A biogeographical study of the threatened ant *Dinoponera lucida* Emery (Hymenoptera: Formicidae: Ponerinae) using a cytogenetic approach

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**Abstract.** 1. Ants of the genus *Dinoponera* belong to a convergent group, in which there is no morphologically specialized caste of reproducing females and reproduction is by fertilized workers known as gamergates. *Dinoponera lucida* Emery, which is native to Brazilian Atlantic rain forest, is included on the official list of Brazilian fauna species threatened with extinction, due to habitat fragmentation, loss of their natural habitat and to peculiarities in their biology.

2. Karyotype variation was studied among *D. lucida* populations in the states of Bahia and Espírito Santo, Brazil. The cytogenetic study was carried out on brain ganglia and/or on male and/or female gonads. Banding techniques, such as sequential staining with DA/CMA3/DAPI fluorochromes and FISH, were also applied.

3. The diploid chromosome numbers ranged from 106 to 120. Variations in the karyotype were detected in the populations from Bahia, while in Espírito Santo the karyotypes were the same everywhere. Most of the chromosomes were small in size and acrocentric, except for a differentiated pair (AMt). This pair exhibited polymorphism in the different populations.

4. The karyotype variation detected in *D. lucida* suggested that the original population has previously been divided into two allopatric populations distributed in two different refugial areas of Atlantic rain forest in the early Quaternary. They probably developed in parallel and differentiated in karyotype composition. When the Atlantic rain forest regained its continuous distribution along with the Brazilian coast, the two groups came to occupy the continuous area of occurrence of the species that is seen today.

**Key words.** Ant, Atlantic rainforest, biogeography, cytogenetic, extinction, Neotropical region.

**Introduction**

Cytogenetic studies on insects have contributed significantly to understanding the evolutionary history and geographic dispersion of various groups that have been studied (Marescalchi & Scali, 1993; Soldán & Putz, 2000; Pellegrino *et al*., 2005). The contribution of cytogenetics to interpreting the speciation processes is a controversial matter (White, 1973; King, 1993; Spirito, 1998) because to play an effective role in speciation, the chromosome rearrangements need to be fixed so that they are transmitted to future generations. However, natural selection tends to reject the majority of these rearrangements, so that most are eliminated (King, 1993). Thus, the significance of the chromosome rearrangements in speciation has always been studied in diploid sexual organisms that perform normal
meiosi. As with other Hymenoptera, ants exhibit arrhenotokous
parthenogenesis in the production of haploid male individuals, 
so, meiosis is sometimes regarded as ‘atypical’ (Bull, 1981; Da 
Cruz Landim & Beig, 1981; Perondini et al., 1981). Thus, there 
are few speciation studies based on chromosome variation
in this insect order (Mariano et al., 2006b), so the role that
chromosome rearrangements may play is still obscure in 
Hymenoptera speciation.

Several examples of chromosomal variation in ant species
are reported in the genera Rhytidoponera (Ectatomminae) 
(Crozier, 1969; Imai et al., 1977), Myrmecia (Myrmeciinae) 
(Imai et al., 1994) and Typhlomyrmex (Ectatomminae) (Mariano 
et al., 2006a). The diploid number observed in species of the 
Formicidae ranges from 2n = 2 to 2n = 120, with Myrmecia 
rosulandi (Myrmeciinae) being at the bottom (Croslan & 
Crozier, 1986) and Dinoponera lucida (Ponerinae) being at 
the top of the range (Mariano et al., this paper, and unpublished results). 

