



Genetic variability in *Melipona scutellaris* from Recôncavo, Bahia, Brazil

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ABSTRACT. Bees play a key role in pollination and thereby help maintain plant diversity. The stingless bee *Melipona scutellaris* is an important pollinator in northeastern Brazil because it is endemic to this region. Both deforestation and timber harvesting have reduced the nesting sites for this species, thus reducing its population and range. Genetic studies may help reverse this process by providing important tools for their proper management with a view to conservation of this species. Microsatellite markers have proven to be ideal for mapping genes and population genetic studies. Our aim was to study, using microsatellite markers, the interpopulation genetic variability of *M. scutellaris* in different parts of the Recôncavo region in Bahia State, Brazil. In all, 95 adult workers from 11 localities in Recôncavo Baiano (Amargosa, Cabaceiras do Paraguaçu, Conceição da Feira, Conceição do Almeida, Domingos Macedo Costa, Governador Mangabeira, Jaguaripe, Jiquiriça, Maragojipe, São Felipe, and Vera Cruz) were analyzed using 10 pairs of microsatellite primers developed for different Meliponini species. The total number of alleles, allele richness, and genetic diversity ranged from 2 to 7 per locus (average = 4.4), 1.00 to 4.88, and 0.0 to 0.850, respectively. The expected and observed

heterozygosities varied from 0.0 to 0.76 and 0.0 to 0.84, respectively. No locus showed deviation from the expected frequencies in the chi-square test or linkage disequilibrium. The fixation index, analysis of molecular variance, and unweighted pair-group method using the arithmetic average revealed the effects of human activities on the populations of *M. scutellaris*, as little genetic structure was detected.

Key words: Recôncavo Baiano; Meliponini tribe; Pollinators; Microsatellite markers; Genetic diversity; Conservation

INTRODUCTION

Within stingless bees, the species *Melipona scutellaris*, popularly known as “uruçu verdadeira”, “uruçu do nordeste”, or “uruçu do campo”, stands out for being endemic to northeastern Brazil. Its range comprises the area from Bahia to Rio Grande do Norte States, particularly transition zones between the Atlantic Forest and caatinga (semi-arid region). *M. scutellaris* is one of the most raised species for honey and propolis production, representing an important income in familial agriculture (Kerr et al., 1996).

Deforestation and timber harvesting have been destroying the nesting sites of stingless bees (cavities in trees or soil), while agrochemicals affect colonies or visiting flowers in addition to contaminating rivers and ponds in which the bees collect water. These activities have effectively reduced their population size and, consequently, their geographical range. Population reduction eventually leads to the loss of genetic variability. In bees, this process is particularly damaging once the lack of genetic variation interferes with sex determination, potentially increasing the formation of diploid males. Therefore, genetic studies might be helpful to counteract this process by providing relevant information to design proper management techniques focused on the conservation of bee species (Carvalho-Zilse and Kerr, 2006).

The region named Recôncavo Baiano encompasses an area of 5250.51 km² in Bahia State with a human population of 576,672 inhabitants, according to the Brazilian Institute of Geography and Statistics. This region includes an intricate landscape that includes coastal zones with several river basins, waterfalls, beaches, and mangroves, and a few relicts of the Atlantic forest. Indeed, most of the Atlantic forest, one of the largest original biomes in Brazil, has been intensively deforested and replaced by sugarcane plantations, pastures for cattle, and other crops that, coupled with an inefficient recovery policy, determined the maintenance of a few forest patches (Oliveira and Albuquerque, 2010).

Therefore, the aim of this study was to estimate the interpopulation genetic variation of *M. scutellaris* from Recôncavo Baiano based on microsatellite markers in order to understand the effects of environmental changes in this region on the population of this important and intensively raised pollinator in northeastern Brazil.

MATERIAL AND METHODS

The samples comprised adult workers of *M. scutellaris* obtained from bee keepers or from nests collected in the forests. Specimens from 95 colonies from 11 localities in Recôncavo Baiano [Amargosa (AM), Cabaceiras do Paraguaçu (CBP), Conceição da Feira

(CF), Conceição do Almeida (CA), Domingos Macedo Costa (DMC), Governador de Mangabeira (GOV), Jaguaripe (JG), Jiquiriça (JQ), Maragojipe (MGJ), São Felipe (SF), Vera Cruz (VC)] (Table 1 and Figure 1) were used. DNA extraction was performed according to Waldschmidt et al. (1997) using 1 worker per colony.

