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Research Article

Genetic divergence between populations of the stingless bee uruçu amarela (*Melipona rufiventris* group, Hymenoptera, Meliponini): Is there a new *Melipona* species in the Brazilian state of Minas Gerais?

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Abstract

Allozyme, microsatellite and random amplified polymorphic DNA (RAPD) molecular markers were used to investigate the within and between population genetic variability and between population genetic differentiation of the Brazilian stingless bee uruçu amarela (nominally *Melipona rufiventris* Lepeletier, 1836) present in savanna and Atlantic forest habitats of the Brazilian state of Minas Gerais (MG). We found low levels of within population variability, although there were a large number of private alleles that specifically characterized these populations. The F_{ST} values indicated a high level of genetic diversity between populations. Analysis of molecular variance (AMOVA) showed a high degree of population differentiation between the savanna and Atlantic forest habitats, confirmed by population pairwise F_{ST} data. Principal coordinates analysis and unweighted pair-group method using an arithmetic average (UPGMA) dendrograms also confirmed that in Minas Gerais the savanna populations (*M. rufiventris*) were genetically distinct from those present in the Atlantic forest (*M. mondury*). In addition, populations from locations near the towns of Dom Bosco and Brasilândia de Minas were genetically different from those collected in other localities in the savanna. Our data indicate that populations of uruçu amarela found in the savanna and Atlantic forest habitats of Minas Gerais state should be treated separately for conservation purposes and that special attention should be given to the populations found in the region of Dom Bosco and Brasilândia de Minas until their taxonomic status is clarified.

Key words: endangered species, genetic divergence, molecular markers, stingless bees, uruçu amarela.

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Introduction

In Brazil there are approximately 192 species of stingless bees (Silveira *et al.*, 2002), which play an important role in the pollination of a large number of wild plants and crops in Brazilian ecosystems (Kerr *et al.*, 1996). However, Campos (1998) stated that populations of the stingless bee *Melipona rufiventris* (uruçu amarela) were being drastically reduced due to deforestation, predatory honey collecting and widespread burning, leading to the inclusion of these bees in the endangered species list of the Brazilian state of Minas Gerais.

Populations of uruçu amarela occur in southeastern Brazil from the state of Bahia to the state of Santa Catarina and have usually been considered as a single species, *M. rufiventris* (Moure, 1975) but such an extensive distribution encompasses diverse environmental conditions and associated habitat types and it is possible that many locally adapted ecotypes have arisen. For instance, variation in relation to the color of the posterior legs of *M. rufiventris* was found when different populations were analyzed by Moure and Kerr (1950) and Moure (1975), who reported that there were some specimens almost without black spots on the posterior tibia in the states of Minas Gerais, Rio de Janeiro, São Paulo and Paraná while specimens from Bahia, Goiás, São Paulo and Santa Catarina presented a large black spot on the apical portion of the posterior tibia and in all basitarsus. The corbicula and tergal hairs also presented variability in the specimens analyzed, which led to the suggestion that there was much to be done for a better knowledge of the different forms attributed to this species (Moure, 1992).

In 2002, our laboratory initiated a project to analyze the genetic variation among populations of uruçu amarela collected in the savanna and in the Atlantic forest of Minas Gerais state. Biochemical analysis demonstrated different migration rates of some enzymatic loci (*Est-1*, *Est-2*, *Est-3*,

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Est-4, *Pep-1,* and *Mdh-1*) between colonies collected in different regions (Costa *et al.*, 2005).

Melo (2003) reported morphological evidence to suggest that uruçu amarela populations from southeastern Brazil in fact comprised two different species, *M. rufiventris* and *M. mondury*. According to this author, the typical savanna species *M. rufiventris* occurs in Mato Grosso, northwestern São Paulo, western Minas Gerais and southern Goiás, while *M. mondury* is distributed across the Atlantic forest where it is found in the states of Bahia, Espírito Santo, Rio de Janeiro, Minas Gerais, São Paulo, Paraná and Santa Catarina. As argued by Melo (2003), however, the recognition of these species based on the morphology of worker bees is very difficult.

