GENETIC VARIABILITY AND SOCIAL STRUCTURE OF COLONIES IN *Acromyrmex heyeri* AND *A. striatus* (HYMENOPTERA: FORMICIDAE)

DIEHL, E.,[1] ARAÚJO, A. M. de[2] and CAVALLI-MOLINA, S.[2]

[1]Laboratório de Genética, Setor de Insetos Sociais, Universidade do Vale do Rio dos Sinos, C.P. 275, CEP 93001-970, São Leopoldo, RS, Brazil

[2]Departamento de Genética, Universidade Federal do Rio Grande do Sul, C.P. 15053, CEP 91501-970, Porto Alegre, RS, Brazil

Correspondence to: Elena Diehl, Laboratório de Genética, Setor de Insetos Sociais, Universidade do Vale do Rio dos Sinos, C.P. 275, CEP 93001-970, São Leopoldo, RS, Brazil, e-mail: ediehl@cirrus.unisinos.br

Received August 2, 2000 – Accepted September 21, 2000 – Distributed November 30, 2001

ABSTRACT

The breeding structure of both colony and population of social insects can be examined by genetic analysis. Colonies of the leaf-cutting ants *Acromyrmex heyeri* and *A. striatus* (Myrmicinae, Attini) were thus analyzed for isoenzyme systems MDH, a-GPDH, and AMY to describe genotype variability and social structure. A total of five loci were investigated (three for amylase and one for each other system). Ninety-seven colonies of *A. heyeri* and 103 of *A. striatus* were sampled in different localities in Southern Brazil (State of Rio Grande do Sul). The genotypes found show the occurrence of monogyny and polygyny associated or not with polyandry, which indicates that the social organization is colony-specific. The polygyny and polyandry observed are likely to be responsible for the great genotypic diversity of the colonies. The average inbreeding coefficient per colony was higher in *A. striatus* than in *A. heyeri,* which may reflect the different patterns of production of sexual individuals and nuptial flight of those two species.

Key words: Attini, genetic polymorphism, breeding structure, leaf-cutter ants.

RESUMO

Variabilidade genética e estrutura social das colônias de *Acromyrmex heyeri* e *A. striatus* (Hymenoptera, Formicidae)

A estrutura de cruzamento de colônias e populações de insetos sociais pode ser observada por análise genética. Assim, colônias de formigas cortadeiras *Acromyrmex heyeri* e *A. striatus* (Myrmicinae, Attini) foram analisadas para os sistemas isoenzimáticos MDH, a-GPDH e AMY, a fim de descrever sua variabilidade genotípica e estrutura social. Foram investigados cinco locos (três para amilase e um para cada outro sistema), em 97 colônias de *A. heyeri* e 103 de *A. striatus,* amostradas em diversas localidades do Rio Grande do Sul. Os genótipos encontrados indicaram a ocorrência de monoginia e poliginia associadas ou não à poliandria, indicando que a organização social é colônia específica. Tanto a poliginia quanto a poliandria são responsáveis pela grande diversidade genotípica das colônias. O coeficiente de endocruzamento médio por colônia foi mais alto em *A. striatus* do que em *A. heyeri* e pode refletir os diferentes padrões de produção dos indivíduos sexuados e de vôo nupcial das duas espécies.

Palavras-chave: Attini, polimorfismo genético, estrutura de cruzamento, formigas cortadeiras.

INTRODUCTION

Understanding the reproductive strategies and social structure of colonies of ant populations is an important issue concerning the sociobiology of these social insects, as related to genetic and ecological components of their adaptive strategies (Hölldobler & Wilson, 1990). Social structure characteristics include: alternative existence of only one functional queen (monogyny) or two or more queens in each colony (polygyny) with the occurrence or not of a hierarchy of dominance between them; single (monoandry) or multiple (polyandry) fertilization of the queen; and the existence or not of worker reproduction. Different reproduction strategies observed in ants involve dispersion of the reproductive forms; colony foundation by one queen only (haplometrosis) or by many queens together (pleometrosis); colony fission; and recruitment of new queens (Heinze & Tsuji, 1995; Rüppell *et al.*, 1998). Sexual dispersion determines the breeding structure of the population, thus influencing the degree of genetic relatedness (Seppä, 1992). Knowledge of these aspects is essential to studies about the origin and maintenance of sterile castes as well as to foresee evolution of colony characteristics (Pamilo, 1991a, b, c).

Early studies about the social structure of Hymenoptera pointed to monogyny happening with monoandry as the most common breeding mechanism, as occurs in *Melipona subnitida* (Meliponidae) (Contel & Kerr, 1976), *Aphaenogaster rudis* (Formicidae) (Crozier, 1973), and *Solenopsis invicta* (Formicidae) (Hung & Vinson, 1976). However, later studies showed polygyny as the principal social structure of many species (Pamilo & Rosengren, 1984; Kaufmann *et al.*, 1992; Pamilo, 1993). In some species polygyny can be facultative (Seppä, 1992; Aron *et al.*, 1999).

