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Are the Neotropical ants *Pachycondyla crenata* (ROGER, 1861) and *Pachycondyla mesonotalis* (SANTSCHI, 1923) (Hymenoptera: Formicidae: Ponerinae) good species? A cytogenetic approach

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Abstract

The Neotropical ants *Pachycondyla crenata* (ROGER, 1861) and *Pachycondyla mesonotalis* (SANTSCHI, 1923) (Hymenoptera: Formicidae: Ponerinae) nest in pre-existing hollow cavities on trees in native and planted forests where they form discrete colonies. Their morphology is very similar; only their body size appears different. They are sympatric in Brazil, but *P. crenata* can be found further north of the Neotropical Region than *P. mesonotalis*. Aiming to evaluate the character differentiation in these taxa, cytogenetic studies were carried out and complemented by morphometric observations. Cytogenetic preparations were made using prepupae gathered from two Brazilian sites. Both taxa have a diploid karyotype composed of 26 chromosomes. The karyotype formula for *P. crenata* is 2K = 2M + 24A, while for *P. mesonotalis* it is 2K = 26A. A pericentric inversion AM on a single pair of chromosomes may have given rise to the difference between the species. Although it is not possible to pinpoint the more ancestral karyotype between *P. crenata* and *P. mesonotalis*, we speculate that the reported chromosome rearrangement was the differentiation mechanism that prompted their species divergence, resulting only in morphometric differences with a minimum impact on the ecology and morphology, yet allowing them to live sympatrically.

Key words: Chromosome, karyotype, speciation, ponerine ants, *Pachycondyla*.

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Introduction

The ponerine ant species *Pachycondyla crenata* (ROGER, 1861) and *Pachycondyla mesonotalis* (SANTSCHI, 1923) are arboreal and generally nest in pre-existing cavities on trees where they actively forage for prey (concerning *P. crenata*, see LONGINO 2001). The larger species, *P. crenata*, is distributed from southeastern Mexico to northeastern Argentina (KEMPF 1972, LONGINO 2001). *Pachycondyla mesonotalis*, described from Santa Catarina State in southern Brazil, has rarely been recorded in literature, though it has been found living sympatrically with *P. crenata* in several Brazilian areas, according to unpublished data from the ant collection of the Myrmecology Laboratory, Cocoa Research Center, Ilheus, Bahia, Brazil (CPDC).

Cytogenetic studies can contribute a range of new information which is independent from the characters usually used for phylogenetics. Like biochemical data, cytogenetic information, varying from morphological to molecular data according to the kind of methodology used (SESSIONS 1996), can reveal differences or similarities that cannot be detected through simple observation. Cytogenetics alone cannot resolve questions about species isolation, but, more importantly, is a powerful tool for systematic and phylogenetic studies developing strong arguments that allow inferences about evolution processes. This paper is part of a recent series of research studies carried out on the karyotypes of Neotropical ants belonging to several subfamilies (MARIANO & al. 1999, 2001, 2003, 2004, BORGES & al. 2004a, b).

In its current form, the genus *Pachycondyla* sensu Brown in BOLTON (1995, 2003) doesn't inspire unanimity among ant taxonomists and is quite likely polyphyletic, comprising several unrelated ant groups with plesiomorphic traits (see SCHMIDT 2005). Neotropical species of this genus, formerly distributed in half a dozen different genera (*P. crenata* and *P. mesonotalis* were both included in the genus *Neoponera*, according KEMPF 1972), appear to be a complex mosaic of species groups based largely on morphological evidence, but in fact containing some biological species separated only through chemical, behavioral or cytogenetic information (see LUCAS & al. 2002, MARIANO 2004, WILD 2005, CPDC Collection, unpubl.).

In an unpublished manuscript (a provisional key for Neotropical *Pachycondyla*, for which several versions exist; we refer to the most recent, dated XI.1987), written before all the information we currently have on the Brazilian sympatric populations of *P. crenata* and *P. mesonotalis* became available, W. Brown Jr. (deceased in 1997) considered *P. crenata* to be "exceptionally variable in size" and made no comment on *P. mesonotalis*, which we suppose that he considered as synonym of the first. Hereafter, we present evidence that they are in fact two closely related species, based on the chromosomal events that hypo-

thetically originated the divergence between the two taxa. Even if a future (and urgently necessary) taxonomic revision might reveal numerous synonymies in the genus *Pachycondyla*, this study demonstrates the existence of at least two sympatric taxa in the *P. crenata* species complex.

Material and methods

Specimens of both taxa studied here have been compared, few years ago, directly with the Kempf Collection at the Museum of Zoology at the University of Sao Paulo (MZUSP), and these ants correspond to the same taxa referred by Kempf in his catalogue of 1972.

