Genetic structure of the stingless bee *Tetragonisca angustula*

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Running title: Genetic structure of *T. angustula*

18 **Abstract**

19 The stingless bee *Tetragonisca angustula* Latreille 1811 is distributed from Mexico to
20 Argentina and is one of the most widespread bee species in the Neotropics. However, this
21 wide distribution contrasts with the short distance traveled by females to build new nests.
22 Here we evaluate the genetic structure of several populations of *T. angustula* using
23 mitochondrial DNA and microsatellites. These markers can help us to detect differences in the
24 migratory behavior of males and females. Our results show that the populations are highly
25 differentiated suggesting that both females and males have low dispersal distance. Therefore,
26 its continental distribution probably consists of several cryptic species.

27

28 **Key words** islands; Meliponini; microsatellites; mtDNA; population genetics

Introduction

The stingless bee *Tetragonisca angustula* Latreille 1811 distributed from Mexico to Argentina is one of the most widespread bee species in the Neotropics (Silveira *et al*., 2002; Camargo & Pedro, 2013). It is a small (4-5 mm in length), generalist and highly eusocial bee (Michener, 2007) and highly adaptable to different nest sites. Colonies comprise up to 5,000 individuals (Lindauer & Kerr, 1960), and are usually built in tree trunks or in wall cavities. It swarms frequently and is extremely successful in urban environments (Batista *et al*., 2003; Slaa, 2006; Velez-Ruiz *et al*., 2013). *Tetragonisca angustula* is one of the most popular stingless bees for meliponiculture in Latin America (Nogueira-Neto, 1997; Cortopassi-Laurino *et al*., 2006) and nest transportation and trading is very common among beekeepers.

In general, colony reproduction in stingless bees begins by workers searching for a new nest site within their foraging range (van Veen & Sommeijer, 2000a). Daughter nests are established at most a few hundred meters from the "mother" nest (Nogueira-Neto, 1997). After selecting the site, several workers begin to transport cerumen, propolis and honey from the mother nest to the new one (Nogueira-Neto, 1997). This nest site preparation phase can last from a few days (van Veen & Sommeijer, 2000a) to a few months (Nogueira-Neto, 1997). A virgin queen then leaves the mother nest accompanied by hundreds of workers (van Veen & Sommeijer, 2000b). The next day the virgin queen flies out, mates with presumably one male (Peters *et al*., 1999; Palmer *et al*., 2002), returns to the nest and about a week later begins oviposition (van Veen & Sommeijer, 2000b).

In contrast, little is known about the reproductive behavior of stingless bee males. After emergence from brood cells, they remain in the nest for two to three weeks (Cortopassi-Laurino, 2007). They then leave the nest and never return. There are no data about the behavior of males during their period outside the nest. In the laboratory, males can live up to six weeks (Velthuis *et al*., 2005). Therefore, they likely have two to four weeks for dispersal

and reproduction. Males often form mating aggregations, and these are comprised of males from hundreds of different, and not necessarily nearby colonies (Paxton, 2000; Cameron *et al*., 2004; Kraus *et al*., 2008; Mueller *et al*., 2012). This suggests high male dispersal.

Studies on the genetic structure of populations can help us better understand dispersal behavior and evolutionary history. There are three existing population genetic studies of *T. angustula* (Oliveira *et al*., 2004; Baitala *et al*., 2006; Stuchi *et al*., 2008). These studies are of limited scope due to limited sampling or the use of outdated molecular markers such as RAPDs and isozymes. Thus, the conclusions are ambiguous concerning the extent and direction of gene flow, population differentiation, dispersal and evolutionary history.

Here we evaluate the genetic structure of several populations of *T. angustula* using mitochondrial DNA (mtDNA) and microsatellite markers. Considering the wide distribution of *T. angustula* and the commonness of nest transportation and trading, we expect low genetic differentiation among populations despite the low dispersal distance of females during swarming.

Materials and methods

Sampling

We collected 1,002 *T. angustula* from 457 sites distributed on the mainland and islands in south/south-eastern Brazil (Table S1). Eleven islands all with arboreal vegetation and of area greater than 1.0 km^2 were selected, 10 being land-bridge islands isolated about 12,000 years ago (Suguio *et al*., 2005) and one sedimentary island (Ilha Comprida) which arose about 5,000 years ago (Suguio *et al*., 2003). The islands range in size from 1.1 to 451 km^2 and are 0.1 to 38 km from the mainland (Table S2, Fig. 1). Bees were sampled from nests (n = 125,

one per nest) and flowers (n = 877) (Table S1). Samples were grouped into 17 populations, 14 from the mainland and three from islands (Fig. 1).

We preserved the specimens in 96% ethanol for transport to the laboratory. DNA extraction followed the protocol described in Francisco *et al.* (2014). We dried the specimens at room temperature for 20 min prior to DNA extraction.

Mitochondrial DNA sequencing

Two mitochondrial genes were partially sequenced: cytochrome c oxidase subunit 1 (*COI*) and cytochrome b (*Cytb*). Details about amplification and sequencing are given in Francisco *et al.* (2014).

Microsatellite genotyping

The samples were genotyped for eleven microsatellite loci: Tang03, Tang11, Tang12, Tang17, Tang29, Tang57, Tang60, Tang65, Tang68, Tang70, and Tang77 (Brito *et al*., 2009). PCR conditions for each locus are given in Francisco *et al.* (2014). Electrophoresis, visualization and genotyping were performed according to Francisco *et al.* (2011).

MICRO-CHECKER 2.2.3 (van Oosterhout *et al*., 2004) was used to identify null alleles and scoring errors. COLONY 2.0.1.7 (Jones & Wang, 2010) was used to determine whether individuals collected in the same plant or places nearby were related. Samples were excluded from our data set if matched all of the following three criteria: collected at sites distant less than 2 km, indicated as related by COLONY, and sharing a mtDNA haplotype. Overall, 722 *T. angustula* bees from 17 populations were deemed suitable for further genetic analyses (Table 1).