Table 1. Localities, number of colonies, altitude of samples, and geographic coordinates of *Melipona scutellaris* used in this study.

Municipality	Code	No. of colonies	Altitude (m)	Geographic coordinates
Amargosa	AM	13	400	12°48'03.9"S 39°07'34.1"W
Cabaceiras do Paraguaçu	CBP	5	210	12°32'08"S 39°11'27"W
Conceição da Feira	CF	4	227	12°39'20"S 39°06'10"W
Conceição do Almeida	CA	10	216	12°48'3.9"S 39°07'34.1"W
Domingos Macedo Costa	DMC	5	192	12°51'31.3"S 39°09'28.4"W
Governador Mangabeira	GOV	11	204	12°34'51.2"S 39°06'51.6"W
Jaguaripe	JG	13	11	13°06'31.0"S 39°07'56.3"W
Jiquiriça	JQ	6	342	13°15'24"S 39°34'20"W
Maragojipe	MGJ	11	39	12°50'16"S 38°54'51.8"W
São Felipe	SF	4	195	12°45'39"S 39°03'02.9"W
Vera Cruz	VC	13	13	12°57'37"S 38°36'31"W

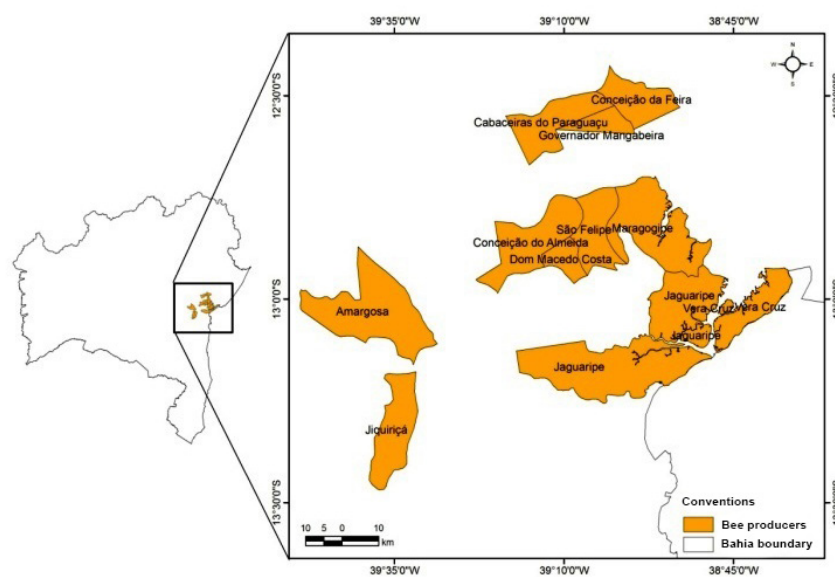


Figure 1. Map of Recôncavo Baiano, Bahia, showing the sampled localities of *Melipona scutellaris*.

Ten heterologous microsatellite primers (Table 2) were selected and amplified as described by Peters et al. (1998). The amplifications were performed in a total volume of 10 μ L containing 1X buffer (Biotools, Spain), 0.1 mM of each dNTP, 0.4 μ M of each primer, 1.5 mM $MgCl_2$, 0.5 U Taq polymerase (Biotools), and 20 ng template DNA. The PCR conditions were as follows: an initial denaturation step at 94°C for 3 min, followed by 39 cycles at 92°C for 30 s, specific annealing temperature of each primer for 1 min, and 72°C for 30 s, plus a final extension step at 72°C for 5 min. The PCRs were carried out in an ATC201 thermocycler (Nyx Technik, USA). The amplification products were run using electrophoresis on an 8% non-denaturing polyacrylamide gel, visualized by 0.2% silver nitrate staining, and photodocumented.

Table 2. Microsatellite primers used in the study originally described in *Melipona bicolor* (Mbi), *Melipona mondury* (Mmo), *Melipona rufiventris* (Mru), and *Trigona carbonaria* (Tc4).

