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Supernumerary chromosome segments in different genera of Formicidae

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Abstract

Supernumerary chromosome segments have been detected in natural populations of *Tapinoma nigerrimum, Aphaenogaster senilis, Aphaenogaster iberica, Plagiolepis schmitzii,* and *Plagiolepis pygmaea.* Chromosomal location, frequencies, response to C-banding techniques, and other aspects of extra segments are studied. Previous hypotheses about the chromosome differentiation processes between related species of the genus *Tapinoma* are examined using this new cytogenetic information. The possible significance of the supernumerary chromosome segments in the process of chromosomal evolution in ants is discussed.

Introduction

One form of chromosomal polymorphism commonly identified in several groups of animals and plants is the presence of supernumerary chromosome segments, attached to one or more chromosomes of the standard complement.

The majority of observed supernumerary segments in both plant and animal species are heterochromatic, although some euchromatic segments have been described also (Camacho *et al.*, 1984; Ruíz-Rejón, Lozano & Ruíz-Rejón, 1989). In grasshoppers, the supernumerary segments may show four types of response to C-banding: those darkly C-banding, those partly C-banding, those lightly C-banding and those not C-banding (Camacho *et al.*, 1984; Garcia de la Vega *et al.*, 1986).

The role that supernumerary segments play in natural populations is still unknown, although the effects they produce in individuals may be very informative in this regard. Extensive literature has been accumulated on supernumerary segments, in which their size and location, meiotic behaviour, response to different banding techniques and mode of inheritance have been studied (John, 1973; Hewitt, 1979; John & King, 1982; Navas-Castillo, Cabrero & Camacho, 1986; Rufas *et al.*, 1988; Santos & Esteban, 1990; De la Torre *et al.*, 1990; López-León, Cabrero & Camacho, 1991; Camacho *et al.*, 1991; López-León, Cabrero & Camacho, 1992). The effects of nucleolar organiser regions (NORs) have also been studied. In grasshoppers, a significant increase in the activity of some NORs caused by the presence of a certain supernumerary segment has been described (Cabrero, Navas-Castillo & Camacho, 1986). A supernumerary segment with activity NOR has also been described in grasshoppers (Camacho, Navas-Castillo & Cabrero, 1986).

In ants, typical supernumerary chromosome segments have not been hitherto described. The aim of this paper is to present the results of a survey of the supernumerary chromosome segments of *Tapinoma nigerrimum, Aphaenogaster senilis, Aphaenogaster iberica, Plagiolepis schmitzii* and *Plagiolepis pygmaea*, and to determine their character-

istics, behaviour and frequencies in the populations analyzed and their possible evolutionary significance. Previous hypotheses (Palomeque *et al.*, 1988) about the chromosome differentiation processes between related species of the genus *Tapinoma* are revised using this new cytogenetic information.

Materials and methods

The material analyzed was collected in different localities in the south of the Iberian Peninsula. All material studied was identified by letters followed by a number. The letters refer to the place of collection (M, Motril, Granada; BSJ, Bosque de San Juan, Granada; CF, Castel de Ferro, Granada; AL, Almería; VQ, Vejar Quesada, Jaén; SC, Sierra de Cazorla, Jaén; MJ, Mojón Blanco, Mancha Real, Jaén; RF, Rio Frio, Jaén; LP, La Pandera, Los Villares, Jaén; LL, Las Lagunillas, Jaén) and the number indicates the year of sampling. Several colonies have been collected in each locality. An equal number of individuals has been analyzed in each colony.

The taxonomic identification of the studied species has been realized by Dr. A. Tinaut. This identification is based in Collingwood (1978) and in other taxonomic literature.

Chromosome preparations were made from cerebral ganglia of all castes and germ cells of early pupae, using the technique described by Meredit (1969). Subsequently, cytological analysis was performed using the C-banding technique of Sumner (1972). The silver impregnation procedure was essentially the same as described by Howell and Black (1980). In certain chromosome preparations, we used the technique described by Rufas *et al.* (1982). Like Fernández-Piqueras *et al.* (1983), using the same criteria, a distinction was made between 'primary' nucleolar organizing regions, which are active in all prophase plates of all individuals of a given taxon, and 'secondary' NORs, which are active only occasionally.

In some species, specifically in *Tapinoma nigerrimum* and *Aphaenogaster senilis*, we have studied the frequencies of the supernumerary segment carrier individuals during two consecutive years. To apply the Hardy-Weinberg law, we made use of the methods usually employed in the study of equilibrium in sex-linked genes. In this study, the standard chromosomes and the chromosomes that carry supernumerary segments are identified by the letters H and B respectively. In *T. nigerrimum*, the frequency of segment carrier chromosomes has been determined using the method usually employed when the observed number of females and males is different. The studied males and queens have been collected in different colonies. In *A. senilis* we have studied the frequencies of segment carrier males during the years 1988 and 1989. The frequencies of the different karyomorphs existing in queens have been determined in accordance with the Hardy-Weinberg assumptions. In both species the same number of individuals has been analyzed in each colony.

Results

Tapinoma nigerrimum

The chromosome number of *Tapinoma nigerrimum* is n = 9 in males and 2n = 18 in females and workers. The standard diploid karyotype formula is 2n = 10m + 4sm + 4st (Palomeque *et al.*, 1988).

A supernumerary chromosome segment on chromosome 8 is identified in certain populations. It was found that 48% of the studied males carried a supernumerary segment (Table 1). This segment is situated on the terminal region of the short arm (Fig. 1a and 1b). The presence or absence of a segment makes chromosome 8 either submetacentric (sm) or subtelocentric (st).

We have observed the presence of a supernumerary segment in somatic cells of males (Fig. 1a) and testes cells (Fig. 1b). Similarly, supernumerary segment carrier queens and workers were observed. Both diploid castes are homomorphic (BB) or heteromorphic (BH) for the chromosomes of pair no. 8 (Fig. 1c and 1e) in the segment carrier queens and workers.

C-banding analysis showed the presence of paracentromeric constitutive heterochromatin in all chromosomes. Chromosomes 6, 7, 8 and 9 also carry heterochromatin in part or almost all of their short arms (Palomeque *et al.*, 1988).

