

Allozyme variation and genetic relatedness in a population of *Camponotus chilensis* (Hymenoptera: Formicidae) in Chile

Variación alozímica y parentesco genético en una población de *Camponotus chilensis*
(Hymenoptera: Formicidae) en Chile

LAFAYETTE C. EATON and RODRIGO G. MEDEL

Departamento de Ciencias Ecológicas, Facultad de Ciencias,
Universidad de Chile, Casilla 653, Santiago, Chile

ABSTRACT

We present data on allozyme variability of 7 loci in 16 colonies of a population of *Camponotus chilensis*. With this information, we infer the reproductive structure of the colonies and calculate the coefficient of relatedness of workers within colonies. Colonies of *C. chilensis* may have from one to at least four queens, based upon both number of genotypes and degree of relatedness among workers. Our results suggest an association between the body mass of workers and their genotype for MDH-2, with more large heterozygous individuals.

Key words: allozyme variation, *Camponotus*, ants, social insects.

RESUMEN

Presentamos datos de variabilidad alozímica de 7 loci en 16 colonias de una población de *Camponotus chilensis*. Sobre la base de esta información, inferimos la estructura reproductiva de las colonias y calculamos el coeficiente de parentesco de las obreras al interior de las colonias. El número de genotipos y el grado de parentesco entre las obreras revelaron que las colonias de *C. chilensis* pueden tener desde una hasta al menos cuatro reinas. Además, nuestros resultados sugieren una asociación entre la masa corporal de las obreras y su genotipo para MDH-2, en que los individuos más grandes tienden a ser heterocigotos.

Palabras claves: variación alozímica, *Camponotus*, hormigas, insectos sociales.

INTRODUCTION

Identification of allozymes by gel electrophoresis has served as a useful tool to infer multiple mating of queens or polygyny, and the coefficient of relatedness among individuals (Ross 1988). Although there are several problems in inferring the number of reproductive individuals from the coefficient of relatedness (reviewed in Seppä 1992), the use of allozyme analysis has the advantage that it permits distinguishing all genotypes by their phenotypes, and that it focuses on loci neutral to social evolution (Pamilo 1984, Hagen *et al.* 1988). In a recent review, Hölldobler & Wilson

(1990) indicated that monogynic colonies of the genera *Rhytidoponera*, *Aphaenogaster*, *Harpagoxenus* and *Solenopsis* exhibited a coefficient of relatedness about 0.75 as expected from the kin selection hypothesis. However, polygynic colonies in a number of species present degrees of relatedness among workers well below 0.75, thus raising the question of the mechanisms that prevent workers from producing their own offspring.

The situation where two or more queens coexist in the same nest but remain separated from each other has been reported in two species of the genus *Camponotus* (Hölldobler 1962). The genus *Camponotus*

TABLE 1

Putative-genotypes for 7 variable loci in 16 colonies of *Camponotus chilensis* and average relatedness values (r) in each colony.

Genotipos putativos para 7 loci variables en 16 colonias de *Camponotus chilensis* y valores de parentesco genético (r) entre las obreras de cada colonia.

colony	r	PGM		PGI			MDH				6-PGD												
		11	12	11	12	22	11	12	22	13	33	24	34	11	12	22	13	23	33	24	44		
1	0.968	6	0	5	1	0	6	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	
2	0.825	6	0	2	4	0	0	0	6	0	0	0	0	0	0	0	3	3	0	0	0	0	0
3	0.953	6	0	0	6	0	0	0	6	0	0	0	0	0	0	0	6	0	0	0	0	0	0
4	0.745	6	0	6	0	0	0	2	4	0	0	0	0	0	0	0	6	0	0	0	0	0	0
5	0.823	6	0	6	0	0	0	3	3	0	0	0	0	0	0	6	0	0	0	0	0	0	0
6	1.000	6	0	0	6	0	0	6	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
7	0.258	5	0	-	-	-	2	0	1	2	0	0	0	0	0	0	0	1	3	1	0	0	0
8	0.765	6	0	-	-	-	6	0	0	0	0	0	0	0	0	0	0	1	0	3	2	0	0
9	0.750	6	0	-	-	-	0	2	4	0	0	0	0	0	0	0	0	0	4	0	1	0	0
10	1.000	3	0	-	-	-	0	3	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
11	0.388	6	0	-	-	-	0	1	2	0	0	1	2	0	0	0	0	0	1	3	0	0	0
12	0.898	5	0	-	-	-	0	0	0	0	0	2	3	0	0	0	0	0	0	5	0	0	0
13	0.851	6	0	-	-	-	0	0	6	0	0	0	0	0	0	3	0	3	0	0	0	0	0
14	0.047	32	7	20	15	4	4	14	15	6	0	0	0	10	11	7	9	0	0	0	0	0	0
15	0.560	28	0	28	0	0	0	0	0	12	12	2	5	0	0	0	14	0	5	3	1	0	0
16	0.899	30	0	30	0	0	0	0	0	19	0	13	-	-	-	-	-	-	-	-	-	-	-