Distribution of the leaf-cutting ants *Acromyrmex heyeri* and *A. striatus* (Myrmicinae, Attini) is restricted to Uruguay, Argentina, Bolivia, and Southern Brazil. In Rio Grande do Sul, the southernmost state in Brazil, they occur in high concentration. These species present differences in nest building, patterns of seasonal and daily activity, production of sexual individuals, and nuptial flight (Diehl-Fleig, 1993). The objective of this investigation was to describe the genetic variability of colonies as a way to establish the social structure of *A. heyeri* and *A. striatus*.

MATERIAL AND METHODS

Colony samples

In the fall and summer of two consecutive years adults of *A. heyeri* and *A. striatus* were collected from 11 localities in the State of Rio Grande do Sul (Table 1). Each locality was assumed to comprise a population; groups of colonies from one population occupying areas of up to 1 km^2, distant from other groups by least 5 km, were considered demes. Males, gynes, and workers were collected from colonies of each deme for each species. Individuals from 97 colonies of *A. heyeri* and from 103 colonies of *A. striatus* were analyzed by electrophoresis for malate dehydrogenase (MDH – EC 1.1.1.37) and a-glycerophosphate dehydrogenase (a-GPDH – EC1.1.1.8) systems. The amylase (AMY – EC3.2.1.1) system was analyzed in individuals from 79 of the 97 *A. heyeri* colonies and in individuals from 88 colonies of the 103 *A. striatus* colonies. Seven to ten individuals per colony were analyzed.

Electrophoresis

Variation at isoenzyme loci was resolved using horizontal electrophoresis. Eight percent polyacrylamide gels and Poulik (1957) buffers were used for MDH; 6% and 7% polyacrylamide gels and Roose & Gottlieb (1976) buffers were used for a-GPDH and AMY, respectively. Gels were run at a voltage gradient of 10V/cm until the front line was 9 cm from the origin. Staining procedures for MDH and a-GPDH were performed as described by Ayala *et al.* (1972); AMY was stained according to Chao & Scandalios (1972).

Measures of genetic and genotype diversity

Genetic variability was calculated for different hierarchic levels: colony, deme, population, and species. For each level, genetic diversity was estimated based on five-loci data using the following criteria of assessment: proportion of polymorphic colonies, number of alleles per locus, observed heterozygosity, expected heterozygosity, and inbreeding coefficient. For each species, the proportion of polymorphic colonies (PC) for each locus was calculated by means of the number of colonies with two or more alleles per locus, in which the frequency of the most common allele was inferior to 0.99, divided by the total number of colonies analyzed.

TABLE 1
Geographical localization of the sampling areas and number of *Acromyrmex heyeri* and *A. striatus* colonies analyzed in each population.

Population	Geographical latitude (south) /longitude (west)	Deme	Number of colonies	
			A. heyeri	*A. striatus*
São Leopoldo	29º45'/51º08'	SLNC	15	9
		SLME	9	5
		SLSJ	1	8
		SLCA	10	1
		SLCP	1	3
Gravataí	29º56'/50º59'	GCE	3	–
		GPR	10	–
Viamão	30º05'/51º01	VAGRO	4	1
		VURG	6	–
Osório	29º53'/50º15'	OPO	2	14
		OR	1	–
Arroio do Sal	29º38'/49º56'	ASR	3	6
		ASC	4	6
Torres	29º20'/49º43'	TP	4	5
		TI	11	11
		TCI	10	9
Barra do Ribeiro	30º17'/51º17'	BRHS	3	2
Picada Verão	29º33'/51º02'	PVC	–	2
Nova Petrópolis	29º22'/51º07'	NPE	–	15
Eldorado do Sul	30º06'/51º20'	ESBR	–	4
São Pedro da Serra	29º26'/51º31'	SPAS	–	2

At the colony level, the average number of alleles per locus (A'col) was estimated by means of the total number of different alleles found in each locus, per colony, divided by the number of colonies analyzed. Observed heterozygosity and expected heterozigosity were calculated based only on genotypes detected in diploid individuals (gynes and workers). Average observed heterozygosity per locus per colony (h_o'col) was calculated using the sum of heterozygosity observed in each locus (h_o), per colony (h_o = number of observed heterozygotes, per locus in each colony, divided by the number of diploid individuals analyzed) divided by the number of colonies analyzed. Average expected heterozygosity per locus per colony (h_e'col) was calculated using the sum of expected heterozygosity per colony, in each locus [using the formula $h_e = 1 - \sum x_i^2$, where x_i is the frequency of the i^{th} allele in each locus (Nei, 1972)] divided by the number of colonies analyzed. The inbreeding coefficient per locus per colony (F'col) was calculated as h_e'col – h_o'col/h_e'col.

At deme and population level, average values of heterozygosity observed (H_o) and heterozygosity expected (H_e) were calculated over all loci and over all colonies of each deme or population by the sum of h_o or h_e obtained in each locus from all colonies of each deme or population divided by the total number of loci analyzed. The average inbreeding coefficient (F) was calculated over all loci and over all colonies of the deme or population as $H_e - H_o / H_e$. Tests of hypotheses for the results obtained for F were performed using chi-square statistics, $\chi^2 = F^2N$, where N is the number of individuals analyzed (Li & Horvitz, 1953).

Social structure

The social structure of the colonies was inferred from the analysis of the diploid (workers and gynes) and haploid (males) genotypes found in each colony.