GENEPOP 4.1.2 (Rousset, 2008) was used to verify Hardy-Weinberg equilibrium (HWE) in populations and loci and to detect linkage disequilibrium (LD). Markov chain was

103 set for 10,000 dememorizations, 1,000 batches and 10,000 iterations per batch. In cases of
104 multiple comparisons, *P*-values were corrected by applying Sequential Goodness of Fit test by
105 the program SGOF 7.2 (Carvajal-Rodríguez *et al*., 2009). This method is advantageous over
106 other correction methods because it increases its statistical power with the increasing of the
107 number of tests (Carvajal-Rodríguez *et al*., 2009).
108
109 *Genetic diversity*
110 ARLEQUIN 3.5.1.3 (Excoffier & Lischer, 2010) was used to calculate mtDNA haplotype (*h*)
111 and nucleotide (π) diversity. GENALEX 6.5 (Peakall & Smouse, 2006, 2012) was used to
112 calculate microsatellite allelic richness (*A*) and expected heterozygosity (H_E). Since sample
113 sizes were different, allelic richness was standardized by rarefaction (*Ar*) using the program
114 HP-RARE 1.0 (Kalinowski, 2005). Differences in *Ar* among populations were estimated by
115 Mann-Whitney two-tailed U Test (Mann & Whitney, 1947). Inbreeding coefficients (F_{IS})
116 were calculated for each population with 10,000 permutations using ARLEQUIN.
117
118 *Population differentiation and gene flow*
119 MEGA 5.2.1 (Tamura *et al*., 2011) was used to calculate the number of base substitutions per
120 site from mitochondrial sequences by averaging over all sequence pairs between populations
121 using the Kimura 2-parameter (K2p) model (Kimura, 1980). Population pairwise θ values (an
122 F_{ST} analogue, Weir & Cockerham 1984) were calculated with 10,000 permutations by
123 ARLEQUIN using microsatellite alleles. When heterozygosity is high, F_{ST} and its analogues
124 may not be appropriate measures of genetic differentiation (Hedrick, 2005; Jost, 2008; Heller
125 & Siegismund, 2009). For this reason, Jost's D_{est} (Jost, 2008) was calculated. This statistc is
126 not influenced by heterozygosity (Jost, 2008) and is more appropriate for microsatellite data
127 (Heller & Siegismund, 2009). Global D_{est} was calculated with 9,999 permutations for mtDNA

and microsatellite data using GENALEX. Pairwise D_{est} was calculated only for microsatellite data. Mantel tests between genetic and geographical distances among populations were performed with 9999 permutations by GENALEX to verify isolation by distance for both molecular markers.

BAPS 6 (Corander *et al*., 2008; Cheng *et al*., 2013) was used to infer population structure using microsatellites and the geographic coordinates of the sampled individuals to spatially cluster them. BAPS 6 provides a Bayesian analysis of genetic population structure that creates *K* groups of individuals based on the similarity of their genotypes. The program was initially ran 5 times for each of $K = 1$ to 17 and then 10 times for each of $K = 5$ to 14. These results were used for admixture analysis with 200 iterations to estimate the admixture coefficients for the individuals, 200 simulated reference individuals per population and 20 iterations to estimate the admixture coefficients of the reference individuals.

Estimates of rates and direction of current and/or recent migration (*m*) between populations were determined by the program BAYESASS 3 (Wilson & Rannala, 2003) using microsatellites multilocus genotypes through Markov chain Monte Carlo (MCMC) techniques. We performed five independent runs with 10^7 MCMC iterations, burn-in of 10^6 iterations and sampling frequency of 2,000. The delta values used were 0.25 (migration), 0.40 (allele frequencies) and 0.55 (inbreeding).

Assessment of population demography

To detect any recent bottleneck events we used the program BOTTLENECK 1.2.02 (Piry *et al*., 1999). We used the two-phased model (TPM) of mutation which is suggested as the most appropriate for microsatellites (Di Rienzo *et al*., 1994). The variance among multiple steps was 12 and the proportion of stepwise mutation model in the TPM was 95% as suggested by

Piry *et al.* (1999). Altogether 10,000 iterations were performed. The significance of any deviation was determined with a Wilcoxon sign-rank test.

Results

Island occurrences

Tetragonisca angustula was found and collected on five of the 11 islands visited (Table 1). However, only the samples from Ilha Grande, Ilha de São Sebastião and Ilha Comprida were included in the analyses. The other collections were not included due to small sample size (Ilha do Cardoso, $n = 1$), and to individuals being highly related, with anecdotal reports of introduced nests (Ilha de Santa Catarina, see Francisco *et al.* (2014)).

MtDNA diversity

The *COI* gene sequences were 417 bp long (GenBank accession numbers KF222891-KF223893) and 32 haplotypes were identified. The *Cytb* sequences were 391 bp long (KF223894-KF224896) and generated 43 haplotypes. Most differences among haplotypes were synonymous substitutions, since the number of distinct amino acid sequences were four for *COI* and 15 for *Cytb*. We concatenated the nucleotide sequences (808 bp) for population analyses.

The 722 concatenated sequences defined 73 haplotypes. Since h and π were positively correlated ($r = 0.510$, $P = 0.036$, $n = 17$) we hereafter use π as our measure of mtDNA diversity. Nucleotide diversity ranged from 0.0006 ± 0.0019 (Passa Quatro) to 0.0407 ± 0.0251 (Ilha Comprida) (Table 1).

