Chromosome Numbers in Spanish Formicidae (Hymenoptera)

IV. New data of Species from the Genera *Camponotus*, *Formica*, *Lasius*, *Messor,* and *Monomorium*

by

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ABSTRACT

In this paper we report new karyological data from seven species belonging to subfamilies Formicinae and Myrmicinae. Among them we include two that are considered as endemic Iberian species, *Formica frontalis* and *Formica subrufa*. Also the chromosome number of *Formica gerardi* is reported. In *Lasius brunneus*, a variation on chromosome number probably due to the presence of B-chromosomes was detected. For two other species (*Camponotus cruentatus* and *Messor barbarus*) we found different chromosome numbers from those previously published. Also we confirm the chromosome number reported for *Monomorium subopacum*.

INTRODUCTION

Hymenoptera form one of the most distinct and well-defined insect orders and have long been perceived as a natural group. Haplo-diploidy or male haploidy is the main characteristic of the order (Crozier 1975, Gauld & Bolton 1988).

Several studies have been carried about cytogenetic aspects of ants. A wide variation has been observed in relation to the chromosome number (n=1 to n=42). Karyological analysis has proved to be useful to determine the karyotypic relationship and evolution between related species (Imai 1971, Loiselle *et al.* 1990, Palomeque *et al.* 1988, 1993) as well for the establishment and characterization of new species (Imai *et al.* 1994).

In recent years our group have performed studies in Spanish Formicidae. In relation to this, we have published several reviews of chromosome numbers (Lorite et al. 1998a, 1998b, 2000). In this paper we report new karyological data from seven species from the Formicinae

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and Myrmicinae subfamilies.

MATERIAL AND METHODS

Haploid metaphase plates have been obtained from testes of male pupae using the technique described in Lorite *et al.* (1996). Diploid metaphase plates have been obtained from worker prepupae cerebral ganglia cells according to the technique described in Imai et al. (1977).

For chromosomic morphology, we use the classification system proposed by Levan *et al.* (1964) that differentiates five types of chromosomes; metacentrics (M), submetacentrics (SM), subtelocentrics (ST), acrocentrics (A) and telocentrics (T).

RESULTS AND DISCUSSION

Subfamily Formicinae

Camponotus cruentatus

The males from the analyzed population present a haploid chromosome number of n=20. The haploid karyotype formula is n=12SM+5ST+3T (Fig. 1a). Hauschteck-Jungen & Jungen (1983) reported a chromosome number of n=18 for another population also collected in Spain. Unfortunately, in this paper photographs are not provided, so it is not possible to perform comparisons. However, the existence of intraspecific karyotypic differences is not unusual in ants.

In Indian species, Imai *et al.* (1984) observed that the genus *Camponotus* is karyotypically heterogeneous, with divergent chromosome numbers ranging between n=10 to n=22. The increase in the chromosome numbers was related to the increase in the numbers of subtelocentric to telocentric chromosomes in relation to submetacentric or metacentric chromosomes. The following haploid karyotype formulae have been described: n=10 (10M), n=12 (12A), n=13 (10M+3A or 13M), n=16 (5M+11A), n=17 (5M+12A), n=20 (20A or 1M+19A) y n=21 (1M+21A). These results could indicate that Robertsonian processes could be involved in the evolution of the *Camponotus* genus. The karyotype of *C. cruentatus*, described here, is in concordance with this hypothesis since it is enriched by subtelocentric-telocentric chromosomes. Robertsonian changes seem to be the most important processes for the karyotypic evolution in ants (Imai *et al.* 1988, 2001) and are probably the reason for the differences found in *C. cruentatus*.

Genus *Formica*

In this paper we have analyzed three species from this genus. Two of them (*F. frontalis* and *F. subrufa*) are endemic Iberian species (Tinaut

