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Genetic Variability of Native Populations of *Wasmannia* Forel (Hymenoptera: Formicidae) and their Biogeographical Implications

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Variabilidade Genética de Populações Nativas de Espécies de *Wasmannia* Forel (Hymenoptera: Formicidae) e suas Implicações Biogeográficas

RESUMO - *Wasmannia auropunctata* (Roger) e *Wasmannia rochai* Forel são duas formigas economicamente importantes no Sudeste e Sudoeste da Bahia. Marcadores de RAPD (polimorfismo de DNA amplificado ao acaso) foram utilizados para distinguir e analisar a variabilidade genética de populações de ambas as espécies, provenientes de Ilhéus e Jequié, BA, e da Guiana Francesa. As distâncias genéticas entre populações de *W. auropunctata* e *W. rochai* (55,8-71,4%) evidenciam a diferenciação genética entre elas. As populações de *W. auropunctata* presentes em canaviais e bananais de Jequié mostraram-se geneticamente mais distantes (30,1-46,3%), podendo representar populações restritas a fragmentos isolados. As elevadas distâncias genéticas entre as populações de *W. auropunctata* das áreas experimentais da CEPLAC, em Ilhéus (26,8-34,6%), assim como as demais populações de Ilhéus (23,3-40,8%), sugerem uma estrutura multicolonial de *W. auropunctata* no Sudeste da Bahia. A proximidade genética entre as populações de *W. auropunctata* dos cacauais (14,1%) e coqueirais de Ilhéus (18,5%) com as populações de floresta da Guiana Francesa sugere que houve expansão grande e recente de populações oriundas de uma única população a partir de um centro não-determinado, estando hoje distribuídas em habitats com características ambientais semelhantes. O elevado polimorfismo e os valores das heterozigosidades estimadas para as duas espécies sugerem que foram analisadas populações nativas de ambas as espécies.

PALAVRAS-CHAVE: *W. auropunctata*, RAPD, polimorfismo, heterozigosidade

ABSTRACT - *Wasmannia auropunctata* (Roger) and *Wasmannia rochai* Forel are economically important ants in the Southeast and Southwest regions of Bahia State, Brazil. Randomly Amplified Polymorphic DNA (RAPD) markers were used to distinguish and analyze the genetic variability of populations of both species from Ilhéus, Jequié, BA and French Guyana. The genetic distances among *W. auropunctata* and *W. rochai* populations (55.8-71.4%) suggest genetic differentiation among them. *Wasmannia auropunctata* populations from sugarcane and banana plantations in Jequié were the most distant genetically (30.1-46.3%) and may represent populations restricted to isolated fragments. The high genetic distances among *W. auropunctata* populations from CEPLAC experimental areas, in Ilhéus (26.8-34.6%) and the other populations from Ilhéus (23.3-40.8%), suggest a multicolonial structure of *W. auropunctata* in southeast Bahia. The genetic proximity among the *W. auropunctata* populations from cocoa (14.1%) and coconut plantations (18.5%) in Ilhéus with the populations from the French Guyanan forests suggest that there was recent and large expansion of populations derived from a single population, that are today distributed in habitats with similar environmental characteristics. The high polymorphism and the estimated heterozygosity values for the two species suggest that we studied native *W. auropunctata* and *W. rochai* populations.

KEY WORDS: *W. auropunctata*, RAPD, polymorphism, heterozygosity

The genus *Wasmannia* is exclusively Neotropical. The occurrence of *Wasmannia auropunctata* (Roger) (Myrmicinae) out of this region result of recent introductions. Currently, ten species are recognized and six occur in Brazil: *W. affinis* Santschi, *W. auropunctata*, *W. iheringi* Forel, *W. lutzi* Forel, *W. rochai* Forel, and *W. villosa* Emery (Longino & Fernandez 2007).

The little fire ant *W. auropunctata* is found in Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guyana, Paraguay and Peru (Fernald 1947, Kempf 1972, Lubin 1984, Delabie 1988). Its occurrence has also been recorded in Costa Rica (Tennant 1994), the Galapagos islands (Brandão 1991), Jamaica (Wheeler 1908), Panama (Tennant 1994), Puerto Rico (Torres 1984), Uruguay (Brandão 1991) and Venezuela (Goitia *et al* 1992). In Brazil, *W. rochai* Forel is found from São Paulo through the south of Ceará and in the Amazonas state, also ocurring in Venezuela, Guianas, Panama, Trinidad, Guatemala and Costa Rica (Kempf 1972, Longino & Fernandez 2007).