Locus	Size	Repeat	Ta ¹ (°C)	Ta ² (°C)	Primer sequence (5'-3')
Mbi215	92	(TTC) ₆	57.5	60.0	F: AGAGACGAAAAGTGGCGG R: GCTGTTCCCTGTTCGAG
Mbi218	131	(CCT) ₃ (TCT) ₇	60.0	60.0	F: CTCGACTTAATTTCCATCGGC R: GCAATTTCAATCGGACC
Mbi232	128	(CTT) ₁₃	50.0	53.0	F: TTTTCTCTTAAATTTCTTCT R: CTTACTCGACGACTTATTT
Mbi233	119	(GAA) ₁₅	57.5	53.0	F: ACGAGCACGGGCCATAA R: GATCCATCGACCGTCTT
Mbi254	213	(AAG) ₁₁	55.0	53.0	F: CAATCGTTGGAAGGGAAC R: GGACCTATACCCAAGTCCAT
Mbi256	127	(AGA) ₉	57.5	53.0	F: GGATTCAAGCAGACGATA R: GATCAAAGTCCCATCTT
Mmo15	100	(GAAC) ₅	54.0	53.0	F: GCAAAATGAGCAAACGGACA R: GAGAGAGAGGGAGGTTC
Mru03	120	(AG) ₆ (CGTT) ₆	63.0	57.0	F: AGAGAGAGAGAGAGACGC R: GGTTGAAAGGAGAACGAAC
Mru14	136	(TCG) ₈ TT(GTC) ₄	62.5	58.0	F: GCTGTTCCCTGTTCGAG R: CCCCTATCTTTATGCCG
Tc4.287	179	(GAA) ₉	55.0	53.0	F: TCCACCGCGATACGATGGTAC R: GTAATACAACCGCGCTTCTC

Ta¹ (°C) = annealing temperature. Ta² (°C) = optimized annealing temperature.

The diversity estimators, i.e., number of alleles per locus, allele richness (Petit et al., 1998), and genetic diversity (Nei, 1978), were calculated using the FSTAT software (Goudet, 1995). Analyses of observed heterozygosity (H_o) and expected heterozygosity (H_e) (Nei, 1978) per locus in each population were performed using the TFPGA software version 1.3 (Miller, 1997).

The GENEPOP 4.0 software (Raymond and Rousset, 1995) was used to estimate the deviation of microsatellite loci from Hardy-Weinberg equilibrium (HWE) using the chi-square test and to verify linkage disequilibrium (LD) between the loci. The FreeNA program (Chapuis and Estoup, 2007) was used to generate a corrected dataset of null alleles that might influence the values of genetic differentiation. The estimated values in the chi-square test were adjusted by the sequential Bonferroni's correction $P \leq 0.05$ (Rice, 1989).

Analysis of molecular variance (AMOVA) was used to verify the genetic structure within and among populations. The fixation index F_{ST} (Weir and Cockerham, 1984) was used to estimate the pairwise genetic distance between localities. These analyses were performed using the Arlequin 3.01 software (Excoffier et al., 2005). Grouping analysis was carried out with the unweighted pair-group method using the arithmetic average (UPGMA) (Dias, 1998) in the TFPGA software version 1.3 (Miller, 1997).

The Structure 2.1 software (Pritchard et al., 2000) was used to identify the population structure based on individual genotypes. Models with K from 1 to 10 populations, replicated 10 times, were tested assuming “admixture” and “correlated allele frequencies”. These tests were performed via Markov-Monte Carlo Chains, with a burn-in of 500,000 and 1,000,000 replicates. The K values were verified using the Harvester software (Earl and vonHoldt, 2012).

RESULTS

The total number of alleles in all loci was equal to 44, ranging from 20 in SF to 38 in AM, with a mean value of 29.36 alleles per locality. The number of alleles, allele richness, and genetic diversity within each locus are shown in Table 3. In those localities with genetic diversity equal to zero, the loci were monomorphic. Both H_E and H_O are listed in Table 4.

