Intersecting effects of landscape and body size on dispersal in bee populations

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Abstract

Quantifying genetic structure and levels of genetic variation are fundamentally important to predicting the ability of populations to persist in human-altered landscapes and adapt to future environmental changes. Genetic structure reflects the dispersal of individuals over generations, which can be mediated by species-level traits or environmental factors. Dispersal distances are commonly positively associated with body size and negatively associated with the amount of degraded habitat between sites, motivating investigation of these potential drivers of dispersal concomitantly. We quantified genetic structure and genetic variability within populations of ten bee species in the tribe Euglossini across fragmented landscapes. We genotyped bees at thousands of SNP loci and tested the following predictions: (1) larger species disperse farther; (2) species with greater resource specialization disperse farther; (3) deforested areas restrict dispersal; and (4) sites surrounded by more intact habitat have higher genetic diversity. Body size was a strong predictor of genetic structure, but, surprisingly, larger species showed higher genetic structure than smaller species. The way that deforestation affected dispersal varied with body size, such that larger species dispersed less far in areas with more forest. There was no effect of geographic distance on dispersal, and sites with more intact habitat had higher genetic diversity. These results challenge the dominant paradigm that individuals of larger species disperse farther, motivating further work into ecological drivers of dispersal for bees.

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Running title: Landscape, body size, and dispersal in bees

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Abstract:

Quantifying genetic structure and levels of genetic variation are fundamentally important to predicting the ability of populations to persist in human-altered landscapes and adapt to future environmental changes. Genetic structure reflects the dispersal of individuals over generations, which can be mediated by species-level traits or environmental factors. Dispersal distances are commonly positively associated with body size and negatively associated with the amount of degraded habitat between sites, motivating investigation of these potential drivers of dispersal concomitantly. We quantified genetic structure and genetic variability within populations of ten bee species in the tribe Euglossini across fragmented landscapes. We genotyped bees at thousands of SNP loci and tested the following predictions: (1) larger species disperse farther; (2) species with greater resource specialization disperse farther; (3) deforested areas restrict dispersal; and (4) sites surrounded by more intact habitat have higher genetic diversity. Body size was a strong predictor of genetic structure, but, surprisingly, larger species showed higher genetic structure than smaller species. The way that deforestation affected dispersal varied with body size, such that larger species dispersed less far in areas with more forest. There was no effect of geographic distance on dispersal, and sites with more intact habitat had higher genetic diversity. These results challenge the dominant paradigm that individuals of larger species disperse farther, motivating further work into ecological drivers of dispersal for bees.

Keywords: genetic structure, dispersal, body size, Euglossine, habitat, deforestation, bee

Introduction

As much as 75% of the global land surface has been modified by humans (Luyssaert et al., 2014). One of the most concerning forms of land modification is deforestation, which typically leads to fragmented landscapes that are characterized by small, isolated patches of forest surrounded by agriculture or human infrastructure. Deforestation is a leading cause of biodiversity loss worldwide, due to negative effects on abundance, species diversity, and genetic diversity (Schlaepfer et al., 2018).

Theory suggests that populations persisting in fragmented areas may experience genetic erosion before changes in abundance can be detected (Pflüger, Signer, & Balkenhol, 2019). Therefore, quantifying the genetic variability and genetic structure of populations living in fragmented areas is fundamental to understanding their ability to persist in human-altered landscapes and adapt to future environmental changes. Genetic structure reflects a non-random spatial distribution of genotypes, which occurs when gene flow is limited across space (Wright, 1943). Gene flow occurs via dispersal and maintains genetic diversity within populations (Franklin, Ian Robert, 1980). Spatially limited gene flow often results in a pattern whereby populations become more genetically distinct as the distance between them increases, a pattern termed "isolation by distance" (Wright, 1943). Landscape features such as water bodies or mountains can also impede gene flow, a pattern called "isolation by resistance" (McRae, 2006). Populations that are isolated and for which dispersal is limited may be at higher risk of extinction due to loss of alleles via genetic drift, which lowers evolutionary potential (Frankel, Otto Herzberg & Soulé, Michael E., 1981).

Dispersal distances may be mediated both by individual characteristics and environmental effects (Baguette et al., 2012). Dispersal scales linearly with body size across many clades, including birds and mammals (Dawideit, Phillimore, Laube, Leisler, & Böhning-Gaese, 2009; Ottaviani, Cairns, Oliverio, & Boitani, 2006), moths (Beck & Kitching, 2007), plants (Thomson et al., 2010), butterflies (Stevens et al., 2013), and bees (López-Uribe, Jha, & Soro, 2019). However, dispersal-body size associations often show

high variability within the groups assessed, and other species-level characteristics may also be important such as life history traits (McCoy, Richmond, Mushinsky, Britt, & Godley, 2010; Stevens et al., 2013), dispersal capacity (Hillman, Drewes, Hedrick, & Hancock, 2014), diet breadth (Stevens et al., 2014), and other resource requirements (Bowler & Benton, 2005).