C-banding analysis carried out in segment carrier individuals showed a darkly C-banded segment (Fig. 1d).

In *T. nigerrimum* there is primary or secondary

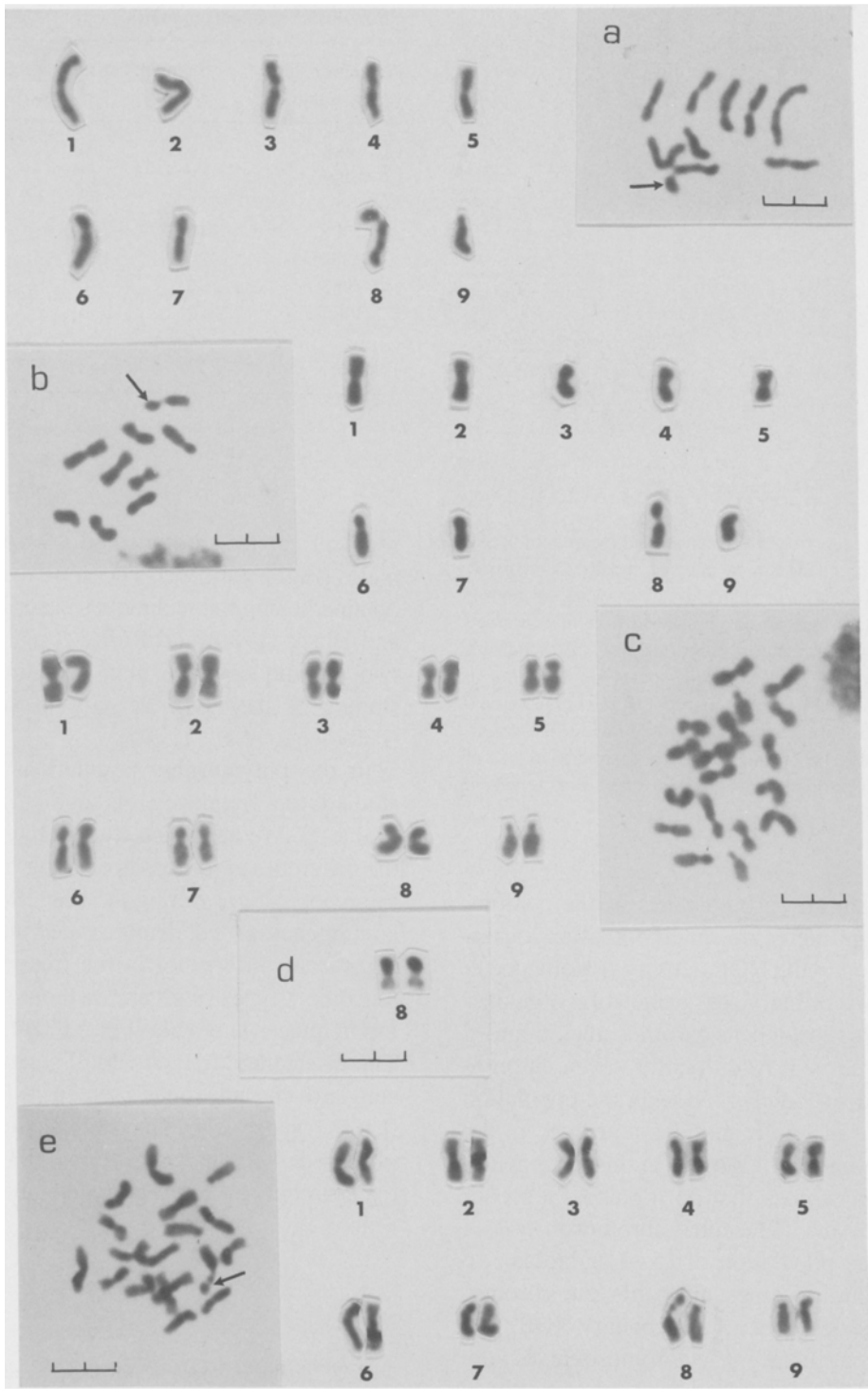

Fig. 1. Tapinoma nigerrimum. (a and b). Haploid karyotypes and metaphase plates showing a supernumerary chromosome segment from somatic cells of males and from testes cells respectively. (c and e). Diploid karyotypes and metaphase plates from cerebral ganglia cells of females, homomorphic (BB) and heteromorphic (BH) respectively for the chromosome of pair no. 8 (d). Selected chromosomes 8 showing a supernumerary chromosome segment darkly C-banded. Arrows point to the supernumerary chromosome segment on chromosome 8. Bars represent 5 μm.

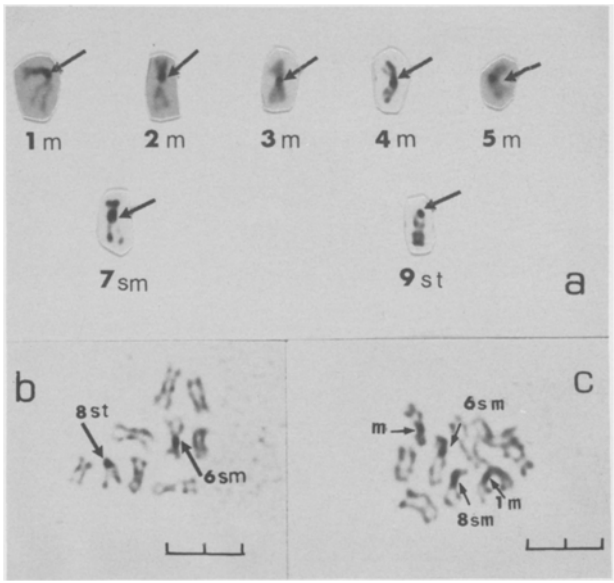

Fig. 2. Tapinoma nigerrimum. Chromosomal location of active NOR by silver impregnation in the male germ cells. Comparison between standard males and segment carrier males. (a and b) Standard males. (a) Selected chromosomes from silver stained metaphases showing secondary NORs (arrows). (b) Metaphase plate showing a primary NOR in chromosomes 6 and 8 (arrows). (c) Metaphase plate showing a primary NOR in chromosome 6 and another in segment carrier chromosome 8 (arrows). A secondary NOR is present in chromosome 1 and another in one of the smallest metacentric chromosomes (arrows). Bars represent 5 μm.

