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Gene Variation, Population Differentiation, and Sociogenetic Structure of Nests of *Partamona seridoensis* (Hymenoptera: Apidae, Meliponini)

Carlo Rivero Moura Fernandes · Celso Feitosa Martins · Kátia Maria Ferreira · Marco Antonio Del Lama

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Abstract Gene variation and the differentiation of two populations of *Partamona seridoensis* (Hymenoptera: Apidae: Meliponini) from the Caatinga biome, a semi-arid ecosystem unique to Brazil, were estimated through allozymic and microsatellite analyses. These populations exhibited similar low degrees of enzyme gene variation. Observed genotype frequencies at the allozyme and microsatellite loci were in accordance with Hardy–Weinberg equilibrium in the two populations. Both markers demonstrated that the two populations are not genetically homogeneous and must be considered distinct populations. The occurrence of private alleles at the allozyme and microsatellite loci corroborates this differentiation, sustaining the hypothesis of a low level of interpopulation gene flow. The phenotypic segregations clearly demonstrated that the progeny inside each nest were the result of mating between the queen of the colony and only one male.

Keywords Genetic variability · Interpopulation differentiation · Sociogenetic structure · *Partamona seridoensis* · Brazilian Caatinga

Introduction

Flowering plants often depend on bees for pollination, and bees in turn are dependent on flowering plants for resources. Therefore, the conservation of flowering plants depends on the preservation of bee populations. About 70% of

C. R. M. Fernandes · C. F. Martins
Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil

K. M. Ferreira · M. A. Del Lama (✉)
Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luis km 235, 13565-905 São Carlos, São Paulo, Brazil
e-mail: dmdl@ufscar.br

angiosperms depend on pollination, and a third of the plant species cultivated by humans are pollinated by bees (Kevan and Imperatriz-Fonseca 2006; Klein et al. 2007; Kremen et al. 2007).

In Brazil, eusocial species of Meliponini, also called stingless bees, visit 40–90% of the native tree species, depending on the ecosystem, and 60–75% of savanna species are pollinated by this group of bees (Silberbauer-Gottsberger and Gottsberger 1988). These bees are currently threatened as a result of environmental changes caused by deforestation, the indiscriminate use of pesticides, and predation by honey hunters (Brosi 2009).

Partamona is a Neotropical genus of stingless bee with 33 described species, the adult workers of which are very similar in morphology and color patterns. The low number of morphological traits appropriate for encoding and polarization hinders the establishment of phylogenetic relationships within the group. Therefore, nesting behavior and the structure of nest entrance are essential traits for species identification (Camargo and Pedro 2003). There are few data on the ecology of species of *Partamona* (Barreto and Castro 2007), and the genetics of these species is poorly known; however, the findings on the cytogenetics of some species of the genus (Tosta et al. 2007; Martins et al. 2009) and the genetic structure of populations of *P. helleri* and *P. mulata* have been reported (Brito and Arias 2008, 2010).

Partamona seridoensis occurs mainly in the Caatinga biome and builds its home only in termite nests (Pedro and Camargo 2003). The Caatinga biome, a semiarid ecosystem unique to northeastern Brazil, is made up of a mosaic of deciduous forest with thorny scrub vegetation, covering nearly 800,000 km^2 of the country (ca. 8.6%) (Andrade-Lima 1981). The mean annual rainfall index of the Cariri region in the state of Paraíba (northeastern Brazil) is one of the lowest in the country (300 mm/year), and the rainy season is highly unpredictable. More than 50% of the Caatinga is altered by human activities, and desertification is estimated to be threatening 15% of the region (Castelletti et al. 2004; Leal et al. 2005). As most species of Meliponini rely on forests for survival and the Caatinga vegetation depends on bees for pollination, the analysis of gene variation among populations is a useful tool for monitoring the status and assisting in the management of this threatened ecosystem.

Observations on provisioning activities suggest that the dispersing abilities of stingless bees seem to be limited (Engels and Imperatriz-Fonseca 1990). Flight capacity, however, may not be directly correlated with natural foraging distances. For example, some studies have shown that euglossine bees do not forage for fragrances or nectar distances at sites 400 m away (Kroodsman 1975; Schemske 1981). Studies have also shown that euglossine bees have the capacity for long-distance flight (Janzen 1971; Dressler 1982; Cerântola et al. 2011). The analysis of genetic differentiation among populations and the resulting estimates of gene flow indirectly allow investigation of the dispersal ability of these insects for mating.