RESULTS

Three alleles were detected for the MDH locus: *Mdh-1*^{100} present only in *A. heyeri*; *Mdh-1*^{74}, rare, but shared by both species; and *Mdh-1*^{57}, only in *A. striatus*. For a-GPDH, five alleles were detected in both species. The AMY system detected three loci: *Amy-1* which was monomorphic in both species; *Amy-2* with two alleles: *Amy-2*^{100} restricted to *A. heyeri* and *Amy-2*^{92} detected in all individuals of *A. striatus* and only in the individuals from three colonies of *A. heyeri*. The *Amy-4* locus presented seven alleles common to both species. The frequency of each allele by species is shown in Table 2.

The proportion of polymorphic colonies varied greatly between the different loci analyzed (Table 3). Although *Amy-2* presented two alleles in *A. heyeri,* no polymorphism within-colony was observed. Few colonies exhibited polymorphism for the *Mdh-1* locus, only 1% in *A. heyeri,* and 8% in *A. striatus,* because a different allele was predominant in each species.

The number of alleles per locus detected in individual colonies ranged from one to three in both species (Table 3). This higher number was verified in the *Amy-4* locus, which presented seven alleles considering all individuals analyzed.

TABLE 2
Allele frequencies of the five isoenzyme loci in *Acromyrmex heyeri* and *A. striatus*, calculated from the genotypes of diploid (gynes and workers) and haploid (males) individuals of all colonies analyzed. The denomination given to each allele corresponds to the relative electrophoretic migration (RM) of the respective alloenzyme.

		Acromyrmex heyeri		*Acromyrmex striatus*	
Locus	**Alelle**	**N**	**Freq.**	**N**	**Freq.**
Amy-1	100	900	1.0	1,200	1.0
Amy-2	100	879	0.977	0	–
	92	21	0.023	1,200	1.0
Amy-4	100	3	0.003	53	0.043
	87	7	0.007	99	0.079
	80	56	0.061	790	0.633
	67	345	0.375	192	0.154
	60	240	0.261	49	0.039
	53	208	0.226	61	0.049
	33	61	0.067	4	0.003
a-Gpdh-1	100	100	0.078	1,007	0.619
	83	201	0.156	100	0.062
	75	260	0.201	423	0.260
	64	684	0.529	86	0.053
	48	47	0.036	10	0.006
Mdh-1	100	1,269	0.957	0	–
	74	57	0.043	26	0.016
	57	0	–	1,627	0.984

TABLE 3
Polymorphism data for *A. heyeri* and *A. striatus* per locus. N = number of colonies analyzed; PC = proportion of polymorphic colonies; A'col = average number of alleles per colony; h_o'col = average observed heterozygosity per colony; h_e'co = average expected heterozygosity per colony; F'col = inbreeding average coefficient per colony.

Species	Locus	N	PC (%)	A'col x – limits		h_o'col x – limits		h_e'col x – limits		F'col x – limits		% demes with F'col > 0
A. heyeri	*Mdh-1*	97	1.0	1.1	(1.0-1.5)	0.2	(0-5.0)	0.1	(0-4.8)	0	(–0.042-0)	0
	a-Gpdh-1	97	49.0	1.7	(1.0-3.0)	19.9	(0-80.0)	20.0	(0-54.0)	0.005	(–1.464-1.0)	17.6
	Amy-1	79	0	1.0	–	0	–	0	–	0	–	0
	Amy-2	79	0	1.0	–	0	–	0	–	0	–	0
	Amy-4	79	63.0	2.0	(1.3-3.0)	13.1	(0-80.0)	28.3	(8.4-48.8)	0.152	(–0.667-1.0)	66.7
A. striatus	*Mdh-1*	103	8.0	1.1	(1.0-1.5)	2.3	(0-19.1)	3.0	(0-13.3)	0.233	(–0.667-1.0)	0.06
	a-Gpdh-1	103	54.0	1.7	(1.0-2.5)	25.7	(0-71.4)	24.8	(0-49.9)	–0.036	(–0.555-0.5)	0
	Amy-1	88	0	1.0	–	0	–	0	–	0	–	0
	Amy-2	88	0	1.0	–	0	–	0	–	0	–	0
	Amy-4	88	51.0	1.8	(1.1-3.0)	2.9	(0-8.5)	20.1	(4.1-53.1)	0.856	(–0.171-1.0)	86.7

The average heterozygosity observed per locus per colony (h_o'col) ranged from 0% to 80% in *A. heyeri* and 0% to 71.4% in *A. striatus.* The average heterozygosity expected (h_e'col) varied between 0% and 54% and between 0% and 49.9% in each species, respectively. With regard to the inbreeding coefficient per locus per colony (F'col), the variation was very significant between loci, with many colonies of both species showing a significant deviation of panmixis (Table 3).

Table 4 presents average observed (H_o) and expected (H_e) heterozygosities as well as average inbreeding coefficient (F) for demes and populations when all five loci were taken into account. For *A. heyeri,* a total of six populations, comprising sixteen demes, and more five isolated demes were analyzed for those parameters.

