Myrmecological News	13	89-102	Vienna, April 2010

Karyotype evolution in ants (Hymenoptera: Formicidae), with a review of the known ant chromosome numbers

Pedro LORITE & Teresa PALOMEQUE

Abstract

Ants (Hymenoptera: Formicidae) constitute a diversified insect group with more than 12,000 species described. Like other hymenopterans, they are haplodiploid whereby fertilized eggs develop into diploid females (workers and queens) whereas unfertilized eggs develop into haploid males. A large number of species have been cytogenetically studied. The chromosome number is currently known for more than 750 species. All these data are summarized in this paper. Formicidae is one of the insect groups with the most variable chromosome number. The haploid chromosome numbers are known to range from n = 1 to n = 60. This chromosome diversity suggests that karyotype modifications have accompanied ant diversification. Karyotype evolution has followed chromosome-mutation processes able to change not only chromosome number but also chromosome morphology. We review the different chromosome mutations observed in ants and the possible role of such mutations in karyotype evolution in these insects, and we examine the hypotheses proposed to explain how this karyotype evolution may have occurred. Among chromosome rearrangements, Robertsonian centric fusions and fissions, besides inversions and translocations, seem to be the main processes that generate changes in ant karyotypes. Other processes altering chromosome numbers, such as polyploidy or aneuploidy, do not appear to be important in ant evolution. Ant subfamilies present different levels of variation in relation to chromosome number. The highest variation has been found in primitive subfamilies such as Ponerinae (n = 3 - 60) and Myrmeciinae (n = 1 - 47) whereas in less primitive subfamilies the chromosome numbers are less variable, as in Dolichoderinae (n = 5 - 16), Formicinae (n = 8 - 28), and Myrmicinae (n = 4 - 35). Few data are available for other subfamilies. Primitive ants present not only the highest range of variation in chromosome number but also the most complex chromosome polymorphisms. In contrast, less primitive genera show lower variation in chromosome number, and generally only simple polymorphisms have been detected. We conclude with an outlook on future research avenues.

Key words: Formicidae, cytogenetics, chromosome number, karyotype, evolution, genetics, haplo-diploid, review.

Myrmecol. News 13: 89-102 (online 20 January 2010)
ISSN 1994-4136 (print), ISSN 1997-3500 (online)

Received 2 October 2009; revision received 30 November 2009; accepted 1 December 2009

Dr. Pedro Lorite (contact author) & Dra. Teresa Palomeque, Departamento de Biología Experimental, Área de Genética, Universidad de Jaén, 23071 Jaén, Spain. E-mail: plorite@ujaen.es

Introduction

Ants (Hymenoptera: Formicidae) form one of the most distinct and well-defined insect families and have long been perceived as a natural group. Being social insects, ants live in colonies, with two female castes (workers and queens) and males which present morphological and genetic differences. The main genetic differences are that males are haploid while queens and workers are diploid. Haplo-diploidy or male haploidy is the main genetic characteristic of the order Hymenoptera.

Of more than 12,000 described ant species (BOLTON & al. 2007), many have been cytogenetically analysed, although the majority of these studies have been made only in order to determine the chromosome number and the karyotype. These studies have shown that ants are among those eukaryotes most variable in chromosome number, ranging from n (haploid chromosome number) = 1 to n = 60. This wide variation could be due to the evolution of this old family, which appears to have diversified more than 100 million years ago (BRADY & al. 2006, MOREAU & al. 2006, MOREAU 2009).

In ant cytogenetics, the groundbreaking work was performed by Hirotami T. Imai and Rossiter H. Crozier, who, with co-workers, analysed the majority of ant karyotypes. Crozier published a monograph in 1975, reviewing Hymenoptera cytogenetics (CROZIER 1975). This study included the chromosome numbers in ants based on the data known up to 1975. In 1977, IMAI & al. published a paper on Australian ants in which karyotypes of a total of 150 species were presented. Later, another key paper was published on Indian ants with the data of 94 species (IMAI & al. 1984). These works are important not only for the large number of species analysed but also because chromosome polymorphism in ants is analysed and a hypothesis for chromosome evolution in this group, the "minimum-interaction theory", is given for the first time. This theory seeks to establish the possible mechanisms governing ant karyotype evolution (IMAI & al. 1986, 1988a).

Karyological analysis has proven useful to determine the karyotypic relationships between related species and their evolution (IMAI 1971, PALOMEQUE & al. 1988, LOISELLE

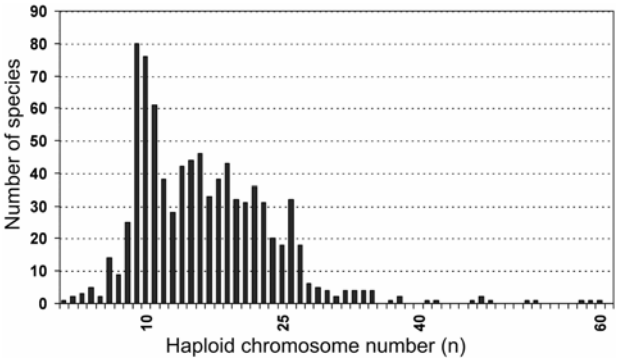

Fig. 1: Frequency distribution of known haploid chromosome numbers in species of the family Formicidae.

& al. 1990, PALOMEQUE & al. 1993b). Karyological analysis has also proven useful to establish and characterise new species (IMAI & al. 1994), since speciation can occur with a modification in chromosome number or with changes in chromosome morphology.

Since Crozier's work, several reviews on chromosome numbers have been made. These studies, however, did not include a large number of species and are limited to countries, regions, subfamilies, tribes or genera (HAUSCHTECK-JUNGEN & JUNGEN 1976, ROSENGREN & al. 1980, GOÑI & al. 1983, HAUSCHTECK-JUNGEN & JUNGEN 1983, FISCHER 1987, LOISELLE & al. 1990, LORITE & al. 1998a, b, 2000, MARIANO & al. 2003, 2006, 2007). Thus, ant cytogenetics as a whole has not been reviewed after Crozier's review (CROZIER 1975), which included less than 200 ant species whereas today more than 750 ant chromosome numbers are known.

Chromosome numbers in Formicidae

All or at least the majority of the ant chromosome studies are reviewed in this paper (see Appendix, as digital supplementary material to this article, at the journal's web pages). Formicidae are highly variable in terms of chromosome number. The lowest chromosome number was found in *Myrmecia croslandi*, i.e., n = 1 (CROSLAND & CROZIER 1986, TAYLOR 1991), and the highest in *Dinoponera lucida*, i.e., n = 60 (MARIANO & al. 2008). Figure 1 shows the distribution of haploid chromosome numbers in the 750 ant taxa analysed. This distribution is basically the same as reported by IMAI & al. (1988a) using 500 ant species.

The majority of the karyotyped species belong to the subfamilies Myrmicinae, Formicinae, Dolichoderinae, and Ponerinae. In Myrmicinae, with more than 400 karyotyped species, the chromosome numbers range from n = 4 to n = 35. As most karyotyped ants belong to Myrmicinae, the histogram of chromosome numbers in this family (Fig. 2a) is similar to the one reported by IMAI & al. (1988a) and to the histogram for all 750 ant species analysed (Fig. 1).

In Formicinae, with about 100 karyotyped species, chromosome numbers range from n = 8 to n = 28 (Fig. 2b). The histogram differentiates four groups of species with different "modal" values (i.e., groups that differ in the values that are most frequent). The species with n = 8 - 10 are mainly from the genera *Camponotus*, *Lepisiota*, and *Plagiolepis*. The group with n = 14 - 15 includes mainly species from the genera *Lasius*, *Paratrechina*, and some *Camponotus.* The group with n = 19 - 21 also includes species from the genus *Camponotus* and the majority of *Polyrhachis* species. The last group, with n = 26 - 27, is formed by *Formica*, *Polyergus*, and *Cataglyphis* species.

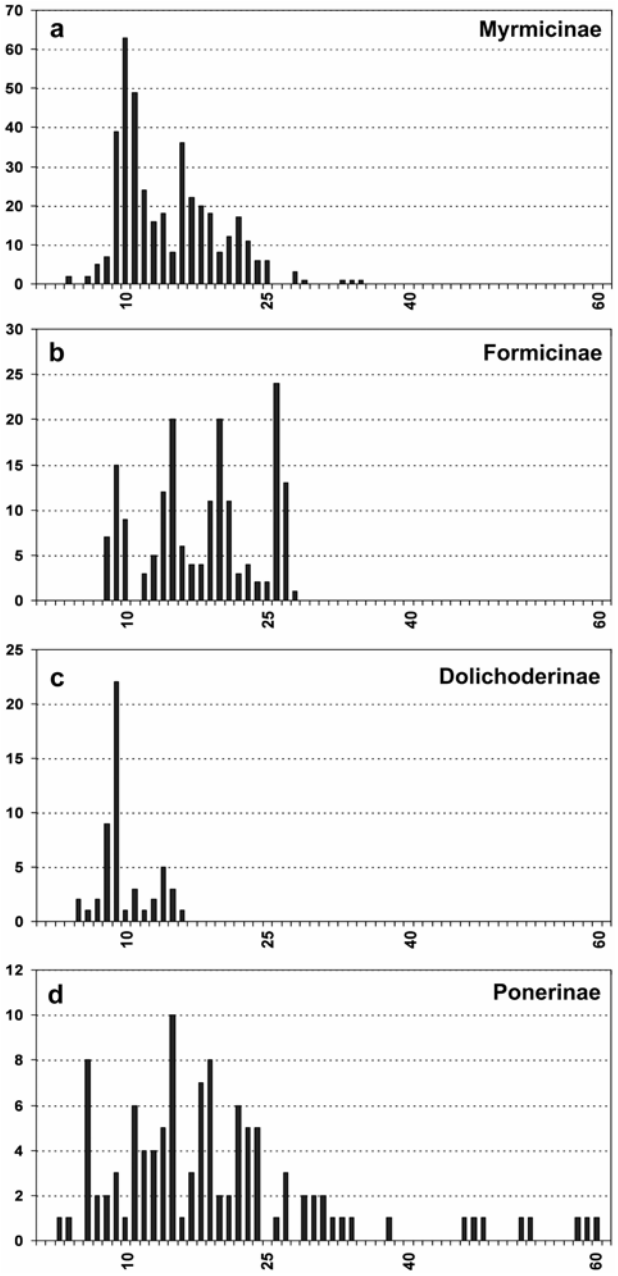

Fig. 2: Distribution of haploid chromosome numbers in species of the subfamilies (a) Myrmicinae, (b) Formicinae, (c) Dolichoderinae, and (d) Ponerinae.

