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Berry Brosi, Emory University
SS Suni, University of Arizona
JL Bronstein, University of Arizona

Journal Title: Biotropica
Volume: Volume 46, Number 2
Publisher: WILEY | 2014-03-01, Pages 202-209
Type of Work: Article
Publisher DOI: 10.1111/btp.12084
Permanent URL: https://pid.emory.edu/ark:/25593/tw9sq

Final published version: http://dx.doi.org/10.1111/btp.12084

Accessed September 26, 2019 6:22 PM EDT
Spatio-temporal Genetic Structure of a Tropical Bee Species Suggests High Dispersal Over a Fragmented Landscape

Sevan S. Suni1,1,3,5, Judith L. Bronstein2, and Berry J. Brosi4
1Center for Insect Science, University of Arizona, Tucson AZ 85721, U.S.A
2Department of Ecology and Evolutionary Biology, University of Arizona, Tucson AZ 85721, U.S.A
3Organismic and Evolutionary Biology Program, University of Massachusetts, Amherst MA 01003, U.S.A
4Department 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 \( \varphi_{PT} \), 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.
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 \((\text{Div})\) as \((1/n)[1 - \Sigma p_i^2]\), where \(p_i\) is the frequency of allele \(i\), and \(n\) is the number of loci. \(\text{Div}\) is the average over all loci of the probability that two individuals will be genetically different at one locus. We calculated \(\text{Div}\), as well as the actual \((\text{Na})\) and effective \((\text{Nef})\) 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, \(\phi_{pt}\), which is appropriate for use with haploid data, such as the male bees used in this study.

We estimated \(\phi_{pt}\) among all pairs of forest fragments using Genalex, and calculated the probability that \(\phi_{pt}\) was significantly different from zero using 9999 permutations. We estimated the global \(\phi_{pt}\) 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 \(\phi_{pt}\), \(G'_{st}\), 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 programming 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 ($\lambda$ 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 ($D_g$) 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 $\phi_{PT}$ value was 0.015 and was marginally significantly different from zero ($P = 0.082; 95\% \text{ CI } [-0.01, 0.027])$. Pairwise $\phi_{PT}$ 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 $\phi_{PT}$ value was 0.01 and was not significantly different from zero ($P = 0.31; 95\% \text{ CI } [-0.03, 0.05])$. Pairwise $\phi_{PT}$ values among forest fragments were also low (Table 2). The global $G'_{ST}$ and $D_{est}$ values were again higher than the $\phi_{PT}$ 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, $\phi_{PT}$ values were consistently lower than $G'_{ST}$ and $D_{est}$ values for levels of differentiation over all fragments between years. $\phi_{PT}$ 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, $\phi_{PT}$ 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; $R_{xy} = 0.14; P = 0.31$). This pattern was strengthened, but remained non-significant, when overland distance was used (Mantel test; $R_{xy} = 0.19; P = 0.22$).

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; $D_g = 3.3 \times 10^{-4}; P = 0.97$).
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, Eupriesea, 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 $\phi_{PT}$ 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, $\phi_{PT}$, 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 *Eupriesea violacea*, and Dick et al. (2004) found identical mitochondrial haplotypes spanning the Andes mountains for some bees in the genera *Euglossa* and *Eupriesea*. 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 Schindwein 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*.
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).

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Biotropica. Author manuscript; available in PMC 2015 March 01.


## 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.

<table>
<thead>
<tr>
<th>Forest fragment</th>
<th>Year</th>
<th>$n$ males</th>
<th>$Na \pm SE$</th>
<th>$Nef \pm SE$</th>
<th>$Div \pm SE$</th>
<th>Allelic richness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Las Alturas</td>
<td>2009</td>
<td>11</td>
<td>2.1 ± 0.44</td>
<td>1.8 ± 0.38</td>
<td>0.31 ± 0.12</td>
<td>2.12</td>
</tr>
<tr>
<td>LC Station</td>
<td>2009</td>
<td>25</td>
<td>4.6 ± 1.1</td>
<td>2.5 ± 0.72</td>
<td>0.41 ± 0.12</td>
<td>3.83</td>
</tr>
<tr>
<td>Romero II</td>
<td>2009</td>
<td>19</td>
<td>3.9 ± 0.72</td>
<td>2.3 ± 0.51</td>
<td>0.45 ± 0.11</td>
<td>3.65</td>
</tr>
<tr>
<td>Average</td>
<td>------</td>
<td>---</td>
<td>3.5 ± 0.49</td>
<td>2.2 ± 0.31</td>
<td>0.39 ± 0.06</td>
<td>3.20</td>
</tr>
<tr>
<td>Osa</td>
<td>2010</td>
<td>59</td>
<td>5.4 ± 1.4</td>
<td>2.5 ± 0.72</td>
<td>0.39 ± 0.11</td>
<td>2.82</td>
</tr>
<tr>
<td>Las Alturas</td>
<td>2010</td>
<td>17</td>
<td>3.4 ± 0.96</td>
<td>2.6 ± 0.79</td>
<td>0.42 ± 0.13</td>
<td>2.79</td>
</tr>
<tr>
<td>LC Station</td>
<td>2010</td>
<td>8</td>
<td>2.3 ± 0.45</td>
<td>1.9 ± 0.39</td>
<td>0.40 ± 0.14</td>
<td>2.23</td>
</tr>
<tr>
<td>Romero II</td>
<td>2010</td>
<td>12</td>
<td>3.4 ± 0.60</td>
<td>2.3 ± 0.43</td>
<td>0.50 ± 0.11</td>
<td>2.97</td>
</tr>
<tr>
<td>Average</td>
<td>------</td>
<td>---</td>
<td>3.6 ± 0.48</td>
<td>2.3 ± 0.29</td>
<td>0.43 ± 0.06</td>
<td>2.65</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>2009</td>
<td>2010</td>
<td>2009</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Osa</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Las Alturas</td>
<td>0.007</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>LC Station</td>
<td>0.004</td>
<td>0.046*</td>
<td>0.000</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Romero II</td>
<td>0.024</td>
<td>0.001</td>
<td>0.008</td>
<td>0.004</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Levels of genetic differentiation calculated as $\phi_{PT}$ for each pair of fragments in 2009 and 2010. Asterisks represent $P$-values values less than 0.05 based on 9999 permutations.
TABLE 3
Temporal genetic differentiation calculated as $\phi_{PT}$, $G'_{ST}$, and $D_{ST}$, for individuals in three fragments, as well as over all fragments between 2009 and 2010. Asterisks represent $P$-values values less than 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Las Alturas</th>
<th>LC Station</th>
<th>Romero II</th>
<th>Pooled fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi_{PT}$</td>
<td>0.07* [0.01, 0.13]</td>
<td>0.024 [-0.03, 0.07]</td>
<td>0.009 [-0.04, 0.05]</td>
<td>0.032* [0.01, 0.03]</td>
</tr>
<tr>
<td>$G'_{ST}$</td>
<td>0.18* [0.13, 0.26]</td>
<td>0.093* [0.05, 0.17]</td>
<td>0.11* [0.05, 0.17]</td>
<td>0.067* [0.04, 0.13]</td>
</tr>
<tr>
<td>$D$</td>
<td>0.15* [0.11, 0.22]</td>
<td>0.086* [0.05, 0.16]</td>
<td>0.09* [0.05, 0.17]</td>
<td>0.058* [0.03, 0.11]</td>
</tr>
</tbody>
</table>
### 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.

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