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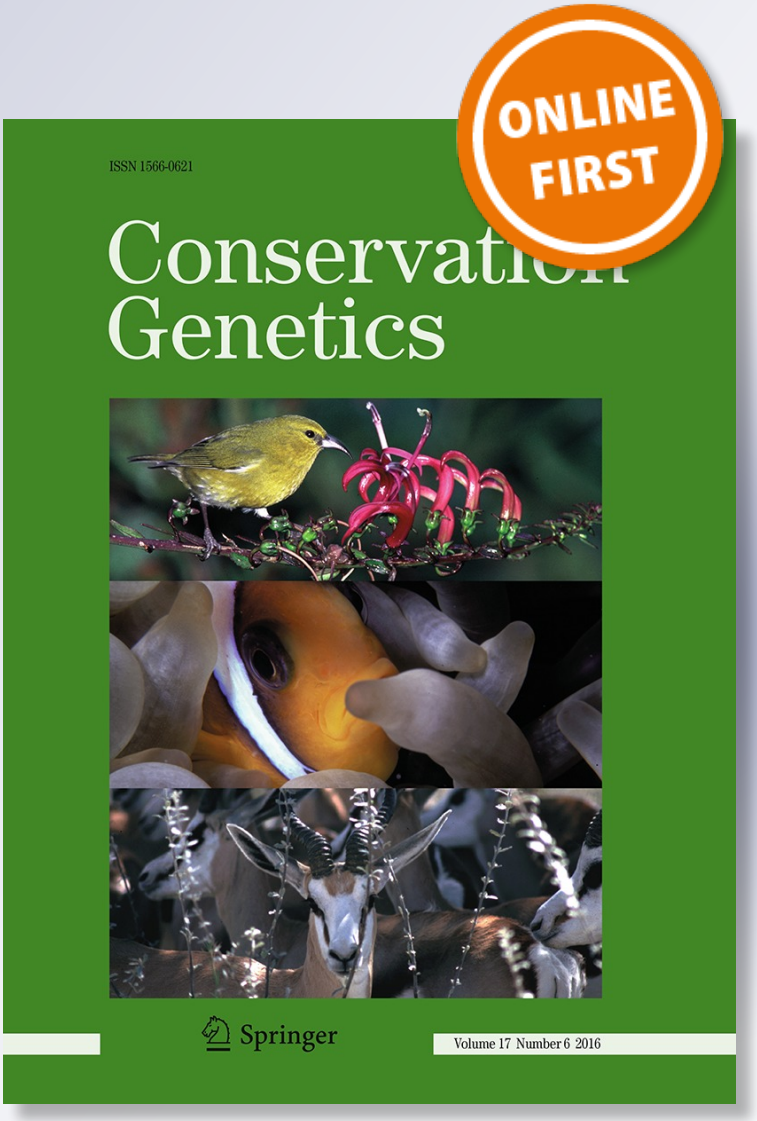

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RESEARCH ARTICLE

Dispersal of the orchid bee *Euglossa imperialis* over degraded habitat and intact forest

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Abstract Fragmentation of habitat can decrease resource availability and restrict movement among geographic areas. Persistence in fragmented landscapes depends on the maintenance of connectivity among populations, without which genetic diversity may decrease and lead to population declines. Bees are particularly vulnerable to the negative effects of low genetic diversity so it is important to understand patterns of dispersal for native bees living in fragmented areas. I used population genetic techniques to characterize patterns of genetic diversity and dispersal for the orchid bee *Euglossa imperialis* within and among forest fragments in southern Costa Rica, in which the furthest two fragments were 226 km from one another. In addition, I compared results of population genetic analyses conducted with all bees sampled, and results from analyses conducted with a reduced dataset containing only one individual per full sibling family from each site. For both datasets genetic diversity was low within forest fragments, with expected heterozygosity averaging 0.28 for the full dataset and 0.29 for the dataset containing only one full sibling per site. I found no evidence that deforested areas restricted dispersal; pairwise estimates of genetic differentiation F'_{ST} among forest fragments averaged 0.01 for the full dataset, and 0 for the dataset containing only one full sibling per site. Genetic distance among sites within forest fragments was significantly correlated to geographic distance for the full dataset, but there was no significant correlation for the dataset that contained only one individual from each full sibling family. This suggests that family structure can drive results of analyses of genetic structure, although reductions in sample sizes following removal of full siblings may have reduced power to detect genetic structure. Despite no evidence for restricted dispersal, the low genetic diversity found suggests that *E. imperialis* may be an important candidate for future conservation monitoring.