The known chromosome numbers in Dolichoderinae are less variable than in other subfamilies, i.e., n = 5 - 16 (Fig. 2c). In this subfamily, the number of karyotyped species is about 50. The modal value is n = 9. This modal value is due to the fact that this chromosome number is common in most of *Iridomyrmex* species, although n = 9 also occurs in other genera.

About 100 species of Ponerinae have been analysed. This subfamily presents the most variable chromosome number, i.e., n = 3 - 60, with a modal value of n = 15 (Fig. 2d). The subfamily also contains one of the genera most variable in chromosome number, namely *Pachycondyla*, with n = 6 to n = 52 (MARIANO & al. 2007).

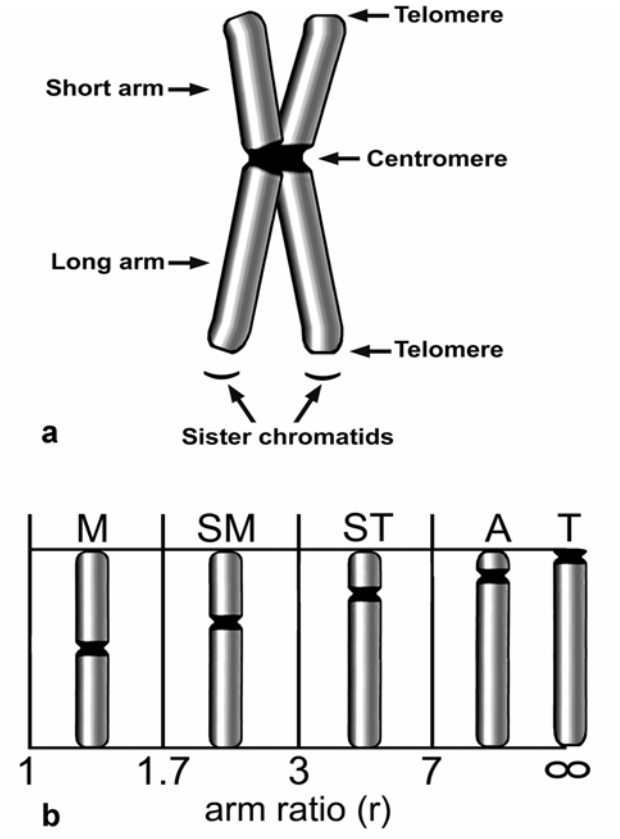

Fig. 3: (a) Morphology of a mitotic chromosome. (b) Chromosome classification. Five different chromosome morphologies were defined according to the arm ratio, r, i.e., the ratio of the length of the long arm vs. that of the short arm. Metacentric chromosomes (M) have r = 1 to 1.7, submetacentric (SM) r = 1.7 - 3, subtelocentric (ST) r = 3 - 7 and acrocentric (A) r > 7. Telocentric (T) chromosomes are those with the centromere in a terminal position.

Within the Myrmeciinae subfamily, species from its two genera (*Myrmecia* and *Nothomyrmecia*) have been karyotyped. The species of *Myrmecia* are highly variable in chromosome number, with n = 1 to n = 42. *Nothomyrmecia macrops,* the only species of the genus *Nothomyrmecia*, has been karyotyped and the chromosome number is n = 47 (IMAI & al. 1990). For the remaining ant subfamilies, a low number of species have been karyotyped and some even completely lack cytogenetic data (see Appendix).

Mechanisms of chromosomal changes found in Formicidae

The genome of eukaryotes is organised into discrete pieces that are the chromosomes, which contain a single extremely long DNA molecule packaged with proteins. Each species has a characteristic number of chromosomes, and the length and morphology of these chromosomes are conserved among individuals of the same species. The chromosome complement is represented in the karyotype. In a karyotype, the homologous chromosomes are placed together and arranged according to their length and morphology. Since some organisms are haploid such as the males of ants and other hymenopteran species, also haploid karyotypes can be found. The classification of chromosome morphology is based on the classification proposed by LEVAN & al. (1964), which takes into consideration the size of the short and long arms of the chromosomes (Fig. 3). Although alternatives have been proposed (IMAI 1991), the classification of LEVAN & al. (1964) remains most commonly used.

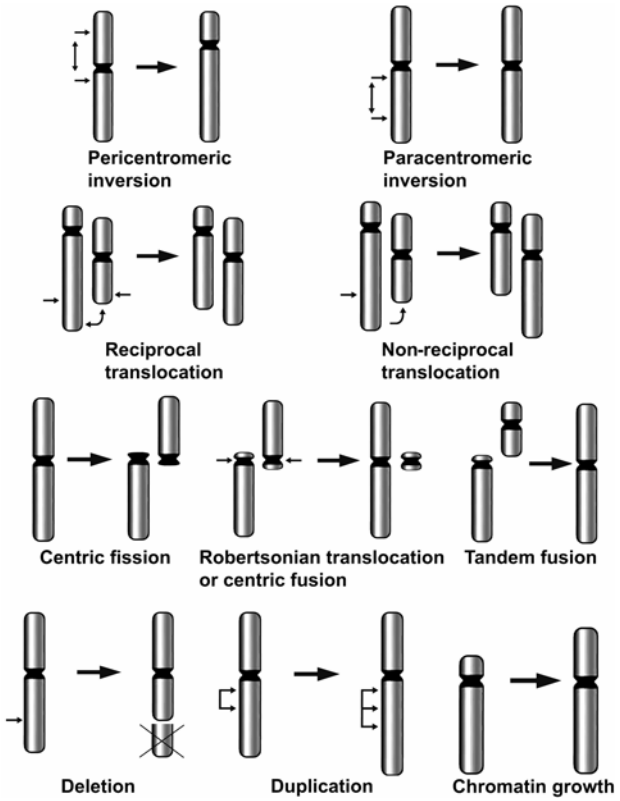

Fig. 4: Chromosome rearrangements observed in ants.

Although each species has a characteristic chromosome number, there are mechanisms that cause changes in the karyotype. These mechanisms are chromosome rearrangements that can change the number and morphology of chromosomes. These changes have been detected in ants at several levels. First, there are differences at the population level, which involves the maintenance of different karyotypes in the same population, with a high frequency to be explained by recurrent mutations. Second, there are differences between populations of the same species with fixed karyotypic differences, and finally there are differences between sister or closely related species (also see SEIFERT 2009, for a taxonomic perspective). Chromosome mutations could be important in speciation processes. Sometimes, species evolution has been accompanied by changes in the karyotype. Changes in the karyotype could be generated by several types of processes (see Fig. 4). In the following, we review the different chromosome rearrangements observed in ants and the possible role of such mutations in ant-karyotype evolution.

Chromosome rearrangements

Inversions: In a single chromosome, a segment breaks off and is reversed end to end. Two different inversion processes can be defined: **paracentromeric inversions**, if the two breaking points occur in one arm of the chromosome; and **pericentromeric inversions**, if the two breaking points occur in different chromosome arms and therefore include

the centromere (i.e., the most constricted region of a chromosome that holds together the two chromatids and divides the chromatid into two chromosome arms, Fig. 3a). Pericentromeric inversions can be more easily detected than paracentromeric ones since they often alter chromosome morphology (Fig. 4). Inversions usually do not cause phenotypic effects. However, in heterozygote individuals, nonviable gametes can be generated when single crossing-over occurs within the inverted regions. This leads to reduced fertility due to the production of recombinant chromosomes with duplications of deletions (WHITE 1973).

Paracentromeric inversions have not been reported in ants, although paracentromeric inversions are the most common chromosome rearrangement in other insects, such as *Drosophila* (AULARD & al. 2004). In fact, they may also be numerous in ants, but they are difficult to detect in ants, as opposed to *Drosophila*, where the presence of polytenic chromosomes (i.e., giant chromosomes that result from several successive DNA replications without chromosome separation by cell division and that thus have high numbers of sister chromatids) facilitates cytogenetic studies. Generally, chromosome changes, especially in vertebrates, can be easily detected using chromosome G-banding techniques, which generate specific band-interband patterns throughout the chromosomes. G-banding has been achieved in one ant species (LORITE & al. 1996), but the size of the chromosomes and the number of bands are smaller in ants than in vertebrates and therefore less useful for the analysis of chromosome rearrangements. Consequently, detection of paracentromeric inversions in ants will require the application of molecular techniques.

In ants, pericentromeric inversions have been described as inter- and intrapopulation polymorphisms. CROZIER (1968a) detected interpopulation differences in *Iridomyrmex gracilis*. In this species, intrapopulation polymorphism has been detected in cytogenetic comparative studies of three Australian populations: Cranbourne, Melton and Pooncarie. The Cranbourne population of *I. gracilis* shows the karyotype n = 6M + 1SM + 1A (M denoting metacentric, SM submetacentric, and A acrocentric; for details, see Fig. 3b), whereas in the Melton population it is n = 5M + 1SM + 2A. In the latter, the karyotype shows an acrocentric chromosome that, according to the author, comes from a pericentromeric inversion of one of the medium-sized metacentric chromosomes. Females from the Pooncarie population show a heteromorphic chromosome pair, one metacentric and the other one acrocentric. Pericentromeric inversions have also been detected in other dolichoderine species (CROZIER 1970a). In the ponerine species complex of *Pachycondyla crenata* and *P. mesonotalis*, pericentromeric inversions could act as a possible speciation mechanism (MARIANO & al. 2006).

IMAI & al. (1977, 1984) detected polymorphisms by inversion in different Australian populations of the ants *Rhytidoponera metallica* and *Myrmecia pilosula*, as well as in Indian populations of *Camponotus variegatus* and *Meranoplus bicolor*. A polymorphism by inversion is also present in *Pheidole pallidula.* In Southern Spain, we found two different karyotypes coexisting within populations of this species (P. Lorite & T. Palomeque, unpubl.): As shown in Figure 5, the two smaller chromosomes of *Pheidole pallidula* are generally metacentric but in some individuals one of them is subtelocentric (for a definition, see Fig. 3b).