Studies concerning the genetic variability of these different forms of uruçu amarela may provide relevant data on their population structure, kin relationships and gene flow between remnant populations. These data are very important for elaborating strategies for their conservation. It is also important to bear in mind that less than 9% of the original Atlantic forest remains and that a great number of tropical tree species are pollinated by *Melipona* bees.

Genetic analysis of populations has been greatly enhanced with the progress of molecular techniques. Considering that various classes of molecular markers are now available, it is possible to compare the patterns of structure revealed by them in order to verify how gene flow, drift and selection govern the distribution of genetic variation within and between populations of a given species.

This paper describes the use of allozyme, microsatellite and random amplified polymorphic DNA (RAPD) markers to investigate the genetic variability within and between uruçu amarela populations present in the savanna and in Atlantic forest habitats of Minas Gerais state and also to analyze the genetic differentiation between the populations.

Materials and Methods

Genetic material

Samples were collected in ten savanna habitats and seven Atlantic forest habitats localities within Minas Gerais, the geographic distribution and the number of colonies sampled in each locality being show in Figure 1 and Table 1. For all statistical analyses, colonies were grouped according to locality and each locality was considered as representing a population. Bees were collected at the entrance of their colonies and transferred, alive, to our Laboratory where they were stored at -70 °C until need for electrophoretic analyses. Voucher specimens from both biomes were deposited in the insect collection at the Entomology Museum of the Universidade Federal de Viçosa (UFVB) (Viçosa, Minas Gerais, Brazil) and in the bee collection of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

Allozyme analysis

We analyzed 87 colonies with allozyme markers using eight bees per colony. Individual worker bees were divided into two parts, head/mesosoma and metasoma, which were homogenized in 100 μL of 0.2% (v/v) β-mercaptoethanol solution and kept on ice. For allozyme analysis the homogenates were absorbed with Whatman N° 3 filter papers and applied to standard horizontal 14% (w/v) starch gels and electrophoresis conducted according to the procedures of Harris and Hopkinsons (1978). The following en-

Figure 1 - Map of Brazil (A) and Minas Gerais (B) state (B) showing the geographic location of the 17 natural populations of uruçu amarela bees analyzed.

Table 1 - Location and number of uruçu amarela colonies sampled in the savanna and the Atlantic forest of the Brazilian state of Minas Gerais. Data ordered by increasing latitude.

Habitat and location (code)	Latitude and longitude	Number of colonies
Savanna habitat		
Dom Bosco (DB)	16°39' S, 46°16' W	2
Brasilândia de Minas (BR)	17°00' S, 46°00' W	5
Patos de Minas (PA)	18°34' S, 46°31' W	6
Guimarânia (GU)	18°50' S, 46°47' W	20
Patrocínio (PT)	18°56' S, 46°59' W	2
Pequi (PE)	19°37' S, 44°39' W	2
Uberaba (UB)	19°44' S, 47°55' W	2
Córrego Danta (CD)	19°49' S, 45°54' W	4
Arcos (AR)	20°16' S, 45°32' W	4
Formiga (FO)	20°27' S, 45°25' W	2
Subtotal for savanna colonies		49
Atlantic forest habitat		
Poté (PO)	17°48' S, 41°47' W	5
Itamarandiba (IT)	17°51' S, 42°51' W	5
Coluna (CO)	18°14' S, 42°50' W	2
Rio Vermelho (RV)	18°17' S, 43°00' W	25
Marliéria (MA)	19°42' S, 42°43' W	1
Diogo Vasconcelos (DV)	20°29' S, 43°11' W	4
Resende Costa (RC)	20°55' S, 44°14' W	6
Subtotal for Atlantic forest colonies		48
Total colonies sampled		97

zyme systems were assayed: hexokinase (E.C.2.7.1.1, HK), glucose phosphate isomerase (E.C.5.3.1.9, GPI), esterase (E.C.3.1.1.1, EST), aminopeptidase A (E.C.3.4.11, PEP-A), hydroxybutyrate dehydrogenase (E.C.1.1.1.30, HBDH), isocitrate dehydrogenase (E.C.1.1.1.42, ICD), malate dehydrogenase (E.C.1.1.1.37, MDH), phosphoglucomutase (E.C.2.7.5.1, PGM), α-glycerophosphate dehydrogenase (E.C.1.1.1.8, GPD), diaphorase (E.C.1.6.4.3, DIA), alcohol dehydrogenase (E. C. 1.1.1.1, ADH) and acid phosphatase (E. C. 3.1.3.2, ACP).