As for the populations, only Osório and Torres showed inbreeding coefficients equivalent to zero; the other four presented significant values of F. The Arroio do Sal population exhibited a significant negative F, indicating an excess of heterozygotes, and there are good reasons to think that this finding is reliable as discussed later in this article. When the analysis is made at the level of the demes, F was positive in some of them, negative or zero in others, indicating a greater heterogeneity between demes than between populations.

The picture for the species *A. striatus* is not strictly comparable to that of *A. heyeri,* since most data obtained refers to demes and to only three populations as a whole (São Leopoldo, Arroio do Sal, and Torres).

These populations always presented F > 0, and out of the 17 demes studied, only two showed F = 0 (SLCP and VAGRO).

Analyses of the diploid (gynes and workers) and haploid (males) genotypes of individual colonies allowed evaluation of the genetic structure and inferences about the social structure of each species, particularly regarding the minimum number of queens present in each colony and the minimum number of males which fertilizated the queen or queens. The results concerning the genotype structure of each colony and the frequencies of each type were similar for both species (Table 5). The *Mdh-1* locus was of little use because each species has only two alleles, one of them with low frequency. However, for a-*Gpdh-1* and *Amy-4* loci, a great variability of genotypes in each colony was found, which ranged from one to four homozygous types per colony.

TABLE 4
Average observed (H_o) and expected (H_e) heterozygosities, and average inbreeding coefficient (F) per colony, in each deme (capital letters) and population (small letters) of *Acromyrmex heyeri* and *A. striatus* for data from the five loci analyzed.

	Acromyrmex heyeri					*Acromyrmex striatus*				
Deme/Pop.	**H_o**	**H_e**	**F**	**c^2 total**	**d.f.**	**H_o**	**H_e**	**F**	**c^2 total**	**d.f.**
SLNC	0.040	0.056	0.286	16.423[a]	7	0.072	0.082	0.122	20.173[b]	6
SLME	0.100	0.106	0.057	1.735	9	0.082	0.088	0.068	40.313[c]	4
SLSJ	0	0.090	1.0	7.000[a]	2	0.040	0.083	0.518	27.829[c]	6
SLCA	0.046	0.100	0.540	54.418[c]	7	0.143	0.173	0.173	9.160[a]	2
SLCP	0	0	0	0	0	0.111	0.074	–0.500	3.000	2
S. Leopoldo	0.058	0.086	0.326	50.476[c]	11	0.065	0.087	0.253	98.462[c]	9
GCE	0.094	0.133	0.293	11.988[a]	4	–	–	–	–	–
GPR	0.068	0.114	0.404	24.000[b]	8	–	–	–	–	–
Gravataí	0.067	0.115	0.417	24.163[b]	8	–	–	–	–	–
VAGRO	0.014	0.072	0.806	18.217[b]	4	0.125	0.235	0.468	0.872	1
VURG	0.048	0.110	0.564	28.122[c]	4	–	–	–	–	–
Viamão	0.035	0.096	0.635	44.021[c]	5	–	–	–	–	–
OPO	0.170	0.164	–0.037	20.251[c]	4	0.056	0.107	0.476	38.649[c]	9
OR	0.400	0.270	–0.482	1.161	2	–	–	–	–	–
Osório	0.219	0.177	–0.237	4.077	5	–	–	–	–	–
ASR	0.157	0.143	–0.098	3.660	5	0.029	0.118	0.754	36.917[c]	5
ASC	0.137	0.114	–0.202	61.993[c]	4	0.152	0.105	–0.448	10.466[a]	3
A. do Sal	0.140	0.124	–0.129	50.278[c]	6	0.091	0.112	0.188	59.029[c]	5
TP	0.089	0.112	0.205	8.713[a]	2	0.065	0.104	0.375	24.526[c]	4
TI	0.039	0.083	0.530	33.764[c]	6	0.006	0.038	0.842	59.760[c]	4
TCI	0.084	0.089	0.056	2.458	7	0.097	0.138	0.297	24.160[b]	7
Torres	0.049	0.088	0.443	9.309	8	0.051	0.087	0.414	99.441[c]	8
BRHS	0.029	0.104	0.721	19.454[c]	4	0.072	0.085	0.153	8.565[a]	3
PVC	–	–	–	–	–	0	0.049	1.000	7.000[b]	1
NPE	–	–	–	–	–	0.099	0.101	0.020	94.540[c]	4
ESBR	–	–	–	–	–	0	0.043	1.000	17.000[c]	1
SPAS	–	–	–	–	–	0.068	0.228	0.702	35.878[c]	6
Species	0.066	0.097	0.320	10.342	11	0.062	0.096	0.354	405.376[c]	10

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.

The possible social structure of *A. heyeri* and *A. striatus* colonies was inferred from these data by analyzing the occurrence of different types of genotypes in each colony (Table 6). Considering all the genotypes together, for the *Mdh-1*, a-*Gpdh-1,* and *Amy-4* loci, 15% and 24% of the *A. heyeri* and *A. striatus* colonies, respectively, can be explained by monogyny and monoandry; 68% of

A. heyeri and 57% of *A. striatus* colonies can descend from one necessarily heterozygous queen and multiple mates. Moreover, 17% and 19% of the colonies of *A. heyeri* and *A. striatus,* respectively, can only be explained by polygyny associated with polyandry.

