

Beekeeping practices and geographic distance, not land use, drive gene flow across tropical bees

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Abstract

Across the globe, wild bees are threatened by ongoing natural habitat loss, risking the maintenance of plant biodiversity and agricultural production. Despite the ecological and economic importance of wild bees and the fact that several species are now managed for pollination services worldwide, little is known about how land use and beekeeping practices jointly influence gene flow. Using stingless bees as a model system, containing wild and managed species that are presumed to be particularly susceptible to habitat degradation, here we examine the main drivers of tropical bee gene flow. We employ a novel landscape genetic approach to analyse data from 135 populations of 17 stingless bee species distributed across diverse tropical biomes within the Americas. Our work has important methodological implications, as we illustrate how a maximum-likelihood approach can be applied in a meta-analysis framework to account for multiple factors, and weight estimates by sample size. In contrast to previously held beliefs, gene flow was not related to body size or deforestation, and isolation by geographic distance (IBD) was significantly affected by management, with managed species exhibiting a weaker IBD than wild ones. Our study thus reveals the critical importance of beekeeping practices in shaping the patterns of genetic differentiation across bee species. Additionally, our results show that many stingless bee species maintain high gene flow across heterogeneous landscapes. We suggest that future efforts to preserve wild tropical bees should focus on regulating beekeeping practices to maintain natural gene flow and enhancing pollinator-friendly habitats, prioritizing species showing a limited dispersal ability.

Keywords: beekeeping, dispersal, land use, landscape genetics, pollination, stingless bees

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Introduction

Wild bees are essential for the reproduction of nearly 90% of plant species (Ollerton *et al.* 2011) and are also

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critical providers of pollination services for more than 60% of agricultural crops species, contributing about one-third of the global food production (Gallai *et al.* 2009). In the past decade, the role of wild bees as crop pollinators has gained substantial attention because they can compensate for the reported declines in honeybee populations (Brown & Paxton 2009; Jaffé *et al.* 2010) by assuring enough pollinators (Aizen & Harder 2009) and by pollinating some crops more effectively than managed honeybees (Garibaldi *et al.* 2013). However, wild bees have proven susceptible to the degradation of natural habitats, as their abundance and diversity are negatively affected by habitat loss and landscape homogenization (Kennedy *et al.* 2013; Brown & Oliveira 2014). The human-mediated modification of natural habitats can also affect the long-term viability of wild bee populations, fragmenting them, reducing gene flow and increasing Allee effects (Allendorf *et al.* 2012). Indeed, a handful of recent studies conducted in temperate regions have shown that urbanization (Davis *et al.* 2010; Jha & Kremen 2013) and agricultural land use (Jha 2015) can restrict gene flow in individual bee species.

As tropical pollinators seem more susceptible to habitat loss than those of temperate regions (Ricketts *et al.* 2008), there is a pressing need for studies assessing the impact of land use on wild bee gene flow in tropical ecosystems. An estimated 2101 km² of tropical forest is 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). However, the influence of land use on the population dynamics of wild tropical bees remains largely unknown (Viana *et al.* 2012), and only one previous study quantified land use impacts on gene flow for a tropical species (Jaffé *et al.* 2015a). Past tropical studies have largely investigated genetic diversity and isolation by distance in wild native bees (Zayed 2009; Zimmermann *et al.* 2011; Freiria *et al.* 2012; Suni *et al.* 2014) and thus do not provide insights into the impacts of topography, land use or environment factors possibly influencing wild bee gene flow.

Widely distributed across tropical regions, stingless bees (Apidae: Meliponini) are key pollinators of both native flora (Vit *et al.* 2013) and commercial crops (Slaa *et al.* 2006; Giannini *et al.* 2015) and therefore of great biological and economic importance. Like honeybees, stingless bees are eusocial bees and many species are managed for honey production and enhanced crop pollination (Vit *et al.* 2013). However, the commercial use of stingless bees across the developing world remains essentially informal as technical knowledge is still scarce, and management practices lack the standardization found in apiculture (Jaffé *et al.*

2015b). The transportation of colonies across large geographic areas (above 1000 km), a frequent practice among stingless beekeepers (Byatt *et al.* 2016), could strongly influence patterns of gene flow in stingless bees. For instance, migratory beekeeping has led to substantial admixture between different European honeybee subspecies and in some cases resulted in the complete replacement of a native by an introduced subspecies (De la Rúa *et al.* 2009). While one study found a low genetic differentiation between managed populations of the stingless bee *Melipona scutellaris*, presumably due to the exchange of colonies between beekeepers (Carvalho-Zilse *et al.* 2009), a comparison between managed and wild colonies of *Tetragonisca angustula* revealed similar nuclear genetic diversity, albeit lower mitochondrial genetic diversity in managed colonies (Santiago *et al.* 2016). Yet, the general effect of management on patterns of stingless bee genetic differentiation has never been assessed across species and biomes.

Dispersal is believed to be restricted in this group of eusocial bees with perennial colonies. Daughter colonies rely on resources from their maternal ones during their initial establishment, and hence, they do not establish far from each other (van Veen & Sommeijer 2000; Roubik 2006; Vit *et al.* 2013). As gene flow is mediated by queen and male dispersal, genetic differentiation in wild stingless bee populations is expected to increase rapidly with geographic distance (i.e. isolation by geographic distance, or IBD). In addition, given that foraging range is related to body size across bees (Araújo *et al.* 2004; Greenleaf *et al.* 2007), smaller species are expected to show a more restricted dispersal than larger ones (assuming the foraging range of workers is a good proxy of queen and male dispersal ability) and thus higher genetic differentiation.

As a restricted dispersal implies a diminished ability to relocate to high-quality habitats, stingless bees might be extremely susceptible to the human-mediated modification of natural habitats. Habitat degradation could also hinder stingless bee gene flow (Brosi *et al.* 2008; Jha 2015), and thus drive the depletion of genetic diversity through the action of genetic drift, and increase the risk of population extinctions (Zayed 2009; Allendorf *et al.* 2012). Additionally, mountain ranges (Lozier *et al.* 2011), temperature (Coroian *et al.* 2014) and precipitation gradients (El-Niweiri & Moritz 2011) have also been shown to influence patterns of genetic differentiation in bee populations. Finally, highways are known to facilitate mosquito dispersal across broad spatial scales (Medley *et al.* 2015), so they could influence bee gene flow as well. However, no landscape genetic study has yet assessed how genetic differentiation is jointly influenced by deforestation, elevation, climatic factors and

transport routes (i.e. isolation by landscape resistance, or IBR), across different species.

