Evolutionary insights into the genomic organization of major ribosomal DNA in ant chromosomes

G. A. Teixeira*†, H. J. A. C. de Aguiar‡, F. Petitclerc§, J. Orivel§, D. M. Lopes† and L. A. C. Barros‡

*Programa de Pós-graduação em Biologia Celular e Estrutural, Universidade Federal de Viçosa, Viçosa, Brazil; †Laboratório de Citogenética de Insetos, Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Brazil; ‡Universidade Federal do Amapá, Campus Binacional, BR 156, n° 3051, Bairro Universidade, Oiapoque, 68980-000, Brazil; and §CNRS, UMR EcoFoG, AgroParisTech, CIRAD, INRA, Université de Guyane, Université des Antilles, Campus Agronomique, Kourou, France

Abstract

The major rDNA genes are composed of tandem repeats and are part of the nucleolus organizing regions (NORs). They are highly conserved and therefore useful in understanding the evolutionary patterns of chromosomal locations. The evolutionary dynamics of the karyotype may affect the organization of rDNA genes within chromosomes. In this study, we physically mapped 18S rDNA genes in 13 Neotropical ant species from four subfamilies using fluorescence in situ hybridization. Furthermore, a survey of published rDNA cytogenetic data for 50 additional species was performed, which allowed us to detect the evolutionary patterns of these genes in ant chromosomes. Species from the Neotropical, Palearctic, and Australian regions, comprising a total of 63 species from 19 genera within six subfamilies, were analysed. Most of the species (48 out of 63) had rDNA genes restricted to a single chromosome pair in their intrachromosomal regions. The position of rDNA genes within the chromosomes appears to hinder their dispersal throughout the genome, as translocations and ectopic recombination are uncommon in intrachromosomal regions because they can generate meiotic abnormalities. Therefore, rDNA genes restricted to a single chromosome pair seem to be a plesiomorphic feature in ants, while multiple rDNA sites, observed in distinct subfamilies, may have independent origins in different genera.

Keywords: evolution, ribosomal DNA, gene dispersion, Formicidae, chromosome rearrangements, physical mapping.

Introduction

Eukaryotic genomes have repetitive tandem sequences such as in the major ribosomal RNA genes (45S = 18S + 5.8S + 28S), herein denominated rDNA, which contain highly conserved genic sequences and are therefore useful as molecular genetic markers, allowing comparisons across distant taxa. However, intergenic spacers vary in both sequence and length (Long and Dawid, 1980; Sumner, 2003; Symonová, 2019). The 45S ribosomal genes are part of the nucleolus organizing regions (NORs) and are located in portions of the DNA that, after their condensation, usually appear as secondary constric-
chromosomes, which makes using this procedure unsatisfactory and inconclusive (Imai et al., 1992; Hirai et al., 1994; Lorite et al., 1997; Barros et al., 2009, 2015; Aguiar et al., 2017).

Since the 1980s, molecular cytogenetic tools have been used to study karyotypes. For example, fluorescence in situ hybridization (FISH) has proven to be an effective and precise tool for physically mapping specific DNA sequences within chromosomes (reviewed by Levsky and Singer, 2003; Liehr, 2017). The rDNA genes can be located in single or multiple chromosome pairs (Sochorová et al., 2018). In several organisms, studies of these genes have pointed to chromosomal differences within species complexes (Mantovani et al., 2005; Barbosa et al., 2017; Dutra et al., 2020) and between species with similar karyotypes (Panzer et al., 2012; Golub et al., 2015; Gokhman et al., 2016). As a consequence, inferences can be made based on chromosomal rearrangements that shape the chromosomal evolution of a species (Roy et al., 2005; Nguyen et al., 2010; Britton-Davidian et al., 2012; Cabral-de-Mello et al., 2011; Dutrillaux and Dutrillaux, 2012; Roa and Guerra, 2012; Menezes et al., 2019; Degrandi et al., 2020).

In ants, the physical mapping of rDNA genes using the FISH technique was first described in Australian ants by Hirai et al. (1994, 1996). Since then, the number of studies mapping these genes has increased (Mariano et al., 2008; Santos et al., 2016; Miclino et al., 2019a; Teixeira et al., 2020) and other repetitive sequences, such as telomerases (Meyne et al., 1995; Pereira et al., 2018; Miclino et al., 2020; Castro et al., 2020), satellite DNA (Lorite et al., 2004; Huang et al., 2016), 5S ribosomal genes (Aguiar et al., 2017), and microsatellites (Barros et al., 2018; Miclino et al., 2019b), have been mapped in the chromosomes using the FISH technique. To date, molecular cytogenetic studies on rDNA genes in ants have improved understanding of chromosomal evolution and phylogeny and provided taxonomic resolutions for different ant groups (Hirai et al., 1994, 1996; Santos et al., 2010, 2016).

In this study, we physically mapped 18S rDNA clusters using the FISH technique and verified if they were GC-rich in 13 Neotropical ant species from four subfamilies (Ectatomminae, Formicinae, Myrmicinae, and Ponerinae). In addition, we reviewed previous molecular cytogenetic data related to rDNA gene clusters (45S, 18S, or 28S) in ants. Using these data, we investigated whether the number and location of the ribosomal gene clusters followed a specific pattern or were randomly distributed in order to understand the genomic organization and evolutionary dynamics of these genes in ants.

Results

Chromosome mapping of 18S rDNA clusters in 13 Neotropical ant species

All of the studied ant species presented only a single 18S rDNA site that was colocalized with GC-rich regions (CMA3) (Table 1), while AT-rich regions (DAPI+) were not detected in any species. Most of the species presented GC-rich rDNA genes in the intrachromosomal regions (pericentromeric or interstitial), including Pseudoponera gilberti (Kempf, 1960) (Fig. 1A, S1A), Anochetus targinii Emery, 1894 (Fig. 1B, S1B), Odontomachus haematodus (Linnaeus, 1758) (Fig. 1C, D, S1C), Odontomachus bauri Emery, 1892 (Fig. 1E, S1D), Pheidole germaini Emery, 1896 (Fig. 2A, S2A), Cremaetogaster longispinosa Emery, 1890 (Fig. 2B, S2B), Solenopsis giminata (Fabricius, 1804) (Fig. 2C, D, S2C), Myrmicrypta sp. (Fig. 2E, S2D), and Acromyrmex echinatior (Forel, 1899) (Fig. 2F). Additional GC-rich bands only occurred in P. gilberti in the pericentromeric region of the 3rd and 4th metacentric chromosome pairs (Fig. S1B). Differences in rDNA cluster sizes between homologous chromosomes were observed in heterozygous O. bauri individuals. One of the homologous chromosomes showed clusters approximately twice the size of those in the other chromosome (Fig. 1F, S1E). A male individual bearing a chromosome with minor GC-rich bands was analysed (Fig. S1F). Homozygous individuals with duplicated clusters were not observed.

The remaining species showed GC-rich 18S rDNA clusters across the entire chromosome arm, occupying either the long arm, as in Gnamptogenys tortuolosa (Smith, 1858) (Fig. 3A, S3A), or residing in the short arm, as in Strumigenys diabola Bolton, 2000 (Fig. 2G, S2E), Camponotus atriceps (Smith, 1858) (Fig. 3B, S3B), and Gigantopios destructor (Fabricius, 1804) (Figs. 3C, S3C, D). Heteromorphism of 18S rDNA clusters was detected in all of the analysed C. atriceps and G. tortuolosa individuals. In the latter species, the heteromorphism of the NOR resulted in differences in total size between homologous chromosomes, which changed their morphology such that one was submetacentric while the other was subtelocentric. In G. destructor, additional GC-rich bands were located in the interstitial region of the long arm of the largest subtelocentric chromosome pair (Fig. S3C, D).