Keywords Euglossine · Orchid bee · Dispersal · Genetic structure · Genetic diversity · Fragmentation

Introduction

Over 50% of the earth's surface has been converted from natural habitat for agricultural use or human infrastructure (Hooke and Martín-Duque 2012). Short-term consequences of habitat conversion include crowding of individuals into smaller fragments, which can lead to deprivation of resources and population declines (Ewers and Didham 2005). Over the longer-term, consequences of land conversion can include reduced genetic diversity, due to population reduction, restricted dispersal, or genetic drift within isolated fragments (Gaggiotti and Hanski 2004). Reduced genetic diversity can lead to further population declines and increase extinction risk (Taylor 2003), so it is important to characterize the extent to which dispersal is restricted for populations living in fragmented areas.

Understanding the consequences of habitat fragmentation for native bee pollinators is particularly salient for several reasons. First, bees are responsible for the pollination of almost 90% of all angiosperm species (Ollerton

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et al. 2011). Second, wild bees will visit nearby agricultural crops (Kremen et al. 2002; Ricketts et al. 2008), and pollination by bees increases seed set in about two-thirds of crops (Kremen and Chaplin-Kramer 2007). Finally, bees may be particularly vulnerable to negative impacts of habitat loss (Winfree et al. 2009). Bees are haplodiploid, so they theoretically have lower effective population sizes relative to other groups (Crozier 1976; Hedrick and Parker 1997). In addition, their system of sex determination operates such that genotype at the *csd* locus determines sex, with heterozygotes developing into females and hemizygotes developing into males (Beye et al. 2003). In this single-locus sex determining system, homozygous individuals develop into males (van Wilgenburg et al. 2006). Reduced genetic diversity could theoretically lead to population declines because diploid males are sterile (Zayed and Packer 2005).

Orchid bees (also known as Euglossine bees; Hymenoptera: Apidae, tribe Euglossini) are emerging as an important group for the study of bee responses to land use change. They are a Neotropical clade that is well known as important pollinators of over 700 species of orchids and other tropical plants due to their extraordinary dispersal abilities and the unique behavior of males (Roubik and Hanson 2004). Males collect perfumes in flowers for later use in courtship behavior (Eltz et al. 2005a), so they can be easily captured using similar chemical baits.

Fragmentation seems to have mixed effects on the abundance, species diversity, and dispersal of orchid bees. Some studies report significant reductions following experimental deforestation (Powell and Powell 1987), in fragments with smaller cores (Nemésio and Silveira 2010), or in smaller fragments (Brosi 2009). Other studies report no association between fragment size and abundance and species diversity (Becker et al. 1991; Tonhasca et al. 2002a,b; Brosi et al. 2007), but these studies had smaller sample sizes and (Becker et al. 1991); Tonhasca et al. (2002a,b) and took place in landscapes that were dominated by large tracts of forest. In areas where forest remnants are present throughout the landscape, they may act as corridors for orchid bees (Silviera et al. 2015). Given that orchid bees have the potential to disperse tens of kilometers (Janzen 1971; Dressler 1982; Wikelski et al. 2010; Pokorny et al. 2015), effects of fragmentation may become apparent only when fragments are far from large tracts of forest (Moura De Aguiar et al. 2015). Population genetic work suggests that dispersal is restricted for some species (Zimmermann et al. 2011; Freiria et al. 2012; Suni and Brosi 2012; Boff et al. 2013), and genetic diversity may be lower (Rosa et al. 2016), and diploid male load higher in smaller, more isolated populations (Giangarelli et al. 2015; but see Souza et al. 2010). However, some studies found no evidence for restricted dispersal over relatively large geographic areas, in which the furthest sites were more than 50 km from one another (Sofia et al. 2005; Cerântola et al. 2010; López-Uribe et al. 2014; Suni et al. 2014). Given these mixed results, our understanding of responses of orchid bees to land use change will benefit from additional population genetic studies over fragmented areas.