NOR activity in all chromosomes of the complement, although there are interpopulation differences in relation to the NOR activity (Palomeque *et al.*, 1990a). Two primary active nucleolar organizer regions (NORs) located in chromosomes 6 and 8 are usually observed. Additionally, seven chromosomes showed a secondary NOR in the population of Motril, five in Las Lagunillas and three in the population of Sierra de Cazorla. In this last population, the chromosomes that do not show a secondary NOR were the metacentric chromosomes 2, 3, 4 and 5. In the population of Las Lagunillas two metacentric chromosomes, probably the chromosomes 2 and 3, do not show a secondary NOR. The size of these four metacentric chromosomes is very similar. It is difficult to identify them correctly in the chromosome preparation stained with silver.

The NOR staining techniques showed that the activity of NORs does not change when there is a supernumerary segment present (Fig. 2a, 2b and 2c). Silver staining showed the existence of a primary NOR in chromosome 6 and another in the segment carrier chromosome 8 as in the standard males (Fig. 2b and 2c). The same results have been obtained using the techniques described by Howell and Black (1980) and Rufas *et al.*, (1982). These two techniques were also used in the previous studies of the NOR activity in standard males (Palomeque *et al.*, 1990a).

Table 1. Tapinoma nigerrimum. Frequency of supernumerary chromosome segments in males.

Population and year of sampling	Segment carrier No.	%	Standard No.	%
LL-1988	9	50	9	50
LL-1989	15	45	18	55
M-1989	11	55	9	45
AL-1988	17	100	0	0
AL-1989	20	100	0	0
MJ-1988	0	0	19	100
SC-1989	0	0	23	100
TOTAL	72	48	78	52

In the polymorphic populations LL, we have studied the frequency of segment carrier males (Table 1). We have also studied the frequencies of the different karyomorphs existing in queens in the same populations during the year 1988 (Table 2). A contingency χ^2 test demonstrated that there are not significant differences in the frequencies of chromosome 8 types (with and without segment) existing in queens and males ($\chi^2 = 0.011$, P: 0.90-0.95). The determined frequencies of segment carrier and standard chromosomes were p = 0.462 and q = 0.538 respectively. The frequencies of the three observed karyomorphs (HH, HB and BB) in queens conform to a Hardy-Weinberg distribution (Table 2).

Table 2. Tapinoma nigerrimum. Frequencies of the different karyomorphs in queens. Application to the Hardy-Weinberg distribution.

Karyomorphs	HH	HB	BB	χ^2	P
Observed	8	17	5	0.30	0.50 - 0.70
Expected	8.7	14.9	6.4		not. sign.