There was a non-significant positive correlation between the size of the sampled area and mtDNA diversity ($r = 0.135$, $P = 0.606$, $n = 17$). The correlation between median

177 elevation and mtDNA diversity was negative but non-significant (r = -0.428, P = 0.087, n =
178 17).
179
180 *MtDNA differentiation*
181 Population structure was high. Of 73 haplotypes 67 were population-specific. We built a
182 haplotype network where the frequency and distribution of haplotypes are shown (Fig. S1).
183 The network shows a 'star-pattern' centered on four haplotypes. It illustrates the high number
184 of endemic haplotypes, and the great number of nucleotide substitutions that separate the
185 Porto União/Foz do Iguaçu populations from the others. The populations of Teresópolis,
186 Resende, Prudentópolis, Angra dos Reis, and Ilha Grande all feature unique haplotypes.
187 Global D_{est} was 0.772 (P < 0.001) indicating a highly significant population structure.
188 The highest K2p values were found for Porto União/Foz do Iguaçu with respect to all other
189 populations (2.809% to 3.306%) (Table 2).
190
191 *Microsatellite diversity*
192 After the Sequential Goodness of Fit correction, deviation from HWE was occasional, likely
193 arising from type 1 error, and therefore no locus was removed from the analyses (Table S3).
194 No significant LD was found between any pair of loci (all P > 0.05).
195 Microsatellite diversity was moderate to high. *Ar* and H_E were positively correlated (r
196 = 0.787, P < 0.001, n = 17). Hereafter we use *Ar* as our measure of microsatellite diversity. *Ar*
197 was standardized for 22 individuals and ranged from 5.37 (Porto União) to 9.45 (Resende)
198 (Table 1). *Ar* was significantly different only between Porto União and Resende (U = 93, P =
199 0.033) and Porto União and Teresópolis (U = 29, P = 0.039).
200 There was a negative but non-significant correlation between *Ar* and size of the
201 sampled area (r = -0.114, P = 0.662, n = 17) and between *Ar* and median elevation (r = -

202 0.084, $P = 0.748$, $n = 17$).

203 Six populations had inbreeding coefficients (F_{IS}) significantly different from zero ($P <$
204 0.05). The highest F_{IS} (0.2177) was found in São José (Table 1).

205

206 *Microsatellite differentiation*

207 Global D_{est} was high (0.375, $P < 0.001$) and indicates population structure. Pairwise
208 comparisons also detected population structure, since most θ values were between 0.05 and
209 0.15 (Table S4) and most D_{est} values were higher than 0.25 (Table 3). Pairwise θ and D_{est}
210 were positively correlated ($r = 0.977$, $P < 0.001$, $n = 136$) and we use D_{est} as our measure of
211 microsatellite differentiation hereafter. D_{est} ranged from 0.0204 (Guaratuba × Blumenau) to
212 0.8464 (Prudentópolis × Foz do Iguaçu). High D_{est} values were always detected in
213 comparisons between Porto União/Foz do Iguaçu and other populations. Low differentiation
214 was observed in some populations near the coast (Iguape, Apiaí, Guaratuba, Blumenau, and
215 São José) but also inland (Porto União × Foz do Iguaçu and Prudentópolis × Teodoro
216 Sampaio).

217 Population structure was also suggested by the spatial cluster approach used by BAPS,
218 which determined $K = 10$ as the most likely optimal number of clusters (probability of
219 98.99%). The clusters were [Foz do Iguaçu/Porto União], [Iguape/Apiaí/Guaratuba/
220 Blumenau/SãoJosé], [Ilha Comprida], [São Sebastião], [Ilhabela], [Ilha Grande], [Passa
221 Quatro], [Teodoro Sampaio/Prudentópolis], [Teresópolis], [Resende/Angra dos Reis] (Fig. 1).
222 D_{est} results are in good agreement with these clusters.

223 The results of the migration rates estimated in BAYESASS suggested a low level of gene
224 flow throughout the studied area (Table S5). Only 15 out of 272 comparisons showed $m > 0$
225 between two populations. Most of the populations that showed evidence of gene flow are near
226 the coast (Fig. 1), but inland populations such as Porto União × Foz do Iguaçu also showed

227 evidence of gene flow. Migration is directional. For instance, the non-differentiation detected
228 between Prudentópolis × Teodoro Sampaio is apparently due to a high migration rate (0.2486
229 migrants per generation) from Prudentópolis to Teodoro Sampaio, whereas migration in the
230 opposite direction was not detected (Table S5). The results obtained by BAYESASS are in good
231 agreement with the population structure indicated by θ, D_{est} and BAPS.
232
233 *Isolation by distance*
234 There was a positive and significant correlation between geographic and genetic distance for
235 both mitochondrial (r = 0.415, P = 0.004, n = 136) and microsatellite markers (r = 0.464, P <
236 0.001, n = 136).
237
238 *Population demography*
239 We did not detect recent bottlenecks in any of the 17 populations (all P > 0.1392, Table S6).

Discussion

Our results show that *T. angustula* populations are highly differentiated as demonstrated by mtDNA and microsatellite markers. This suggests that both females and males have low dispersal distance.

Per population mtDNA nucleotide diversity (π) ranged from low to high. High π suggests that a population had a long evolutionary history and a large effective population size. Low π may be explained by lineage sorting or suggest that a population bottleneck has occurred in the past (Avise, 2000). The characteristic star shape of the *T. angustula* haplotype network provides evidence of relatively recent local extinction, re-colonization, and population expansion. Several phylogeographic studies of vertebrate and invertebrate populations, conducted in some of the areas that we studied, also found low mtDNA nucleotide diversity (Cabanne *et al*., 2007; Carnaval *et al*., 2009; Batalha-Filho *et al*., 2010; Brito & Arias, 2010; Francisco & Arias, 2010; D'Horta *et al*., 2011; Bell *et al*., 2012). As argued in these papers, changes in sea level during the Pleistocene generated population bottlenecks followed by species expansion, and this is reflected in localized low nucleotide diversity to this day. Therefore, it is likely that populations that have high mtDNA diversity (e.g. Angra dos Reis) did not experience recent bottlenecks, while populations with low mtDNA diversity (e.g. Passa Quatro) are in regions that likely arose by a recent population expansion.

Overall, we found high mitochondrial genetic differentiation between populations. Similar population structuring has been observed for other stingless bee species (Brito & Arias, 2010; Francisco & Arias, 2010; Quezada-Euán *et al*., 2012; Brito *et al*., 2013; Francisco *et al*., 2013). The mtDNA population structure of stingless bees probably arises from their reproductive behavior. Nonetheless, some populations are not well differentiated

from others. This is likely due to gene natural flow, although human transportation also likely plays a role. For instance, haplotypes 34, 35, and 36 all found in Ilha Comprida, were similar to those found in Passa Quatro/Teodoro Sampaio (34 and 36) and Teresópolis (35) (Fig. S1). Due to the high frequency of endemic haplotypes, and the physical distance between these populations, we suggest that nests have been transported to Ilha Comprida causing an artificial increase in this population's mtDNA diversity.