Chromosome mapping review of rDNA clusters in ants

Cytogenetic data available in the literature related to the rDNA genes of 50 ant species from 12 genera and six subfamilies were reviewed (Table 1; Fig. 4). Most data were concentrated on Neotropical ants, with information on 33 species, while the Palearctic and Australian regions had data on only one and 16 species, respectively. A single rDNA site localized in the intrachromosomal region was observed in most species (Fig. 4A, B). However, Camponotus renggeri Emery, 1894, Dinoponera gigantea (Perty, 1833), and most of the studied Myrmecia species presented multiple rDNA sites over the entire short chromosome arm. The subfamily Myrmicinae possessed most of the rDNA data, and the Myrmecinae subfamily showed
Table 1. Summary of the available molecular cytogenetic data concerning major ribosomal genes (45S, 28S or 18S) detected by FISH in ants [Colour table can be viewed at wileyonlinelibrary.com].

| Ant species | 2n(n) | Localization of rDNA genes in karyotype | Ideogram | Co-localization CMA3/rDNA | Reference CMA3/rDNA |
|-------------|-------|----------------------------------------|----------|--------------------------|---------------------|
| **Neotropical ants** |       |                                        |          |                          |                     |
| **Subfamily Myrmicinae** |       |                                        |          |                          |                     |
| Acromyrmex aspersus | 38 | Largest subtelocentric pair | | Yes | Teixeira et al. (2017) |
| Acromyrmex coronatus | 38 | Largest subtelocentric pair | | Yes | Barros et al. (2016) |
| Acromyrmex disciger | 38 | Largest subtelocentric pair | | Yes | Barros et al. (2016) |
| Acromyrmex echinatior | 38 | Largest subtelocentric pair | | Yes | Barros et al. (2016) |
| Acromyrmex niger | 38 | Largest subtelocentric pair | | Yes | Barros et al. (2016) |
| Acromyrmex striatus | 22 | 2nd metacentric pair | | Yes | Cristiano et al. (2013)/ Teixeira et al. (2017) |
| Acromyrmex subterraneus molestans | 38 | Largest subtelocentric pair | | Yes | Teixeira et al. (2017) |
| Atta bisphaerica | 22 | 4th metacentric pair | | Yes | Barros et al. (2014)/Teixeira et al. (2017) |
| Atta laevigata | 22 | 4th metacentric pair | | Yes | Barros et al. (2014)/Teixeira et al. (2017) |
| Atta robusta | 22 | 4th metacentric pair | | Yes | Barros et al. (2015) |
| Atta sexdens rubropilosa | 22 | 4th metacentric pair | | Yes | Barros et al. (2014)/Teixeira et al. (2017) |
| Crematogaster longispina | 24 | Largest metacentric pair | | Yes | Present study |
| Mycetophylax conformis | 30 | 11th metacentric pair | | Yes | Cardoso et al. (2014)/ Micolino et al. (2019a) |
| Mycetophylax morschi | 26 | 2nd submetacentric pair | | – | Micolino et al. (2019a) |
| Mycetophylax morschi | 28 | 7th metacentric pair | | – | Micolino et al. (2019a) |
| Mycetophylax morschi | 30 | Acrocentric pair | | – | Micolino et al. (2019a) |
| Mycetophylax simplex | 36 | Smallest metacentric pair | | Yes | Cardoso et al. (2014)/ Micolino et al. (2019a) |
| Mycocepurus goeldii | 8 | 2nd metacentric pair | | Yes | Barros et al. (2010)/Barros et al. (2012) |
| Myrmicocyrtus sp. | 30 | 9th metacentric pair | | Yes | Present study |
| Pheidole germaini | 22 | Subtelocentric pair | | Yes | Present study |
| Solenopsis geminata | 32 | Smallest submetacentric pair | | Yes | Present study |
| Strumigenys diabola | 40 | 4th submetacentric pair | | Yes | Present study |
| Mycetomoellerius holmgreni | 20 | 4th metacentric pair | | Yes | Barros et al. (2018) |
| **Subfamily Formicinae** |       |                                        |          |                          |                     |
| Camponotus atriceps c | 40 | 2nd submetacentric pair | | Yes | Present study |
| Camponotus cingulatus c | 40 | 2nd submetacentric pair | | Yes | Aguiar et al. (2017) |
| Camponotus renggeri | 40 | 2nd submetacentric pair and medium-sized subtelocentric pair | | Yes | Aguiar et al. (2017) |
| Camponotus rufipes c | 40 | 2nd submetacentric pair | | Yes | Aguiar et al. (2017) |
| Gigantopus destructor | (39) | 8th metacentric pair | | Yes | Present study |
| **Subfamily Ponerinae** |       |                                        |          |                          |                     |
| Anochetus altisquamis c | 30 | 3rd submetacentric pair | | – | Santos et al. (2010) |
| Anochetus horridus c | 46 | 4th telocentric pair | | – | Santos et al. (2010) |
| Anochetus targionii | 30 | 7th metacentric pair | | Yes | Present study |
| Dinoponera gigantea | 82 | Multiple pairs | | – | Aguiar et al. (2011) |
| Dinoponera lucida | (59) | Largest pair | | Yes | Mariano et al. (2008) |
| Odontomachus bauri d | 44/(22) | 2nd subtelocentric pair | | Yes | Present study |
| Odontomachus haematodus | 44 | 3rd subtelocentric pair | | Yes | Present study |

(Continued)
Table 1. Continued

| Ant species              | 2n/2n (n) | Localization of rDNA genes in karyotype | Ideogram | Co-localization CMA3/rDNA | Reference CMA3/rDNA |
|--------------------------|-----------|----------------------------------------|----------|--------------------------|---------------------|
| Pseudoponera gilberti    | 22        | Largest metacentric pair               |          | Yes *                    | Present study       |
| Subfamily Ectatomminae   |           |                                        |          |                          |                     |
| Gnamptogenys moelleri    | 34, 44    | 4th metacentric pair                   |          | Yes                      | Teixeira et al. (2020) |
| Gnamptogenys regularis   | 26        | Metacentric and submetacentric         |          | Yes                      | Teixeira et al. (2020) |
| Gnamptogenys striatula   | 32        | 5th metacentric pair                   |          | Yes                      | Teixeira et al. (2020) |
| Gnamptogenys striatula   | 34        | 4th metacentric pair                   |          | Yes                      | Teixeira et al. (2020) |
| Gnamptogenys triangularis| 24        | Largest metacentric pair               |          | Yes                      | Teixeira et al. (2020) |
| Gnamptogenys tortuolosa  | 44        | Subtelocentric and submetacentric      |          | Yes                      | Present study       |
| Subfamily Dolichoderinae |           |                                        |          |                          |                     |
| Dolichoderus attelaboides| 58        | Largest submetacentric                 |          | Yes                      | Santos et al. (2016) |
| Dolichoderus biden       | 18        | Largest metacentric pair               |          | Yes *                    | Santos et al. (2016) |
| Dolichoderus decollatus  | 38        | 2nd metacentric pair                   |          | Yes                      | Santos et al. (2016) |
| Dolichoderus diversus    | 22        | Largest metacentric pair               |          | Yes *                    | Santos et al. (2016) |
| Dolichoderus imitator    | 38        | Largest metacentric pair               |          | Yes                      | Santos et al. (2016) |
| Dolichoderus lutosus     | 10        | 2nd metacentric pair                   |          | Yes *                    | Santos et al. (2016) |
| Dolichoderus voraginosus | 20        | Largest metacentric pair               |          | No *                     | Santos et al. (2016) |
| Australian ants          |           |                                        |          |                          |                     |
| Subfamily Myrmeciinae    |           |                                        |          |                          |                     |
| Myrmecia banksi          | 10        | Smallest acrocentric pair              |          | –                        | Hirai et al. (1994), (1996) |
| Myrmecia chasei          | 47        | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |
| Myrmecia croslandi       | 2, 3, 4 b | Acrocentric pair                       |          | –                        | Hirai et al. (1994), (1996) |
| Myrmecia forficata       | 52 b, 54  | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |
| Myrmecia fulvipes        | 48, 50    | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |
| Myrmecia gulosa          | 38 b      | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |
| Myrmecia haskinsorum     | 18        | Multiple pairs                         |          | –                        | Hirai et al. (1994), (1996) |
| Myrmecia imaii           | 8         | Largest acrocentric pair               |          | –                        | Hirai et al. (1994), (1996) |
| Myrmecia mandibularis    | 56 b      | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |
| Myrmecia michaelseni     | 27        | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |
| Myrmecia occidentalis    | 64 b      | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |
| Myrmecia pavidia         | 44        | Multiple pairs                         |          | –                        | Hirai et al. 1996    |
| Myrmecia pilosula        | 22 b      | Acrocentric pair                       |          | –                        | Hirai et al. (1996)  |
| Myrmecia pilosula        | 19, 23 b, 27, 32 | Multiple pairs |          | –                        | Hirai et al. (1994), (1996) |
| Myrmecia simulima        | 70 b      | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |
| Myrmecia arnoldi         | 53, 55, 57, 59 | Multiple pairs |          | –                        | Hirai et al. (1996)  |
| Myrmecia vindex          | 74, 76    | Multiple pairs                         |          | –                        | Hirai et al. (1996)  |