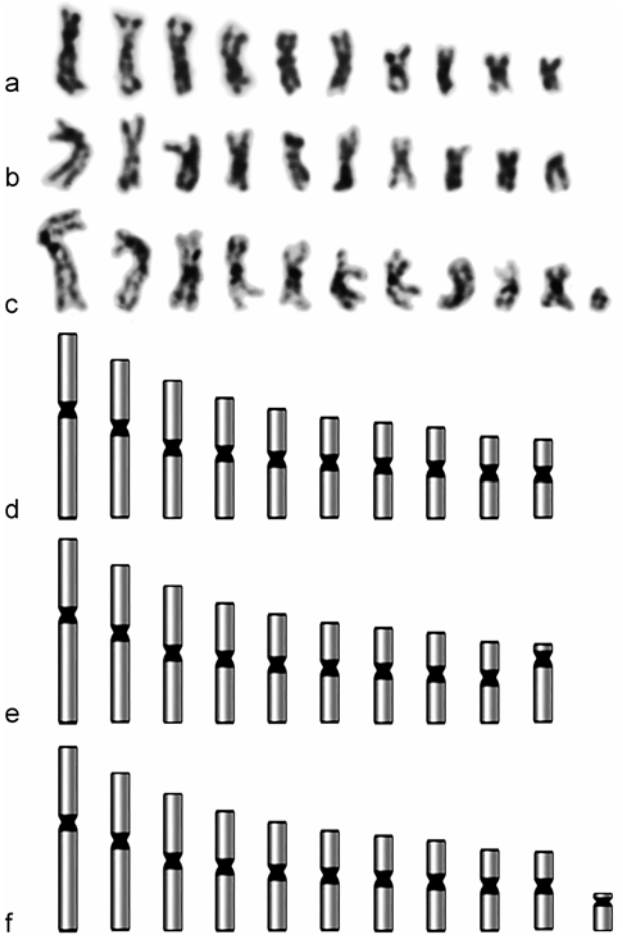

Fig. 5: Karyotypes and idiograms of a polymorphic species. In Spanish populations, different karyotypes have been observed for the species *Pheidole pallidula*. The standard haploid karyotype presents 10 metacentric chromosomes (a, d). In some individuals a pericentromeric inversion changes one of the metacentric chromosomes into subtelocentric (b, e) and others have a haploid number of n = 11 by the presence of a small B-chromosome (c, f).

Translocations: In **translocations**, a chromosome fragment is broken and attaches to another site, generally a nonhomologous chromosome (Fig. 4). When two different chromosomes interchange a fragment, this process is called **reciprocal translocation**.

Individuals with balanced translocation (no loss of chromatin) have a normal phenotype but result in reduced fertility in heterozygosis. In meiosis, the two chromosome pairs involved in the translocation may form quadrivalent configurations (i.e., structures generated by the pairing of four chromosomes during meiosis) and the chromosome segregation could generate genetically unbalanced and nonviable gametes (WHITE 1973). Thus, polymorphisms due to translocation are rare in animals. Consequently, it is relatively frequent that, when related species show karyotypic differences due to this type of chromosomal rearrangement, it acts as a possible reproductive isolation mechanism.

Unlike the general situation described, it has been argued that translocations could play an important role in ant chromosome evolution (IMAI & al. 1988a). Those authors considered the higher frequency of translocations in

ants when compared to other animal groups, and argued that this could be the case because haploid males cannot produce unbalanced gametes. Another possible cause of the relatively high frequency of translocations could be mechanisms that reduce the chromosome pairing or chromosome-crossover rate in translocation heterozygotes (SHERIZEN & al. 2005). In fact, it was suggested that translocations have participated in the evolution of the genera *Myrmecia*, *Tetramorium*, *Iridomyrmex*, *Pheidole*, *Camponotus*, *Monomorium*, *Rhytidoponera* and *Pachycondyla*, among others (IMAI & KUBOTA 1972, IMAI & al. 1977, 1984, CROSLAND & CROZIER 1986, IMAI & al. 1988a). IMAI & al. (1977, 1984) reported that translocation polymorphisms are more frequent in ant species with low chromosome number ($n \leq 12$).

The best case study of translocation polymorphism in ants is one in *Ponera scabra*. IMAI & KUBOTA (1972) and IMAI & al. (1988a) described some populations of workers with a chromosome number of $2n = 7$ or 8 and males with $n = 3$ or 4. These authors suggested that the karyotype with $n = 3$ derived from one with $n = 4$ by a translocation between the two chromosomes, with a small loss of chromosomal segments in both chromosomes. The authors observed this translocation for 15 years, indicating that the heterozygous queens are viable and fertile.

Chromosome fission and fusion, Robertsonian rearrangements: In **chromosome-fission** processes, a biarmed chromosome breaks apart at the centromere, producing two telocentric chromosomes (Fig. 4). In the reverse process, **chromosome fusion**, two different chromosomes (acrocentric or telocentric) join together to form a single biarmed chromosome (Fig. 4). In **Robertsonian translocations**, two acrocentric chromosomes are involved. The breaks occur at the centromeres and the long arms join to form a single chromosome. The short arms of the involved chromosomes form a very small chromosome that is generally lost after the rearrangement (Fig. 4). In **tandem fusion**, the end of one chromosome is fused to the end or to the centromere of another one, following the inactivation of one of the centromeres (Fig. 4).

In general, centric fusions and fissions appear to be important processes in the karyotype evolution of many animal groups while tandem fusion would have a less prominent role (WHITE 1973). The importance of centric fusions and fissions was evident very early. Thus, in 1916, ROBERTSON suggested that in different taxonomic groups, chromosome number varies but that the number of chromosomal arms (fundamental number) remains more constant.

Polymorphisms due to these types of chromosome rearrangements have been observed in a large number of ant species, especially in species with high chromosome numbers ($n > 12$) (IMAI & al. 1988a), although these polymorphisms have also been observed in species with $n = 9$ (IMAI & al. 1977) and $n = 11$ (IMAI & KUBOTA 1975). Robertsonian changes appear to be involved in the karyotype evolution and speciation of various ant genera (GOÑI & al. 1983, PALOMEQUE & al. 1988, 1993b).

The most extreme karyotype variation due to fusion-fission rearrangements has been described by CROZIER (1969) in the Australian Ponerine *Rhytidoponera metallica*. Different populations of this species have haploid chromosome numbers from 17 to 22. The author suggested that the karyotypes are well-related via fusion processes. Each decrease in chromosome number is associated with the smaller of two acrocentric chromosomes and the presence of new large, metacentric chromosomes. In the karyotype with $n = 22$, the formula is $n = 1M + 20A$, and in the one with $n = 17$, it is $n = 6M + 11A$. The finding of diploid females with odd chromosome numbers, such as $2n = 41$ or 43, also suggests the existence of individuals having a heteromorphic chromosome pair with a metacentric chromosome and the two acrocentric ones corresponding to the homologous arms. In another species of *Rhytidoponera* (*R. maniae*) and in *Pheidole nodus*, a similar polymorphism was detected (IMAI & KUBOTA 1972, 1975, IMAI & al. 1977).

As in other animal groups, few polymorphisms caused by tandem fusion have been reported in Formicidae. Only IMAI & al. (1977, 1988b, 1994), and IMAI & TAYLOR (1989) suggested the involvement of this type of fusion, in the complex polymorphism found in the species complex of *Myrmecia pilosula*; clearly, this is not a common process of chromosomal differentiation between related species. Despite this, CROZIER (1975) also suggested its possible participation in the evolution of the genus *Iridomyrmex*, although changes in this taxon can also be explained by pericentromeric inversions followed by centric fusions or fissions (IMAI & al. 1977), processes thought to be more common in the evolution of ants.

Loss and growth of chromatin: Changes in the amount of chromatin (Fig. 4) are common in animal and plant species. However, the exact nature of these variations is not completely known. Sometimes they have been associated with duplications, faulty DNA replication, non-homologous pairings in meiosis, and mobile genetic elements, among other molecular processes (see GREGORY 2005 for a review). In general, chromatin growth is considered to be more frequent than chromatin loss. In theory, the loss of codified DNA could produce a deleterious or a lethal effect in the individuals carrying the mutations. Still, changes in the amount of heterochromatin (i.e., chromosomal material that cytologically is intensely stained, that is tightly packed, and that is, for the most part, genetically inactive) seem not to be harmful and are quite frequent in fact. Changes in the amount of constitutive heterochromatin are considered to be among the main mechanisms involved in the karyotype evolution of ants (CROZIER 1975, IMAI & al. 1977, 1988a, GOÑI & al. 1983). These changes could happen very quickly, i.e., in one or a few generations, resulting in the presence of polymorphisms in certain chromosome pairs.

Another cause of addition of chromosomal material is the presence of extra chromosome segments or blocks of heterochromatic chromatin, usually at distal locations (PALOMEQUE & al. 1993a). These chromosome segments appear as inter- and intrapopulation polymorphisms. Extra chromosome segments have been reported in numerous plant and animal species. They are also called supernumerary segments, since their presence as population polymorphism has no apparent effect on the viability of the organisms that carry them. Such segments often are heterochromatic, but in some instances, the C-banding technique (i.e., a chromosome-banding technique that reveals the heterochromatin) has shown that part of the supernumerary segment contain euchromatin (i.e., chromosomal material that cytologically is lightly stained and that is, for the most part, genetically active) (PALOMEQUE & al. 1993a).

Polyploidy: Polyploidy is generated by duplications of

a complete haploid genome, resulting in organisms with chromosome numbers of 3n, 4n, etc.

In general, polyploidy is an evolutionary mechanism that is rare in animals, although it is frequent in plants (OTTO & WHITTON 2000). This has been explained by its incompatibility with the chromosome-determined sex mechanism and by the complex morphogenetic development of animals, as compared to plants. In insects, the presence of polyploid species is restricted to parthenogenetic species (OTTO & WHITTON 2000).

Reviewing the available data, CROZIER (1975) found that, in general, ants with low chromosome numbers have larger chromosomes than species with high chromosome numbers, suggesting that the total size of the genome in this group remains almost constant. IMAI & al. (1977, 1988a) analysed the relation between the average length of chromosomes and chromosome number. If the variation in chromosome number is derived from polyploidy, the ratio between these data would be more or less constant. However, if Robertsonian changes are responsible for the numerical changes, the average lengths would vary inversely with the chromosome number. The cytogenetic data support the idea that the most important changes in chromosome evolution of ants are Robertsonian rearrangements. Also it should be considered that increases in genome size are generally due to heterochromatin growth and that the amount of euchromatin is relatively constant in ants. Recent studies support this contention. TSUTSUI & al. (2008) have recently determined the genome size in 40 ant species. They have observed that, in general, ants have small genomes in comparison to other insect groups, that species belonging to the same genera have very similar genome sizes, and that the size of the genome is less variable among species from the same subfamily than among subfamilies. Unfortunately, the chromosome number is known only in less than half of the species studied (see Appendix) but the results become noteworthy when the genome size and karyotype data are taken together. The largest genome sizes are not accompanied with high chromosome numbers. Ponerinae is one of the subfamilies with the largest genome size but, as commented above, Ponerinae is the subfamily with the most variable chromosome number (n = 3 to 60). The ponerine species analysed by TSUTSUI & al. (2008) belong to the genera *Ponera*, *Dinoponera* and *Odontomachus*. These genera present very different chromosome numbers. One of the species analysed, *Ponera pennsylvanica*, has a low chromosome number of n = 6 (HAUSCHTECK-JUNGEN & JUNGEN 1983). The other species used in the study of TSUTSUI & al. (2008) have not been karyotyped but the known chromosome numbers in other *Odontomachus* species range from n = 15 to n = 22 (IMAI & al. 1977, GOÑI & al. 1982, IMAI & al. 1983). Finally, the species with the highest chromosome number known in ants belongs to the genus *Dinoponera* (*D. lucida*), with n = 57 - 60 (MARIANO & al. 2008). Thus, the large size of the genome in this subfamily does not seem to be related to the chromosome number. Another example of lack of correlation between chromosome number and genome size occurs in the genus *Solenopsis*. The genome sizes of *S. invicta* (753.3 Mb) and *S. xyloni* (472.3 Mb) markedly differ (JOHNSTON & al. 2004, TSUTUSI & al. 2008) but both species have the same chromosome number, i.e., n = 16 (GLANCEY & al. 1976, TABER & COKENDOLPHER 1988).

