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Polimorfismo isoenzimático em *Tetragonisca angustula* (Hymenoptera; Meliponinae, Trigonini) do Sul do Brasil

Isoenzyme Polymorphism in *Tetragonisca angustula* (Hymenoptera; Meliponinae, Trigonini) from Southern Brazil

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Resumo

Abelhas sem ferrão são insetos nativos brasileiros e que desempenham um elevado potencial polinizador para inúmeras culturas agrícolas e plantas nativas. *Tetragonisca angustula* é uma das espécies mais manejadas pelos meliponicultores, deste modo, conhecer estrutura genética dessas populações é de grande importância, principalmente, para futuros projetos de conservação. Para isso, neste estudo foi realizado a caracterização bioquímica e a relação genética por eletroforese das isoenzimas esterase (EST), isocitrato desidrogenase (IDH), enzima málica (ME) e malato desidrogenase (MDH). Abelhas adultas foram coletadas em meliponários de Maringá e Astorga na região Noroeste do estado do Paraná, Brasil. *T. angustula* de Maringá apresentou dois locos polimórficos (22,22%) e oito locos polimórficos foram observados em *T. angustula* de Astorga (88,89%). A heterozigosidade média estimada para *T. angustula* foi de 0,1260. O valor de F_{IS} foi de 0,4036 e indicou um excesso de homozigose. O valor F_{IT} mostrou que as duas populações não foram diferenciadas, sem nenhum fluxo gênico entre elas. Os resultados sugerem a mesma origem para as duas populações de acordo com a distância genética de Nei.

Palavras-chaves: Eletroforese. Caracterização Genética. Genética de Populações. Isoenzimas. *Tetragonisca angustula*.

Abstract

Stingless bees are native Brazilian insects and have a high pollinating potential for countless agricultural crops and native plants. Tetragonisca angustula bees are one of the species most managed by honey farmers and knowing about the genetic structure of these populations is of great importance, especially for future conservation projects. For this, the biochemical characterization and genetic relationship by electrophoresis of esterase (EST), isocitrate dehydrogenase (IDH), malic enzyme (ME), and malate dehydrogenase (MDH) isozymes was analyzed. Adult bees were collected from meliponaries in Maringá and Astorga in the northwestern region of the state of Paraná, Brazil. T. angustula from Maringá presented two polymorphic loci (22.22%) and eight polymorphic loci were observed in T. angustula from Astorga (88.89%). Average heterozygosity estimated for T. angustula was 0.1260. F_{IS} value was 0.4036 and indicated an excess of homozygous. F_{IT} value showed that the two populations were not differentiated, without any gene flow between them. The results suggest the same origin for the two populations in agreement with Nei's genetic distance.

Keywords*: Electrophoresis. Genetic Characterization. Isoenzymes. Population Genetics. Tetragonisca angustula.*

1 Introduction

Tetragonisca angustula (LATREILLE, 1811) (Hymenoptera; Meliponinae, Trigonini), locally known "Jataí", is one of the commonest neotropical Trigonini and with great importance for the ecology of several ecosystems. *T. angustula* is widely distributed from south of Mexico to Argentina and in the whole Brazilian territory and well adapted to a wide area of occurrence (CAMARGO; PEDRO, 2008). The Trigonini constitutes a diversified group, with many species building their nests in living or dead tree trunks (NOGUEIRA-NETO, 1997).

Although *T. angustula* is not endangered, the increase in environmental degradation due to the intensification of agriculture represents a serious threat (JHA, 2015). Essentially to pollinators of native flora (LICHTENBERG *et al*., 2017) and commercial crops (HALINSKI *et al*., 2020). Human activities such as habitat destruction due to losses, degradation, fragmentation, and increased use of pesticides are causing population declines in abundance and diversity worldwide (DIRZO *et al*., 2014; OLLERTON *et al*., 2014,). Leading to a decline in pollinator populations (POTTS *et al*., 2010; OLLERTON *et al*., 2014, GIANNINI *et al*., 2020).