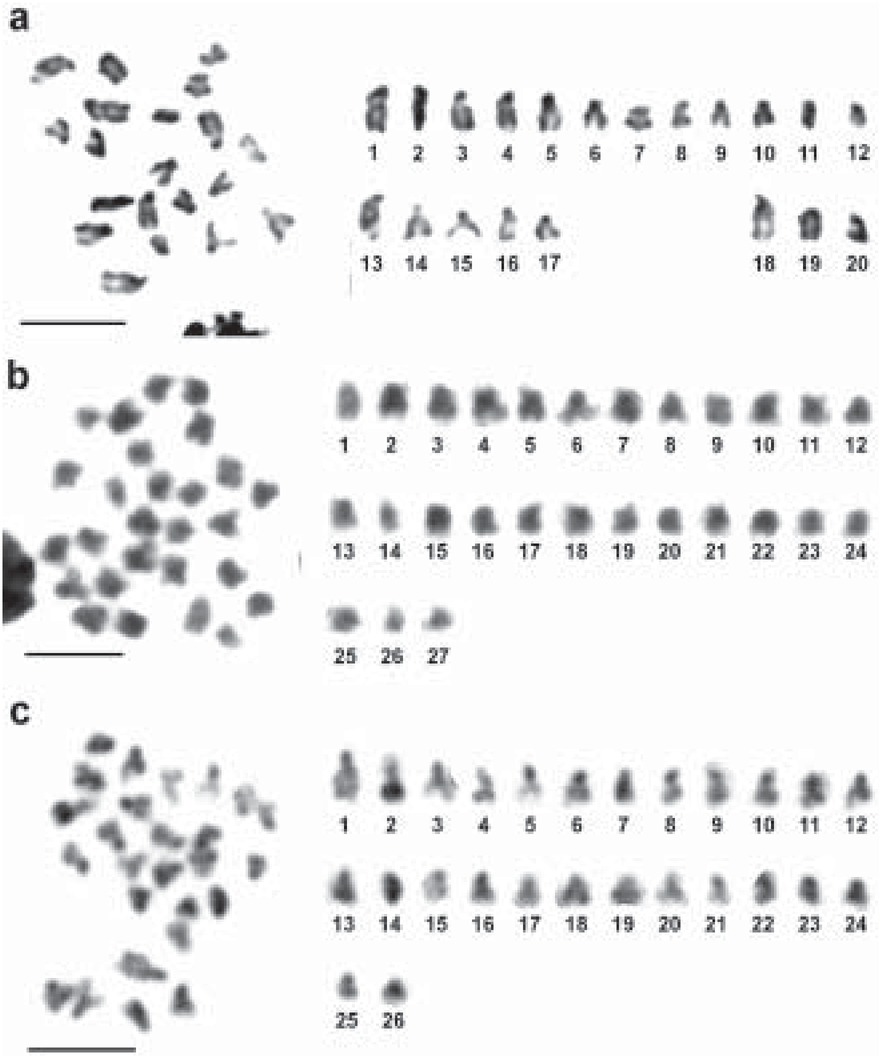

Fig. 1. Haploid metaphase plates and karyotypes of male pupae from *Camponotus cruentatus* (a), *Formica gerardi* (b) *Formica subrufa* (c). Bar=5µm.

1990, Tinaut & Martínez-Ibáñez 1998).

Formica frontalis

We found in worker ganglion cells a diploid number of 2n=52 (Fig. 2).