Nuclear genetic diversity was moderate to high in all populations. Microsatellite diversity was not significantly different between populations except for Porto União. This result shows that the ecological features of each sampling site are not influencing the molecular diversity. Indeed, variables such as size of the sampled area and median elevation were not significantly correlated with genetic diversity for both mtDNA and microsatellites. Moreover, our results did not detect recent bottleneck (e.g. due to habitat fragmentation) in any of the studied populations. However, it is worth emphasizing that theoretical and practical studies have shown that habitat fragmentation affects immediately the genetic structure by increasing it, while the reduction of genetic diversity may take longer (Varvio *et al*., 1986; Keyghobadi *et al*., 2005). According to this statement, it will be necessary to monitor the genetic diversity of *T. angustula* populations studied over years, since currently high structure was detected.

Evidence of inbreeding was found in six populations (Angra dos Reis, Blumenau, São José, Porto União, Foz do Iguaçu and Teodoro Sampaio). This might be an artifact caused by Wahlund effect (Hartl & Clark, 2007) and/or a consequence of the low dispersal of *T. angustula*. If the latter is true, the persistence of these populations is at risk (Keller & Waller, 2002).

Microsatellite data also indicated high genetic structuring and low gene flow among populations. This suggests that like females, the dispersal distance of males is also quite

limited even between populations separated by 34 km of continuous forest. It is interesting here to note that all island populations were differentiated from their mainland counterparts, indicating that males do not cross water for distances as short as 300 m. The program BAYESASS suggested that the highest migration rate is from Prudentópolis to Teodoro Sampaio, populations separated by more than 300 km. These two populations do not share any mtDNA haplotypes suggesting that this gene flow is mediated only by males following the stepping stone model (Kimura & Weiss, 1964).

For both markers population clusters appear to be unrelated to physical barriers (such as rivers or mountain ranges) or forest presence, indicating that genetic connectivity demands more than just habitat connectivity (Marsden *et al*., 2012). Populations may diverge even when there are no apparent obstacles to gene flow due to low dispersal, geographic distance and genetic drift (isolation by distance). Overall, the population structure of *T. angustula* is shaped by isolation by distance.

The highest genetic divergence observed was between Porto União/Foz do Iguaçu and the remaining populations. At least 15 mtDNA mutation steps separate these two populations from the others. This represents about 2.8 to 3.3% divergence, which is as high as the divergence between lineages A and Y of *Apis mellifera* (Franck *et al*., 2001), which are thought to have diverged over one million years ago (Whitfield *et al*., 2006). Francisco *et al.* (2014) suggested that bees from Porto União and Foz do Iguaçu might belong to the subspecies *T. angustula fiebrigi* while the others to *T. angustula angustula.*

Among the islands we visited only Ilha do Mel (Zanella, 2005), Ilha de Santa Catarina (Steiner *et al*., 2006) and Ilha Grande (Lorenzon *et al*., 2006) had been previously surveyed for bees and *T. angustula* was reported on all of them. We did not locate *T. angustula* on six of the 11 islands we visited. Our failure to verify *T. angustula* on most islands may be due its ancestral absence on the islands when they became isolated or to its extinction after isolation.

The constraint on queen dispersal prevents (re)colonization of islands whose distance from the mainland is greater than a few hundred of meters. Even if (re)colonization has occurred, its establishment may not have been successful. With low dispersal, *T. angustula* has low effective population size and high extinction rate. Island size may be critical to the survival of viable *T. angustula* populations – we were unable to locate them on any island less than 28 km^2. Competition among colonies doubtless limits the number of colonies an island can support so that small islands may not be able to maintain viable populations of *T. angustula*. The rarity of stingless bee species on islands has been noted elsewhere (Schwartz-Filho & Laroca, 1999; Zanella, 2005).

Our results indicate that *T. angustula* is not genetically homogeneous across the studied area. Considering that this species has a continental distribution, we speculate this species is ancient and includes wide range of genetically different taxa with the same (or similar) morphology. Sampling across its entire distribution range is needed to elucidate its taxonomic status as well as its evolutionary history.

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Disclosure

The authors have no conflict of interest.

References

Avise, J.C. (2000) Phylogeography: *The History and Formation of Species*. Harvard University Press, Cambridge, MA.

Baitala, T.V., Mangolin, C.A., Toledo, V.A.A. and Ruvolo-Takasusuki, M.C.C. (2006) RAPD polymorphism in *Tetragonisca angustula* (Hymenoptera; Meliponinae, Trigonini) populations. *Sociobiology*, 48, 1–13.

Batalha-Filho, H., Waldschmidt, A.M., Campos, L.A.O., Tavares, M.G. and Fernandes-Salomão, T.M. (2010) Phylogeography and historical demography of the neotropical stingless bee *Melipona quadrifasciata* (Hymenoptera, Apidae): incongruence between morphology and mitochondrial DNA. *Apidologie*, 41, 534–547.

Batista, M.A., Ramalho, M. and Soares, A.E.E. (2003) Nesting sites and abundance of Meliponini (Hymenoptera: Apidae) in heterogeneous habitats of the Atlantic Rain Forest. *Lundiana*, 4, 19–23.

Bell, R.C., Brasileiro, C.A., Haddad, C.F.B. and Zamudio, K.R. (2012) Evolutionary history of *Scinax* treefrogs on land-bridge islands in south-eastern Brazil. *Journal of Biogeography*, 39, 1733–1742.

Brito, R.M. and Arias, M.C. (2010) Genetic structure of *Partamona helleri* (Apidae, Meliponini) from Neotropical Atlantic rainforest. *Insectes Sociaux*, 57, 413–419.

Brito, R.M., Francisco, F.O., Domingues-Yamada, A.M.T., Gonçalves, P.H.P., Pioker, F.C., Soares, A.E.E. and Arias, M.C. (2009) Characterization of microsatellite loci of *Tetragonisca angustula* (Hymenoptera, Apidae, Meliponini). *Conservation Genetics Resources*, 1, 183–187.