Using stingless bees as a model system, containing wild and managed species that are presumed to be particularly susceptible to habitat degradation, here we aim to help fill the important knowledge gap regarding the main drivers of tropical bee gene flow. We employ a comparative landscape genetic approach to analyse data from 135 populations of 17 stingless bee species for which microsatellite-based genetic distance estimates were available. We first test for isolation by distance across species and assess whether stingless bee gene flow is determined by body size, a long-assumed yet never explicitly tested hypothesis for bee pollinators (Greenleaf *et al.* 2007). Second, we evaluate whether managed species show weaker isolation by distance than wild ones, as expected if beekeepers frequently exchange or transport colonies. Finally, we quantify the environmental drivers by assessing how stingless bee genetic differentiation is affected by deforestation, elevation, climate and transport routes.

Material and methods

Data set

To compile our data set, we performed an extensive literature search looking for works reporting pairwise genetic and geographic distances in stingless bees. Different combinations of search criteria like 'stingless bees', 'genetic distance' and 'microsatellites' were used in the platforms Web of Science, Google Scholar and Scielo. In addition, we searched for graduate theses on the databases of selected Brazilian Universities, including Universidade de São Paulo, Universidade Federal do Piauí and Universidade Federal do Amazonas. We only included works using microsatellite markers, as genetic distances estimated from microsatellites reflect recent geneflow processes (Allendorf *et al.* 2012; Balkenhol *et al.* 2016) and are thus more relevant to assess any impact of recent land use changes. In all cases, authors genotyped worker offspring and calculated genetic distances by pooling the genotypes of colonies from the same sampling location (or population). We chose Weir and Cockerham's Fixation Index (F_{ST}) as a measure of genetic distance, because more than half of the analysed studies used this estimate. In cases when the papers did not report pairwise F_{ST} , or when they provided other genetic distance estimates, we either used F_{STAT} (Goudet 2001) to compute pairwise F_{ST} from the raw data or asked the authors to provide pairwise F_{ST} values when the raw data were unavailable. Geographic coordinates of all studied populations were either retrieved from the papers or calculated from the

centroids of the municipalities or cities in cases when they were unavailable. Data were retrieved from a total of 15 articles, containing information for 135 populations of 17 stingless bee species (Table 1, and Figs 1 and S1, Supporting information). While samples were collected across diverse tropical biomes within the Americas, including tropical rain forests (Brazil and Mexico), dry forests (Brazil and Mexico) and semi-arid forests (Brazil), the distance separating study populations ranged between 1 and 2600 km.

We determined whether the studies employed samples from managed or wild colonies, using the information provided in the manuscripts and asking the authors when this information was not reported. When the majority of samples analysed in a given study came from managed colonies of unknown origin, the species was classified as managed. Species represented by samples collected from wild colonies, or from beekeepers rearing exclusively local bees, were classified as wild. All species were thus classified as either managed or wild. We also retrieved the number of colonies analysed, the number of loci used for genotyping, the mean expected heterozygosity and the mean number of alleles per locus.

As foraging range is related to body size across bees (Greenleaf *et al.* 2007), including stingless bees (Araújo *et al.* 2004), intertegular distance was used as a proxy of dispersal range (see Appendix S1, Supporting information). In addition, we quantified occurrence area and the maximum distance separating each study population. To estimate occurrence area, we retrieved the coordinates of all reported occurrences for each species from *speciesLink* (<http://splink.cria.org.br/>), CONABIO (<http://www.conabio.gob.mx/>) and GBIF (<http://www.gbif.org/>). We then calculated the area of a convex hull polygon (containing the outermost coordinates) for each species. We also gathered all available life-history characteristics, but found that data were frequently unavailable or showed little variation across the studied species (see Appendix S4, Supporting information).

Spatial analyses

We considered two geneflow patterns: Isolation by geographic distance (IBD) and isolation by landscape resistance (IBR), which took into consideration deforestation, elevation, climatic features and transport routes. To do so, we obtained high-resolution maps for each one of the 17 species, including forest cover (University of Maryland: <http://earthenginepartners.appspot.com/science-2013-global-forest/download.html>), elevation, mean annual temperature and mean annual precipitation (WorldClim: <http://www.worldclim.org/>). All maps were continuous rasters, containing a given value

Table 1 Data set summary. The number of populations studied (NP) is shown along the number of colonies analysed (NC), whether they were represented by managed or wild colonies (Type), the source of F_{ST} estimates (F_{ST}^*), the number of loci employed for genotyping (NL), the mean expected heterozygosity (H_e), the mean number of alleles per locus (NA), the maximum distance separating study populations (MD), intertegular distance (ITD) and occurrence area (OA). References containing the original data are also provided