DNA extraction and microsatellite analysis

The protocol described by Waldschmidt *et al.* (1997) was used to extract DNA from five bees per colony for 57 colonies, the genetic material from each bee being analyzed separately. Nine microsatellite loci (Mbi32 (GenBank AF002827), Mbi215 (AF002837), Mbi218 (AF002838), Mbi232 (AF002841), Mbi233 (AF002842), Mbi254 (AF002843), Mbi256 (AF002844), Mbi259 (AF 002845) and Mbi278 (AF002846)) were analyzed using the amplifications conditions described by Peters *et al.* (1998) for *Melipona bicolor*. The amplification products were subjected to electrophoresis on 8% (w/v) non-denaturing polyacrylamide gel and visualized by staining with 0.2% (w/v) silver nitrate. The lengths of amplified alleles were checked using a 10 bp DNA ladder (Invitrogen, São Paulo, Brazil).

RAPD analysis

For the RAPD marker analysis we used one bee from each of 70 colonies. The PCR-RAPD analyses were performed as described by Williams *et al.* (1990), with some modifications. Each PCR reaction used a total volume of 25 µL with 1x reaction buffer, 0.1 mM dNTPs, 0.4 µM 10-base primer (Operon Technologies, Alameda, CA, USA), 0.5 U *Taq* polymerase (Phoneutria, Belo Horizonte, Minas Gerais, Brazil) and 2 µL template DNA (25 ng). Amplification was in a PTC-100 thermocycler (MJ Research) programmed for 40 cycles, each cycle consisting of 15 s at 94 °C, 30 s at 35 °C and 1 min at 72 °C, with a final extension after the 40th cycle for 7 min at 72 °C. The amplification products were separated by electrophoresis in 1.2% (w/v) agarose gels in TBE (90 mM Tris-borate pH 8.0, 10 mM EDTA) buffer and stained with ethidium bromide (0.2 µg/mL). Bands were visualized under UV light and the images analyzed using the AlphaDigidoc 1201 system (AlphaDigidoc). A negative control with no genomic DNA was used in each amplification to check for possible contaminants.

Data analysis of gene diversity

For each locus and in Minas Gerais state intrabiome level, allelic frequencies, observed (A_o) and effective number of alleles (A_e) were computed according to Hartl and Clark (1989). The observed (H_o) heterozygosity and Nei's (1978) unbiased expected (H_e) heterozygosity were also estimated using the Popgene program (Yeh *et al.*, 1999). Tests for conformation to Hardy-Weinberg (H-W) equilibrium (test of non-random association of alleles within diploids individuals) and genotypic linkage disequilibrium were performed using the Genepop program (Raymond and Rousset, 1995).

To estimate allele frequencies from RAPD loci, assuming H-W equilibrium, bees were scored for the presence (1) or absence (0) of RAPD fragments and Lynch and Milligan's (1994) procedure was applied on a 347-band matrix.

Differentiation statistics

The Popgene program (Yeh *et al.*, 1999) yielded Wright's (1978) F_{ST} estimates, the significance of which was tested by a permutation procedure. Population pairwise genetic distances were calculated. Pairwise F_{ST} values were used as short-term genetic distances between populations, the Arlequin program (Excoffier *et al.*, 2005) being used to apply a minor transformation which linearized the distance and the population divergence time. G_{ST} values were estimated from RAPD markers, a measure of population genetic differentiation for binary data that is analogous to F_{ST}.

