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Cytogenetic studies of ant *Linepithema humile* Shattuck (= *Iridomyrmex humilis* Mayr) in European populations

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SUMMARY — This paper is a cytogenetic study of the ant *Linepithema humile* Shattuck (= *Iridomyrmex humilis* Mayr) in Spanish populations. The karyotypes from Australian and Spanish populations of this species are compared. C-banding and silver staining is performed for the first time in *Linepithema humile*. The C-banding and Ag-staining pattern of this species is also compared with the corresponding patterns of other species of Formicidae. The possible processes of chromosomal evolution between related species of the genus are discussed according to the new cytogenetic data and to the different taxonomical classifications proposed about these species.

Key words: *Linepithema humile* Shattuck (= *Iridomyrmex humilis* Mayr), Formicidae, karyotypes, Ag-NORs, C-banding.

INTRODUCTION

The «Argentine ant» *Linepithema humile* Shattuck (= *Iridomyrmex humilis* Mayr) is native to Southern America although it is widely distributed throughout the world. This species is an important pest species in Northern America, Europe, Southern Africa, Hawaii and parts of Australia (SHATTUCK 1992*a*).

There is an extensive literature about the cytogenetics of the ants, but in the majority of cases, only the karyotypes obtained by means of standard staining with Giemsa have been analyzed. C-banding and Ag-staining have been performed only in some species (CROSLAND and CROZIER 1986; PALOMEQUE *et al.* 1987, 1988, 1990*a*, 1990*b*, 1990*c*, 1993*a*, 1993*b*; IMAI *et al.* 1988, 1992, 1994; IMAI and TAYLOR 1989; HIRAI *et al.* 1994; among others).

C-banding techniques permit us to determine and locate the constitutive heterochromatin and are extensively used for the study of karyotype changes of related species. rDNA on a given chromosome is located at a nucleolar organizer region (NOR) which consist of a cluster of tandemly repeated rDNAs. Some NORs can be visualized by the silver staining method and these NORs (Ag-NORs) are considered to be transcriptionally active clusters of rDNA (GOODPASTURE and BLOOM 1975). Trascriptionally active and inactive

populations of rDNAs coexist in the same cell, even though the biological meaning of this phenomenon and an explanatory molecular mechanism remain to be elucidated.

Other chromosome banding techniques have been recently applied to ants, like «in situ» hybridization (IMAI *et al.* 1992; HIRAI *et al.* 1994), staining with specific fluorochromes and G-banding (LORITE *et al.* 1996). Chromosome banding allowing accurate identification of all pairs of chromosomes and their linkage gene groups seems to be essential to more advanced genetic studies. It also allows a better comparison between different populations or different species.

The «Argentine ant» *Linepithema humile* has been previously studied by CROZIER (1968*a*, 1975) in Australian populations. In this paper European populations of this species were analyzed using Giemsa staining, C-banding and Ag-staining. C-banding and Ag-staining techniques have been applied for the first time. The possible processes of chromosomal evolution between related species of the genus are discussed using the new cytogenetic data and the different taxonomical classifications proposed about these species.

MATERIALS AND METHODS

The material analyzed was collected from various localities in the Southern part of the Iberian Peninsula. Haploid metaphases were obtained from testes of early pupae using the technique described by MEREDITH (1969) with some modifications. Ants pupae were dissected in distilled water and the testes were removed and put on a clean slide with distilled water for 45 min. The water was removed and the fixative solution (acetic acid: ethanol, 3:1) was added and incubated for 45 min. The material was macerated with a drop of 60% acetic acid. New fixative solution was added to the slides and dyed at 65° C. Diploid metaphases were obtained from cerebral ganglia of worker prepupae using the technique of IMAI *et al.* (1977). C-banding was performed according to the technique of SUMNER (1972). The silver impregnation procedure was essentially the same as described by HOWELL and BLACK (1980). The chromosome lengths were measured using an image analyzer (Videoplan Kontron).