Species	NP	NC	Type	F_{ST}^*	NL	H_e	NA	MD (km ²)	ITD (cm)	OA (km ²)	Reference
<i>Frieseomelitta varia</i>	5	69	Managed [†]	Article	5	0.75	16.40	1851.33	1.41	3.48×10^6	Gonçalves (2010)
<i>Melipona compressipes</i>	11	13	Managed	Raw data	5	0.33	4.00	1187.10	3.13	2.80×10^6	Pinto (2007)
<i>Melipona marginata</i>	5	54	Managed	Article	4	0.56	8.00	1266.10	2.16	4.00×10^6	Moresco (2009)
<i>Melipona quadrifasciata</i>	15	127	Wild	Author	9	0.35	7.20	2620.79	2.91	1.34×10^6	Tavares <i>et al.</i> (2013)
<i>Melipona scutellaris</i>	4	149	Managed	Raw data	7	0.48	5.29	704.00	2.83	1.04×10^6	Carvalho-Zilse & Kerr (2006)
<i>Melipona seminigra</i>	10	18	Managed	Raw data	4	0.35	5.00	789.20	2.67	2.50×10^6	Pinto (2007)
<i>Melipona subnitida</i>	5	231	Managed	Article	9	0.49	8.44	473.08	2.42	3.49×10^5	Da Silva & Diniz (2012)
<i>Melipona yucatanica</i>	4	11	Wild	Raw data	7	0.42	3.10	667.00	2.61	2.95×10^5	de Jesús May-Itzá <i>et al.</i> (2010)
<i>Nannotrigona testaceicornis</i>	6	32	Wild	Article	8	0.41	4.75	653.90	1.28	2.14×10^6	Fonseca (2010)
<i>Partamona helleri</i>	5	47	Wild	Article	9	0.16	1.42	1667.60	1.67	3.67×10^6	Brito (2005)
<i>Partamona mulata</i>	5	58	Wild	Article	9	0.2	1.88	580.50	1.67	2.71×10^5	Brito (2005)
<i>Plebeia remota</i>	4	65	Managed [‡]	Raw data	15	0.66	6.65	704.09	1.46	3.67×10^5	Francisco <i>et al.</i> (2013)
<i>Scaptotrigona bipunctata</i>	8	20	Wild	Article	13	0.46	4.54	1118.20	1.86	1.53×10^6	Alvarenga (2012)
<i>Scaptotrigona hellwegeri</i>	3	41	Wild	Article	7	0.75	5.73	510.00	1.75	3.53×10^5	Quezada-Euán <i>et al.</i> (2012)
<i>Scaptotrigona xanthotricha</i>	25	42	Wild	Article	12	0.81	28.16	1605.00	1.75	4.88×10^5	Duarte <i>et al.</i> (2014)
<i>Tetragonisca angustula</i>	17	722	Wild	Author	11	0.78	18.60	1204.45	1.07	7.49×10^6	Francisco (2012)
<i>Trigona spinipes</i>	3	43	Wild	Author	7	0.62	6.11	2270.00	1.69	2.35×10^7	Jaffé <i>et al.</i> (2014)

* F_{ST} values were either provided in the article (Article), calculated from raw data (Raw data), or provided by the author (Author).

[†]Wild nests were collected inside the University of São Paulo's campus in Riberão Preto, which hosts two large stingless bee apiaries (meliponaries). These meliponaries contain many managed colonies of *F. varia*; some brought from remote locations. We thus considered this a managed population, as 'wild' colonies inside the campus are likely to be swarms from managed colonies or at least hybrids.

[‡]Although many samples of this species came from wild colonies, a large number of samples were collected from managed colonies, including some from beekeepers that confirmed the transportation of colonies from remote locations. We thus considered this a managed population, affected by human-mediated dispersal.

for each pixel. We cropped all rasters to the extent of the study regions, which comprised a buffer area of 10 km around our sampling locations to minimize border effects. To test for the effect of transport routes on stingless bee gene flow, we obtained shapefiles of the Brazilian-wide road network and the Brazilian Amazon basin river network, because waterways constitute the main transportation mean in the Amazon (IBGE: ftp://geofp.ibge.gov.br/cartas_e_mapas/bases_cartograficas_continuas/bcim/). We then combined the roads and rivers shapefiles, joined neighbouring rivers and roads (located within 0.5 km) and converted the resulting line shapefile into a network, where every turn was considered a node. We extracted the geographic coordinates of each node and calculated the geographic distance separating each pair of neighbouring nodes (consisting of rivers or roads). To incorporate

our study populations into this network, we introduced one additional node for each study population and created a link connecting it to the closest network node (usually within 3 km).

We tested whether deforestation, elevation, temperature, precipitation and transport routes influence stingless bee gene flow. To do so, we created resistance surfaces for each one of these variables except rivers and roads. As we assumed higher gene flow across forested areas than between agricultural or other non-forested landscapes (Jha 2015), we inverted the forest cover rasters, using the absolute values after subtracting 100 from every pixel (Jaffé *et al.* 2015a). We thus obtained resistance surfaces where forested pixels had lower resistance values. Given that mountain ranges also constitute a potential barrier to bee gene flow, we created resistance surfaces where pixels with higher

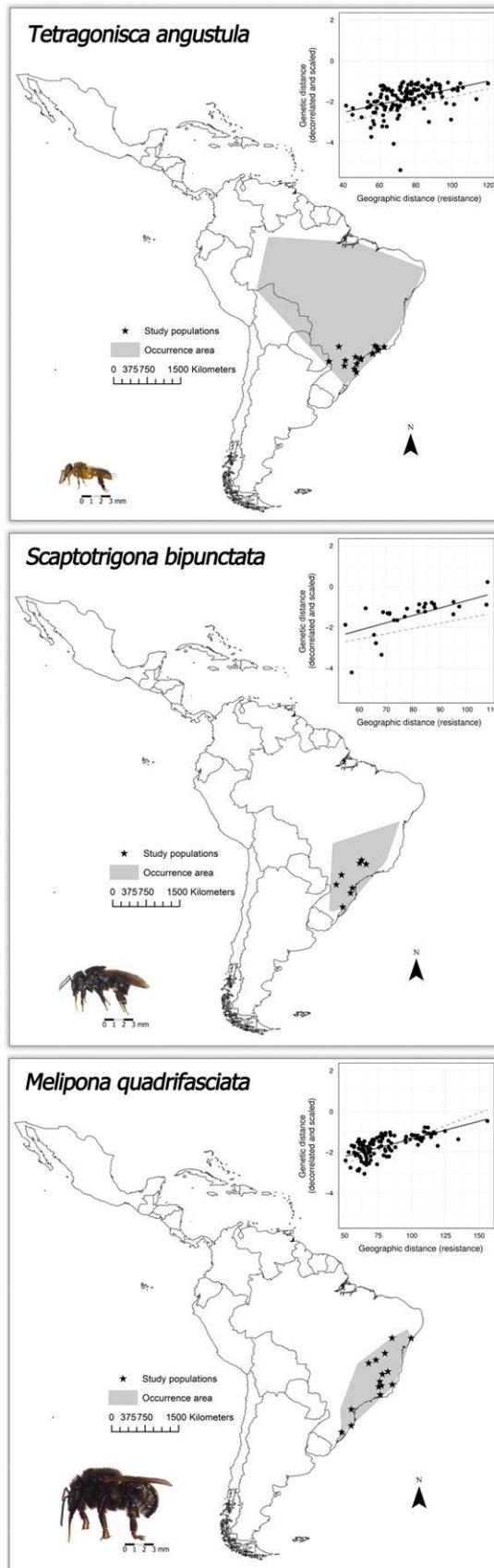


Fig. 1 Isolation by geographic distance (IBD) in three stingless bee species with different body size. For each species, a scaled picture of a specimen is followed by a map showing its natural distribution range and study populations. IBD plots on upper-right corners show the species-specific IBD slopes (solid lines) in relation to the general IBD slope across all species (dashed lines). See Fig. S1 (Supporting information) for the maps and plots of all 17 species and Fig. S2 (Supporting information) for the IBD slopes of all species sorted by body size.