Environmental drivers of dispersal include resource availability (Baguette, Michael et al., 2012) and the extent of landscape connectivity among sites (Baguette et al., 2013). Larger organisms tend to have higher resource requirements than smaller organisms, so resource availability may more strongly influence dispersal propensity of larger organisms than smaller ones (Byers, 2000). In terms of landscape connectivity, physical barriers to movement and poor matrix quality can both restrict dispersal (Manel & Holderegger, 2013). There has therefore been much interest into the extent to which anthropogenically-altered landscapes constrain dispersal. Restricted dispersal across anthropogenic habitat has been found for a range of species including small mammals (Ribeiro et al., 2021), birds (Björklund et al., 2010), bees (Jha & Kremen, 2013) and butterflies (Crawford, Desjardins, & Keyghobadi, 2011; Takami et al., 2004). This may be due to higher mortality for animals that travel farther in between habitat fragments (Bonelli et al., 2013; Lucas et al., 1994; Mennechez et al., 2003). Other studies reveal little evidence of restricted dispersal across anthropogenically-altered areas for organisms including bats (Richardson et al., 2021), plants (Culley, Sbita, & Wick, 2007), and other bee species (Suni, 2017). Urban areas may even act as a conduit for movement in some species (Ballare & Jha, 2021; Miles et al., 2019). Therefore, understanding how trait-mediated dispersal distances intersect with landscape effects on dispersal is critical given ongoing and projected anthropogenic landscape changes.

Bee pollinators may be particularly vulnerable to negative effects of habitat fragmentation due to their haplodiploid genetic systems, which render their effective population sizes no more than 75% that of equally-sized diploid populations (Whiting & Whiting, 1925). Widespread population declines due to habitat loss have been reported for many bee species (LeBuhn & Vargas Luna, 2021; Potts et al.,

2010), and these may occur via the loss of floral resources or nesting areas (Carvell et al., 2006; Cohen et al., 2020), greater energetic costs associated with travel (Andrieu, Dornier, Rouifed, Schatz, & Cheptou, 2009), or heat stress (Aguirre-Gutiérrez et al., 2017; Suni & Dela Cruz, 2021). Body size has been proposed as an important potential driver of responses of bees to habitat loss, with larger bees potentially being able to cross larger degraded areas but also requiring larger areas of forage to persist (Harrison & Winfree, 2015). Meta analyses based on mark-recapture and genetic data suggest larger bees travel farther (Greenleaf, Williams, Winfree, & Kremen, 2007; López-Uribe et al., 2019), but explicit tests of how body size and landscape may jointly influence dispersal in bees are lacking.

Here, we examined drivers of genetic structure and genetic diversity for ten species of bees in the tribe Euglossini that vary widely in body size. Euglossine bees (also called Orchid Bees) are important pollinators of over 700 species of orchids and other tropical plants (Roubik & Hanson, 2004). Male Euglossine bees exhibit a unique behavior whereby they visit orchids and other plants to collect volatile compounds that are used in sexual chemical signaling when emitted during courtship behavior (Thomas Eltz, Sager, & Lunau, 2005). To understand if species-level traits and landscape characteristics are associated with dispersal genetic diversity, we developed thousands of SNP loci for each species. We then tested the following predictions: (1) larger species disperse farther; (2) species with greater resource specialization disperse farther; (3) deforested areas restrict dispersal; and (4) sites surrounded by more intact habitat have higher genetic diversity. Our joint analysis of individual traits with landscape effects on dispersal reveals patterns that contradict the dominant paradigm found for bees, and suggests future areas of inquiry regarding drivers of dispersal in fragmented landscapes.

Materials and Methods

Field sampling

We sampled bees of ten species that range in body length from 9 mm to 28 mm (Figure 1) at six sites throughout southern Costa Rica in May and June of 2019 (Figure 2, Table S1). Sites included the Las

Alturas Biological Research Station, the Las Cruces Biological Research Station, the La Gamba Biological Research Station, the Saladero Ecolodge, and the Bromelias Ecolodge, and a site at the northern part of the Osa Peninsula at which local landowners provided permission to sample (Agua Buena, see Figure 2). These species sampled vary in their resource specialization, with the number of orchid morphospecies visited ranging from 6 to 44 (Roubik & Hanson, 2004; Table S2). The landscape in this area is comprised of forest fragments, pastureland, palm oil plantations, and small towns. Extensive deforestation occurred in the 1950s following European settlement and reduced forest cover to 25% by the 1990s, but pollen and charcoal analyses from lake-sediment cores suggest continuous occupation and some forest clearing by indigenous people over a 3,000-year period (Clement & Horn, 2001).

To attract bees we used the chemical baits 1,8-cineole and methyl salicylate. These chemical baits mimic the natural fragrances emitted by orchids (Janzen, 1981). Baits were placed approximately 1.5 m off the ground on tree trunks between the hours of 9 am and 12 pm on sunny days, and in forest fragments between zero and 93 m from forest edges. We netted bees as they arrived at baits, and we stopped sampling when no more bees arrived after 15 minutes. Bees were killed using ethyl acetate, and samples were then transported back to the University of San Francisco for curation and DNA extraction. Bees were pinned and then identified by examining the velvet area, a patch of dense hair on the tibial tuft, as well as other species-specific characteristics (Roubik & Hanson, 2004). After genotyping and quality control (see below), our final sample included 539 bees, with an average of 89.8 bees per site (range 26 - 140) that represented 12.8 bees per species per site (range 2 - 53).