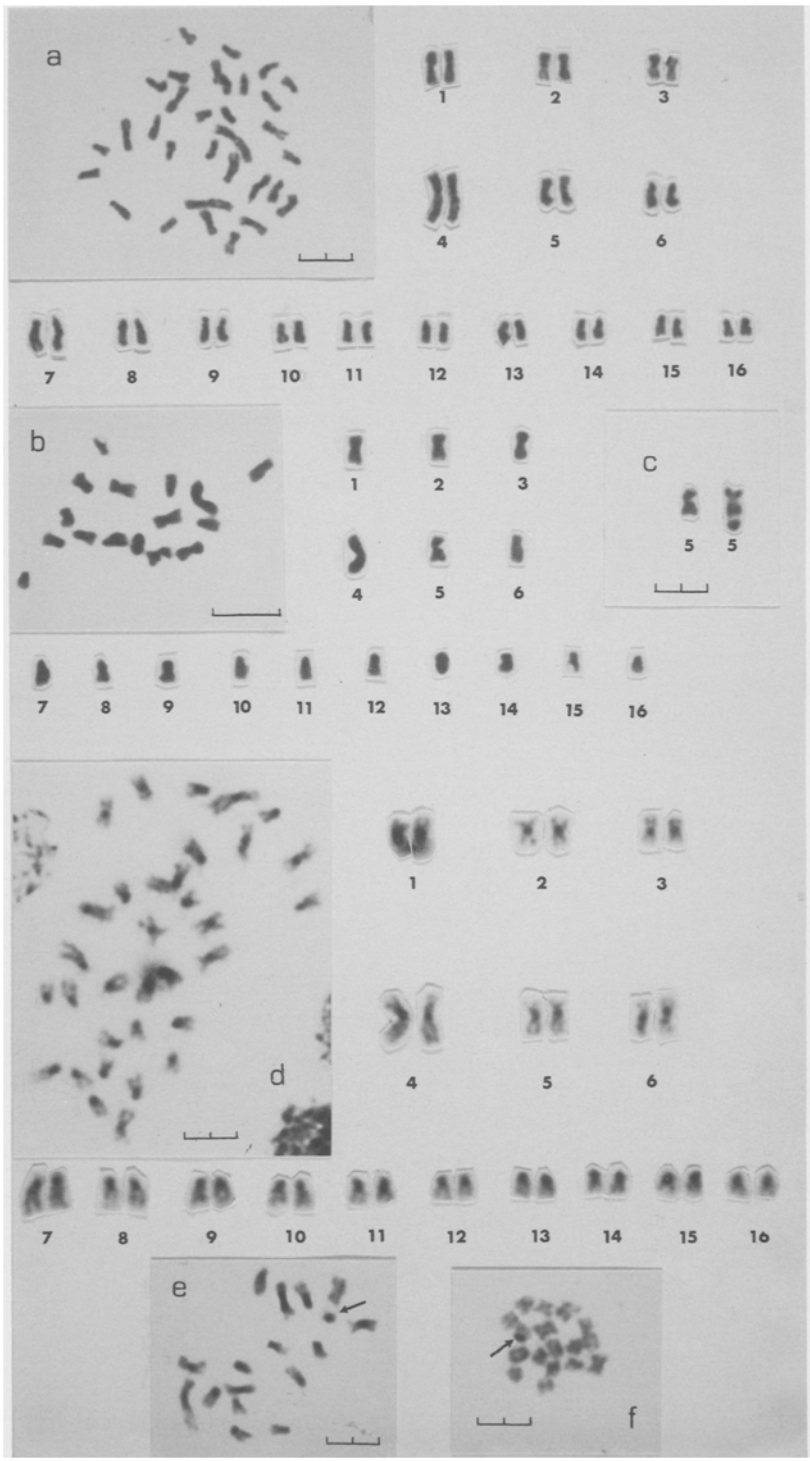

Fig. 3. Aphaenogaster senilis. (a) Standard diploid karyotype and metaphase plate from ganglia cells of worker prepupae. (b) Standard haploid karyotype and metaphase plate (c) Chromosomes 5 selected from metaphase plate of standard male and segment carrier male respectively (d) C-band karyotype and metaphase plate (e) C-band metaphase plate showing a supernumerary chromosome segment only partly C-banded (arrow) (f) Chromosomal location of active NOR by silver impregnation (arrow). Bars represent 5 μm.

Table 3. Aphaenogaster senilis. Frequency of supernumerary chromosome segments in males.

Population and year of sampling	Segment carrier No.	%	Standard No.	%
RF-1988	15	56	12	44
RF-1989	26	59	18	41
LP-1989	15	75	5	25
TOTAL	56	62	35	38

Aphaenogaster senilis

The diploid chromosome number is 2n = 32 in females and workers and n = 16 in males (Fig. 3a and 3b). The corresponding karyotype formula is 2n = 6m + 6sm + 20st (Fig. 3a).

The germ cells of 91 males were examined, and 62% of studied males carried a supernumerary segment (Table 3). The supernumerary segment is situated on chromosome 5, in the terminal region of the long arm (Fig. 3c).

We observed paracentromeric C-bands in all chromosomes. Chromosome 6 carried a second interstitial C-band in the long arm. In addition, all subtelocentric chromosomes carry heterochromatin in part or almost all of the short arms (Fig. 3d).

C-banding analysis carried out in segment carrier individuals showed a segment that was only partly C-banded (Fig. 3e).

The silver staining carried out in standard males showed the existence of a primary NOR in one of the smallest subtelocentric chromosomes, probably in chromosome 12, near its centromeric region (Fig. 3f).

We have studied the frequencies of segment carrier males in the years 1988 and 1989. The observed frequencies of segment carrier and standard chromosomes in 1988 were p = 0.56 and q = 0.44 respectively. The chromosome frequencies in males and females have to be the same in a genetic equilibrium. In accordance with the Hardy-Weinberg assumptions, the frequencies of the different karyomorphs in queens will be 0.31 (BB), 0.49 (BH) and 0.19 (HH). In the next year, the frequencies of the two observed karyomorphs in males, B and H, conform to a Hardy-Weinberg distribution (Table 4).

Aphaenogaster iberica

The chromosome number is 2n = 34 in females and workers and n = 17 in males. The corresponding karyotype formula is 2n = 6m + 4sm + 8st + 16t (Fig. 4a).

A supernumerary chromosome segment on chromosome 4 is identified in certain populations;

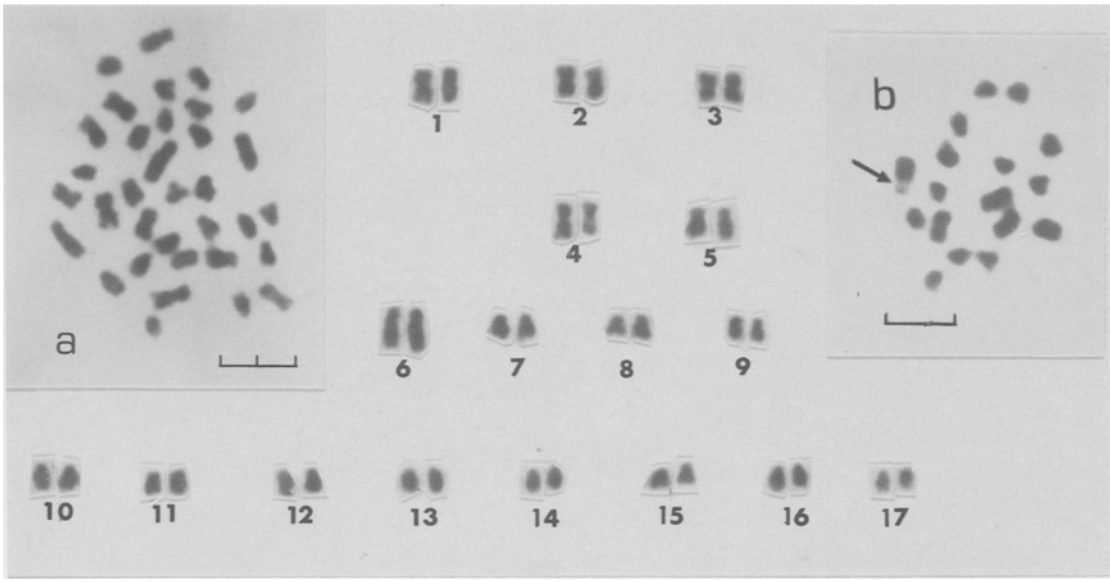

Fig. 4. Aphaenogaster iberica. (a) Standard diploid karyotype and metaphase plate from cerebral ganglia cells of worker male pupae showing a supernumerary chromosome segment with a differential stain (arrow). Bars represent 5 μm.