Ants of the Dinoponera genus belong to a convergent group 
that includes a hundred poneromorph species (sensu Bolton, 
2003) and rare Myrmicinae, in which there is no distinct 
reproducing female in the worker caste, while reproduction is
performed by a fertilized worker known as a gamergate (Peeters,
1993, 1997; Monnin & Peeters, 1998; Heinze et al., 1999;
Hölldobler et al., 2002). Dinoponera lucida Emery is on the 
oficial list of Brazilian fauna species threatened with extinction 
(Ministério do Meio Ambiente, 2003). This threat mainly results 
from fragmentation and loss of the ants’ natural habitat and from 
the peculiarities of its biology, which predispose the isolated 
populations to endogamy and/or extinction (De Souza et al., 
2001). This species is endemic to the Atlantic rainforest and
occurs in both wet and semideciduous forest fragments (sensu 
Thomas, 2003) stretching from the South of Bahia to Espirito 
Santo and extending into the inland state of Minas Gerais, an area 
currently known as the Atlantic Rainforest Central Corridor.

In this study, we assessed, by means of cytogenetics, the biogeo-
graphic significance of the karyotype variation among D. 
locuica populations in the States of Bahia and Espirito Santo, Brazil.

Material and methods

Materials for cytogenetic analysis were gathered from D. lucida 
colonies that were collected in areas along the Atlantic 
Rainforest Central Corridor from 15 sites in the States of Bahia 
and Espirito Santo, Brazil, between March 2004 and January 
2005 (Table 1, Fig. 1).

For each site, one to three ant nests were opened and from each one of these, six to eight juveniles were removed at the
pre-pupa stage (before meconium elimination) for cytogenetic
analysis following the protocol of Imai et al. (1988). The
metaphases were obtained from brain ganglia and/or male and/
or female gonads, following the same protocol. After 24 h, the
slides were stained with Giemsa in order to characterize the
number and morphology of the chromosomes. The chromosome 
characterization was based on the nomenclature of Imai et al. 
(1988). For sequential staining with DA/CMA/DAPI fluorescent,
the slides were submitted to sequential staining treatment
according to the protocol of Schweizer (1980), and

Table 1. Dinoponera lucida populations cytogenetically studied in the States of Bahia (BA) and Espirito Santo (ES), number of analyzed colonies, individuals and cells per population; chromosome

| Location | State/City | No. of colonies | No. of individuals | No. of analyzed cells per individual | Total number of cells analyzed |
|----------|------------|-----------------|--------------------|-------------------------------------|-------------------------------|
| ES/CEPLAC | Teixeira de Freitas | 1 | 8 | 728 | 728 |
| ES/Lignedores | Bahia Belontie – Barrolândia | 2 | 12 | 728 | 728 |
| ES/AL | Itamaraju | 3 | 24 | 728 | 728 |
| ES/Lignedores | Lagedão | 4 | 36 | 728 | 728 |
| ES/INCAPER | Santa Teresa – Santo Antonio do Canaã | 5 | 48 | 728 | 728 |
| ES/FLONA | São Luiz | 6 | 60 | 728 | 728 |
| ES/INCAPER | S. Brás de Pinda | 7 | 72 | 728 | 728 |
| ES/Lignedores | Linhares | 8 | 84 | 728 | 728 |
| ES/INCAPER | Linhares | 9 | 96 | 728 | 728 |
| ES/FLONA | Cariacica | 10 | 108 | 728 | 728 |
| ES/INCAPER | Marechal Floriano | 11 | 120 | 728 | 728 |
| ES/INCAPER | Viana | 12 | 132 | 728 | 728 |
were later analyzed under an epifluorescent microscope. The fluorescent in situ hybridization (FISH) technique was applied according to the protocol of Viégas-Péquignot (1992) using the rDNA pDm 238 probe, which contains a ribosomal cistron unit of *Drosophila melanogaster* (18S, 5.8S and 28S plus the intergenic sequences) inserted into a pBR 322 plasmid (Roiha et al., 1981), which consisted of marking the probe, denaturation, hybridization and immunological detection.

**Results and discussion**

**Cytogenetic information**

The cytogenetic analysis consisted of the determination of the chromosome number and the localization of the regions rich in G + C and ribosomal genes by in situ hybridization. The results obtained are shown in Table 1 and Figs 2–5.