No loci deviated from the expected frequencies. However, the HWE could not be estimated for loci Mbi215 in VC, Mru03 in CBP, CF, GOV, and SF, Mru14 in CF, Mmo15 in CF, DMC, JG, JQ, MGJ, SF, and VC, and Tc4.287 in CBP, CF, DMC, JQ, MGJ, SF, and VC, once these loci were monomorphic in these localities. The P values in relation to HWE are listed in Table 4. In relation to LD, only locus Mru03 Tc4.287 was found in LD after sequential Bonferroni's correction $P \leq 0.05$ (Rice, 1989) (Table 5).

The pairwise values for the F_{ST} were low, ranging from -0.01 between AM and DMC and between CA and DMC to 0.23 between JQ and MGJ. Moreover, the F_{ST} values were significant in all comparisons using the GOV locality in most of the comparisons involving JQ, MGJ, SF, and VC (9 of 10 comparisons) and in some of the comparisons encompassing AM, CBP, CA (6 of 10), and CF (4 of 10) (Table 6). The total F_{ST} value in all populations and all loci was low, but positive (F_{ST} total = 0.10) (Table 7). Likewise, AMOVA revealed that 89.33% of the total genetic variation in *M. scutellaris* was found within localities (Table 7).

Grouping analysis via UPGMA (Figure 2) based on the mean values of genetic distances between colonies showed the occurrence of 4 clusters. The first group comprised the populations from DMC, VC, CA, AM, CBP, CF, JG, and SF, the second group was composed of samples from MGJ, and the 3rd and 4th groups were exclusively formed by GOV and JQ samples. Nonetheless, the bootstrap values were inconsistent. Analyses using Structure revealed a single group (Figure 3), although the K value determined using Harvester was K = 10 (Figure 4).

Table 3. Number of individuals analyzed (N), number of alleles (N_A), number of private alleles (Pa), allele richness (Ar), and Nei's genetic diversity (Nd) obtained in *Melipona scutellaris* per locus in each locality.

	AM	CBP	CF	CA	DMC	GOV	JG	JQ	MGJ	SF	VC
Mbi215											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	4	3	3	4	3	3	4	3	2	2	2
Pa	0	0	0	0	0	1	0	0	0	0	0
Ar	3.05	2.80	3.00	3.42	2.60	2.29	2.76	2.58	1.86	2.00	1.31
Nd	0.62	0.60	0.58	0.67	0.38	0.43	0.53	0.43	0.31	0.50	0.08
Mbi218											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	2	2	2	2	2	3	3	3	3	2	2
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	1.87	2.00	2.00	1.98	1.98	2.47	2.40	2.67	2.23	2.00	2.00
Nd	0.32	0.55	0.42	0.44	0.35	0.45	0.44	0.60	0.39	0.58	0.51
Mbi232											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	6	3	3	5	5	4	4	5	3	4	4
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	4.25	2.98	3.00	3.40	4.56	3.15	2.81	4.52	2.84	4.00	3.39
Nd	0.80	0.68	0.71	0.66	0.83	0.64	0.59	0.85	0.65	0.75	0.67
Mbi233											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	6	4	4	6	5	3	5	6	6	3	5
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	3.86	3.40	4.00	4.59	4.58	2.85	3.67	4.89	4.12	3.00	4.16
Nd	0.74	0.53	0.75	0.83	0.83	0.66	0.73	0.85	0.75	0.75	0.80
Mbi254											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	5	3	4	3	4	3	4	4	3	2	3
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	3.55	2.60	4.00	2.61	3.78	2.13	3.56	3.32	2.92	2.00	2.26
Nd	0.67	0.40	0.75	0.48	0.80	0.32	0.75	0.63	0.67	0.50	0.46
Mbi256											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	3	2	2	3	2	2	3	2	2	2	2
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	2.29	2.00	2.00	2.87	2.00	2.00	2.82	2.00	1.98	2.00	1.99
Nd	0.52	0.50	0.50	0.66	0.55	0.49	0.63	0.47	0.46	0.42	0.48
Mru03											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	4	2	2	3	3	2	4	4	4	1	3
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	3.19	1.80	2.00	2.46	2.80	1.36	2.15	3.32	2.93	1.00	2.40
Nd	0.62	0.20	0.25	0.43	0.60	0.09	0.29	0.63	0.60	0.00	0.44
Mru14											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	4	2	2	3	4	4	4	3	4	2	4
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	3.10	2.00	2.00	2.89	3.78	3.39	3.16	2.82	2.96	2.00	3.41
Nd	0.64	0.45	0.25	0.67	0.80	0.66	0.69	0.55	0.65	0.50	0.72
Mmo15											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	2	2	1	2	1	2	1	1	1	1	1
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	1.87	2.00	1.00	1.95	1.00	1.61	1.00	1.00	1.00	1.00	1.00
Nd	0.32	0.45	0.00	0.39	0.00	0.17	0.00	0.00	0.00	0.00	0.00
Tc4.287											
N	13	5	4	10	5	11	13	6	11	4	13
N_A	2	2	2	2	1	3	3	1	1	1	1
Pa	0	0	0	0	0	0	0	0	0	0	0
Ar	1.80	1.80	2.00	1.65	1.00	2.86	2.33	1.00	1.00	1.00	1.00
Nd	0.27	0.20	0.25	0.19	0.00	0.66	0.39	0.00	0.00	0.00	0.00
Total N_A per locality	38	25	25	33	30	29	35	32	29	20	27
Mean N_A per locality	3.8	2.5	2.5	3.3	3.0	2.9	3.5	3.2	2.9	2.0	2.7
Mean Ar per locality	2.88	2.34	2.50	2.78	2.81	2.41	2.66	2.81	2.38	2.00	2.29
Mean Nd per locality	0.55	0.46	0.45	0.54	0.51	0.46	0.50	0.50	0.45	0.40	0.42

