Published in final edited form as:
Biotropica. 2014 March 1; 46(2): 202–209. doi:10.1111/btp.12084.

Spatio-temporal Genetic Structure of a Tropical Bee Species Suggests High Dispersal Over a Fragmented Landscape

Sevan S. Suni[1,1,3,5], **Judith L. Bronstein**[2], and **Berry J. Brosi**[4]

[1]Center for Insect Science, University of Arizona, Tucson AZ 85721, U.S.A

[2]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson AZ 85721, U.S.A

[3]Organismic and Evolutionary Biology Program, University of Massachusetts, Amherst MA 01003, U.S.A

[4]Department of Environmental Studies, Emory University, Atlanta GA 30322, U.S.A

Abstract

Habitat destruction threatens biodiversity by reducing the amount of available resources and connectivity among geographic areas. For organisms living in fragmented habitats, population persistence may depend on dispersal, which maintains gene flow among fragments and can prevent inbreeding within them. It is centrally important to understand patterns of dispersal for bees living in fragmented areas given the importance of pollination systems and recently documented declines in bee populations. We used population and landscape genetic techniques to characterize patterns of dispersal over a large fragmented area in southern Costa Rica for the orchid bee species *Euglossa championi*. First, we estimated levels of genetic differentiation among forest fragments as ϕ_{PT}, an analog to the traditional summary statistic F_{ST}, as well as two statistics that may more adequately represent levels of differentiation, G'_{ST} and D_{est}. Second, we used a Bayesian approach to determine the number and composition of genetic groups in our sample. Third we investigated how genetic differentiation changes with distance. Fourth, we determined the extent to which deforested areas restrict dispersal. Finally, we estimated the extent to which there were temporal differences in allele frequencies within the same forest fragments. Within years we found low levels of differentiation even over 80 km, and no effect of land use type on level of genetic differentiation. However, we found significant genetic differentiation between years. Taken together our results suggest that there are high levels of gene flow over this geographic area, and that individuals show low site fidelity over time.

Keywords

genetic differentiation; euglossine; dispersal; fragmentation; orchid bee; landscape genetics

INTRODUCTION

Habitat loss is one of the most serious threats to biodiversity (*e.g.*, Sala *et al*. 2000). It reduces connectivity among geographic locations (Primack 1993) and genetic diversity within populations (Ledig & Conkle 1983), potentially leading to higher extinction risk

[5]Corresponding author: sssuni@eco.umass.edu.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

FIGURE S1. Map of sampling locations.

TABLE S1. Number of bees sampled per site, and geographic locations of sites sampled in 2010.

(Taylor 2003). For organisms living in fragmented habitats, persistence may depend on dispersal potential because movement among fragments can maintain gene flow among them and prevent inbreeding within them.

Understanding patterns of connectivity for wild bee populations in fragmented areas is important for several reasons. First, bees are essential for the pollination of about 90 percent of angiosperm species (Ollerton *et al*. 2011) and about two-thirds of agricultural crops (Kremen 2007). Second, bees have experienced significant declines in abundance in recent years (Biesmeijer *et al*. 2006, Goulson *et al*. 2008, Grixti *et al*. 2009, Kosior *et al*. 2007), and these declines are likely due in part to habitat fragmentation (Winfree *et al*. 2009). Third, bees are haplodiploid, which theoretically lowers their effective population sizes because males are usually hemizygous at all loci (Crosier 1976, Hedrick & Packer 1997). Fourth, bees operate under a system of single locus sex determination in which individuals develop as females only if they are heterozygous at the *csd* locus (Beye *et al*. 2003). If genetic diversity is low in populations and bees are homozygous at the *csd* locus, they will develop into diploid males, which are effectively sterile. This makes bees theoretically more vulnerable to negative effects of inbreeding than haplodiploidy alone (Zayed & Parker 2005), though diploid males do not seem to be a problem in some groups such as orchid bees (Souza *et al*. 2010).

Conservation genetic data has emerged as a powerful tool for monitoring the conservation status of populations and detecting responses to environmental changes. To date the majority of conservation genetic studies on bees have focused on temperate, eusocial species, in particular bumble bees (Cameron *et al*. 2011, Goulson *et al*. 2008, Zayed 2009). This is unfortunate because 90 percent of all bee species are non-eusocial, a greater percentage of tropical versus temporal plants rely on animals for pollination (Ollerton 2011), and pollen limitation is generally greater for tropical plants (Vamosi *et al*. 2006).

Orchid bees (Hymenoptera: Apidae, tribe Euglossini; also known as Euglossine bees) are the sole pollinators of over 700 species of orchids and are important pollinators of many other tropical plants (Ramírez *et al*. 2002, Roubik & Hanson 2004), and they are thought to be particularly susceptible to deforestation (Roubik & Hanson 2004, but see Cerântola *et al*. 2010). Results from previous work on effects of forest fragmentation on abundance and species diversity of orchid bees have been inconclusive. No significant relationship between fragment size and abundance and species diversity was found in four studies (Becker 1991, Tonhasca *et al*. 2002a, b, Brosi *et al*. 2007). However, Brosi (2009) did find significant reductions in abundance and species diversity in smaller and more isolated fragments, possibly due to this study's larger sample sizes. Likewise, Powell and Powell (1987) found a similar pattern immediately following an experimental deforestation event. Declines in abundance in smaller fragments may not necessarily reflect extreme vulnerability if dispersal among fragments occurs. On the other hand, if dispersal is restricted, orchid bees may have limited access to resources such as food or mates, and genetic diversity may decline, which may reduce chances for long-term persistence.