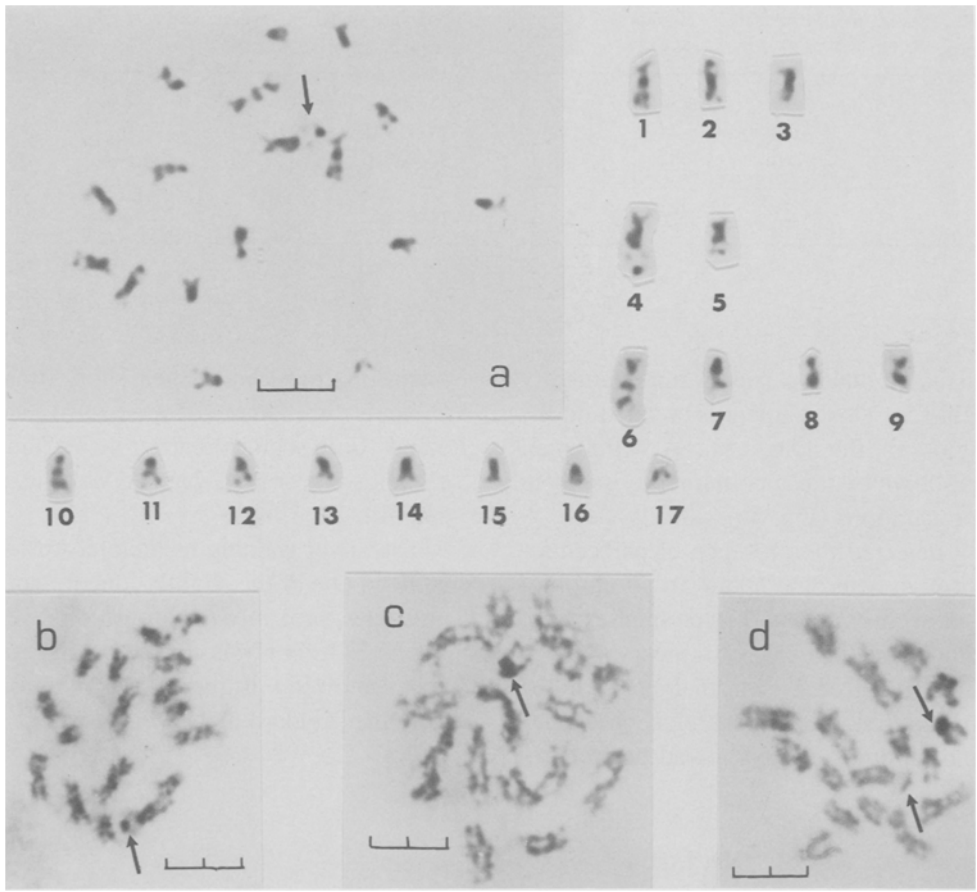

Fig. 5. Aphaenogaster iberica. (a and b) Haploid C-band karyotype and metaphase plates. Chromosome 4 showing a supernumerary chromosome segment only partly C-banded (arrow). (c and d) Chromosomal location of active NOR by silver impregnation (arrows): (c) Standard males. (d) Segment carrier males. Bars represent 5 μm.

Table 4. Aphaenogaster senilis. Frequencies of the different karyomorphs in males. Application to Hardy-Weinberg distribution.

Karyomorphs	H	B	χ^2	P
Observed	26	18	0.18	0.50 - 0.70
Expected	24.6	19.4		not. sign.

Table 5. Aphaenogaster iberica. Frequency of supernumerary chromosome segments in males.

Population and year of sampling	Segment carrier No.	%	Standard No.	%
SC-1989	10	45	12	55
BSJ-1989	0	0	12	100
AL-1989	12	38	20	59
M-1989	7	41	10	59
TOTAL	29	35	54	65

Table 6. Plagiolepis schmitzii. Frequency of supernumerary chromosome segments in males.

Population and year of sampling	Segment carrier No.	%	Standard No.	%
ALM-1990	16	32	34	68
SR-1990	0	0	41	100
RF-1991	0	0	51	100
TOTAL	16	11	126	89

Table 7. Plagiolepis pygmaea. Frequency of supernumerary chromosome segments in males.

Population and year of sampling	Segment carrier No.	%	Standard No.	%
CF-1991	22	34	42	66
VQ-1991	0	0	48	100
SC-1991	0	0	39	100
TOTAL	22	15	129	85

35% of the studied males carried a supernumerary segment (Table 5). This segment is situated on the terminal region of the long arm. A differential staining was shown by the supernumerary segment in Giemsa preparations (Fig. 4b).

C-banding detected the presence of paracentromeric constitutive heterochromatin in all chromosomes, except in chromosome 17, the smallest telocentric chromosome (Fig. 5a). Chromosome 2 carried a second terminal heterochromatic band in the short arm. In addition, the four subtelocentric chromosomes and chromosome 5 showed heterochromatin in almost all of their short arms. Interstitial C-bands are also observed in chromosomes 6 and 10. In segment carrier males, the segment on chromosome 4 is C-banded only in part (Fig.5a and 5b).

The silver staining technique showed a primary NOR in one of the slightly longer telocentric chromosomes, probably in chromosome 12 (Fig. 5c).

The NOR analysis carried out in segment carrier males showed a primary NOR in chromosome 12, as in the standard males (Fig. 5d).