colony	FI-Est1				FI-Est2								FI-Est3						
	11	12	22		11	12	22	13	23	24	33	34	44	12	13	22	23	33	
1	0	0	6		0	0	0	-	6	-	0	0	-	-	-	-	-	-	-
2	6	0	0		0	0	0	-	6	-	0	0	-	-	-	-	-	-	-
3	6	0	0		0	2	4	-	0	-	0	0	-	6	0	-	0	0	-
4	3	3	0		0	0	0	-	3	-	2	1	-	0	0	-	6	0	-
5	6	0	0		0	0	0	-	0	-	3	3	-	-	-	-	-	-	-
6	6	0	0		0	6	0	-	0	-	0	0	-	0	0	-	0	6	-
7	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	24	8	8		2	5	0	5	6	4	6	7	3	2	12	4	4	7	-
15	28	0	0		0	0	0	0	0	0	26	0	0	-	-	-	-	-	-
16	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

is represented in Chile by 6 species, of which one of the most abundant and widely distributed is *Camponotus chilensis* (Snelling & Hunt 1975). In spite of being one of the numerically dominant species in central Chile (Ipinza *et al.* 1983), nothing is known about its genetic and reproductive structure. In this paper we present data on allozyme variability in a population of this species. Using this information, we infer the deviation of colonies from the mono-

gynic reproductive structure, calculate the coefficient of relatedness of workers within nests, and assess an association between genotype and body mass in two colonies.

MATERIALS AND METHODS

During April and September of 1991 and March 1992, we took samples of a total of 16 colonies of *C. chilensis* in El Manzano,

located in the canyon of the Maipo River (33°34' S, 70°24' W) about 50 km SE of Santiago. Ants were sampled from each colony only on one occasion. The colonies were separated by distances of about 10 m, and always found under rocks in the edge of shrub vegetation. We did not detect any apparent barriers between colonies. The insects were maintained alive until processed for electrophoresis. A preliminary test demonstrated that the presence or absence of the gaster did not affect the detection of variable systems, thus entire individuals were ground in a 0.2 M phosphate extraction buffer pH 7.5, with the addition of 2 drops of mercaptoethanol, 40 mg of bovine albumin and 100 mg of dithiothreitol per 100 ml of buffer. Extracts were adsorbed on 2 mm x 5 mm filter paper wicks. Horizontal starch gel electrophoresis was performed according to Conkle *et al.* (1982), using the systems: A (lithium borate) for octanol dehydrogenase (ODH), menadione reductase (MNR), and phosphoglucosmutase (PGM); system D (morpholine citrate) for malic enzyme (ME), malate dehydrogenase (MDH), 6-phosphogluco dehydrogenase (6-PGD) and shikimate dehydrogenase (SKDH); and 0.005 histidine (Brewer 1970) for glyceraldehyde phosphate dehydrogenase (GAPD), phosphogluco isomerase (PGI) and fluorescent esterase (Fl-EST). Staining recipes were from Conkle *et al.* (1982) and Richardson *et al.* (1986). Mendelian interpretation of variable systems was confirmed by the positions of bands in haploid males. Five or 6 workers were examined from 13 colonies, a total of 40 workers, virgin queens and males from 3 additional colonies. Coefficient of relatedness among workers was estimated according to Queller & Goodnight (1989, equation 6).