Colonies of *W. auropunctata* are polydomic with fragmented nests, so that the same colony is subdivided into numerous nests (Wetterer & Porter 2003). They are also polygynic, with a high number of fertile queens, although the correct number of queens per nest is difficult to quantify due to the absence of clear boundaries between colonies (Passera 1993, 1994). Nests of this ant are found in different habitats such as semi arid and wet climates (Spencer 1941, Kusnezov 1952, Lubin 1984). They are generally established at soil level, close to the surface and located under stones, among fallen leaves on the ground, in hollow branches or rotten stumps (Clark *et al* 1982, Torres 1984, Naumann 1994), around roots and in plant cavities (Spencer 1941, Ulloa-Chacon & Cherix 1988). They are also found under bryophytes and thick tree bark and under the base of palm tree leaves (Wheeler 1908, Kusnezov 1952, Fabres & Brown 1978).

In the canopy of cocoa trees, *W. auropunctata* is considered a dominant ant (Majer *et al* 1994, Medeiros *et al* 1995), since in this habitat it is numerically predominant, mutually exclusive regarding other dominant ant species and occupies large areas (Leston 1973). In this case, *W. auropunctata* is a serious problem of ecological imbalance and causes agricultural damage (Delabie & Cazorla 1991, Souza *et al* 1998) or discomfort to the human populations (Hölldobler & Wilson 1990, Passera 1994, Holway *et al* 2002). It may even be responsible for the changes of the native ant fauna (Clark *et al* 1982, Lubin 1984, Meier 1985), and the most successful competitor compared to other ant species in the Bahia cocoa agricultural system (Delabie *et al* 2007). However, in primary tropical forest, where this ant occurs naturally, it has not dominant characteristic, whose lower density is determined by coexistence with other species of ants (Levings & Franks 1982, Tennant 1994).

Wasmannia rochai is a subdominant species (Majer *et al* 1994) among the ants that form the mosaic in the cocoa canopy in southeast Bahia, considering that it also forms numerous populations, but it does not usually occupy large areas of the tree canopies and may be associated with other ant species (Majer *et al* 1994). However, this ant is also responsible for important agricultural damage, although in smaller proportions than that caused by *W. auropunctata* (Souza *et al* 1998).

Morphologically, *W. auropunctata* and *W. rochai* differ by the petiole aspect and the hairiness of the body. In *W. auropunctata*, the petiole is almost rectangular, whereas in *W. rochai* it is triangular; the body hairs in *W. auropunctata* are long and scarce, while in *W. rochai* they are shorter and truncated (Wetterer & Porter 2003). Therefore differentiating the *W. auropunctata* and *W. rochai* workers is difficult and can be aggravated by the fact that these ants are only 1.5 mm long. Thus new procedures are needed to distinguish the populations of these two species.

The Randomly Amplified Polymorphic DNA (PCR-RAPD) is one of the molecular procedures available that has been successfully used for genetic differentiation of morphologically closely ant species (Shoemaker *et al* 1994, Julian *et al* 2002). Furthermore, as this technique permits the detection of numerous polymorphisms and requires a small quantity of DNA (Ferreira & Grattapaglia 1998), it has been much used to study genetic variability in eusocial hymenopteran populations that generally present low genetic variability (Metcalf *et al* 1975, Shoemaker *et al* 1992).

This study used RAPD molecular markers to distinguish native populations of *W. auropunctata* and *W. rochai*, and to analyze the genetic variability among populations within each species.

Material and Methods

Insects. Twenty-four colonies of *W. auropunctata* and four of *W. rochai* were collected between January 2004 and January 2006 in different agricultural systems in Bahia, Brazil, [municipalities of Jequié (13º54'S, 40º01'W) and Ilhéus (14º37'S, 39º04'W)] and in forest areas in French Guyana [Pointe Combi (05º19'N, 52º57'W) and Petit Saut (05º04'N, 52º01'W)] (Table 1). The geographic distance between French Guyana and Ilhéus is approximately 4000 km and 200 km between Ilhéus and Jequié. Within Ilhéus, the geographic distance among the CEPLAC experimental areas (square D´, F and H) and the farm Cordilheira is approximately 4 km; between these areas and the district of Ponta da Tulha (areas I, II and III) it is about 50 km, while the areas within this district are 100 m apart. The Jequié areas (A, B, C, km 14 and km 15), located in the village Cajueiro, on the fringes of BR 330, are 50 m to 1000 m apart. The distances among the colonies collected ranged from 50 m to 1 km in the same Brazilian location, and there are approximately 27 km between the French Guyana localities.

