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RESEARCH ARTICLE

Landscape genetics of a tropical rescue pollinator

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Abstract Pollination services are increasingly threatened by the loss and modification of natural habitats, posing a risk to the maintenance of both native plant biodiversity and agricultural production. In order to safeguard pollination services, it is essential to examine the impacts of habitat degradation on the population dynamics of key pollinators and identify potential "rescue pollinators" capable of persisting in these human-altered landscapes. Using a landscape genetic approach, we assessed the impact of landscape structure on genetic differentiation in the widely-distributed tropical stingless bee *Trigona spinipes* (Apidae: Meliponini) across agricultural landscape mosaics composed of coffee plantations and Atlantic forest fragments in southeastern Brazil. We genotyped 115 bees at 16 specific and highly polymorphic microsatellite loci, developed using next-generation sequencing. Our results reveal that *T. spinipes* is capable of dispersing across remarkably long distances, as we did not find genetic differentiation across a 200 km range, nor fine-scale spatial genetic structure. Furthermore, gene flow was not affected by forest cover, land cover, or elevation, indicating that reproductive individuals are able to disperse well through agricultural landscapes and across altitudinal gradients. We also found evidence of a recent population expansion, suggesting that this opportunistic stingless bee is capable of colonizing degraded habitats. Our results thus suggest that *T. spinipes* can persist in heavily-altered landscapes and can be regarded as a rescue pollinator, potentially compensating for the decline of other native pollinators in degraded tropical landscapes.

Keywords Agricultural landscapes · Tropical forest cover · Gene flow · Landscape resistance · Pollination services · Stingless bees

Introduction

Pollination services are increasingly threatened by the human modification of natural habitats (González-Varo et al. 2013; Kremen et al. 2007; Potts et al. 2010; Vanbergen and The Insect Pollinators Initiative 2013). A disruption in pollination services could have important negative ecological and economic consequences, because the cessation of these services could reduce wild plant diversity, narrow ecosystem stability, reduce crop production, and decrease food security and human welfare (Aizen and Harder 2009; Gallai et al. 2009; Garibaldi et al. 2011). This is especially true if key native pollinators cannot withstand habitat modifications in pollinator-dependent landscapes, such as agricultural systems.

Wild bees are a particularly valuable asset within agricultural landscapes, because they can compensate for the worldwide decline in honeybee populations (Brown and

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Paxton 2009; Jaffé et al. 2010; vanEngelsdorp and Meixner 2010) by assuring a sufficient pollinator density for crop production (Aizen and Harder 2009) and by pollinating many crops more efficiently than honeybees (Garibaldi et al. 2013). However, wild bee populations have proven susceptible to the degradation of natural habitats, as multiple studies indicate that bee abundance and richness are negatively affected by habitat loss and fragmentation (Brown and Oliveira 2013; Kennedy et al. 2013; Winfree et al. 2009). While past studies document declines in bee communities, much remains unknown about the population dynamics and dispersal processes of native bees in human-altered landscapes. For instance, some native pollinators exhibit reduced nesting densities (Goulson et al. 2010; Jha and Kremen 2013a) and reduced gene flow across human-altered habitats (Davis et al. 2010; Jha and Kremen 2013b). Research devoted to better understanding the influence of forest cover loss and land use expansion on wild bee population dynamics is therefore essential to safeguard bee populations and ensure pollination services in a changing world (Hadley and Betts 2011; Lautenbach et al. 2012; Viana et al. 2012; Wratten et al. 2012).

While a few studies have utilized landscape genetic approaches to quantify land use impacts on wild bee gene flow within temperate regions (Davis et al. 2010; Jha and Kremen 2013b), no such efforts have been undertaken in the tropics to date. In contrast, past molecular work in the tropics has largely focused on looking at genetic diversity and isolation by distance in native bees (Freiria et al. 2012; Suni et al. 2014; Zimmermann et al. 2011). Given that rates of pollinator loss seem to be faster in the tropics than in temperate regions (Ricketts et al. 2008; Viana et al. 2012), there is a pressing need to understand land use impacts on wild bee gene flow in tropical ecosystems. Additionally, the loss of native pollinators within tropical systems is particularly critical given that most tropical plant species are biotically pollinated and self-incompatible (Ollerton et al. 2011), and thus likely to be more vulnerable to pollinator declines. Moreover, an estimated 2101 km^2 of tropical forest are destroyed every year (Hansen et al. 2013), and the rate of land conversion to agriculture is expected to further increase in response to a growing human population (Laurance et al. 2014), with major potential negative impacts for native pollinators. Finally, many tropical crops are pollinator-dependent (Giannini et al. 2015a), making pollination a critical ecosystem service within tropical agricultural landscapes (Klein et al. 2008).

One strategy to safeguard pollination services in tropical agro-ecosystems is to examine the impact of habitat degradation on the population dynamics of key pollinators and to identify potential "rescue pollinators" (Jaffé et al. 2010). Capable of colonizing degraded habitats and dispersing through heterogeneous landscapes, rescue pollinators like honeybees (*Apis mellifera*) can both compensate for a decline in visits by other pollinators (e.g. Aizen and Feinsinger 1994), and ensure plant gene flow across fragmented landscapes (e.g. Dick 2001). These pollinators are able to persist in degraded habitats by maintaining high gene flow levels across heterogeneous landscapes, which allows them to retain large effective population sizes, remain largely unaffected by genetic drift, and thus avoid inbreeding and the fitness reductions associated to Allee effects (Allendorf et al. 2012; Stephens and Sutherland 1999).