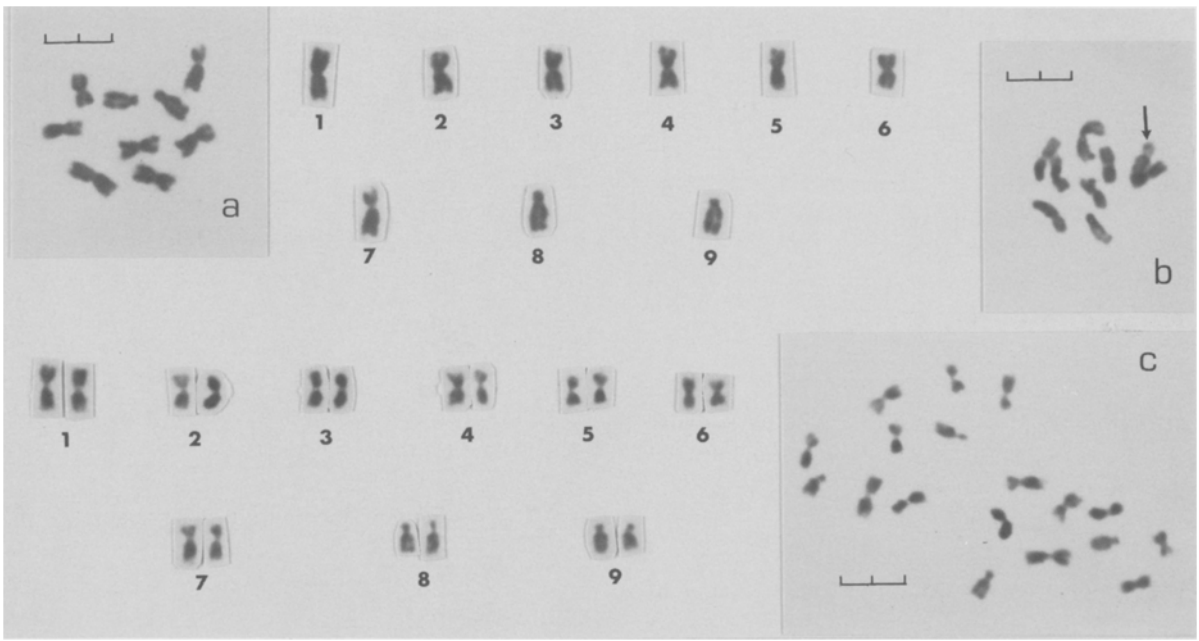

Fig. 6. Plagiolepis schmitzii. (a) Standard haploid karyotype and metaphase plate from germ cells of early male pupae. (b) Metaphase plate showing a supernumerary chromosome segment with a differential stain (arrow) (c) Standard diploid karyotype and metaphase plate. Bars represent 5 μm.

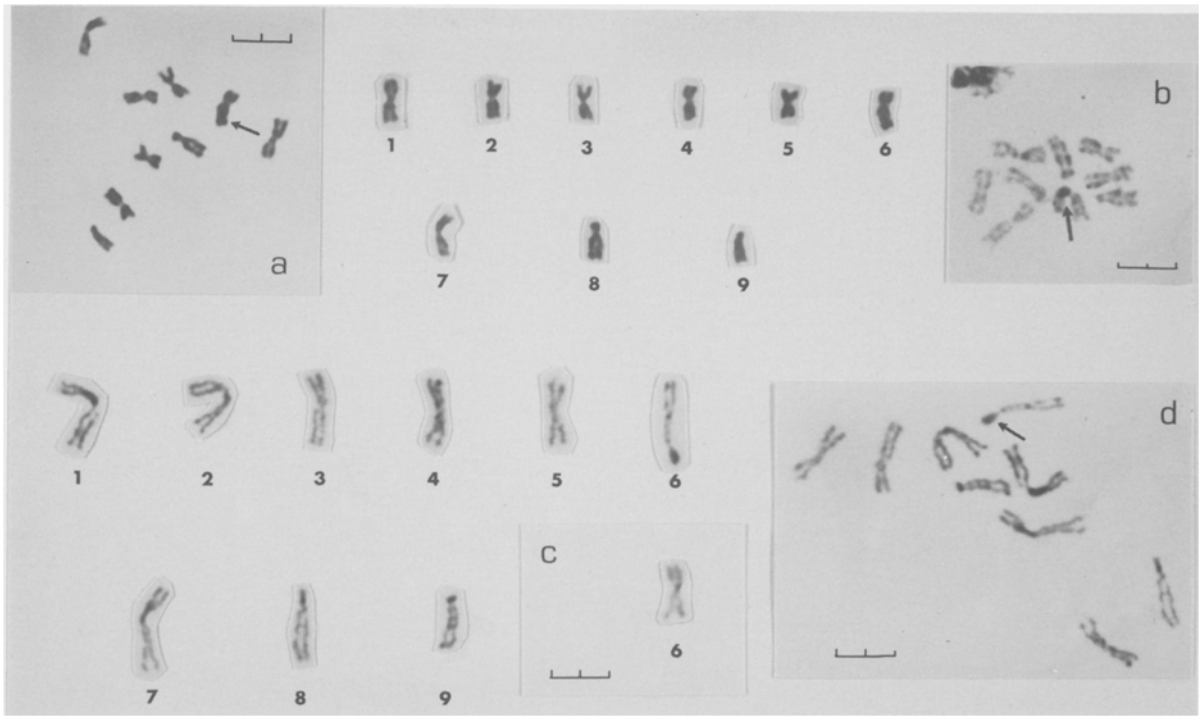

Fig. 7. Plagiolepis schmitzii. (a) Haploid karyotype and metaphase plate showing a supernumerary chromosome segment on chromosome 6 (arrow). (b) Chromosomal location of Ag-NOR (arrow). (c) Selected C-banded chromosome 6 without supernumerary segment. (d) C-band karyotype and metaphase plate showing a supernumerary segment darkly C-banded (arrow). Bars represent 5 μm.

Plagiolepis schmitzii

The chromosome number is n = 9 in males and 2n = 18 in females and workers (Fig. 6a and 6c). The diploid karyotype formula is 2n = 12m + 2sm + 2st + 2t (Fig. 6c).

A supernumerary chromosome segment has only been observed in one of the studied populations (Table 6); 32% of the analyzed males of this population carried a supernumerary segment.

The segment is located on chromosome 6. The absence or presence of a segment makes the chromosome 6 either metacentric (m) or submetacentric (sm) (Fig. 6a and Fig. 7a). A differential stain was shown by the supernumerary segment in Giemsa preparations (Fig. 6b).

C-banding analysis showed the presence of paracentromeric constitutive heterochromatin in chromosomes 1, 2, 6, 7, 8 and 9. In addition, chromosome 2 carried a second interstitial heterochromatic band. The remaining chromosomes did not show paracentromeric C-bands. C-banding carried out in segment carrier individuals showed a darkly C-banded segment (Fig. 7c and 7d).

In standard males we observed a primary NOR in chromosome 1, adjacent to the centromeric regions (Fig. 7b).

Plagiolepis pygmaea

The chromosome number is n = 9 in males and 2n = 18 in females and workers. The haploid karyotype formula is n = 7m + 1sm + 1st (Fig. 8a).