Formica gerardi

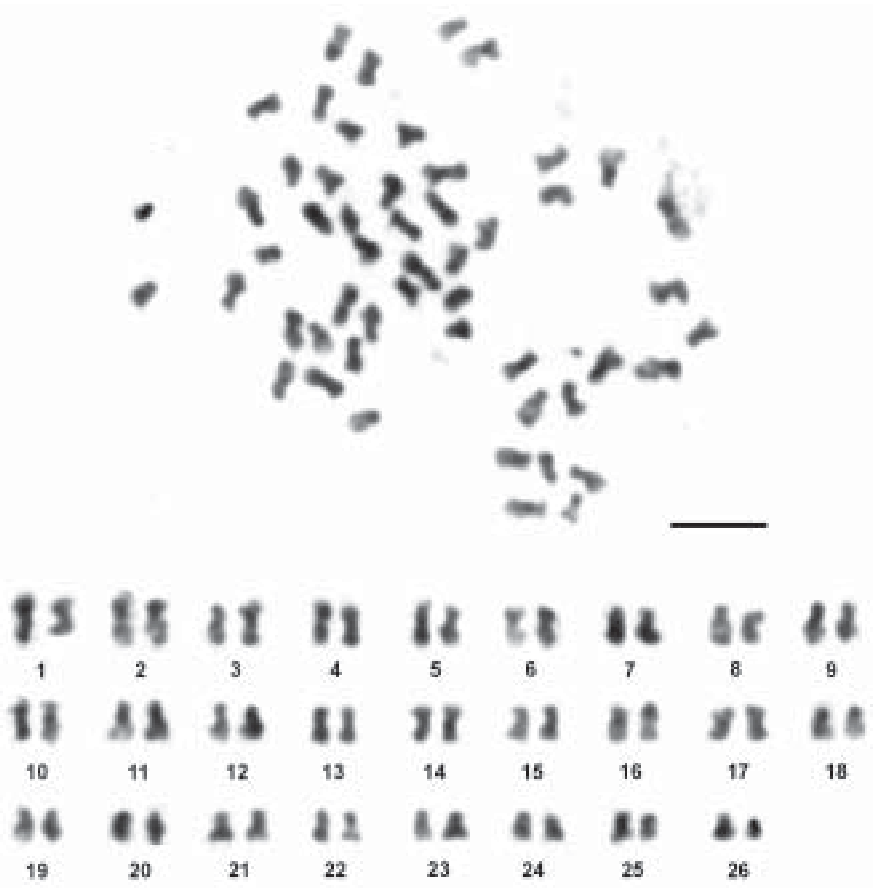

Fig. 2. Diploid metaphase plate and karyotype of female prepupae from *Formica frontalis*. Bar=5µm.

The haploid chromosome number of this species is n=27 (Fig. 1b).

Formica subrufa

In males, the haploid number is n=26 (Fig. 1c).

The three species present similar chromosome numbers and karyotypes to those found in other species from the genus *Formica*. However, the karyotype analysis is very difficult since the exact chromosome morphology cannot be clearly determined by the small size of the majority of them. In spite of this, the karyotypes are composed basically of metacentric to submetacentric chromosomes. As mentioned above, in ant species with high chromosome numbers, subtelocentrics and telocentrics increase gradually. The situation is different within the genus *Formica*, in which the karyotypes are enriched by metacentric to submetacentric chromosomes. Imai (1969) considers that this alteration in relation to the general tendency found in ants appears to be brought about by the partial or whole duplications of the chromosome set. Other authors (Hauschteck-Junken & Junken 1976) consider that it is hardly possible to identify individual chromosomes except the first ones, which are larger. These authors analyzed 14 species of *Formica* and in all of them the first chromosome is well defined, as happens with the species analyzed in the present paper, especially for the largest one in *Formica subrufa*.

The genus *Formica* was subdivided by Wheeler (1913) into several subgenera: *Coptoformica*, *Serviformica*, *Raptiformica* and *Formica* sensu stricto. A new subgenus (*Iberoformica*) was defined by one of us (Tinaut 1990) with a unique species, *Formica subrufa*, an endemic Iberian species. Until now the chromosome numbers from 24 species have been reported, the majority of them belonging to the *Serviformica* and *Formica* subgenera (9 and 10 species respectively).

The accumulated data show that within the genus *Formica* the chromosome number appears to be very uniform with n=26-27, 2n=52-54 (revision in Hauschteck-Jungen & Jungen 1976, Rosengren *et al.* 1980, Lorite *et al.* 1998a). In spite of this, a non-homogeneous distribution of chromosome numbers between the different subgenera has been reported. The analyzed species belonging to the subgenus *Serviformica* present n=27 (2n=54) with the exception of *F. transcaucasica* with n=26 (2n=52) (Rosengren *et al.* 1980). In the subgenus *Formica* all analyzed species present n=26 (2n=52). There are few data for the subgenera *Coptoformica* and *Raptiformica* (3 and 2 species respectively). In *Coptoformica* and in *Raptiformica* all analyzed species have n=26.

The data presented in this paper are in concordance with this unequal distribution. So *F. gerardi* has n=27 as other *Serviformica* ants and *F. frontalis* n=26 as all analyzed species belonging to the subgenus *Formica*.

F. subrufa had been included in the subgenus *Serviformica* until the definition of the subgenus *Iberoformica* (Tinaut 1990). In *Serviformica* n=27 seems to be the typical chromosome number, although *F. subrufa* presents n=26. This data, although not definitive, in addition to the morphological particularities of *F. subrufa* (Tinaut 1990) could justify the maintenance of the subgenus *Iberoformica* for this species.

The low variation in relation to the chromosome number in this genus could indicate that species differentiation in this genus is not conducted by changes in chromosome number, as was suggested by Hauschteck-Jungen & Jungen (1976). So it seems to be clear that new and different genetic data are necessary to clarify the difficult classification in this genus.