For all statistical analyses, colonies were grouped according to locality and each locality was considered as representing a population.

DNA extraction and amplification. The genomic DNA of five individuals from each nest was individually extracted according to Waldschmidt *et al* (1997) and amplified by PCR-RAPD according to Williams *et al* (1990), with some modifications. The reaction mixture (25 µl) consisted of 3 µl DNA, 2.5 µl enzyme buffer, 1.0 µl triphosphate deoxyribonucleotide mixture (dATP, dTTP, dCTP, dGTP), 2.5 µl primer, 4 µM, 15.8 µl miliQ

Table 1 Sampling localities of the colonies of *Wasmannia auropunctata* and *W. rochai* in Brazil (all in Bahia) and French Guyana.

Localities (code)	Codes	Number of colonies	Habitat
W. auropunctata			
Jequié - km 14 (Cajueiro)	1	2	Banana plantations
Jequié - km 14 (Cajueiro)	2	2	Sugarcane plantations
Jequié - km 15 (Cajueiro)	3	2	Sugarcane plantations
Jequié - Area B (Cajueiro)	4	2	Cocoa plantations
Jequié - Area A (Cajueiro)	5	2	Cocoa plantations
Ilhéus - Square H (Ceplac)	6	2	Cocoa plantations
Ilhéus - Square F (Ceplac)	7	2	Cocoa plantations
Ilhéus - Square D' (Ceplac)	8	2	Cocoa plantations
Ilhéus - Area I (Ponta da Tulha)	9	2	Coconut plantations
Ilhéus - Area II (Ponta da Tulha)	10	2	Coconut plantations
Ilhéus - Area III (Ponta da Tulha)	11	2	Coconut plantations
Pointe Combi/French Guyana - Experimental Station	12	1	Forest
Petit Saut/French Guyana - Dam		1	Forest
W. rochai			
Ilhéus - Farm Cordilheira	13	2	Cocoa plantations
Jequié - Area C (Cajueiro)	14	2	Cocoa plantations

water and 0.2 μl *Taq* polymerase. The mixture was incubated in a PTC-100 thermocycler (MJ Research) programmed for 40 cycles, each one consisting of a denaturation step (15 seconds at 94ºC), one annealing step (30 seconds at 35ºC) and an extension step (1 min at 72ºC). An extra extension step was performed (7 min at 72ºC).

Nineteen primers were used in the amplifications: OPA13, OPAA6, OPAA11, OPAA16, OPAB19, OPB12, OPG3, OPK01, OPM05, OPN08, OPO13, OPP04, OPW01, OPW04, OPX06, OPY07, OPY14, OPY16 and OPZ11 (Operon Technologies, Alameda, AL, USA – http://www.operon.com/products/RAPD/overview.aspx). A pilot study showed that these primers generated at least seven bands and a clear amplification pattern, and for this reason they were used in the amplifications. The reaction products were separated by electrophoresis in 1.2% agarose gel containing 10 μg/ml ethidium bromide. The DNA bands obtained were visualized under ultraviolet light and the images generated were captured using the AlphaDigiDoc 1201 software.

Statistics. For the analysis of the molecular data a binary matrix was constructed based on the presence (1) and absence (0) of amplified DNA fragments. The data matrix was used to calculate the heterozygosity and the Nei & Li coefficient (S_{ij}) (Dias 1998), where the genetic similarity between the i-eth and j-eth individual was obtained by the equation:

$$S_{ij} = 2a/(2a + b + c)$$

where:

a = number of bands present simultaneously in the two samples

b = number of bands present in the first and absent in the second sample

c = number of bands absent in the first and present in the second sample

The genetic similarity obtained was converted into genetic distance (D_{ij}) (Dias 1998) using the arithmetical complement of the similarity, calculated by the equation:

$$D_{ij} = 1 – S_{ij}$$

The genetic distances obtained were used in the cluster analysis using UPGMA algorithm. The analysis of molecular variance (AMOVA – Excoffier *et al* 1992) was carried out to estimate the genetic variability among and within the populations. All the statistical analyses were carried out using the Genes computer program (Cruz 2001).