Hierarchical analyses of molecular variance (AMOVA) (Excoffier *et al.*, 1992) for investigating the genetic structure of populations were performed using the Arlequin program, which allowed the hierarchical partitioning of the total genetic variation between biomes, between populations within biomes and within populations and the estimation of the *F*-statistics, which was tested by random permutation. Thus, two separate methods were used for the calculation of the *F*-statistics, one based on the frequency and the other using AMOVA. To study the genetic affinity between individual bees we used the Arlequin program to perform the assignment test (Paetkau *et al.*, 1995).

Gene flow between populations was estimated based on the mean number of migrants per generation as calculated using the Genepop program.

Principal coordinates analysis plots and unweighted pair-group method using an arithmetic average (UPGMA) dendrograms from Nei's (1978) genetic distance matrix were constructed to investigate the relationships between populations using the STATISTICA program (Statsoft, 2000). The stress statistic (Kruskall, 1964) was calculated to determine the goodness of fit of the display generated by the principal coordinates analysis.

Results

The molecular analysis results demonstrated that populations of uruçu amarela from the Minas Gerais savanna were genetically distinct from those present in the Atlantic forest. Our data also revealed that uruçu amarela populations found near Dom Bosco and Brasilândia de Minas were genetically different from those collected in other localities in the Minas Gerais savanna, because of which the data will be presented separately for this group.

Gene diversity

Most of the allozyme loci analyzed (*Pgm-1*, *Gpi-1*, *Icd-1*, *Hk-1*, *Gpdh-1*, *Gpdh-2*, *Mdh-2*, *Est-5*, *Est-6*, *Est-7*, *Dia-1*, *Adh-1* and *Acp-1*) were monomorphic for the same allele in all populations. The *Est-1*, *Est-2* and *Est-3* loci were also monomorphic but presented private alleles, that is, alleles found in either savanna or in Atlantic forest populations from the sampled areas. Only the *Mdh-1, Pep-1* and *Est-4* loci were polymorphic.

Null alleles were detected at the *Hbdh-1* locus, with the bees from the Dom Bosco and Brasilândia de Minas colonies being the only bees from savanna colonies to show hydroxybutyrate dehydrogenase activity. The samples were analyzed several times for the *Hbdh-1* locus but the bands for this enzyme were consistently absent in bees from the savanna colonies, indicating that our results were not due to technical problems.

Bees from Dom Bosco and Brasilândia de Minas exhibited the *Est-4* private alleles from both savanna and Atlantic forest populations but showed a profile similar to populations from the Atlantic forest for the *Mdh-1* and *Hbdh-1* loci. Bees from Dom Bosco exhibited the typical savanna *Pep-1*^{2} allele, while bees from Brasilândia de Minas presented the typical Atlantic forest *Pep-1*^{1} allele.

Allozyme loci revealed low levels of genetic diversity, as revealed by the number of alleles and the observed (H_o) and expected (H_e) heterozygosity per locus (Table 2).

Table 2 - Allozyme, microsatellite and random amplified polymorphic DNA (RAPD) loci diversity for uruçu amarela sampled in the savanna and the Atlantic forest of Minas Gerais state. The values show the number of alleles observed (A_o) and the effective number of alleles (A_e) along with the observed heterozygosity (H_o) and Nei's unbiased expected heterozygosity (H_e).

	Savanna				Atlantic forest				BRA/DB*			
Locus	A_o	A_e	H_o	H_e	A_o	A_e	H_o	H_e	A_o	A_e	H_o	H_e
Allozyme												
Est-4	1.00	1.00	0.00	0.00	2.00	1.03	0.03	0.03	3.00	2.20	0.92	0.57
Pep-1	2.00	1.01	0.01	0.01	1.00	1.00	0.00	0.00	2.00	1.67	0.00	0.41
Mdh-1	2.00	1.08	0.07	0.08	1.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00
Over all loci	1.10	1.00	0.00	0.00	1.05	1.00	0.00	0.00	1.15	1.10	0.05	0.05
Microsatellite												
Mbi215	1.00	1.00	0.00	0.00	1.00	1.00	0.00	0.00	2.00	1.22	0.20	0.18
Mbi218	3.00	1.29	0.21	0.23	5.00	1.15	0.10	0.13	1.00	1.00	0.00	0.00
Mbi232	1.00	1.00	0.00	0.00	4.00	1.06	0.06	0.06	2.00	1.92	0.49	0.48
Mbi233	2.00	1.01	0.02	0.02	5.00	2.82	0.40	0.65	1.00	1.00	0.00	0.00
Mbi254	4.00	1.80	0.39	0.45	4.00	3.22	0.55	0.69	1.00	1.00	0.00	0.00
Over all loci	1.67	1.12	0.07	0.08	2.56	1.47	0.12	0.17	1.22	1.12	0.02	0.07
RAPD	1.82	1.38		0.23	1.79	1.34		0.21	1.43	1.30		0.17