I used population genetic techniques to assess patterns of dispersal of the orchid bee *Euglossa imperialis* over a fragmented area in southern Costa Rica in which the furthest two fragments were 226 km from one another. First, I investigated how genetic distance among individuals changes with geographic distance. Second, I investigated if deforested areas restrict dispersal. Finally I used a Bayesian clustering approach to investigate if individuals are partitioned into distinct genetic groups or if the individuals comprise a genetically homogeneous population.

Methods

Species and sampling

In April of 2010 I sampled *E. imperialis* males from seven forest fragments in Southern Costa Rica, focusing on the Osa Peninsula and sites surrounding the Gulfo Dulce, and radiating out west and north from the Peninsula (Fig. 1). Sites C, D, F, H, L, M, and O were in primary forest, and the other sites were in fragments that had been disturbed and were in earlier successional stages. *E. imperialis* is a medium-sized orchid bee, averaging about 15 mm in length, and has been observed pollinating orchids in at least 18 genera (Roubik and Hanson). I focused on *E. imperialis* for the current study for three reasons. First this bee is intermediate in body size relative to two other orchid bees on which pervious population genetic work was conducted over a similar geographic area (Suni and Brosi 2012). Second, it was one of the species most commonly caught while sampling was being conducted for a different study (Suni et al. 2014). Third, Costa Rica is in the middle of this species' range, which spans much of Central and South America.

The landscape in the sampling area contains a mixture of forest, agricultural areas, oil palm plantations, rural areas, and towns. High levels of forest fragmentation occurred primarily the 1960s and reduced forest cover to about 15% of what it had been, though pollen records suggest there has been a long history of forest clearing, agriculture, and fire by indigenous peoples (Clement and Horn 2001). Within each of the seven forest fragments I caught bees using the chemical baits 1,8-cineole and methyl salicylate (Janzen 1981). I sampled at 1–6 sites within each fragment, for a total of 17 sites. Some of these sites were also sampled in two previous studies on different