Brito, R.M., Francisco, F.O., Françoso, E., Santiago, L.R. and Arias, M.C. (2013) Very low mitochondrial variability in a stingless bee endemic to cerrado. *Genetics and Molecular Biology*, 36, 124–128.

Cabanne, G.S., Santos, F.R. and Miyaki, C.Y. (2007) Phylogeography of *Xiphorhynchus fuscus* (Passeriformes, Dendrocolaptidae): vicariance and recent demographic expansion in southern Atlantic forest. *Biological Journal of the Linnean Society*, 91, 73–84.

Camargo, J.M.F. and Pedro, S.M. (2013) Meliponini Lepeletier, 1836. *Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region - online version* (eds J.S. Moure, D. Urban & G.A.R. Melo), available at http://www.moure.cria.org.br/catalogue. Accessed Apr/02/2015.

Cameron, E.C., Franck, P. and Oldroyd, B.P. (2004) Genetic structure of nest aggregations and drone congregations of the southeast Asian stingless bee *Trigona collina*. *Molecular Ecology*, 13, 2357–2364.

Carnaval, A.C., Hickerson, M.J., Haddad, C.F.B., Rodrigues, M.T. and Moritz, C. (2009) Stability predicts genetic diversity in the Brazilian Atlantic Forest hotspot. *Science*, 323,

785–789.

Carvajal-Rodríguez, A., Uña-Alvarez, J. and Rolán-Alvarez, E. (2009) A new multitest correction (SGoF) that increases its statistical power when increasing the number of tests. *BMC Bioinformatics*, 10, 209.

Cheng, L., Connor, T.R., Sirén, J., Aanensen, D.M. and Corander, J. (2013) Hierarchical and spatially explicit clustering of DNA sequences with BAPS software. *Molecular Biology and Evolution*, 30, 1224–1228.

Corander, J., Sirén, J. and Arjas, E. (2008) Bayesian spatial modeling of genetic population structure. *Computational Statistics*, 23, 111–129.

Cortopassi-Laurino, M. (2007) Drone congregations in Meliponini: what do they tell us? *Bioscience Journal*, 23, 153–160.

Cortopassi-Laurino, M., Imperatriz-Fonseca, V.L., Roubik, D.W., Dollin, A., Heard, T., Aguilar, I.B., *et al*. (2006) Global Meliponiculture: challenges and opportunities. *Apidologie*, 37, 1–18.

D'Horta, F.M., Cabanne, G.S., Meyer, D. and Miyaki, C.Y. (2011) The genetic effects of Late Quaternary climatic changes over a tropical latitudinal gradient: diversification of an Atlantic Forest passerine. *Molecular Ecology*, 20, 1923–1935.

Excoffier, L. and Lischer, H.E. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567.

Francisco, F.O. and Arias, M.C. (2010) Inferences of evolutionary and ecological events that influenced the population structure of *Plebeia remota*, a stingless bee from Brazil. *Apidologie*, 41, 216–224.

Francisco, F.O., Brito, R.M., Santiago, L.R., Gonçalves, P.H.P., Pioker, F.C., Domingues-Yamada, A.M.T. and Arias, M.C. (2011) Isolation and characterization of 15 microsatellite loci in the stingless bee *Plebeia remota* (Apidae: Meliponini). *Conservation Genetics Resources*, 3, 417–419.

Francisco, F.O., Santiago, L.R. and Arias, M.C. (2013) Molecular genetic diversity in populations of the stingless bee *Plebeia remota*: A case study. *Genetics and Molecular Biology*, 36, 118–123.

Francisco, F.O., Santiago, L.R., Brito, R.M., Oldroyd, B.P. and Arias, M.C. (2014) Hybridization and asymmetric introgression between *Tetragonisca angustula* and *Tetragonisca fiebrigi*. *Apidologie*, 45, 1–9.

Franck, P., Garnery, L., Loiseau, A., Oldroyd, B.P., Hepburn, H.R., Solignac, M. and Cornuet, J.-M. (2001) Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. *Heredity*, 86, 420–430.

425 Hartl, D.L. and Clark, A.G. (2007) *Principles of Population Genetics*, 4th edn. Sinauer
426 Associates, Inc., Sunderland, MA.

427 Hedrick, P.W. (2005) A standardized genetic differentiation measure. *Evolution*, 59, 1633–
428 1638.

429 Heller, R. and Siegismund, H.R. (2009) Relationship between three measures of genetic
430 differentiation GST, DEST and G'ST: how wrong have we been? *Molecular Ecology*,
431 18, 2080–2083.

432 Jones, O.R. and Wang, J. (2010) colony: a program for parentage and sibship inference from
433 multilocus genotype data. *Molecular Ecology Resources*, 10, 551–555.

434 Jost, L. (2008) G_{ST} and its relatives do not measure differentiation. *Molecular Ecology*, 17,
435 4015–4026.

436 Kalinowski, S.T. (2005) hp-rare 1.0: a computer program for performing rarefaction on
437 measures of allelic richness. *Molecular Ecology Notes*, 5, 187–189.

438 Keller, L.F. and Waller, D.M. (2002) Inbreeding effects in wild populations. *Trends in*
439 *Ecology and Evolution*, 17, 230–241.

440 Keyghobadi, N., Roland, J., Matter, S.F. and Strobeck, C. (2005) Among- and within-patch
441 components of genetic diversity respond at different rates to habitat fragmentation: an
442 empirical demonstration. *Proceedings of the Royal Society B*, 272, 553–560.

443 Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions
444 through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*,
445 16, 111–120.

446 Kimura, M. and Weiss, G.H. (1964) The stepping stone model of population structure and the
447 decrease of genetic correlation with distance. *Genetics*, 49, 561–576.

448 Kraus, F.B., Weinhold, S. and Moritz, R.F.A. (2008) Genetic structure of drone congregations
449 of the stingless bee Scaptotrigona mexicana. *Insectes Sociaux*, 55, 22–27.

450 Lindauer, M. and Kerr, W.E. (1960) Communication between the workers of stingless bees.
451 *Bee World*, 41, 29–41, 65–71.