RESULTS AND DISCUSSION

The chromosome number of *Linepithema humile* Shattuck (*Iridomyrmex humilis* Mayr) is $n = 8$ in males and $2n = 16$ in females (Figs. 1a, 1b). The karyotype formula of this species is 5M + 2SM + 1A according to the chromosomic measurement taken at several metaphases (Table 1). The data shown in Table 1 were the mean values of the values observed. These results coincide with the data given by other authors. CROZIER (1968*a*, 1975) reports the same

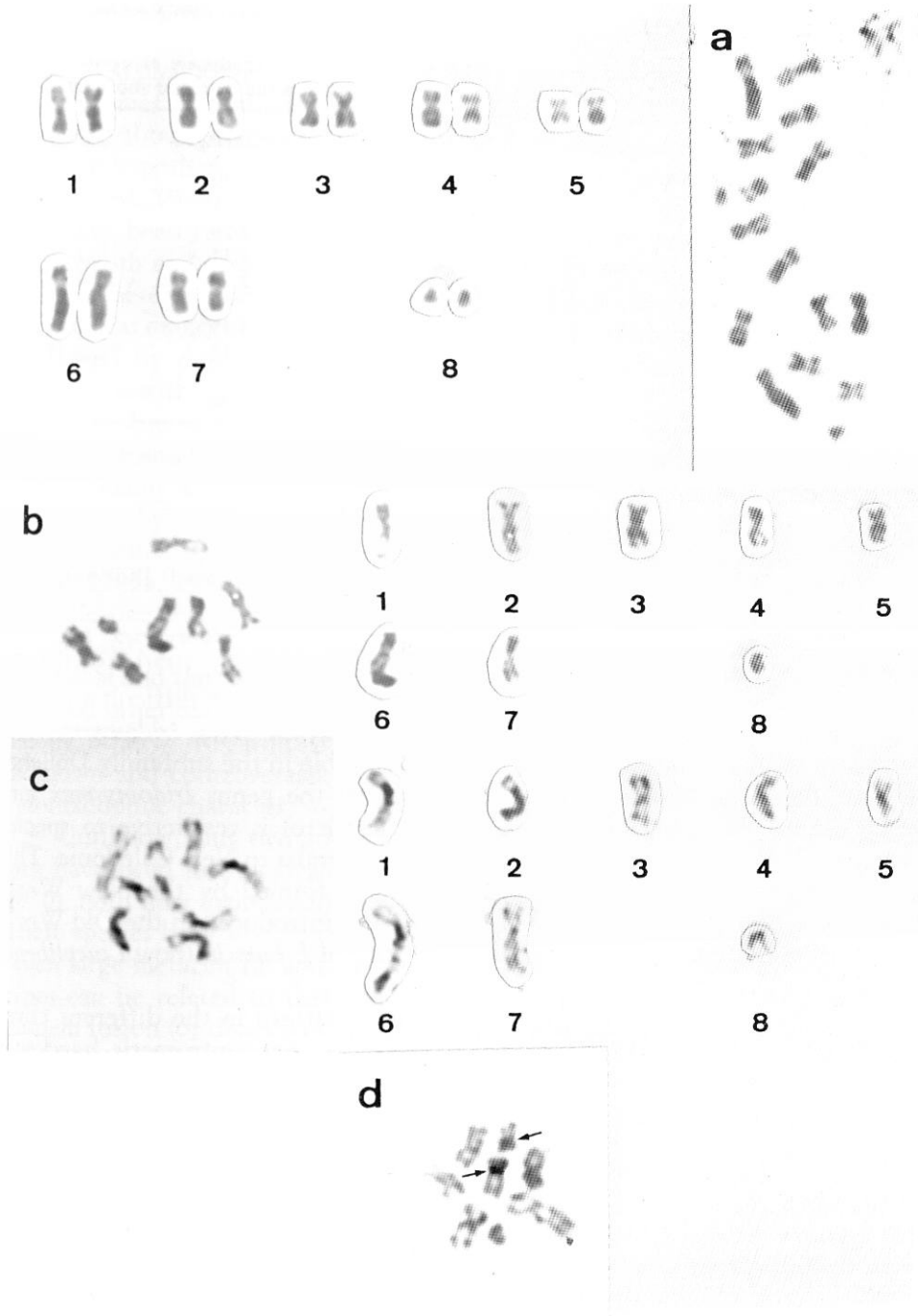

Fig. 1. — *Linepithema humile*. (a) Diploid karyotype and metaphase plate from cerebral ganglia cell of worker prepupae. (b) Haploid karyotype and metaphase plate from germ cells of early male pupae (c) C-band karyotype and metaphase plate. (d) Ag-staining showing a site with positive reaction in chromosome 7 and another in a metacentric chromosome (arrows).

TABLE 1 - Analysis of the standard karyotype of *Linepithema humile*. Lengths are expressed in µm. L.A./S.A. = ratio between the lengths corresponding to the long and short arms.