*Bees from Brasilândia de Minas and Dom Bosco (q.v. Table 1).

Significant deviations from H-W equilibrium were found for a reduced number of loci and populations at the 5% level. In turn, complete linkage disequilibrium was found for all 210 pairs of loci. Allozyme data (21 loci) allowed correct assignment of 77% of the bees to their population origin, with a much higher assignment efficiency (98%) for the Atlantic forest populations.

The nine analyzed microsatellite loci generated 31 alleles. The Mbi32, Mbi232, Mbi233 and Mbi278 loci generated private alleles for populations from savanna and Atlantic forest, *i.e.*, allele 1 at these loci was absent from the Atlantic forest populations. Populations from Dom Bosco and Brasilândia de Minas presented different alleles from the other savanna populations for the Mbi215, Mbi218, Mbi232 and Mbi233 loci.

Microsatellite loci showed moderate intrabiome variation in terms of observed alleles per locus, observed (H_o) and expected (H_e) heterozygosity (Table 2), although significantly higher than allozyme loci. Significant deviations from H-W equilibrium were found for some populations and loci at the 5% level. Tests for linkage disequilibrium between microsatellite loci within populations gave 17 significant P-values at the 5% level out of 36 pairs of loci, concentrated mainly on Atlantic forest areas. Microsatellite data showed lower assignment efficiency than allozyme, with 40% of bees correctly assigned to their population of origin, ranging from 60% for Dom Bosco/Brasilândia de Minas to 26% for the Atlantic forest populations.

The RAPD technique was also an effective means for identifying markers that were unique to savanna populations (*e.g.* OPB14, OPD20, OPE07, OPF06, OPG19 and OPU16) or Atlantic forest populations (*e.g.* OPB14, OPD20, OPE07, OPF06, OPG07, OPG19 and OPT18). However, populations from Dom Bosco and Brasilândia de Minas sometimes presented molecular markers specific to the savanna and sometimes molecular markers specific to the Atlantic forest. Additionally, molecular markers diagnostic for these two populations were detected with primers OPE07, OPJ15, OPK01, OPM15, OPN16, OPO06, OPP04 and OPZ06. Allele frequency tables for all loci are available on request from the authors.

Population differentiation

For the allozymes, the overall F_{ST} was 0.18 for savanna populations and 0.27 for Atlantic forest populations (Table 3). For the microsatellites, the multilocus F_{ST} was 0.25 for savanna populations and 0.21 for Atlantic forest populations (Table 3), confirming the high genetic diversity found among the populations sampled. Additionally, the N_m parameter estimated from allozyme and microsatellites was 0.004 and 0.055 bees per generation, respectively, indicating a low migration rate between populations.

The AMOVA (Table 4) demonstrated a high degree of population differentiation between the Minas Gerais savanna and Atlantic forest. Significant genetic differences between biomes as well as between populations were revealed by the allozyme and microsatellite markers, 89% of the total genetic variation for allozymes and 66% for the microsatellites loci being attributable to biome divergence. The AMOVA F_{ST} values for both allozyme and microsatellite loci were very high.

Table 3 - Single-locus Wright's F_{ST} values estimated from allozyme and microsatellite markers and G_{ST} values estimated from random amplified polymorphic DNA (RAPD) markers for uruçu amarela bees sampled in the savanna and the Atlantic forest of Minas Gerais state.