Increased environmental degradation, with consequent habitat destruction, poses a serious threat to the Meliponinae and can result in the isolation of subpopulations (LOZIER; ZAYED, 2017). Thus, a knowledge of the genetic variability and genetic structure of stingless bee species is essential for the control and preservation of these species (FRANCISCO *et al*., 2014, 2017).

Isoenzyme analysis provides an important source of information for species differentiation (CASTANHEIRA; CONTEL, 1995; STUCHI *et al*., 2012; RONQUI *et al*., 2016). The use of these isoenzymes in studies on the characterization

of bee population genetics (STUCHI *et al*., 2008) and also acts as possible bioindicators of the presence of pesticides (FERMINO *et al*., 2011, MOREIRA *et al*., 2018).

Isoenzymes studies in the Apidae have been done by several investigators (AIDAR *et al*., 2001; CASTANHEIRA; CONTEL, 2005; COSTA *et al*., 2005; RUVOLO-TAKASUSUKI *et al*., 1997, 1998, 2006). However, only a few studies have investigated isoenzyme variability in *T. angustula.* Castanheira e Contel (1995) described polymorphism for the hexokinase enzyme locus and showed rare variants in two loci for glycerol-3-phosphate dehydrogenase. Castanheira e Contel (2005) observed a highly significant correlation between the amount of ferruginous color displayed in the mesepisternum of the *T. angustula* and the Hk^{88} allele frequency. Subsequently, RAPD (restriction fragment-length polymorphism) molecular markers, distinguished two groups of *T. angustula* based on their natural geographic distribution (OLIVEIRA *et al*., 2004). Despite this technique having great genetic variability between populations, just as isoenzymes were not able to separate *T. angustula* and *T. fiebrigi* in two species (BAITALA *et al*., 2006).

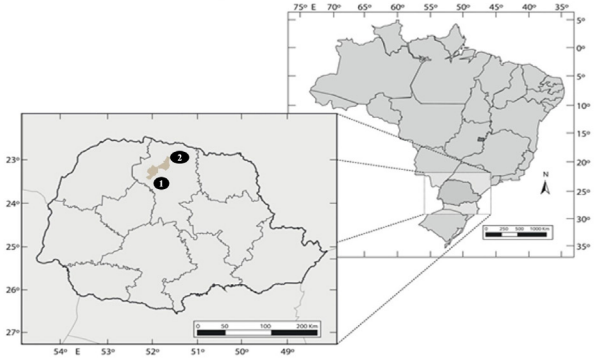
New methods have been developed, such as genomics, which are the most effective markers for genetic-population studies (LOZIER; ZAYED, 2017). Other simpler methods such as the use of mitochondrial DNA (mtDNA) and microsatellites (KOLING, MORETTO, 2010; FRANCISCO *et al*., 2014; SANTIAGO *et al*., 2016; Francisco *et al*., 2017), PCR-RAPD (polymerase chain reaction - random amplified polymorphic DNA) (OLIVEIRA *et al*., 2004), and PCR-RFLP (restriction fragment length polymorphism) (SANTOS *et al*., 2015) efficient in evaluating population genetics. Therefore, more informative systems are necessary to the understanding of the population genetic structure of these important stingless bees.

This research applied the isoenzyme analyses for the evaluation of two populations, demonstrating that it is possible to characterize and quantify genetic variability used the esterase, isocitrate dehydrogenase, malic enzyme, and malate dehydrogenase isoenzymes of *T. angustula* which occur in Northwest of Paraná.

2 Material and Methods

T. angustula adults were collected from two areas in the Northwest of Paraná, Southern Brazil, both areas located in semideciduous seasonal forest in the Atlantic Forest biome. Six workers were sampled from each of 10 colonies from Maringá (23º 25' 31" S, 51º 56' 19" W) and 23 colonies from Astorga (23º 13' 57" S, 51º 39' 56" W) (Figure 1). The distance between the two populations was 50 ± 10 km.

Figure 1 - Map of Brazil (right), shows the location of the state of Paraná, on the map of Paraná (left) showing the geographic sampling locations, 1 - Maringá and 2 – Astorga



Source: the authors.