452 Lorenzon, M.C.A., Conde, M.M.S. and Barbosa, C.G. (2006) Eusocial Apidae in tropical
453 insular region. *Brazilian Archives of Biology and Technology*, 49, 733–738.

454 Mann, H.B. and Whitney, D.R. (1947) On a test of whether one of two random variables is
455 stochastically larger than the other. *The Annals of Mathematical Statistics*, 18, 50–60.

456 Marsden, C.D., Woodroffe, R., Mills, M.G.L., McNutt, J.W., Creel, S., Groom, R., *et al*.
457 (2012) Spatial and temporal patterns of neutral and adaptive genetic variation in the
458 endangered African wild dog (*Lycaon pictus*). *Molecular Ecology*, 21, 1379–1393.

459 Michener, C.D. (2007) *The Bees of the World*, 2nd edn. Johns Hopkins University Press,
460 Baltimore, MD.

461 Mueller, M.Y., Moritz, R.F.A. and Kraus, F.B. (2012) Outbreeding and lack of temporal
462 genetic structure in a drone congregation of the neotropical stingless bee *Scaptotrigona*
463 *mexicana*. *Ecology and Evolution*, 2, 1304–1311.

464 Nogueira-Neto, P. (1997) *Vida e Criação de Abelhas Indígenas Sem Ferrão*. Nogueirapis,
465 São Paulo, SP.

466 Oliveira, R.C., Nunes, F.M.F., Campos, A.P.S., Vasconcelos, S.M., Roubik, D., Goulart, L.R.
467 and Kerr, W.E. (2004) Genetic divergence in *Tetragonisca angustula* Latreille, 1811
468 (Hymenoptera, Meliponinae, Trigonini) based on RAPD markers. *Genetics and*
469 *Molecular Biology*, 27, 181–186.

470 van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. and Shipley, P. (2004) micro-checker:
471 software for identifying and correcting genotyping errors in microsatellite data.
472 *Molecular Ecology Notes*, 4, 535–538.

473 Palmer, K.A., Oldroyd, B.P., Quezada-Euán, J.J.G., Paxton, R.J. and May-Itzá, W.J. (2002)
474 Paternity frequency and maternity of males in some stingless bee species. *Molecular*
475 *Ecology*, 11, 2107–2113.

476 Paxton, R.J. (2000) Genetic structure of colonies and a male aggregation in the stingless bee
477 *Scaptotrigona postica*, as revealed by microsatellite analysis. *Insectes Sociaux*, 47, 63–
478 69.

479 Peakall, R. and Smouse, P.E. (2006) genalex 6: genetic analysis in Excel. Population genetic
480 software for teaching and research. *Molecular Ecology Notes*, 6, 288–295.

481 Peakall, R. and Smouse, P.E. (2012) GenAlEx 6.5: Genetic analysis in Excel. Population
482 genetic software for teaching and research – an update. *Bioinformatics*, 28, 2537–2539.

483 Peters, J.M., Queller, D.C., Imperatriz-Fonseca, V.L., Roubik, D.W. and Strassmann, J.E.
484 (1999) Mate number, kin selection and social conflicts in stingless bees and honeybees.
485 *Proceedings of the Royal Society B*, 266, 379–384.

486 Piry, S., Luikart, G. and Cornuet, J.-M. (1999) BOTTLENECK: a computer program for
487 detecting recent reductions in the effective size using allele frequency data. *Journal of*
488 *Heredity*, 90, 502–503.

489 Quezada-Euán, J.J.G., May-Itzá, W.J., Rincón, M., De la Rúa, P. and Paxton, R.J. (2012)
490 Genetic and phenotypic differentiation in endemic *Scaptotrigona hellwegeri* (Apidae:
491 Meliponini): implications for the conservation of stingless bee populations in contrasting
492 environments. *Insect Conservation and Diversity*, 5, 433–443.

493 Di Rienzo, A., Peterson, A.C., Garza, J.C., Valdes, A.M., Slatkin, M. and Freimer, N.B.
494 (1994) Mutational processes of simple-sequence repeat loci in human populations.
495 *Proceedings of the National Academy of Sciences of the United States of America*, 91,
496 3166–3170.

497 Rousset, F. (2008) Genepop'007: a complete re-implementation of the genepop software for

498 Windows and Linux. *Molecular Ecology Resources*, 8, 103–106.

499 Schwartz-Filho, D. and Laroca, S. (1999) A comunidade de abelhas silvestres (Hymenoptera,
500 Apoidea) da Ilha das Cobras (Paraná, Brasil) aspectos ecológicos e biogeográficos. *Acta*
501 *Biológica Paranaense*, 28, 19–108.

502 Silveira, F.A., Melo, G.A.R. and Almeida, E.A.B. (2002) *Abelhas Brasileiras: Sistemática e*
503 *Identificação*. Fundação Araucária, Belo Horizonte, MG.

504 Slaa, E.J. (2006) Population dynamics of a stingless bee community in the seasonal dry
505 lowlands of Costa Rica. *Insectes Sociaux*, 53, 70–79.

506 Steiner, J., Harter-Marques, B., Zillikens, A. and Feja, E.P. (2006) Bees of Santa Catarina
507 Island, Brazil - a first survey and checklist (Insecta: Apoidea). *Zootaxa*, 1220, 1–18.

508 Stuchi, A.L.P.B., Ruvolo-Takasusuki, M.C.C. and Toledo, V.A.A. (2008) Análise da genética
509 de populações em abelhas jataí (*Tetragonisca angustula* Latreille) por meio de
510 isoenzimas. *Magistra*, 20, 68–77.

511 Suguio, K., Angulo, R.J., Carvalho, A.M., Corrêa, A.M., Tomazelli, L.J., Willwock, J.A. and
512 Vital, H. (2005) Paleoníveis do mar e paleolinhas de costa. *Quaternário do Brasil* (eds
513 C.R.G. Souza, K. Suguio, A.M.S. Oliveira & P.E. Oliveira), pp. 114–129. Associação
514 Brasileira de Estudos do Quaternário, São Paulo.

515 Suguio, K., Tatumi, S.H., Kowata, E.A., Munita, C.S. and Paiva, R.P. (2003) Upper
516 Pleistocene deposits of the Comprida Island (São Paulo State) dated by
517 thermoluminescence method. *Anais da Academia Brasileira de Ciências*, 75, 91–96.