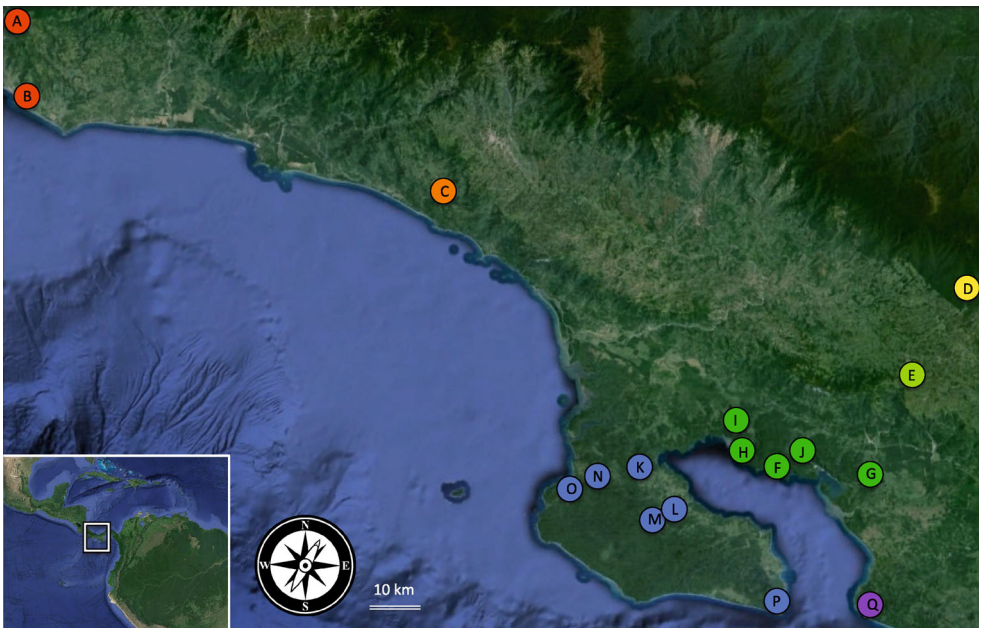

Fig. 1 Sites throughout southern Costa Rica with *colors* representing forest fragments and letters representing sites. Forest fragment 1 is represented by *red* (sites *A* and *B*), fragment 2 by *orange* (site *C*), fragment 3 by *yellow* (site *D*), fragment 4 by *light green* (site *E*), fragment 5 by *dark green* (sites *G–J*), fragment 6 by *blue* (sites *K–P*), and fragment 7 by *purple* (site *Q*). (Color figure online)

orchid bee species (Sites D & E, Suni and Brosi 2012; Sites D–L, N, and O, Suni et al. 2014). In the latter study, sites F–L, N, and O were considered part of one forest fragment, but due to updated satellite imagery (Google Earth version 7.1.5.1557), and the presence of a road, I chose to designate sites F–J as a distinct forest fragment for population genetic analysis in the current study (see below). The furthest two sites were 226 km apart, and the closest two were 1.8 km apart. At each site, I netted male bees between 7 and 10 am and spent at least one hour sampling. Bees tended to arrive within 15 min of the initiation of sampling, and I waited at least 15 min after the last bee was caught before ceasing sampling. I visited sites G to K on two different days, and visited the remainder of sites during only one day. Bees typically arrived at baits within 15 min of sampling, and new arrivals tended to cease after about thirty minutes. The final sample included 1–65 bees per fragment, for a total of 158 *E. imperialis* (Table 1; Online Resource 1).

Table 1 Sample sizes and number of alleles averaged over loci pre and post-removal of full siblings for each site within each fragment

Fragment	Site	Full	No-sib	Full N_a	No-sib N_a
1	A	2	2	1.1	1.1
1	B	15	7	2.9	2.7
2	C	4	2	1.5	1.3
3	D	1	1	1	1
4	E	1	1	1	1
5	F	17	7	2.4	2.3
5	G	8	4	1.6	1.6
5	H	17	7	2.1	2.1
5	I	8	4	2.2	1.8
5	J	12	4	2.0	1.7
6	K	14	5	2.0	1.9
6	L	15	6	2.0	1.7
6	M	11	5	1.6	1.6
6	N	10	6	2.6	2.1
6	O	8	5	2.3	2.3
6	P	7	2	1.5	1.3
7	Q	8	4	1.9	1.7

See Online Resource 1 for genetic diversity measures at the level of the fragment

Genetic analyses

I performed genetic analyses at the Genomics Core facility at the University of Arizona. First I extracted DNA using a phenol–chloroform extraction procedure (Sambrook et al. 1989) and then amplified DNA at nine microsatellite loci that were unlinked in the species for which they were developed, and that were labeled with fluorescent dyes (Applied Biosystems, Foster City, CA): Egc 17, Egc 18, Egc 24, Egc 26, Egc 30a, Egc 35, Egc 37, Egc 51 (Souza et al. 2007), and Ann28 (Paxton et al. 2009). I determined optimal PCR conditions for each primer pair and then multiplexed loci together in two batches. Egc 26 and Egc 30a did not amplify during PCR optimization and were dropped from the analysis. The PCR conditions for the remaining loci were as follows: 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 individuals were genotyped more than once to verify that genotyping error rates were negligible. PCR products were run on an

ABI 3730 (Applied Biosystems) automated DNA sequencer, and the microsatellite lengths were analyzed using GENEMAPPER (Applied Biosystems, Carlsbad, CA, USA), and Geneious software v7.0 (Biomatters Ltd, Auckland, New Zealand). All samples were scored twice (Online resource 4 contains a table of genotypes generated from non-full siblings, see below).