Paleartic ant

Subfamily Dolichoderinae

| Tapinoma nigerrimum      | 18        | Largest submetacentric pair            |          | Yes                      | Lorite et al. (1997) |

*Additional markers CMA3.*

*Data showed by Hirai et al. (1996).*

*Heteromorphism of rDNA clusters between the homologues.*

*Poly morbism of rDNA clusters between the homologues — unavailable data.*

© 2021 Royal Entomological Society, 30, 340–354
a different pattern in relation to other subfamilies with multiple rDNA sites observed in the majority of species (Fig. 4C). *Dolichoderus voraginosus* Mackay, 1993 did not show any co-localization of 18S rDNA clusters and GC-rich bands. Fig. 5 summarizes the available data on the number and position of NORs in ant species and the phylogenetic relationships among these species and is based on the published molecular phylogenies.

**Discussion**

**Patterns of rDNA clusters in the karyotypes of specific ant groups**

Specific rDNA patterns can be observed in the karyotypes of some ant groups where several species have been studied. In species of the genus *Dolichoderus*, the chromosome numbers range from $2n = 10$ to $58$, and a single rDNA site in the interstitial region has been observed in the majority of the investigated species. However, there are two exceptions: *D. voraginosus* and *Dolichoderus atte-laboides* (Fabricius, 1775), where the rDNA clusters are located in the terminal region of the long arm and in the short arm, respectively (Santos *et al.*, 2016).

Despite the chromosomal variation observed in *Gnantogenys* spp. ($2n = 24$–$44$), they all have a single rDNA site in the intrachromosomal region (Teixeira *et al.*, 2020). Even *Gnantogenys moelleri* (Forel, 1912), a species with differing chromosome numbers between two populations ($2n = 34$ and $44$), has a single intrachromosomal rDNA site. *Gnantogenys tortuolosa* is an exception, with rDNA clusters occurring over the entire long arm (this study). In the genus *Anochetus*, the chromosome number ranges from $2n = 30$ to $46$, and a single pericentromeric rDNA site has been observed (Santos *et al.*, 2010).

All analysed fungus-farming ants (*Attina*) had a single rDNA site (Table 1). In the genus *Mycetophylax*, although chromosome number differs among species ($2n = 26$–$36$), a single rDNA site is located in the pericentromeric or terminal region in all the species of the genus (Micolino *et al.*, 2019a). The leaf-cutting ants are considered the most derived among the *Attina* species (Schultz and Brady, 2008). *Atta* spp. ($2n = 22$) have a single rDNA site in the interstitial region and *Acromyrmex* spp. ($2n = 38$)
have a single rDNA site in the terminal region (Barros et al., 2015, 2016; Teixeira et al., 2017). Acromyrmex striatus (Roger, 1863), a sister group of the leaf-cutting ants, has $2n = 22$ and pericentromeric rDNA clusters that are not located on the same chromosome pair relative to that in Atta spp. (Cristiano et al., 2013; Teixeira et al., 2017). In A. echinatior ($2n = 38$), rDNA clusters are located in the interstitial region of the same pair as observed in other Acromyrmex spp. (this study).

The chromosome number of the Australian bulldog ants is highly variable, ranging from $2n = 2$ to 76 (Imai et al., 1994). In addition, Myrmecia spp. present remarkable patterns of multiple 28S rDNA clusters that are highly dispersed throughout their genomes. The number of rDNA sites increases with the chromosome number of the species. This suggests several ribosomal gene amplification events have occurred in the different species of Myrmecia and that they have accumulated in karyotypes throughout the evolution of the genus. Only four out of 16 species from this monophyletic genus have the entire arm or intrachromosomal rDNA clusters restricted to a single pair of chromosomes (Hirai et al., 1994, 1996; Hirai, 2020). This pattern is observed in species with small chromosome numbers, which suggests that a single NOR is plesiomorphic among Myrmecia (Hirai, 2020).

Carpenter ants (Camponotus) from the subgenus Myrmothrix have $2n = 40$ chromosomes, with its studied species having a single rDNA site in the terminal region. Camponotus renggeri is the only exception, having an additional rDNA cluster at the terminal region of a medium-sized subtelocentric pair (Aguiar et al., 2017).

In the giant ants of the genus Dinoponera, two contrasting patterns have been observed: D. gigantea ($2n = 82$) has multiple rDNA sites located on its short chromosome arms (Aguiar et al., 2011), whereas Dinoponera lucida Emery, 1901 has a higher chromosome number ($2n = 120$), but only a single rDNA site restricted to the.

Figure 2. Fluorescence in situ hybridization with 18S rDNA probe (red blocks) of Myrmicinae ants: (A) Pheidole germaini ($2n = 22$), (B) Crematogaster longispina ($2n = 24$), (C) Solenopsis geminata ($2n = 32$) and (D) Solenopsis geminata ($n = 16$), (E) Myrmicocrypta sp. ($2n = 30$), (F) Acromyrmex echinatior ($2n = 38$), (G) Strumigenys diabola ($2n = 40$). Bars = 5 μm. [Colour figure can be viewed at wileyonlinelibrary.com].
intrachromosomal region of its largest chromosome pair (Mariano et al., 2008).

Homeology patterns among chromosomal pairs bearing ribosomal genes can be detected in a few ant genera, such as Gnamptogenys (striatula group), Camponotus (Myrmothrix), Atta, and Acromyrmex (Table 1). However, it is speculative to infer such homeology patterns for the entire Formicidae family. Ants constitute an ultra-diverse monophyletic group with more than 13 800 described species (Bolton, 2020). They show a wide range of karyotype variation both in number (2n = 2–120) and chromosomal morphology (reviewed by Lorite and Palomeque, 2010; Mariano et al., 2019).

Insights concerning the organizational patterns of ribosomal gene clusters in the ant genome

The mapping of the ribosomal gene clusters of 63 species distributed in 19 genera and six subfamilies, together with information on their phylogenetic relationships, demonstrated that a single pair of chromosomes bearing the GC-rich rDNA clusters is the most frequent trait among the studied species, regardless of the chromosome number (Table 1, Fig. 4A). Genomes carrying rDNA clusters in more than a single chromosome pair have been observed in non-related taxa, such as D. gigantea (Aguiar et al., 2011), C. renggeri (Aguiar et al., 2017), and Myrmecia spp. (Hirai et al., 1994, 1996). We hypothesize that having a single rDNA site should be considered a plesiomorphic trait because multiple rDNA sites were observed in different non-related lineages that do not share exclusive common ancestry and appear de novo throughout the Formicidae family.