DNA sequencing and SNP calling

Genomic DNA was extracted from one or two middle legs of each specimen (two legs for the smallest species) using DNeasy Blood and Tissue Extraction Kits (Qiagen). DNA concentration was quantified using a Qbit 2.0 fluorometer (Thermo-Fisher) and then 100 ng of DNA per individual was used to prepare ddRADseq libraries using a protocol modified from Poland et al. (2012), as follows. DNA was

digested with the enzymes PstI and MspI (New England Biolabs), and then unbarcoded adaptors that were synthesized by IDT (Integrated DNA Technologies) were ligated onto the sticky ends. Ligation products were then cleaned with Agencourt Ampure XP beads (Beckman Coulter), and were then used as templates for PCR. PCR was performed in 96 well plates with each well containing one sample and one of 285 uniquely barcoded TrueSeq primer pairs that had been synthesized by the University of California San Francisco Center for Advanced Technology (UCSF CAT). An AccuBlue DNA Concentration Kit (Biotium) was used to quantify DNA, and then 40 ng of each sample was pooled. Pooled DNA was cleaned using Agencourt Ampure XP beads, and it was then size-selected (300-500 bp) using a Blue Pippin Prep (Sage Science). Success in obtaining accurate target fragment size distributions was confirmed using a Tapestation 4200 (Agilent). The pooled, size-selected DNA was then cleaned using a Monarch PCR & DNA cleanup kit (NEB) before 150-bp paired-end sequencing was performed on a NovaSeq 6000 (Illumina) at the UCSF CAT.

Samples were demultiplexed at the UCSF CAT and quality control of the sequencing run was assessed using the software FastQC v.0.11.8 (Andrews, 2010). Raw Illumina reads were cleaned using the *process_radtags* program in STACKS v. 1.57 (Catchen et al. 2011, 2013). Reads with quality scores (Phred33) below 10 within a sliding window of 15% of the read length, those with Illumina TruSeq adapter contamination, or those for which the restriction enzyme cut-site for MspI or PstI was not intact were discarded. An average of 2,057,810 raw reads was recovered across samples and after quality control filtering an average of 1,286,243 were retained. This resulted in 13,412 - 153,924 SNPs per species. The *denovo_map.pl* pipeline was used to identify orthologous loci across individuals for each species separately. We performed STACKS parameter optimization following (Paris et al. 2017), and chose the following parameter combination: m = 3, M = 2, n = 3 for each species. The maximum observed heterozygosity required to process a locus was set to 0, as in Alonso-Garcia et al. (2021), because samples were haploid. We limited analyses to the first SNP per locus using --*write-single-snp*,

and we used the *--fstats* option in the populations program to estimate expected heterozygosity and the percent of loci that were polymorphic for each species within each site. We estimated allelic differentiation (F_{ST}, Wright, 1943), and absolute genetic divergence D_{XY} (Nei, 1987; Cruickshank & Hahn, 2014) among site pairs for each species. Unlike F_{ST}, D_{XY} is not sensitive to levels of within-population genetic diversity (Charlesworth, 1998; Nei, 1973) though it does depend on ancestral levels of genetic diversity (Cruickshank & Hahn, 2014).

Landscape analyses

To estimate the percent forest surrounding each sampling location and between locations we used ArcGIS v.2.4 (Esri, Redlands, CA). We used the Esri 2020 Land Cover dataset that corresponded to scene 17P (Karra et al. 2021) to obtain forest cover of the study region. We quantified the amount of forest cover within a circle of radius 24 km for each sampling location (Figure S1). We chose this radius because Euglossine bees are capable of travel over tens of kilometers in a single day (D. H. Janzen, 1971). To estimate the amount of forest between pairs of sampling locations we first used ArcGIS to calculate Euclidian (straight-line) geographic distances between all possible site pairs. Euclidian distances are the shortest distance between sites, and may traverse water. We also calculated “Broken-stick” geographic distances as in Davis et al. (2010), which are the shortest overland distances between two sites. For both types of distances we overlaid rectangles of width 1000 m and calculated the amount of forest between each pair of sites. We centered rectangles at each pair of sites and the percent forested area was quantified within that rectangle (Figure S1). Many sites are located near the coastlines of the Golfo Dulce or the Pacific Ocean. We did not clip the circular or rectangular buffers to the coastline if they extended into the water, so water was included as deforested area. We did this to obtain a realistic estimate of the proportion of forest cover relative to other land cover types and to reflect possible Euglossine bee flight paths, since some Euglossine species seem to have restricted dispersal over large bodies of water (da Rocha Filho et al., 2013).

Statistical analyses

To determine if body size predicts dispersal we ran linear mixed models implemented using the lme4 package in R (Bates et al., 2014) with F_{ST} or D_{XY} as the as the dependent variable, body size as the independent variable, and the pair of sites between which F_{ST} was calculated as the random effect. We also ran a model that included genus as an independent variable, to determine if the association we found between body size and dispersal held within genera or was driven by genus. We tested for statistical significance of the independent variable using likelihood ratio tests on nested models. In the results section we report estimates from the best model chosen via backward model selection, and chi-square and associated P-values from likelihood ratio tests. We used species-site combinations with at least four sampled individuals in analyses that used F_{ST} or D_{XY}, resulting in the removal of 10 individuals from these analyses (Table S1).

To determine if diet breadth predicts dispersal we compiled the number of morphospecies and genera of orchids visited for each species in the dataset from records reported in Roubik and Hanson (2004). We ran linear mixed models with F_{ST} or D_{XY} as the dependent variable, the number of orchid morphospecies or genera as the independent variable, and bee genus, species, and the sites between which F_{ST} or D_{XY} was calculated as random effects. We used likelihood ratio tests on nested models to assess the significance of independent variables.

