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Genetic relationship among *Camponotus rufipes* Fabricius (Hymenoptera: Formicidae) nests by RAPD molecular markers

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ABSTRACT. Random amplified polymorphic DNA (RAPD) markers were used to investigate the genetic relationship among nests of the carpenter ant, *Camponotus rufipes*, located in the same area. Five random oligodecamers were used to amplify DNA from 108 ant workers collected from six nests. A total of 47 RAPD markers were identified, which revealed low levels of genetic differentiation among nests (Φst = 0.00218) and a low average Shannon index (0.3727) among workers within nests. These results together suggest that the *C. rufipes* nest may be formed by a single, once-mated queen and that nests produced by queens that are genetically related tend to keep their nests in close proximity to one other.

Keywords: carpenter ants, monogyne, genetic polymorphism, genetic distance.

Relação genética entre ninhos de *Camponotus rufipes* Fabricius (Hymenoptera: Formicidae) usando marcadores moleculares RAPD

RESUMO. Marcadores moleculares RAPD (*Random Amplified Polymorphic* DNA) foram usados para investigar a relação genética entre os ninhos da formiga carpinteira, *Camponotus rufipes*, localizados em uma mesma área. Cinco oligodecâmeros aleatórios foram utilizados para amplificar o DNA de 108 operárias coletadas em seis ninhos. Um total de 47 marcadores RAPD foi identificado, indicando baixa diferenciação genética entre os ninhos (Φst = 0,00218) e um baixo índice de Shannon (0,3737) entre operárias de um mesmo ninho. Os resultados sugerem que a colônia de *C. rufipes* pode ser formada por uma única rainha e, ninhos produzidos por rainhas geneticamente relacionadas possuem tendência de serem fundados em locais próximos.

Palavras-chave: formiga carpinteira, monogínica, polimorfismo genético, distância genética.

Introduction

The number of queens in an ant colony is an important characteristic to understand the genetic structure of this social insect (ROSS, 2001). *Camponotus* species have been associated with monogyny, i.e., nests headed by only one queen where the nestmates are usually full or half siblings (HEINZE et al., 1994; HÖLLDOBLER; WILSON, 1990). However, other studies have revealed cases of polygyny or polyandry in this genus (AKRE et al., 1994; FRASER et al., 2000; GADAU et al., 1996; GERTSCH et al., 1995).

Camponotus rufipes is a wood-harvester ant that inhabits areas ranging from sea level up to 3,000 m altitude in the neotropical region (FERNÁNDEZ, 2003). Different studies of *C. rufipes* have been published regarding the nestmate-recognition system (JAFFÉ; SANCHEZ, 1984), morphometric patterns of their castes (DINIZ et al., 1994), karyotypes (MARIANO et al., 2001), foraging activities (TAVARES et al., 2008), neural plasticity and behavior (SOARES et al., 2008), sucrose solution intake (SCHILMAN; ROCES, 2008), as biological indicators of environmental conditions (SILVA et al., 2006) or even protection against ectoparasites in which tufted capuchins (*Cebus apella*) rub their fur with carpenter ants (VERDERANE et al., 2007). However, as far as we know, no study has examined the genetic relationship of *C. rufipes* nests built in the same geographical area. Elucidating the genetic relationship among nests is important for understanding other aspects of ant biology such as kin discrimination, division of labor, reproductive success of individual queens in polygynous societies, and the organization of founder groups (HEINZE et al., 1994; HÖLLDOBLER; WILSON, 1990). Thus, the present study aims to present a preliminary investigation of the genetic variability

within and among *C. rufipes* nests built near each other, and verify genetic evidence of monogyny, since this species displays worker polymorphism and soldier caste aggression typical of monogyny ants (BUENO; CAMPOS-FARINHA, 1999; LAMON; TOPOFF, 1981).

Material and methods

Camponotus rufipes specimens were collected from six nests found along a cross-section in the Tietê Ecological Park (Figure 1), which is located on the east side of the city of São Paulo (23° 25' S; 46° 28' W). The distances between the nests were the following: 23 m between nests 1 and 2, 0.56 m between 2 and 3, 57 m between 3 and 4, 37 m between 4 and 5, 85 m between 5 and 6, and 202 m between 6 and 1.