In eukaryotes, it is common to observe variations in the number of rDNA clusters and the location of these genes in the chromosomes within genera (Sánchez-Gea et al., 2000; Gross et al., 2010; Cabral-de-Mello et al., 2011; Gokhman et al., 2014; Mazzoleni et al., 2018), among populations (Panza et al., 2014; Ferreti et al., 2019; Menezes et al., 2019), and sexes of the same species (Nakayama et al., 2001; Šťáhlavský et al., 2018). The possession of terminal rDNA clusters seems to be a common trait among mammals, fish, and molluscs, but less so in arthropods (Sochorová et al., 2018). Within the ultra-diverse insect group, the location of rDNA clusters may follow distinct patterns in its two largest orders; terminal rDNA sites are more abundant in Coleoptera, whereas pericentromeric rDNA clusters are more frequent in Orthoptera (Sochorová et al., 2018). In Formicidae, terminal rDNA sites are a less common feature and all species with multiple rDNA clusters show these genes in the entire short chromosome arms including terminal/subterminal

Figure 3. Fluorescence in situ hybridization with 18S rDNA probe (red blocks) of an Ectatomminae ant (A) Gnamptogenys tortuolosa (2n = 44), and Formicinae ants (B) Camponotus atriceps (2n = 40) and (C) Gigantiops destructor (n = 39). In G. tortuolosa, the box shows a remarkable size heteromorphism of 18S rDNA clusters between homologues. Bars: 5 μm. [Colour figure can be viewed at wileyonlinelibrary.com].
regions, such as C. renggeri, D. gigantea, and Myrmecia spp. (Aguiar et al., 2011, 2017; Hirai et al., 1994, 1996).

Different hypotheses have been proposed to explain the cytogenetic pattern (conservative or variable) of these rDNA clusters in the genome of several organisms. Such hypotheses are linked to the specific locations of these rDNA clusters on the chromosomes. Rearrangements, such as translocations, unequal exchange, and ectopic recombination mechanisms (i.e., between non-homologous chromosomes), which can lead to gene dispersion or increases in number in the genome, are more likely in the terminal/subterminal regions of chromosomes and are uncommon in the intrachromosomal regions (Martins and Wasko, 2004; Mantovani et al., 2005; Nguyen et al., 2010; Roa and Guerra, 2012; Hirai, 2020).

Based on rDNA mapping data in fish karyotypes, Martins and Wasko (2004) proposed that translocations are more likely to occur in telomeric regions because of their proximity within the interphase nucleus, which originates from the ordering of chromosomes according to Rabl’s model. Effects due to the location of ribosomal genes in relation to their dispersion in the karyotype were also observed in Coleoptera (Dutrillaux et al., 2016) and primates (Gerbault-Seureau et al., 2017). These authors argued that translocations in the interstitial position could result in abnormal meiosis and, therefore, unbalanced gametes. In contrast, translocations in terminal positions may increase the number of rDNA genes in the genome. This would lead to fewer meiotic abnormalities and highlights the selection for interstitial rDNA site stability (Dutrillaux et al., 2016; Gerbault-Seureau et al., 2017).

Ectopic recombination is another mechanism suggested to explain the rDNA patterning in moths and butterflies (Nguyen et al., 2010) and plants (Roa and Guerra, 2012). It is also included in the recent model proposed by Hirai (2020). In this model, two mechanisms are important: the “site effect” and the “molecular effect.” The former allows terminal region associations due to the proximity of these regions in a meiotic bouquet. The “site effect” is a precondition for the “molecular effect,” which refers to systems of affinity/non-affinity due to the similarity between rDNA sequences with other repetitive sequences. Thus, rDNA clusters in the terminal regions tend to associate with other repetitive sequences of non-homologous chromosomes more easily, facilitating the occurrence of ectopic recombination and dispersion of these genes in the genome (Hirai, 2020).

There are reports of species with multiple rDNA clusters associated with the centromeres of acrocentric chromosomes (Cazaux et al., 2011). In the recent model proposed by Hirai (2020), the centromeric region of acrocentric chromosomes (chromosomes with a short and heterochromatic arm) that have rDNA genes associated with the centromere may behave as subterminal regions. Therefore, such an arrangement would also facilitate eventual associations of rDNA genes with other repetitive sequences and the occurrence of ectopic recombination, which leads to their dispersal in the genome (for details, see Hirai, 2020).

The ant rDNA chromosome evolution seems to be in accordance with the above-mentioned hypothesis about dispersal and NOR location because the single rDNA clusters of most studied species are interstitial or pericentromeric (Fig. 4B). In ant species, terminal rDNA clusters are
Figure 5. Summary of available data (this study and literature) concerning the number and position of rDNA genes in ant species, along with their degree of relatedness and diploid chromosome numbers. Ideograms of rDNA-bearing chromosomes show number and location (terminal, interstitial, pericentromeric or centromeric) of rDNA clusters (red) in the haploid complement. Phylogenetic relationships are based on 1—Moreau and Bell (2013), 2—Schmidt (2013), 3—Larabee et al. (2016), 4—Santos et al. (2016), 5—Hasegawa and Crozier (2006), 6—Ward et al. (2015), 7—Solomon et al. (2019), 8—Micolino et al. (2019a), 9—Bacci et al. (2009), 10—Queiroz EC (2015, unpublished data). Colours of phylogenetic branches indicate the following subfamilies: red, Ponerinae; purple, Dolichoderinae; pink, Myrmeciinae; blue, Formicinae; green, Myrmicinae; grey, Ectatomminae. [Colour figure can be viewed at wileyonlinelibrary.com].
prone to rearrangements that lead to their dispersal. *Camponotus reneggeri* (Aguiar et al., 2017), *D. gigantea* (Aguiar et al., 2011), and *Myrmecia* spp. present multiple NORs in the entire short chromosome arms including terminal/subterminal regions, which facilitates the association of these genes with the heterochromatic sequences of other non-homologous acrocentric chromosomes during meiosis and the subsequent occurrence of ectopic recombination (Hirai, 2020). This pattern may be applicable to different ant groups. In addition, inversions have been shown to change the position of rDNA genes in *A. echinator* (this study), *Dolichoderus* spp. (Santos et al., 2016), and *Myrmecia* spp. (Hirai et al., 1996).

A single rDNA site located in the terminal region or entire chromosomal arm was observed in some ant species. The repetitive sequences in the subterminal/terminal chromosome regions probably do not form affinity systems with ribosomal genes (the so-called molecular effect; for details, see Hirai, 2020) in these species. Therefore, rDNA clusters are restricted to a single chromosomal pair. Future studies focusing on the characterization of repetitive sequences that make up the heterochromatin of these species will help clarify this hypothesis.

Size heteromorphisms are frequent in karyotypes where the rDNA clusters have terminal positions in the chromosomes, as reported in this study (*G. tortuolosa* and *C. atriceps*) as well as in other ants (Aguiar et al., 2017) and insects in general (Cabral-de-Mello et al., 2011; Maryańska-Nadachowska et al., 2016; Andrade-Souza et al., 2018). Subtle variations in the size of the rDNA clusters between homologous chromosomes can be observed as a result of late condensation during cell division (Sumner, 2003). However, large variations, such as those mentioned above, at the terminal region on the chromosome are usually related to duplications/deletions as a result of unequal exchange (Schubert and Lysack, 2011). It is believed that exchanges are less common in intrachromosomal regions (Hirai, 2020).

Size variations in the rDNA clusters can be observed when these genes are located in the interstitial/pericentromeric region of the chromosomes, as seen in *Gnamptogenys regularis* Mayr, 1870 (Teixeira et al., 2020) and in *O. bauri* (this study). A different path seems to be involved in the evolution of these karyotypes compared to the rearrangements involved in terminal rDNA heteromorphisms. In these cases, the mechanism may be associated with the formation of extrachromosomal circular DNA (eccDNA), which is likely to form tandem repetitive sequences similar to the rDNA genes (Cohen and Segal, 2009). These eccDNAs may be lost, leading to deletions in the original rDNA sequences, or they may be replicated via a rolling circle mechanism and reinserted into the original chromosome, producing duplications of these repetitive sequences (Cohen and Segal, 2009).