elevations had higher resistance values. To do so, we used the elevation rasters, adding the minimum elevation of each raster to all pixels to make all elevation values positive. We assumed resistance to gene flow increased with increasing temperature (Jaffé *et al.* 2010) and precipitation (El-Niweiri & Moritz 2011), because warmer temperatures and higher precipitation are more likely to represent a barrier to gene flow in the tropical and subtropical regions where our study species occur. We thus employed the raw, untransformed temperature and precipitation rasters to construct resistance surfaces. Finally, to test for isolation by geographic distance, we created null-model rasters where all pixels had the same value (Jaffé *et al.* 2015a; Jha 2015). All spatial analyses were done using the R package RASTER (Hijmans 2014) and GRASS GIS.

We used circuit theory (McRae *et al.* 2008) to estimate resistance to gene flow between populations for each explanatory variable separately (deforestation, elevation, temperature, precipitation, rivers and roads, and geographic distance). We used the program CIRCUITSCAPE v4.0 to estimate pairwise 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, we decreased the resolution of resistance surfaces by aggregating blocks of pixels (Jaffé *et al.* 2015a; Jha 2015). As the analysis of 85 rasters (five per species, for 17 species) involved considerable computing demands, we used the Amazon Elastic Compute Cloud (EC2) and the Texas Advanced Computing Center (TACC) from The University of Texas to run spatial and CIRCUITSCAPE analyses. To calculate resistance distance of rivers and roads, we used a graph where the edge conductance was the inverse of the geographic distance between nodes.

Landscape genetic analyses

Our analyses had two aims: to assess how isolation by distance varies across species, and how it is influenced by bee body size, management and other confounding factors; and to quantify how genetic differentiation is affected by deforestation, elevation, climatic factors, and rivers and roads. Because resistance distance always

contains some element of geographic distance, there is an intrinsic collinearity between geographic and resistance distance, so we could not include both in a single model. We thus separated our analyses in two parts: (i) we first modelled IBD across species, including species-specific covariates such as bee body size, management and other sources of variation like the number of loci employed for genotyping and their level of polymorphism; (ii) we then performed a regression of the residuals from the first analysis (genetic distance detrended for geographic distance) onto the residuals of a linear regression between resistance distance and geographic distance (resistance distance detrended for geographic distance). This second procedure was designed to detect an influence of resistance distance on genetic differentiation beyond that of geographic distance and is analogous to a partial Mantel test.

Modelling isolation by geographic distance across species. We modelled genetic distance (F_{ST}) as a function of geographic distance and species-specific variables (with interactions between species-specific variables and geographic distance). The logit-transformation was used to linearize F_{ST} , given that it has advantages in power and interpretability over the arcsin-transformation (Warton & Hui 2011). Specifically, we fit a full model expressing the logit-transformed F_{ST} as a function of geographic distance, number of colonies analysed, number of loci used for genotyping, mean expected heterozygosity, mean number of alleles per locus, type of colonies sampled (managed or wild), intertegular distance, maximum distance separating study populations, occurrence area and the interactions between the last four predictors and geographic distance (fixed effects). Our model thus accounted for methodological variations among studies (due to differences in the number of colonies analysed, the number of loci employed and their level of polymorphism), as well as possible species-specific effects. For instance, we allowed the intercepts and slopes for geographic distance to vary across species (random effects), to account for interspecific variations in patterns of IBD. This model decomposes the isolation-by-distance slope into three components: an 'overall' slope which is shared across species; a modification to the slope which is a linear function of intertegular distance, type of colonies sampled, maximum species range and occurrence area; and a species-specific deviation (a random slope) which captures the variation among species that is not explained by the measured covariates (see Appendix S2, Supporting information, for a detailed description of the model structure). We fit mixed-effects regression models using penalized least squares and used a correlation structure designed to account for nonindependence of pairwise

distances (maximum-likelihood population effects, or MLPE). The MLPE correlation structure treats the residual for each pairwise distance as the sum of two random population-level effects and an observation-level error (Clarke *et al.* 2002). Code implementing the MLPE correlation structure within the R package NLME (Pinheiro *et al.* 2014) is provided at <https://github.com/nspope/corMLPE>.

After fitting the full model, we fit models with all possible combinations of the fixed effects, always including the main effect of geographic distance. We did not attempt to find the best model but rather averaged the fixed effects and predicted values from the resulting set of possible models using Akaike weights, with shrinkage (Burnham & Anderson 2002). This procedure has several advantages over simple model selection: model-averaging ameliorates overfitting by shrinking the effect sizes of unimportant variables towards zero, is robust to model selection error (Johnson & Omland 2004) and avoids an arbitrary choice between multiple models which are essentially equally well supported by the data. Model generation and calculation of variable importance and Akaike weights were done using the MUMIN package (Kamil 2014).

As described above, the 'overall' isolation-by-distance slope for each species is the (model-averaged) sum of fixed effects and the best linear unbiased predictor of a random slope. As such, there is no closed-form approximation for the standard error of the IBD slopes for each species. To calculate standard errors (and associated parametric 95% confidence intervals) for each slope, we used parametric bootstrapping (Efron & Tibshirani 1986). To generate a single bootstrap sample, we simulated a new data set using the model-averaged predicted values and their associated model-averaged covariance matrix (Burnham & Anderson 2004), where the predictions from each model are calculated conditional on the estimated random effects. We then used the model-fitting and model-averaging procedure described above on this synthetic data set and calculated the isolation-by-distance slopes for each species from the synthetic model-averaged predictions. Standard errors were calculated as the standard deviation of bootstrap replicates for each species-specific slope. To estimate standard errors for the fixed effects, we used a similar procedure but model-averaged the marginal predicted values and associated covariance matrix prior to generating synthetic data sets. In this case, each simulated data set essentially incorporates new values for the random slopes and intercepts, and the distribution of bootstrap replicates effectively integrates over uncertainty in the point estimates of the random slopes and intercepts. The breadth of the confidence intervals for a given species (e.g. the precision of the IBD slope

estimate) was influenced by the number of populations (i.e. the number of observations for each species). Thus, species with few populations showed IBD slopes with broader confidence intervals, and point estimates that are shrunk towards the overall (fixed) IBD slope.