Coffee is one tropical crop that benefits from pollinators, as it exhibits increased per-bush fruit set and increased field-level crop yields when exposed to insect pollination (De Marco Jr and Coelho 2004; Klein 2009; Klein et al. 2003; Ricketts 2004). It is also one of the most widely cultivated and economically valuable crops in the tropics (Donald 2004; Jha et al. 2014), and one of Brazil's main export commodities, generating more than US$ 3 billion per year (ABIC 2012). The primary coffee-producing region in Brazil is the southeastern coastal Atlantic forest region, a biodiversity hotspot that has suffered severe deforestation during the past decades due to agricultural expansion (Joly et al. 2014; Ribeiro et al. 2009). Although previous research on coffee agro-ecosystems indicates that the abundance and diversity of native bees declines with decreasing landscape complexity and forest proximity (Jha and Vandermeer 2010; Ricketts 2004), the influence of landscape structure on the population genetics of wild coffee pollinators has not been studied to date.

Using landscape genetic tools we assessed the impact of landscape structure on genetic differentiation in the tropical stingless bee *Trigona spinipes* (Apidae: Meliponini), across agricultural landscape mosaics composed of coffee plantations and Atlantic forest fragments. In an attempt to identify a potential rescue pollinator, we selected *T. spinipes* because it is a generalist and opportunistic pollinator, dominant in most pollinator networks, broadly distributed across South America, and considered the ecological equivalent of the honeybee *Apis mellifera* (Biesmeijer and Slaa 2006; Giannini et al. 2015b). *T. spinipes* is an effective pollinator of important crops, including carrot, sunflower, orange, mango, strawberry, squash, bell pepper (Giannini et al. 2014), and coffee (Ngo et al. 2011). Based on the limited natural history and ecological data available for *T. spinipes* (Biesmeijer and Slaa 2006; Nogueira-Neto 1997), we hypothesize that this generalist pollinator is capable of colonizing degraded habitats and maintain high gene flow levels across agricultural landscapes. Specifically, we predict that we will find: (1) Higher gene flow across agricultural landscapes than between regions predominantly covered by preserved forest remnants; and 2) A

genetic signature of a population expansion in a region where native vegetation has been recently replaced by crop fields or urban areas.

Materials and methods

Sampling

Our main study region was the area surrounding Poços de Caldas, between the States of São Paulo and Minas Gerais, one of the most traditional and productive coffee plantation regions in Brazil (Fig. 1). In addition, we collected bee samples from two outgroup study regions, one located in the city of São Paulo (200 km distant) and one located in the city of Mossoró, Rio Grande do Norte (2000 km distant, Fig. 1). Sampling in our main study region took place during the coffee flowering season, between September and October 2013. Sampling in São Paulo and Mosoró took place between August 2012 and August 2013.

Ten coffee farms from our main study region were selected to maximize variation in forest cover. The minimum distance between coffee farms was 6 km. Bees were collected across all ten coffee farms using entomological nets and colored pan-traps (da Unesc 2008), and by collecting directly from nests. In each farm, 10 randomly chosen coffee bushes, separated by at least 100 m, were surveyed with entomological nets for 10 min to collect all bees visiting coffee flowers. In addition, three sampling stations containing three 11 cm diameter colored pan-traps (yellow, white, and blue) were placed at forest-coffee border sites spaced throughout each farm. Pan-traps were left in the field from sunrise to sunset. We searched for nests within each coffee farm, but since not all farms contained nests, we also collected samples from nests located in regions surrounding and between the farms. Nests were individually disturbed by vibrating tree branches and throwing small stones, and attacking bees were collected from hair and clothes. In São Paulo and Mossoró, bees were only collected from wild nests. All totaled, 115 bee samples were identified, georeferenced, stored in