In ants as well as in other eukaryotes, rDNA clusters are located in GC-rich regions (Symonová, 2019) and, therefore, usually coincide with CMA3+ bands, possibly as a result of GC-biased gene conversion (gBGC) over the course of evolutionary time (Escobar et al., 2011). This co-localization of GC-rich regions and rDNA was observed for all the ants studied to date (Table 1), with the exception of *D. voraginosus* (Santos et al., 2016). However, GC-rich chromatins are not always an indication of ribosomal genes, as seen here in *G. destructor*, *P. gilberti*, and in some *Dolichoderus* spp. and fungus-farming ants (Table 1).

In insects, a compilation of previous data concerning rDNA genes has been conducted, for example, in moths and butterflies (Lepidoptera; Nguyen et al., 2010), beetles (Coleoptera; Dutrillaux and Dutrillaux, 2012), and kissing bugs (Heteroptera; Panzera et al., 2012). However, this is the first survey of Hymenoptera species. We have compiled available information and new data on 13 Neotropical ant species. In different organisms, including ants, the number and location of chromosomes bearing rDNA clusters within the genome follow general patterns that govern the modes of evolution for these genes (Martins and

### Table 2. The relationship between Neotropical ant species studied using 18S rDNA FISH and their chromosome number

| Ant species                  | Subfamily   | Locality                        | 2n  |
|------------------------------|-------------|---------------------------------|-----|
| Acromyrmex echinatior (Forel, 1899) | Myrmicinae | Barro Colorado—Panamá           | 38  |
| Anochetus targioni Emery 1894 | Formicinae  | Campus Agronomique, Kourou—FG   | 30  |
| Camponotus atriceps (Smith, 1858) | Formicinae | Viçosa—Minas Gerais—BR          | 40  |
| Crematogaster longispina Emery, 1890 | Myrmicinae | La Montagne des Singes—FG       | 24  |
| Gigantopsis destructor (Fabricius, 1804) | Formicinae | Sinnamary—FG                    | 78  |
| Gnamptogenys tortuolosa (Smith, 1858) | Ectatomminae | Sinnamary—FG                    | 44  |
| Myrmicocrypta sp. | Myrmicinae | Sinnamary—FG                    | 30  |
| Odontomachus bauri Emery, 1892 | Ponerinae   | Aquilândia—Maranhão—BR          | 44  |
| Odontomachus haematodus (Linnaeus, 1758) | Ponerinae | Campus Agronomique, Kourou—FG and Ubá—Minas Gerais—BR | 44 |
| Pheidole gemmae Emery, 1896 | Myrmicinae  | Viçosa—Minas Gerais—BR          | 22  |
| Pseudoponera gilberti (Kempf, 1960) | Ponerinae | Sinnamary—FG                    | 12  |
| Solenopsis geminate (Fabricius, 1804) | Myrmicinae | Sinnamary—FG                    | 32  |
| Strumigenys diabola Bolton 2000 | Myrmicinae | Sinnamary—FG                    | 40  |

FG, French Guiana; BR, Brazil.
Wasko, 2004; Nguyen et al., 2010; Dutrillaux et al., 2016; Gerbault-Seureau et al., 2017; Hirai, 2020; this study). We can conclude that having only a single pair of chromosomes bearing rDNA clusters is more common in the ant genome because of the pericentromeric/interstitial location of these genes on the chromosomes. Intrachromosomal regions are sites with low frequencies of rearrangements, such as non-Robertsonian translocations and ectopic recombination, and are therefore less prone to meiotic abnormalities. It should be assumed that the chromosomal location of rDNA clusters influences the dispersion of these genes within the karyotype.

Future studies will allow the mapping of rDNA genes in more ant taxa, including the other remaining subfamilies. Other repetitive sequences, such as 5S rDNA and histone genes, may also be mapped in ant species as a tool to investigate further patterns that reflect the relationship between chromosomal location and dispersion in the genome. Finally, a solid understanding of the evolutionary patterns of ribosomal gene dispersal in ant chromosomes may provide a comparative model for other insects.

Experimental procedures

Obtaining samples for analysis

Field surveys to collect ant colonies were performed in French Guiana, Brazil, and Panamá (Table 2) from the following locations: La Montagne des Singes, Kourou (5.07225, −52.69407), Campus Agronomique, Kourou (5.17312, −52.65480), and Sinnamary (5.28482, −52.91403), all in French Guiana; Viçosa (−20.757041, −42.873516) and Ubá (−21.128880, −42.937646), both in Minas Gerais, Brazil, and Açailândia (−4.842000, −47.29667) in Maranhão, Brazil; and Barro Colorado Island (9.150000, −79.833333) in Panama. Sampling permit in Brazil was provided by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBIO accession numbers 32 459 and 350 G. A. Teixeira et al. 2018). The chromosomes were used. For comparative analysis, the following traits were considered for each species: chromosome number, number of rDNA-bearing chromosomes, location of rDNA clusters in the karyotype, and co-localization of CMA3 and DAPI staining in this species was performed by these authors.

Fluorescence in situ hybridization with rDNA 18S probe

The 18S rDNA probes were obtained by amplification via polymerase chain reaction (PCR) using Melpionia quinquemaculata Lepeletier, 1836, rDNA primers 18SF1 (5′−GTC ATA GCT TTG TCT CAA AGA−3′) and 18SR1.1 (5′−CGC AAA TGA AAC TTT AAT CT−3′) Pereira JOP (2006, unpublished data) in the genomic DNA from the ant Camponotus rutipes (Fabricius, 1775). Gene amplification was performed following Pereira JOP (2006, unpublished data). The probes were labelled by an indirect method using digoxigenin-11-dUTP (Roche Applied Science, Mannheim, Germany), and the FISH signals were detected with anti-digoxigenin-rhodamine (Roche Applied Science), following the manufacturer’s protocol.

The rDNA 18S genes were mapped by FISH, following the protocol of Pinkel et al. (1986). The slides were treated with RNase A (100 μg/ml) and kept in a moist chamber at 37°C for 1 h. After that, they were washed in 2 × SSC for 5 min, incubated in 5 μg/ml pepsin in 0.01 N HCl for 10 min, washed in 1 × PBS for 5 min, and dehydrated in 50%, 70%, and 100% alcohol series for 3 min each. After this pretreatment, metaphase chromosomes were denatured in 70% formamide/2 × SSC at 75°C for 3 min, and 20 μl of hybridization mix containing 200 ng of labelled probe, 2 × SSC, 50% formamide, and 10% dextran sulfate was denatured for 10 min at 85°C and added on preparations. The slides were kept in a moist chamber at 37°C overnight. Then, the slides were washed in 2 × SSC for 5 min; the detection solution including anti-digoxigenin-rhodamine was added on slides that were kept in a moist chamber at 37°C for 1 h. The slides were washed in 4 × SSC/Tween and dehydrated in an alcohol series. Finally, counterstaining with DAPI (DAPI Fluoroshield, Sigma Aldrich) was performed.

Chromosomal analysis

Chromosomes were arranged in order of decreasing size and based on the ratio of the chromosomes arm lengths (r = long arm/short arm), according to the classification proposed by Levan et al. (1964). The chromosomes were classified as m = metacentric (r = 1−1.7), sm = submetacentric (r = 1.7−3), st = subtelocentric (r = 3−7), and a = acrocentric (r > 7); they were organized using Adobe Photoshop® 21.1.1 and measured using Image Pro Plus®. Ideograms of the NOR-bearing chromosome/chromosomes (i.e., graphical representation of the chromosomes concerning the rDNA clusters) of the ant species were then designed with the Easy Idio software (Diniz and Xavier, 2006).

For the fluorochrome staining and FISH 18S rDNA technique, 30 metaphases from at least three individuals of each species were analysed. In the case of O. bauri, which presented a chromosomal polymorphism involving rDNA clusters, seven individuals...
were analysed (six females and one male). The metaphases were analysed and photographed using a fluorescence microscope, Olympus BX60, attached to an image system, QColor Olympus®, with the filters WB (450–480 nm), WU (330–385 nm), and WG (510–550 nm) for the fluorochromes CMA₃, DAPI, and rhodamine, respectively.