Sibship reconstruction

The presence of family structure within data sets can bias estimates of population genetic structure (Rodriguez-Ramilo and Wang 2012). Therefore, I used the following approach to circumvent any potential problems caused by family structure, following Whiteley et al. (2013) and Whiteley et al. (2014). I used the program COLONY version 1.2 (Wang 2004) to assign individuals to full-sibling families. I then generated a data set with one randomly chosen one individual from each family (hereafter the no-sib dataset; Table 1 shows sample sizes for each site pre and post-removal of full siblings). I then conducted the population genetic analyses described below both with the full data set (containing full-siblings) and with the no-sib dataset, and compared the resulting patterns of genetic structure. The results from the full data set may be more comparable to previous population genetic studies on orchid bees in which the authors did not follow the approach described above and may have had full siblings in their samples.

Genetic diversity

I used the genetic software program Genalex (Peakall and Smouse 2006) to calculate two measures of genetic diversity for each forest fragment. First, I calculated the number of alleles (N_a). Second, I calculated average haploid genetic diversity per fragment (h) as $(1/n)[1 - \sum pi^2]$, where p_i is the frequency of allele i, and n is the number of loci. Thus, h is the probability that two individuals will be genetically different at one locus, averaged over all loci, and is analogous to expected heterozygosity. Third I used Genalex to calculate unbiased haploid genetic diversity (uh), which includes a correction for sample size. I also used the R package hierfstat (Goudet and Jombart 2015) to calculate allelic richness (A_r), which is based on rarefaction and thus allowed for comparison of genetic diversity among samples of different sizes. I estimated null allele or allelic dropout frequency for each locus among individuals at each site. For haploid individuals, both null alleles and allelic dropout manifest as the lack of a genotype at a given locus. Therefore, I estimated the rates of null alleles and allelic dropout as the proportion of individuals for whom no microsatellite amplified as in Souza et al. (2010). I tested for linkage disequilibrium among loci using the program Genepop (Rousset 2008).

Genetic structure

I performed several population genetic analyses to characterize patterns of dispersal over the geographic area sampled and understand what may be driving those patterns. First, I characterized genetic differentiation among all pairs of sites, and investigated if these patterns of genetic differentiation were explained by geographic distance or by landscape characteristics. Because sample sizes within sites were small (Table 1), I estimated genetic distance among pairs of sites following Suni et al. (2014). I calculated a measure of genetic distance among all pairs of individuals from sites at which more than one individual was sampled ($n = 70$ from 15 sites; see Table 1 for reduction in sample sizes after removing full siblings). I calculated haploid genetic distance (HGD) using the program Genalex, where alleles shared between two individuals yield a distance of one and two alleles that are different yield a distance of 0. The total distance between two individuals is the sum over all loci. The genetic distance between each pair of sites was then the average of the HGD values that had been calculated among all individuals between that pair of sites. To determine if there was an association between genetic and geographic distance, that is, whether there was isolation by distance (IBD), I ran Mantel tests implemented in Genalex with 9999 permutations. I used the logarithm of geographic distance in Mantel tests, following Rousset (1997). I determined if there was significant IBD over all sites, using a genetic distance matrix containing average HGD among all pairs of individuals between every pair of locations. Using different cutoffs for the minimum number of individuals sampled per site in the genetic distance calculation did not qualitatively change results, therefore I report results for analyses in which two or more individuals were sampled. I also investigated if there was IBD over geographic areas dominated by forest by testing for IBD using sites within the three southerly most fragments, and within the sites in fragments 5 and 6. I ran the Mantel tests with both Euclidian (straight line) distances between all location pairs and also with 'broken stick' distances as in Davis et al. (2010), which are the shortest overland distances between two locations. I used both types of distances because the study area surrounded Costa Rica's Golfo Dulce (Fig. 1), making the shortest distance between some site pairs over water.