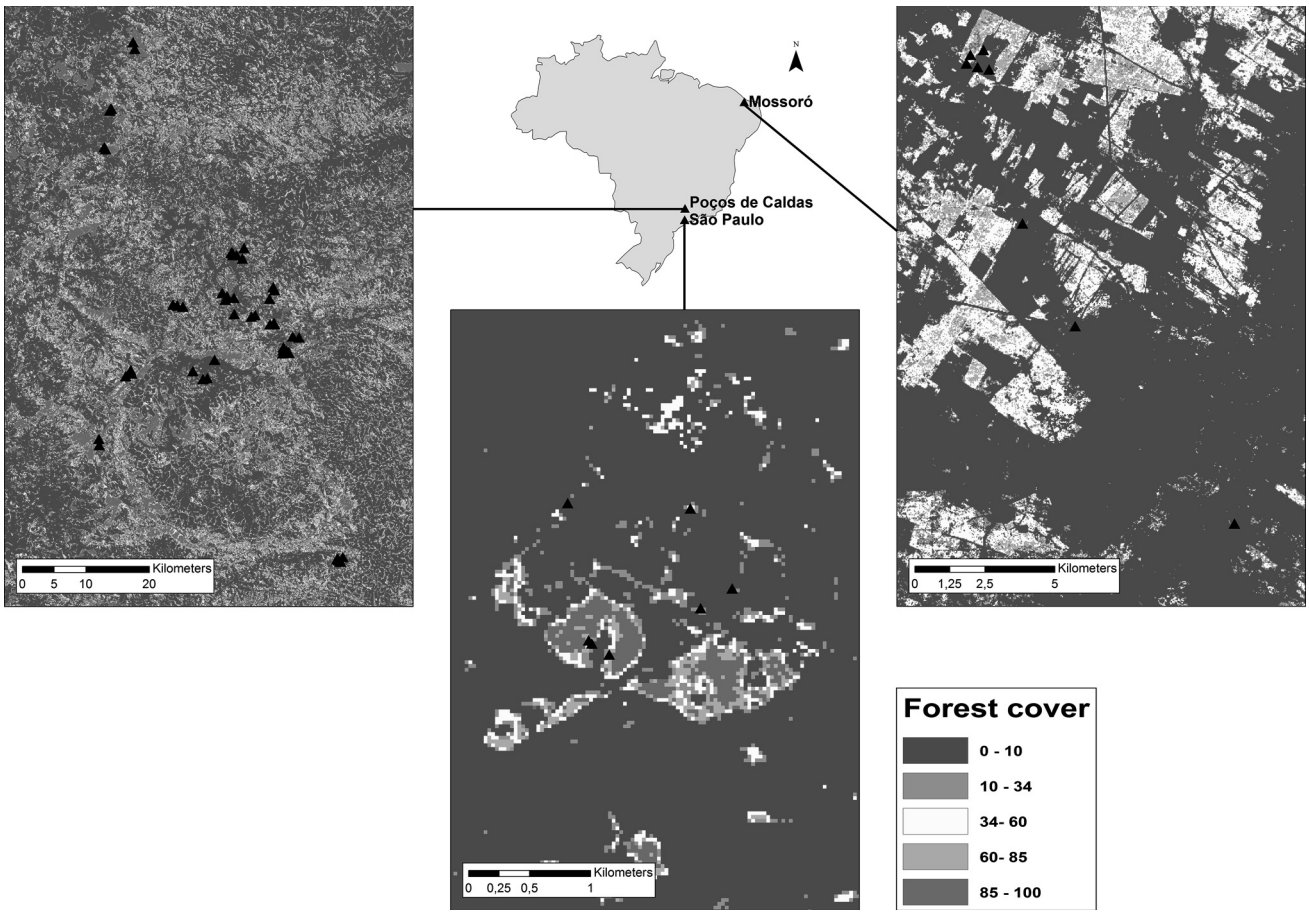

Fig. 1 Map of Brazil showing the three study regions (Mossoró, São Paulo, and Poços de Caldas), and a zoomed image of these regions showing the spatial distribution of bee samples (*triangles*) and forest cover. Only samples of individuals representing unique colonies are shown

absolute ethanol and later frozen at −20 °C. To verify species identity, Dr. Silvia Pedro (USP-Riberão Preto) and Dr. Airton Carvalho (UFERSA-Mossoró) helped identify a subset of specimens to the species level.

Microsatellite development

DNA was extracted from a single bee using the Qiagen DNeasy Blood & Tissue Kit. The DNA was then sent to the ESALQ-Piracicaba (Laboratório Multiusuários Centralizado) for MiSeq Illumina sequencing and bioinformatic processing. Reads were visualized using FastQC, and filtered using Seqyclean. Different genome assemblies were done using Velvet v1.2.09 and compared with Cd-hit, removing redundant contigs. Finally, microsatellites were identified using QDD v 3.1.1 and primers designed with Primer3. We followed QDD guidelines to select 36 microsatellites from more than 500 loci containing at least ten tandem repeats. These 36 microsatellites were then tested for amplification quality and polymorphism employing M13-tagged forward primers (Schuelke 2000) and a DNA pool from 18 individuals scattered across all three study regions. Each primer was tested on a gradient of annealing temperatures ranging between 56 and 63 °C.

Genotyping

From the 36 tested loci, 16 showed good amplification and high polymorphism and were thus selected for subsequent genotyping (GeneBank accession numbers and detailed information for all loci are provided in Online Resource 1). Multiplex PCRs were conducted using fluorescent marked primers (FAM, VIC, and PET). We ran 26 µl PCR reactions containing four primers and 2.5 µl Buffer 10X, 2.5 µl BSA, 1.5 µl forward-primer 10 µM, 1.5 µl reverse-primer 10 µM, 2.0 µl dNTPs 2.5 mM, 1 µl Taq Polymerase 5U/µl and 6 µl DNA. PCRs began with a 5 min denaturation step at 95 °C, followed by 34 cycles of 30 s at 95 °C, 50 s at the primer-specific annealing temperature determined in the previous step, and 45 s at 72 °C, followed by a final extension at 72 °C for 20 min. PCR products were resolved on an ABI 3730 Sequencer and alleles were scored manually using GeneMarker (Softgenetics).

Genetic analyses

We excluded all individuals with less than 11 successfully amplified loci. To compute unbiased allele frequencies and avoid the pseudo-replication of samples, we first assigned all bee samples into colonies using the program COLONY V2 (Jones and Wang 2010). This program was run under the assumption that colonies are headed by one singly-mated queen, based on the recent characterization of the *T.spinipes*'s mating system (Jaffé et al. 2014). We then selected one individual from each of the identified colonies, to construct a dataset from which to compute unbiased allele frequencies. We used this dataset to check for null alleles using Micro-Checker (Van Oosterhout et al. 2004), and test for Hardy–Weinberg equilibrium and linkage disequilibrium using Genepop (Rousset 2008). While one locus showed null alleles (TS-22), five loci showed a significant departure from Hardy–Weinberg equilibrium in our main study region (TS-10, TS-16, TS-21, TS-22, and TS-29). All of these loci were therefore excluded from subsequent analyses. No loci combination was found to show significant linkage disequilibrium. We then ran COLONY again, without the excluded loci, and selected one individual from each colony to construct a final dataset (N = 75 individuals from unique colonies, spatial coordinates and genotypes are provided in Online Resource 2).