Modelling isolation by resistance across species. To estimate an effect of isolation by resistance that is additional to that of isolation by distance, we use a procedure analogous to a partial Mantel test. There exists an intrinsic correlation between resistance and geographic distance, because resistance distance always contains some element of geographic distance, to a greater or lesser degree depending on the spatial heterogeneity of the resistance surface. Therefore, resistance distance could be viewed as a landscape resistance to movement 'contaminated' with geographic distance. To try to isolate the effect of landscape resistance on genetic differentiation, we first detrended genetic distance for geographic distance by calculating the residuals from the averaged model described above. We then detrended resistance distance for geographic distance by regressing the former on the latter and calculating the residuals. These two sets of residuals represent the following: (i) a measure of genetic differentiation that is not explained by geographic distance and (ii) a measure of resistance distance which is additional to geographic distance. To relate these two quantities, we regressed detrended genetic distance on detrended resistance distance for each landscape variable (altitude, deforestation, precipitation, temperature, and rivers and roads). We included random slopes in these regression models, to capture species-specific variation in genetic differentiation with regard to landscape resistance, and the MLPE correlation structure to account for correlation among the detrended pairwise measures.

This approach of detrending resistance distance assumes that the 'contamination' with geographic distance has a simple linear form and is quite similar to the procedure used in partial Mantel tests. In a partial Mantel test, the two variables of interest (i.e. resistance distance and genetic distance) are independently regressed on geographic distance, and the two sets of residuals are then correlated (with a permutation test to assess significance). We used the same procedure with a more complex model than a simple regression of genetic on geographic distance, and we used the MLPE correlation structure rather than permutation to address dependencies among pairwise distances. Note that because we regress residuals from both the covariate and the response, the estimated regression coefficients are unbiased (Arbia & Baltagi 2008). In contrast, the related but erroneous procedure of detrending the response variable and regressing it on the raw covariate

will result in substantial bias (Freckleton 2002). To assess the power, error rates and estimation bias of our approach, we ran simulations under varying levels of collinearity between resistance and geographic distance (see Appendix S3, Supporting information).

Results

As expected, genetic differentiation was explained by geographic distance across the 17 analysed stingless bee species (Fig. 1, Table 2). However, the magnitude of the species-specific IBD effect greatly varied across species (parametric bootstrap likelihood ratio test – LRT – of models with and without a random slope: LR = 41.22, $P < 0.0001$). Mean IBD slope overall species was 1.30 (95% CI: 0.95–1.66), while species-specific IBD slope coefficients ranged between –0.23 and 3.36, and in 9 out of the 17 analysed species, confidence intervals did not overlap zero (Table S1, Supporting information, Fig. 2A). Interestingly, bee body size (intertegular distance), a proxy of foraging range, did not explain IBD patterns (Fig. S2, Supporting information). Instead, management did have an important contribution to explaining the strength of IBD (Table 2). Specifically, wild species showed stronger IBD than managed ones (Fig. 2B).

Overall, we did not find evidence for isolation by resistance (LRT of all IBR models against a null model containing no predictor: Altitude LR = 0.50, $P > 0.1$; deforestation LR = 0.23, $P > 0.1$; precipitation LR = 2.20, $P > 0.1$; temperature LR = 1.14, $P > 0.1$; and rivers and roads LR = 1.12, $P > 0.1$). Species-specific IBR effects were generally weak and did not vary between species, except in the case of temperature where we detected interspecific variation in IBR (LRT of models with and without a random slope: Altitude LR = 2.22, $P > 0.1$; deforestation LR = 0.02, $P > 0.1$; precipitation LR = 0.61, $P > 0.1$; temperature LR = 7.97, $P = 0.02$; and rivers and roads LR = 1.79, $P > 0.1$; Figs 3 and S3, Supporting information). Only in one species (*Partamona helleri*), we detected significant IBR, specifically isolation by altitude and by roads (Table S2, Supporting information).

Discussion

Our work provides the first comparative overview of the patterns of gene flow of different managed and wild stingless bee species occurring across diverse tropical biomes. Our results show that (i) stingless bee gene flow is limited by geographic distance, although the strength of IBD varies across species; (ii) bee body size is not related to IBD patterns; (iii) managed species show weaker IBD than wild ones; and (iv) deforestation, elevation, precipitation, temperature, and rivers

Table 2 Summary statistics for the full model of isolation by geographic distance. Variable importance and the number of models containing it, model-averaged estimates for fixed effects, standard errors, 95% and 90% confidence intervals (CIs) are shown

Parameter	Importance (N. models)	Model-averaged estimate	Bootstrap SE	Lower 95% CI	Upper 95% CI	Lower 90% CI	Upper 90% CI
Intercept	–	–1.74*	0.12	–1.98	–1.5	–1.94	–1.54
Geographic distance resistance	1.00 (1296)	1.30*	0.18	0.95	1.66	1	1.6
Type of colonies (managed)	0.86 (864)	0.24	0.22	–0.18	0.67	–0.11	0.6
Interaction between type of colonies (managed) and geographic distance resistance	0.81 (432)	–1.19*	0.51	–2.19	–0.19	–2.03	–0.35
Intertegular distance	0.70 (864)	0.26	0.15	–0.04	0.56	0.01	0.52
Maximal range	0.49 (864)	–0.03	0.1	–0.23	0.17	–0.19	0.14
Occurrence area	0.40 (864)	0.03	0.07	–0.1	0.17	–0.08	0.15
Interaction between intertegular distance and Geographic distance resistance	0.39 (432)	0.19	0.2	–0.2	0.59	–0.14	0.52
Number of loci	0.30 (648)	0.04	0.06	–0.09	0.16	–0.07	0.14
Interaction between maximal range and geographic distance resistance	0.29 (432)	–0.13	0.19	–0.51	0.25	–0.45	0.19
Number of alleles	0.29 (648)	–0.02	0.05	–0.11	0.07	–0.09	0.05
Expected heterozygosity	0.27 (648)	–0.01	0.06	–0.12	0.11	–0.11	0.09
Number of colonies	0.27 (648)	0.02	0.08	–0.13	0.18	–0.11	0.15
Interaction between occurrence area and geographic distance resistance	0.15 (432)	–0.02	0.04	–0.09	0.05	–0.08	0.04

Effects where 95% CIs did not overlap zero are highlighted by stars (*).