**Phylogenetic relationships**

The phylogenetic relationship among ant species was determined by associating with previously published molecular phylogenies. The resultant cladogram topology at the subfamily level was determined following Moreau and Bell (2013). The Poneroid clade topology was determined according to Schmidt (2013) and Larabee et al. (2016); the clade topology for the subfamily Dolichoderinae was determined according to Santos et al. (2016), and that for the subfamily Myrmicinae was determined according to Bacci et al. (2009), Ward et al. (2015), Queiroz EC (2015, unpublished data), Solomon et al. (2019), and Micilino et al. (2019a). The topology of the species groups within the subfamily Myrmicinae was determined according to Hasegawa and Crozier (2006).

**Acknowledgements**

We would like to thank Dr. Cléa S. F. Mariano for kindly providing Acromyrmex echinatior samples, Dr. Jacques H. C. Delabie for species identification, and Dr. Luiz Fernando Gomes for his valuable assistance in the laboratory with Odontomachus bauri chromosome preparations. We also acknowledge Laboratório de Biologia Molecular de Insetos of the Universidade Federal de Viçosa (UFV) for technical support. GAT thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarships granted. Financial support for this study was provided by “Investissement d’Avenir” grants managed by the French Agence Nationale de la Recherche (DRIIIH ref. ANR-11-LABX-0010 and CEBA, ref. ANR-10-LABX-25-01), by the PQ-FEDER 2014-2020, Région Guyane (BING, ref. HY0007194), and by the Programa de Auxilio ao Pesquisador – PAPESQ/UNIFAP/2019.

**Data availability statement**

All relevant data are within the paper.

**References**

Aguilar, H.J.A.C., Barros, L.A.C., Mariano, C.S.F., Delabie, J.H.C. and Pompolo, S.G. (2011) 45S rDNA localization for the giant Ant Dinoponera gigantea with evolutionary inferences for the Dinoponera genus (Formicidae: Ponerinae). *Sociobiology, 57* (3), 607–620.

Aguilar, H.J.A.C., Barros, L.A.C., Alves, D.R., Mariano, C.S.F., Delabie, J.H.C. and Pompolo, S.G. (2017) Cytogenetic studies on populations of Camponotus rufipes (Fabricius, 1775) and Camponotus reneggeri Emery, 1894 (Formicidae: Formicinae). *PLoS One, 12*(5), e0177702.

Andrade-Souza, V., Duarte, O.M.P., Martins, C.C.C., Santos, I.S., Costa, M.G.C. and Costa, M.A. (2018) Comparative molecular phylogenetics of Melipona Illiger species (Hymenoptera: Apidae). *Sociobiology, 65*(4), 696–705.

Bacci, M., Jr., Solomon, S.E., Mueller, U.G., Martins, V.G., Carvalho, A.O.R., Vieira, L.G.E. et al. (2009) Phylogeny of leafcutter ants in the genus Atta Fabricius (Formicidae: Attini) based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution, 51*(3), 427–437.

Barbosa, P., Leal, E.V., Silva, M., Almeida, M.C., Moreira-Filho, O. and Antoni, R.F. (2017) Variability and evolutionary implications of repetitive DNA dynamics in genome of Astyanax scabripinnis (Teleostei, Characidae). *Comparative Cytogenetics, 11*(1), 143–162.

Barros, L.A.C., Mariano, C.S.F., Pompolo, S.G. and Delabie, J.H. C. (2009) Hsc-FA and NOR bandings on chromosomes of the giant ant Dinoponera lucida Emery, 1901 (Hymenoptera: Formicidae). *Comparative Cytogenetics, 3, 97–102.

Barros, L.A.C., Aguier, H.J.A.C., Mariano, C.S.F., Delabie, J.H.C. and Pompolo, S.G. (2010) Cytogenetic characterization of the lower-Attine Mycocepurus goeldii (Formicidae: Myrmicinae) and Chromosomal Inferences. *Florida Entomologist, 97*(4), 1694–1701.

Barros, L.A.C., Aguier, H.J.A.C., Andrade-Souza, V., Mariano, C.S.F., Delabie, J.H.C. and Pompolo, S.G. (2012) Occurrence of pre-nucleolar bodies and 45S rDNA location on the chromosomes of the ant Mycocepurus goeldii (Forel) (Formicidae, Myrmicinae, Attini). *Hereditas, 149*, 50–54.

Barros, L.A.C., Teixeira, G.A., Aguier, H.J.A.C., Mariano, C.S.F., Delabie, J.H.C. and Pompolo, S.G. (2014) Banding patterns of three leafcutter ant species of the genus Atta (Formicidae: Myrmicinae) and Chromosomal Inferences. *Florida Entomologist, 97*(1), 186–190.

Barros, L.A.C., Aguier, H.J.A.C., Teixeira, G.A., Mariano, C.S.F., Teixeira, M.C., Delabie, J.H.C. et al. (2015) Cytogenetic data on the threatened leafcutter ant Atta robusta Borgmeier, 1939 (Formicidae: Myrmicinae: Attini). *Comptes Rendus Biologies, 338*(10), 660–665.

Barros, L.A.C., Aguier, H.J.A.C., Mariano, C.S., Andrade-Souza, V., Costa, M.A., Delabie, J.H.C. et al. (2016) Cytogenetic data on six leafcutter ants of the genus Acromyrmex Mayr, 1865 (Hymenoptera, Formicidae, Myrmicinae): insights into chromosome evolution and taxonomic implications. *Comparative Cytogenetics, 10*(2), 229–243.

Barros, L.A.C., Teixeira, G.A., Aguier, H.J.A.C., Lopes, D.M. and Pompolo, S.G. (2018) Cytogenetic studies in Trachymyrmex holmgreni Wheeler, 1925 (Formicidae: Myrmicinae) by conventional and molecular methods. *Sociobiology, 65*(2), 185–190.

Bolton, B. (2020) An online catalog of the ants of the world. https:// antcat.org (accessed May 15, 2020).

Britton-Davidian, J., Cazaux, B. and Catalan, J. (2012) Chromosomal dynamics of nucleolar organizer regions (NORs) in the house mouse: micro-evolutionary insights. *Heredity, 108*(1), 68–74.

Cabral-de-Mello, D.C., Oliveira, S.G., Moura, R.C. and Martins, C. (2011) Chromosomal organization of the 18S and 5S rRNAs and histone H3 genes in Scarabaeinae coleopterans: insights
into the evolutionary dynamics of multigene families and heterochromatin. BMC Genetics, 12, 88.

Cardoso, D.C., Pompolo, S.G., Cristiano, M.P. and Tavares, M.G. (2014) The role of fusion in ant chromosome evolution: insights from cytogenetic analysis using a molecular phylogenetic approach in the genus Mycetophyllum. PLoS One, 9(4), e95408.

Castro, C.P.M., Cardoso, D.C., Micolino, R. and Cristiano, M.P. (2020) Comparative FISH-mapping of TTAGG telomeric sequences to the chromosomes of leafcutter ants (Formicidae, Myrmicinae): is the insect canonical sequence conserved? Comparative Cytogenetics, 14(3), 369–385.

Cazaux, B., Catalan, J., Veyrunes, F., Douzery, E.J. and Britton-Davidian, J. (2011) Are ribosomal DNA clusters rearrangement hotspots? A case study in the genus Mus (Rodentia, Muridae). BMC Evolutionary Biology, 11(1), 124.

Cholak, L.R., Haddad, C.F. and Parise-Maltempi, P.P. (2020) Cytogenetic and molecular analyses reveal a divergence of 18S rDNA sites and absence of the canonical TTAGG insect telomeric repeat in parasitoid Hymenoptera. Genetica, 142, 317–322.