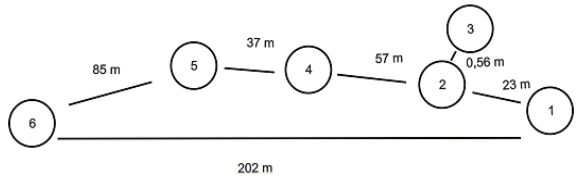

Figure 1. Distance between each nest *C. rufipes*, in the Tietê Ecological Park.

Eighteen ants were collected alive from each of the six nests and brought to the laboratory for DNA isolation. Total DNA was extracted using the modified protocol of Taggart et al. (1992), in which an STE buffer was used (0.1 M NaCl, 0.05 M Tris-HCl and 0.001 M EDTA, pH 8.0). In the RAPD reactions, 2 ng μL^{-1} of DNA template, 10 pmol of the primer, 3.0 mM of $MgCl_2$, 1U of *Taq* DNA Polymerase, 2.5 mM of dNTP and ultrapure water were used, for a total of 10 µL. The following five RAPD primers were used for genetic evaluation: OPB-10, OPB-12, OPA-2, OPA-3 and OPA-5, from Operon Technologies, USA. The RAPD reactions were performed in a thermocycler (PTC-100 MJ Research) under the following conditions: 94 for 5, 92 for 1, 31 - 35 for 1, and 72° for 2 minutes, and the final extension at 72° for 5 minutes. The samples were subjected to electrophoresis in 2% agarose gel. All RAPD reactions were carried out twice to ensure the repeatability of the bands. The bands selected to be used in the analysis were based on their resolution, signal strength and reliability. The presence (1) and absence (0) matrices were obtained through analysis of the agarose gels as performed with the following programs: ARLEQUIN version 2.0 (SCHNEIDER et al., 2000) to quantify the genetic differentiation between nests, TFPGA 1.3 (MILLER, 1997) to estimate Nei's genetic distance (NEY, 1978) and POPGENE 1.31 (YEH et al., 1999) to produce the Shannon index to calculate intra-nest genetic diversity.

Results and discussion

The UPGMA dendrogram (Figure 2) based on Nei's genetic distance showed a pattern of genetic differentiation among the six nests. AMOVA (Table 1) analysis was implemented considering all nests as one group. A total of 47 bands using five RAPD primers were identified. The genetic differences between nests was low (Φst = 0.00218) which may be a result of the low number of polymorphic bands produced by the RAPD marker. Heinze et al. (1994), using multilocus DNA fingerprinting for *C. floridanus* (Buckley) found a considerable amount of genetic variation (*F*st = 0.19), although using a more polymorphic molecular marker.

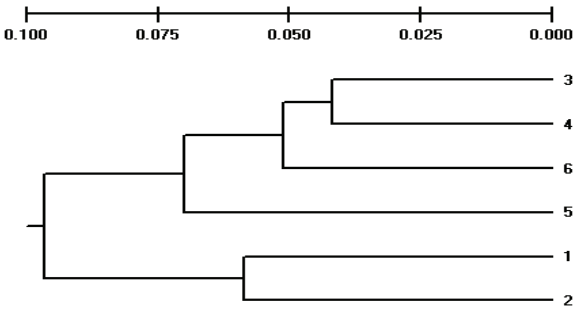

Figure 2. Dendrogram generated using UPGMA Nei's genetic distance from samples of *C. rufipes* workers inferred from RAPD. Bootstrap values based on 1000 replications.

Table 1. AMOVA analysis from the RAPD data of *Camponotus rufipes* workers collected from six nests built in the same geographical area.

Source of variation	Variance components	Percentage of variation	Φ-statistics	*P*-value
Among nests	0.00109	0.22	Φst = 0.00218	0.02542
Within nests	0.49891	99.78		

The RAPD evaluation of the six nest workers showed a high degree of genetic similarity among them. This finding suggests that the *C. rufipes* queens of the nests analyzed herein were derived from the same genetic origin and were possibly females from the same colony. Another hypothesis for this low level of genetic differentiation is the presence of satellite nests, which are usually found in this species (BUENO; CAMPOS-FARINHA, 1999).