Lasius brunneus

The majority of studied males present a haploid chromosome number of n=15 with a karyotype formula of n=1M+3ST+11ST (Fig. 3a). Some of the analyzed males showed a variable chromosome number. These males showed cells with n=15 and n=16 (Fig. 3b). We suggest that the observed variation on chromosome number could be due to the presence of B-chromosomes, as it has been reported in other species of the same genus. In *Lasius niger* intra- and interindividual numerical variation of the numbers by the presence of B-chromosomes have been reported (Palomeque et al. 1990). In *Lasius brunneus*, due to the small size of the chromosomes, we were not able to determine which of them is the B-chromosome. The presence of B-chromosomes in ants was reported for the first time in *Leptothorax spinosior* (Imai 1974). Since then B-chromosomes have been detected in at least ten species (Crozier 1975, Imai *et al.* 1977, 1985, Goñi *et al.* 1982, Taber & Cokendolpher 1988, Palomeque *et al.* 1990, Lorite *et al.* 1998a).

As in the genus *Formica*, the chromosome numbers in *Lasius* are very uniform. The nine analyzed species present chromosome numbers of

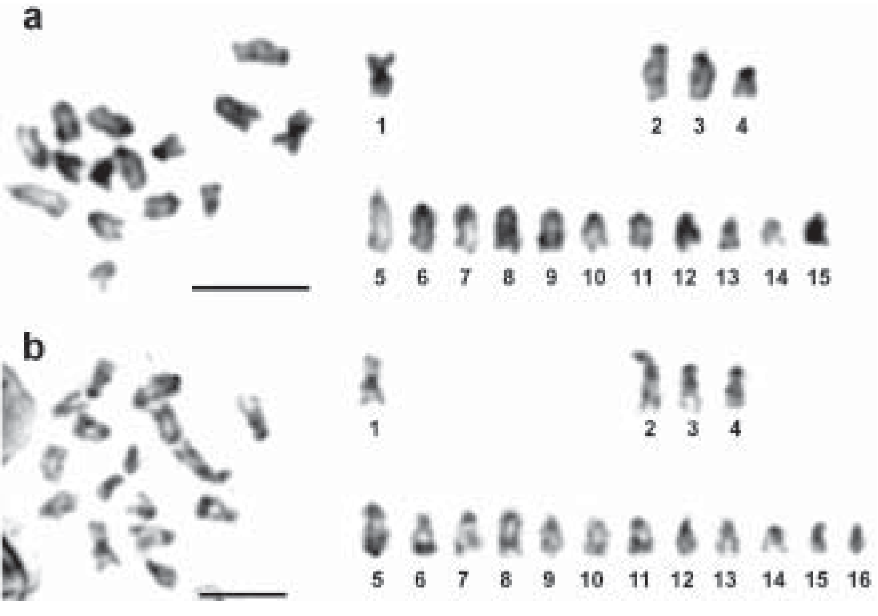

Fig. 3. (a) Metaphase plate and standard haploid karyotype (n=15) from *Lasius brunneus*. (b) Haploid metaphase plate with one B-chromosome (n=16). Bar=5µm.

n=14-15. Hauschteck-Jungen & Jungen (1983) with the data available at that time, observed that in the case of n=14 chromosomes one large metacentric chromosome was found which is absent in all n=15 *Lasius* karyotypes. Since then, only two new species have been analyzed; *L. sakagamii*, n=15 (Yamauchi *et al.* 2001) and *L. brunneus*, n=15 (this paper). Both karyotypes lack the large metacentric chromosome and present only a medium-sized one. The karyotypes present in different species from the genus *Lasius* could be easily related by Robertsonian processes of centric fusion or fission involving the large metacentric chromosome and two small subtelocentric or telocentric ones.

Subfamily Myrmicinae

Messor barbarus

The haploid chromosome number in this species is n=21. As in the majority of ant species with high chromosome numbers, the chromosome size is too small to determine exactly their morphology (Fig. 4a). The haploid karyotype presents at least nine metacentric to submetacentric chromosomes. The remaining ones seem to be subtelocentric. As in *Camponotus cruentatus* a different chromosome number was previously reported, n=22 (Hauschteck-Jungen, in Crozier's 1975 revision). This is the only reference to this species. No photography or karyotype description is available, so it is impossible to carry out a comparative analysis.