We determined if deforested areas restrict dispersal while taking geographic distance into account by performing multiple regression on distance matrices (Wang, 2013) using the *tseries* R package (Trapletti et al., 2022). For each species, we performed MMRR four times using 10,000 permutations. F_{ST} and D_{XY} were highly correlated in our dataset (correlation coefficient = 0.97, 95% CI = [0.96, 0.98]), so we performed MMRR using only F_{ST} values. A matrix containing pairwise F_{ST} values among sites was the dependent variable, and the independent variables included a matrix containing pairwise geographic distances among sites, and a matrix specifying what percent of that distance was

forested. For each species, we ran MMRR using predictor matrices that included Euclidian geographic distances and Broken-stick distances. We estimated the overall statistical significance of multiple comparisons using a modified false discovery rate procedure (Benjamini and Yekutieli 2001; Narum 2006).

We examined if the way that deforested areas affected dispersal is mediated by body size by running linear mixed models in which the dependent variable was F_{ST} or D_{XY} between site pairs, the interaction between body size and the percent forest between pairs of sites was the independent variable, the geographic distance between sites was a covariate, and genus and the site pair were random effects. We ran separate models using the percent forested area and geographic distance between site pairs calculated using Euclidian and Broken-stick geographic distances. The significance of the interaction between body size and the percent forest between pairs of sites was assessed using likelihood ratio tests on nested models.

To determine if sites that were surrounded by more forest had higher genetic diversity we ran linear mixed models implemented using the *lme4* package in R (Bates et al., 2014; R Core Team, 2019). Either expected heterozygosity, the percent of loci that were polymorphic, or the number of private alleles was the dependent variable, the percent forest surrounding sites at a radius of 24 km was the independent variable, sample size was a covariate, and species was a random effect. We found differences in dispersal between bees in different genera (see results), so we also added genus as a predictor variable in the model. Significance of the independent variables was assessed using likelihood ratio tests on nested models.

Results

Larger bees had higher genetic structure than smaller bees (For F_{ST}: Est. = 0.019, $\chi^2 = 88.1$, $P < 0.001$; For D_{XY}: Est = 0.00011, $\chi^2 = 80$, $P < 0.001$; Figure 3, Figure S2). This negative relationship between body size and dispersal distances was driven by bees of the larger genus *Eulaema* having higher F_{ST} and

D_{XY} values than bees of the smaller genus *Euglossa*. Genus significantly predicted dispersal estimates (For F_{ST}: Est. = 0.34, χ^2 = 141, P < 0.001; For D_{XY}: Est. = 0.0021, χ^2 = 133, P < 0.001). F_{ST} ranged from 0.28 - 0.55 for bees in the genus *Eulaema* and 0.015 - 0.19 for bees in the genus *Euglossa* across sites. D_{XY} ranged from 0.0025 - 0.47 for bees in the genus *Eulaema* and 0.00067 - 0.0017 for bees in the genus *Euglossa* across sites (Table S2).

There was evidence that resource specialization predicted dispersal distances. Species that were reported to visit more orchid morphospecies or genera had higher estimates of F_{ST} between site pairs (For morphospecies: Est. = 0.0076, χ^2 = 5.4, P = 0.02; For genera: Est. = 0.0091, χ^2 = 4.9, P = 0.028; Figure S3). Species that visited more orchid morphospecies also had higher estimates of D_{XY} (Est. = 0.000052, χ^2 = 5.9, P = 0.015), and there was a trend towards species that visited more orchid genera having higher genetic differentiation (Est. = 0.000034, χ^2 = 3.1, P = 0.081).

The way that the amount of forested area among sites affected genetic differentiation depended on body size. The interaction between body size and the percent forest between pairs of sites was a significant predictor of F_{ST} among site pairs, such that increasing forest between sites was associated with higher F_{ST} between them for large bees but not for smaller bees (Figure S4). Broken-stick distance was a stronger predictor of genetic differentiation (For Euclidian distance: Interaction est. = 0.00016, χ^2 = 6.5, P = 0.011; for Broken-stick distance: Interaction est. = 0.00024, χ^2 = 10.0 , P = 0.0016). However, for D_{XY} the association between the amount of forest between sites and genetic differentiation was not mediated by body size (For Euclidian distacne: χ^2 = 0.033, P = 0.86; for Broken-stick distance: χ^2 = 0.024, P = 0.88).

When modeling each species separately, there was no evidence that deforested areas restricted dispersal. The percent of land that was deforested between pairs of sampling locations did not predict genetic differentiation for any species (Table S3). Geographic distance was not a predictor of dispersal for any species (Table S3).

There was some evidence that sites with more intact habitat had higher genetic diversity, and that genetic diversity was lower for larger bees. Expected heterozygosity was positively associated with the percent of land that was forested around sites (Range 0 - 0.41, Est. = 0.0031, $\chi^2 = 6.6$, $P = 0.01$, Figure 4, Table S1). The percent of loci that were polymorphic was not influenced by the percent of land that was forested ($\chi^2 = 0.22$, $P = 0.63$). There were more private alleles in sites surrounded by more forest (Est. = 10.1, $\chi^2 = 5.5$, $P = 0.019$). Expected heterozygosity did not differ among genera (Chisq = 0.007, $P = 0.93$), but the percent of polymorphic loci was lower for bees in the larger genus *Eulaema* (Est. = -0.75, Chisq = 6.6, $P = 0.01$).

Discussion

We present a systematic investigation of morphological and landscape drivers of genetic structure for ten bee species within a clade, as well as an assessment of how genetic diversity varies with the amount of intact habitat surrounding sites. Body size was inversely related to genetic structure, and this was driven by differences between genera in the genetic differentiation among sites. Within genera, there were no associations of genetic structure and body size. There was evidence that floral fragrance resource specialization was associated with higher dispersal. Contrary to predictions, dispersal was not lower among sites separated by less forest. For larger bees, the presence of more forest among sites was associated with lower dispersal. Deforested landscapes were associated with lower genetic diversity.

