



Isoenzyme variation in the leaf-cutting ants *Acromyrmex heyeri* and *Acromyrmex striatus* (Hymenoptera, formicidae)

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Abstract

This is the first study of isoenzyme variability in the leaf-cutting ants (Myrmicinae, Attini) *Acromyrmex heyeri* (Forel, 1899) and *A. striatus* (Roger, 1863) which are common throughout the southern Brazilian state of Rio Grande do Sul. We studied the alloenzyme variability of malate dehydrogenase (MDH), α -glycerophosphate dehydrogenase (α -GPDH) and amylase (AMY) in 97 colonies of *A. heyeri* and 103 colonies of *A. striatus*. Five loci were found for these enzyme systems, one locus (*Amy-1*) being monomorphic in both species and four loci (*Mdh-1*, α -*Gpdh-1*, *Amy-2*, and *Amy-4*) being polymorphic. For each species there were exclusive alleles for the *Mdh-1* and *Amy-2* loci and differences were also found in the allele frequencies for the other polymorphic loci. Ontogenetically different gene activity was detected for the MDH and α -GPDH systems, with between-caste differences, probably related to flight activity, also being found for α -GPDH.

Key words: ants, allozyme variation, fungus grower ants, genetic polymorphism, haplodiploid.

Received: August 27, 1999; accepted: June 19, 2002.

Introduction

Leaf-cutting ants of the genus *Acromyrmex* are drawing increasing attention because of the damage they cause to crops. Due to the great morphological polymorphism of the species and the considerable variability found among individuals, *Acromyrmex* is one of the most complex genera of the family Formicidae. The head and thorax spines are important systematic characters, but their proportions can vary between and within colonies, individuals with bilateral asymmetry of the head being very common. According to Gonçalves (1961), this variability was referred to by Emery (1905) and Santschi (1925) who revised the genus. There are also behavioral differences between these two species, including nest architecture and stability, seasonal and daily activity, and worker mobility. In Brazil, *A. heyeri* and *A. striatus* are restricted to the southern states, with the greatest concentration of these species being in the Rio Grande do Sul, the southernmost Brazilian state.

Ants in general provide unique opportunities for the investigation of various biological, populational and evolutionary questions. This is because their colonies are highly

complex and contain various morphological castes with different functions. Ants are especially suitable for studies in behavioral ecology and genetic structure and provide rich material for investigating and documenting phenomena and theories on kin selection and altruism, group selection, and competition at the individual, colony and between-colonies organizational levels (Brian, 1983; Sudd and Franks, 1987; Hölldobler and Wilson, 1990). Ants are haplodiploid organisms, *i.e.* males result from parthenogenesis and females from fertilized eggs, the occurrence of these two ploidy levels providing an important tool for genetic analyses.

Some authors have suggested that diploid organisms have more genetic variability than haplodiploid organisms (for reviews see Snyder, 1974; Nevo, 1978; Lester and Selander, 1979; Pamilo *et al.*, 1978a, b; Falcão and Contel, 1990). Studies on haplodiploids have mainly been done with Hymenoptera, especially wasps, bees and ants. Graur (1985) argued that the level of sociality is more important than haplodiploidy in determining gene diversity in the Hymenoptera. Gene variability in the Formicidae has been widely studied in some species from Australia (Crozier *et al.*, 1984; Herbers, 1991), Europe (Pamilo, 1978b; Sjöppa, 1992) and North-America (Ward, 1980; Ross and Fletcher,

1985a), but only a few studies concern South-American ants (Ross and Trager, 1990; Diehl-Fleig and Souza, 1999).

In spite of their economic importance and the potential usefulness of genetic and evolutionary studies of this group, the genetics of the fungus-growing leaf-cutting ants *A. heyeri* and *A. striatus* are unknown. In this paper we report on the isoenzyme patterns, genetic control and alloenzyme variability of malate dehydrogenase, α -glycerophosphate dehydrogenase and amylase in these ants.