Locus	Savanna	Atlantic forest	BRA/DB*
Allozyme F_{ST} values			
Est-4	-	0.27	0.07
Pep-1	0.11	-	1.00
Mdh-1	0.19	-	-
Over all loci	0.18	0.27	0.46
Microsatellite F_{ST} values			
Mbi215	-	-	0.14
Mbi218	0.30	0.12	0.00
Mbi232	-	0.20	0.50
Mbi233	0.09	0.25	-
Mbi254	0.23	0.19	-
Over all loci	0.25	0.21	0.40
RAPD G_{ST} values	0.40	0.52	0.47
Population pairwise F_{ST} values	Savanna	Atlantic forest	BRA/DB
Savanna	0.0000	0.9186†	0.9917†
Atlantic forest	0.78259‡	0.0000	0.9527†
BRA/DB*	0.73700‡	0.65161‡	0.0000

*Bees from Brasilândia de Minas and Dom Bosco (q.v. Table 1).
†,†Pairwise F_{ST} values estimated from allozyme (†) or microsatellite (‡) frequency data and significant at the 5% level after 110 permutations.

The UPGMA dendrogram and principal coordinates analysis plot (Figure 2) constructed from the allozyme, microsatellite and RAPD genetic distance matrices showed three distinct clusters, one containing the Atlantic forest populations, another the savanna populations and a third containing the Dom Bosco and Brasilândia de Minas populations. The principal coordinates analysis stress-values yielded an excellent goodness of fit according to the classification proposed by Kruskal (1964).

Discussion

The allozyme, microsatellite and RAPD molecular marker data set used in our study all produced the same clustering pattern for the populations analyzed, supporting the differences noted by visual examination of the molecular profiles and revealing the genetic differences between the Minas Gerais savanna and Atlantic forest populations. Although populations shared most of the alleles, there was a large number of private alleles that specifically character-

Table 4 - Analysis of molecular variance (AMOVA) and degrees of freedom (df) for allozyme and microsatellite markers in populations of uruçu amarela bees sampled in the savanna and the Atlantic forest of Minas Gerais state.

	Allozyme			Microsatellite		
Sources of variation†	df	% variation	*F* statistics	df	% variation	*F* statistics
Biomes	1	89.91	F_{CT} = 0.8990*	1	66.27	F_{CT} = 0.6627*
Populations	15	8.76	F_{SC} = 0.8673*	15	11.30	F_{SC} = 03350*
Within populations	1375	1.34	F_{ST} = 0.9866*	551	22.43	F_{ST} = 0.7757*
Sources of variation‡	df	% variation	*F* statistics	df	% variation	*F* statistics
Biomes	2	97.37	F_{CT} = 0.9737*	2	72.34	F_{CT} = 0.7234*
Populations	14	1.18	F_{SC} = 0.4494*	14	4.09	F_{SC} = 0.1479*
Within populations	1375	1.45	F_{ST} = 0.9855*	551	23.57	F_{ST} = 0.7643*

†Savanna and Atlantic forest biomes.
‡Savanna, Atlantic forest and, Brasilândia de Minas and Dom Bosco localities.
*p < 0.00000, after 1023 permutations.

ized savanna and Atlantic forest populations. Differences in allelic frequencies of the same alleles in different populations (*e.g. Mdh-1* allele 1 and Mbi218 alleles 2, 3 and 4) also helped to differentiate populations.

These results indicate that Minas Gerais savanna populations of uruçu amarela constitute a different species from that present in the Minas Gerais Atlantic forest, the savanna populations being *M. rufiventris* and the Atlantic forest populations *M. mondury*, as originally proposed by Melo (2003).

Our data also suggests that populations found in the Dom Bosco and Brasilândia de Minas areas are genetically different from populations collected in other localities in the Minas Gerais savanna and form a separated group in our statistical analysis, as shown in Tables 2, 3 (the pairwise population F_{ST} values) and 4 and in Figure 2. However, because of the small sample size of these populations, additional studies are needed with larger samples from Dom Bosco and Brasilândia de Minas before a definitive conclusion can be made on the taxonomic status of the bees from these localities.