Genetic diversity measures were estimated using Genepop (Rousset 2008) and HP-rare (Kalinowski 2005). We used SPAGeDi (Hardy and Vekemans 2002) to calculate Rousset's inter-individual genetic distance (*a*, Rousset 2000), along with relatedness (R_{ij}) and kinship (F_{ij}) coefficients. We also used the R package *gstudio* (Dyer 2014) to compute the AMOVA-distance between individuals, the proportion of shared alleles (D_{ps}), and the pairwise genetic distances G_{ST} and D_{est} (Jost 2008). To estimate the most probable number of subpopulations represented in our sample (optimal K), we ran STRUCTURE (Pritchard et al. 2000), performing 10 replicates of each simulation with K = 1–6, a burn-in of 50,000 and 100,000 post burn-in Markov chain Monte Carlo (MCMC) iterations, and assuming admixture and correlated allele frequencies. To test for recent population bottleneck events we ran the program BOTTLENECK (Piry et al. 1999) with 10000 replications and under the assumption of the Stepwise Mutation Model (SMM) and the Two-Phase Mutation Model (TPM, variance = 12, Proportion of SMM = 95 %), as they are more appropriate for microsatellites (Piry et al. 1999). Per-locus and multi-locus allele frequency distributions were also examined for a mode shift, which indicates a recent genetic bottleneck (Piry et al. 1999). Finally, we employed the program MSVAR v1.3 (Beaumont 1999) to infer ancient demographic changes and estimate ancestral and current effective population size (N_e) (Girod et al. 2011; Williamson-Natesan 2005). We include a convergence diagnostic (Rhat, Gelman and Rubin 1992), details on the number and thinning of the MCMC simulations, and a sensitivity analysis which assesses results of the posterior simulations from MSVAR across various priors (see Online Resource 6 for details). In order to avoid any potential biases arising from the Wahlund effect when inferring past demographic changes (Allendorf

et al. 2012), we only included samples from our main study region (Poços de Caldas) in the BOTTLENECK and MSVAR analyses.

Spatial analyses

In order to assess the influence of landscape structure on genetic differentiation, we obtained the following high resolution rasters: 1) A continuous forest cover map for 2000 (University of Maryland: http://earthenginepartners.appspot.com/science-2013-global-forest/download.html), where every pixel contained a forest cover value ranging from zero to 100; 2) A categorical Global Land Cover Map for 2009 (GlobCover: http://due.esrin.esa.int/globcover), where every pixel contained a land cover class code (legends are provided in Online Resource 2); 3) A continuous high resolution digital elevation map (DEM) for the main study area (USGS-EROS: http://eros.usgs.gov/elevation-products), where every pixel contained an elevation value expressed in meters; and 4) A continuous high resolution digital elevation map (DEM) for the whole study area (WorldClim: http://www.worldclim.org/), where every pixel contained an elevation value expressed in meters. We created two sets of rasters to assess local and broad genetic differentiation: One covering our main study region (Poços de Caldas), and one covering all three regions (Mossoró, São Paulo, and Poços de Caldas). For each set, we cropped all rasters to the extent of the study regions, which comprised a buffer area of at least 10 km around our sampling locations, to minimize border effects. Spatial analyses were done using the R package *raster* (Hijmans 2014).

We then used circuit theory (McRae et al. 2008) to estimate the resistance to gene flow between samples for each explanatory variable (geographic distance, forest cover, land cover, and elevation). We used the program Circuitscape v4.0 (McRae 2006) to estimate pairwise resistance distances. Because we hypothesized higher gene flow across agricultural or non-forested landscapes than between regions predominantly covered by preserved forest remnants, we created resistance surfaces where forested pixels had higher resistance values. Two separate resistance surfaces were generated, one using forest cover and one using land cover maps. To this end, we used the raw forest cover rasters and transformed the land cover rasters (assigning a maximal resistance of 0.9 to all forested land cover classes, and a minimal resistance of 0.1 to all other classes). To test the contrasting hypothesis (higher gene flow across forested landscapes), we inverted the forest cover rasters (using the absolute values after subtracting 100 from every pixel), and re-transformed the land cover rasters (assigning a minimal resistance of 0.1 to all forested land cover classes, and a maximal resistance of 0.9 to all other classes). Since genetic differentiation has been found to be influenced by elevation in other bees (Lozier et al. 2011), we also examined the independent effect of elevation. We hypothesize that mountain ranges constitute a potential barrier to bee gene flow, so we created resistance surfaces were pixels with higher elevations had higher resistance values. To do so we used the raw elevation from the DEMs as resistance values for each pixel. Finally, to test for isolation by geographic distance, we created null-model rasters by replacing all values of the forest cover rasters with 0.5, and calculated resistance distances between sampling locations. Because Circuitscape does not accept zero resistance values, we replaced zero values in all rasters with 0.0001. To achieve a reasonable computing time for each Circuitscape run (<2 h), we decreased the resolution of resistance surfaces by aggregating blocks of pixels (Shirk et al. 2010, final raster resolutions are presented in Online Resource 2).

Landscape genetic analyses

To relate genetic distance to resistance distances, we ran regressions using maximum-likelihood population effects (MLPE) parameterization (Clarke et al. 2002). The MLPE model uses a residual covariance structure to account for the non-independence of pairwise distances, and is becoming a standard approach in landscape genetic studies since it accounts for the non-independence of pairwise distances within a likelihood framework, which is compatible with model selection (Peterman et al. 2014; Van Strien et al. 2012). Code implementing the MLPE correlation structure within the R package *nlme* (Pinheiro et al. 2014) is provided at (https://github.com/nspope/corMLPE). In all models we used inter-individual genetic distance estimates as response variables and the different resistance distances (geographic distance, forest cover, land cover, and elevation) as predictors. We thus ran all analyses on the level of individuals from unique colonies (see above), which increased the number of observations and statistical power (Shirk et al. 2010). We only ran simple regressions (containing a single predictor) because predictors were collinear with geographic resistance distance and with each other (Online Resource 3). To assess local and broad genetic differentiation we ran two separate model selection analyses, one using the samples from our main study region (Poços de Caldas), and one using the samples from all three regions (Mossoró, São Paulo, and Poços de Caldas). The Akaike Information Criterion corrected for finite sample size (AICc) was used to select the best models.