A handful of population genetic studies have attempted to characterize the conservation status of orchid bees. Souza *et al* (2010) found that orchid bee populations may not be at risk of extinction due to genetic factors, as was previously implied by prior work by Zayed *et al*. (2004). Results from Freiria *et al*. (2011) also suggest that populations of some orchid bees are healthy; these authors found high levels of genetic diversity within Atlantic forest fragments in Brazil. Levels of gene flow seem to differ among species and genera (Cerântola *et al*. 2010, Freiria *et al*. 2011. Suni and Brosi 2011, Zimmerman *et al*. 2011), and little is known about the extent to which dispersal differs between forested and deforested areas. Zimmerman *et al*. (2011) found low levels of genetic differentiation over 130 km of

agricultural land for eight species, but were unable to distinguish between possible reasons for their observations. The authors proposed that the low estimates of genetic differentiation either reflected dispersal among sites, or fragmentation in their study site occurred too recently for populations to evolve genetic differences. In the latter case the signature of restricted dispersal would not have been detectable. Another possibility is that the estimator they used to gain insight into levels of differentiation underestimated true levels (see *Methods* section).

We explored patterns of genetic differentiation among forest fragments for the orchid bee *Euglossa championi*. We characterized: (1) the number of genetic groups; (2) the level of isolation by distance; (3) the impact of landscape matrix on genetic differentiation; and (4) temporal changes in genetic composition of forest fragments. Previous work found no significant differentiation between forest fragments separated by 14 km (Suni & Brosi 2011). The current study expanded the geographic scale of sampling to include forest fragments separated by as much as 80 km, and included data from two years. Understanding how the genetic composition of sampling locations changes over time may be a good way to determine if high levels of dispersal occur, *i.e.*, differences in the genetic composition of individuals in the same site over time would be consistent with high levels of dispersal in that geographic area.

METHODS

Species And Sampling

Using the chemical baits cineole and methyl salicylate (Janzen 1981) we caught 55 male *Eug. championi* in three forest fragments (1 sampling location in each) in July 2009 and 96 *Eug. championi* in four forest fragments (12 total sampling locations) in April 2010 in Southern Costa Rica (Fig. S1). The fragments sampled from in 2009 were four to 26 km from one another, in 2010 they were four to 81 km from one another, and they surrounded by a mixture of towns, rural areas, and agricultural areas. The number of bees within each forest fragment ranged from 8 to 59, with an average of 18 in 2009 and 24 in 2010 (Table S1). We focused on *Eug. championi* because it is one of the few orchid bee species found along an elevational gradient from cloud forest to sea-level tropical rain forest. Despite being present across this wide habitat range, it is not highly abundant in each of these habitats. Our aim was to capture at least 20 individuals per forest fragment. Orchid bees arrived at the baits within about 15 min and the number of arriving bees tapered off typically after 30 min. In some cases we sampled for more than three hours, but no new bees arrived at our baits, suggesting that the population sizes in those fragments were small.

Molecular Analyses

We extracted DNA using a phenol-chloroform extraction procedure (Sambrook *et al*. 1989). All samples collected were haploid males, which we genotyped at eight microsatellite loci: Egc 17, Egc 18, Egc 24, Egc 26, Egc 35, Egc 37, Egc 51 (Souza *et al.* 2007), and Ann28 (Paxton *et al.* 2009) that were labeled with fluorescent dyes (Applied Biosystems). The loci were multiplexed together in two sets of four loci using the following PCR procedure: 94°C for 4 min, 35 cycles of 94°C for 30 s, 58°C for 30 s, 72 °C for 30 s, 72°C for 6 min, and then 4°C for 4 min. Five percent of the bees were re-genotyped to verify that genotyping error rates were negligible. Previous work showed that these loci are not linked in the species for which they were developed (Souza *et al.* 2007, Paxton *et al.* 2009). We ran PCR products on an ABI 3730 (Applied Biosystems) automated DNA sequencer in the Genomics Core Facility at the University of Arizona, and analyzed the microsatellite lengths using GENEMAPPER software (Applied Biosystems).

GENETIC DIVERSITY

We calculated measures of genetic diversity within each fragment using the software program Genalex (Peakall & Smouse 2006). We calculated average haploid genetic diversity per fragment (*Div*) as $(1/n)[1 - \sum p_i^2]$, where p_i is the frequency of allele *i*, and *n* is the number of loci. *Div* is the average over all loci of the probability that two individuals will be genetically different at one locus. We calculated *Div*, as well as the actual (*Na*) and effective (*Nef*) number of alleles (Kimura and Crow 1964), for individuals within each forest fragment. We also calculated allelic richness using the program Fstat (Goudet 1995). Allelic richness is based on rarefaction and thus is appropriate for comparisons among samples of different sizes.

Genetic Differentiation Among Forest Fragments

G_{ST}-like estimators such as F_{ST} (Wright 1951), are the most widely used measures of genetic differentiation in population and conservation genetic studies, but they may underestimate true levels of differentiation (Jost 2008). Though these estimators theoretically have a maximum of one, their values are often close to zero even when populations have non-overlapping sets of alleles. This is because when heterozygosity is high, G_{ST} -like estimators approach zero regardless of the true genetic differences among populations. When using markers that have high heterozygosity, two other estimators may more adequately capture levels of differentiation: G'_{ST} (Hedrick 2005) and D_{est} (Jost 2008). There are often large differences between G_{ST} -like estimators and G'_{ST} and D_{est} (Heller & Siegismund 2009). Therefore, we calculated these three estimators and compared them to an F_{ST} -like estimator, ϕ_{PT}, which is appropriate for use with haploid data, such as the male bees used in this study.