The karyological information in this genus is very limited since only two species have been previously analyzed, *M. aciculatum* (Imai 1966, 1969) and an Indian non-determined species, *Messor* sp. (Imai *et al.* 1984), with chromosome numbers of 2n=44 and 2n=41 respectively. The karyotype of *M. barbarus* is comparable with the latter. In this species the chromosome 1 is heteromorphic due to Robertsonian polymorphism (M/2T). If the heteromorphic pair has been generated by centric fusion of the two telocentric chromosomes, two medium-sized chromosomes (one SM and the other ST) and 19 small-sized ones formed the karyotype. In *M. barbarus* the karyotype presents two medium-sized chromosomes (also SM and ST, formally denominated by us as 1 and 10) and 19 small-sized ones.

Monomorium subopacum

This species presents a diploid number of 2n=34. The diploid karyotype is formed by nine pairs of M to SM chromosomes and 8 pairs of ST-T ones (Fig. 4b). In Crozier's revision (1975) the haploid chromosome of n=17 is reported in this species. We confirm this chromosome number in females and provide for the first time metaphase plate photography and karyotype description of this species.

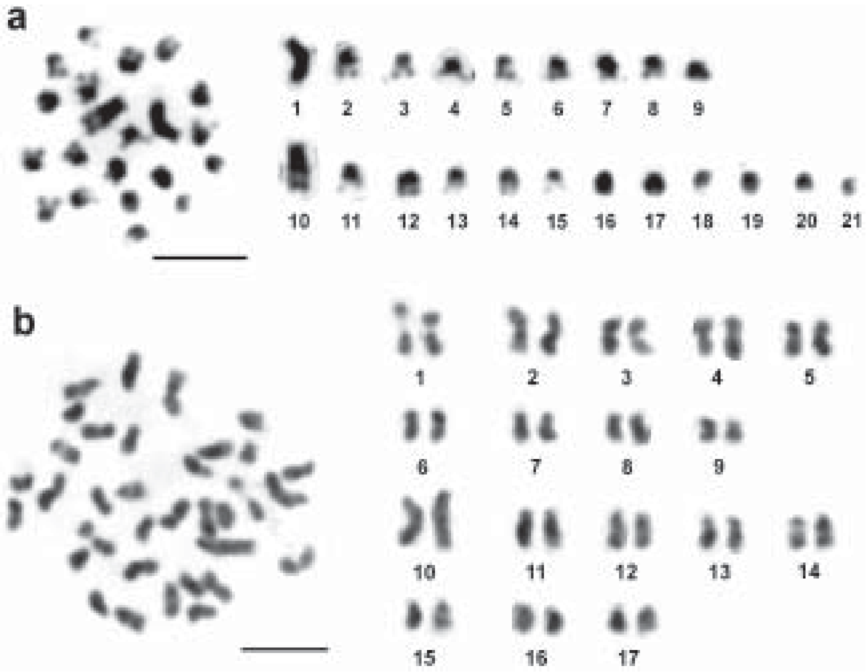

Fig. 4. (a) Haploide metaphase plate and karyotype from *Messor barbarus*. (b) Diploid metaphase plate and karyotype *from Monomorium subopacum*. Bar=5µm.

Monomorium is one of the most variable genera in relation to its chromosome number, 2n=16 to 70 (Imai *et al*. 1984). So in this genus very variable karyotype organization could be present. However, the only analyzed species from this genus that presents the same chromosome number as *M. subopacum* also presents a similar karyotype. This Indian species (*Monomorium* sp.5) was also analyzed by Imai *et al.* (1984). Its karyotype formula is n=10 M-SM + 7 ST-T, similar to that of *M. supopacum* (n=9 M-SM + 8 ST-T) and could be easily related by Robertsonian processes.

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Table 1. Analyzed species, chromosome numbers and population location.

Species	**n (2n)**	**Population**
Subfamily Formicinae		
Camponotus cruentatus	20	Sierra de Cazorla (Jaén)
Formica frontalis	(52)	Sierra Nevada (Granada)
Formica gerardi	27	Rio Frío (Jaén)
Formica subrufa	26	Canena (Jaén)
Lasius brunneus	15	Fuente del Rey (Jaén)
Subfamily Myrmicinae		
Messor barbarus	21	Pegalajar (Jaén)
Monomorium subopacum	(34)	Cabo de Gata (Almería)