Material and Methods

Adults and pupae of males (haploids) and gynes (diploid reproductive females), and adult workers (diploid sterile females) were collected from 97 *A. heyeri* and 103 *A. striatus* colonies at several localities in Rio Grande do Sul, Brazil. The samples were kept alive at 10 °C during transport to the laboratory, where they were frozen at -15 °C.

Seven to 10 individuals per colony were analyzed by horizontal electrophoresis for three enzymatic systems: malate dehydrogenase (MDH: Enzyme Commission 1.1.1.37), α -glycerophosphate dehydrogenase (α -GPDH: EC 1.1.1.8) and amylase (AMY: EC 3.2.1.1). Each individual was homogenized with 0.03 mL of distilled water, samples being absorbed onto filter-paper wicks which were then inserted into slots in polyacrylamide gels (see below). For both species, samples from males and gynes of known genotypes were applied as controls at six different gel positions and for a better comparative analysis individuals of both species were distributed on the same gel. Electrophoresis was carried out in polyacrylamide gels of the following concentrations: 6% for α -GPDH, 7% for AMY and 8% for MDH, the gel and electrode buffer systems being those of Poulik (1957) for MDH and Roose and Gottlieb (1976) for α -GPDH and AMY. The gels were run at 4 °C and 10 V/cm until the front line was 9 cm from the origin. Staining was by the Ayala *et al.* (1972) method for MDH and α -GPDH and the method of Chao and Scandalios (1972) for AMY.

Since male ants are haploids they give only one principal band for each locus, so band patterns observed in the gynes and workers were compared to their brothers' bands to determine how alloenzymes of the MDH, α -GPDH and AMY systems are genetically controlled. Isoenzyme loci were sequentially numbered from anode (lowest) to cathode (highest). The fastest alloenzyme of each enzyme system was assigned number 100, the other electromorphs being assigned numbers by dividing their migration distance by that of allele 100 and multiplying by 100.

Results

Malate dehydrogenase

For MDH, three principal isoenzymes (all with one to three secondary bands, depending on principal band inten-

sity and storage time) were detected in adults and pupae of both species (Figure 1). The occurrence of three phenotypes, two with a principal band only and a third with three bands, is evidence that MDH is controlled by one locus with three co-dominant alleles (*Mdh-1*¹⁰⁰, *Mdh-1*⁷⁴ and *Mdh-1*⁵⁷). Males, gynes, and adult workers gave similar band intensity patterns, indicating identical MDH activity in the reproductive and worker castes. In male and gyne pupae, besides *Mdh-1* locus bands, a group of three additional anodic bands was detected that could have been due to a second locus (*Mdh-2*) which may only be active during the immature stages. This isoenzyme group was poorly resolved, which made it difficult to determinate its genetic control. The MDH isoenzyme system allowed us to distinguish between the two species because the *Mdh-1*¹⁰⁰ allele occurred only in *A. heyeri* and the *Mdh-1*⁵⁷ allele only in *A. striatus*. The *Mdh-1*⁷⁴ allele, although found in both species, occurred at very low frequencies (Table 1).

α -glycerophosphate dehydrogenase

For the α -GPDH system, two zones of activity (α -Gpdh-1 and α -Gpdh-2) were observed on the gels, the first one was active in both adults and pupae while the second was only detected in the pupae of reproductives. We interpreted the α -Gpdh-1 region as being controlled by one locus (α -Gpdh-1). Five principal alloenzymes, all with both faster and slower secondary bands, were also detected (Figure 1). As there was only one principal band in the males and one or two principal bands in the gynes and workers it appears that α -glycerophosphate dehydrogenase is a monomeric enzyme. Males and gynes gave α -Gpdh-1 bands with a much higher intensity than did workers. All five alloenzymes of the α -Gpdh-1 locus were detected in both species, although they occurred at different frequencies (Table 1). The α -Gpdh1¹⁰⁰ allele was most common in *A. striatus* and the α -Gpdh1⁶⁴ allele in *A. heyeri*, while the α -Gpdh-2 isoenzymes showed the least mobility and was poorly resolved, making it difficult to determinate its genetic control.