We estimated ϕ_{PT} among all pairs of forest fragments using Genalex, and calculated the probability that ϕ_{PT} was significantly different from zero using 9999 permutations. We estimated the global ϕ_{PT} over all forest fragments in each year using the Analysis of Molecular Variance framework (AMOVA; Excoffier *et al.* 1992). We also estimated global G'_{ST} (Hedrick 2005) and D_{est} (Jost 2008) and tested for significance using 1,000 permutations using the online genetic software program SMOGD (Crawford 2010). Data were diploidized prior to analysis because SMOGD does not accept haploid data.

To estimate temporal genetic differentiation we calculated ϕ_{PT}, G'_{ST} and D_{est} for individuals caught in the three fragments sampled in both 2009 and 2010. We also calculated these measures between individuals pooled from the three fragments each year. For this population-based estimate of genetic differentiation among forest fragments our minimum sample size was eight. We used sites within forest fragments with smaller samples only in our individual-based estimates, such as our analysis of isolation by distance (see below).

Isolation By Distance

To test for correlations between genetic and geographic distances, we ran Mantel tests using one genetic distance matrix and two kinds of geographic distance matrices. We did this only for the samples obtained in 2010 because we sampled in only three locations in 2009. To generate a genetic distance matrix we first calculated haploid genetic distance *HGD* (Huff *et al*. 1993), using the program Genalex (Peakall & Smouse 2006). To calculate *HGD*, alleles shared between two individuals yield a distance of one and two alleles that are different yield a distance of 0. Distances are summed over loci to give a total distance between two individuals. The matrix we used for the Mantel test contained average *HGD* among all pairs of individuals between every pair of locations. We included locations that had more than four individuals for these analyses (10 locations). The first geographic distance matrix contained Euclidian (straight line) distances between all location pairs. Due to the geography of our study area surrounding Costa Rica's Golfo Dulce (Fig. S1), the shortest distance

between some location pairs was over water. Thus, the second geographic distance matrix was comprised of *overland* distances between locations. Overland distances were determined using the 'broken stick' method implemented in Davis *et al*. (2010), in which a series of straight lines are fitted to the coastline to estimate distance between two locations. We also estimated the scale of isolation by distance (IBD) using $F_{ST}/(1- F_{ST})$ as suggested by Rousset (1997), and the pattern was the same as when HGD was used; thus we report only the results based on HGD.

Landscape Genetics

Partial Mantel tests—To determine the extent to which levels of genetic differentiation can be explained by the type of environmental matrix we grouped location pairs into two groups: one in which they were separated by forest and another in which they were separated by deforested areas (Table S1). We then performed partial Mantel tests on three matrices, a genetic distance matrix with average pairwise HGD values among individuals between locations, a geographic distance matrix, and a matrix that indicated whether each pair of locations was separated by forest or not. We ran partial Mantel tests using the VEGAN package (Oksanen *et al*. 2011) for the R statistical programing language (R development core team, 2008). For this analysis we used the broken-stick estimate of geographic distance because the IBD analysis described above revealed that this measure of distance accounted for a greater proportion of the variation in genetic differentiation among sampling locations (see Results).

Bayesian Analysis Of Population Structure

To detect population structure we also used a Bayesian clustering method implemented in the program STRUCTURE v. 2.3.2 (Pritchard *et al*. 2000), which operates without *a priori* population assignment. STRUCTURE identifies the number of populations (K) that individuals belong to, and assigns individuals to populations. The resulting boundaries among genetic groups can then be matched to physical aspects of the landscape. We ran STRUCTURE for the individuals caught in 2010, without incorporating prior information, under the admixture model. We used the 'infer ALPHA' option (where ALPHA is the Dirichlet parameter for degree of admixture), and ran the program with correlated allele frequencies, using the $\lambda = 1$ option (λ parameterizes the allele frequency prior with a Dirichlet distribution of allele frequencies). We performed ten runs with a burn-in of 100,000 followed by 500,000 iterations. We also varied burn-in length and number of iterations to check the consistency of the results, performing one longer run with a burn-in of 200,000 followed by 1,000,000 iterations.

Detection Of Geographically Restricted Alleles

When studying highly mobile species, standard population and landscape genetic statistical tests often fail to detect small amounts of population subdivision even if it exists (Palumbi 2003). Therefore, we determined the extent to which alleles are distributed randomly through space, implemented using the program SAShA (Kelly & Oliver *et al*. 2010) and the MATLAB environment (Mathworks, Inc.). Assuming that alleles are identical by descent, non-random distributions of alleles can be considered departures from panmixia, and occurrences of the same allele in different locations can be considered evidence of gene flow. SAShA generates the observed distribution of geographic distances among instances of each allele, as well as a null distribution generated from the same data. The null distribution is the distribution of geographic distances between all pairs of samples in the data set regardless of allelic identity (the expectation under panmixia). SAShA then tests for a significant deviation of the arithmetic mean of the observed distribution (*OM*) from that of the null distribution (*EM*). An *OM* significantly less than *EM* indicates that alleles are

underdistributed, and that gene flow is somewhat restricted. We tested for significance of the difference between *OM* and *EM* (*Dg*) using 1000 permutations, as well as by using a jackknife procedure in which the data set was repeatedly reanalyzed after excluding each allele in turn.