The diploid chromosome numbers (2n) detected ranged from 106 to 120 (reports by Mariano et al. 2004 are included) (Table 1). With the exception of the AMt pair, the chromosomes are extremely small in size compared to those of other ant species (Mariano et al., 2004). The AMt pair is larger than the other chromosomes and is characterized by having a heterochromatic short arm and a large heterochromatin block at the end of a long arm. According to Imai (1991), this kind of chromosome may result from centric fission and heterochromatin addition. In Bahia, most of the karyotypes ranged between 2n = 116 to 120. Two locations that exclusively exhibited 2n = 120 were Teixeira de Freitas and Mucuri, both located in the extreme south of the State. Results from the other locations in Bahia indicated that there was great karyotypic heterogeneity between populations.
Fig. 2. Mitotic and meiotic metaphases of *Dinoponera lucida* after conventional staining; populations from the States of Bahia (BA) and Espírito Santo (ES): (a) Viana (ES), worker, $2n = 118$; (b) Cariacica (ES), worker, $2n = 118$; (c) Mucuri (BA), worker, $2n = 120$; (d) Ibirapuã (BA), worker, $2n = 118$; (e) Teixeira de Freitas (BA), male, $n = 60$; (f) Barrolândia (BA), male, $n = 58$. Bar = 5 μm. A $M_t$ pairs indicated by arrows.

Fig. 3. Polymorphism of size in the AM pair of *Dinoponera lucida* for populations from the States of Bahia (BA) and Espírito Santo (ES), in mitotic and meiotic cells after Giemsa staining: (a) Barrolândia (BA), worker, $2n = 106$ (Mariano et al., 2004); (b) Teixeira de Freitas (BA), male, $n = 60$; (c) Linhares (ES), male, $n = 59$; (d) Cariacica (ES), worker, $2n = 118$; (e) Viana (ES), worker, $2n = 118$; (f) Marechal Floriano (ES), worker, $2n = 118$; (g) Santo Antônio do Canaã (ES), worker, $2n = 118$. Bar = 5 μm.
In Barrolândia alone, for example, four different karyotypes occurred together (2n = 106, 116, 118, 120) in a population distributed in a single forest fragment where there was no evidence of any recent occurrence of habitat fragmentation. However, this population is today completely isolated from the other populations of the same species situated in that State by extensive eucalyptus plantations, in which *D. lucida* is completely absent. In Lagedão, the males in the populations sampled had n = 57 (corresponding to a diploid karyotype of 2n = 114). The populations in Espírito Santo exhibited uniformly haploid karyotypes (male) with n = 59 and diploid karyotypes (female) with 2n = 118 chromosomes.

As already stated, except the larger AMt pair, the karyotype consists of very small-sized chromosomes, probably all acrocentric. This makes their morphological classification difficult (Fig. 2). This problem, already pointed out by Mariano et al. (2004) for this species, limits the AMt pair to fine-scale cytogenetic analysis because the analytical protocols that we used do not allow the detailed study of the small chromosomes structure, nor their individual recognition by pairs.

The analysis of the AMt pair indicated polymorphism in the different populations, with variations in the heterochromatin distribution (Fig. 3). Staining with Cromomicin A3 fluorochrome indicated positive marking for CMA3 in the AMt in all populations (Fig. 4), while the DAPI fluorochrome marked the telomere region, indicated the richness of AT sequences in this region. The FISH technique using a DNAr probe, in which we explicitly stained the AMt pair, was only conducted on individuals collected from Linhares (ES) and Teixeira de Freitas (BA). In the two populations analyzed, there were positive signals for the

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**Fig. 4.** Mitotic metaphases stained with CMA3: (a) worker, 2n = 118, Barrolândia (BA); (b) worker, 2n = 118, Ibirapuã (BA); (c) worker, 2n = 118, Linhares CVRD (ES); (d) male, n = 59, Linhares FLONA (ES). Bar = 5 μm, the AMt chromosomes are signalized by more intense staining.