For locality and locus abbreviations, see Tables 1 and 2, respectively.

Table 4. Expected heterozygosity (H_E), observed heterozygosity (H_O), and probability of Hardy-Weinberg equilibrium deviation (P) in *Melipona scutellaris* from Recôncavo Baiano.

	AM	CBP	CF	CA	DMC	GOV	JG	JQ	MGJ	SF	VC
H_E											
Mbi215	0.55	0.54	0.53	0.52	0.34	0.42	0.46	0.40	0.30	0.50	0.07
Mbi218	0.31	0.50	0.38	0.42	0.32	0.24	0.31	0.44	0.17	0.50	0.50
Mbi232	0.69	0.62	0.63	0.35	0.66	0.63	0.65	0.63	0.70	0.69	0.71
Mbi233	0.74	0.66	0.63	0.70	0.76	0.48	0.73	0.72	0.76	0.66	0.75
Mbi254	0.55	0.34	0.63	0.47	0.58	0.31	0.72	0.40	0.64	0.50	0.45
Mbi256	0.45	0.48	0.47	0.48	0.48	0.48	0.45	0.44	0.43	0.38	0.47
Mru03	0.51	0.18	0.22	0.26	0.32	0.09	0.28	0.40	0.57	0.00	0.31
Mru14	0.48	0.42	0.22	0.64	0.62	0.53	0.53	0.38	0.54	0.47	0.62
Mmo15	0.31	0.42	0.00	0.38	0.00	0.17	0.00	0.00	0.00	0.00	0.00
Tc4.287	0.26	0.18	0.22	0.18	0.00	0.50	0.20	0.00	0.00	0.00	0.00
H_O											
Mbi215	0.57	0.60	0.61	0.54	0.38	0.44	0.48	0.44	0.31	0.57	0.08
Mbi218	0.32	0.56	0.43	0.44	0.36	0.25	0.32	0.48	0.17	0.57	0.52
Mbi232	0.72	0.69	0.71	0.36	0.73	0.66	0.68	0.68	0.73	0.79	0.74
Mbi233	0.77	0.73	0.71	0.74	0.84	0.51	0.76	0.79	0.80	0.75	0.78
Mbi254	0.58	0.38	0.71	0.49	0.64	0.32	0.75	0.44	0.68	0.57	0.46
Mbi256	0.47	0.53	0.54	0.51	0.53	0.51	0.47	0.48	0.45	0.43	0.49
Mru03	0.53	0.20	0.25	0.27	0.36	0.09	0.29	0.44	0.59	0.00	0.32
Mru14	0.50	0.47	0.25	0.67	0.69	0.55	0.55	0.41	0.57	0.54	0.64
Mmo15	0.32	0.47	0.00	0.39	0.00	0.17	0.00	0.00	0.00	0.00	0.00
Tc4.287	0.27	0.20	0.25	0.19	0.00	0.52	0.21	0.00	0.00	0.00	0.00
P											
Mbi215	0.71	0.63	1.00	0.28	1.00	1.00	0.13	1.00	1.00	0.31	-
Mbi218	1.00	1.00	1.00	1.00	1.00	0.61	0.66	1.00	0.20	1.00	0.60
Mbi232	0.52	1.00	1.00	0.49	0.89	0.69	1.00	0.88	0.31	1.00	0.68
Mbi233	0.46	1.00	0.32	0.52	0.84	0.48	0.83	0.03	0.86	0.06	0.77
Mbi254	0.52	0.11	0.32	1.00	0.70	1.00	0.80	0.77	0.30	0.31	0.16
Mbi256	1.00	0.43	1.00	0.84	1.00	0.07	1.00	1.00	1.00	1.00	0.08
Mru03	0.86	-	-	0.49	0.62	-	1.00	0.74	0.11	-	0.67
Mru14	0.87	1.00	-	0.22	0.69	0.71	0.66	0.51	0.78	1.00	0.51
Mmo15	1.00	1.00	-	1.00	-	1.00	-	-	-	-	-
Tc4.287	1.00	-	-	1.00	-	1.00	0.52	-	-	-	-