Results

The 19 primers used generated 249 RAPD bands for *Wasmannia* populations with 100 (40.2%) present only in *W. auropunctata* and 47 (18.9%) only in *W. rochai*. The mean number of bands per primer was 10.5 and 7.7 for *W. auropunctata* and *W. rochai*, respectively. In *W. auropunctata*, 99.5% of the bands were polymorphic, while in *W. rochai* they were 77.5%. The amplification pattern obtained with the OPW01 primer (Fig 1) in the two *Wasmannia* species, and the pattern obtained with the other primers used showed the viability of the PCR-RAPD to differentiate *W. auropunctata* from *W. rochai* populations.

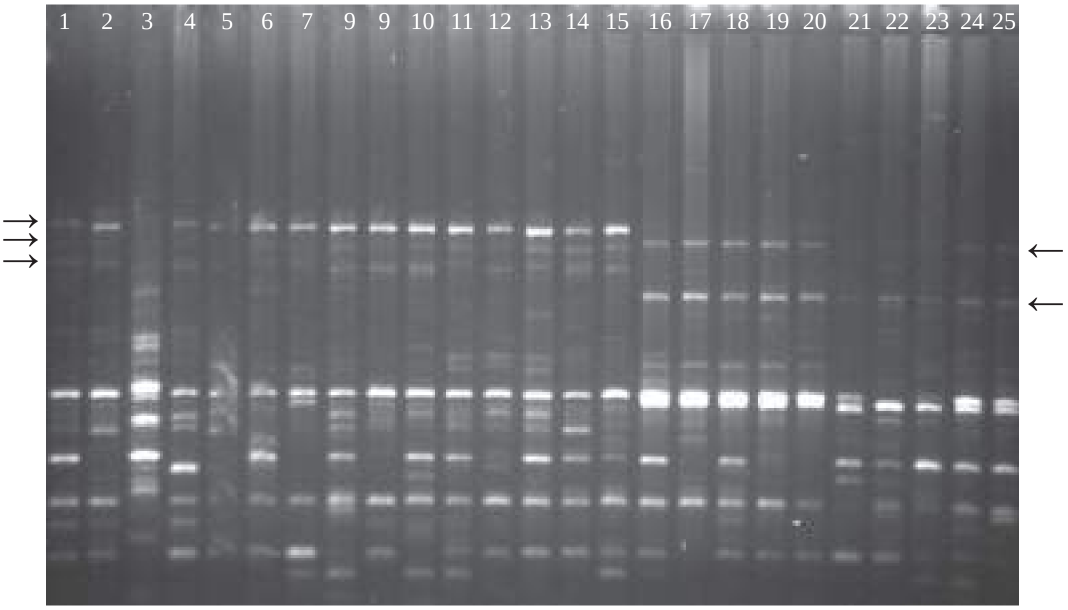

Fig 1 Representative RAPD amplification pattern of genomic DNA of *Wasmannia auropunctata* (1-15) and *W. rochai* workers (16-25) obtained with the "primer" OPW01. Each five lines represent a different colony. Diagnostic bands for both species are indicated by arrows.

The mean heterozygosity values estimated for *W. auropunctata* populations were: 0.407 (Ilhéus), 0.415 (Jequié) and 0.386 (French Guyana). For *W. rochai*, the mean heterozygosities were 0.434 and 0.398 for the populations from Ilhéus and Jequié, respectively. In spite of the criticisms regarding the calculation of this parameter when using dominant markers and assuming the Hardy-Weinberg equilibrium, it was used in the present study to verify the segregation of the loci in the two species.

The genetic distance estimated among the *W. auropunctata* populations ranged from 10.6% to 46.3% (Table 2). The shortest distance was detected between the populations from sugarcane and banana plantations from Jequié (10.6%). Small genetic distances were also found among the populations from coconut trees in Ilhéus (12.2%) and among the populations from coconut trees and cocoa trees in Ilhéus (12.4%). Small genetic distance values, ranging from 14.1% to 18.5%, were also detected among *W. auropunctata* populations from

Table 2 Genetic distances (%) among *Wasmannia auropunctata* populations (1 to 12) and *W. rochai* populations (13 and 14).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1														
2	10.6													
3	25.0	24.9												
4	41.5	40.2	30.6											
5	41.2	40.7	30.1	19.5										
6	37.7	37.1	32.3	31.1	30.6									
7	43.1	42.6	35.1	34.2	35.3	26.8								
8	46.3	45.7	40.6	40.2	41.1	32.2	34.6							
9	39.3	38.6	34.1	30.9	32.6	12.4	31.6	35.5						
10	39.8	39.7	32.2	27.0	22.8	23.3	32.5	38.2	23.0					
11	38.5	38.6	33.9	30.4	26.1	27.4	36.6	40.8	26.4	12.2				
12	39.1	38.1	32.6	29.4	32.0	14.1	26.6	32.4	18.5	26.8	30.9			
13	61.7	62.5	61.3	55.8	59.0	64.7	64.4	63.6	65.2	58.3	58.7	62.7		
14	62.3	63.8	63.5	63.2	68.4	71.4	70.1	70.3	70.7	66.9	65.4	68.0	41.7	

Ilhéus and French Guyana (Table 2).