518 Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5:
519 Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary
520 distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28,
521 2731–2739.

522 Varvio, S.-L., Chakraborty, R. and Nei, M. (1986) Genetic variation in subdivided
523 populations and conservation genetics. *Heredity*, 57, 189–198.

524 van Veen, J.W. and Sommeijer, M.J. (2000a) Colony reproduction in *Tetragonisca angustula*
525 (Apidae, Meliponini). *Insectes Sociaux*, 47, 70–75.

526 van Veen, J.W. and Sommeijer, M.J. (2000b) Observations on gynes and drones around
527 nuptial flights in the stingless bees *Tetragonisca angustula* and *Melipona beecheii*
528 (Hymenoptera, Apidae, Meliponinae). *Apidologie*, 31, 47–54.

529 Velez-Ruiz, R.I., Gonzalez, V.H. and Engel, M.S. (2013) Observations on the urban ecology
530 of the Neotropical stingless bee *Tetragonisca angustula* (Hymenoptera: Apidae:
531 Meliponini). *Journal of Melittology*, 15, 1–8.

532 Velthuis, H.H.W., Koedam, D. and Imperatriz-Fonseca, V.L. (2005) The males of *Melipona*
533 and other stingless bees, and their mothers. *Apidologie*, 36, 169–185.

534 Weir, B.S. and Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population
535 structure. *Evolution*, 38, 1358–1370.

536 Whitfield, C.W., Behura, S.K., Berlocher, S.H., Clark, A.G., Johnston, J.S., Sheppard, W.S.,
537 *et al*. (2006) Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis*
538 *mellifera*. *Science*, 314, 642–645.

539 Wilson, G.A. and Rannala, B. (2003) Bayesian inference of recent migration rates using
540 multilocus genotypes. *Genetics*, 163, 1177–1191.

541 Zanella, F.C.V. (2005) Abelhas da Ilha do Mel: estrutura da comunidade, relações
542 biogeográficas e variação sazonal. *História Natural e Conservação da Ilha do Mel* (eds
543 M.C.M. Marques & R.M. Britez), pp. 189–208. Editora da UFPR, Curitiba.

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Table 1 Population characteristics and genetic diversity in *Tetragonisca angustula* populations. SA: sampled area in square kilometers. ME: median elevation in meters. N: sample size. NH: number of haplotypes. h ± sd: haplotype diversity and standard deviation. π ± sd: nucleotide diversity and standard deviation. Ar ± se: allelic richness after rarefaction for 22 individuals and standard error. H_E ± se: expected heterozigosity and standard error. F_{IS}: inbreeding coefficient.

Population	SA (km^2)	ME (m)	N	NH	h ± sd	π ± sd	Ar ± se	H_E ± se	F_{IS}
1. Teresópolis (TERE)	253	939	46	5	0.207 ± 0.079	0.0087 ± 0.0080	9.07 ± 1.27	0.770 ± 0.045	0.0251
2. Resende (RESE)	10	444	44	3	0.394 ± 0.072	0.0065 ± 0.0066	9.45 ± 1.40	0.773 ± 0.054	0.0248
3. Passa Quatro (PASQ)	385	911	48	2	0.042 ± 0.039	0.0006 ± 0.0019	6.02 ± 0.97	0.626 ± 0.082	-0.0185
4. Angra dos Reis (ANGR)	1208	12	57	7	0.642 ± 0.049	0.0369 ± 0.0225	8.97 ± 1.47	0.769 ± 0.041	0.0463*
5. Ilha Grande (IGRA)	193[&]	11	57	5	0.687 ± 0.032	0.0153 ± 0.0116	6.50 ± 1.26	0.682 ± 0.057	0.0247
6. São Sebastião (SSEB)	117	14	46	3	0.478 ± 0.052	0.0075 ± 0.0072	8.05 ± 1.32	0.712 ± 0.050	0.0088
7. Ilha de São Sebastião (IBEL)	336[&]	25	49	3	0.190 ± 0.072	0.0036 ± 0.0047	6.11 ± 0.98	0.636 ± 0.061	0.0062
8. Iguape (GUAP)	67	13	38	3	0.152 ± 0.077	0.0070 ± 0.0070	6.75 ± 1.13	0.637 ± 0.080	-0.0353
9. Ilha Comprida (ICOM)	200[&]	10	22	4	0.398 ± 0.122	0.0407 ± 0.0251	5.64 ± 0.69	0.659 ± 0.054	0.0381
10. Apiaí (APIA)	504	161	45	6	0.625 ± 0.068	0.0152 ± 0.0115	7.35 ± 1.40	0.634 ± 0.086	0.0142
11. Guaratuba (GUAR)	341	15	36	6	0.636 ± 0.061	0.0233 ± 0.0159	7.02 ± 1.28	0.611 ± 0.084	0.038
12. Blumenau (BLUM)	389	36	45	8	0.628 ± 0.062	0.0127 ± 0.0102	7.38 ± 1.48	0.594 ± 0.102	0.0857**
13. São José (SJOS)	841	11	24	5	0.493 ± 0.116	0.0084 ± 0.0080	7.29 ± 1.48	0.604 ± 0.092	0.2177**
14. Prudentópolis (PRUD)	885	811	42	5	0.302 ± 0.089	0.0049 ± 0.0056	6.35 ± 0.97	0.621 ± 0.070	0.0404
15. Porto União (PUNI)	1521	913	35	5	0.506 ± 0.090	0.0086 ± 0.0080	5.37 ± 0.94	0.513 ± 0.072	0.0832**
16. Foz do Iguaçu (FOZI)	5148	543	43	9	0.632 ± 0.074	0.0157 ± 0.0119	6.59 ± 1.15	0.555 ± 0.064	0.1389**
17. Teodoro Sampaio (TSAM)	5550	440	45	9	0.716 ± 0.058	0.0179 ± 0.0130	7.22 ± 1.13	0.616 ± 0.081	0.0965**

[&]: island area; *: $P < 0.05$; **: $P < 0.01$.