Biochemical studies have already demonstrated the usefulness of enzyme phenotypes in the taxonomic characterization of several other Hymenoptera (Wagner and Brisco, 1983; Kuenzi and Coppel, 1986). Studies reporting on the detection of species-specific patterns that permit distinguishing closely related organisms using RAPD (Waldschmidt *et al.*, 2000; Oliveira *et al.*, 2004) or microsatellite markers (Estoup *et al.*, 1995; Haavie *et al.*, 2000; Dhuyvetter *et al.*, 2004) are also well documented.

In our study, the F_{ST} values and the AMOVA results (Tables 3 and 4) confirmed that there were significant genetic differences between the samples analyzed. Such genetic heterogeneity can be explained by reduced gene flow and selective forces that act upon subpopulations in different habitats and lead to significant differentiation among them. Furthermore, it has been suggested that active dispersal in *Melipona* species is restricted to a few hundred meters at the most (Engels and Imperatriz-Fonseca, 1998), which may contribute to this high differentiation. Moreover, the genetic differentiation between subpopulations could be enhanced by habitat fragmentation, which isolate different *Melipona* subpopulations in small fragments (Brown and Albrecht, 2001) and decrease the number of nests found in each locality. This reinforces the importance of attempts to preserve the greatest possible number of colonies of these bees in Minas Gerais, if possible, in the region of their natural occurrence.

Our molecular analysis results also showed that both species are characterized by low variability in terms of allele number. The single-locus allozymes and microsatellite expected heterozygosity values estimated for *M. rufiventris* and *M. mondury* were also low in comparison with those reported for other bee species (Snyder, 1974, Metcalf *et al.*, 1975, Pamilo *et al.*, 1978; Berkelhamer, 1983, Packer and Owen, 1992, Estoup *et al.*, 1995; Widmer *et al.*, 1998; Widmer & Schmid-Hempel, 1999). Environmental degradation with the consequent subdivision and reduction of populations is one explanation for the low levels of variability observed in our study. Indeed, it was difficult to find colonies in several localities because *M. rufiventris* and *M. mondury* populations in Minas Gerais consist of small local populations, with these species being so depleted that they are now considered endangered species in Minas Gerais (Campos, 1998). The small number of populations of these species could be contributing to the reduction in their genetic variability by inbreeding and/or genetic drift. Inbreeding could also have contributed to the reduction in populations size due to the deleterious effects of homozygosis in loci responsible for sex determination in these bees, leading to the appearance of diploid drones in colonies. Packer and Owen (2001) have already showed that endangered species of some Lepidoptera had significantly lower levels of heterozygosity when compared with non-endangered species of the same order. The effective reduced population size and the occurrence of population