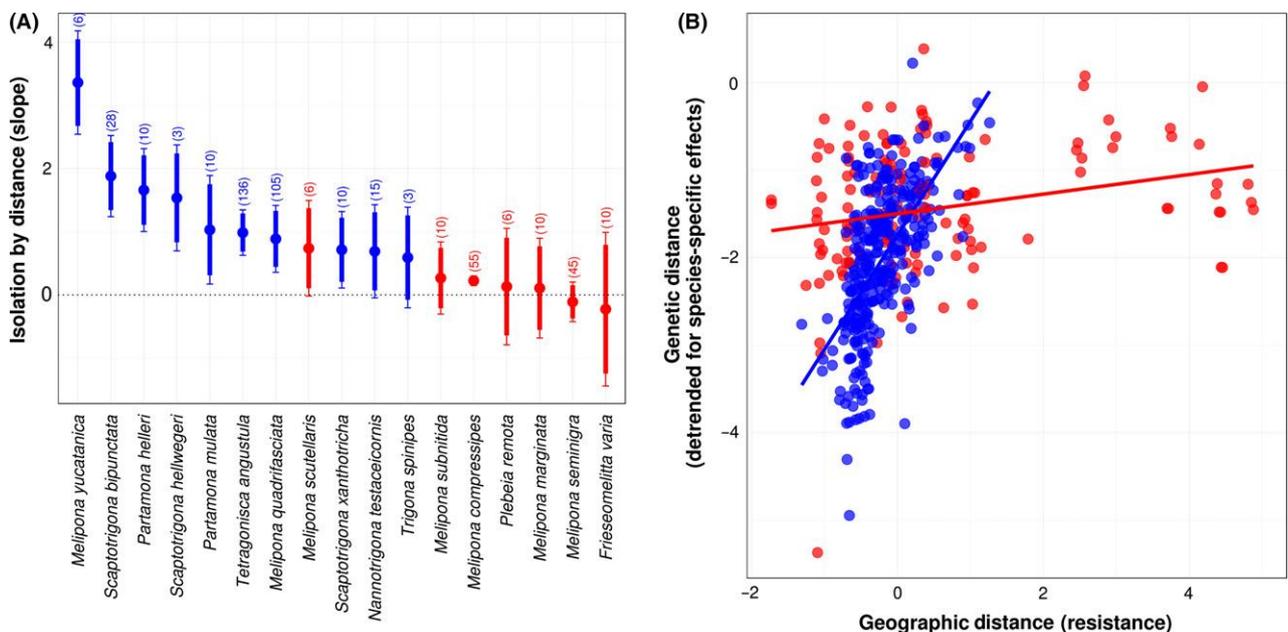


Fig. 2 (A) Isolation by distance (IBD) estimates across wild (blue) and managed (red) stingless bee species. Model-averaged isolation-by-distance slope coefficients (solid dots) are shown in addition to bootstrap 90% (thicker lines) and 95% (thinner lines) confidence intervals. Slopes are ordered by strength and sample sizes (number of pairwise distances) are shown inside brackets. (B) Effect of management on isolation by distance. The figure shows IBD across wild (blue) and managed (red) species. Genetic distance was detrended, meaning that species-specific effects have been subtracted out. See Table S2 (Supporting information) for full summary statistics.