Chromosome	Long arm	Short arm	Total length	LA/SA
1	1.82	1.47	3.29	1.24
2	1.67	1.31	2.98	1.27
3	1.52	1.26	2.78	1.21
4	1.31	1.06	2.37	1.24
5	1.37	0.91	2.28	1.51
6	3.13	1.26	4.39	2.48
7	1.92	0.91	2.83	2.11
8	1.26	—	1.26	—

chromosome number and karyotype formula for the Australian population of this species.

The chromosome numbers found in the genus *Iridomyrmex* are very variable (n = 6, 7, 8, 9, 11 and 14) (CROZIER 1968*a*, 1968*b*, 1970, 1975; IMAI *et al.* 1977; IMAI and YOSIDA 1964) and its karyotypes are difficult to relate (CROZIER 1975). Some authors have considered that the genus *Iridomyrmex* has proven to be one of the most taxonomically variable in the subfamily Dolichoderinae. Shattuck (1992*a*, 1992*b*) has separated the genus *Iridomyrmex* into seven genera. The genus *Iridomyrmex* (sensu stricto) is restrictive to species from east India to China, and from Southern Australia to New Caledonia. This author considers that the genus *Linepithema* is formed by the New World species as well as by other species of *Iridomyrmex* introduced in the Old World but originally native to America. This is the case of *I. humilis* (now *Linepithema humile*).

C-banding detected a different C-banding pattern in the different chromosomes. On chromosomes 1, 5 and 6 a big C+ pericentromeric band was detected. In the others only a small C+ pericentromeric band was evident. There are also two interstitial bands in the long arm of chromosome 6 (Fig. 1c). Until now, only one non-determined Australian species of the genus, *Irydomyrmex* sp. 13, has been studied by C-banding techniques (IMAI *et al.* 1977). *Irydomyrmex* sp. 13 with a karyotype formula $2n$ = 10 (M or SM) + 8A = 18 has a typical animal C-banding pattern in which all M or SM chromosomes have distinct pericentromeric blocks and all acrocentric chromosomes have totally heterochromatic short arms (IMAI *et al.* 1977). Some species of the genus *Tapinoma* also included in the subfamily Dolichoderinae, have also been studied using C-banding techniques. In these species a variable size in the pericentromeric C-bands is also found, as well as interstitial and telomeric C-bands (PALOMEQUE *et al.* 1988). In other genera of ants, C-banding negative chromosomes have also been observed (IMAI *et al.* 1977).

The above mentioned variability of C-banding patterns is in accordance with the hypotheses suggested about the chromosomic evolution in ants. IMAI *et al.* (1988, 1994) considered that the principal rearrangements in ants evolution have been centric fission, centric fusion, pericentric inversions and tandem growth and deletion of heterochromatin. In several species of ants occurrence of heterochromatic supernumerary chromosome segments has been described (PALOMEQUE *et al.* 1993*a*). Probably, some of these segments have been originated by duplication of heterochromatic material. This fact is also in accordance with IMAI *et al.* (1988, 1994).

We observed a site with positive Ag-stain in chromosome 7 near the centromere and another in a metacentric chromosome which, on the basis of its size, probably corresponds to chromosome 5 (Fig. 1d). Occurrence of Ag-stained or Ag-NOR sites in pericentromeric regions and coincident with C-positive bands seems to be common in ants (PALOMEQUE *et al.* 1988, 1990*b*, 1990*c*, 1993*a*, 1993*b*; IMAI *et al.* 1992; HIRAI *et al.* 1994). Of these Ag-NORs, only the one in chromosome 7 was invariably stained with silver. However staining of Ag-NOR on chromosome 5 was variable depending on both the individual and the cell. Similar variability has been observed in other genera of ants and other animals (PALOMEQUE *et al.* 1993*a*, 1993*b*; SÁNCHEZ *et al.* 1995; among others). Regardless of biological significance of silver staining, still unknown and controversial, the study of Ag-NORs has proven to be a good cytotaxonomic character.

Until now, only two Southern American species of the old genus *Iridomyrmex* have been karyologically studied, *I. pilifer* and *I.* sp. nr. *pilifer* (CROZIER 1970), both now included in the genus *Linepithema* (SHATTUCK 1992*a*, 1992*b*). These species have n = 9, their karyotypes are very similar and are formed by seven large metacentric and two small acrocentric chromosomes. These karyotypes can be related to that one in *I. humilis* (now *Linepithema humile*) by a tandem fusion (or dissociation) of one small chromosome and a metacentric one (yielding the large submetacentric chromosome in *L. humile*). This hypothesis is in accordance with the principal rearrangement considered in ants chromosome evolution by various authors (IMAI *et al.* 1977, 1988, 1994; PALOMEQUE *et al.* 1988, 1993*b*).

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