2.1 Preparing the samples and electrophoresis

For electrophoretic analysis, we used the horizontal technique on 14% starch gels (Penetrose 30, Corn Products of Brazil S.A.) as described by Smithies (1955). The samples were homogenized in 30 μL of a 0.1% 2-mercaptoethanol solution and then centrifuged at 16,000 x g for 10 minutes at 2 ºC. Supernatants from the samples were then loaded singly into gel wells.

The zones of enzymatic activity visualized were the conventional ones for each isozyme studied: esterase – EST (EC 3.1.1.1), isocitrate dehydrogenase – IDH (EC 1.1.1.42), malic enzyme – ME (1.1.1.40), and malate dehydrogenase – MDH (EC 1.1.1.37). 4-Methylumbelliferyl esters (acetate and butyrate) and α-naphthyl esters (acetate and propionate) were used to study the substrate preferences of esterase. Staining with 4-Methylumbelliferyl esters and naphthol esters was performed according to the procedure described by Bitondi, Mestriner (1983). Acetate and propionate 4-methylumbelliferyl solutions were prepared immediately before use by the method of Hopkinson *et al*. (1973).

The population structure of the *T. angustula* was analyzed using the POPGENE 1.31 software (Yeh *et al*. 1999). Polymorphic loci were submitted to chi-square tests for deviation from Hardy-Weinberg equilibrium. The differentiation within and among subpopulations was estimated by the contingency test, F-statistics (WRIGHT, 1951, 1965; NEI, 1977, 1978).

3 Results and Discussion

3.1 Isoenzyme characterization

The number of esterase bands detected and their behavior with the substrates employed was presented in Table 1. The best substrates for the detection of esterase activity were those derived from 4-methilumbelliferyl butyrate. It can be seen that Est-1 was not detected with the α-naphthol derivatives. Est-2 presented activity in all substrates employed but presented more intense activity in the presence of 4-methilumbelliferyl acetate and α-naphthol acetate and propionate. While Est-3

was not detected with the 4-methilumbelliferyl acetate and α-naphthol derivatives, suggesting more specificity than the other esterase zones.

Table 1 - Specificity of *Tetragonisca angustula* esterases for substrates derived from 4-Methylumbelliferyl and α-Naphthyl

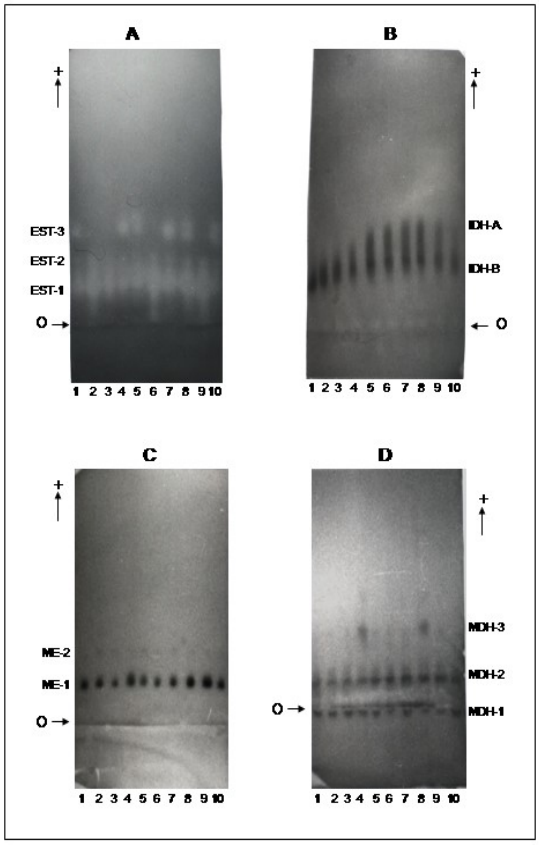
	Esterase		
Substrate	1	2	3
4-Methylumbelliferyl acetate	+	+	--
4-Methylumbelliferyl butyrate	++	++	++
a-Naphthyl acetate	--	++	--
a-Naphthyl propionate	--	++	--

* ++ maximum esterase activity; + 50% esterase activity; - no esterase activity

Source: Research data

Seven loci of isozyme activity were analyzed in *T. angustula* collected from Maringá and nine loci were observed in *T. angustula* from Astorga. For all of the enzymatic systems that presented polymorphism, the observed alleles were denominated according to electrophoretic mobility starting from the anode (Figure 2).