Fine-scale spatial genetic structure in our main study region was then examined using spatial autocorrelation analysis. Using randomization tests and nonparametric smoothing, we evaluated whether *T. spinipes* showed a greater degree of spatial genetic structure than what would

be expected by chance. To detect spatial genetic structure at different scales, we used a local polynomial fitting (LOESS) of pairwise kinship (F_{ij}) to pairwise geographic distance (Bruno et al. 2008; Castilla et al. *in review*). In order to test if the average observed kinship predicted by LOESS at a given distance differed from the null model, we permuted row and column indices for the kinship matrix 999 times; and at each permutation we re-fitted the LOESS model using the permuted kinship and geographic distance matrix. We used the 95 % percentiles of the permutation-derived LOESS predictions to generate a confidence envelope around the null expectation of $F_{ij} = 0$. Finally, we estimated bee flight distance as an additional measure of dispersal ability. To do so, we calculated the geographic distance separating samples collected in different locations but assigned to the same colony by COLONY.

Results

From the 16 loci employed for genotyping, eleven followed the Hardy–Weinberg equilibrium, and showed no null alleles nor linkage disequilibrium. These newly developed microsatellite loci proved highly polymorphic, with a total number of alleles per locus ranging between 11 and 16 (across the three study regions), a mean number of alleles per locus ranging between 3.73 and 11.64, and an expected heterozygosity (H_e) ranging between 0.55 and 0.83 (Table 1). Genetic diversity was particularly high in Poços de Caldas, our main study region. STRUCTURE analyses showed that the most likely number of populations represented in our sample (optimal K) was two when including all regions, one when including São Paulo and Poços de Caldas, and one when including Poços de Caldas alone (Online Resource 4). Pairwise genetic distances also revealed lower genetic differentiation between São Paulo and Poços de Caldas than between these two regions and Mossoró (Table 2). We did not find evidence of a recent population bottleneck, neither when testing for a heterozygosity exess nor when examining allele frequencies, which exhibited L-shaped distributions (Online Resource 5). However, we found a significant heterozygosity deficiency in our main study region (Wilcoxon tests one tail p value for the SMM and the TPM = 0.002 and 0.03 respectively), indicative of a recent population expansion (Cornuet and Luikart 1996). On the other hand, MSVAR revealed a population contraction starting around 678 years ago (CIs: 92–6295), with a mean ancestral $N_e = 26915$ (CIs: 8433–86298), and a mean current $N_e = 3327$ (CIs: 356–42073; Online Resource 6).

Table 2 Pairwise genetic distance between all three study regions

G_{ST} \ D_{est}	Mossoró	São Paulo	Poços de Caldas
Mossoró		0.140	0.189
São Paulo	0.610		−0.034
Poços de Caldas	0.775	0.107	

The lower diagonal shows Hedrick's G_{ST}, while the upper diagonal shows Jost's D_{est} (Jost 2008)

In our main study region, neither geographic distance, forest cover, land cover, nor elevation explained genetic distance (Tables 3, 4; Fig. 2). These results hold when including the samples from São Paulo (data not shown). Across all three study regions, however, geographic distance was found to explain genetic distance better than the other predictors (Table 3), and it showed a significant positive association with genetic distance ($\beta = 0.21$, SE = 0.008, $t_{2773} = 24.77$, $p < 0.001$; Table 4, Fig. 3). All results hold when using the different genetic distance measures (data not shown), as well as when testing the contrasting hypothesis that gene flow was higher across forested landscapes (inverted resistance values, Online Resource 7).

Spatial autocorrelation analysis also confirmed a lack of fine-scale spatial genetic structure, as pairwise kinship of neighboring colonies was not higher than the null expectation (Fig. 4). Geographic distances separating samples assigned to the same colony ranged between 28 m and 8700 m (Mean ± SD = 1742 ± 3419 m, N = 6).

Table 1 Genetic diversity statistics for the three study regions, where N is the number of individuals representing unique colonies, A the number of alleles, A_r the sample-size corrected allelic richness, PA_r the sample-size corrected private allelic richness, H_o the observed heterozygosity, and H_e the expected heterozygosity

Study region	N	A	Ar	PA_r	H_o	H_e
Mossoró	8	3.73 ± 1.27	3.55 ± 1.15	1.26 ± 1.08	0.66 ± 0.05	0.55 ± 0.07
São Paulo	7	5.82 ± 1.17	5.70 ± 1.09	1.58 ± 0.81	0.83 ± 0.04	0.83 ± 0.02
Poços de Caldas	60	11.64 ± 1.63	6.19 ± 0.76	1.78 ± 0.82	0.87 ± 0.01	0.83 ± 0.01

Mean and standard deviation are reported for each estimate

Table 3 Model selection summary, showing MLPE regressions using Rousset's inter-individual genetic distance (*a*) as response variable and the different resistance distances (RD) as predictors