Gokhman, V.E., Anokhin, B.A. and Kuznetsova, V.G. (2014) Distribution of 18S rDNA loci in four lace bug species (Hemiptera, Tingidae) with the same chromosome number. Comparative Cytogenetics, 9(4), 513–522.

Golub, N.V., Golub, V.B. and Kuznetsova, V.G. (2015) Variability of 18rDNA loci in four lace bug species (Hemiptera, Tingidae) with the same chromosome number. Phylogenetic and Evolutionary Biology, 136, 2561–2575.

Falciome, C., Hernando, A. and Bressa, M.J. (2018) Comparative cytogenetic analysis in Erythrolamprus snakes (Serpentes: Dipsadidae) from Argentina. Anais da Academia Brasileira de Ciências, 90(2), 1417–1429.

Ferreti, A.B.S.M., Ruiz-Ruano, F.J., Milani, D., Loreto, V., Marti, D.A., Ramos, E. et al. (2019) How dynamic could be the 45S rDNA cistron? An intriguing variability in a grasshopper species revealed by integration of chromosomal and genomic data. Chromosoma, 128, 165–175.

Gerbault-Seureau, M., Cacheux, L. and Dutrillaux, B. (2017) The relationship between the (In-) stability of NORs and their chromosomal location: the example of Cercoptethicidae and a short review of other primates. Cytogenetic and Genome Research, 153(3), 138–146.

Gokhman, V.E., Anokhin, B.A. and Kuznetsova, V.G. (2014) Distribution of 18S rDNA sites and absence of the canonical TTAGG insect telomeric repeat in parasitoid Hymenoptera. Genetica, 142, 317–322.

Gokhman, V.E., Bolsheva, N.L., Govind, S. and Muravenko, O.V. (2016) A comparative cytogenetic study of Drosophila parasitoids (Hymenoptera, Figitidae) using DNA-binding fluorochromes and FISH with 45S rDNA probe. Genetica, 144(3), 335–339.

Hirai, H. (2020) Chromosome dynamics regulating genomic dispersion and alteration of Nucleolus Organizer Regions (NORs). Cells, 9(4), 971–984.

Hirai, H., Yamamoto, M.T., Ogura, K., Satta, Y., Yamada, M., Taylor, R.W. et al. (1994) Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the Myrmecia pilosula species complex. Chromosoma, 103(3), 171–178.

Hirai, H., Yamamoto, M.T., Taylor, R.W. and Imai, H.T. (1996) Genomic dispersion of 28S rDNA during karyotypic evolution in the ant genus Myrmecia (Formicidae). Chromosoma, 105, 190–196.

Hirai, H. (2020) Chromosome dynamics regulating genomic dispersion and alteration of Nucleolus Organizer Regions (NORs). Cells, 9(4), 971–984.

Hirai, H., Yamamoto, M.T., Ogura, K., Satta, Y., Yamada, M., Taylor, R.W. et al. (1994) Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the Myrmecia pilosula species complex. Chromosoma, 103(3), 171–178.

Hirai, H., Yamamoto, M.T., Taylor, R.W. and Imai, H.T. (1996) Genomic dispersion of 28S rDNA during karyotypic evolution in the ant genus Myrmecia (Formicidae). Chromosoma, 105, 190–196.

Hirai, H. (2020) Chromosome dynamics regulating genomic dispersion and alteration of Nucleolus Organizer Regions (NORs). Cells, 9(4), 971–984.

Hirai, H., Yamamoto, M.T., Ogura, K., Satta, Y., Yamada, M., Taylor, R.W. et al. (1994) Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the Myrmecia pilosula species complex. Chromosoma, 103(3), 171–178.

Hirai, H., Yamamoto, M.T., Taylor, R.W. and Imai, H.T. (1996) Genomic dispersion of 28S rDNA during karyotypic evolution in the ant genus Myrmecia (Formicidae). Chromosoma, 105, 190–196.

Hirai, H. (2020) Chromosome dynamics regulating genomic dispersion and alteration of Nucleolus Organizer Regions (NORs). Cells, 9(4), 971–984.

Hirai, H., Yamamoto, M.T., Ogura, K., Satta, Y., Yamada, M., Taylor, R.W. et al. (1994) Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the Myrmecia pilosula species complex. Chromosoma, 103(3), 171–178.

Hirai, H., Yamamoto, M.T., Taylor, R.W. and Imai, H.T. (1996) Genomic dispersion of 28S rDNA during karyotypic evolution in the ant genus Myrmecia (Formicidae). Chromosoma, 105, 190–196.

Hirai, H. (2020) Chromosome dynamics regulating genomic dispersion and alteration of Nucleolus Organizer Regions (NORs). Cells, 9(4), 971–984.
organizer regions (NORs) and kinetochores in the Australian ant Myrmecia croslandi. The Japanese Journal of Genetics, 67(6), 437–447.

Imai, H.T., Taylor, R.W. and Crozier, R.H. (1994) Experimental bases for the minimum interaction theory. I. Chromosome evolution in ants of the Myrmecia pilosula species complex (Hymenoptera: Formicidae: Myrmeciinae). The Japanese Journal of Genetics, 69(2), 137–182.

Larabee, F.J., Fisher, B.K., Schmidt, C.A., Matos-Maraví, P., Janda, M. and Suarez, A.V. (2016) Molecular phylogenetics and diversification of trap-jaw ants in the genera Anochetus and Odontomachus (Hymenoptera: Formicidae). Molecular Phylogenetics and Evolution, 103, 143–154.

Levan, A., Fredga, K., and Sandberg, A.A. (1964) Nomenclature for centromeric position on chromosomes. Hereditas, 52(2), 201–220. http://dx.doi.org/10.1111/j.1601-5223.1964.tb01953.x.

Levsy, J.F. and Singer, R.H. (2003) Fluorescence in situ hybridization: past, present and future. Journal of Cell Science, 116, 2833–2838.

Liehr, T. (2017) “Classical cytogenetics” is not equal to “banding cytogenetics”. Molecular Cytogenetics, 10, 1–3.

Long, E.O. and Dawid, I.B. (1980) Repeated genes in eukaryotes. Annual Review of Biochemistry, 49(1), 727–764.

Lorite, P. and Palomeque, T. (2010) Karyotype evolution in ants (Hymenoptera: Formicidae), with a review of the known ant chromosome numbers. Myrmecological News, 13(1), 89–102.

Lorite, P., Aráñega, A.E., Luque, F. and Palomeque, T. (1997) Analysis of the nucleolar organizing regions in the ant Tapinoma nigerrimum (Hymenoptera, Formicidae). Heredity, 78, 578–582.

Lorite, P., Carrillo, J.A., Tinaut, A. and Palomeque, T. (2004) Evolutionary dynamics of satellite DNA in species of the genus Formica (Hymenoptera, Formicidae). Gene, 332, 159–168.

Malimpensa, G.D.C., Traldi, J.B., Martínez, J.D.F., Deon, G., Azambuja, M., Nogaroto, V. et al (2020) Chromosomal diversification in two species of Pimelodus (Siluriformes: Pimelodidae): Comparative cytogenetic mapping of multigene families. Zebrafish, 17(4), 278–286.

Mantovani, M., Abel, L.D. and Moreira-Filho, O. (2005) Conserved 5S and variable 45S rDNA chromosomal localization revealed by FISH in Astyanax scabripinnis (Pisces, Characidae). Genetica, 123, 211–216.

Mariano, C.S.F., Pompolo, S.G., Barros, L.A.C., Mariano-Neto, E., Campiolo, S. and Delabie, J.C.H. (2008) A biogeographical study of the threatened ant Dinoponera lucida Emery (Hymenoptera: Formicidae: Ponerinae) using a cytogenetic approach. Insect Conservation and Diversity, 1(3), 161–168.