RESULTS

We found high levels of dispersal among fragments separated by as much as 80 km. Levels of genetic diversity were moderate, which is consistent with high levels of dispersal. Haploid genetic diversity within fragments averaged 0.39 ± 0.06 in 2009 and 0.43 ± 0.06 in 2010, similar to the other estimators of genetic diversity (reported in Table 1). Allelic richness was moderate in both years, and differed between sites over years (Table 1).

Genetic Differentiation Among Forest Fragments

Our analysis of genetic differentiation among forest fragments revealed low spatial genetic structure within years, and differences among the measures used. For the 2009 samples, the global ϕ_{PT} value was 0.015 and was marginally significantly different from zero ($P = 0.082$; 95% CI [−0.01, 0.027]). Pairwise ϕ_{PT} values among fragments were also low (Table 2). The global G'_{ST} and D_{est} values were higher and differed significantly from zero: 0.083 (95% CI [0.054, 0.17]), and 0.056 (95%CI [0.022, 0.012]), respectively. For the 2010 samples, the global ϕ_{PT} value was 0.01 and was not significantly different from zero ($P = 0.31$; 95% CI [−0.03, 0.05]). Pairwise ϕ_{PT} values among forest fragments were also low (Table 2). The global G'_{ST} and D_{est} values were again higher than the ϕ_{PT} values, and were significantly different from zero: 0.15 (95% CI [0.11, 0.22]) and 0.10 (95% CI [0.07, 0.16]), respectively.

Temporal genetic differentiation between individuals caught in the same fragments in 2009 and 2010 was low for all estimators but, unlike spatial genetic differentiation within years, was significantly different from zero for all three measures. Similar to differentiation within years, ϕ_{PT} values were consistently lower than G'_{ST} and D_{est} values for levels of differentiation over all fragments between years. ϕ_{PT} between years over all fragments was 0.032 (95% CI [0.01, 0.28]), G'_{ST} was 0.067 (95% CI [0.04, 0.13], and D_{est} was 0.058 (95% CI [0.03, 0.11]). For each fragment between years, ϕ_{PT} values were also lower than G'_{ST} and *D* (Table 3).

ISolation By Distance

Our analysis of isolation by distance also suggested high levels of genetic exchange among locations. We found a positive but non-significant correlation between genetic and Euclidian geographic distance over the area sampled (Mantel test; Rxy = 0.14; $P = 0.31$). This pattern was strengthened, but remained non-significant, when overland distance was used (Mantel test; Rxy = 0.19; $P = 0.22$).

Landscape Genetics

Our landscape genetic analyses revealed no effect of forest cover on level of genetic differentiation between locations. For the individual-level analysis, partial Mantel tests showed no significant correlation between genetic distance and forest cover when geographic distance was held constant (r = 0.23; $P = 0.26$). The results from the Bayesian analysis of population structure implemented using STRUCTURE suggest that there is little genetic subdivision among bees within the area sampled; K = 1 received the highest support. The analysis using SAShA revealed that the average distance between co-occurring alleles was 34.1 m, and no evidence that alleles were either geographically restricted or overdispersed (OM was not significantly different from EM; $Dg = 3.3\times10^{-4}$; $P = 0.97$).

DISCUSSION

This study found significant temporal—but not spatial—genetic differentiation for the orchid bee *Eug. championi*. Orchid bees are among the largest bees and some species have been observed to travel over a kilometer in a single flight (Wikelski *et al*. 2010) and tens of kilometers in a day (Janzen 1971). However, it is unknown how flight ability translates into dispersal dynamics, *i.e.*, if some species of orchid bees have large home ranges but show site fidelity, or if they disperse far from their natal areas. It is thought that males are the dispersers and females are philopatric (Cocom Pech *et al*. 2008, Augusto & Garófalo 2010), but some genera-specific differences in dispersal may exist, at least for males. Mark-recapture studies have confirmed that some species – particularly in the genera *Eulaema*, *Eufriesea*, and *Exaerete* – have high recapture rates, which suggests some site fidelity. Other species—particularly in the genus *Euglossa*—have lower recapture rates, suggestive of lower site fidelity and possible longer distance dispersal (Ackerman & Montalvo 1985).

It is likely that *Eug. championi* sometimes disperses far from natal habitat. Previous work found no significant genetic structuring for *Eug. championi* sampled from forest fragments separated by up to 14 km in southern Costa Rica (Suni & Brosi 2011). The significant G'_{ST}, and *D* values found in this study indicate that over this area dispersal is restricted to some extent. However, for several fragments ϕ_{PT} values were not significant and G'_{ST} and *D* values were very low even between sites separated by 80 km. Furthermore, the results from the STRUCTURE and SAShA analyses suggest that the individuals sampled belong to one genetic group. This indicates that overall, there is a large amount of gene flow over this area. Our results differ from those of a mark-recapture study of orchid bees in an urban area, which found lower levels of dispersal than would be expected given the bees flight capability (López-Uribe *et al*. 2008).

Values of our F_{ST} -like estimator, ϕ_{PT}, were much lower than G'_{ST} and *D* values, often differing by an order of magnitude. These differences among estimators used are consistent with previous work on orchid bees (Freiria *et al*. 2011, Suni & Brosi 2011), and also with a suite of studies of genetic differentiation for a range of organisms (Heller & Siegismund 2009). This is because F_{ST} -like estimators sometimes underestimate true differentiation (*i.e.*, differences in allele frequencies across populations) particularly when heterozygosity is high (Jost 2008).