Dataset	Predictor	logLik	AICc	ΔAICc	Weight
Poços de Caldas	Forest cover RD*	2077.02	−4146.01	0.00	0.31
	Land cover RD*	2076.76	−4145.50	0.51	0.24
	Geographic distance RD*	2076.75	−4145.48	0.53	0.23
	Elevation RD*	2076.71	−4145.39	0.62	0.22
Mossoró, São Paulo, and Poços de Caldas	Geographic distance RD *	3187.66	−6367.31	0.00	1.00
	Land cover RD	3152.04	−6296.07	71.24	<0.001
	Elevation RD	3099.31	−6190.60	176.71	<0.001
	Forest cover RD	2983.46	−5958.91	408.41	<0.001

The sample-size corrected Akaike information criterion (AICc) is provided for each model along with ΔAICc and the weight of each model. Best models (ΔAICc < 2) are highlighted by *

Table 4 Summary statistics of the best MLPE models (ΔAICc < 2) using Rousset's inter-individual genetic distance (*a*) as response variable and the different resistance distances (RD) as predictors. For each model we provide estimates, standard errors (SE), degrees of freedom (df), *t*-values, *p*-values and confidence intervals (CI)

Dataset	Predictor	Estimate	SE	df	*t*	*p*	CI (min/max)
Poços de Caldas	Forest cover RD	6.94×10^{-4}	8.46×10^{-4}	1770	0.82	0.41	$-9.64 \times 10^{-4}/2.35 \times 10^{-3}$
	Land cover RD	2.15×10^{-2}	5.61×10^{-2}	1770	0.38	0.70	$-8.85 \times 10^{-2}/0.13$
	Geographic distance RD	6.86×10^{-3}	1.90×10^{-2}	1770	0.36	0.72	$-3.03 \times 10^{-2}/4.40 \times 10^{-2}$
	Elevation RD	1.84×10^{-6}	9.23×10^{-6}	1770	0.20	0.84	$-1.62 \times 10^{-5}/1.99 \times 10^{-5}$
Mossoró, São Paulo, and Poços de Caldas	Geographic distance RD	0.21	8.44×10^{-3}	2773	24.77	<0.001*	0.19/0.23*

Significant relations ($p < 0.05$, CI not containing zero) are highlighted by *

Discussion

This study presents the first landscape genetic analysis of a key tropical pollinator in an economically important agricultural landscape. Our results reveal that *T. spinipes* is capable of long-distance dispersal across human-altered landscapes. Specifically, we did not find genetic differentiation across a 200 km range, nor fine-scale spatial genetic structure. Furthermore, gene flow was not affected by forest cover, land cover or elevation, indicating that these bees are able to disperse well through agricultural landscapes and across an elevation gradient ranging between 660 and 1800 m. Finally, we found evidence of a recent population expansion and an ancient population contraction.

Our results reveal extensive gene flow across a large and heterogeneous region. Specifically, STRUCTURE analyses did not show population differentiation across a 200 km range (between Poços de Caldas and São Paulo), and only revealed two different genetic clusters when considering Mossoró, a distant population located 2000 km away (Online Resource 4). Supporting this result, pairwise genetic distance between Poços de Caldas and São Paulo was low (Table 2). Moreover, we were only able to detect isolation by geographic distance (IBD) when analyzing all three study regions. Our findings thus reveal that *T. spinipes* is capable of dispersing and maintaining high gene flow across large distances (at least 200 km), and that IBD appears only at a large geographic scale (2000 km). This result is in line with studies assessing gene flow in other stingless bees. For instance, using 12 specific microsatellite markers, Duarte et al. found weak isolation by distance in the stingless bee *Scaptotrigona xanthotricha* across a region spanning nearly 2000 km (Duarte et al. 2014). Similarly, running STRUCTURE with samples of *Tetragonisca angustula* genotyped at 11 specific loci, Francisco and colleagues found two genetic clusters, each spanning regions exceeding 500 km (Francisco et al. 2014). On the other hand, another study using 5 microsatellites and 10 ISSR primers to genotype samples of *Melipona scutellaris* (Tavares et al. 2013), found a significant isolation by distance across a region of 400 km, as well as two genetic clusters associated to different elevations (although the effect of elevation was not formally tested). Some stingless bee species thus seem to have a remarkable dispersal ability, but it remains unclear how natural history, other landscape features, or climate, influence dispersal across species (Giannini et al. 2012, 2015c; Roubik 1992).