TABLE 5
Frequencies of colonies of *Acromyrmex heyeri* and *A. striatus* according to the genotypes in *Mdh-1*, a-*Gpdh-1* and *Amy-4* loci.

Locus	Colony genotype structure	*A. heyeri*		*A. striatus*	
		N	Freq.	N	Freq.
Mdh-1	1 homozygous	96	0.99	94[a]	0.91
	1 homo-1 heterozygous	1	0.01	8[a]	0.08
	2 homozygous	–	–	1[a]	0.01
a-Gpdh-1	1 homozygous	48[a]	0.49	44[a]	0.43
	1 heterozygous	4[a]	0.04	2[a]	0.02
	1 homo-1 heterozygous	18[a]	0.19	22[a]	0.21
	1 homo-1 hemizygous	2[a]	0.02	3[a]	0.03
	1 homo-2 heterozygous	2[b]	0.02	3[b]	0.03
	1 homo-5 heterozygous	0	0	1[b]	0.01
	2 homozygous	3[b]	0.03	4[b]	0.04
	2 heterozygous	0	0	1[b]	0.01
	2 homo-1 heterozygous	16[b]	0.17	14[b]	0.13
	2 homo-2 heterozygous	1[b]	0.01	5[b]	0.05
	3 homozygous	2[c]	0.02	0	0
	3 homo-1 heterozygous	0	0	1[b]	0.01
	2 homo-3 heterozygous	0	0	1[c]	0.01
	2 homo-1 hetero-1 hemizygous	0	0	1[c]	0.01
	3 heterozygous	0	0	1[c]	0.01
	2 homo-1 hemizygous	1[c]	0.01	0	0
Amy-4	1 homozygous	25[a]	0.32	36[a]	0.41
	1 heterozygous	2[a]	0.02	0	0
	1 homo-1 heterozygous	6[a]	0.08	6[a]	0.07
	2 homozygous	19[a]	0.24	26[b]	0.30
	2 homo-1 heterozygous	13[b]	0.16	2[b]	0.02
	1 homo-1 hemizygous	5[b]	0.06	9[b]	0.10
	2 homo-1 hemizygous	0	0	6[c]	0.07
	1 homo-2 heterozygous	2[b]	0.03	0	0
	1 hetero-1 hemizygous	1[b]	0.01	0	0
	3 homozygous	6[c]	0.08	2[c]	0.02
	4 homozygous	0	0	1[c]	0.01

[a] Monogyny and monoandry; [b] monogyny and polyandry; [c] polygyny and polyandry.

DISCUSSION

The results obtained in the present investigation showed that the proportion of polymorphic colonies varied greatly between the different loci. When all loci are considered together, the proportion of polymorphic colonies was high and very much the same in both species, 80.4% in *A. heyeri* and 80.6% in *A. striatus.* This result is interesting since both species showed a great number of demes (and populations) with F > 0 (Table 4). Thus, despite the high levels of inbreeding in the species, *A. heyeri* and *A. striatus* are able to maintain a considerable number of polymorphic colonies. The polymorphism found in several colonies shows the Mendelian ratios expected, in accordance with a model of monogynic and monoandric colonies. Other findings fitted a monogynic but polyandric model, where as for many colonies only polygyny with polyandry can explain the polymorphism (Table 5).

Monogynic species usually exhibit a high within-colony relatedness, near 75%, which can be reduced only by polyandry (Ross & Fletcher, 1985a, b; Sundström, 1989).

In the case of polygynic species, within-colony relatedness is smaller, though it depends on relatedness between the queens (Stille *et al.*, 1991; Ross, 1993). In polygynic colonies a hierarchy between the queens can occur (Ross, 1988; Heinze, 1990; Heinze & Smith, 1990), or a queen multiple mate, not necessarily using the sperm of all males in the same proportion (Ross, 1986). In these situations, the degree of within-colony relatedness can be higher than expected because a smaller number of females and males effectively contribute to brood production; in other words, the effective size of the colony is smaller than the actual size.

The estimate of relatedness sheds no light on this. It can only indicate an average number of reproductive individuals necessary to produce the gene variability observed. Of course, the degree of relationship between queen and males increases the degree of intracolony relatedness. However, this cannot be recognized through analysis of the brood.

The average inbreeding coefficients per colony varied according to locus and with the number of colonies sampled. When the data from the five loci were considered, 62.5% and 82.4% of the demes of *A. heyeri* and *A. striatus*, respectively, showed significant levels of inbreeding. Nevertheless, the ASC demes of both species presented negative inbreeding coefficients, indicating a significant excess of heterozygotes per colony.

The high heterozygosity observed in ASC demes could be related to: a large initial number of individuals founding the colonies of these demes, outbreeding occurring in a large population, a recent mixture of populations, preferential crosses, or even differences in the gene frequencies between sexes. Concerning the latter, if in ant colonies specialization occurs in the production of sexual individuals and if in one population only a small number of colonies produce males, then gene frequencies between sexes could differ only by chance.

TABLE 6
Number of *Acromyrmex heyeri* and *A. striatus* colonies whose social structure can be inferred from the genotypes of workers, gynes, and males.