Amylases

Four anodic amylase activity zones were detected and interpreted as resulting from four amylase loci, although isoenzyme detection for the *Amy-3* zone (probably representing the *Amy-3* locus) was not easy and therefore omitted (Figure 1). The *Amy-1* locus was monomorphic in both species. Two *Amy-2* alleles were detected, *Amy-2*¹⁰⁰ being restricted to *A. heyeri* and *Amy-2*⁹² to *A. striatus*. The *Amy-2*⁹² allele was only found in individuals from three of the 97 *A. heyeri* colonies, and determination of the enzyme structure produced by this locus was not possible because all individuals had only one band, which suggests homozygosity. The *Amy-4* locus had seven co-dominant alleles according to the electrophoretic phenotypes. Since the

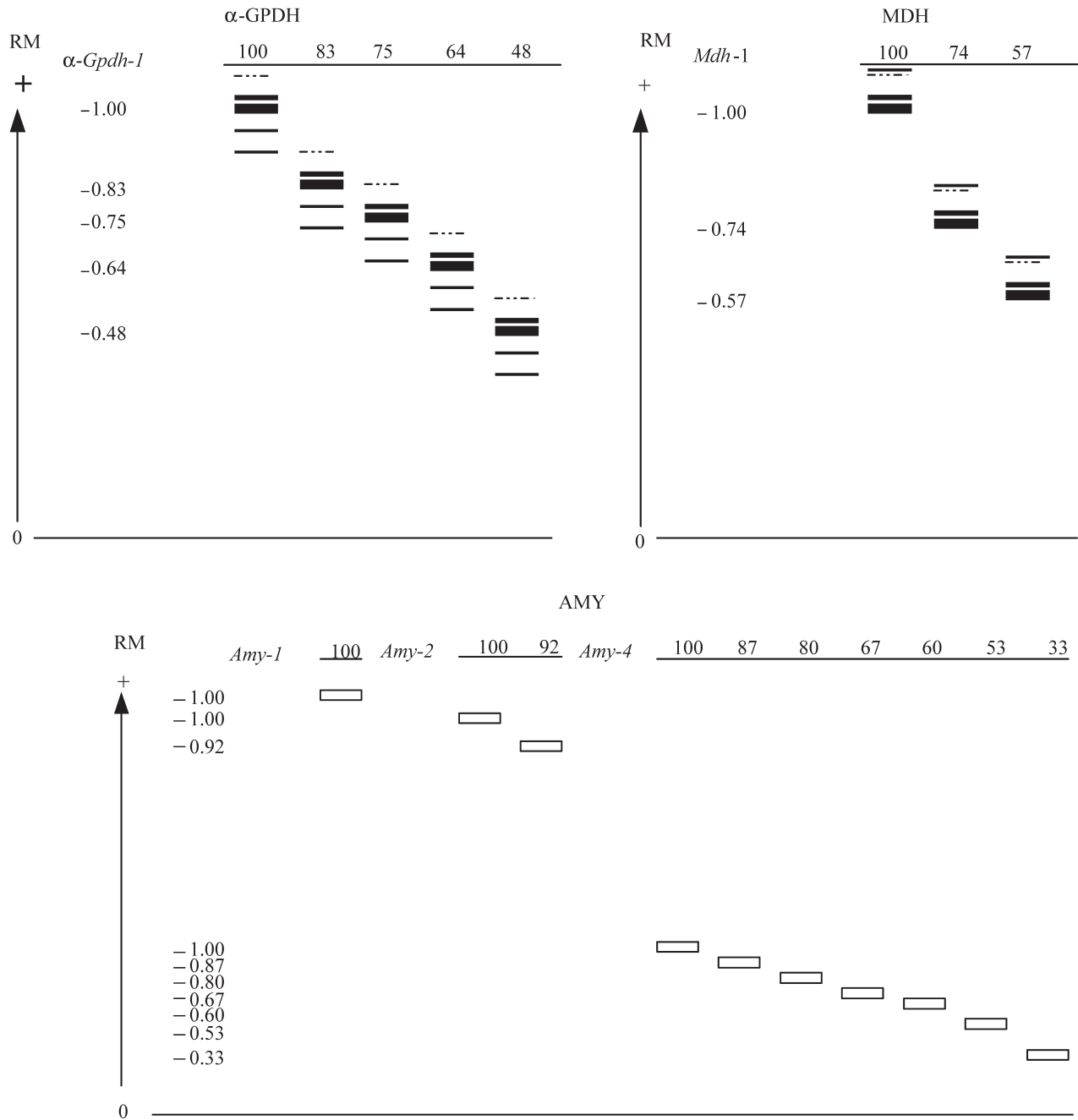


Figure 1 - Electrophoretic pattern of malate dehydrogenase (MDH), α -glycerophosphate dehydrogenase (α -GPDH) and amylase (AMY) in *Acromyrmex heyeri* and *Acromyrmex striatus*. The gene locus and its alleles are indicated at the top of each pattern and the relative migration (RM) of each primary isoenzyme is shown on the left.

males gave only one band and the females one or two bands, this enzyme was interpreted as being monomeric. The seven alleles were common to both species but occurred at different frequencies (Table I). The most frequent allele in *A. striatus* was *Amy-4*⁸⁰, while no allele occurred at a high frequency in *A. heyeri*. In a comparison of males, gynes and adult workers, band intensities were similar for the three loci, although male and gyne pupae did not show amylase activity.

Discussion

Analyses of alloenzyme variation in respect of the MDH, α -GPDH and AMY enzyme systems of *A. heyeri* and *A. striatus* made it possible for us to identify and characterize five loci with several co-dominant alloenzyme markers for these species. Of these five loci, *Amy-1* was monomorphic, *Mdh-1* and *Amy-2* gave exclusive alleles for each species, and *α -Gpdh-1* and *Amy-4* gave different