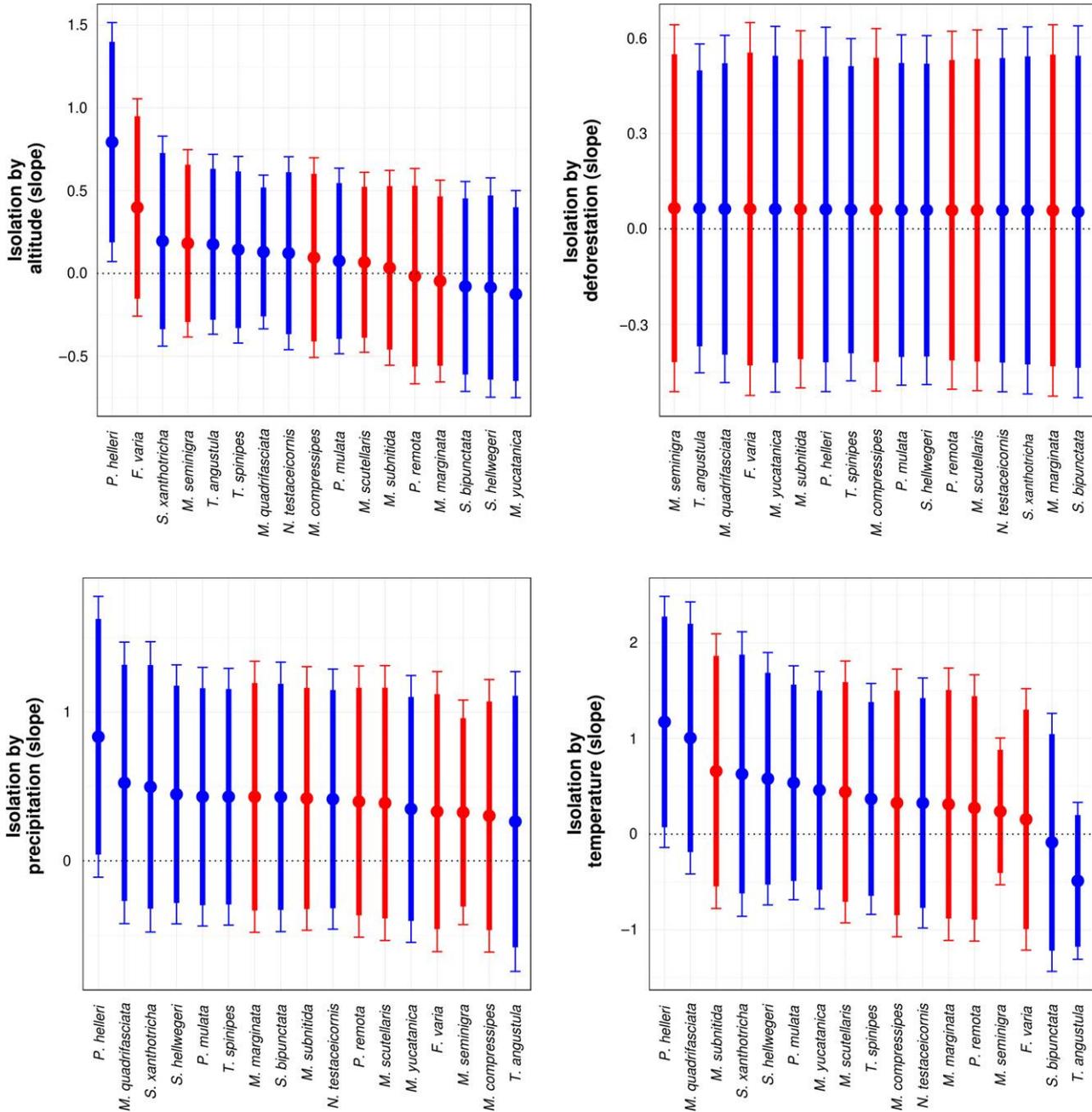


Fig. 3 Isolation by resistance (IBR) estimates. Model-averaged IBR slope coefficients (solid dots) are shown in addition to bootstrap 90% (thicker lines) and 95% (thinner lines) confidence intervals. Slopes are ordered by strength and grouped by wild (blue) and managed (red) species. See Table S2 (Supporting information) for full summary statistics.

and roads do not appear to influence gene flow across stingless bee species. Given the importance of stingless bees as pollinators of wild and cultivated plants across the tropics, and their assumed sensitivity to habitat loss, our results reveal important and unexpected insights into the processes driving spatial genetic structure in tropical bees. In contrast to previously held beliefs, gene flow was not related to body size or deforestation.

Rather, IBD was distinct between managed and wild species, revealing the critical importance of beekeeping practices in shaping the patterns of stingless bee genetic differentiation.

Geographic distance was a key variable explaining genetic differentiation across stingless bees. This result was expected, as dispersal is known to be restricted in this group of bees (van Veen & Sommeijer 2000; Roubik

2006; Vit *et al.* 2013). However, we also found significant interspecific variation in IBD, which suggests that dispersal ability greatly varies among species, probably due to life-history differences (Roubik 2006; Vit *et al.* 2013). Given that foraging range is related to body size across bees (Araújo *et al.* 2004; Greenleaf *et al.* 2007), we hypothesized that smaller bees would show a more restricted dispersal than larger ones and thus a steeper IBD slope. Our results demonstrate that this is not the case, indicating that the common proxy for foraging range (body size) is not a good indicator of gene flow across bee species. This is likely due to the fact that foraging ranges reflect the flight capability of workers, not the dispersal ability of queens and males. While there is variation in the ratio queen/worker size across the group (Tóth *et al.* 2004), long-distance dispersal of reproductive individuals (Jaffé *et al.* 2010), a high rate of colony reproduction (Jaffé *et al.* 2009) or the incidence of intraspecific queen parasitism (Wenseleers *et al.* 2011) could substantially increase gene flow regardless of worker foraging range.

As an accentuated IBD slope suggests restricted dispersal, we posit that species with a steep IBD may not easily colonize new habitats, as suggested for other bees (Carvell *et al.* 2012; Vanbergen 2014). Such species are expected to be more susceptible to habitat degradation than species showing a low IBD, indicative of long-distance dispersal. Our results reveal that wild populations of *Melipona yucatanica* may be extremely susceptible to habitat degradation, as this species shows the most accentuated IBD (Fig. 2A). This is a rare species found only in preserved Mesoamerican forests (Fig. S1, Supporting information). Previous work suggests this is indeed an endangered species, given its restricted distribution and apparent reproductive isolation between Mexico and Guatemala (de Jesús May-Itzá *et al.* 2010).

Our results show that gene flow in stingless bees is more affected by geographic distance than by deforestation, elevation, precipitation, temperature, or rivers and roads. This result is surprising given the assumed dependence of stingless bees on pristine forest patches for food and nesting (Brosi *et al.* 2008; Kennedy *et al.* 2013) and the high forest loss across all the studied biomes (Hansen *et al.* 2013), especially in the Atlantic Forest (Ribeiro *et al.* 2009). Thus, even if natural habitats are needed to fulfil foraging and nesting requirements, reproductive individuals seem to maintain gene flow across heterogeneous human-altered landscapes. The only other landscape genetic study analysing gene flow in a wild tropical bee, the stingless bee *Trigona spinipes*, also found that this species is capable of dispersing across remarkably long distances (200 km), and through degraded habitats and altitudinal gradients (Jaffé *et al.* 2015a). Our findings suggest that such an ability to

disperse across human-altered landscapes is not exclusive to the widely distributed generalist species (like *T. spinipes*), but is perhaps a common pattern in stingless bees. This effect does not seem to be caused by a temporal mismatch between sampling date and map date, because we employed the most recent available maps, and recent land use changes are expected to be minimal (Hansen *et al.* 2013). Moreover, we should be able to detect an effect of the forest cover present a decade ago, as a previous landscape genetic work on bumblebees found that bees respond to land use even decades later (Jha & Kremen 2013). Among our studied species, the only possible exception to the general pattern of extensive gene flow across environmental gradients was *Partamona helleri*, which showed significant isolation by altitude and by roads (rivers outside the Amazon basin were not considered). This is a widely distributed species (Fig. S1, Supporting information) that builds semi-exposed clay nests in a variety of substrates, including roof eaves, wall crevices and abandoned bird nests (Brito & Arias 2010). Although previous studies have reported low genetic variability in this species (Borges *et al.* 2010; Brito & Arias 2010), little is known about its flight physiology or dispersal behaviour. Our results suggest that high elevations hinder *P. helleri* gene flow, but further studies are needed to determine why this species is more sensitive to altitude than other species building exposed nests (e.g. *Partamona mulata* and *T. spinipes*). The effect of roads, on the other hand, might be related to the altitude effect (fewer roads in mountains imply larger road resistances between populations).