The significant differentiation between some locations, but no significant IBD even over 80 km suggests that patterns of movement of orchid bees are complex. It is likely that at least to some extent genetic differentiation is governed by distance; the IBD model (Rousset 1997) seems to hold for many ectothermic organisms if enough populations are sampled over a large enough spatial scale (Jenkins *et al*. 2010). It is possible that had we sampled over a larger area we would have found a significant association of genetic and geographic distance. However, population genetic studies of other orchid bee species over larger geographic areas have found inconsistent associations of genetic and geographic distance. Zimmerman *et al*. (2011) found significant IBD over 1000 km for *Eug. dilemma*. However, Freiria *et al*. (2011) did not find significant IBD over 850 km for *Eufriesea violacea*, and Dick *et al*. (2004) found identical mitochondrial haplotypes spanning the Andes mountains for some bees in the genera *Euglossa* and *Eufriesea*. Genetic differentiation may be governed by factors other than geographic distance. For example, in landscapes that are made up of discontinuous habitat, dispersal may occur via corridors that result in animals not following the most direct route (Townsend and Levey 2005). Indeed, the stronger pattern of IBD observed when overland rather than Euclidian geographic distance was used is consistent with the idea that bees travel more frequently over land and not over Costa Rica's Gulfo Dulce, which is about 20 km wide in some places.

Our data suggest that deforested areas in our study area do not restrict dispersal for *Eug. championi*. We found no significant difference in differentiation among locations separated by forest or deforested areas when distance among locations was taken into account. This suggests that there is some dispersal over deforested areas for *Eug. championi* in Costa Rica, though we cannot rule out the possibility that fragmentation occurred too recently to have captured a genetic signature of population isolation. For much of the area studied fragmentation occurred in the 1960s, though there has long been a history of fire, and clearing and agriculture by indigenous peoples (Clement & Horn 2001). It is also possible that in areas where there are fewer patches of forest or where the patches are farther apart, dispersal would be more restricted for these bees. Orchid bee species seem to differ in their propensity to leave forested areas (Milet-Pinheiro and Schlindwein 2005). It would therefore be worthwhile to sample *Eug. championi* in landscapes in which deforestation is more severe, such as on either side of large palm oil plantations—which are increasingly common in Costa Rica—to determine the extent to which large tracts of potentially inhospitable habitat affect dispersal.

Interestingly, we found significant temporal genetic differentiation over one year for *Eug. championi*. Explanations for temporal differences in allele frequencies between bees sampled in 2009 and 2010 include evolution within the population or migration to the site. Genetic bottlenecks resulting from selective sweeps could have caused temporal differences in the genetic composition of fragments. However, given that orchid bees have one or two generations a year, it is unlikely that evolutionary processes such as natural selection or genetic drift are responsible for the genetic differences between years. A more plausible explanation is that the temporal differentiation is due to migration into/out of the fragments of genetically different individuals after the first sampling and before the second. This explanation is consistent with our finding that allelic richness differed between pairs of sites sampled in 2009 and 2010 (Table 1). This explanation is also consistent with our population genetic data within years. We found low levels of differentiation among fragments that were tens of kilometers from one another, suggesting that orchid bees disperse considerable distances within their lifetime.

The small number of bees sampled in some fragments and small number of comparisons between 2009 and 2010 warrant additional investigations into patterns of genetic structure in *Eug. championi.* Hale *et al*. (2012) showed that for population genetic studies based on microsatellite genotypes, 25–30 diploid individuals is usually sufficient to accurately estimate allele frequencies. Our sample sizes were smaller than this, and may not have adequately captured the allele frequencies in the population. Had we increased our sample size we may have found significant IBD. However, *Eug. championi* seemed to be rare in some fragments, so obtaining larger sample sizes in a future study may be difficult and/or could have negative impacts on local populations. Despite our sample sizes, the temporal genetic differentiation we found here is consistent with results from non-genetic studies suggesting that at least a few species of male orchid bees have large home ranges (Janzen *et al*. 1981, Dressler 1982, Wikelski *et al*. 2010).

To what extent do our results provide insight into levels of dispersal for orchid bees as a clade? Orchid bees vary greatly in body size, coloration, behavior, and resource requirements (Roubik & Hanson 2004). Species also seem to vary in their levels of gene flow (Table 4). Freiria *et al*. (2011) and Zimmerman *et al*. (2011) found significant F_{ST} values over 130-850 km while Cerântola *et al*. (2011) did not find significant F_{ST} values over 440 km. Thus, patterns of dispersal may be species-specific. However, the number of population genetic studies on orchid bee species is still low; more work needs to be done to determine the extent to which interspecific or intergeneric differences exist. For example, to date there has been only one population genetic study of a species from the genus *Eulaema*

(Suni & Brosi 2011). This study found high levels of genetic structure, which seems to be an exception among the orchid bees. Furthermore, Suni and Brosi found that body size, which is sometimes used as a proxy for dispersal ability (Cane *et al*. 2006, Greenleaf *et al*. 2007), was not a good predictor of dispersal. In addition, F_{ST} values are not necessarily comparable among studies because they are heavily dependent on the level of heterozygosity of the markers used. It will be helpful for future studies to also use G'_{ST} *or D* so that more informative comparisons among studies can be made, and insight gained into possible behavioral, ecological or physiological factors that restrict or promote dispersal.