Species	Monogyny and monoandry		Monogyny[a] and polyandry		Polygyny and polyandry	
	N	Freq.	N	Freq.	N	Freq.
Acromyrmex heyeri	12	0.15	54	0.68	13	0.17
Acromyrmex striatus	21	0.24	50	0.57	17	0.19

[a] Obligatory heterozygous queens; [b] considering the five loci together were analyzed 79 and 88 colonies of *A. heyeri* and *A. striatus*, respectively.

As a consequence, deviations will occur in the genotypic proportions with an excess of homozygotes or heterozygotes (Pamilo & Varvio-Aho, 1979). However, this is not the case of *A. heyeri* and *A. striatus* since in these species there is no specialization in the production of sexual individuals (Diehl-Fleig, 1993).

The hypothesis for the increase in heterozygote frequency as an immediate consequence of a recent mixture of populations, as defended by Crow & Kimura (1970), seems to be the best explanation for the high heterozygosity of the above-mentioned ASC demes. This would involve the fact that the occupied by these colonies was altered in recent years after receiving thousands of cubic meters of sand of different origins.

Thus, it is reasonable to suppose that this change favored the introduction of a great number of individuals from different colonies of *A. heyeri* and *A. striatus* of different origin and, consequently, presenting different genotypes.

A smaller number of demes with significant average inbreeding coefficient per colony was found in *A. heyeri* than in *A. striatus.* One factor that could be contributing to this difference is the pattern of nuptial flight observed in these species. In *A. heyeri,* it concerns thousands of sexual individuals from several colonies which fly together, favoring panmixis and reducing inbreeding. Compared to *A. striatus,* there is low dispersion of alate individuals as well as a small number of individuals participating in that flight (Diehl-Fleig, 1993). As the alate individuals do not go far away, gene flow is restricted, increasing the probability of mating between related individuals. Moreover, analysis of the social structure indicates a greater frequency of monogyny associated with monoandry in *A. striatus* (Table 6). These facts lead to less intracolony genotype variation in this species, which could increase the inbreeding coefficient.

Parental genotypes can be inferred from the brood genotypes. However, when the workers are heterozygous it is impossible to establish with full certainty the genotypes of the queen and the male, because they could be daughters of a homozygote queen inseminated by one or more hemizygous males for the other allele. This situation can be reverted when data about the male sibship are available since the males are haploids and have a singler allele originated from the mother. Considering the data for a-*Gpdh-1* and *Amy-4* loci, males with different alleles from their sisters were found in two colonies of *A. heyeri* and in seven of *A. striatus* (Table 5). Therefore, in 2.1% and 6.8% of *A. heyeri* and *A. striatus* colonies, respectively, the presence of at least two queens is confirmed. Moreover, the occurrence of two homozygotes together with heterozygotes for different alleles, or the occurrence of three or four homozygotes in only one colony suggests polygyny with polyandry.

When the three polymorphic loci are considered, the number of colonies whose social structure is inferred to be monogynic (the queen being heterozygous) and polyandric increases in relation to the analysis of each locus independently. The same is true in the case of polygynic and polyandric colonies. Thus, polygyny and polyandry can be more easily detected when more loci are studied. The results obtained in the present investigation support the assertion that no more than 15% of *A. heyeri* and 24% of *A. striatus* colonies are monogynic and monoandric. Higher (47.8%) and lower (8.7%) frequencies were found, respectively, in *Acromyrmex crassispinus* and *Acromyrmex balzani*, two other leaf-cutter ant species (Diehl-Fleig & Souza, 1999). These are maximum values because in the absence of polymorphism, multiple queens, each inseminated by more than one male, are not detected by alloenzyme analysis. In this case, polygyny can only be detected during nest excavation when two or more queens are found. However, it is important to consider that this orthodox form of polygyny detection can lead to an overestimate, because many queens are not functional. Delabie (1989) showed that the developmental degree of ovarioles indicated only one or two reproductive queens, though 50% of *Acromyrmex subterraneus brunneus* colonies were polygynic. In most of the *A. heyeri* and *A. striatus* colonies, apparently with monogyny or polygyny associated with polyandry, the genotype frequencies in each colony suggest a male differential contribution and/or a hierarchy of reproductive queens.

Although ants are characterized by haplodiploidy in terms of sex determination system, loci responsible for sex determination have been described in some species (Ross & Fletcher, 1985b). The homozygotes for these loci are sterile or non-viable males, which cause an extra load to the colony. Polyandry affects the distribution

of these males between the colonies, but does not change their frequency in the population (Page, 1980, 1986; Crozier & Page, 1985). The increased genetic variation among all diploid offspring may decrease the deleterious effect of non-functional diploid male survival and colony productivity (Pedersen & Boomsma, 1999). Out of the 118 males of *A. heyeri* and 140 of *A. striatus* analyzed in this work, 2.5% and 0.7%, respectively, were diploid. It is important to emphasize that such males can only be detected when they are heterozygotes, which implies that the values obtained could be underestimates.