Figure 2 - Starch gel eletrophoretic pattern of *Tetragonisca angustula* isoenzymes; (A) Esterase activity stained with 4-Methylumbelliferyl Butyrate of the Astorga population; (B) Isocitrate dehydrogenase of the Astorga population; (C) Malic enzyme of the Maringá population; (D) Malate dehydrogenase of the Astorga population



Source: the authors.

The quaternary structure of the enzymes can be often inferred from the electrophoretic pattern of acceptable heterozygotes. The electrophoretic analysis of extracts of heterozygous *T. angustula* for EST, IDH, ME presented a two bands pattern, suggesting that these enzymes possess a quaternary monomeric structure.

The staining techniques revealed the esterase isozyme patterns of the *T. angustula* adults and indicated three esterase loci, referred to as *Est-1*, *Est-2,* and *Est-3*. *Est-1* was monomorphic for individuals collected from Maringá and *Est-3* presented activity only in *T. angustula* collected from Astorga.

T. angustula populations collected from two localities (Maringá and Astorga) presented one activity zone for IDH, with two alleles ($Idh\text{-}1^A$, $Idh\text{-}1^B$) being detected, with the $Idh\text{-}1^A$ allele being the most anodic. Two zones of ME activity were detected in both populations, with *Me-1* the most anodic and *Me-2* the least anodic. The population of Maringa it was monomorphic for *Me-1* and *Me-2*. While the population from Astorga presented *Me-2* polymorphism, with two alleles being detected and *Me-1* considered monomorphic.

Three activity zones for MDH, with *Mdh-1* being the most anodic and *Mdh-3* exhibited cathodic migration. Population from Maringá does not present polymorphism for the *Mdh* loci, but in populations from Astorga were detected polymorphism for all *Mdh* loci. *Mdh-1* zone was only observed in individuals collected from Astorga.

3.2 Population genetics

The allele frequencies of each subpopulation are given in Table 2. *T. angustula* collected in Maringá presented two polymorphic loci (Est-2 and Idh) out of seven analyzed, totalized 22.22%. While *T. angustula* collected in Astorga presented 88.89%, with eight polymorphic loci.

Estimates of genetic variability for each subpopulation and for the total population were calculated on the basis of polymorphic loci (Table 2) and mean heterozygosity (Table 3). The two populations are in Hardy-Weinberg equilibrium for *Idh* ($\chi^2_{(1)}$ = 3.309, p= 0.0689), *Me-2* ($\chi^2_{(1)}$ = 0.004, p= 0.9484), *Mdh-1* ($\chi^2_{(1)}$ = 0.047, p= 0.8277), *Mdh-2* ($\chi^2_{(1)}$ = 1784, p= 0.1816). The observed heterozigozity for *T. angustula* populations was 0.0775 and expected heterozigosity was 0.1632 (Table 3).

Table 2 - Isozyme allele frequency in *Tetragonisca angustula* populations from Maringá and Astorga

	Maringá Population		**Astorga Population**	
Locus	**Allele A**	**Allele B**	**Allele A**	**Allele B**
Est-1	1.0000	---	0.9259	0.0741
Est-2	0.4595	0.5405	0.9200	0.0800
Est-3	---	---	0.8038	0.1962
Idh	0.7250	0.2750	0.9220	0.0780
Me-1	1.0000	---	1.0000	---
Me-2	1.0000	---	0.9889	0.0111
Mdh-1	---	---	0.9773	0.0227
Mdh-2	1.0000	---	0.8636	0.1364
Mdh-3	1.0000	---	0.7941	0.2059

Source: Research data.