On the other hand, TSUTUSI & al. (2008) suggested that in the genome evolution of two genera (*Ectatomma* and *Apterostigma*), genome duplication could have been involved, since the species analysed from these genera (*E. tuberculatum* and *A. dentigerum*) present a genome size that is twice that of the other ant species included in their study. No cytogenetic information is available from *A. dentigerum*, but the known chromosome numbers for other species of the genus *Apterostigma* are not especially high, with n = 10 - 12 (MURAKAMI & al. 1998). The species with the highest genome size included in the study of TSUTUSI & al. (2008) is *Ectatomma tuberculatum*. This species has recently been karyotyped and its chromosome number is n = 18 (BARROS & al. 2008). The analysis of its karyotype did not reveal signs of polyploidy intervention and further studies are needed, especially at the molecular level, to determine the mechanisms that have caused this increased genome size in this species.

A most plausible explanation for the observed variation in genome size among species is the existence of differences in the amount of heterochromatin. Satellite DNA (i.e., highly repetitive non-coding DNA organised as tandem repeats) is the major DNA component of heterochromatin. The analysis of satellite DNA in different species of insects shows that its proportion in the genome is highly variable, also among related species (see PALOMEQUE & LORITE 2008, for a review). In ants, differences in the amount of satellite DNA have been also detected among castes of the same species. In the ant *Aphaenogaster subterranea*, satellite DNA in queens represents 25% of the satellite DNA found in workers (LORITE & al. 2002c). The differences in the amount of satellite DNA could explain the differences noted in the genome size in related species (see BIÉMONT 2008, for a review). Unfortunately, there are few studies on satellite DNA and other repetitive DNAs or on other aspects of the structure and organization of ant genomes (see GOODISMAN & al. 2008, for a review).

Although polyploidy appears not to play a major role in the genome evolution of ants, the presence of diploid males in ants is not a rare phenomenon. In fact, diploid males were detected early in *Pseudolasius* (HUNG & al. 1972) and *Solenopsis invicta* (GLANCEY & al. 1976). CROZIER (1975) cited the existence of occasional polyploid individuals in other genera. IMAI & al. (1977) reported triploid and tetraploid individuals in the genera *Crematogaster* and *Camponotus*. In *Leptothorax muscorum,* diploid and triploid males were also found (LOISELLE & al. 1990). It remains unclear whether or not diploid males are fertile. HUNG & al. (1974) found that testes of diploid males of *Solenopsis invicta* were often atrophied, but this is not the case with *Leptothorax muscorum*, where diploid males apparently have normal testes (LOISELLE & al. 1990). In *Lasius sakagamii*, diploid males seem to be fertile and they produce diploid spermatocytes (YAMAUCHI & al. 2001). In fact, the viability of diploid sperm or oocytes could be the cause of triploid individuals found in several ant species.

Polyploid cells are not rare in germinal or somatic tissues. Polyploid cells were detected early by HAUSCHTECK (1961) in the cerebral ganglia of *Camponotus ligniperda* and *Pheidole pallidula* and later by LORITE & al. (1998b) in *Tapinoma nigerrimum* (which currently is a junior synonym of *T. erraticum*, see BOLTON & al. 2007, but which we plan to lift from synonymy, based on morphological

and karyological evidence; P. Lorite, T. Palomeque & A. Tinaut, unpubl.). Germinal tissues in *Aphaenogaster osimensis* and "*Leptothorax*" (now *Temnothorax*) *albipennis* were also found to have polyploid cells (IMAI & YOSIDA 1966, ORLEDGE 1998).

Aneuploidy: Aneuploidy is another type of numerical chromosomal change detected in ants. This change implies the addition or loss of a whole chromosome from the genome. Loss of one of the chromosomes from a homologous pair results in monosomy, and the addition of one in trisomy.

In general, monosomy is lethal, but in *Myrmecia pilosula*, monosomic individuals have been reported (IMAI & al. 1988a). The diploid standard karyotype of this species has a chromosome number of 2n = 32, but in one population of this species, 50% of the workers have only 31 chromosomes. No morphological alterations were detected in these workers. The authors suggested that the karyotype with 2n = 31 derived from the karyotype with 2n = 32 by deletion of one chromosome. This polymorphism is not in the form of mosaics and, consequently, the authors suggested that the chromosomes would have been lost during the meiotic process, and not after fertilisation.

Complex karyotypes have been detected in *Monomorium indicum*. The standard karyotype appears to be 2n = 22, the presence of reciprocal translocations and non-disjunction processes resulting in the presence of individuals with partial monosomy, trisomy and tetrasomy (IMAI & al. 1984).

Supernumerary chromosomes or B-chromosomes: In addition to the normal karyotype, individuals or populations sometimes may carry supernumerary or so-called B-chromosomes. These chromosomes are not essential since there are individuals lacking them, but it has been observed that these elements have an impact on various characteristics, such as the frequency of chiasmata (i.e., the points that hold together the paired homologous chromosomes when they separate from one another after meiosis crossing-over and that thus in fact are the cytological manifestation of crossing-over) and the formation rate of abnormal spermatids (CAMACHO 2005). In general, B-chromosomes are mainly or entirely heterochromatic and their origin and evolution remains enigmatic at present, although they probably derived from a normal chromosome complement (see CAMACHO 2005, for a review).

In ants, B-chromosomes were first described in *Temnothorax spinosior*. In this species, the standard chromosome number is n = 12, 2n = 24 (IMAI 1966). Later, IMAI (1974) detected a polymorphism in this species, i.e., B-chromosomes. The number of B-chromosomes ranges from one to 12 in males and their number is variable among individuals from the same population. In *Prenolepis jerdoni*, a high number of B-chromosomes was found. The standard karyotype has 16 chromosomes and the presence of 4 to 11 B-chromosomes was detected (IMAI & al. 1988a).

The elimination of B-chromosomes from somatic-line cells has been reported in several ant species. For example, in *Lasius niger*, B-chromosomes were found in male and female germ cells but they are absent in cerebral ganglion cells (PALOMEQUE & al. 1990b). In *Temnothorax spinosior* the B-chromosomes are absent in somatic cells of both sexes and they are partially unstable in female germ cells (IMAI 1974). Nevertheless, in other species, B-chromosomes are present in somatic cells, as it has been reported by MARIANO & al. (2001) for the cerebral ganglion cells of *Camponotus* sp.

Typical B-chromosomes have been also observed by LOISELLE & al. (1990) and LORITE & al. (2000, 2002b) in other ant species such as *Leptothorax muscorum*, *Lasius brunneus,* and *Pheidole pallidula* (Fig. 5). Species such as *Aphaenogaster rudis*, *Podomyrma adelaidae*, and *Temnothorax rugatulus* have numerical variations that have been considered possible cases of B-chromosomes (CROZIER 1975, IMAI & al. 1977, TABER & COKENDOLPHER 1988).

Hypotheses of karyotype evolution in Formicidae

Formicidae is one of the insect groups with the greatest variation in chromosome number. It is tentatively assumed that species differentiation has been accompanied by changes in the karyotype.

Three early hypotheses were presented to explain karyotype evolution in ants by IMAI & al. (1977): the fusion hypothesis, the fission hypothesis, and the modal hypothesis. According to the **fusion hypothesis**, the ancestral karyotype had a high chromosome number. This karyotype was composed basically of acrocentric chromosomes. During Formicidae evolution, chromosome numbers were then reduced, mainly by chromosome fusions. Only by this mechanism could an ancestral karyotype of n = 40 acrocentric chromosomes generate a karyotype with n = 20 metacentric, submetacentric or subtelocentric chromosomes. These chromosomes could become telocentric by pericentromeric inversions. Other chromosome fusions of these new chromosomes could again result in a reduction of chromosome number. Under this hypothesis, the mechanisms that augment chromosome numbers would have low frequency. The same authors later discarded this hypothesis for several reasons, one of the most important being the loss of chromatin. Centric fusion of acrocentric chromosomes results in the loss of the short arms (WHITE 1973). IMAI & al. (1977) calculated, based on data for other organisms (IMAI 1975), that the repetition of these fusion processes would lead to a loss of about 3.4 - 20.4% of the initial genome. This loss would probably be deleterious. Even assuming that the missing segments include only telomeric and / or heterochromatic subtelomeric DNA, an unlikely prospect, it would be necessary to assume that the chromosome inversions basically take the direction of transforming biarmed chromosomes into acro- or telocentric chromosomes.

Under the **fission hypothesis**, the ancestral karyotype had a very low chromosome number, namely n = 3 (IMAI & al. 1977). The subsequent increase in chromosome number resulted from the combined action of centric fissions and pericentromeric inversions. Under this model, centric fission changed the meta-, submeta-, or subtelocentric chromosomes into two telocentric chromosomes each. By the growth of the constitutive heterochromatin they could transform into acrocentrics and by pericentromeric inversions into other biarmed chromosomes, starting a new cycle again. According to this scheme, each original metacentric chromosome could form two new ones. This model assumes that centric fusions will occur occasionally but evolution tends to augment the chromosome number.

Under the **modal hypothesis**, the ancestral chromosome number was the modal number of today. Hence, karyotype evolution was bidirectional, both raising and lowering the

chromosome number from n = 11 (IMAI & al. 1977). However, even under this scenario, the authors considered fission processes to be more frequent than chromosome fusion.

Later, IMAI & al. (1986) reviewed previous theories attempting to explain the non-random distribution of translocations and Robertsonian polymorphisms. The authors differentiated two groups in relation to their chromosome number, i.e., species with $n \leq 12$ and species with $n > 12$. These groups are based on the distribution of chromosome numbers, since this distribution is bimodal with an antimodal value of n = 12 - 13. Also, the frequencies of karyotype polymorphism differ in each group. Thus, the polymorphisms by translocation are present in species with low chromosome numbers ($n \leq 12$), while Robertsonian polymorphisms occur in species with high chromosome numbers ($n > 12$) (IMAI & al. 1986).