Table 2 Estimates of evolutionary divergence over sequence pairs between populations of *Tetragonisca angustula*. The number of base substitutions per site from averaging over all sequence pairs between populations are shown. Analyses were conducted using the Kimura 2-parameter model (Kimura, 1980) and involved 722 nucleotide sequences. Population abbreviations as in Table 1.

	TERE	RESE	PASQ	ANGR	IGRA	SSEB	IBEL	GUAP	ICOM	APIA	GUAR	BLUM	SJOS	PRUD	PUNI	FOZI
RESE	0.0071															
PASQ	0.0085	0.0109														
ANGR	0.0077	0.0101	0.0067													
IGRA	0.0079	0.0103	0.0069	0.0042												
SSEB	0.0094	0.0118	0.0083	0.0064	0.0076											
IBEL	0.0084	0.0108	0.0074	0.0056	0.0066	0.0010										
GUAP	0.0088	0.0111	0.0077	0.0058	0.0070	0.0012	0.0004									
ICOM	0.0082	0.0111	0.0067	0.0062	0.0072	0.0028	0.0020	0.0000								
APIA	0.0084	0.0107	0.0078	0.0057	0.0073	0.0016	0.0008	0.0022	0.0025							
GUAR	0.0089	0.0113	0.0073	0.0061	0.0072	0.0020	0.0012	0.0014	0.0028	0.0017						
BLUM	0.0083	0.0111	0.0078	0.0060	0.0071	0.0015	0.0007	0.0009	0.0024	0.0013	0.0017					
SJOS	0.0089	0.0113	0.0079	0.0059	0.0071	0.0012	0.0005	0.0007	0.0023	0.0011	0.0015	0.0010				
PRUD	0.0099	0.0123	0.0015	0.0081	0.0083	0.0097	0.0088	0.0090	0.0081	0.0092	0.0087	0.0092	0.0092			
PUNI	0.0319	0.0317	0.0295	0.0315	0.0339	0.0329	0.0319	0.0319	0.0321	0.0317	0.0314	0.0323	0.0323	0.0308		
FOZI	0.0311	0.0309	0.0287	0.0308	0.0331	0.0321	0.0312	0.0312	0.0313	0.0310	0.0306	0.0315	0.0315	0.0301	0.0012	
TSAM	0.0088	0.0111	0.0009	0.0070	0.0072	0.0085	0.0076	0.0079	0.0071	0.0081	0.0076	0.0081	0.0081	0.0023	0.0297	0.0289

Table 3 Pairwise index of differentiation (D_{est}) from microsatellite data of *Tetragonisca angustula*. Population abbreviations as in Table 1.

	TERE	RESE	PASQ	ANGR	IGRA	SSEB	IBEL	GUAP	ICOM	APIA	GUAR	BLUM	SJOS	PRUD	PUNI	FOZI
RESE	0.1074															
PASQ	0.2821	0.2177														
ANGR	0.1761	0.0988	0.2842													
IGRA	0.3183	0.2389	0.4053	0.1735												
SSEB	0.2866	0.2082	0.4191	0.1710	0.1186											
IBEL	0.4341	0.3806	0.4261	0.3119	0.2912	0.2144										
GUAP	0.3914	0.3053	0.4394	0.2455	0.1733	0.0975	0.2064									
ICOM	0.3129	0.2739	0.3395	0.2152	0.1883	0.1594	0.2254	0.0992								
APIA	0.3826	0.3016	0.4209	0.2389	0.1904	0.1042	0.1802	0.0300	0.1356							
GUAR	0.3787	0.3153	0.4012	0.2914	0.2115	0.1251	0.2069	0.0912	0.1568	0.0445						
BLUM	0.3866	0.3101	0.4063	0.2943	0.1854	0.1298	0.2300	0.0551	0.1550	0.0328	0.0204					
SJOS	0.3928	0.3339	0.4518	0.3108	0.2159	0.1234	0.1959	0.0834	0.2113	0.0468	0.0375	0.0212				
PRUD	0.2772	0.2424	0.1387	0.3213	0.4331	0.4518	0.5061	0.4647	0.3608	0.4803	0.4253	0.4341	0.4854			
PUNI	0.7951	0.7547	0.8380	0.6959	0.7074	0.6485	0.6971	0.6693	0.7351	0.6764	0.7051	0.6824	0.6344	0.8281		
FOZI	0.7863	0.7375	0.8439	0.6896	0.6589	0.5886	0.6663	0.6296	0.7007	0.6154	0.6276	0.6061	0.5685	0.8464	0.0274	
TSAM	0.2649	0.2257	0.1173	0.3242	0.3981	0.4218	0.4545	0.4396	0.3552	0.4504	0.4043	0.4052	0.4466	0.0308	0.8015	0.8169

Colours highlight D_{est} values. Green: $D_{est} < 0.05$; yellow: $0.05 < D_{est} < 0.15$; orange: $0.15 < D_{est} < 0.25$; red: $D_{est} > 0.25$. All $P < 0.0054$.

Figure legend

Fig. 1 Posterior probability assignment (vertical axis) of individual genotypes (horizontal axis) for $K = 10$ (*Tetragonisca angustula*) according to the program BAPS (upper panel). Below, map of the studied area with the approximate location of the sampled populations. Population names are 1: Teresópolis, 2: Resende, 3: Passa Quatro, 4: Angra dos Reis, 5: Ilha Grande, 6: São Sebastião, 7: Ilha de São Sebastião, 8: Iguape, 9: Ilha Comprida, 10: Apiaí, 11: Guaratuba, 12: Blumenau, 13: São José, 14: Prudentópolis, 15: Porto União, 16: Foz do Iguaçu, and 17: Teodoro Sampaio. Detailed location of all islands visited (lower panels). IGRA: Ilha Grande; IANC: Ilha Anchieta; ITMD: Ilha do Tamanduá; IVIT: Ilha da Vitória; IBUZ: Ilha de Búzios; IBEL: Ilha de São Sebastião; IMTG: Ilha Monte de Trigo. ICOM: Ilha Comprida; ICAR: Ilha do Cardoso; IMEL: Ilha do Mel. ISCA: Ilha de Santa Catarina.