Table 3 - Average heterozygosity for *Tetragonisca angustula* populations from Astorga and Maringá

Locus	Sample Size	Observed heterozygosity	Expected heterozygosity	Average heterozygosity
Est-1	148	0.0000	0.1023	0.068
Est-2	137	0.0000	0.3252	0.3220
Est-3	79	0.0380	0.3154	0.1577
Idh	149	0.1946	0.2275	0.2713
Me-1	150	0.0000	0.0000	0.0000
Me-2	121	0.0165	0.0164	0.0110
Mdh-1	110	0.0455	0.0444	0.0222
Mdh-2	150	0.2000	0.1800	0.1178
Mdh-3	138	0.2029	0.2580	0.1635
Mean	131.5	0.0775	0.1632	0.1260
St. Dev		0.0927	0.1269	0.1146

Source: Research data.

Population differentiation was also examined by calculating F_{IS}, $F_{IT,}$ and F_{ST} values for each locus and the mean value across all loci (Table 4). F_{IS} values correspond to mean deviation from random mating within subpopulations (0.4036), F_{IT} values correspond to mean deviation from random mating over all subpopulations (0.5827) and F_{ST} values correspond to the measure of the degree of genetic differentiation among subpopulations (0.5827). There are also three loci (*Me-2*, *Mdh-1*, *Mdh-2*) in heterozygosis.

Table 4 - Fixation index (F_{IS}), F_{IT}, F_{ST} of *Tetragonisca angustula* populations. Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$

Locus	Allele number	F_{IS}	F_{IT}	F_{ST}	Nm
Est-1	148	1.0000	1.0000	0.0385	6.2500
Est-2	137	1.0000	1.0000	0.2478	0.7590
Est-3	79	0.8796	0.9771	0.8097	0.0587
Idh	149	0.0429	0.1068	0.0668	3.4944
Me-1	150	*****	*****	0.0000	*****
Me-2	121	-0.0112	-0.0056	0.0056	44.5000
Mdh-1	110	-0.0233	0.9701	0.9708	0.0075
Mdh-2	150	-0.1579	-0.0732	0.0732	3.1667
Mdh-3	138	0.1605	0.2568	0.1148	1.9286
Mean	131.5	0.4036	0.5827	0.3002	0.5827

Source: Research data.

Genetic identities, for the two populations, were calculated using the gene frequencies (Table 2), and Nei's genetic distance (1978) was determined from the genetic identity values. Nei's unbiased measures of genetic identity and genetic distance were 0.8401 and 0.1742, respectively (Table 5).

Three esterase activity zones were detected in *T. angustula* using 4-methylumbelliferyl butyrate. However, *Est-3* was specific for a population from Astorga. Differences in several zones of esterase activity and substrate specificity detected here showed that 4-methylumbelliferyl butyrate was the best substrate for the detection of esterase activity since they permitted the detection of a larger number of esterase activity zones.

Table 5 - Genetic distance (NEI, 1978) for two populations of *Tetragonisca angustula.* Superior and inferior diagonals indicate respectively genetic identity and genetic distance

Population	Maringá	Astorga
Maringá	*****	0.8401
Astorga	0.1742	*****

Source: Research data

Three esterase activity zones were detected in *T. angustula* using 4-methylumbelliferyl butyrate. However, *Est-3* was specific for a population from Astorga. Differences in several zones of esterase activity and substrate specificity detected here showed that 4-methylumbelliferyl butyrate was the best substrate for the detection of esterase activity since they permitted the detection of a larger number of esterase activity zones. Methylumbelliferyl esters preference was found in other Trigonini stingless bees. Eight esterase activity zones were detected with umbelliferyl esters in *Nannotrigona testaceicornis*, *Plebeia droryana* presented five esterase zones, all zones detected with umbelliferyl esters, and *Scaptotrigona postica*, *S. xantotricha*, *S. bipunctata* presented nine activity zones detected with umbelliferyl butyrate (LIMA; MESTRINER, 1985). Starch gel electrophoresis of esterase in *Apis mellifera* showed an enzyme preference for 4-methylumbelliferyl butyrate and α-naphthyl butyrate as a substrate (BITONDI; MESTRINER, 1983; RUVOLO-TAKASUSUKI, *et al*., 1998). Substrate preference studies in *Apis mellifera* showed that esterase-1a was visualized only when 4-methylumbelliferyl propionate or butyrate was used as substrate (RUVOLO-TAKASUSUKI *et al*., 1997).