Colonies of *P. crenata* and *P. mesonotalis* were collected in the Mata Córrego do Paraíso Reserve (20° 45' S, 45° 52' W), which belongs to the Federal University of Viçosa (UFV) at Viçosa, Minas Gerais, Brazil, and at the experimental fields of the Cocoa Research Center (14° 45' S, 39° 13' W), CEPEC / CEPLAC, at Ilheus, Bahia, Brazil. Both taxa were found in hollow trunks or in dried cocoa pods still hanging on the trees. Collection in the UFV Reserve was carried out in November 2000, and it was conducted in the CEPLAC fields in April 2001. In both areas, several colonies were located, but available material for cytogenetic studies (prepupae) was only found in three of them (see Tab. 1). Attempts at keeping colonies until new prepupae matured were unsuccessful. Field populations from both taxa ranged from one (a single gyne) to fewer than 50 (including one gyne, monomorphic workers, and immature individuals). The colonies were always monogynous. Voucher specimens of the colonies are deposited in the CPDC Collection.

Cytogenetic preparations were made following the procedure established by IMAI & al. (1988). Chromosomes were ranked as acrocentric (A) and metacentric (M) for karyotype study, using IMAI's (1991) nomenclature. The haploid karyotype formula of males (when available) is designated by n [K = A + M], while the diploid female one is designated by 2n [2K = 2A + 2M].

To avoid any doubt about the characters that separate the two taxa, a simple morphometric analysis was performed, where scape length was compared to head width in a series of individuals (n = 15 for each, originating from an equivalent number of Brazilian sites) from the CPDC collection (Fig. 1).

Results

Although the two taxa have highly similar morphological characteristics, a morphometric analysis (Fig. 1) showed that both of them are perfectly defined and that their size is a reliable element for their identification.

Both have a rather similar karyotype, with the same chromosome number (2n = 26, and also n = 13 for a small number of haploid male *P. mesonotalis* prepupae from Ilheus), and have a majority of acrocentric chromosomes (Tab. 1, Figs. 2, 3). The karyotypes of *P. mesonotalis* from the both sites, about 1,000 km apart, are exactly the same. The only obvious difference between the karyotypes of the two species is the substitution of an acrocentric pair (A) in *P. mesonotalis* by a metacentric one (M) in *P. crenata*. Their karyotype formulas, following the nomenclature of IMAI & al. (1994), are then 2K = 2M + 24A in *P. crenata* and 2K = 26A in *P. mesonotalis*, respectively. Within approximately 30 species studied cytogenetically (MARIANO 2004), there is no record of other *Pachycondyla* with the same chromosome number (2n = 26), except for an unidentified Neotropical species of the *Pachycondyla villosa* (FABRICIUS, 1804) group. Although also formerly classified in the *Neoponera* genus, this ant is obviously not close related to *P. crenata* and *P. mesonotalis* and has a completely different karyotype (MARIANO 2004).

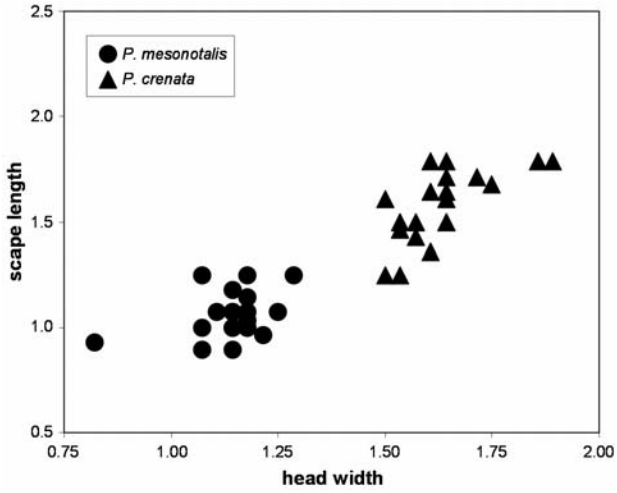

Fig. 1: Comparison of *Pachycondyla crenata* and *Pachycondyla mesonotalis* (Brazil; several sites) through morphometric analysis: head width (mm) vs. scape length (mm).

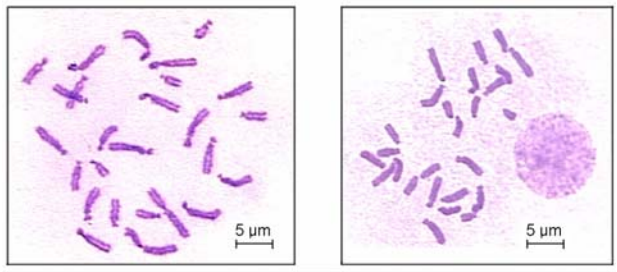

Fig. 2: Metaphases of *Pachycondyla crenata* (left) and *P. mesonotalis* (right). Giemsa staining.

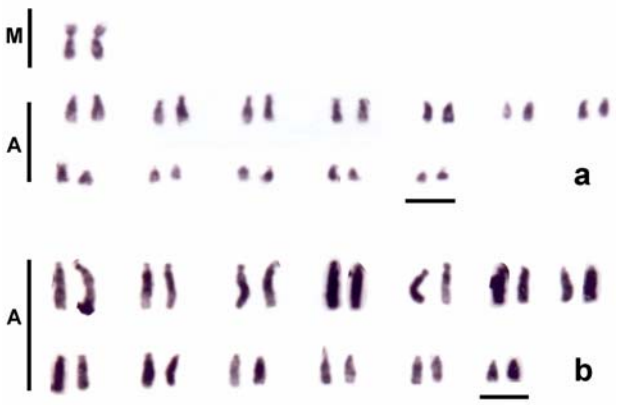

Fig. 3: Diploid karyotypes of: a) *Pachycondyla crenata*, 2n = 26; b) *P. mesonotalis*, 2n = 26. Bar = 5 µm.