To search for associations between genotype and body mass, we prepared contingency tables for the variable enzymes in two colonies, one polygynic (colony 15), and one monogynic (colony 16). We used the G-test with Williams' correction (Sokal & Rohlf 1981). Inspection of colonies had revealed two size classes of workers; large workers weigh between 0.023 and 0.042 g, while small workers weigh between 0.0075

and 0.015 g. We weighed all sampled individuals for colonies 15 and 16 on an AND precision scale (0.0001 g), and the largest and smallest of collected workers were used for electrophoresis.

RESULTS AND DISCUSSION

Of the 18 presumptive loci found (see below), seven showed variation that could be given a consistent Mendelian interpretation. The putative genotypes for the 16 colonies are summarized in Table 1. Not all systems were scorable for all runs. ODH and GAPD appeared to stain the same thing, two invariant isozymes. ME also presented two isozymes, one invariant and the other

TABLE 2

Contingency tables for associations among malate dehydrogenase genotype and body size in workers of *Camponotus chilensis*. a) Colony 15, offspring of one queen. b) Colony 15, comparison of offspring of two queens. c) Colony 16, offspring of one queen.

Tablas de contingencia para las asociaciones entre el genotipo para malato deshidrogenasa y masa corporal en obreras de *Camponotus chilensis*. a) Colonia 15, descendencia de una reina. b) Colonia 15, comparación de la descendencia de dos reinas. c) Colonia 16, descendencia de una reina.

a)

		genotype	
		33	13
large	small	2	8
	large	10	4

b)

		genotype	
		13 + 33	24 + 34
large	small	10	7
	large	14	0

c)

		genotype	
		33	34
large	small	8	10
	large	11	3

identical to the variable system reported in MDH. MNR presented three invariant isozymes. SKDH was variable, but streaking prevented an adequate interpretation. Two isozymes were present in PGM and 6-PGD, and three in MDH, PGI and FI-EST. It is interesting to note that a second (invariant) system of 6-PGD was observed only in queens.

Since a maximum of two genotypes may be present in the worker offspring of a haplodiploid cross, more than one queen must be present in at least colonies 4, 7, 8, 11, 14 and 15. In colony 14, eight different genotypes were observed in workers for FI-EST₂, which implies a minimum of 4 queens. The number of inferred queens in this colony is consistent with the extremely low coefficient of relatedness among workers (0.047, Table 1). Relatedness values for variable loci should approach 0.75 for colonies with one queen. Since we included all results in the calculations, including loci homozygous for all individuals of some colonies, observed relatedness values are often greater than 0.75. For example, colony 1 had one individual heterozygous for PGI and was homozygous for the four other loci scored, giving an r of 0.968 and colony 4, which must have more than one queen since three alleles were observed for MDH, gave an r of 0.745, because no variation was observed at four of the six loci scored. Since the observed pattern may also be determined by occasional foundress association with varying queen mortality, we can not be sure of the polygynic reproductive structure of *C. chilensis*. However, in some but not all colonies where the genotypes indicate more than one queen, the relatedness values are much less than 0.75, which is in accord with the low values previously reported in polygynic species. *C. chilensis* may present polygyny in some, but not all colonies. Sample sizes of 5 or 6 are too small to be certain that there is not more than one queen, but the 30 workers, 6 males and 4 virgin queens of colony 16 showed genotypes consistent with one singly mated queen. That some colonies present patterns consistent with a monogynic and others with a polygynic reproductive structure suggests that the

polygyny is secondary, that is, supernumerary queens may be added by adoption or by fusion with other colonies (Hölldobler & Wilson 1990).

Analysis of the presumably polygynic colonies revealed a significant association between genotype and body mass only for MDH. Colony 15 most likely has two queens, one of which produces 13 and 33 offspring, the other producing 24 and 44 workers. Among the offspring of the former, there is an excess of small 33 and large 13 individuals ($G = 6.11$, $P < 0.05$, Table 2a). All 7 of the 24 and 44 workers were large, which is probably not due to chance ($G = 9.35$, $P < 0.01$, Table 2b). In colony 16, the association between MDH genotype and body mass was of borderline significance ($G = 3.86$, $P = 0.05$, Table 2c), again with an excess of large heterozygous individuals.