Esterase patterns for 10 species of Brazilian stingless bees were presented by Falcão and Contel (1990) but they did not get to establish appropriate the electrophoretic conditions for the esterase investigation in *T. angustula*. Ruvolo-Takasusuki *et al*. (2006) detected two esterase activity zones (*Est-1* and *Est-2*) in *T. angustula* by polyacrylamide gel. The evaluation of isoenzymes in polyacrylamide gels was observed two loci for esterase (*Est-1* and *Est-2*) and acid phosphatase (*Acp-1* and *Acp-2*) and one locus for carbonic anhydrase (*Ca*) (STUCHI *et al*., 2008). The later study presented another esterase profile. Where for *T. fiebrigi* three esterases

were observed: EST-1 (β-esterase, cholinesterase I), EST-2 (α-esterase, cholinesterase II), and EST-4 (αβ-esterase, carboxylesterase). Two more esterases were detected in *T. angustula*: EST-3 (β-esterase, acetylesterase) and EST-4 (αβ-esterase, carboxylesterase) (STUCHI *et al*., 2012). And the electrophoretic profile of *T. weyrauchi* showed six stereo regions (RONQUI *et al*., 2016), exceeding those observed for *T. angustula* and *T. fiebrigi*.

Allele variation for *Idh* did not get observed in a previous study (FALCÃO; CONTEL, 1991b). *Idh* polymorphism was detected in three species of Brazilian stingless bees: *Partamona helleri*, *Scaptotrigona bipunctata* and *Friesella schrottkyi* and three electrophoretic phenotypes were detected in *F. schrottkyi* females (FALCÃO; CONTEL, 1991b). Analysis of the electrophoretic profiles of IDH and genetic segregations obtained for the hives analyzed indicate that IDH molecule has a dimeric structure and that the control of this polymorphic system depends on the activity of two alleles at one locus. *Idh* allele frequencies indicate a high polymorphism degree in *T. angustula.*

Considering the ME isoenzyme, two loci were detected, but only the Astorga *Me-2* was polymorphic. The *Me* polymorphism did not detect by Falcão and Contel (1991b) in studies with 10 species of Brazilian stingless bees, including *T. angustula*.

No genetic polymorphism for *Mdh* was observed in *T. angustula* from Maringá, and these results were observed by Falcão and Contel (1991b). The variation in MDH observed in *T. angustula* from Astorga indicates that two alleles at three loci are responsible for these polymorphisms. The MDH data obtained in the present study were not sufficient to permit inferences about the structure of the molecule of this enzyme, despite the complexity of electrophoretic patterns. The MDH isozyme study in the *P. droryana* showed individual variation which suggests the presence of four alleles at a single locus (MACHADO *et al*., 1992).

Population genetics research conducted is helping to explain general patterns of population structure and gene flow, mating systems and effective population sizes of common species (JHA, 2015). Genetics also played a key role in conservation biology, since the molecular tools allowed studies of the main conservation parameters that were previously very difficult, including species identity, gene flow levels, genetic diversity, inbreeding and kinship (ALLENDORF *et al*., 2013).

The heterozygosity observed (0.077) in this study, despite the few loci analyzed, was higher than that observed in other eusocial hymenopterans (0.05) (METCALF *et al*., 1975; PAMILO *et al*., 1978). However, it was considered low when compared to studies with *T. angustula* from different regions. Where population samples from Ivatuba (PR) were considered high (0.247), whereas, for the population of Dracena (SP) average heterozygosity (0.099) was observed, despite the distance of one population from the other, they were considered as a single population (STUCHI *et al*., 2006).