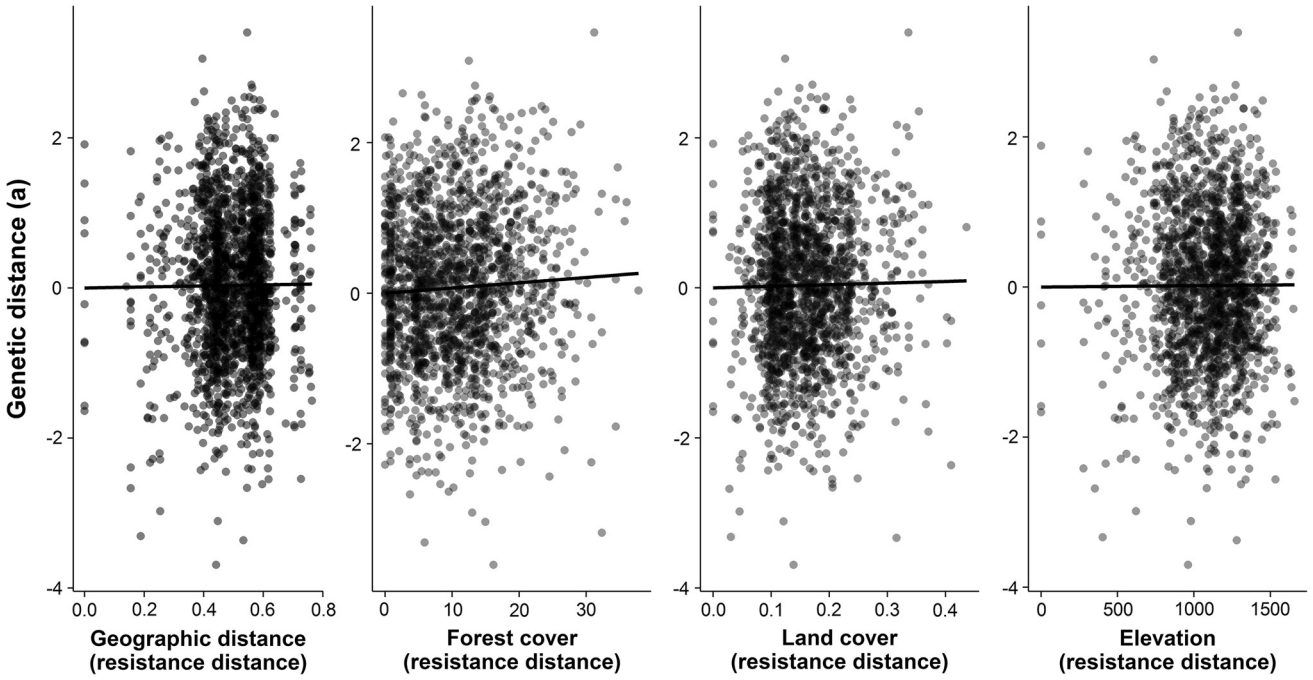

Fig. 2 Relationship between Rousset's inter-individual genetic distance (*a*) and geographic distance resistance distance, forest cover resistance distance, land cover resistance distance and elevation resistance distance, in the Poços de Caldas region. Genetic distance is de-correlated for the MLPE correlation structure

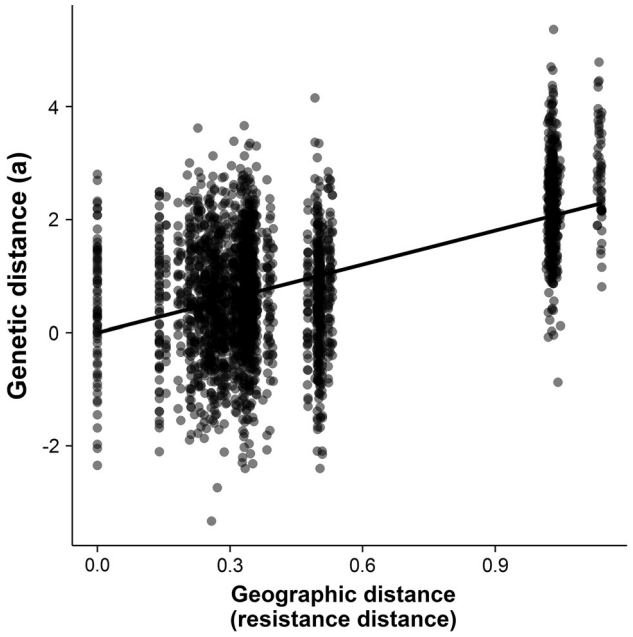

Fig. 3 Relationship between Rousset's inter-individual genetic distance (*a*) and geographic distance resistance distance across all three study regions (Mossoró, São Paulo, and Poços de Caldas). Genetic distance is de-correlated for the MLPE correlation structure

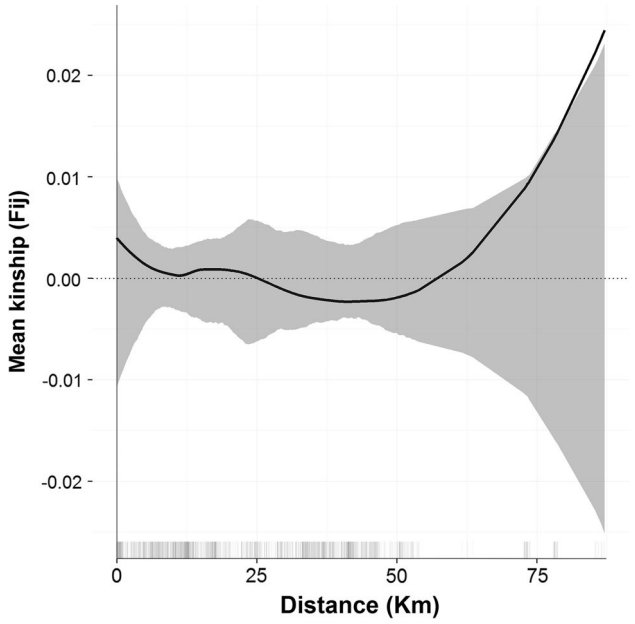

Fig. 4 Spatial autocorrelation analysis. The black solid line is the LOESS fit to the observed kinship, while the grey shaded regions are 95 % confidence bounds around the null expectation (*black dotted line*). Short *vertical lines* at the bottom of the figure are observed pairwise distances

While stingless bees are thought to have a restricted dispersal, because daughter colonies depend on their maternal ones during their initial establishment (Inoue et al. 1984; van Veen and Sommeijer 2000), we did not find evidence of spatial genetic structure in our main study region. Our results indicate that pairwise kinship was not

substantially higher between colonies separated by a few Kilometers than between distant colonies, separated by more than 50 km (Fig. 4). This finding matches those of a recent study performing similar analyses on African honeybees (Gruber et al. 2013), and suggests: (1) A high colony reproduction rate, leading to many daughter colonies spreading away from their maternal colonies, and an enhanced gene flow via males; (2) A high dispersal capacity of virgin queens and daughter colonies; (3) A high male dispersal capacity; (4) A combination of these alternatives. A recent study showed that *T. spinipes* colonies are usually headed by one singly-mated queen, thus ruling out queen multiple mating (polyandry) as a mechanism to increase effective population size (Jaffé et al. 2014). However, very little is known about colony reproduction rate or colony dispersal in this species (Engels and Imperatriz-Fonseca 1990; Inoue et al. 1984; Nogueira-Neto 1997; van Veen and Sommeijer 2000). Given that we found bees from the same colony separated by more than 8 km, our results suggest that *T. spinipes* workers have a higher flight capacity than previously reported estimates (Araújo et al. 2004). Whether virgin queens and males have a similar flight capacity remains an open question, but our results suggest they do.