While high levels of polyandry were detected in monogynic colonies of some bees and wasps (Page, 1980; Ross, 1986), the same does not seem to be true for monogynic ants (Ward, 1983; Ross & Fletcher, 1985a; Have *et al.,* 1988; Pamilo, 1991d; Keller & Reeve, 1994). The absence of polyandry or only slight polyandry (oligoandry) in monogynic colonies can be in agreement with the load hypothesis occasioned by diploid males. Monogynic colonies of *Solenopsis invicta* when producing diploid males suffer such intense disturbance that extinction may ensue (Ross & Fletcher, 1986). In the case of polygynic colonies, polyandry produces a few diploid males, but they do not cause upset (Pamilo & Rosengren, 1984; Ross & Fletcher, 1986; Pamilo, 1993; Pamilo *et al.*, 1994).

The diversity hypotheses emphasize the adaptive significance of the increase of gene variability among workers; so polyandry would be particularly important and adaptive in monogynic species (Crozier & Page, 1985; Page, 1986; Sherman *et al.*, 1988; Keller & Reeve, 1994). In polygynic species, the existence of many queens in one colony reduces the effects of polyandry (Pamilo, 1993), at the same time that it increases gene diversity (Keller & Reeve, 1994; Pedersen & Boomsma, 1999).

Another interesting fact observed in *A. heyeri* and *A. striatus* colonies was the absence of heterozygotes in some colonies with two or more homozygotes. Crozier & Consul (1976), when hypothesizing on selection at the colony level, suggested that, in colonies with heterozygous queens, defense and foraging tasks would be performed more by heterozygous than by homozygous workers. As a consequence, these would be more subject to loss, a situation causing a deficiency of heterozygotes in these colonies. Although this hypothesis can explain the results obtained in the present study, its confirmation would need the comparison between genotypes of workers that execute external tasks with those responsible for the internal tasks of the colony.

With regard to the genotype diversity found in most colonies of the two *Acromyrmex* species, it is likely that polyandry, associated either with monogyny or polygyny, is largely responsibility for the genotype diversity in their colonies. The results suggest that the social structure (mono- or polygyny with mono- or polyandry) is characteristic to each colony and not to the species as a whole. The small degree of variation found between populations suggests that the organization of the social structure of colonies is similar in the different populations studied.

Acknowledgments — We thank Martina da Silva and Senilda Fritsch for their technical assistance. This research was supported by grants from CAPES, CNPq, FAPERGS, FINEP, PROPESP-UFRGS and PROENPE-UNISINOS.

REFERENCES

ARON, S., CAMPAN, E., BOOMSMA, J. J. & PASSERA, L., 1999, Social structure and split sex ratios in the ant *Pheidole pallidula. Ethol. Ecol. & Evol., 11*: 209-227.

AYALA, F. J., POWELL, J. R., TRACEY, M. L., MOURÃO, C. A. & PEREZ-SALAS, S., 1972, Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. *Genetics, 70*: 113-139.

CHAO, S. E. & SCANDALIOS, J. G., 1972. Developmentally Dependent Expression of Tissue Specific Amylases in Maize. *Molec. Gen. Genetics, 115*: 1-9.

CONTEL, E. P. B. & KERR, W. E., 1976, Origin of males in *Melipona subnitida* estimated from data of an isozymic polymorphic system. *Genetica, 46*: 271-277.

CROW, J. F. & KIMURA, M., 1970, *An Introduction to Population Genetics Theory*. Harper & Row Publishers, NY, 591p.

CROZIER, R. H., 1973, Apparent differential selection at an isozyme locus between queens and workers of the ant *Aphaenogaster rudis*. *Genetics, 73*: 313-318.

CROZIER, R. H. & CONSUL, P. C., 1976, Conditions for genetic polymorphism in social Hymenoptera under selection as the colony level. *Theor. Popul. Biol., 10*: 1-9.

CROZIER, R. H. & PAGE, R. E., 1985, On being the right size: male contributions and multiple mating in social Hymenoptera. *Behav. Ecol. Sociobiol., 18*: 105-115.

DELABIE, J. H. C., 1989, Observações sobre a ocorrência de poliginia em colônias de *Acromyrmex subterraneus brunneus* Forel, 1893, em cacauais. *An. Soc. Ent. Brasil., 18*: 193-197.

DIEHL-FLEIG, E., 1993, Sex ratio and nuptial flight pattern of the leaf-cutting ants *Acromyrmex heyeri* and *A. striatus* (Hymenoptera, Formicidae). *Insectes Soc., 40*: 111-113.

DIEHL-FLEIG, E. & SOUZA, F. M., 1999, Variabilidade isoenzimática e organização social de *Acromyrmex crassispinus* Forel e *A. balzani* Emery (Hymenoptera, Formicidae). *Rev. Brasil. Entomol., 43*: 55-59.

HAVE, T. M. Van Der, BOOMSMA, J. J. & MENKEN, S. B. J., 1988, Sex-investment ratios and relatedness in the monogynous ant *Lasius niger* (L.). *Evolution, 42*: 160-172.

HEINZE, J., 1990, Dominance behavior among ant females. *Naturwissenschaften, 77*: 41-43.

HEINZE, J. & SMITH, T. A., 1990, Dominance and fertility in a functionally monogynous ant. *Behav. Ecol. Sociobiol., 27*: 1-10.

HEINZE, J. & TSUJI, K., 1995, Ant reproductive strategies. *Res. Popul. Ecol., 37*: 135-149.