Table 1 - Allelic frequencies of five isoenzyme loci of the leaf-cutting ants *Acromyrmex heyeri* and *Acromyrmex striatus*.

Locus	Alleles	<i>A. heyeri</i>		<i>A. striatus</i>	
		N	Frequencies	N	Frequencies
<i>Mdh-1</i>	100	1269	0.957	0	—
	74	57	0.043	26	0.016
	57	0	—	162	0.984
α - <i>Gpdh-1</i>	100	100	0.078	100	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
<i>Amy-1</i>	100	900	1.000	120	1.000
<i>Amy-2</i>	100	879	0.977	0	—
	92	21	0.023	120	1.000
<i>Amy-4</i>	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

¹Malate dehydrogenase (MDH), α -glycerophosphate dehydrogenase (α -GPDH) and amylase (AMY).

allelic frequencies for these two species and allowed their differentiation.

It is well established that genetic polymorphisms vary according to the species and loci studied, *i.e.* in some species a locus can be monomorphic while in others it can have many alleles. For example, we found two alleles for the *Mdh-1* locus for each of the *Acromyrmex* species analyzed, while only one allele has been reported for the *Mdh-1* locus in *Nothomyrmecia macrops* (Ward and Taylor, 1981) and *Acromyrmex balzani* (Diehl-Fleig and Souza, 1999), two in *Aphaenogaster rudis* (Crozier, 1973), *Formica pressilabris* (Pamilo and Rosengren, 1984), *Tapinoma minutum* (Herbers, 1991) and *Acromyrmex crassispinus* (Diehl-Fleig and Souza, 1999), three in *Formica exceta* (Pamilo *et al.*, 1978a; Pamilo, 1991) and five in *Formica sanguinea* (Pamilo, 1981) and *Rhytidoponera chalybaea* (Ward, 1980).

We found five alleles for the α -*Gpdh-1* locus, all common to *A. heyeri* and *A. striatus*, but occurring at differ-

ent frequencies. Both species gave a larger number of alleles for the α -*Gpdh* locus than have other ant species, there being one allele for *N. macrops* (Ward and Taylor, 1981), two for *F. exceta* (Pamilo *et al.* 1978a), *Solenopsis invicta* (Ross and Fletcher, 1985a, b) and *A. crassispinus* (Diehl-Fleig and Souza, 1999) and four for *Myrmica ruginodis* (Seppä, 1992) and *A. balzani* (Diehl-Fleig and Souza, 1999).