The aim of the present study was to estimate gene variation and the degree of differentiation between two populations of *P. seridoensis* sampled in the state of Paraíba, Brazil, through allozymic and microsatellite analyses. The sociogenetic

structure of the colonies was analyzed to determine the mating system and resulting familial sociogenetic structure.

Materials and Methods

Adult workers from 49 nests of *P. seridoensis* were sampled from two regions of Cariri located 60 km apart in the semiarid region of the state of Paraíba (northeastern Brazil). The first region (São João do Cariri) is located in the eastern portion of Cariri at 350 m above sea level and has Caatinga vegetation. Specimens sampled from 23 nests were obtained from Moreiras farm (7°23′36.3″ S; 36°24′53.9″ W), which has 499.5 ha. The second region (Sumé) is located in western Cariri at 650 m above sea level and also has Caatinga vegetation. Specimens were sampled from 26 nests on Almas farm (7°28′23.7″ S; 36°54′17.1″ W), which has 6000 ha. Almas farm has a private reserve covering 3305 ha in a very good state of conservation. The farm also has altered vegetation areas previously used for livestock farming (cattle and goats) and cotton crops. Both regions have a similar climate, with highly seasonal rainfall of 300 mm/year, annual mean temperature of 25°C, and mean relative humidity of 65%.

Samples were obtained from March 2008 to June 2009 along delimited transects in each area. The nests were labeled and geo-referenced. The specimens were kept frozen (for allozyme analysis) or in absolute alcohol (for microsatellite analysis). Voucher specimens were deposited in the Entomological Collection of the Department of Systematics and Ecology of the Universidade Federal da Paraíba after species identification by a specialist.

Allozyme Analysis

Head–thorax extracts of adult workers from 45 nests of *P. seridoensis* were obtained in 0.2 ml 0.2% 2-mercaptoethanol after centrifugation of the homogenates at $6000 \times g$ for 15 min at 4°C. The supernatants were analyzed in horizontal electrophoresis in 14% starch gel (Penetrose 30, Corn Brazil). Samples from two nests from Almas farm (N41 and N48) and two nests from Moreiras farm (N45 and N46) were not submitted to allozyme analysis, as these samples were immediately stored in alcohol after collection.

Fourteen enzyme systems were analyzed: aconitase, *mtAcon* (Tris–citric acid, pH 7.5, TC 7.5); α-glycerophosphate dehydrogenase, *Gpdh-1* and *Gpdh-2* (TC 7.5); esterases, *Est-1* and *Est-2* (TC 7.5); hexokinase, *Hk* (Tris–EDTA–maleic acid–magnesium, pH 7.4, TEMM 7.4); β-hydroxybutyrate dehydrogenase, *Hbdh* (TC 7.5); isocitrate dehydrogenase, *Icd* (TC 7.5); leucine aminopeptidase, *Lap* (Tris–citric acid–borate, pH 8.0–8.3, TCB 8.0–8.3); malate dehydrogenase, *cMdh* and *mtMdh* (TC 7.5); malic enzyme, *Me* (TC 7.5); phosphoglucomutase, *Pgm* (TEMM 7.4); 6-phosphogluconate dehydrogenase, *6-Pgd* (TC 7.5); glucose phosphate isomerase, *Gpi* (Tris–citric acid, pH 8.0, TC 8.0); peptidase A (leu–tyr), *Pep-A* (TCB 8.0–8.3); and superoxide dismutase, *Sod* (TCB 8.0–8.3). Buffers and

reaction mixtures were prepared based on the standard protocols described by Harris and Hopkinson (1976).

Microsatellite Analysis

Total DNA was extracted from the mesosoma using the phenol–chloroform method (Sheppard and McPheron 1991) or from a leg using Chelex 100 (Walsh et al. 1991) from two adult workers of each of the 49 nests of *P. seridoensis* sampled in the two study areas. For the analysis of gene variation at microsatellite loci, 12 pairs of heterologous oligos designed for *Trigona carbonaria* Tc3 (Green et al. 2001), *Scaptotrigona postica* Latreille, 1807, T4 (Paxton et al. 1999a), and *Melipona bicolor* Lepeletier, 1836, Mbi (Peters et al. 1998) were tested in females from several nests. Polymerase chain reaction (PCR) amplification occurred in a final volume of 25 µl containing 1 µl extracted DNA, 1 µM each primer, 2.5 mM $MgCl_2$, 250 µM each dNTP, 1× reaction buffer, 1 U *Taq* DNA polymerase (BioTools), and sterilized water. The reaction was allowed to develop for 35 cycles at 94°C for 30 s, 20 s at the hybridization temperature set for each primer pair (48–54°C), and 70°C for 1 min. Positive amplification with the pairs of oligos was visualized in 9% nondenaturing polyacrylamide gel stained with silver. The phenotyping of individuals, however, was performed using the *forward* primer of each pair marked with a fluorophore. In this case, the PCR product was diluted 10×, and an aliquot of 2 µl was mixed with 7.75 µl of 0.1% Tween 20 and 0.25 µl ET550-R (standard size) and subsequently run in the sequencer MegaBACE 1000. The results were analyzed with the MegaBACE Genetic Profiler version 1.2.