High genetic distances were detected among the *W. auropunctata* populations from the CEPLAC experimental areas in Ilhéus (26.8% to 34.6%) and among the other populations in Ilhéus (23.3% to 40.8%). On the other hand, the higher genetic distance (46.3%) was detected among Jequié (banana plantations) and Ilhéus (cocoa plantations) populations. For the populations of *W. rochai*, high genetic distance (41.7%) was found between Jequié and Ilhéus (cocoa plantations).

Genetic distances ranging from 55.8% to 71.4% were detected when populations of the two *Wasmannia* species were compared (Table 2) and the cluster analysis (Fig 2) showed the formation of two groups: [G1] *W. auropunctata* from Brazil and French Guyana forests and [G2] *W. rochai* (Brazil).

The molecular variance showed the genetic variation was greater among the populations than within each population for both *W. auropunctata* (55.5% and 44.5%, respectively) and *W. rochai* (59.1% and 40.9%, respectively).

Discussion

The RAPD markers showed clear discrimination between the *W. auropunctata* and *W. rochai* populations, for all primer pairs tested, indicating their use to distinguish the two species in addition to morphological characters. The results of the present study showed that the two species analyzed presented high genetic variability in terms of polymorphic bands and average heterozygosity rate. The analysis of molecular variance indicated that most of this variability occurred among populations.

The occurrence of intranidal mating, the nest reproduction mechanism by budding and polydomy are strategies adopted by these two species that might explain the high variability found among populations.

Colony foundation by budding is a general pattern in polydomic species of invasive ants, such as the *Wasmannia* genus, and is facilitated by intranidal mating (Passera 1993, 1994). Thus, the genetic variability within colonies would decrease. However, budding may coexist with the nuptial flight in *W. auropunctata*, as in other ants of different subfamilies, such as in the Dolichoderinae *Tapinoma sessile* (Say), and in the Formicinae *Formica yessensis* Forel and *F. lugubris* Zetterstedt (Passera 1993, 1994). Thus, high rate of polygyny and occurrence of nuptial flight that permits random mating far from the origin nests, also reported for *Wasmannia* species in their native range (Souza *et al* unpublished), allow that even with the occurrence of budding, the populations of the two species analyzed here still show high intranidal genetic variability.

The high genetic distances found between the *W. auropunctata* populations in CEPLAC areas (Ilhéus) and among the other populations from Ilhéus compared to the former areas may justify the existence of aggressive behavior between non-nestmate *W. auropunctata* workers (Le Breton *et al* 2004, Errard *et al* 2005, Souza 2007), suggesting a multicolonial structure for *W. auropunctata* in Southeastern Bahia. High aggressiveness among and within the Ilhéus populations is due to a multicolonial structure of this species in native areas (Le Breton *et al* 2004, Errard *et al* 2005), because in multicolonies workers from different colonies are aggressive (Pedersen *et al* 2006).

Genetic mechanisms may be directly involved with the ecological aggressiveness and different behavior in *W. auropunctata* populations distributed in native and non-native