IMAI & al. (1986, 1988a, 1994, 2001) proposed the **minimum-interaction theory** to explain the mechanisms that have been selected for to reduce the risk of occurrence of certain chromosomal mutations that lower the adaptive value of heterozygotes. The theoretical and experimental bases of this hypothesis are complex (see original papers) but its main points are summarized here. In meiosis, bivalents are telomere-attached to the nuclear membrane in the so-called bouquet configuration or "hammock structure". According to the minimum-interaction theory, some spontaneous chromosomal mutations could be the result of crossing-over or mis-resolution of chromosome interlocking in meiotic chromosomes (Figs. 6a, b). Logically, these exchanges need a proximity of DNA fibres to each other. Consequently, the mechanisms that tend to reduce the proximity among chromosomes will be selected for.

The minimum-interaction theory takes into consideration the tendency in eukaryotes to increase the amount of DNA, mainly by duplication or polyploidy. This increase of genomic DNA could be an evolutionary tool since new gene functions could be acquired. Increases could accelerate the rate of spontaneous chromosomal mutations and deleterious mutations, so that mechanisms that reduce the risk of these mutations could have been selected for in eukaryotes. Among these mechanisms are the improvement of DNA-repair processes, the hammock structure, the enlargement of the nuclear volume, and a greater degree of chromosome contraction. A factor related to the probability of chromosome interactions is chromosome number. Generally, mean chromosome size is larger with low chromosome numbers than with high chromosome numbers. For the same genome size a low chromosome number results in a higher possibility of interaction between non-homologous chromosomes. This effect is probably weaker if the size of the chromosomes is reduced (Fig. 6c), since the proximity among chromosome fibres is also reduced.

According to IMAI & al. (1986, 1988a, 1994, 2001), the minimum-interaction theory would explain the asymmetric distribution of translocations and Robertsonian polymorphisms in Formicidae, and also a more important role of fissions over that of fusions. According to this scenario, the probability of translocations would be very high in species with low chromosome numbers and large chromosomes. The fission processes, with a corresponding increase in chromosome number, are then favoured by selection. Specifically, the authors of the theory suggested that karyotype evolution in Formicidae is leading towards an

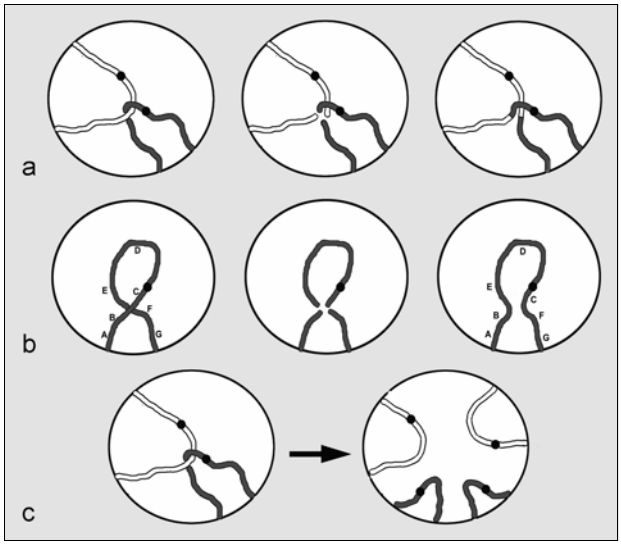

Fig. 6: The minimum-interaction theory. Chromosome mutations induced by the proximity between chromosomes follow from crossing-over or mis-resolution of chromosome interlocking in pachytene chromosomes: (a) reciprocal tranlocation and (b) inversion; (c) reduction of the risk of mutation by increases in chromosome number via centric fission. Re-drawn from IMAI & al. (1986).

increase rather than a decrease in chromosome number, with fissions and pericentromeric inversions being the most common chromosome mutations. Occasionally, some centric fusions could occur, especially in karyotypes which have pseudo-acrocentric chromosomes (i.e., chromosomes with an extraordinarily elongated heterochromatic short arm, IMAI & al. 1988a), as a mechanism leading to the elimination of heterochromatin, thereby reducing the possibility of non-specific associations.

Karyotype evolution and speciation

As commented above, chromosome rearrangements could be involved in speciation, since they reduce the fitness of structural hybrids due to the deleterious effect in the meiosis of the heterozygous individuals. Therefore, chromosome mutations could be involved in speciation, as they have the potential to generate postmating isolation mechanisms. However, the relationships between chromosomal changes and speciation are not always clear and often the result of a chromosomal mutation is only chromosome polymorphism (KING 1987). An important aspect in chromosome evolution in ants is their haploid-diploid nature. Because males are haploid, there is no meiosis for gamete production (PALOMEQUE & al. 1990a). Thus, it is possible that Formicidae are more tolerant of chromosome mutations than are other groups of organisms.

A relation between karyotype evolution and speciation processes has been suggested in several ant genera. CROZIER (1970b) found that different chromosomal groups are present in *Iridomyrmex* and that these karyotypic differences were correlated with morphological differences. Twenty-two years later, SHATTUCK (1992a, b) revised the subfamily Dolichoderinae and divided the former *Iridomyrmex* genus into seven genera: *Anonychomyrma*, *Doleromyrma*, *Iridomyrmex* s.str., *Linepithema*, *Ochetellus*, *Papyrius*, and *Philidris*. The majority of species of the former *Iridomyrmex*

genus have a chromosome number of n = 9, but species with n = 6 - 8, 11, and 14 have also been detected. CROZIER (1970b) suggested that the species with n = 14 are not closely related to other *Iridomyrmex*. SHATTUCK (1992a, b) placed these species into the genus *Ochetellus*, and considered Old World species with n = 9 to belong to the genus *Iridomyrmex* s.str. Other Old World species have n = 8. Although both karyotypes could easily be related by Robertsonian processes, morphological differences between these groups of species (CROZIER 1968b) have caused them to be assigned to different genera, such as *Anonychomyrma* or *Papyrius* (SHATTUCK 1992a, b). There are also morphological differences between species with n = 8 - 9 from the Old World and species with n = 8 - 9 from the New World (CROZIER 1970b). SHATTUCK (1992b) placed the New World species into the genus *Linepithema*.

A possible relationship between karyotype evolution and speciation processes has also been suggested for *Camponotus*. MARIANO & al. (2003), reviewing the cytogenetic data for the genus, reported that karyological differentiation has been clearly involved in the diversification of *Camponotus*, especially by centric fusions-fissions and inversions. This genus has the second largest number of described species and also contains a large number of karyotyped species. Nearly 70 *Camponotus* species have been studied and the chromosome number is rather variable, ranging from n = 9 to n = 26 (see Appendix). The observed karyotypic variation found in this genus could also be a consequence of its polyphyletic origin (BRADY & al. 2006).

Probably the best example of the application of cytogenetic data to the relationship among ant species are studies performed in the genus *Myrmecia*. In the *Myrmecia pilosula* complex, the diploid chromosome number ranges from 2n = 2 to 2n = 32 (IMAI & al. 1977, CROSLAND & CROZIER 1986). IMAI & al. (1994) defined five different species in this species complex according to their karyotypes. A complex polymorphism has been observed in other species of the same genus, such as *Myrmecia piliventris* with n = 2 - 4, 32 and 2n = 4, 6, 64 (IMAI & al. 1988a), or *Myrmecia fulvipes* with n = 6, 2n = 12, 48, 50, 60 (IMAI & al. 1977), so that it is possible that there are several sibling species under these taxa.

However, there are other ant genera in which species differentiation is not explained by important karyotypic variations. Thus, there are well-diversified genera with a high number of species but without visible changes in their karyotypes. One of these genera is *Formica.* In this genus, 32 species have been karyotyped and the chromosome numbers appear to be quite uniform, i.e., n = 26 - 27 (HAUSCHTECK-JUNGEN & JUNGEN 1976, ROSENGREN & al. 1980, LORITE & al. 1998a). Other examples of genera having a very constant chromosome number are *Lasius* and *Pogonomyrmex* (see Appendix). Karyotype conservation is also observed among related genera. For example, MOREAU & al. (2006), using molecular techniques, have shown that *Formica*, *Polyergus*, and *Cataglyphis* are very closely related. Although only four *Polyergus* and *Cataglyphis* species have been karyotyped, their chromosome numbers are, as in *Formica*, n = 26 - 27 (see Appendix). One of the genera with the largest number of karyotyped species is *Pheidole*, which is distributed worldwide and also contains the largest number of described species, with over 1100 species (BOLTON & al. 2007). The molecular phylogenetic studies strongly support the monophyly of this genus (MOREAU 2008), in which about 75 species have been karyotyped, 65 having a chromosome number of n = 9 - 10 and all chromosomes being metacentric. Most species with chromosome numbers different from n = 9 - 10 are unidentified species, and are treated as *Pheidole* spp. in the publications (GOÑI & al. 1982, IMAI & al. 1983, 1984).

Conclusions

Cytogenetic studies in Formicidae have determined that changes in the karyotype have accompanied genus and species differentiation. Often when several species of one genus have been karyotyped, a variable chromosome number as well as changes in chromosome morphology have been found. The numerical variations have been brought about mainly by Robertsonian changes of centric fusion or fission. Other processes that have altered chromosome numbers, such as polyploidy or aneuploidy, may not play an important role in the evolution of ants. Robertsonian changes besides inversions and translocations seem to be the main processes that generate changes in ant karyotypes. As commented above, there is an asymmetric distribution of chromosome mutations (IMAI & al. 1986), and thus translocations are more frequent in species with low chromosome numbers while Robertsonian changes take place mainly in species of high chromosome numbers.

According to the minimum-interaction theory, the chromosome number in ants generally tends to increase. However, despite this possible trend, the increase of chromosome numbers appears to have some limits. Ant karyotype evolution results in a wide diversity of karyotypes, but probably an optimal range in chromosome number exists. The haploid chromosome numbers in ants range from n = 1 to n = 60 but most ant species have a chromosome number of between n = 8 and n = 27 (Fig. 1). This idea is not incompatible with the minimum-interaction theory. In fact, this theory proposes mechanisms by which selection could act against low-chromosome-number karyotypes.

As commented above, ants with low chromosome numbers have larger chromosomes than species with high chromosome numbers (CROZIER 1975, IMAI & al. 1977, 1988a). Consequently, chromosome number and chromosome size are inversely related. Chromosome size is variable but it has been suggested that there are upper and lower tolerance limits, since beyond certain size limits cell-division processes could falter (see SCHUBERT 2007 for a review). In fact, when large numbers of species of a biological group are analysed, the chromosomal-number distribution is similar to a normal symmetric distribution where the majority of the species show intermediate chromosome numbers. This is the pattern of chromosome-number distribution in mammals (IMAI 1986, PARDO-MANUEL DE VILLENA & SAPIENZA 2001), parasitic Hymenoptera (GOKHMAN 2006), and ants (this paper). Thus, selection against extreme chromosome numbers could make the karyotype less variable if the chromosome number remains within the optimal range. Therefore, high or low chromosome numbers and high chromosome-number variability could be basal features.