(-) = monomorphic loci for the sampled locality. For locality and locus abbreviations, see Tables 1 and 2, respectively.

Table 5. Results of pairwise probabilities of linkage disequilibrium considering all 10 loci in samples of *Melipona scutellaris* from Recôncavo Baiano.

Locus pair	Probability (P)	Locus pair	Probability (P)
Mbi215 and Mbi218	0.95	Mbi232 and Mru14	0.99
Mbi215 and Mbi232	0.97	Mbi233 and Mru14	1.00
Mbi218 and Mbi232	0.99	Mbi254 and Mru14	0.99
Mbi215 and Mbi233	0.92	Mbi256 and Mru14	0.97
Mbi218 and Mbi233	0.99	Mru03 and Mru14	0.85
Mbi232 and Mbi233	0.97	Mbi215 and Mmo15	0.49
Mbi215 and Mbi254	0.96	Mbi218 and Mmo15	0.47
Mbi218 and Mbi254	0.96	Mbi232 and Mmo15	0.89
Mbi232 and Mbi254	0.61	Mbi233 and Mmo15	0.57
Mbi233 and Mbi254	1.00	Mbi254 and Mmo15	0.98
Mbi215 and Mbi256	0.74	Mbi256 and Mmo15	0.99
Mbi218 and Mbi256	0.99	Mru03 and Mmo15	0.78
Mbi232 and Mbi256	0.91	Mru14 and Mmo15	0.66
Mbi233 and Mbi256	1.00	Mbi215 and Tc4.287	0.40
Mbi254 and Mbi256	0.66	Mbi218 and Tc4.287	0.94
Mbi215 and Mru03	0.18	Mbi232 and Tc4.287	0.94
Mbi218 and Mru03	0.71	Mbi233 and Tc4.287	0.49
Mbi232 and Mru03	0.85	Mbi254 and Tc4.287	0.98
Mbi233 and Mru03	1.00	Mbi256 and Tc4.287	0.98
Mbi254 and Mru03	1.00	Mru03 and Tc4.287	0.04*
Mbi256 and Mru03	0.99	Mru14 and Tc4.287	0.60
Mbi215 and Mru14	0.95	Mmo15 and Tc4.287	0.69
Mbi218 and Mru14	0.41		

*Significant after sequential Bonferroni's correction ($P \leq 0.05$). For locus abbreviations, see Table 2.