Average intra-nest genetic variability based on the Shannon index was low (0.3727). This indicates

a genetic similarity among workers within the nests. This finding, associated with low genetic differences between nests according to AMOVA (Table 1) and the aggressive behavior of *C. rufipes*, suggest that this species appears to be a monogyne with a single mated queen. This same monogyne social structure was demonstrated for *C. ocreatus* (GOODISMAN; HAHN, 2004) and *C. floridanus* (GADAU et al., 1996). Yet, in a study of *C. herculeanus*, different populations showed different relatedness values among worker nest mates, which could not distinguish whether *C. herculeanus* nests were headed either by multiple mating queens or multiple queens reproducing in the nest (SEPPÄ; GERTSCH, 1996).

Nests produced by queens that are genetically related can keep their nests close to each other, even if the workers recognize conspecifics from other nests as alien (JAFFÉ; SANCHEZ, 1984). This situation seems to be happening with the *C. rufipes* nests analyzed herein because of the proximity in which they were built.

Further investigations using other more polymorphic markers such as microsatellites (BOOTH et al., 2009; CROZIER et al., 1999; SEPPÄ; GERTSCH, 1996) should be carried out to verify the sociogenetic structure found herein and develop a better understanding of the social genetic structure of *C. rufipes*.

Conclusion

Genetic differentiation was low between ant workers from different nests and among workers within the same nest. This suggests that the queens of the colonies analyzed herein have the same genetic origin, or that they came from satellite nests.

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References

AKRE, R. D.; HANSEN, L. D.; MYHRE, E. A. Colony size in carpenter ants (Hymenoptera: Formicidae). **Journal of the Kansas Entomological Society**, v. 67, n. 1, p. 1-9, 1994.

BOOTH, W.; YOUNGSTEADT, E.; SCHAL, C.; VARGO, E. L. Characterization of 8 polymorphic microsatellite loci in the neotropical ant-garden ant, *Camponotus femoratus* (Fabricius). **Conservation Genetics**, v. 10, n. 5, p. 1401-1403, 2009.

BUENO, O. C.; CAMPOS-FARINHA, A. E. As formigas domésticas. In: MARICONI, F. A. M. (Ed.). **Insetos e outros invasores de residências**. Piracicaba: Fealq, 1999. p 135-180.

CROZIER, R. H.; KAUFMANN, B.; CAREW, M. E.; CROZIER, Y. C. Mutability of microsatellites developed for the ant *Camponotus consobrinus*. **Molecular Ecology**, v. 8, n. 2, p. 271-276, 1999.

DINIZ, J. A. F.; VON ZUBEN, C. J.; FOWLER, H. G.; SCHLINDWEIN, M. N.; BUENO, O. C. Multivariate morphometrics and allometry in a polymorphic ant. **Insectes Sociaux**, v. 41, n. 2, p. 153-163, 1994.

FERNÁNDEZ, F. **Introducción a las hormigas de la región Neotropical**. Bogotá: Instituto de Investigacion de Recursos Biológicos Alexander von Humboldt, 2003.

FRASER, V. S.; KAUFMANN, B. P. O. B.; CROZIER, R. H. Genetic influence on caste in the ant *Camponotus consobrinus*. **Behavioral Ecology and Sociobiology**, v. 47, n. 3, p. 188-194, 2000.

GADAU, J.; HEINZE, J.; HÖLLDOBLER, D. Population and colony structure of the carpenter ant *Camponotus floridanus*. **Molecular Ecology**, v. 5, n. 6, p. 785-792, 1996.

GERTSCH, P.; PAMILO, P.; VARVIO, S. L. Microsatellites reveal high genetic diversity within colonies of *Camponotus* ants. **Molecular Ecology**, v. 4, n. 2, p. 257-260, 1995.

GOODISMAN, M. A. D.; HAHN, D. A. Colony genetic structure of the ant *Camponotus ocreatus* (Hymenoptera: Formicidae). **Sociobiology**, v. 44, n. 1, p. 21-33, 2004.

HEINZE, J.; GADAU, J.; HÖLLDOBLER, B. Genetic variability in the ant *Camponotus floridanus* detected by multilocus DNA fingerprinting. **Naturwissenschaften**, v. 81, n. 1, p. 34-36, 1994.