CONCLUSIONS

Overall we found low levels of genetic differentiation within years but significant genetic differentiation between years for *Eug. championi*, suggesting that there are high levels of dispersal within and among fragments. *Euglossa championi* may be resistant to negative effects of fragmentation due to an ability to fly over non-forested areas and utilize resources in other fragments or habitat types. Alternatively, deforestation may result in higher levels of physical stress on bees as they travel farther, or through open areas with higher temperatures. Higher levels of dispersal could lead to outbreeding depression or disease spread. Likewise, the downstream effects on plants could be negative, and include increased outbreeding depression, or could be positive, and include the maintenance of gene-flow in populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the staff at the Las Cruces Biological Station near San Vito, Costa Rica, H. Woodard at Saladero Lodge in Costa Rica, M. Kaplan and the staff of University of Arizona's genomics core facility for help with laboratory work, T. Brookhart for fieldwork assistance, and I. Hinojosa for translating the abstract into Spanish. SSS was supported by the Center for Insect Science at the University of Arizona (NIH grant 5K12 GM000708).

REFERENCES

Ackerman JD, Montalvo AM. Longevity of Euglossine bees. Biotropica. 1985; 17:79–81.

Augusto SC, Garofalo CA. Task allocation and interactions among females in *Euglossa carolina* nests (Hymenoptera, Apidae, Euglossini). Apidologie. 2010 DOI: 10.1051/apido/2010040.

Becker JJ, Dandwell DT, Smith WHF, Braud J, Binder B, Depner J, Fabre D, Factor R, Von Rosenberg J, Wallace G, Weatherall P. Global bathymetry and elevation data at 30 arc seconds resolution: Srtm30_Plus. Geodesy. 2009; 32:355–371.

BEye M, Hasselmann M, Fondrk MK, Page RP JR. Omholt SW. The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. Cell. 2003; 114:419–429. [PubMed: 12941271]

Biesmeijer JC, Roberts SPM, Reemer M, et al. Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. Science. 2006; 313:351–354. [PubMed: 16857940]

Brosi B, Daily GC, Shih TM, Ovideo F, Durán G. The effects of forest fragmentation on bee communities in tropical countryside. J. App. Ecol. 2007; 45:773–783.

Brosi B. The effects of forest fragmentation on euglossine bee communities: (Hymenoptera: Apidea: Euglossini). Biol. Conserv. 2009; 142:414–423.

Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswald TL. Patterns of widespread decline in North American bumble bees. Proc. Nat. Acad. Sci. USA. 2011; 108:662–667. [PubMed: 21199943]

Cane JH, Minckley RL, Kervin LJ, Roulston TH, Williams NM. Complex responses within a desert bee guild (Hymenoptera: Apiformes) to urban habitat fragmentation. Ecol. App. 2006; 16:632–644.

Cerântola N, Oi CA, Cervini M, Del Lama MA. Genetic differentiation of urban populations of Euglossa chordata from the state of São Paulo, Brazil. Apidologie. 2010 DOI: 10.1051/apido/2010055.

Clement R, Horn S. Pre-Columbian land-use history in Costa Rica: a 3000-year record of forest clearance, agriculture and fires from Laguna Zoncho. Holocene. 2001; 11:419–426.

Cocom Pech ME, De W, May-Itza J, Medina Medina LA, Quezada-Euan JJG. Sociality in *Euglossa* (*Euglossa*) *viridissima* Friese (Hymenoptera, Apidae, Euglossini). Insect. Soc. 2008; 55:428–433.

Crawford NG. smogd: software for the measurement of genetic diversity. Mol. Ecol. Res. 2010; 10:556–557.

Dick CW, Roubik DW, Gruber KF, Bermingham E. Long distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae: Euglossini) revealed by comparative mitochondrial DNA phylogeography. Mol. Ecol. 2004; 13:3775–3785. [PubMed: 15548290]

Dressler RL. Biology of the orchid bees (Euglossini). Ann. Rev. Ecol. System. 1982; 13:373–394.

Eberhard WG. Group nesting in two species of *Euglossa* bees. Journal of the Kansas Entomological Society. 1989; 61:406–411.

Eltz T, Sager A, Lunau K. Juggling with volatiles: exposure of perfumes by displaying male orchid bees. J. Comp. Physiol. A. 2005; 7:575–581.

Excoffier L, Smouse PE, Quattro JM. Analysis of Molecular Variance Inferred From Metric Distances Among DNA Haplotypes: Application. Genetics. 1992; 131:479–491. [PubMed: 1644282]

Goudet J. Fstat (Version 1.2): A computer program to calculate F-statistics. J. Heredity. 1995; 86:485–486.

Goulson D, Lye GC, Darvill B. Decline and conservation of bumble bees. Ann. Rev. Ent. 2008; 53:191–208.

Greenleaf SS, Williams NM, Winfree R, Kremen C. Bee foraging ranges and their relationship to body size. Oecologia. 2007; 153:589–596. [PubMed: 17483965]

Grixti JC, Wong LT, Cameron SA, Favret C. Decline of bumble bees (Bombus) in the North American Midwest. Biol. Conserv. 2009; 142:75–84.

Hedrick P. A standardized genetic differentiation measure. Evolution. 2005; 59:1633–1638. [PubMed: 16329237]

Hedrick PW, Parker JD. Evolutionary genetics and genetic variation of haplodiploids and x-lined genes. Ann. Rev. Ecol. Syst. 1997; 28:55–83.