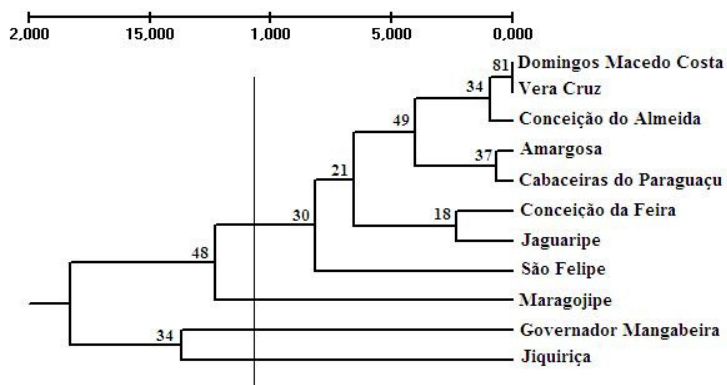
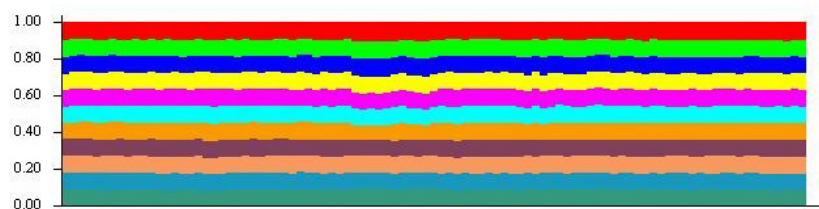
Table 6. Values of pairwise F_{ST} in samples of *Melipona scutellaris* from Recôncavo Baiano.

F_{ST}	AM	CBP	CF	CA	DMC	GOV	JG	JQ	MGJ	SF	VC
AM	0										
CBP	0.05 ^{NS}	0									
CF	0.07 ^{NS}	0.07 ^{NS}	0								
CA	0.00	0.06 ^{NS}	0.10 ^{NS}	0							
DMC	-0.01	0.10 ^{NS}	0.06	-0.01	0						
GOV	0.11*	0.19*	0.20*	0.09*	0.09*	0					
JG	0.08*	0.12*	0.03	0.07*	0.03	0.15*	0				
JQ	0.12*	0.19*	0.18 ^{NS}	0.17*	0.15*	0.13*	0.18*	0			
MGJ	0.14*	0.15*	0.16*	0.13*	0.10 ^{NS}	0.18*	0.12*	0.23*	0		
SF	0.10*	0.12*	0.13*	0.09*	0.06 ^{NS}	0.15*	0.09*	0.22*	0.10*	0	
VC	0.06*	0.12*	0.16*	0.05*	0.01 ^{NS}	0.14*	0.08*	0.20*	0.07*	0.08*	0

*Significant after sequential Bonferroni's correction ($P \leq 0.05$). ^{NS}Non-significant after sequential Bonferroni's correction ($P \leq 0.05$). For locality abbreviations, see Table 1.

Table 7. Analysis of molecular variance (AMOVA) and fixation index (F_{ST}) values obtained in all microsatellite loci analyzed of *Melipona scutellaris* from Recôncavo Baiano.

Source of variation	Components of variance	Percentage variation
Among populations	0.28	10.67
Within populations	2.41	89.33
Total	2.70	
F_{ST}	0.10	

**Figure 2.** UPGMA dendrogram showing the mean genetic distances among the sampled localities of *Melipona scutellaris*.**Figure 3.** Graph of Bayesian analysis using the Structure software showing no genetic structure in *Melipona scutellaris* samples.

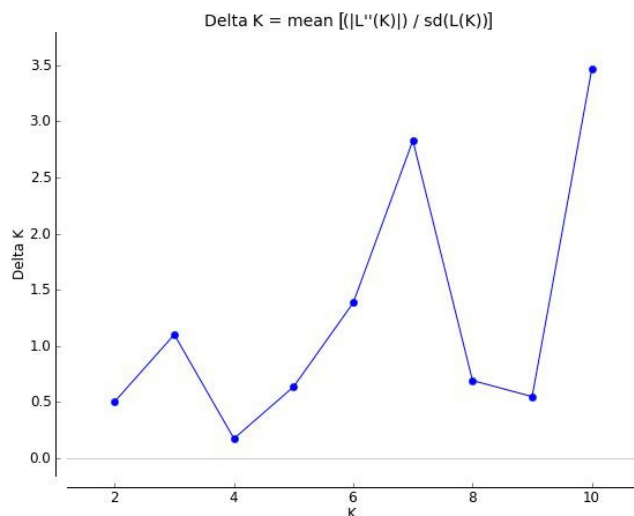


Figure 4. Graph showing the K values based on the genetic data of *Melipona scutellaris* samples using the Harvester software.