Data Analysis

Genetic variability at enzyme and microsatellite loci was estimated through intralocus heterozygosity ($H_i = 1 - \sum p^2$) and expected average heterozygosity ($H_e = \sum H_i/n$) based on the genotypes of five (allozymes) and three (microsatellites) randomly chosen females from each nest. The significance of differences in genetic diversity between study areas was tested using a paired *t*-test of arcsine-transformed H_e values (Archie 1985). Deviations from Hardy–Weinberg equilibrium and pairwise linkage disequilibrium among loci were assessed with the Monte Carlo approximation of the exact test described by Guo and Thompson (1992) and implemented using the Arlequin program (Excoffier et al. 2005). *F*-statistics and analysis of molecular variance (AMOVA) values were determined and assignment tests were conducted using the GenAlex 6.4 program (Peakall and Smouse 2006).

Results

Among the 14 enzymes corresponding to 17 enzyme gene loci, 5 exhibited electrophoretic variants. Considering the frequency criterion (≤95%), only the *Est*-2 and *cMdh* loci exhibited polymorphism in both populations, although the electrophoretic variants of Gpi, 6-Pgd, and Hbdh were detected at low frequencies

in the samples from Almas. The observed intralocus heterozygosity of the variable loci ranged from 2.1% (*Gpi* and *β-Hbdh* loci of Almas) to 47.9% (*cMdh* locus of Almas), resulting in an average heterozygosity for all loci of 5.6% for Almas and 5.0% for Moreiras. The observed genotypes at the polymorphic loci in both populations were distributed in accordance with Hardy–Weinberg equilibrium (Table 1). Evidence of pairwise linkage disequilibrum was detected between the *Est-2* and *Gpi* loci in the Almas population ($\chi^2 = 4.85$; $P = 0.027$) and between the *Mdhc* and *Est-2* loci in the Moreiras population ($\chi^2 = 9.46$; $P = 0.002$).

Eleven microsatellite loci were tested. There was no amplification for the locus *Mbi278*. In another eight loci (*Mbi522*, *Mbi218*, *Mbi28*, *Mbi215*, *Mbi11*, *Mbi201*, *Tc3.320,* and *T4-171*), females of all the nests exhibited only one allele for each locus. Genetic variation was detected at the loci *Mbi254*, *Mbi232,* and *Mbi32*, with six, eight, and two alleles in the Almas population and three, four, and two alleles in the Moreiras population. The observed intralocus heterozygosity ranged from 19.6% (locus *Mbi254*, Moreiras) to 59.6% (locus *Mbi232*, Almas), resulting in an average heterozygosity of 13.6% in the Almas population and 8.3% in the Moreiras population, considering the 11 loci analyzed. The observed genotypes at the polymorphic loci in both populations were distributed according to Hardy–Weinberg equilibrium (Table 1). Evidence of pairwise linkage disequilibrium was detected between the *Mbi232* and *Mbi32* loci in the Almas ($\chi^2 = 17.71$; $P = 0.013$) and Moreiras ($\chi^2 = 8.89$; $P = 0.030$) populations.

Both populations exhibited similar heterozygosities averaged over enzyme and microsatellite loci ($t = 0.661$; $P = 0.259$). Low but significant F_{st} values were estimated among the enzyme and microsatellite loci analyzed, resulting in an average value of 0.045 for allozyme loci and 0.039 for microsatellite loci (Table 2). As expected, these results were corroborated by the AMOVA, which revealed that 4.54% of the variation at the allozyme loci and 3.95% of the variation at the microsatellite loci accounted for the gene variation not shared by the two populations (Table 3). The assignment test indicated that only 61% of the samples were correctly identified in their population of origin.

Phenotypic segregation data were analyzed at the enzyme loci in progenies from the 16 colonies with more than six individuals per colony (Table 4). Phenotypic segregations were also observed in two colonies at locus *Mbi254* and three colonies at locus *Mbi232*, obtaining the expected ratio of 1:1 for the condition of monandry in all cases (data not shown).