A supernumerary chromosome segment has only been observed in one of the studied populations (Table 7); 34% of the analyzed males of this population carried a supernumerary chromosome segment.

The segment is located on chromosome 6. The absence or presence of a segment makes chromosome 6 either metacentric (m) or submetacentric (sm) (Fig. 8a and 8b).

C-banding detected the presence of paracentromeric constitutive heterochromatin in all chromosomes, except in chromosome 3 and 5. Chromosome 1 also carried two interstitial heterochromatic

bands and chromosomes 3 and 5 one interstitial band. Extraordinarily marked pericentromeric heterochromatin blocks are found in chromosome 4. In addition, chromosome 9 carried a telomeric C-band (Fig. 8c).

In segment carrier males, the segment on chromosome 6 is darkly C-banded (Fig. 8d).

The silver staining technique carried out in standard males showed a primary NOR in chromosome 1 and a secondary NOR in chromosome 4 (Fig. 8b).

Discussion

Previous cytogenetic studies in species of the genera *Tapinoma*, *Aphaenogaster* and *Plagiolepis* have been carried out by Crozier (1975), Hauschteck-Jungen and Jungen (1983), Imai *et al.*. (1984), and Imai *et al.* (1988).

Robertsonian rearrangement, pericentric inversion and translocation are considered the most important modes of spontaneous chromosomal muta-

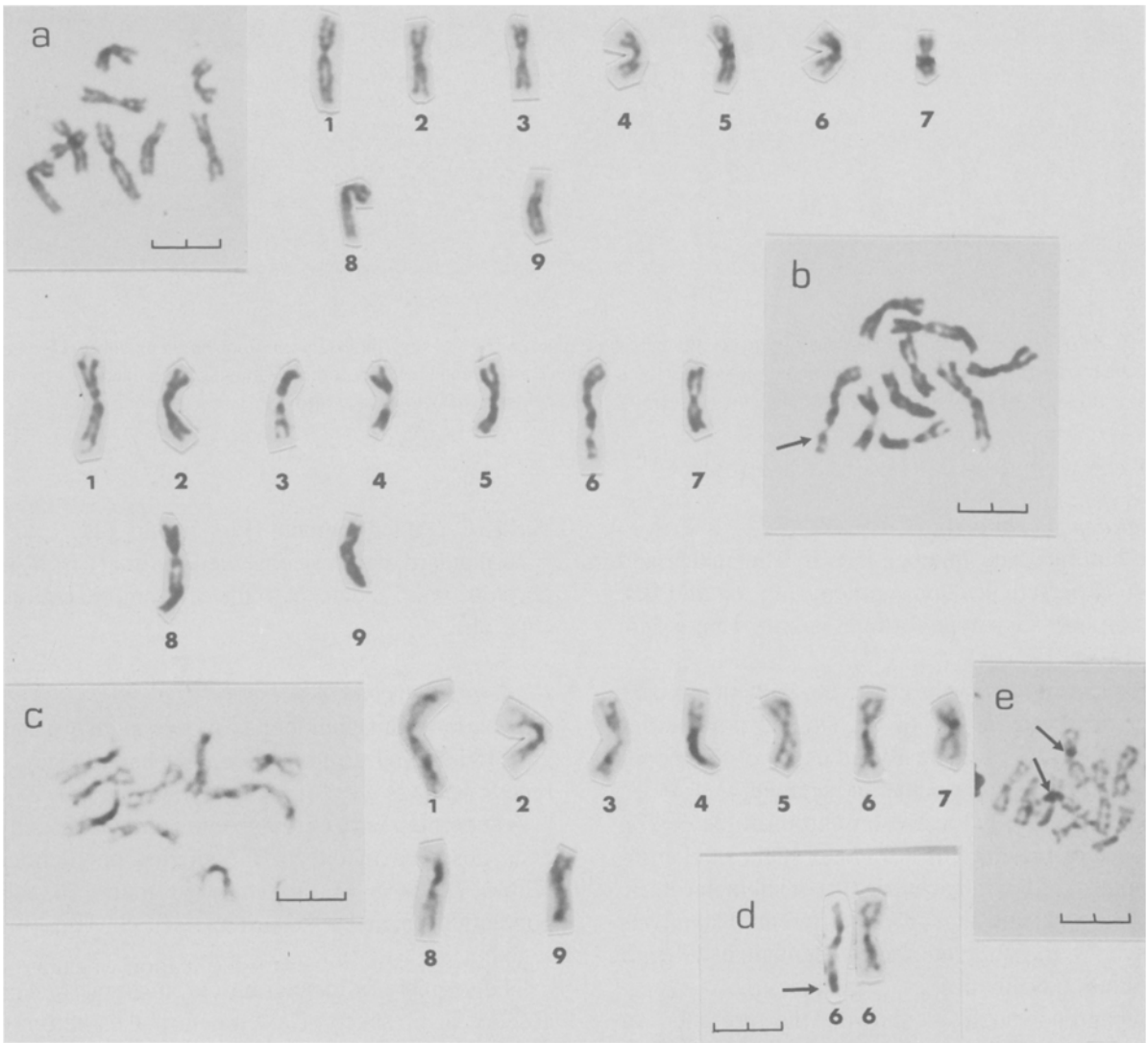

Fig. 8. Plagiolepis pygmaea. (a) Standard haploid karyotype and metaphase plate from germ cells of early male pupae. (b) Haploid karyotype and metaphase plate showing a supernumerary chromosome segment on chromosome 6 (arrow) (c) Standard C-band karyotype and metaphase plate. (d) Selected C-banded chromosomes 6 with (arrow) and without a supernumerary chromosome segment. (e) Chromosomal location of Ag-NOR (arrows). Bars represent 5 μm.

tion in ants (Crozier, 1975; Imai *et al.*, 1988). The same authors considered that the translocations and Robertsonian polymorphisms are non-randomly distributed; the former are found at high frequencies in species with low chromosome numbers (n < 12), while the latter predominate in those with high numbers (n > 12). The recognition of this dichotomy in relation to the chromosome number is essential for the analysis of karyotype evolution in ants (Imai *et al.*, 1988).