The inverse association between body size and dispersal distance across genera, and the lack of association within genera contrasts with what has been found previously for bees. A significant positive relationship was found between body size and homing or foraging distance for 62 bee species from six families (Greenleaf et al., 2007). That study compiled observational data of short-term movement patterns, and did not include estimates of realized dispersal. A meta-analysis that examined associations between body size, and estimates of genetic structure based on microsatellites, found an overall

negative relationship between body size and genetic differentiation across 42 species of bees (López-Uribe et al., 2019). Despite that negative relationship overall, there was high variation in that dataset, suggesting traits other than body size are also likely important drivers of dispersal. Indeed, social species exhibited lower genetic structure than solitary species, which could be due to higher levels of kin competition for social species when compared to solitary species (West et al., 2002). In our case, it is possible that avoidance of kin competition contributes to the low genetic structure found for some species examined. However, we posit that kin competition is unlikely responsible for the higher genetic structure found in bees of the genus *Eulaema* because reports of nest sharing have been reported for species within both genera (Augusto & Garófalo, 2004; Cameron & Ramírez, 2001).

We outline several speculations for the higher genetic structure found in bees of the genus *Eulaema*. First, a greater degree of territoriality has been described for species in *Eulaema* than *Euglossa* (Kimsey, 1980). Second, it is possible that bees in the genus *Eulaema* experience higher predation risk when flying over deforested areas (Roubik, 1993). *Eulaema* tend to be black or very dark in coloration, while bees in the genus *Euglossa* tend to be brightly colored and iridescent. Iridescence may increase camouflage in open areas, which are becoming more prevalent with ongoing deforestation in the study area (Stan & Sanchez-Azofeifa, 2019). Third, *Eulaema* may experience a greater risk of overheating when flying over deforested areas. Iridescence reflects light (Seago., 2009), which may reduce heat gain for bees in the genus *Euglossa* as they travel over open areas (Mossakowski, 1979). The darker coloration of *Eulaema* may also contribute to their being more susceptible to overheating in open areas.

The extent to which species are generalized or specialized in their resource requirements may also influence dispersal distances. For example, species that are more generalized in their resource requirements are expected to be able to disperse farther due to their ability to refuel *en route* (Bowler & Benton, 2005). However, an empirical survey of 740 species of varying tropic levels found no association between diet breadth and dispersal (Stevens et al., 2014). In addition, work specifically on bees also

found no evidence that dispersal distances are associated with the degree of dietary specialization across 42 species (López-Uribe et al., 2019). Other types of resources requirements may also be important drivers of dispersal (Bowler & Benton, 2005). Our examination of the extent of floral generalization for fragrance collection revealed a negative association between the number of orchid morphospecies or genera visited and dispersal distances. Many tropical plants are locally rare (Wills et al., 2006), and it is possible that the positive association between floral specialization in orchids visited for fragrance collection and dispersal occurs because species that are more specialized travel farther to acquire specific resources.

We stress that our data do not suggest that the association between resource specialization and dispersal is a general pattern for Euglossine bees. Rather, the pattern was driven by a single species that had both the highest genetic differentiation and was also reported to visit the most genera and morphospecies of orchids (*Eul. meriana*; Figure S3). When this species was removed from the data set, resource specialization no longer predicted genetic differentiation (Table S4).

Our findings suggest that male Euglossine bees in the genus *Euglossa* maintain long distance travel even over deforested landscapes, and that species in *Eulaema* may show more restricted dispersal. This is somewhat surprising given that mark-recapture observations have documented high recapture rates over a monthly time period for species in *Euglossa* (T. Eltz et. al., 1999; López-Uribe et. al., 2008). However, other mark-recapture efforts documented male bees traveling tens of kilometers within a period of days through intact forest (Pokorny et al., 2015). In addition, past population genetic studies have typically found restricted dispersal for *Euglossa* species only for island populations (Boff et. al., 2014; da Rocha Filho et al., 2013). For populations separated by land, mitochondrial COI genotyping found identical haplotypes on both sides of the Andes mountains for bees in *Euglossa* and some genetic structuring in for *Eulaema* species (Dick et al., 2004). Microsatellite genotyping found low genetic structure for *Eug. dilemma* across 130 km (Zimmermann et al., 2011), *Eug. dilemma* and *Eug. viridissima*

across 114 km (Soro, Quezada-Euan, Theodorou, Moritz, & Paxton, 2017), *Eug. imperialis* across 226 km (Suni, 2017), and *Eug. championi* across 80 km (Suni et al., 2014), but significant genetic structuring for *Eul. bombiformis* across just 14 km (Suni & Brosi, 2012). Taken together, the results of these studies and the current study suggest that there may be stronger barriers to movement for larger species, and they motivate future work on additional biotic and abiotic drivers of dispersal.