In ants, the extreme chromosome numbers have been found in primitive groups. Molecular phylogenies have confirmed the basal nature of the poneroid clade, which includes the Ponerinae and Amblyoponerinae subfamilies, among others (BRADY & al. 2006, MOREAU & al. 2006).

Few data are available from Amblyoponerinae species but Ponerinae includes some of the species with some of the highest and lowest chromosome numbers, such as *Ponera* (n = 3 - 6) and *Dinoponera* (n = 53 - 60). Ponerinae also includes some of the genera with the greatest range of variation in chromosome number, such as *Pachycondyla* (n = 6 - 52) or *Platythyrea* (n = 9 - 47). In the formicoid clade, the basal subfamily Myrmeciinae includes a genus with one of the highest chromosome numbers (*Nothomyrmecia*, n = 47) and one with a highly variable chromosome number (*Myrmecia*, n = 1 - 42). It would be informative to increase the cytogenetic data in other subfamilies considered basal as a way to confirm whether the trend indeed is a general one in ants, although current data seem to point in this direction (see Appendix). In the less primitive subfamilies of the formicoid clade, such as Dolichoderinae, Myrmicinae or Formicinae, the chromosome numbers are less variable. These subfamilies include the majority of species analysed to date (about 50 species from Dolichoderinae, 100 from Formicinae, and more than 400 from Myrmicinae). The global variation in chromosome number in each of these subfamilies is less than that found for some of the genera of the Ponerinae or Myrmicinae (n = 5 - 16 in Dolichoderinae, n = 8 - 28 in Formicinae, and n = 4 - 35 in Myrmicinae).

The suggested trend towards karyotype optimisation is also observed at the species level. As has been commented in this review, different chromosome polymorphisms have been found in species from all subfamilies analysed. However, in species belonging to less primitive subfamilies, these polymorphisms are generally less complex and they originated from simple changes which usually generate only two karyotypic forms. The only known exception is the myrmicine *Pheidole noda*, which presents four polymorphic karyotypes (n = 17 - 20), due to fusion and fission processes (IMAI & KUBOTA 1975). In another Myrmicinae species, *Leptothorax muscorum*, a variable chromosome number was found (n = 16 - 23) (LOISELLE & al. 1990), although the authors suggested that at least four different species belong to this taxon.

Conversely, the majority of the complex intraspecific polymorphisms have been detected in species from more primitive genera. Probably the best examples are the species from the genus *Myrmecia* (Myrmeciinae). In the species of this genus, variable karyotypes are frequent. These variations are due to Robertsonian polymorphisms of centric fusion and fission, although other types of polymorphisms have been detected, such as pericentromeric inversions, translocation, deletions or changes in the amount of heterochromatin (IMAI & al. 1977, 1988a, 1994, IMAI & TAYLOR 1989, MEYNE & al. 1995). Other complex polymorphisms or wide variation in chromosome number have also been detected in *Ponera scabra* and *Dinopononera lucida* (Ponerinae) (IMAI & al. 1988a, MARIANO & al. 2008) and in two species from the genus *Rhytidoponera* (Ectatomminae) (IMAI & al. 1977).

The possible existence of an optimal range in chromosome number does not imply that the karyotypes remain unchanged. As has been commented, several mechanisms alter the number and morphology of chromosomes. Since chromosome rearrangements are generally associated with reduced fertility, their fixation in a population appears unlikely. WHITE (1978) suggested that four factors lead to the fixation of a chromosomal mutation: genetic drift, meiotic drive, inbreeding, and a selective advantage of the new karyotype. Some of these processes, if not all, depend heavily on population size, or more specifically on the effective population size, which is related to the number of reproductive individuals. The probability of fixation of a new mutation due to the effect of genetic drift, meiotic drive, and inbreeding is increased in small populations. In social insects, the relative effective population size is much lower than in other organisms since only a few individuals can reproduce per colony. The effective population size depends not only on the number of colonies but also the level of polygyny (CHAPMAN & BOURKE 2001). Hence, the appearance of mutations and their fixation in populations could be random processes and could vary among species or between populations of the same species. These stochastic phenomena result in different evolutionary pathways in ant karyotype evolution as compared to other, non-social organisms. Thus, it is possible to find cases in which speciation has occurred without karyotype changes and others in which species of the same genus present very different karyotypes, as has been described in animal groups such as mammals (IMAI 1983, 1986).

Future directions

As discussed throughout this review, numerous cytogenetic studies have been made in ants, showing the great karyotypic variability in this group of insects and the suitability of ants for analysing karyotype evolution. However, karyotype research has not yet reached its final goal, and several cytogenetic aspects require a more intensive study. We believe that several lines of research should be developed in the future:

1. Cytogenetic studies need to be completed. Although the number of species studied is very high, some subfamilies lack cytogenetic information and in others only a small number of species has been analysed.

2. More information is necessary concerning ant meiosis. Because male ants are haploid, there is no real meiosis during spermatogenesis. CROZIER (1975) considered meiosis to be abortive in Hymenopteran males. However, the analysis of spermatogenesis in the ant *Tapinoma nigerrimum* (for the taxonomic status of this taxon, see above) has shown that only one division occurs with the formation of a single metaphase plate (PALOMEQUE & al. 1990a). The absence of a first division in ants is supported by the observation of the spermatogenesis process in diploid ants (YAMAUCHI & al. 2001). Homologous chromosomes of diploid ants are not paired into bivalents, and spermatogenesis results in the formation of diploid gametes. The meiosis process has been analysed in a range of hymenopteran species but has been little studied in ants and no recent data are available on this basic issue.

3. New tools are needed for a detailed analysis of chromosomal mutations. As discussed regarding ants, it is not possible to achieve good chromosome banding patterns. It would therefore be necessary to develop molecular markers that enable this type of study. The development of linkage maps with genetic markers across all chromosomes might facilitate the detection and analysis of mutations that are not detectable by standard karyotype analysis. Linkage maps are currently available for some ant species (SIRVIÖ & al. 2006). Linkage maps are one of the first steps necessary to perform complete-genome sequencing. Sequenc-

ing of the first six ant genomes has recently started (www.antgenomics.org), but further such projects would be desirable – ants are especially suitable organisms for such projects due to their small genome size in comparison with other insects (TSUTSUI & al. 2008).

4. The future of ant cytogenetics must be coupled with the application of molecular techniques, mainly in two major lines of research, molecular cytogenetics and the combination of cytogenetic and molecular data. Chromosome location of specific DNA sequences by FISH (fluorescence in situ hybridisation) is a powerful tool in the study of chromosome organization and evolution. However, this technique has yet been poorly applied in the study of the ant genome. FISH has provided knowledge on the nature of ant telomeres (MEYNE & al. 1995, LORITE & al. 2002a). Also study into the chromosome location of other non-coding DNAs such as satellite DNA (LORITE & al. 2004a, b) would be particularly worthwhile to ascertain the location of specific sequences among related species, which is needed to perform comparative karyology. Such studies have been applied to only a few *Myrmecia* species (HIRAI & al. 1994, 1996).

5. Another important line of research will be the even wider application of mitochondrial and nuclear molecular markers to construct molecular phylogenies (cf. MOREAU 2009). The comparison of these phylogenies with cytogenetic data will provide relevant information on the direction of chromosomal mutations in ant evolution.

Acknowledgements

The Spanish CGL2006-04331 project and the PAI CECJA CVI 220 supported this work. We are grateful to Dr. Alberto Tinaut (University of Granada, Spain), for valuable suggestions on the manuscript and for providing information on the taxonomic status of Spanish species, and to Dr. Antonio Sánchez (University of Jaén, Spain), for discussion and valuable comments on the manuscript. We are also gratefully to Dr. Hirotami T. Imai (Japanese Ant Database), Dr. Cléa Mariano and Dr. Jacques Delabie (Laboratório de Mirmecologia, CEPEC/CEPLAC, Brazil), for their valuable suggestions and comments.

Zusammenfassung

Ameisen (Hymenoptera: Formicidae) sind eine diversifizierte Insektengruppe mit mehr als 12000 beschriebenen Arten. Wie andere Hymenopteren sind Ameisen haplodiploid, wobei sich aus befruchteten Eiern diploide Weibchen (Arbeiterinnen und Königinnen), aus unbefruchteten Eiern aber haploide Männchen entwickeln. Eine hohe Zahl von Arten ist zytogenetisch untersucht. Die Chromosomenzahl ist derzeit für über 750 Arten bekannt. Alle diese Daten werden in dieser Arbeit zusammengefasst. Formicidae zählen zu den Insekten mit den am stärksten variablen Chromosomenzahlen. Die haploiden Chromosomenzahlen reichen von n = 1 bis n = 60. Diese Chromosomendiversität legt nahe, dass Karyotypmodifikationen gleichzeitig mit der Diversifizierung von Ameisen stattgefunden haben. Die Karyotypevolution ist aus Chromosomenmutationsprozessen gefolgt, die nicht nur die Chromosomenzahl sondern auch die Chromosomenmorphologie verändern können. Wir geben einen Überblick über die verschiedenen bei Ameisen beobachteten Chromosomenmutationen sowie über die mögliche Rolle solcher Mutationen in der Karyotypevolution bei diesen Insekten und untersuchen die Hypothesen, die zur Erklärung ihrer Karyotypevolution vorgeschlagen worden sind. Unter den Chromosomenrearrangements scheinen Robertsonsche zentrische Fusionen und Fissionen neben Inversionen und Translokationen die Hauptprozesse zu sein, die Veränderungen von Ameisenkaryotypen bedingen. Andere Prozesse, die die Chromosomenzahl verändern, wie Polyploidie und Aneuploidie, scheinen in der Ameisenevolution nicht wichtig zu sein. Die Ameisenunterfamilien weisen unterschiedliche Ausmaße von Variation in Zusammenhang mit der Chromosomenzahl auf. Die größte Variation wurde bei primitiven Unterfamilien wie den Ponerinae (n = 3 - 60) und den Myrmeciinae (n = 1 - 47) gefunden, wohingegen die Chromosomenzahl bei weniger primitiven Unterfamilien weniger variiert, beispielsweise bei den Dolichoderinae (n = 5 - 16), Formicinae (n = 8 - 28) und Myrmicinae (n = 4 - 35). Für die anderen Unterfamilien sind wenige Daten verfügbar. Primitive Ameisen haben nicht nur die größte Bandbreite an Chromosomenzahl sondern auch die komplexesten Chromosomenpolymorphismen. Im Gegensatz dazu weisen weniger primitive Genera eine geringere Variation an Chromosomenzahl auf, und es wurden bei letzteren auch generell nur einfache Polymorphismen gefunden. Wir schließen mit einem Ausblick auf zukünftige Forschungsrichtungen.