Discussion

Observed heterozygosity averaged over all enzyme loci was low (ca. 5%), but this is the expected level of gene diversity for haplodiploid hymenopteran species. Hymenoptera display significantly lower levels of electrophoretic variation than most other insects, possibly due to the exposure of deleterious genes in the haploid sex, the presence of eusociality, and small effective population size resulting in a reduction of polymorphism, among other factors (Berkelhamer 1983; Graur 1985).

Table 1 Intralocus heterozygosity for two populations of *P. seridoensis* in Paraíba, Brazil

Population	Parameter	Enzyme locus					Microsatellite locus		
		Mdh	*Est-2*	*6Pgd*	*Gpi*	*β-Hbdh*	*Mbi254*	*Mbi232*	*Mbi32*
Almas farm	Sample size	48	48	48	48	48	52	52	52
	Number of alleles	2	2	2	2	2	6	8	2
	Number of effective alleles	1.627	1.704	1.064	1.021	1.021	2.004	2.388	1.899
	Observed heterozygosity	0.479	0.375	0.063	0.021	0.021	0.519	0.596	0.385
	Expected heterozygosity	0.389	0.417	0.061	0.021	0.021	0.506	0.587	0.478
	χ^2_{HWE}	2.856^{NS}	0.410^{NS}	0.050^{NS}	0.005^{NS}	0.005^{NS}	8.227^{NS}	35.412^{NS}	1.8328^{NS}
Moreiras farm	Sample size	42	42	42	42	42	46	46	46
	Number of alleles	2	2	1	1	1	3	4	2
	Number of effective alleles	1.930	1.995	1.000	1.000	1.000	1.221	1.553	1.996
	Observed heterozygosity	0.476	0.381	–	–	–	0.196	0.326	0.391
	Expected heterozygosity	0.487	0.505	–	–	–	0.183	0.360	0.504
	χ^2_{HWE}	0.006^{NS}	2.346^{NS}	–	–	–	0.541^{NS}	5.069^{NS}	2.144^{NS}

NS, nonsignificant chi-square value for Hardy–Weinberg equilibrium (χ^2_{HWE})

Table 2 *F*-statistics for enzyme and microsatellite loci in samples of *P. seridoensis*

Locus	F_{is}	*P*	F_{st}	*P*	F_{it}	*P*
Mdh	−0.099	0.891	0.036	0.042	−0.059	0.845
Est-2	0.177	0.072	0.057	0.033	0.224	0.038
6Pgd	−0.021	1.000	0.018	0.229	−0.002	1.000
Gpi	0.0007	1.000	−0.001	1.000	−0.0007	1.000
β-Hbdh	0.0007	1.000	−0.001	1.000	−0.0007	1.000
Mean	0.039	0.342	0.045	0.009	0.083	0.234
Mbi254	−0.037	0.767	0.071	0.001	0.037	0.532
Mbi232	0.023	0.384	0.295	0.025	0.052	0.315
Mbi 32	0.211	0.024	0.025	0.124	0.231	0.023
Mean	0.076	0.061	0.039	0.004	0.113	0.025

Table 3 AMOVA of allozyme and microsatellite loci of *P. seridoensis* populations in Paraíba, Brazil

Type of loci	Source of variation	*df*	% Variation	*P*
Allozyme	Among populations	1	4.54	0.009
	Among individuals within populations	88	3.78	
	Within individuals	90	91.68	
Microsatellite	Among populations	1	3.95	0.005
	Among individuals within populations	96	7.38	
	Within individuals	98	88.67	

The number of alleles observed at the microsatellite loci was relatively low. This result was expected, as no species-specific primers were used. Despite the larger number of alleles at the loci *Mbi254* and *Mbi232*, similar levels of observed heterozygosity were detected with locus *Mbi32*, which exhibited only two alleles.

The F_{st} values and AMOVA revealed that the Almas and Moreiras populations, separated by approximately 60 km, are heterogeneous for allozyme (*Est-2* and *cMdh*) and microsatellite loci. The occurrence of private alleles at the allozyme and microsatellite loci in nests sampled at Almas farm corroborates the differentiation of the two populations, but the frequencies resulted in no significant degree of heterozygosity in comparison with the Moreiras population. This interpopulation differentiation, although significant, is not high, considering the low value of the assignment test (only 61% correct identification of origin). Thus, the low but significant differentiation demonstrates that gene flow between populations (i.e., number of migrants) is not high enough to make these populations homogeneous, as some degree of isolation occurred due to the distance between study areas.