**Fig. 5.** Mitotic metaphase of a male of *Dinoponera lucida* (n = 59) of Linhares (Espírito Santo), after the FISH technique with rDNA probe. The AMt corresponds to the chromosome marked with the DNAr probe. Bar = 5 μm.
Biogeographic inferences

Two complementary processes may explain the current karyotype distribution of *D. lucida*: a Pleistocene process of fragmentation of an ancestral population that was at its peak during the last glacial periods; and a current process of Holocene fragmentation resulting from the impact of human activities in the region of distribution of the species.

Pleistocene fragmentation

The karyotype variation detected in *D. lucida* suggested the division of its ancestral distribution area into, at least, two groups of populations, that existed over a long period of time as two megapopulations totally isolated from each other (Fig. 1). The first group occupied the northern portion of the distribution area and corresponded to the modern populations with the karyotype ranging from 2n = 106 to 120 chromosomes. The second group may have become isolated in the south of the range of the species and corresponded to all the forms found today in the central region of Espírito Santo, where there are completely homogeneous populations with karyotypes of 2n = 118 chromosomes, similar to some of the populations in the extreme south of Bahia. The spatial distribution of these two different cytogenetic groups (chromosome number uniformity in Espírito Santo populations while this number varies from 2n = 106 to 120 in Bahia populations) suggested the occurrence of a large scale biogeographic event on a long timescale and involving many generations.

The data also suggested that the geographical barrier isolated the two groups from each other at some moment, and then ceased to exist, allowing the populations in the south to expand towards the north, so that the populations overlapped. This could explain the variety of karyotypes currently detected in Bahia.

The current distribution of this species (compare Figs 1 and 6) shows similarities to that figured by Câmara (1991), which was adopted from a study by Whitmore and Prance (1987), who recombined distribution data of all of native bird, woody angiosperm, and butterfly species reported in the literature. We hypothesize that, during the coldest period of the last ice age, the two groups of *D. lucida* became separated in what coincided with two of the areas shown by Câmara (1991): one to the north of the River Doce, extending North probably to the River Contas where the 2n = 120 came to predominate, and another to the South of the River Doce, extending to the River Paraíba do Sul where the 2n = 118 came to predominate. The most obvious biogeographic barriers are quite clearly the rivers that cross Atlantic rainforest from the east to west, while the River Doce is the most probable separator of the two population groups during the Pleistocene, as it possibly was for the lizard *Gymnodactylus darwinii* (Pellegrino et al., 2005). Alternative, and not exclusive hypotheses for the biogeographic barrier, are: (i) a dryer area with more open grassland vegetation located in the north of Espírito Santo (that is, north of the River Doce) that would have occurred during the period of climatic fluctuations that affected South America during the Quaternary (Rizzini, 1978; Vanzolini, 1992), and during the Pleistocene, as hypothesized for the crab-eating fox *Cerdocyon thous* (Tchaika et al., 2007); (ii) a semi-arid climate developed over the region under the influence of the Abrolhos Peninsula that emerged during the Pleistocene approximately in the same region, with strong effects on the continental fish fauna and a range of plants (Schafer et al., 2006). The reconnection of the *D. lucida* populations happened during the Holocene, when the growth of populations in the south, with 2n = 118, expanded towards the north, entering into sympatry, and possibly hybridization with populations of the group in the north (initially with 2n = 120). These remained practically in the same region as they were in the Pleistocene, and may have suffered a small regression phenomenon from the south to the north.

Modern fragmentation

In the north of the species’ distribution area, South Bahia and Northeast Espírito Santo, the forest cover where the populations are found consists of semi-deciduous forest and coastal tabeland forest fragments. These fragments are currently extremely reduced and isolated; the ant habitat patches are very distant one from another so that the remaining *D. lucida* populations are small, isolated and discontinuous. This could induce strong rates of endogamy in each population and increase genetic erosion, with a strong probability of local extinction (Fig. 1) (Packer & Owen, 2001). The current isolation prevents movement of individuals among the fragments, which prevents not only genetic exchanges, but also the re-colonization of fragments where the species has become extinct locally. An important characteristic of this species is the fact that there is no mating flight, where the two sexual partners generally meet in flight. This limits to the male the long-distance dispersion of the genes, because this is the only winged element of the species. Furthermore, it has a limited flight capacity and apparently avoids flying in open areas (J.H.C. Delabie, unpublished data). The short radius range of the male, along with the aggregated distribution of the colonies, means that it is highly likely that mating occurs with related females.