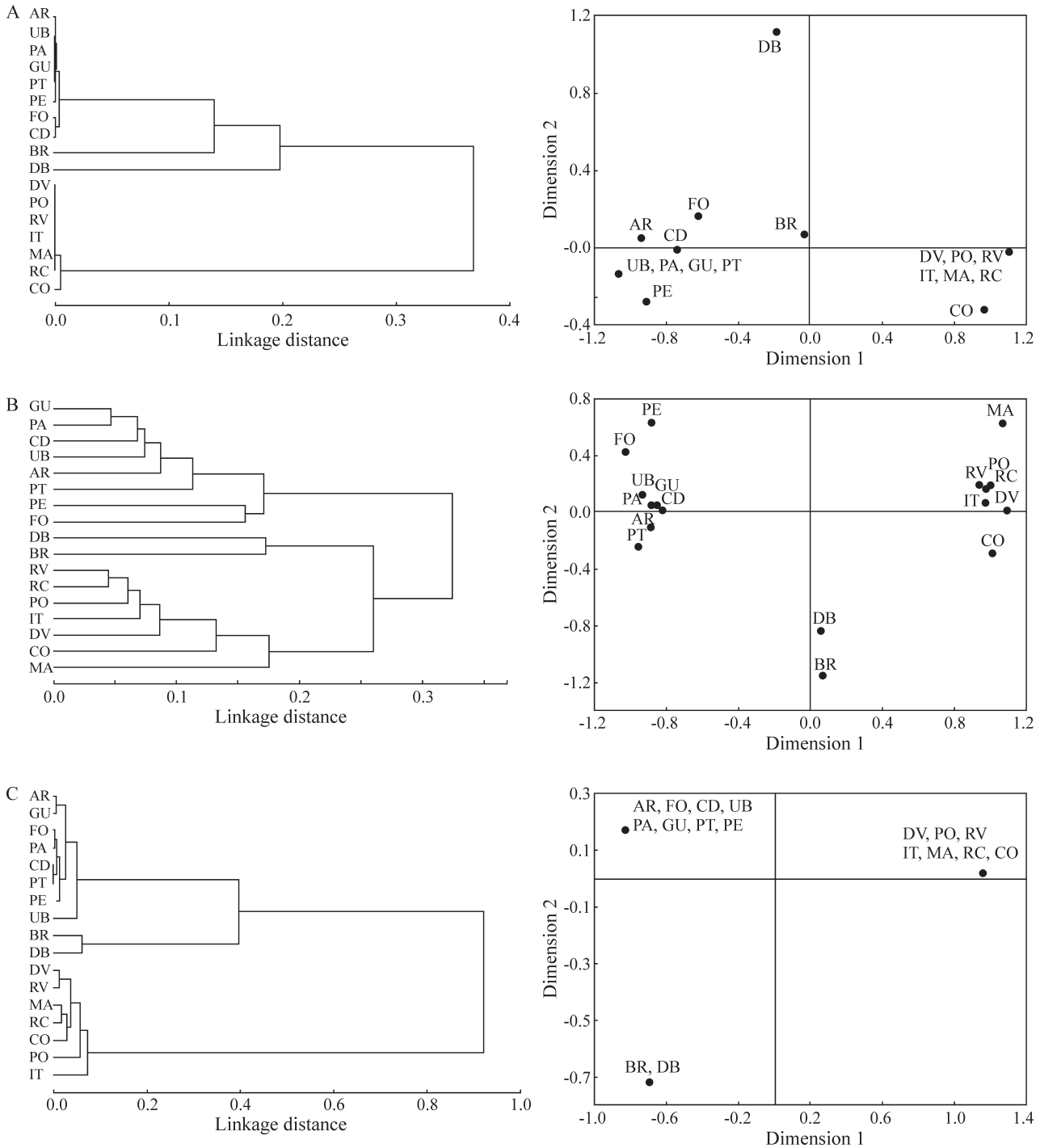

Figure 2 - Unweighted pair-group method using an arithmetic average (UPGMA) dendrogram and principal coordinates analysis plots related to 17 populations of uruçu amarela bees evaluated by allozyme (A, Stress (S) = 0.0043; 100 iterations), microsatellite (B, S = 0.0000042; 100 iterations) and random amplified polymorphic DNA (RAPD) (C, S = 0.0572; 46 iterations) markers.

bottlenecks have also been cited as possible reasons for the low levels of polymorphism found in the ant *Diacamma indicum* (Viginier *et al.*, 2004), and Goropasiinaya *et al.* (2001) reported a reduction in the diversity of microsatellites in small and geographically isolated populations of the ant *Formica cinerea*.

In conclusion, our results suggest that populations of uruçu amarela found in the savanna (*M. rufiventris*) and the Atlantic forest (*M. mondury*) of Minas Gerais should be treated separately for conservation purposes and that colonies should not be translocated from the Atlantic forest to the savanna or vice-versa. Since our study shows that there are two different endangered uruçu amarela species in Minas Gerais, beekeepers and governmental authorities must be aware of the effects of translocations and protection policies and should consider the entire species distribution

area to avoid the loss of region-specific alleles. Additionally, our data suggest that special attention should be given to populations from Dom Bosco and Brasilândia de Minas until their taxonomic status is clarified.

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