We found no support for our prediction that genetic differentiation would be higher between site pairs that were separated by less forest. Rather, for larger bees, a greater amount of forest between sites was associated with greater genetic differentiation between them. We speculate that this pattern could be explained, at least in part, by larger bees having higher resource requirements than smaller bees (Müller et al., 2006). As the amount of forest between sites is diminished, larger bees may travel farther to acquire sufficient resources (Harrison & Winfree, 2015). It is also possible that this pattern is driven by greater generalization of some larger species, which allows them to remain local when there is sufficient forest from which to acquire resources. In particular, *Eul. meriana* is one of the largest bees in our data set and is also reported to be the most generalized in terms of the orchids from which fragrances are collected (Roubik & Hanson, 2004). This species also showed a positive association between the percent of land between sites that was forested and dispersal, although this association was not significant. It is possible that the lack of a significant association was due to limited statistical power, as sample sizes of *Eul. meriana* were rather low. Given that we also hypothesized that predation risks outside of open areas might be higher for larger, more visible species like *Eul. meriana*, an exploration of tradeoffs between resources acquired via travel across open areas and predation risk in open areas would be worthwhile.

While the way that the amount of forest affected F_{ST} was mediated by body size, this was not the case for D_{XY}. These measures both provide insight into gene flow among populations but may reflect different time scales of divergence. D_{XY} is the probability of nonidentity by descent of two alleles drawn

in the two different populations averaged over all loci (Nei, 1987), while F_{ST} is the proportion of the total genetic variance contained in subpopulations. D_{XY} may therefore reflect deeper divergence than F_{ST} (Cruickshank & Hahn, 2014; Nachman & Payseur, 2012). In addition, F_{ST} is affected by within-population levels of genetic variation (expected heterozygosity), while D_{XY} is not. Expected heterozygosity was higher for sites that were surrounded by more forest, and it was is possible that this led to the discrepancy between measures of genetic differentiation. Indeed, the average expected heterozygosity across pairs of sites was associated with F_{ST} between those sites when distance between sites was taken into account and species and genera were random effects (linear mixed model est. = -0.000039, χ^2 = 29.8, $P < 0.001$). Other factors could result in differences between D_{XY} and F_{ST}. D_{XY} is more affected by mutation rates than F_{ST} (Rosenzweig et al., 2016), it may be more susceptible to small sample sizes (Clarkson et al., 2014), and it seems to be more affected by background selection (Matthey-Doret & Whitlock, 2019).

While there was no indication that a lack of forest restricted dispersal among sites, those that were surrounded by less forest had lower genetic diversity. These discordant influences of forest on genetic parameters could be explained by the rate at which inter versus intra-population genetic signatures of habitat fragmentation manifest (Peakall & Lindenmayer, 2006), or by methodological limitations such as small sample sizes (Richardson et al., 2016). With limited dispersal among fragments, genetic drift may quickly cause the loss of rare alleles in small populations (Allendorf, 1986). However, even given limited dispersal, the continued presence of common alleles may result in a lack of isolation by distance or resistance in the short term. Our finding both higher genetic diversity as well as significantly more private alleles in sites with more forest suggests that drift may be lower and effective population sizes higher in fragments surrounded by greater amounts of habitat.

Effects of habitat loss on genetic diversity have been documented across taxa, including mammals (Lino et al., 2019), plants (González et al., 2020), amphibians (Dixo, Metzger, Morgante, &

Zamudio, 2009), and insects (Bickel et al., 2006). The susceptibility of populations to negative effects of habitat fragmentation depends on species-specific characteristics, such as habitat specialization and dispersal capacity (Sekar, 2012; Slade et al., 2013), as well as habitat availability in the surrounding area (Peakall & Lindenmayer, 2006). Species with high dispersal capacity may be less likely to suffer from negative effects of fragmentation if they can utilize other habitat patches. This should result in the maintenance of gene flow among patches and genetic diversity within patches. Lower dispersal capacity but a network of accessible patches should result in a pattern of isolation by distance. Low dispersal capacity and isolated fragments should lead to high genetic drift within patches and the loss of genetic diversity (Louy et al., 2007). Our results therefore suggest genetic drift may be higher in populations of bees in the genus *Eulaema*, as the percent of loci that were polymorphic was significantly lower, and genetic differentiation was higher.

Given that past work has revealed restricted dispersal across water in Euglossine bees (Boff et. al., 2014; da Rocha Filho et al., 2013, we may expect that broken-stick geographic paths may have better reflected patterns of genetic differentiation. However, travel over water as much as 2.5 km from the nearest land was observed for a species in the genus *Eulaema* that was not included in the current study (D. H. Janzen, 1971). Neither Broken-stick nor Euclidian paths predicted patterns of genetic structure in the current study. This suggests that the species examined here may fly short distances over water when traveling, but we cannot rule out that limitations due to small sample sizes of some species, particularly in the genus *Eulaema*, may have limited our ability to detect patters if they indeed exist.

To our knowledge, this work is the first SNP-based assessment of genetic structure in Euglossine bees, and our results highlight risks to populations associated with habitat fragmentation. In particular, genetic diversity was lower in areas with less intact forest, suggesting that these bee species may be at risk of further genetic erosion as habitat fragmentation continues. In addition, our results suggest that large species may need to exert more and more energy traveling through degraded landscapes in the

future. Our findings are largely consistent with patterns found previously for Euglossine bees, which employed mitochondrial haplotypes or microsatellite loci to characterize genetic structure. This contrasts somewhat with what has been found for bumble bees in temperate areas, where investigations of dispersal distances found discrepancies between patterns emerging from microsatellite versus SNP data (J. D. Lozier, 2014; Jeffrey D. Lozier, Jackson, Dillon, & Strange, 2016). The consistency found across studies in low genetic structure for smaller Euglossine bees validates the inverse relationship between dispersal distance and body size that was found in past work (Suni & Brosi, 2012), and motivates investigation into the extent to which species interactions mediate dispersal.