Our results support the hypothesis that *T. spinipes* is capable of colonizing degraded habitats, as we found evidence of a recent population expansion. However, we also found a signal of an ancient population contraction. Previous studies have found that BOTTLENECK is best suited to detect recent demographic events, whereas MSVAR is more appropriate to detect ancient events (Cornuet and Luikart 1996; Girod et al. 2011; Williamson-Natesan 2005). In any case, the ancient population contraction detected by MSVAR (starting before the arrival of European settlers to South America), is not likely related to human-mediated changes in land use. On the other hand, the heterozygosity deficiency detected by BOTTLENECK does not seem to be a consequence of genetic structure within our main study population (Wahlund effect), because we failed to detect any genetic structure, neither when running STRUCTURE nor when performing the spatial autocorrelation analysis. This result thus suggests a recent population expansion (Cornuet and Luikart 1996; Excoffier et al. 2009). The recent conversion of Atlantic forest into agricultural farmland across our study region (Joly et al. 2014) may have facilitated dispersal of *T. spinipes* and resulted in the colonization of new habitats previously dominated by other bee species. Indeed, due to its ability to rapidly establish enormous colonies in recently degraded areas, *T. spinipes* is often regarded as an invasive species, even in places where it is native (Jaffé et al. 2014; Nogueira-Neto 1997). Our results support those of a recent study analyzing plant-bee interaction networks across the main Brazilian biomes, which found that *T. spinipes* performs better in disturbed than in preserved habitats (Giannini et al. 2015b). However, further studies are needed to test if similar population expansions occurred in other areas, and whether their onset matches the time when the forests were replaced by farmland.

Our results also show that *T. spinipes* has an exceptional ability to disperse across large distances and through degraded habitats, fragmented landscapes, and altitudinal gradients. While we were not able to detect higher gene flow across agricultural landscapes than between forested regions, we posit that because our main study region is extremely heterogeneous (about 20 % forest and 80 % agriculture, urban, and pastoral lands) we would only have been able to detect an effect of forest on bee dispersal if such an effect was strong. In other words, because our main study region contains small Atlantic forest remnants scattered across farmland and urban areas, these are likely to act as dispersal barriers only for species that are extremely averse to dispersing through natural habitats. This explanation is further supported by the fact that the results remained unaltered when we tested the contrasting hypothesis that gene flow was higher across forested landscapes. Additional studies are thus needed to test if gene flow in *T. spinipes* is more restricted across large and continuous areas of preserved Atlantic forest, like those found along the coastal regions of Southeastern Brazil (Joly et al. 2014; Ribeiro et al. 2009).

Although previous studies have shown that urbanization (Davis et al. 2010; Jha and Kremen 2013b) and agriculture (Jha 2015), can restrict gene flow in wild bee populations, it is likely that species with greater dispersal abilities are less sensitive to habitat fragmentation (Cerna et al. 2013; Suni et al. 2014; Zimmermann et al. 2011). This seems to be the case of *T. spinipes*, which is capable of colonizing degraded habitats and maintaining high gene flow across vast regions comprising heterogeneous landscapes. Our results thus suggest that *T. spinipes* could serve as a rescue pollinator, being a generalist pollinator able to compensate for the decline of other less resilient pollinators in degraded habitats (Brosi et al. 2008; Dick 2001; Giannini et al. 2015b). Indeed, *T. spinipes* was reported as a frequent visitor of coffee flowers in three different studies (Ngo et al. 2011), and current work performed in the same study region also found *T. spinipes* and *Apis mellifera* are the most abundant visitors of coffee flowers (Saturni et al. unpublished data). Moreover, based on an extensive data base, Giannini et al. (2014) classified *T. spinipes* as an effective pollinator of a large number of agricultural crops. Coffee farms located in heterogeneous landscapes, such as the ones we studied, thus benefit from the pollination services offered by this native bee. Further efforts are needed to quantify the pollination services offered by *T. spinipes*

and its ability to offset pollinator deficits (Boreux et al. 2013; Vaissière et al. 2011). Likewise, extension work is needed to explain the importance of this key native pollinator to farmers, who often regard it as a pest due to its extremely aggressive behavior (Nogueira-Neto 1997; Shackleton et al. 2015).

Our study constitutes an important contribution to understanding the dynamics of a key tropical pollinator in an economically important agro-ecosystem. Our results provide a first insight into the influence of forest cover, land cover, and elevation, on genetic differentiation in an opportunistic stingless bee. Further, we provide resources for 11 highly polymorphic microsatellite loci specific to *T. spinipes,* which we believe will be useful for future studies given its ecological and economic importance. Finally, we believe that this work could serve as a useful reference for studies aiming to assess species-specific patterns of genetic differentiation associated to geographic distance, topography, and land use across complex tropical landscapes. Stingless bees are a critical component of tropical pollinator communities and exhibit a remarkable variation in life history and dispersal ability (Duarte et al. 2014; Francisco et al. 2014; Roubik 1992; Tavares et al. 2013), so further studies are urgently needed to assess how they respond to different patterns of land use.

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