Heller R, Siegismund HR. Relationship between three measures of genetic differentiation GST, DEST and G'ST: how wrong have we been? Mol. Ecol. 2009; 18:2080–2083. [PubMed: 19645078]

Huff D, Peakall R, Smouse PE. Rapd variation within and among natural populations of outcrossing buffalograss *Buchloe dactyloides*. Theor. App. Gen. 1993; 86:927–934.

Janzen DH. Euglossine bees as long-distance pollinators of tropical plants. Science. 1971; 171:203–205. [PubMed: 17751330]

Janzen DH. Reduction in euglossine bee species richness on Isla del Caño, a Costa Rican offshore island. Biotropica. 1981; 13:238–239.

Jost L. Gst and its relatives do not measure differentiation. Mol. Ecol. 2008; 17:4015–4026. [PubMed: 19238703]

Kimura M, CROW J. The number of alleles maintained in a finite population. Genetics. 1964; 49:725–738. [PubMed: 14156929]

Kosior A, Celary W, Olejniczak P, et al. The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of Western and Central Europe. Oryx. 2007; 41:79–88.

Kremen, C.; Chaplin-Kramer, R. Insects as providers of crop pollination and pest control. In: Stewart, AJA.; New, TR.; Lewis, OT., editors. Insect Conservation Biology. CABI; Cambridge, MA: 2007.

Ledig FT, Conkle MT. Genetic diversity and genetic structure in a narrow endemic Torrey pine (Pinus torreyana parry ex. Carr). Evolution. 1983; 37:79–85.

LÓPez-uribe MM, Oi CA, M. A. Del Lama. Nectar foraging behavior of Euglossine bees (Hymenoptera: Apidae) in urban areas. Apidologie. 2008; 39:410–418.

Milet-Pinhiro P, Schlindwein C. Do euglossine males (Apidae, Euglossini) leave tropical rainforest to collect fragrances in sugarcane monocultures? Rev. Brasil. de Zoolog. 2005; 22:853–858.

Oksanen J, Guillaume Blanchet F, Kindt R, Legendre R, et al. vegan: Community Ecology Package. R package version 1.17-10. 2011 http://CRAN.R-project.org/package=vegan.

Ollerton J, Winfree R, Tarrant S. How many flowering plants are pollinated by animals? Oikos. 2011; 120:321–326.

Palumbi SR. Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl. 2003; 13:S146–S158.

Paxton RJ, Zobel MU, Steiner J, Zillikens A, A. Microsatellite loci for Euglossa annectans (Hymenoptera: Apidae) and their variability in other orchid bees. Mol. Ecol. Res. 2009; 9:1221–1223.

Peakall ROD, Smouse PE. genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Not. 2006; 6:288–295.

Powell A, Powell G. Population dynamics of male euglossine bees in Amazonian forest fragments. Biotropica. 1987; 19:176–179.

PRimack, RB. Essentials of conservation biology. Sinauer Associates, Inc; Sunderland Mass, USA: 1993.

Pritchard J, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945–959. [PubMed: 10835412]

RamÍrez S, Dressler RL, Ospina M. Euglossine bees (Hymenoptera: Apidae) from the Neotropical Region: A species checklist with notes on their biology. Biota Colombiana. 2002; 3:7–118.

Roubik, D.; Hanson, P. Orchid bees: biology and field guide. InBio Press; Heredia: 2004.

Rousset F. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics. 1997; 145:1219–1228. [PubMed: 9093870]

Sala OE, Chapin FS III, Armesto JJ, J.J. et al. Global biodiversity scenarios for the year 2100. Science. 2000; 287:1770–1774. [PubMed: 10710299]

Sambrook, J.; Fritsch, EF.; Maniatis, T. Molecular cloning: a laboratory manual. 2nd edn.. Cold Spring Harbor Press; Cold Spring Harbor, New York: 1989.

Souza RO, Cervini M, Del Lama MA, PAxton RJ. Microsatellite loci for euglossine bees (Hymenoptera: Apidae). Mol. Ecol. Not. 2007; 7:1352–1356.

Souza RO, Del Lama MA, Cervini M, et al. Conservation genetics of neotropical pollinators revisited: microsatellite analysis suggests that diploid males are rare in orchid bees. Evolution. 2010 DOI: 10.1111/j.1558-5646.2010.01052.x.

Suni SS, Brosi BJ. Population genetics of orchid bees in a fragmented tropical landscape. Conserv. Gen. 2011; 13:323–332.

Taylor, A. Assessing the consequences of inbreeding for population fitness: past challenges and future prospects. Cambridge University Press; Cambridge, UK: 2003.

Tonhasca A, Blackmer JL, Albuquerque GS. Abundance and diversity of euglossine bees in the fragmented landscape of the Brazilian Atlantic forest. Biotropica. 2002a; 34:416–422.

Tonhasca A JR. Blackmer JL, Albuquerque GS. Within-habitat heterogeneity of euglossine bee populations: a re-evaluation of the evidence. J. Trop. Ecol. 2002b; 18:929–933.

Townsend PA, Levey DJ. An Experimental Test of Whether Habitat Corridors Affect Pollen Transfer. 2005; 86:466–475.