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Data accessibility

Genetic data: Datasets and code used in to produce statistical results and figures, as well as individual genotype data are available on Zenodo.com, DOI: 10.5281/zenodo.6998927. Individual raw sequence reads are deposited in the SRA (BioProject ID: PRJNA880925). *Sample metadata*: Sample metadata, including georeferences in decimal degrees and dates of sampling events are in Table S1.

Benefit-sharing

Benefits generated: Permission of local landowners was obtained prior to sampling. Results of scientific enterprises are being shared with landowners, including biological research stations and ecolodges that promote scientific research and engage with local communities. The contributions of local individuals to research are described in *Methods* and *Acknowledgements*.

Author contributions

MH and SS designed the study, SS collected the specimens, MH curated the specimens, extracted DNA and performed genomic, bioinformatic, and statistical analyses with guidance from SS, and SS wrote the manuscript with critical input from MH.

Tables & Figures

Figure 1. The species sampled, along with their body sizes. From left: *Eulaema bombiformis* (28 mm), *Eulaema meriana* (26 mm), *Eulaema nigrita* (20 mm), *Euglossa imperialis* (15 mm), *Euglossa flammea* (14 mm), *Euglossa championi* (13 mm), *Euglossa maculilabris* (12 mm), *Euglossa mixta* (11 mm), *Euglossa dodsoni* (10 mm), and *Euglossa sapphirina* (9 mm).

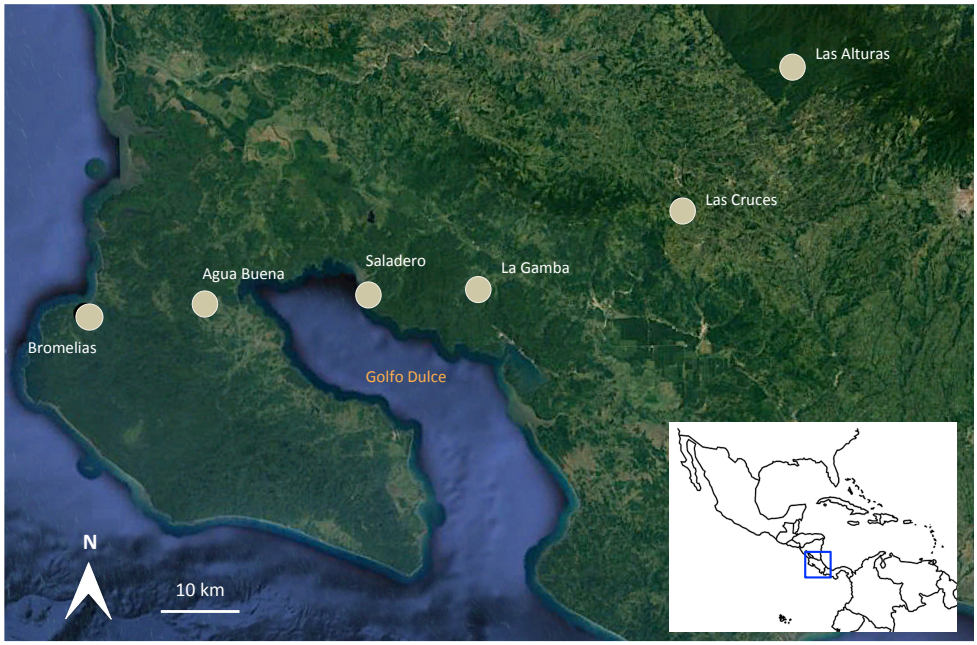


Figure 2. Study area in Southern Costa Rica, extending from costal sites on the Osa Peninsula (bottom left) to a forested site at 1420 meters above sea level (top right). Image from Google Earth Pro v. 7.3.4.8248.