It is also in the South of Bahia that, as a consequence of the historic processes of human occupation, the forest fragments nowadays are smaller and more isolated, which greatly increases the chances of divergent evolution of the karyotypes of the different populations that live there. This karyotype divergence...
may explain the distribution found, as there are colonies with chromosome numbers equal to those of Espírito Santo in the extreme north of the collection area (Barrolândia) that occurred together with others with different chromosome numbers in the same population. This is different from the situation found in the extreme south of Bahia and on the frontier with the State of Minas Gerais, where each population has a different chromosome number, sometimes comprising the only karyotype pattern found in the fragments (Fig. 6). In Australia, the chromosome number in ant populations of Rhytidoponera metallica was \(2n = 22, 24, 36, 42, 46\) (Imai et al., 1977), while in Myrmecia haskinsorum, it varied from \(2n = 12\) to 24 and in Myrmecia pilosula it varied from \(2n = 21\) to 30; these karyotypes originated by centric fissions (Imai et al., 1994). Studies on Brazilian Pachycondyla spp. populations showed that Robertsonian rearrangements (fission and centric fusion) and pericentric inversions were responsible for the karyotypic diversity in the genus (Mariano et al., 2006c, 2007). In several ant genera, such as Camponotus, Pheidole, Atta and Acromyrmex, different populations of a single species, as well as different species in the same taxonomic group, exhibited equal chromosomal numbers, indicating that high karyotypic stability is typical of them (Mariano et al., 2001, 2003; C.D.S.F. Mariano, unpublished data). This is obviously not the case for Dinoponera, although parallel cytogenetic observations on other species of the genus showed that the chromosome number of single populations of Dinoponera gigantea was \(2n = 82\) and Dinoponera quadriceps \(2n = 92\) (C.D.S.F. Mariano, unpublished data).

The forest cover in the central region of Espírito Santo is also fragmented. Because of agricultural activities, which are preferentially established in the lowland areas, most of the forest areas that could be colonized by the ant have been progressively substituted by plantations. However, in spite of this, the forest remnants, most of them in the north of the State or in the mountainous regions, are wide and less isolated one from another in this region than in the South of Bahia. This ensures greater stability in the karyotype structure of the populations, as deduced from the constancy of the chromosome number in that region.

Regardless of how the isolation happened following the differentiation of the population groups, and subsequent overlapping of it, recent phenomena resulting from habitat fragmentation by humans (Franklin et al., 2002) are remodeling their distribution and consequently the karyotype patterns of D. lucida. We hypothesize that the reduction and isolation of habitats and populations may irreversibly lead to the genetic erosion of these populations in the South of Bahia and northeast Minas Gerais, regions most changed by forest conversion, while this phenomenon should not be so acute in the State of Espírito Santo where, although the forest fragmentation also occurs, the D. lucida populations are able to maintain gene flow.

Actions to increase the size of the forest remnants and further re-establish the connections among close forest fragments are necessary and urgent to increase the D. lucida population sizes and that of many other organisms. Further studies will be necessary to verify the viability of breeding among the different population groups in the region of the South of Bahia and possibly to promote contact among some of these populations with each other and with those from the central region of Espírito Santo.

This would only be possible by implementing a management policy to reinforce certain populations from Bahia and Minas Gerais, which are critically threatened, by introduction of societies derived from the stronger Espírito Santo populations, assuming that they are genetically compatible. Our results may yet refute this possibility, unless the purpose of the management is not reinforcement of a threatened population, but rather is re-introduction of new populations in fragments where the ant has been extinguished.

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