Dispersal is a fundamental life-history trait affecting gene flow, and dispersal ability has been demonstrated to be negatively correlated with genetic population structure across a range of taxonomic groups (Meyer et al. 2009). Like other groups of animals, bees are at risk of habitat destruction and fragmentation, especially in tropical lowland forests, where they are particularly species-rich (Silveira et al.

Table 4 Phenotypic segregation in adult workers from nests of *P. seridoensis*

Sampling location	Nest	Locus and phenotype											
		Est-2			*Mdhc*			*Gpi*			*Hbdh*		
		F/F	F/S	S/S	F/F	F/S	S/S	F/F	F/S	S/S	F/F	F/S	S/S
Almas farm, west Cariri, Paraíba, Brazil	A1	4	25	1	2	25	19	46					46
	A2	8	9				19	19					19
	A3	18				**19**		19					19
	A6	9	4			4	9	13					13
	A7	20	15				39		**35**				39
	A10		12	8		8	14	22					22
	A21		12	8		**20**		20					20
	A23	11				4	7	11				7	4
	A28	14				**14**		14					14
	A44		3	6		5	5	5	4				4
Moreiras farm, east Cariri, Paraíba, Brazil	M12	6	3			**9**		9					9
	M15		5	4	5	6		11					11
	M16			6		**6**		6					6
	M18		**6**			3	3	6					6
	M24		**10**				10	10					10
	M26		**11**			**11**		11					11

Only one heterozygous phenotype occurred at the locus and colony indicated in bold

2002; Brosi 2009). In the absence of frequent immigration, local populations of species, such as those persisting in fragmented habitats, have been observed to lose fitness through inbreeding (Takahashi et al. 2008). The social Hymenoptera are particularly vulnerable to the loss of genetic diversity through inbreeding due to the haplodiploid system of sex determination (Pamilo and Crozier 1997; Chapman and Bourke 2001).

High frequencies of homozygotes in any loci may indicate genetic impoverishment resulting from small habitat size, population fragmentation, loss of genetic diversity, or a combination of these factors (Packer and Owen 2001). In the present study, the Almas and Moreiras populations were in accordance with Hardy–Weinberg equilibrium. Most of the pairs of enzyme or microsatellite loci in the two populations exhibited no evidence of linkage disequilibrium. These two conditions suggest that *P. seridoensis* from the Almas and Moreiras farms are, as yet, healthy populations capable of counterbalancing the harmful effects to which they are subjected.

The phenotypic segregations clearly indicate that the progeny analyzed are the result of queens mating with a single male. In only one of the colonies tested (Colony A1), the observed segregation did not match this model. This segregation could be explained, however, by overlapping generations due to the recent replacement of the old queen. The familial segregations observed at the allozyme and microsatellite loci confirm the genetic nature of the variation studied, which was

indirectly demonstrated by the conformity of observed genotypic proportions in the two populations under the condition of genetic equilibrium.

The results of the present study indicate that monandry is the rule in this species. It has been postulated that multiple mating by stingless bee queens is widespread within the large, pantropical taxon Meliponini (Paxton et al. 1999b), but there is no new evidence to support this assumption. Thus, single mating is currently recognized as common among bees (Palmer et al. 2002; Roubik 2006; Soro et al. 2009; Zimmermann et al. 2009) and is likely the ancestral condition for corbiculate bees (Hughes et al. 2008).

No alien workers were detected. This suggests that the rate of workers drifting between colonies is not high among stingless bees, although this behavior can be high in bee yards or colonies that are very close to each other in nature (Palmer et al. 2002; Soro et al. 2009).

Among stingless bees, colony reproduction occurs through swarming. Under this condition, the link between parent and offspring colonies remains for some time, as the new colony depends on resources obtained from the parent colony (Nogueira-Neto 1954). Thus, reproductive females do not migrate very far during swarming (Engels and Imperatriz-Fonseca 1990), and the populations are expected to be highly structured for mitochondrial genes inherited uniparentally (maternal inheritance). If this hypothesis is correct, it is reasonable to assume that a given area is colonized by few female founders (i.e., a small number of maternal lineages). Due to the peculiar system of sex determination in Hymenoptera, it is necessary for these species to have mechanisms that prevent endogamy in order to avoid the generation of diploid males due to homozygosity in the sex locus. If these assumptions are true, the high structuring for maternally inherited mitochondrial genes, indicating sex-biased dispersal in *P. seridoensis*, should oppose a lower population structuring for biparentally inherited nuclear markers (allozymes and microsatellites). Considering the consequences of sex-biased dispersal for inter-population differentiation (Slatkin 1987; Wade and McCauley 1988), this hypothesis merits further investigation.

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