References

AULARD, S., MONTI, L., CHAMINADE, N. & LEMEUNIER, F. 2004: Mitotic and polytene chromosomes: comparisons between *Drosophila melanogaster* and *Drosophila simulans*. – Genetica 120: 137-150.

BARROS, L.A.C., MARIANO, C.S.F., BORGES, D.S., POMPOLO, S.G. & DELABIE, J.H.C. 2008: Cytogenetic studies of the Neotropical ant genus *Ectatomma* (Formicidae: Ectatomminae: Ectatommini). – Sociobiology 51: 555-561.

BIÉMONT, C. 2008: Genome size evolution: within-species variation in genome size. – Heredity 101: 297-298.

BOLTON, B., ALPERT, G., WARD, P.S. & NASKRECKI, P. 2007: Bolton's catalogue of ants of the world 1758-2005. – Harvard University Press, Cambridge, MA, CD-ROM.

BRADY, S.G., SCHULTZ, T.R., FISHER, B.L. & WARD, P.S. 2006: Evaluating alternative hypotheses for the early evolution and diversification of ants. – Proceedings of the National Academy of Sciences of the United States of America 103: 18172-18177.

CAMACHO, J.P.M. 2005: B chromosomes. In: GREGORY, T.R. (Ed.): The evolution of the genome. – Elsevier, San Diego, CA, pp. 223-286.

CHAPMAN, R.E. & BOURKE, A.F.G. 2001: The influence of sociality on the conservation biology of social insects. – Ecology Letters 4: 650-662.

CROSLAND, M.W.J. & CROZIER, R.H. 1986: *Myrmecia pilosula*, an ant with only one pair of chromosomes. – Science 231: 1278.

CROZIER, R.H. 1968a: Cytotaxonomic studies on some Australian dolichoderine ants (Hymenoptera: Formicidae). – Caryologia 21: 241-259.

CROZIER, R.H. 1968b: Interpopulation karyotype differences in Australian *Iridomyrmex* of the "*detectus*" group (Hymenoptera: Formicidae: Dolichoderinae). – Journal of the Australian Entomological Society 7: 25-27.

CROZIER, R.H. 1969: Chromosome number polymorphism in an Australian ponerine ant. – Canadian Journal of Genetics and Cytology 11: 333-339.

CROZIER, R.H. 1970a: Pericentric rearrangement polymorphism in a North American dolichoderine ant (Hymenoptera: Formicidae). – Canadian Journal of Genetics and Cytology 12: 541-546.

CROZIER, R.H. 1970b: Karyotypes of twenty-one ant species (Hymenoptera; Formicidae), with reviews of the known ant karyotypes. – Canadian Journal of Genetics and Cytology 12: 109-128.

CROZIER, R.H. 1975: Hymenoptera. In: JOHN, B. (Ed.): Animal cytogenetics, vol. 3. Insecta 7. – Gebrüder Borntraeger, Berlin, Stuttgart, 95 pp.

FISCHER, K. 1987: Karyotypuntersuchungen an selbständigen und sozialparasitischen Ameisen des Tribus Leptothoracini (Hymenoptera, Formicidae) im Hinblick auf ihre Verwandtschaftsbeziehungen. – PhD thesis, Technical University Darmstadt, 219 pp.

GLANCEY, B.M., ROMAIN, M.K.S. & CROZIER, R.H. 1976: Chromosome numbers of the red and the black imported fire ants, *Solenopsis invicta* and *S. richteri*. – Annals of the Entomological Society of America 69: 469-470.

GOKHMAN, V.E. 2006: Karyotypes of parasitic Hymenoptera: diversity, evolution and taxonomic significance. – Insect Science 13: 237-241.

GOÑI, B., IMAI, H.T., KUBOTA, M., KONDO, M., YONG, H.S. & TSO, Y.P. 1982: Chromosome observations of tropical ants in Western Malaysia and Singapore. – Annual Report of the National Institute of Genetics (Japan) 32: 71-73.

GOÑI, B., ZOLESSI, L.C. DE & IMAI, H.T. 1983: Karyotypes of thirteen ant species from Uruguay (Hymenoptera, Formicidae). – Caryologia 36: 363-371.

GOODISMAN, M.A.D., KOVACS, J.L. & HUNT, B.H. 2008: Functional genetics and genomics in ants (Hymenoptera: Formicidae): the interplay of genes and social life. – Myrmecological News 11: 107-117.

GREGORY, T.R. 2005: The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. – Annals of Botany 95: 133-146.

HAUSCHTECK, E., 1961: Die Chromosomen von fünf Ameisenarten. – Revue Suisse de Zoologie 68: 218-223.

HAUSCHTECK-JUNGEN, E. & JUNGEN, H. 1976: Ant chromosomes. I. The genus *Formica*. – Insectes Sociaux 23: 513-524.

HAUSCHTECK-JUNGEN, E. & JUNGEN, H. 1983: Ant chromosomes. II. Karyotypes of Western Palearctic species. – Insectes Sociaux 30: 149-164.

HIRAI, H., YAMAMOTO, M.T., OGURA, K., SATTA, Y., YAMADA, M., TAYLOR, R.W. & IMAI, H.T. 1994: Multiplication of 28S rDNA and NOR activity in chromosome evolution among ants of the *Myrmecia pilosula* species complex. – Chromosoma 103: 171-178.

HIRAI, H., YAMAMOTO, M.T., TAYLOR, R.W. & IMAI, H.T. 1996: Genomic dispersion of 28S rDNA during karyotypic evolution in the ant genus *Myrmecia* (Formicidae). – Chromosoma 105: 190-196.

HUNG, A.C.F., IMAI, H.T. & KUBOTA, M. 1972: The chromosomes of nine ant species (Hymenoptera: Formicidae) from Taiwan, Republic of China. – Annals of the Entomological Society of America 65: 1023-1024.

HUNG, A.C.F., VINSON, S.B. & SUMMERLIN, J.W. 1974: Male sterility in the red imported fire ant, *Solenopsis invicta*. – Annals of the Entomological Society of America 67: 909-912.

IMAI, H.T. 1966: The chromosome observation techniques of ants and the chromosomes of Formicinae and Myrmicinae. – Acta Hymenopterologica 2: 119-131.

IMAI, H.T. 1971: Karyological studies of Japanese ants. II. Species differentiation in *Aphaenogaster*; with special regard to their morphology, distribution and chromosomes. – Mushi 44: 137-151.

IMAI, H.T. 1974: B chromosomes in the myrmicine ant, *Leptothorax spinosior*. – Chromosoma 45: 431-444.

IMAI, H.T. 1975: Evidence for non-random localization of the centromere on mammalian chromosomes. – Journal of Theoretical Biology 49: 111-123.

IMAI, H.T. 1983: Quantitative analysis of karyotype alteration and species differentiation in mammals. – Evolution 37: 1154-1161.

IMAI, H.T. 1986: Modes of species differentiation and karyotype alteration in ants and mammals. In: IWATSUKI, K., RAVEN, P.H. & BOCK, W.J. (Eds.): Modern aspect of species. – University of Tokyo Press, Tokyo, pp. 87-105.

IMAI, H.T. 1991: Mutability of constitutive heterochromatin (C-bands) during eukaryotic chromosomal evolution and their cytological meaning. – Japanese Journal of Genetics 66: 635-661.

IMAI, H.T., BARONI URBANI, C., KUBOTA, M., SHARMA, G.P., NARASIMHANNA, M.N., DAS, B.C., SHARMA, A.K., SHARMA, A., DEODIKAR, G.B., VAIDYA, V.G. & RAJASEKARASETTY, M. R. 1984: Karyological survey of Indian ants. – Japanese Journal of Genetics 59: 1-32.

IMAI, H.T., BROWN, W.L., KUBOTA, M., YONG, H.S. & THO, Y.P. 1983: Chromosome observations on tropical ants from Western Malaysia. II. – Annual Report of the National Institute of Genetics (Japan) 34: 66-69.

IMAI, H.T., CROZIER, R.H. & TAYLOR, R.W. 1977: Karyotype evolution in Australian ants. – Chromosoma 59: 341-393.

IMAI, H.T. & KUBOTA, M. 1972: Karyological studies of Japanese ants (Hymenoptera: Formicidae). III. Karyotypes of nine species in Ponerinae, Formicinae, and Myrmicinae. – Chromosoma 37: 193-200.

IMAI, H.T. & KUBOTA, M. 1975: Chromosome polymorphism in the ant, *Pheidole nodus*. – Chromosoma 51: 391-399.

IMAI, H.T., MARUYAMA, T., GOJOBORI, Y.I. & CROZIER, R.H. 1986: Theoretical bases for karyotype evolution. 1. The minimum-interaction hypothesis. – American Naturalist 128: 900-920.

IMAI, H.T., SATTA, Y. & TAKAHATA, N. 2001: Integrative study on chromosome evolution of mammals, ants and wasps based on the Minimum Interaction Theory. – Journal of Theoretical Biology 210: 475-497.

IMAI, H.T. & TAYLOR, R.W. 1989: Chromosomal polymorphisms involving telomere fusion, centromeric inactivation and centromere shift in the ant *Myrmecia* (*pilosula*) n = 1. – Chromosoma 98: 456-460.

IMAI, H.T., TAYLOR, R.W., CROSLAND M.W.J. & CROZIER, R.H. 1988a: Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis. – Japanese Journal of Genetics 63: 159-185.

IMAI, H.T., TAYLOR, R.W. & 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). – Japanese Journal of Genetics 69: 137-182.

IMAI, H.T., TAYLOR, R.W., CROZIER, R.H., CROSLAND, M.W.L. & BROWNING, G.P. 1988b: Chromosomal polymorphism in the ant *Myrmecia* (*pilosula*) n = 1. – Annual Report of the National Institute of Genetics (Japan) 38: 82-84.

IMAI, H.T., TAYLOR, R.W., KUBOTA, M., OGATA, K. & WADA, M.Y. 1990: Notes of the remarkable karyology of the primitive ant *Nothomyrmecia macrops*, and of the related genus *Myrmecia* (Hymenoptera, Formicidae). – Psyche 97: 133-140.

IMAI, H.T. & YOSIDA, T.H. 1966: Polyploid cells observed in male and queen ants of *Aphaenogaster osimensis*. – Annual Report of the National Institute of Genetics (Japan) 16: 54.