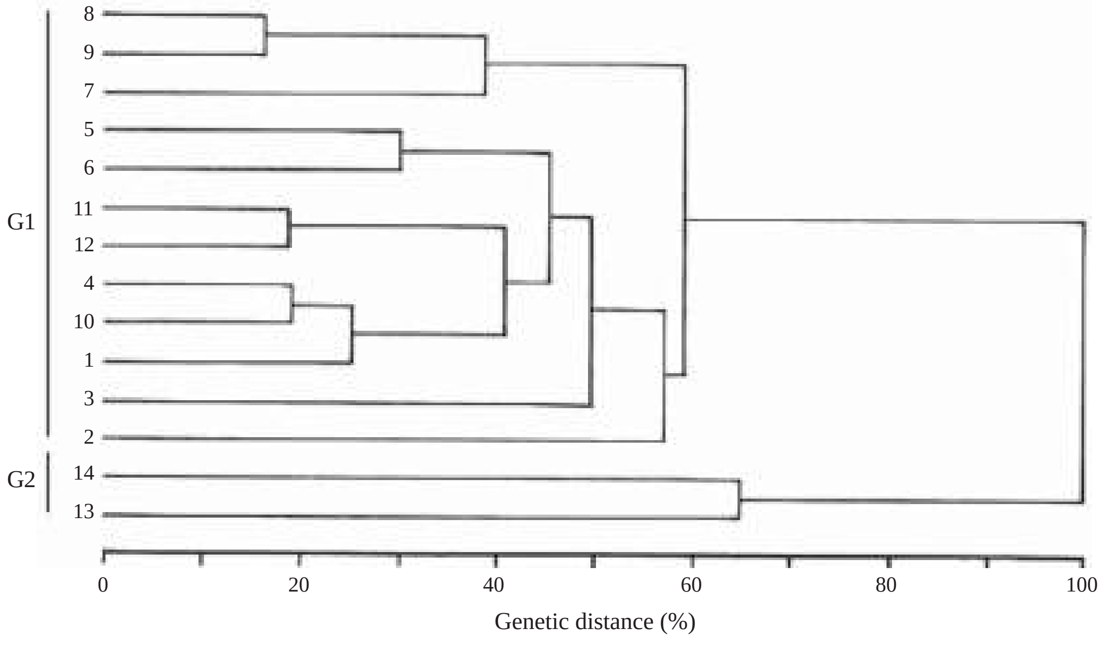

Fig 2 UPGMA from the genetic distances among the *W. auropunctata* (1 to 12) and *W. rochai* (13 and 14) populations.

areas (Delabie *et al* 2007). Similarly, native populations of the Argentine ant *Linepithema humile* (Mayr) have high intraspecific aggressiveness (Tsutsui *et al* 2000), but in areas where it was introduced, there was no aggressive behavior among co-specific individuals (Holway 1999), demonstrating single colonization (Passera 1993, 1994, Holway *et al* 2002, Le Breton *et al* 2004).

The data obtained in the present study showed genetic proximity among *W. auropunctata* populations from cocoa and coconut plantations (Ilhéus) with the forest populations in French Guyana. This can be explained by the recent and large expansion of populations from a single population, which today are distributed in habitats with similar environmental characteristics. In a similar situation, Ahrens *et al* (2005) suggested habitat connectivity events among native populations of the fire ant *Solenopsis invicta* Buren, distributed in Brazil and Argentina, to explain the weak genetic differentiation among them. The similar environmental characteristics of the areas of occurrence of *S. invicta* populations would play a significant role in the genetic flow patterns within their distribution area. Another possible explanation should be that different populations of *W. auropunctata* co-exist, each one with a distinct rate of reproductive or expansion dynamics, where some of them colonized large areas with different habitats, while others remained endemic to a single habitat or region. For example, colonization of new habitats by some *W. auropunctata* populations was directed to fragmented environments and not to its presumed native habitat, the forest. That could be the case of the populations of Jequié (sugarcane and banana plantations) or Ilhéus (coconut trees) since these were the most genetically distinct.

The *W. auropunctata* populations from the area between French Guyana and southeast Bahia, where Ilhéus is located, are in the geographic center of the core area of this species, because localities where the populations are dense and have high genetic diversity may represent the center of genetic differentiation of the taxon (Pielou 1979, Micales *et al* 1998). For example, the number of alleles and expected heterozygosity was found to be higher in native populations of *W. auropunctata* from French Guyana than in introduced populations from New Caledonia (Fournier *et al* 2005b). Similarly, native populations of *L. humile* were genetically variable, while they are probably less diverse where this ant is exotic (Tsutsui *et al* 2000).

Taking into account the genetic proximity among the *W. auropunctata* populations from Brazil and French Guyana, the high polymorphism and the estimated heterozygosity values for the two species, we are convinced that we studied native *W. auropunctata* and *W. rochai* populations because this kind of populations uses to show higher genetic variability than introduced ones (Tsutsui *et al* 2000, Fournier *et al* 2005ab, Foucaud *et al* 2006). However, we do not have enough data or samples yet from distinct localities to affirm that Ilhéus or French Guyana are inserted in the core area, especially because *W. auropunctata* is widely distributed over the Neotropics. Other comparative studies on genetics, population ecology and behavior would be necessary to give a more satisfactory answer.

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