HÖLLDOBLER, B.; WILSON, E. O. **The ants**. Cambridge: Harvard University Press, 1990.

JAFFÉ, K.; SANCHEZ, C. Comportamiento alimentario y sistema de reclutamiento em la hormiga *Camponotus rufipes* (Hymenoptera: Formicidae). **Acta Científica Venezolana**, v. 35, n. 1, p. 270-277, 1984.

LAMON, B.; TOPOFF, H. Avoiding predation by army ants: defensive behaviours of three ant species of the genus *Camponotus*. **Animal Behaviour**, v. 29, n. 4, p. 1070-1081, 1981.

MARIANO, C. S. F.; POMPOLO, S. G.; DELABIE, J. H. C.; CAMPOS, L. A. O. Estudos cariotípicos de algumas espécies neotropicais de *Camponotus* Mayr (Hymenoptera, Formicidae). **Revista Brasileira de Entomologia**, v. 45, n. 4, p. 267-274, 2001.

MILLER, M. P. **Tools for population genetics analyses TFPGA 1.3**: A windows program for the analysis of allozyme and molecular population genetic data. Department of Biological Sciences, Northern Arizona University, 1997.

NEY, M. Estimation of average heterozigosity and genetic distance from a small number of individuals. **Genetics**, v. 89, n. 3, p. 583-590, 1978.

ROSS, K. G. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. **Molecular Ecology**, v. 10, n. 2, p. 265-284, 2001.

SCHILMAN, P. E.; ROCES, F. Haemolymph sugar levels in a nectar-feeding ant: dependence on metabolic expenditure and carbohydrate deprivation. **Journal of Comparative Physiology B: Biochemical, Systemic and Environmental Physiology**, v. 178, n. 2, p. 157-165, 2008.

SCHNEIDER, S.; ROESSLI, D.; EXCOFFIER, L. **Arlequin ver. 2.000**: A software for population genetics data analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva, 2000.

SEPPÄ, P.; GERTSCH, P. Genetic relatedness in the ant *Camponotus hercullanus*. A comparison of estimates from allozyme and DNA microsatellite markers. **Insectes Sociaux**, v. 43, n. 3, p. 235-246, 1996.

SILVA, A. F. D.; MEIRELES, B.; ROSA, J. D.; WINKLER, M.; MORINI, M. S. C.; OLIVEIRA, A. F. Evaluation of the level of metals in *Camponotus rufipes* (Hymenoptera: Formicidae) collected in different environments. **Sociobiology**, v. 47, n. 1, p. 293-304, 2006.

SOARES, P. A. O.; DELABIE, J. H. C.; ZANUNCIO, J. C.; SERRÃO, J. E. Neural plasticity in the brain of workers of the Carpenter ant *Camponotus rufipes* (Hymenoptera: Formicidae). **Sociobiology**, v. 51, n. 3, p. 705-717, 2008.

TAGGART, J. B.; HYNES, R. A.; PRODOHL, P. A.; FERGUSSON, A. A simplified protocol for routine total DNA isolation from salmonid fishes. **Journal of Fish Biology**, v. 40, n. 6, p. 963-965, 1992.

TAVARES, A. A.; BISPO, C. P.; ZANZINI, A. C. Effect of collect time on communities of epigaeic ants (Hymenoptera: Formicidae) in areas of *Eucalyptus cloeziana* and cerrado. **Neotropical Entomology**, v. 37, n. 2, p. 126-130, 2008.

VERDERANE, M. P.; FALOTICO, T.; RESENDE, M. B.; LABRUNA, M. B.; IZAR, P.; OTTONI, E. B. Anting in a semifree-ranging group of *Cebus apella*. **International Journal of Primatology**, v. 28, n. 1, p. 47-53, 2007.

YEH, F. C.; YANG, R. C.; MAO, J.; YE, Z.; BOYLE, T. J. B. **Popgene, the Microsoft Windows-based user-friendly software for population genetic analysis of co-dominant and dominant markers and quantitative traits**. Edmonton: Department of Renewable Resources, University of Alberta, 1996.

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