We have studied eleven different species of ant in previous papers (Palomeque *et al.*, 1987, 1988, 1990, 1990a; Palomeque, Chica & Díaz de la Guardia, 1990b) and here. We have found five cases of chromosomal polymorphisms by supernumerary segments, a type of polymorphism not hitherto described in ants. This polymorphism is found in species with low (*Tapinoma nigerrimum, Plagiolepis schmitzii* and *Plagiolepis pygmaea* with n = 9) and high chromosome numbers (n = 16 in *Aphaenogaster senilis* and n = 17 in *Aphaenogaster iberica*).

We suggest that the supernumerary chromosome segments are probably one frequent form of chromosomal variation in ants. This hypothesis is supported by the high proportion of species with supernumerary segments found in our study. Moreover the supernumerary segments are found in species not taxonomically related. The genera *Tapinoma, Aphaenogaster*, and *Plagiolepis*, are included in three different subfamilies (Dolichoderinae, Myrmicinae and Formicinae respectively). This fact is also in accordance with our hypothesis.

The existence of supernumerary chromosome segments in *Tapinoma nigerrimum*, a species of which we have made an in depth study here and in other papers (Palomeque *et al.*, 1988, 1990, 1990a), is in accordance with the hypothesis about the chromosome differentiation processes between related species of the genus *Tapinoma*, as explained below. Probably other species of ants also possess supernumerary chromosome segments, like grasshoppers, although it has yet not been studied.

For all these reasons the study of the characteristics and behaviour of the supernumerary chromosome segments found in ants and their comparison with such segments in other animal species seems to be a cytogenetically interesting problem.

The majority of the supernumerary segments studied are located on the smallest members of the chromosome set or the short arms of the larger members. Generally, the segments are terminally located; those situated interstitially are rare (Camacho *et al.*, 1984; Navas-Castillo, Cabrero & Camacho, 1986; García de la Vega, Suja & Rufas, 1989 among others). In *Tapinoma nigerrimum* the segment is located on the short arms of the small chromosome; in *Plagiolepis pygmaea* it is located on the small metacentric chromosome. However, the segment in *Aphaenogaster senilis* and *Aphaenogaster iberica* is situated on the long arm of a medium sized chromosome.

The segments observed showed two different types of response to C-banding. The segments of *T. nigerrimum, P. schmitzii* and *P. pygmaea* consist of uniformly darkly C-banded material. However, the segment of *A. senilis* and *A. iberica* is only partly C-banded. Similar results have also been described in other insects. The different reactions to C-banding and other techniques shown by the supernumerary segments have been interpreted as a consequence of their complex and heterogeneous nature (Sentis, Santos & Fernandez-Piqueras, 1986; John, Appels & Contreras, 1986; Gosalvez *et al.*, 1987; Camacho *et al.*, 1991).

The origin of the supernumerary chromosome segments is still unknown and controversial. The extra segments described in this paper could have arisen by repetition of chromosome material, most probably by a duplication process, especially the segment of *T. nigerrimum*. This extra segment may have derived from the duplication of heterochromatic material, since the standard chromosome 8 shows a telomeric C-band (Palomeque *et al.*, 1988). The origin of certain extra segments by massive tandem duplications of some distal chromosome regions has often been assumed in the literature. Such duplication processes are supported by some molecular studies (John, Appels & Contreras, 1986; Rufas *et al.*, 1988). In the remaining species the segment may have also originated by duplication, but in this case from duplication of euchromatic material, since the standard chromosomes lack telomeric C-bands.

Ants are haplodiploids. Consequently only two chromosome forms, with or without a supernumerary segment, are found in haploid males. For this reason, the study of the conditions for equilibrium is carried out by extrapolation from the theory of sex-linked genes. In the studied populations of

Tapinoma nigerrimum and *Aphaenogaster senilis* the observed frequencies of the different karyomorphs did not differ significantly from those expected under Hardy-Weinberg equilibrium conditions. Similar results have been obtained in other insects, especially in the majority of studied species of Orthoptera (Hewitt, 1979; Gosalvez & López-Fernandez, 1981 among others).

Segregation distortion for supernumerary segments has also been described in some species of Orthoptera. In *Eyprepocnemis plorans* a certain supernumerary segment is partly eliminated by the females possessing B chromosomes. However the segment is not eliminated by the males, with or without B chromosomes, nor by the females without B chromosomes. The frequency of the segment remains stable and in accordance with the Hardy-Weinberg law. Several explanations have been suggested by the authors to explain the stability of this segment (López-León, 1991; López-León *et al.*, 1991). Gametic selection and meiotic drive for a supernumerary segment have also been described in *Chorthippus jacobsi* (López-León, Cabrero & Camacho, 1992).

The new cytogenetic data presented in this paper can be used in the analysis of the chromosome differentiation processes between related species of the genus *Tapinoma*. In a previous paper (Palomeque *et al.*, 1988), two possible hypotheses were suggested to explain the karyotype differences between *T. nigerrimum* and *T. erraticum*. The karyotype differences could be due to a Robertsonian exchange between chromosomes 6 and 8 of *T. nigerrimum*, forming chromosome 1 of *T. erraticum* with loss of a partly heterochromatic chromosome fragment. Also, the existing karyotype differences could be due to a fission process involving the same chromosomes. This process would be followed by a tandem growth of heterochromatin until it reached the size of the respective short arms of these chromosomes of *T. nigerrimum*.

The presence of an extra segment in *T. nigerrimum* affecting specifically chromosome 8 can be considered as lending new support to the hypothesis of karyotype changes by means of a fission process. This extra segment has probably originated by a duplication process. Therefore, the duplication of heterochromatic material (also postulated in the fission hypothesis by chromosome 8) seems to be a process which has repeatedly occurred in the karyotype evolution of this taxon, affecting the same chromosome. This pattern of evolution was in accordance with that termed karyotypic orthoselection (White, 1975, 1978). The segment carrier chromosome 8 shows a primary NOR, as does the standard chromosome 8. This fact is also in accordance with the hypothesis of karyotype changes by means of a fission process.

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