JOHNSTON, J.S., ROSS, L.D., BEANI, L., HUGHES, D.P. & KATHIRITHAMBY, J. 2004: Tiny genomes and endoreduplication in Strepsiptera. – Insect Molecular Biology 13: 581-585.

KING, M. 1987: Chromosomal rearrangements, speciation and the theoretical approach. – Heredity 59: 1-6.

LEVAN, A., FREDGA, K. & SANDBERG, A. 1964: Nomenclature for centromeric position on chromosomes. – Hereditas 52: 201-220.

LOISELLE, R., FRANCOEUR, A., FISCHER, K. & BUSCHINGER, A. 1990: Variations and taxonomic significance of the chromosome numbers in the Nearctic species of the genus *Leptothorax* (s.s.) (Formicidae: Hymenoptera). – Caryologia 43: 321-334.

LORITE, P., CARRILLO, J.A., AGUILAR, J.A. & PALOMEQUE, T. 2004a: Isolation and characterization of two families of satellite DNA with repetitive units of 135 bp and 2.5 Kb in the ant *Monomorium subopacum* (Hymenoptera, Formicidae). – Cytogenetic and Genome Research 105: 83-92.

LORITE, P., CARRILLO, J.A., GARCÍA, M.F. & PALOMEQUE, T. 2000: Chromosome numbers in Spanish Formicidae. III. Subfamily Myrmicinae (Hymenoptera). – Sociobiology 36: 555-570.

LORITE, P., CARRILLO, J.A. & PALOMEQUE, T. 2002a: Conservation of (TTAGG)n telomeric sequences among ants (Hymenoptera, Formicidae). – Journal of Heredity 93: 282-285.

LORITE, P., CARRILLO, J.A., TINAUT, A. & PALOMEQUE, T. 2002b: Chromosome numbers in Spanish Formicidae. IV. New data of species from the genera *Camponotus*, *Formica*, *Lasius*, *Messor*, and *Monomorium*. – Sociobiology 40: 331-341.

LORITE, P., CARRILLO, J.A., TINAUT, A. & PALOMEQUE, T. 2004b: Evolutionary dynamics of satellite DNA in species of the genus *Formica* (Hymenoptera, Formicidae). – Gene 332: 159-168.

LORITE, P., CHICA, E. & PALOMEQUE, T. 1996: G-banding and chromosome condensation in the ant *Tapinoma nigerrimum*. – Chromosome Research 4: 77-79.

LORITE, P., CHICA, E. & PALOMEQUE, T. 1998a: Números cromosómicos en los Formícidos españoles. I. Subfamilia Formicinae. – Boletín de la Real Sociedad Española de Historia Natural (Sección Biología) 94: 23-31.

LORITE, P., CHICA, E. & PALOMEQUE, T. 1998b: Chromosome numbers in Spanish Formicidae. II. Subfamily Dolichoderinae. – Sociobiology 32: 77-89.

LORITE, P., RENAULT, S., ROULEUX-BONNIN, F., BIGOT, S., PERIQUET, G. & PALOMEQUE, T. 2002c: Genomic organization and transcription of satellite DNA in the ant *Aphaenogaster subterranea* (Hymenoptera, Formicidae). – Genome 45: 609-616.

MARIANO, C.S.F., DELABIE, J.H.C., CAMPOS, L.A.O. & POMPOLO, S.G. 2003: Trends in karyotype evolution in the ant genus *Camponotus* (Hymenoptera: Formicidae). – Sociobiology 42: 831-839.

MARIANO, C.S.F., DELABIE, J.H.C., SANTOS, J.R.M. & POMPOLO, S.G. 2007: Evolução cariotípica em *Pachycondyla* spp. (Ponerinae) Neotropicais. – O Biológico 69, Suplemento 2: 409-412.

MARIANO, C.S.F., POMPOLO, S.G., BARROS, L.A.C., MARIANO-NETO, E., CAMPIOLO, S. & DELABIE, J.H.C. 2008: A biogeographical study of the threatened ant *Dinoponera lucida* EMERY (Hymenoptera: Formicidae: Ponerinae) using a cytogenetic approach. – Insect Conservation and Diversity 1: 161-168.

MARIANO, C.S.F., POMPOLO, S.G., DELABIE, J.H.C. & CAMPOS, L.A.O. 2001: Estudos cariotípicos de algumas espécies neotropicais de *Camponotus* MAYR (Hymenoptera, Formicidae). – Revista Brasileira de Entomologia 45: 267-274.

MARIANO, C.S.F., POMPOLO, S.G., LACAU, S. & DELABIE, J.H.C. 2006: Questions sur la monophylie du taxon *Pachycondyla* SMITH, 1858: approche cytogénétique sur le sous-genre *Pachycondyla* sensu EMERY, 1901 (Hymenoptera: Formicidae: Ponerinae). – Bulletin de La Societé Entomologique de France 111: 299-304.

MEYNE, J., HIRAI, H. & IMAI, H.T. 1995: FISH analysis of the telomere sequences of bulldog ants (*Myrmecia*: Formicidae). – Chromosoma 104: 14-18.

MOREAU, C.S. 2008: Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae). – Molecular Phylogenetics and Evolution 48: 224-239.

MOREAU, C.S. 2009: Inferring ant evolution in the age of molecular data (Hymenoptera: Formicidae). – Myrmecological News 12: 201-210.

MOREAU, C.S., BELL, C.D., VILA, R., ARCHIBALD, S.B. & PIERCE, N.E. 2006: Phylogeny of the ants: Diversification in the age of angiosperms. – Science 312: 101-104.

MURAKAMI, T., FUJIWARA, A. & YOSHIDA, M.C. 1998: Cytogenetics of ten ant species of the tribe Attini (Hymenoptera, Formicidae) in Barro Colorado Island, Panama. – Chromosome Science 2: 135-139.

ORLEDGE, G.M. 1998: The identity of *Leptothorax albipennis* (CURTIS) (Hymenoptera: Formicidae) and its presence in Great Britain. – Systematic Entomology 23: 25-33.

OTTO, S. & WHITTON, J. 2000: Polyploidy incidence and evolution. – Annual Review of Genetics 34: 401-437.

PALOMEQUE, T., CANO, M.A., CHICA, E. & DÍAZ DE LA GUARDIA, R. 1990a: Spermatogenesis in *Tapinoma nigerrimum* (Hymenoptera, Formicidae). – Cytobios 62: 71-80.

PALOMEQUE, T., CHICA, E., CANO, M.A. & DÍAZ DE LA GUARDIA, R. 1988: Karyotypes, C-banding, and chromosomal location of active nucleolar organizing regions in *Tapinoma* (Hymenoptera, Formicidae). – Genome 30: 277-280.

PALOMEQUE, T., CHICA, E. & DÍAZ DE LA GUARDIA, R. 1990b: Karyotypes, C-banding, chromosomal location of active nucleolar organizing regions, and B-chromosomes in *Lasius niger* (Hymenoptera, Formicidae). – Genome 33: 267-272.

PALOMEQUE, T., CHICA, E. & DÍAZ DE LA GUARDIA, R. 1993a: Supernumerary chromosome segments in different genera of Formicidae. – Genetica 90: 17-29.

PALOMEQUE, T., CHICA, E. & DÍAZ DE LA GUARDIA, R. 1993b: Karyotype evolution and chromosomal relationships between several species of the genus *Aphaenogaster* (Hymenoptera, Formicidae). – Caryologia 46: 25-40.

PALOMEQUE, T. & LORITE, P. 2008: Satellite DNA in insects: a review. – Heredity 100: 564-573.

PARDO-MANUEL DE VILLENA, F. & SAPIENZA, C. 2001: Female meiosis drives karyotypic evolution in mammals. – Genetics 159: 1179-1189.

ROBERTSON, W.R.B. 1916: Chromosome studies. I. Taxonomic relationships shown in the chromosomes of Tettigidae and Acrididae: V-shaped chromosomes and their significance in Acrididae, Locustidae, and Gryllidae; chromosomes and variation. – Journal of Morphology 27: 179-331.

ROSENGREN, M., ROSENGREN, R. & SÖDERLUND, V. 1980: Chromosome numbers in the genus *Formica* with special reference to the taxonomical position of *Formica uralensis* RUZSK. and *Formica truncorum* FABR. – Hereditas 92: 321-325.

SCHUBERT, I. 2007: Chromosome evolution. – Current Opinion in Plant Biology 10: 109-115.

SEIFERT, B. 2009: Cryptic species in ants (Hymenoptera: Formicidae) revisited: we need a change in the alpha-taxonomic approach. – Myrmecological News 12: 149-166.

SHATTUCK, S.O. 1992a: Generic revision of the ant subfamily Dolichoderinae. – Sociobiology 21: 1-181.

SHATTUCK, S.O. 1992b: Review of the Dolichoderinae ant genus *Iridomyrmex* MAYR with descriptions of three new genera (Hymenoptera: Formicidae). – Journal of the Australian Entomological Society 31: 13-18.

SHERIZEN, D.E., JANG, J.K., KATO, N. & MCKIM, K.S. 2005: Translocations are dominant meiotic crossover suppressors due to a defect early in the recombination pathway. – Genetics 169: 767-781.

SIRVIÖ, A., GADAU, J., RUEPPELL, O., LAMATSCH, D., BOOMSMA, J.J., PAMILO, P. & PAGE, R.E. Jr. 2006: High recombination frequency creates genotypic diversity in colonies of the leaf-cutting ant *Acromyrmex echinatior*. – Journal of Evolutionary Biology 19: 1475-1485.

TABER, S.W. & COKENDOLPHER, J.C. 1988: Karyotypes of a dozen ant species from the Southwestern U.S.A. (Hymenoptera: Formicidae). – Caryologia 41: 93-102.

TAYLOR, R.W. 1991: *Myrmecia croslandi* sp.n. a karyologically remarkable new Australian jack-jumper ant (Hymenoptera: Formicidae: Myrmeciinae). – Journal of the Australian Entomological Society 30: 288.

TSUTSUI, N.D., SUAREZ, A.V., SPAGNA, J.C. & JOHNSTON, J.S. 2008: The evolution of genome size in ants. – BiomedCentral Evolutionary Biology 8: 64.

WHITE, M.J.D. 1973: Animal cytology and evolution. 3rd ed. – Cambridge University Press, London and New York, 961 pp.

WHITE, M.J.D. 1978: Chromosomal modes of speciation. In: DAVERN, C.I. (Ed.): Modes of speciation. – W.H. Freeman & Co., San Francisco, CA, pp. 169-276.

YAMAUCHI, K., YOSHIDA, T., OGAWA, T., ITOH, S., OGAWA, Y., JIMBO, S. & IMAI, H.T. 2001: Spermatogenesis of diploid males in the formicine ant, *Lasius sakagamii*. – Insectes Sociaux 48: 28-32.