The variation of the isoenzyme polymorphism observed in this study was different from the polymorphism value of other bees such as *Plebeia remota*, *Partamona mulata*, and *Partamona helleri*, which have reduced numbers of polymorphic loci (FRANCISCO *et al*., 2006). Information similar to this study was observed with markers using mtDNA and microsatellites, where fifteen of the 21 loci proved to be polymorphic, with heterozygosity estimates observed ranging from 0.00 to 0.89 for *T. angustula* (BRITO *et al*., 2009). These polymorphic variations observed in Tetragonisca may be related to factors such as low genetic dispersion, different ecological conditions, and remote isolation shape the population structure of this species (FRANCISCO *et al*., 2017).

They may be associated with queens philopathies, interspecific reproductive parasitism and or artificial maintenance of hives, because evaluations with the species *Melipona mondury* Smith and *Melipona quadrifasciata* Lepeletier using heterospecific markers observe low levels of genetic variability for both species within the same meliponary (KOSER *et al*., 2014). About queens philopathies, the formation of new colonies leads to a reduction in genetic variability, consequently a greater genetic relationship between females (LÓPEZ-URIBE *et al*., 2015). This explains why the increase in nest density in a meliponary due to nest divisions can reduce genetic variability, leading to the production of diploid males and nest losses (SANTIAGO *et al*., 2016). This is due to the division of the colony keeping the same genetics in the meliponary and the reduced area of dispersion of the new nests. Making male dispersion a potentially important mechanism of genetic exchange between populations and prevention of inbreeding (LÓPEZ-URIBE *et al*., 2015). Genetic variation is reported with great importance in natural populations and these populations have a certain degree of genetic heterogeneity that provides adaptive flexibility (FRANCISCO *et al*., 2017).

The positive value of the F_{IS} (0.4036) indicates that there is a homozygous excess in these populations. Through the value of F_{ST} = 0.3002, we can observe that the populations present a high differentiation since the estimated value of F_{ST} = above 0.25 indicates a high genetic differentiation (WRIGHT, 1978). Likewise, it is observed that there may be gene flow between populations, due to the values of Nm. Where Nm = 0.5827 is greater than Nm = 0.5 indicating gene flow (WRIGHT, 1978).

As the *T. angustula* analyzed in this study was collected from meliponaries, this difference can be divided by inbreeding, which would explain the high homozygous values detected. Microsatellite analyzes showed similar genetic variability between wild and captive populations. However, captive populations showed less mitochondrial genetic variability (SANTIAGO *et al*., 2016).

Genetic studies have shown variation both between

colonies within populations and between populations, and the increase in heterogeneity can have positive effects on colonial performance (survival and productivity) (PAMILO *et al*., 1997). For the analyzed populations of *T. angustula*, small genetic distances were observed. The same behavior was observed when evaluating populations in the cities of Maringá and Cianorte, they observed the smallest genetic distances between the populations *T. fiebrigi* (BAITALA *et al*., 2006). The evaluation of populations of *T. angustula* from Ivatuba-PR and Dracena-SP, was not reported the separation of the two populations, forming a single population, despite the geographical distance between them (STUCHI *et al*., 2008).

Thus, due to variations in polymorphic loci added to the adaptation to similar habitats, it may have produced reduced genetic distance between the population analyzed, because about 50 years ago, the Atlantic Forest covered all of these areas, and adaptation to similar habitats may have caused a low genetic distance between populations (BAITALA *et al*., 2006).

More promising studies are being carried out since the discovery of the *A. mellifera* genome (WHITFIELD *et al*., 2006), and are producing contributions to address questions about the evolutionary history of native bees, providing tools to estimate points about species conservation (LOZIER; ZAYED, 2017). This information will be important for the control, preservation, and handling of these stingless bees and to alert about the importance of natural environment preservation for the maintenance of high levels of genetic variability to preserve pollination and production.

4 Conclusion

The application of the isoenzymes established a profile for *T. angustula*, producing good results since genetic variability was observed for the studied loci. The analyzed loci allowed to observe the genetic variability for both populations, showing that both populations have the same origin.

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