Although consequences of the low genetic relatedness within the nest are not well understood, some authors have indicated that high intranest genetic variability may have an adaptive value by decreasing the risk to contracting parasites or infectious diseases (Sherman *et al.* 1988, Shykoff & Schmid-Hempel 1991a, 1991b). On the contrary, others argue that a low relatedness is a mere consequence of female associations due to environmental necessity that do not necessarily convey any adaptive value (e.g., Herbers 1986). The association between genotype and body mass documented in this work is intriguing and suggests that low genetic relatedness within the nest tends to be related with body mass in a non-random fashion. Since the social tasks performed by workers of a single colony are usually related to worker body mass (Oster & Wilson 1978, Wilson 1980), it is quite possible that low genetic relatedness have important consequences on the evolution of task diversity within colonies.

ACKNOWLEDGMENTS

This research was supported by DTI grant B-3048-9011, Universidad de Chile.

LITERATURE CITED

- BREWER GJ (1970) An introduction to isozyme techniques. Academic Press, New York.
- CONKLE MT, PD HODGSKISS, LB NUNNALLY & SC HUNTER (1982) Starch gel electrophoresis of conifer seeds: a laboratory manual. U.S. Department of Agriculture, General Technical Report PSW-64.
- HAGEN RH, DR SMITH & SW RISSING (1988) Genetic relatedness among co-foundresses of two desert ants, *Veromessor pergandei* and *Acromyrmex versicolor* (Hymenoptera: Formicidae). *Psyche* 95: 191-201.
- HERBERS JM (1986) Nest size limitation and facultative polygyny in the ant *Leptothorax longispinosus*. *Behavioral Ecology and Sociobiology* 19: 115-122.
- HÖLDOBLER B (1962) Zur frage der oligogynie bei *Camponotus ligniperda* Latr. und *Camponotus herculeanus* L. (Hym. Formicidae). *Zeitschrift für Angewandte Entomologie* 49: 337-352.
- HÖLDOBLER B & EO WILSON (1990) The ants. Harvard University Press, Cambridge, Massachusetts.
- IPINZA-REGLA JH, R COVARRUBIAS & R FUEYO (1983) Distribución altitudinal de Formicidae en Los Andes de Chile central. *Folia Entomológica Mexicana* 55: 103-128.
- OSTER GF & EO WILSON (1978) Caste and ecology in the social insects. Princeton University Press, Princeton, New Jersey.
- PAMILO P (1984) Genotypic correlation and regression in social groups: multiple alleles, multiple loci and subdivided populations. *Genetics* 107: 307-320.
- QUELLER DC & KF GOODNIGHT (1989) Estimating relatedness using genetic markers. *Evolution* 43: 258-275.
- RICHARDSON BJ, PR BAVERSTOCK & M ADAMS (1986) Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, Sydney.
- ROSS KG (1988) Population and colony-level genetic studies of ants. In: Trager JC (ed) *Advances in myrmecology*: 189-215. EJ Brill, Leiden.
- SEPPÄ P (1992) Genetic relatedness of worker nestmates in *Myrmica ruginodis* (Hymenoptera: Formicidae) populations. *Behavioral Ecology and Sociobiology* 30: 253-260
- SHERMAN PW, TD SEELEY & HK REEVE (1988) Parasites, pathogens, and polyandry in social Hymenoptera. *American Naturalist* 131: 602-610.
- SHYKOFF JA & P SCHMID-HEMPEL (1991a) Genetic relatedness and eusociality: parasite-mediated selection on the genetic composition of groups. *Behavioral Ecology and Sociobiology* 28: 371-376.
- SHYKOFF JA & P SCHMID-HEMPEL (1991b) Parasites and the advantage of genetic variability within social insect colonies. *Proceedings of the Royal Society of London B* 243: 59-62.
- SNELLING RR & JH HUNT (1975) The ants of Chile (Hymenoptera: Formicidae). *Revista Chilena de Entomología* 9: 63-129.
- SOKAL RR & FJ ROHLF (1981) *Biometry: the principles and practice of statistics in biological research*. Second edition, W.H. Freeman and Co., San Francisco.
- WILSON EO (1980) Caste and division of labor in leaf-cutter ants (Hymenoptera: Formicidae: *Atta*). I. The overall pattern in *A. sexdens*. *Behavioral Ecology and Sociobiology* 7: 143-156.