HÖLLDOBLER, B. & WILSON, E. O., 1990, *The ants*. The Belknap Press of Harvard University Press, Cambridge, Mass, 732p.

HUNG, A. C. F. & VINSON, S. B., 1976, Biochemical evidence for queen monogamy and sterile male diploid in the fire ant *Solenopsis invicta*. *Isozyme Bull., 9*: 55.

KAUFMANN, B., BOOMSMA, J. J., PASSERA, L. & PETERSEN, K. N., 1992, Relatedness and inbreeding in a French population of the unicolonial ant *Iridomyrmex humilis* (Mayr). *Insectes Soc., 39*: 195-213.

KELLER, L. & REEVE, H. K., 1994, Genetic variability, queen number, and polyandry in social Hymenoptera. *Evolution, 48*: 694-704.

LI, C. C. & HORVITZ, D. G., 1953, Some methods of estimating the inbreeding coefficient. *Amer. J. Hum. Genet., 5*: 107-117.

NEI, M., 1972, Genetic distance between populations. *Am. Nat., 106.949*: 283-292.

PAGE, R. E., 1980, The evolution of multiple mating behavior by honey bee queens (*Apis mellifera* L.). *Genetics, 96*: 263-273.

PAGE, R. E., 1986, Sperm utilization in social insects. *Ann. Rev. Entomol., 31*: 297-320.

PAMILO, P. & VARVIO-AHO, S. L., 1979, Genetic structure of nests in the ant *Formica sanguinea*. *Behav. Ecol. Sociobiol., 6*: 91-98.

PAMILO, P., 1991a, Evolution of colony characteristics in social insects. 1. Sex allocation. *Am. Nat., 137*: 83-107.

PAMILO, P., 1991b, Evolution of colony characteristics in social insects. 2. Number of reproductive individuals. *Am. Nat., 138*: 412-433.

PAMILO, P., 1991c, Evolution of the sterile caste. *J. Theor. Biol., 149*: 75-95.

PAMILO, P., 1991d, Life span of queens in the ant *Formica exsecta*. *Insectes Soc., 38*: 111-120.

PAMILO, P., 1993, Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity, 70*: 472-480.

PAMILO, P. & ROSENGREN, R., 1984, Evolution of nesting strategies of ants: genetic evidence from different population types of *Formica* ants. *Biol. J. Linn. Soc., 21*: 331-348.

PAMILO, P., SUNDSTRÖM, L., FORTELIUS, W. & ROSENGREN, R., 1994, Diploid males and colony-level selection in *Formica* ants. *Ethol. Ecol. Evol., 6*: 221-235.

PEDERSEN, J. S. & BOOMSMA, J. J., 1999, Positive association of queen number and queen-mating frequency in *Myrmica* ants: a challenge to the genetic-variability hypotheses. *Behav. Ecol. Sociobiol., 45*: 185-193.

POULIK, M. D., 1957, Starch gel electrophoresis in a discontinuous system of buffers. *Nature, 180*: 1477-1479.

ROOSE, M. L. & GOTTLIEB, L. D., 1976, Genetic and biochemical consequences of polyploidy in *Trogopogon*. *Evolution, 30*: 818-830.

ROSS, K. G., 1986, Kin selection and the problem of sperm utilization in social insects. *Nature, 323*: 798-800.

ROSS, K. G., 1988, Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol., 23*: 341-355.

ROSS, K. G., 1993, The breeding system of the fire ant *Solenopsis invicta*: effects on colony genetic structure. *Am. Nat., 141*: 554-576.

ROSS, K. G. & FLETCHER, D. J. C., 1985a, Comparative study of genetic and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol., 17*: 349-356.

ROSS, K. G. & FLETCHER, D. J. C., 1985b, Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and its evolutionary significance. *Evolution, 39*: 888-903.

ROSS, K. G. & FLETCHER, D. J. C., 1986, Diploid male production – a significant mortality factor in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol., 17*: 349-356.

RÜPPELL, O., HEINZE, J. & HÖLLDOBLER, B., 1998, Size-dimorphism in the queens of the North American ant *Leptothorax rugatulus* (Emery). *Insectes Soc., 45*: 67-77.

SEPPÄ, P., 1992, Genetic relatedness of worker nestmates in *Myrmica ruginodis* (Hymenoptera: Formicidae) populations. *Behav. Ecol. Sociobiol., 30*: 253-260.

SHERMAN, P. W., SEELEY, T. D. & REEVE, H. K., 1988, Parasites, pathogens and polyandry in social Hymenoptera. *Am. Nat., 131*: 602-610.

STILLE, M., STILLE, B. & DOUWES, P., 1991, Polygyny, relatedness and nest founding in the polygynous myrmicine ant *Leptothorax acervorum* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol., 28*: 91-96.

SUNDSTRÖM, L., 1989, Genetic relatedness and population structure in *Formica truncorum* (Hymenoptera: Formicidae). *Actes Coll. Insectes Soc., 5*: 93-100.

WARD, P. S., 1983, Genetic relatedness and colony organization in a species complex of ponerine ants. *Behav. Ecol. Sociobiol., 12*: 285-299.