DISCUSSION

The number of alleles varied in the *M. scutellaris* samples, as reported previously by Peters et al. (1998) in *M. bicolor*. The number of alleles per locus and H_o observed in *M. bicolor* ranged from 3 to 6 and from 0.12 to 0.88, respectively. When the primers Mbi were tested in *M. quadrifasciata*, the number of alleles per locus ranged from 1 to 4 and the H_o varied from 0 to 1. Once these primers are heterologous, the amplified regions might be different either in size or base pair composition from the sequences in *M. bicolor*, thus amplifying distinct loci (Carvalho-Zilse and Kerr, 2006). Besides, mutations in the flanking regions might hinder the annealing of primers to template DNA during amplification via PCR, resulting in a null allele (Chapuis and Estoup, 2007), differently from the results in the present study once null alleles were corrected for.

Variation in H_o and H_E was also observed by Lopes et al. (2009) in *M. rufiventris* and by Green et al. (2001) in *Trigona carbonaria*. A significant deviation from HWE was detected when loci Mru03 and Tc4.287 were compared after sequential Bonferroni's correction. The loci Mbi215, Mru03, Mru14, Mmo15, and Tc4.287 were not in proportion to HWE in some localities. The deviation from HWE and the lack of these data in some loci are likely to be caused by the low frequency or absence of heterozygotes in these populations. The small sample size of certain localities, e.g., CF and SF (4 sampled colonies each), CBP and DMC (5 colonies each), and JQ (6 colonies each), might have limited the detection of polymorphisms in these populations. It should be pointed out that low levels of sampling are related to the decreased population size of *M. scutellaris* in the studied region, which also justifies the development of similar studies that might be helpful for the preservation and conservation management of this species.

Another putative explanation for the low frequency of heterozygotes in *Melipona* refers to the swarming behavior of these bees, preferential mating with a single male, and

presence of a single active physogastric queen bee per colony. In *Melipona*, the establishment of new nests is dependent on the mother colony, which results in reduced migration distances within similar environments (Kerr, 1987). Moreover, some studies have shown that the utilization of heterologous primers, although being useful to assess the genetic structure in other species, might lead to lower polymorphism levels than species-specific microsatellite primers (Francisco et al., 2006).

Even though the pairwise F_{ST} comparisons were statistically significant, the values obtained are considered low for meliponins inasmuch as this index was also low when all samples were grouped, revealing little genetic structure among the studied populations of *M. scutellaris*. Furthermore, the UPGMA and Bayesian analysis (using Structure) showed a lack of structuring among these populations. AMOVA showed that 89.32% of the overall genetic variation in this species was found within localities and only 10.67% was found among them, thus corroborating the absence of genetic structure in these samples.

In spite of the $K = 10$ obtained in Harvester, the graph and Q values generated using Structure indicate no genetic structure. According to Pritchard et al. (2000), even when genetic structure is absent, the K values might be higher than 1 when a species undergoes high inbreeding rates.

The results obtained by grouping analyses (UPGMA) and Bayesian inference can be related to the low polymorphic nature of the loci analyzed, commercialization and trade of swarms by bee keepers, changes in larval disks, rational multiplication of colonies, deforestation, and destruction of nesting sites leading to the admixture of genetic material, and the reduction of natural colonies, thus hindering population analysis in *M. scutellaris*. In fact, *M. scutellaris* is the most commercially raised stingless bee species that, if not managed properly, might undergo decreased levels of genetic variation.

Nonetheless, population genetic studies allow the development of efficient management strategies that are able to maintain the genetic variation of populations and to minimize the effects of inbreeding and the loss of alleles. It has been well established that decreased genetic variation as a consequence of inbreeding and allele loss reduces the ability of populations to adapt to environmental changes and, therefore, hinders their long-term survival (Arif and Khan, 2009).

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