of chromosome and increase subtelocentric and acrocentric chromosomes; compare *A. madidiense*, which has a haploid set of chromosomes n = 7m + 10sm + 5 st + 1a, with other species

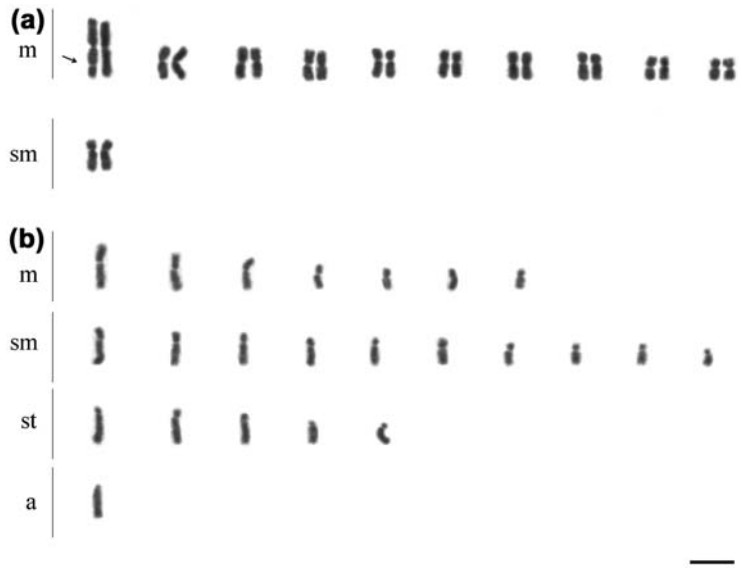

Figure 3. Karyotypes of *Apterostigma* species: (a) female of *A. steigeri* (2*n* = 22); (b) male of *A. madidiense* (n = 23). Arrow indicates secondary constriction suggesting that this pair may carry the nucleolus organizer regions (NORs) Scale bar = 5 µm.

of this genus, e.g. *A. steigery* which has 2n = 20m + 2sm. Furthermore, intermediate diploid number (2n = 32) was reported in *Apterostigma* sp. by Mariano et al. (2011)

Detailed cytogenetic studies including more species of the *Apterostigma* genus are needed to evaluate the chromosome variation in this genus. In addition, chromosome banding techniques and molecular cytogenetics will be useful to detect chromosome rearrangements occurring in this group of ants. Furthermore, karyological data and DNA content studies on particular species will be important to evaluate current hypotheses on ant chromosome evolution, including the occurrence of duplications due to unequal crossing over, translocations, transposable elements, or even an increase in the heterochromatin content (Gregory 2005).

Cytogenetic studies of attine ants are needed to evaluate whether the chromosome number (2n = 50 chromosomes) is maintained in the different species or in groups of *Sericomyrmex* species. Furthermore, cytogenetic studies in other members of the genus *Trachymyrmex* would be helpful in understanding the chromosome rearrangements involved in the evolution of these ants, such as the heterochromatin composition and location. Cytogenetic studies on members of the tribe Attini could contribute to a better understanding of their chromosomal evolution and phylogenetic relationships. It is essential for comparative studies to increase karyotype data information of more ant species. Considering the high biodiversity of Neotropical ant species in current and future studies will be crucial to identifying chromosomal alterations which may have played a direct role in chromosome speciation (White 1970).

Acknowledgments

This research was part of the M.Sc. thesis of the first author. We are grateful to Dr. Jacques Hubert Charles Delabie for the taxonomic identification of species, Rodrigo Feitosa for depositing specimens of *Trachymyrmex* sp. and *Sericomyrmex* sp. in the Museu de Zoologia da Universidade de São Paulo (MZUSP), Danival de Souza for providing *Sericomyrmex* sp., *A. madidiense* and for helpful comments during the experiments, Hilton J.A.C. de Aguiar for helpful suggestions on the manuscript and Manoel José de Souza for support in fieldwork. This research was supported by FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais). LACB acknowledges the grant from Conselho Nacional de Pesquisa (CNPq).

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