Vamosi JC, Knight TM, Steets JA, et al. Pollination decays in biodiversity hotspots. Proc. Nat. Acad. Sci. USA. 2006; 103:956–961. [PubMed: 16418284]

Wikelski M, Moxley J, Eaton-Mordas A, et al. Large-Range Movements of Neotropical Orchid Bees Observed via Radio. PLOS one. 2010; 5:e10738. [PubMed: 20520813]

Winfree R, Aguilar R, Vazquez DP, Lebuhn G, Aizen MA. A meta-analysis of bees' responses to anthropogenic disturbance. Ecology. 2009; 90:2068–2076. [PubMed: 19739369]

Wright S. The genetical structure of natural populations. Ann. Eug. 1951; 15:323–354.

Zayed A, Roubik DW, Packer L. Use of diploid male frequency a an indicator of pollinator decline. Apidologie Suppl. 3:S9–12.

Zayed A, Packer L. Complementary sex determination substantially increases extinction proneness of haplodiploid populations. Proc. Nat. Acad. Sci. USA. 2005; 102:10742–10746. [PubMed: 16020532]

Zayed A. Bee genetics and conservation. Apidologie. 2009; 40:237–262.

Zimmermann Y, Schorkopf DLP, Moritz RFA, Pemberton RW, Quezada-Euan JJG, Eltz T. Population genetic structure of orchid bees (Euglossini) in anthropogenically altered landscapes. Conservation Genetics. 2011 doi:10.1007/s10592-011-0221-1.

TABLE 1

Four measures of genetic diversity for each forest fragment, each year, including the number of alleles, *Na*, the number of effective alleles, *Nef*, unbiased haploid genetic diversity, *Div*, and allelic richness.

Forest fragment	**Year**	***n* males**	***Na* ± SE**	***Nef* ± SE**	***Div* ± SE**	***Allelic richness***
Las Alturas	2009	11	2.1 ± 0.44	1.8 ± 0.38	0.31 ± 0.12	2.12
LC Station	2009	25	4.6 ± 1.1	2.5 ± 0.72	0.41 ± 0.12	3.83
Romero II	2009	19	3.9 ± 0.72	2.3 ± 0.51	0.45 ± 0.11	3.65
Average	------	---	**3.5 ± 0.49**	**2.2 ± 0.31**	**0.39 ± 0.06**	**3.20**
Osa	2010	59	5.4 ± 1.4	2.5 ± 0.72	0.39 ± 0.11	2.82
Las Alturas	2010	17	3.4 ± 0.96	2.6 ± 0.79	0.42 ± 0.13	2.79
LC Station	2010	8	2.3 ± 0.45	1.9 ± 0.39	0.40 ± 0.14	2.23
Romero II	2010	12	3.4 ± 0.60	2.3 ± 0.43	0.50 ± 0.11	2.97
Average	------	---	**3.6 ± 0.48**	**2.3 ± 0.29**	**0.43 ± 0.06**	**2.65**

TABLE 2

Levels of genetic differentiation calculated as ϕ_{PT} for each pair of fragments in 2009 and 2010. Asterisks represent *P*-values values less than 0.05 based on 9999 permutations.

	2010	**2009**	**2010**	**2009**	**2010**
	Osa	**Las Alturas**	**Las Alturas**	**LC Station**	**LC Station**
Osa	------	------	------	------	------
Las Alturas	0.007	------	------	------	------
LC Station	0.004	0.046*	0.000	------	------
Romero II	0.024	0.001	0.008	0.004	0.000

TABLE 3

Temporal genetic differentiation calculated as ϕ_{PT}, G'_{ST}, and D_{est}, for individuals in three fragments, as well as over all fragments between 2009 and 2010. Asterisks represent *P*-values values less than 0.05.

	Las Alturas	LC Station	Romero II	Pooled fragments
ϕ_{PT}	0.07* [0.01, 0.13]	0.024 [−0.03, 0.07]	0.009 [−0.04, 0.05]	0.032* [0.01, 0.03]
G'_{ST}	0.18* [0.13, 0.26]	0.093* [0.05, 0.17]	0.11* [0.05, 0.17]	0.067* [0.04, 0.13]
D	0.15* [0.11, 0.22]	0.086* [0.05, 0.16]	0.09* [0.05, 0.17]	0.058* [0.03, 0.11]

TABLE 4

Summary of population genetic studies utilizing microsatellites on orchid bees to date including species sampled, location, number of microsatellite loci used, the number of sites included in the analysis, average expected heterozygosity, values of F_{ST} -like estimators and Jost's D_{est}, and the associated geographic distances between locations for which those estimates were made. Estimators marked with * were significantly different from zero at the $P < 0.05$ level.

Reference	Species	Location	# loci	# sites	*Hexp*	F_{ST}	Jost'*D*	Distance
Freiria et al (2011)	*Eufriesea violacea*	Brazil	6	6	0.74	0.02 –0.11*	0.05 – 0.32*	130 – 850 km
Cerântola et al (2010)	*Euglossa cordata*	Brazil	9	11	-----	0.003	-----	440 km
Suni and Brosi (2011)	*Euglossa championi*	Costa Rica	8	5	0.32	0.00	0.031*	14 km
Suni and Brosi (2011)	*Eulaema bombiformis*	Costa Rica	9	6	0.67	0.01*	0.28*	14 km
Current study	*Euglossa championi*	Costa Rica	8	4	0.43	0.015	0.10*	81 km
Zimmerman et al (2011)	*Euglossa dilemma*	Mexico, US	3	8	0.82	0.051*	-----	1000 km