The fact that managed species showed weaker IBD than wild ones suggests that management is facilitating stingless bee gene flow. While beekeeping practices are known to have a dramatic influence on the temporal and spatial patterns of honeybee and bumblebee genetic variation (De la Rúa *et al.* 2009, 2013; Kraus *et al.* 2011; Harpur *et al.* 2012), the impact of management on stingless bee genetic differentiation has been rarely examined (Carvalho-Zilse *et al.* 2009; Byatt *et al.* 2016; Santiago *et al.* 2016). Our results reveal the influence of management on stingless bee genetic differentiation across multiple species and biomes and suggest that the artificial transportation of colonies by beekeepers is enhancing gene flow. In contrast to Medley *et al.* (2015), who found that highways facilitate mosquito dispersal at broad spatial scales, our results reveal no effect of rivers and roads, suggesting colony transportation is not restricted to neighbouring regions connected by transport routes. For instance, stingless beekeepers often obtain their colonies from fellow beekeepers (Jaffé *et al.* 2015b) and sometimes transport them over thousands of kilometres (Alves *et al.* 2011). Our findings suggest

that these practices facilitated admixture between introduced colonies and local bee populations. Moreover, the introduction of colonies from different source populations appears to have risen overall genetic differentiation (see intercepts in Fig. 2B). A similar effect was found by Kolbe *et al.* (2008), who reported an increase in genetic diversity driven by admixture between native and invasive lizard populations, and no isolation by geographic distance among introduced populations. In our case, however, genetic homogenization and a decreased genetic differentiation are only likely to occur when these introductions cease, or when all source populations have been admixed (De la Rúa *et al.* 2009). Although comparing IBD in wild and managed populations of the same species would be needed to unequivocally disentangle the effect of management from ecological traits influencing gene flow, such ecological traits do not appear to be driving the pattern documented here. For instance, species with similar life histories (e.g. genus *Melipona* sp.) showed high and low IBD, depending on whether they were wild or managed (Fig. 2A). Nesting habits also seem unrelated to the IBD patterns documented here, because all but three species (*P. mulata*, *P. helleri* and *T. spinipes*) build their nests in cavities from trees or rocky outcrops (see Appendix S4, Supporting information).

Methodological and ecological implications

Our work has important methodological implications. We illustrate how mixed-effects models and MLPE can be applied in a meta-analysis framework to account for multiple factors, and weight estimates by sample size. As our models contained multiple predictors, our IBD slope coefficients are more robust than IBD slopes obtained with simple Mantel tests, which only relate genetic and geographic distance (Jenkins *et al.* 2010). Moreover, because the MLPE correlation structure allows modelling the nonindependence of pairwise distances within a likelihood framework (Clarke *et al.* 2002), compatible with model selection, it is particularly appealing for landscape genetic studies (Peterman *et al.* 2014; Jaffé *et al.* 2015a; Jha 2015). We believe that future landscape genetic studies could greatly benefit from adopting a similar approach.

Our findings also have important implications for the conservation of tropical bees. First, our work reveals that species differ in their ability to relocate to suitable habitats, so future efforts to preserve wild tropical bees should recognize such interspecific differences and prioritize species showing a limited dispersal ability. Second, our findings suggest that reproductive individuals are able to disperse across human-altered landscapes (regardless of body size). Future research is

nevertheless needed to assess how different types of land use influence colonization and nest-establishment success; and how they can be improved by enhancing pollinator-friendly habitats (Garibaldi *et al.* 2014). Finally, our results show that frequent colony transportation by beekeepers is promoting admixture and reducing spatial genetic structure. As indiscriminate transportation of colonies might threaten the conservation of stingless bees, because it could lead to the loss of valuable adaptations to local environmental conditions (Byatt *et al.* 2016), as well as to the introduction of novel pathogens (Graystock *et al.* 2015), colony transportation should be regulated. Promoting beekeeping with regional bees could also create incentives to protect local bee populations and reduce the need to transport colonies from elsewhere.

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R.J. designed the study; R.J., A.L.A., D.A.A. and A.G.C. collected data; F.O.F., M.C.A., M.G.T. and P.D.R. contributed data; R.J., N.P., S.J. and L.G.C. performed the analyses; R.J., N.P., A.L.A., T.C.G. and A.G.C. prepared the figures; R.J. wrote the first draft of the manuscript; and all authors contributed substantially to revisions.

Data accessibility

Original data: See Table 1 for a summary of the information retrieved from the original publications and the full references.

Species occurrence records: speciesLink (<http://splink.cria.org.br/>), CONABIO (<http://www.conabio.gob.mx/>), and GBIF (<http://www.gbif.org/>).

Forest cover data: University of Maryland (<http://earthenginepartners.appspot.com/science-2013-global-forest/download.html>).

Elevation and climate data: WorldClim (<http://www.worldclim.org/current>).

Brazilian-wide road and river network: IBGE (ftp://geofp.ibge.gov.br/cartas_e_mapas/bases_cartograficas_continuas/bcim/).

R implementation of the MLPE correlation structure: GitHub (<https://github.com/nspope/corMLPE>).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Summary statistics for the isolation by distance slopes per species.

Table S2 Summary statistics for the isolation by resistance slopes per species.

Fig. S1 Isolation by geographic distance (IBD) across 17 stingless bee species.

Fig. S2 Isolation by geographic distance (IBD) across 17 stingless bee species sorted by body size.

Fig. S3 Isolation by river and road resistance across 17 stingless bee species.

Appendix S1 Measurement of intertegular distance.

Appendix S2 Model of isolation by distance.

Appendix S3 Detecting isolation by resistance under collinearity.

Appendix S4 Data summary showing information on life history traits for the 17 analysed stingless bee species.

Data S1 Full dataset and R scripts.