The three *Amy* loci in *Acromyrmex* gave different numbers of alleles: *Amy-1* was monomorphic, *Amy-2* had two alleles and *Amy-4* seven alleles. The polymorphism observed for the *Amy-4* locus is as high as that observed for amylase loci in many other species, *e.g.*, nine alleles in *Rhytidoponera mayri* (Crozier *et al.*, 1984), six in *Pogonomyrmex badius* (Tomaszewski *et al.*, 1973), *R. chalybaea* (Ward, 1980) and *A. crassispinus* (Diehl-Fleig and Souza, 1999) and five in *A. balzani* (Diehl-Fleig and Souza, 1999). In *Rhytidoponera confusa* (Ward, 1980), *Iridomyrmex purpureus* (Halliday, 1975, 1983) and *N. macrops* (Ward and Taylor, 1981) four alleles have been detected, while only three have been shown in *Formica fusca*, *F. exceta* and *F. sanguinea* (Pamilo *et al.*, 1978a).

We found differences in gene activity between adult and pupal stages for the MDH, GPDH and AMY enzyme systems. One locus was active in adults and pupae in the MDH and GPDH systems, while a second locus for each system was detected only in the pupae. In the AMY system, although products of the three loci could be detected in adults no amylase activity was observed in pupae. Therefore it appears that there are ontogenetic differences in enzyme activity, indicating differential gene activity during ontogenetic development. Normally isoenzyme patterns are characteristic of particular cellular types and stages of differentiation because they reflect changes in gene regulation patterns (Markert, 1975; McMillin, 1983). Many studies have found variations in the intensity pattern of enzyme bands during ontogenetic development of Hymenoptera, especially bees and wasps (Mestriner, 1969; Contel and Kerr, 1976; Martins *et al.*, 1977; Bitondi and Mestriner, 1983; Falcão and Contel, 1991a, b) although there are no reports of ontogenetic differences in ants, apparently because only adult workers have been studied.

We also observed differences in the activity of some enzyme systems between castes. Although *A. heyeri* and *A. striatus* males, gynes and workers gave similar band intensities for the MDH and AMY systems (suggesting identical enzyme activity in these castes) we observed differences in the intensity of the α -*Gpdh* bands between castes, with α -*Gpdh-1* locus bands having a strong intensity in sexual individuals (pupae and adults) in both species but being weak and frequently undetectable in workers. Two of the most important functions of α -GPDH are to provide glycerol-3-phosphate for lipid biosynthesis and NAD⁺ for wing muscle activity. In insect larvae, this enzyme is found predominantly in the fatty bodies, brain and muscles (Sullivan

et al., 1983). In *A. heyeri* and *A. striatus*, as in many other species of ants, individuals of the reproductive caste are alates. After nuptial flight, the males die and fecundated gynes lose their wings. Since ant workers lack wings, differences in α -GPDH activity would be expected between castes. Thus, when comparing reproductive and non-reproductive castes of *A. heyeri* and *A. striatus* we found differences in band intensities, suggesting differential gene expression. Many studies have been carried out on α -GPDH due to the importance of this enzyme in insect metabolism, especially on the butterfly *Spodoptera* (Lima and Contel, 1990), the fruit fly *Drosophila* (MacDonald and Avise, 1976; Connors and Curtsinger, 1986) and the mosquitoes *Culex* (Pasteur and Stordeur 1976) and *Anopheles* (Scarpassa and Tadei, 1993). From these papers we can conclude that GPDH is a good system for studying changes in gene expression during the ontogenetic development of insects, while our own results indicate that GPDH is an excellent system for studying processes involving caste differentiation in ants.

Acknowledgments

The authors are grateful to the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and to Martina da Silva and Senilda Fritsch for technical assistance.

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