Mariano, C.S.F., Barros, L.A.C., Velasco, Y.M., Guimaraes, I.N., Pompolo, S.G. and Delabie, J.H.C. (2019) Citogenética de hormigas de la región neotropical. In: Fernández, F., Guerrero, R., and Delsinne, T., (Eds.) Hormigas de Colombia. Bogotá: Universidad Nacional de Colombia, pp. 131–157.

Martins, C. and Wasko, A.P. (2004) Organization and evolution of 5S ribosomal DNA in the fish genome. In: Williams, C.R., (Ed.) Focus on genome research. New York: Nova Science Publishers Inc, pp. 335–363.

Maryanska-Nadachowska, A., Anokhin, B.A., Gnezdilov, V.M. and Kuznetsova, V.G. (2016) Karyotype stability in the family Issidae (Hemiptera, Auchenorrhyncha) revealed by chromosome techniques and FISH with telomeric (TTAGG)n and 18S rDNA probes. Comparative Cytogenetics, 10(3), 347–369.

Mazzoleni, S., Rovatsos, M., Schilliaci, O. and Dumas, F. (2018) Evolutionary insight on localization of 18S, 28S rDNA genes on homologous chromosomes in Primates genomes. Comparative Cytogenetics, 12(1), 27–40.

Menezes, R.S.T., Gazoni, T. and Costa, M.A. (2019) Cytogenetics of warrior wasps (Vespidae: Synoeca) reveals intense evolutionary dynamics of ribosomal DNA clusters and an unprecedented number of microchromosomes in Hymenoptera. Biological Journal of the Linnean Society, 126(4), 925–935.

Meyne, J., Hirai, H. and Imai, H.T. (1995) FISH analysis of the telomere sequences of bulldog ants (Myrmecia: Formicidae). Chromosoma, 104, 14–18.

Micolino, R., Cristiano, M.P., Travenzoli, N.M., Lopes, D.M. and Cardoso, D.C. (2019a) Chromosomal dynamics in space and time: evolutionary history of Mycetophylax ants across past climatic changes in the Brazilian Atlantic coast. Scientific Reports, 9(1), 1–13.

Micolino, R., Cristiano, M.P. and Cardoso, D.C. (2019b) Population-based cytogenetic banding analysis and phylogenetic relationships of the neotropical fungus-farming ant Trachymyrmex holmgreni Wheeler, 1925. Cytogenetic and Genome Research, 159(3), 151–162.

Micolino, R., Cristiano, M.P. and Cardoso, D.C. (2020) Karyotype and putative chromosomal inversion suggested by integration of cytogenetic and molecular data of the fungus-farming ant Mycetomellerius iheringi Emery, 1888. Comparative Cytogenetics, 14(2), 197–210.

Moreau, C.S. and Bell, C.D. (2013) Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. Evolution, 67(8), 2240–2257.

Nguyen, P., Sahara, K., Yoshido, A. and Marec, F. (2010) Evolutionary dynamics of rDNA clusters on chromosomes of moths and butterflies (Lepidoptera). Genetica, 138, 343–354.

Panzer, Y.I., Pita, S., Ferreiro, M.J., Ferrandis, I., Lages, C., Pérez, R. et al (2012) High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (Triatominae, Heteroptera). Cytogenetics and Genome Research, 38(1), 56–67.

Panzer, F., Ferreiro, M.J., Pita, S., Calleros, L., Pérez, R., Basmadjian, Y. et al (2014) Evolutionary and dispersal history of Triatoma infestans, main vector of Chagas disease, by chromosomal markers. Infection, Genetics and Evolution, 27, 105–113.

Pereira, T.T.P., Reis, A.C.C., Cardoso, D.C. and Cristiano, M.P. (2018) Molecular phylogenetic reconstruction and localization of the (TTAGG)n telomeric repeats in the chromosomes of Acromyrmex striatus (Roger, 1863) suggests a lower ancestral karyotype for leafcutter ants (Hymenoptera). Comparative Cytogenetics, 12(1), 13–26.

Pinckel, D., Straume, T. and Gray, J.W. (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences of the United States of America, 83, 2934–2938.

© 2021 Royal Entomological Society, 30, 340–354
Roa, F. and Guerra, M. (2012) Distribution of 45S rDNA sites in chromosomes of plants: structural and evolutionary implications. BMC Evolutionary Biology, 12(1), 1–13.

Roy, V., Monti-Dedieu, L., Chaminade, N., Siljak-Yakovenko, S., Aulard, S., Lemeunier, F. et al. (2005) Evolution of the chromosomal location of rDNA genes in two Drosophila species subgroups: ananasae and melanoaster. Heredity, 94(4), 388–395.

Sánchez-Gea, J.F., Serrano, J. and Gallián, J. (2000) Variability in rDNA loci in Iberian species of the genus Zabrus (Coleoptera: Carabidae) detected by fluorescence in situ hybridization. Genome, 43, 22–28.

Santos, I.S., Mariano, C.S.F., Andrade, V., Costa, M.A., Delabie, J.H.C. and Costa, M.A., Delabie, J.H.C., Costa, M.A., Delabie, J. (2012) Distribution of 45S rDNA sites in Chromosomes: Organization and Function. North Benwick, United Kingdom: Blackwell Publishing.

Symonová, R. (2019) Integrative rDNAomics—Importance of the oldest repetitive fraction of the eukaryote genome. Genes, 10(5), 345–360.

Teixeira, G.A., Barros, L.A.C., Aguiar, H.J.A.C. and Pompolo, S.G. (2017) Comparative physical mapping of 18S rDNA in the karyotypes of six leafcutter ant species of the genera Atta and Acromyrmex (Formicidae: Myrmicinae). Genetica, 145, 351–357.

Teixeira, G.A., Barros, L.A.C., Lopes, D.M. and Aguiar, H.J.A.C. (2020) Cytogenetic variability in four species of Gnamptogenys Roger, 1863 (Formicidae: Ectatomminae) showing chromosomal polymorphisms, species complex, and cryptic species. Protoperplasma, 257, 549–560.

Vicari, M.R., Antoni, R.F., Moreira-Filho, O. and Bertollo, L.A.C. (2008) Diversification of a ZZ/ZW sex chromosome system in Characidium fish (Crenuchidae, Characiformes). Genetica, 134(3), 311–317.

Walker, L.I., Soto, M.A. and Spotorno, Á.E. (2014) Similarities and differences among the chromosomes of the wild Guinea pig Cavia tschudii and the domestic Guinea pig Cavia porcellus (Rodentia, Caviidae). Comparative Cytogenetics, 8(2), 153–167.

Ward, P.S., Brady, S.G., Fisher, B.L. and Schultz, T.R. (2015) The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). Systematic Entomology, 40, 61–81.

Zunta, F., Sánchez, A., Burgos, M., Jiménez, R. and de la Guardia, R.D. (1997) Interchromosomal, intercellular and interindividual variability of NORs studied with silver staining and in situ hybridization. Heredity, 78(3), 229–234.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Staining with chromomycin A3 showing GC-rich regions (arrows) of Ponerinae ants: (A) Pseudoponera gigantea (2n = 12), (B) Anogethus tarugoni (2n = 30), (C) Odontomachus haemadus (2n = 44, French Guiana), (D) Odontomachus bauri showing the homoygous and heterozygous states, respectively (2n = 44), and (F) male of Odontomachus bauri with the smaller size regions rich in GC base pairs. Bars = 5 μm.

Figure S2 Staining with chromomycin A3 showing GC-rich regions (arrows) of Myrmicinae ants: (A) Pheidole germaini (2n = 22), (B) Crematogaster longispina (2n = 24), (C) Solenopsis geminata (n = 16), (D) Myrmicocrypta sp. (2n = 30), (E) Strumigenys diabola (2n = 40). Bars = 5 μm.

Figure S3 Staining with chromomycin A3 showing GC-rich regions (arrows) of an Ectatomminae ant (A) Gnamptogenys tortuolosa (2n = 44), and Formicinae ants (B) Camponotus atriceps (2n = 40) and (C, D) Giganticeps destructor (2n = 78, n = 39). Bars = 5 μm.