Tab. 1: Cytogenetical analysis of *Pachycondyla crenata* and *Pachycondyla mesonotalis*: colony origin and number of individuals (prepupae) used in preparations.

Species	Site (Brazil)	Colony number	Individual number	2n, (n)	Karyotype formula 2K, (K)
P. crenata	Viçosa - MG	1	15	26	2M + 24A
P. mesonotalis	Ilhéus - BA	1	8	26, (13)	26A, (13A)
P. mesonotalis	Viçosa - MG	1	12	26	26A

Discussion

The size and volume of *P. crenata* is about twice the volume of *P. mesonotalis*, in spite of W. Brown Jr.'s affirmation in his unpublished manuscript referred earlier, that *P. crenata* is highly variable in size (at least for Brazilian populations). Consequently, we consider that both taxa are distinct, but phylogenetically close, as indicated by the cytogenetical analysis. Furthermore, they also seem very close from an ecological point of view. Since they live sympatrically in Brazil, we suppose that their respective sizes allow them to occupy different niches.

The karyotypical difference between *P. crenata* and *P. mesonotalis* may have originated in a pericentric inversion AM on a single pair of chromosomes, but the assumption that genetic accident was the main cause of the speciation is merely speculative and deserves further verification, since polymorphism for pericentric inversions has been seen in some ant species (IMAI & al. 1977). According to these authors, this is the type of inversion most likely to happen. On the other hand, chromosomal rearrangements, responsible for a range of variation already observed in the karyotypes of many organisms, are thought to be able to trigger a reproductive isolation process (KING 1993), and their function in speciation have been amply discussed by several authors (WHITE 1973, IMAI & CROZIER 1980, KING 1993, SPIRITO 1998, IMAI & al. 2001, 2002, RIESENBERG 2001).

An explanation for low karyotypic difference between the taxa can be found in the coupled model of speciation through morphological and karyotypic alterations proposed by IMAI (1983). Although its morphology is preserved, a species with a definite karyotype can, after a certain period of evolution, present any of three karyotypical states, as follows: a) monomorphic to the original karyotype (Or); b) polymorphic with the original karyotype (Or) and a new karyotype (Ne) induced by chromosomal rearrangements, or c) monomorphic to a new karyotype (Ne), which has replaced the original (Or). The ants *P. crenata* and *P. mesonotalis* illustrate the second possibility (b) where one of the taxa (Or) and (Ne) would have effectively diverged as true species from the ancestral karyotypical situation (Or) or (Ne).

Some species can differentiate morphologically without karyotype alteration (such as in some groups of Neotropical bees; see, for example, ROCHA & POMPOLO 1998). In others, chromosomal differences can be higher between races than between true species (RIESENBERG 2001). In the case of *P. crenata* and *P. mesonotalis*, as in a few other ponerines (MARIANO 2004), these ants have possibly undergone speciation through karyotype alteration, with either little or no morphological alteration, but this hypothesis deserves further examination.

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Zusammenfassung

Die neotropischen Ameisen *Pachycondyla crenata* (ROGER, 1861) und *Pachycondyla mesonotalis* (SANTSCHI, 1923) (Hymenoptera: Formicidae: Ponerinae) legen ihre Nester in vorhandenen Hohlräumen von Bäumen sowohl in ursprünglichen als auch in gepflanzten Wäldern an. Morphologisch sind die beiden einander sehr ähnlich, nur die Körpergröße ist verschieden. In Brasilien leben sie sympatrisch, aber *P. crenata* reicht in der Neotropischen Region weiter nach Norden als *P. mesonotalis*. Ziel der vorliegenden Untersuchung war es, die beiden Taxa für eine Bewertung der Merkmalsunterscheidung cytogenetisch zu charakterisieren sowie ergänzende morphometrische Daten zu liefern. Die cytogenetischen Präparate stammen von Präpuppen von zwei brasilianischen Lokalitäten. Beide Taxa haben einen diploiden Karyotyp mit 26 Chromosomen. Die Karyotypformel von *P. crenata* ist 2K = 2M + 24A, jene von *P. mesonotalis* ist 2K = 26A. Eine perizentrische Inversion AM auf einem einzigen Chromosomenpaar könnte der Ursprung der Unterschiede zwischen den beiden Taxa gewesen sein. Es ist nicht möglich zu entscheiden, welcher der Karyotypen, jener von *P. crenata* oder jener von *P. mesonotalis*, der ursprüngliche ist. Dennoch vermuten wir, dass die erwähnte Chromosomen-Umordnung jener Differenzierungsmechanismus gewesen ist, der die Aufspaltung der beiden Arten verursacht hat. Der Einfluss auf Ökologie und Morphologie ist dabei minimal gewesen, was den beiden Arten trotzdem ein sympatrisches Vorkommen ermöglicht.

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