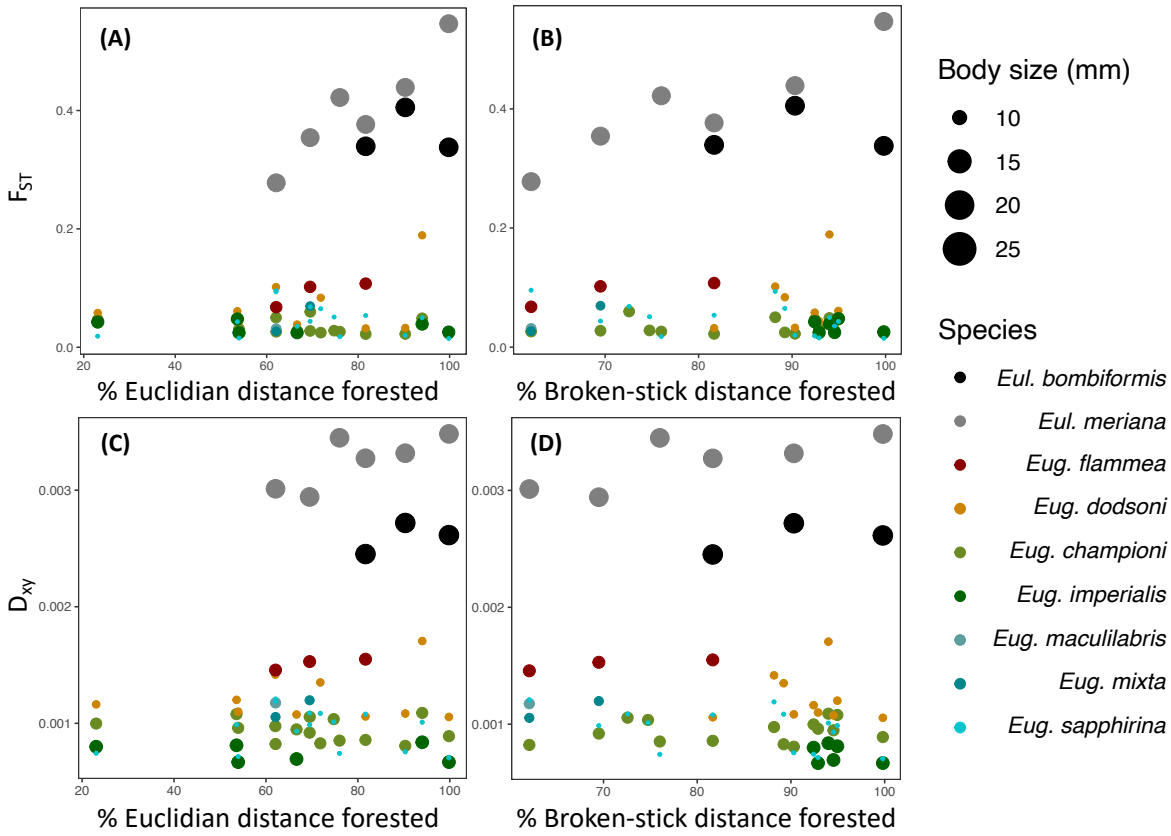
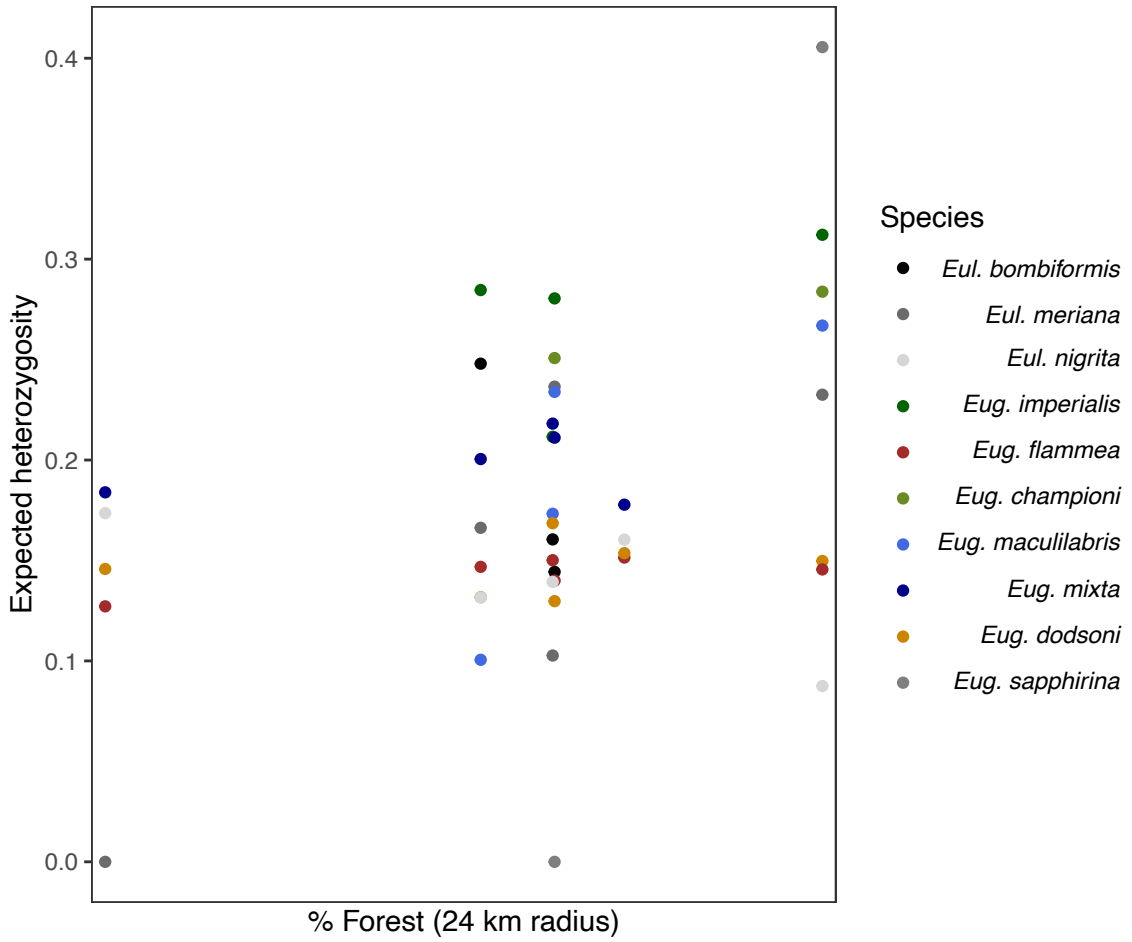


Figure 3. For each species, F_{ST} (panels A & B) or D_{XY} (panels C & D) between each pair of sites is plotted against the percent of forest between those sites that was forested. Panels A & C reflect Euclidian forest paths and panels B & D reflect Broken-stick forest paths. Colors represent different species and the size of the points is proportional to body size. See *Figure S1* for a description of the difference between Euclidian and Broken-stick paths.



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697 **Figure 4**. For each species, expected heterozygosity within sites is plotted against the percent of forest
698 surrounding sites at a radius of 24 km from the sampling location. Colors represent different species.
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