Second, to determine the independent contribution of geographic distance and land cover to variation in genetic distance (HGD) among sites I used a multiple regression approach with a randomized permutation procedure

(MMRR). MMRR is conceptually similar to a partial Mantel test, which is a standard test for assessing the significance of a correlation between distance matrices while controlling for effects of a third matrix. Mantel tests have been the subject of recent controversy due to low power and high Type I error rates (Legendre and Fortin 2010; Guillot and Rousset 2013; Meirmans 2015). MMRR may be preferable to Mantel tests because of more appropriate Type I error rates than Mantel tests, and because MMRR allows for variables to be ranked in terms of their relative effect on the dependent variable (Wang 2013). I implemented MMRR using the R function from Wang (2013), with 100,000 permutations. The three distance matrices included a matrix of pairwise HGD among sites, a matrix of pairwise overland distances among sites, and for the shortest overland distance between two sites, a matrix specifying the percent of the distance that was deforested. Forest cover was assessed using the path tool in Google Earth version 7.1.5.1557, and all deforested areas greater than 0.25 km were included. Agricultural areas, including palm oil plantations, a common crop in southern Costa Rica, were considered deforested areas.

Third, I estimated genetic differentiation among forest fragments using the program Genodive (Meirmans and Van Tienderen 2004). I pooled individuals caught within forest fragments and used Nei's unbiased estimator of G_{ST} (Nei 1987) as an estimate of F_{ST} (Wright 1951) and G'_{ST} (Meirmans and Hedrick 2011) as an estimator of $F'_{ST} \cdot F'_{ST}$ was developed to account for the bias that F_{ST}—like estimators have when heterozygosity is low (Jost 2008). I included only the three southerly most fragments in these analyses because these fragments had at least 9 individuals that were not full siblings (range 9–29; average 21), and the remaining fragments had far smaller sample sizes (range 1–4). I tested for locus-specific allele frequency (genic) differences among sites using Fisher's exact probability tests implemented in Genepop, as described in Ryman et al. (2006). I used a method that corrects for increased Type I error while also minimizing the level of falsely rejected null hypotheses to estimate the overall statistical significance of the multiple comparisons (Narum 2006).

Finally, I estimated the number of genetic groups in the dataset using a Bayesian clustering method implemented in Structure version 2.3.2 (Pritchard et al. 2000). For a given number of groups (K), STRUCTURE assigns a portion of each individual's genome to these groups. I ran Structure with correlated allele frequencies, under the admixture model, using the $\lambda = 1$, and the "infer ALPHA" option. I performed 15 runs with a burnin of 50,000 followed by 100,000 iterations, which included three replicates in which I allowed K to vary from one to five, using both the no LOCPRIOR and LOCPRIOR models. LOCPRIOR uses location information in the prior and may detect subtle population structure (Hubisz et al. 2009). In LOCPRIOR runs, I confirmed that the parameter r was close to or <1, meaning that locations were informative, as in Hubisz et al. (2009). To infer the K value that best fit the data I used the ad hoc ΔK method implemented using the program Structure Harvester (Earl and vonHoldt 2012).

Results

There was low to moderate genetic diversity within forest fragments for *E. imperialis* (Table 1; Online Resource 1). I caught only one *E. imperialis* in fragments 3 and 4 so I did not estimate genetic diversity for these fragments. For the remaining fragments, for the full data set, the number of alleles per locus averaged 2.5, haploid genetic diversity averaged 0.28, unbiased haploid genetic diversity averaged 0.32, and allelic richness averaged 1.9. Measures of genetic diversity were similar for the no-sib data set; the number of alleles per locus averaged 2.3, haploid genetic diversity averaged 0.29, unbiased haploid genetic diversity averaged 0.35, and allelic richness averaged 1.4 (Online Resource 1). In the full data set the proportion of individuals for whom no microsatellite allele amplified, averaged over sites, ranged from 0 to 0.49 among loci (average 0.11; Online Resource 2). There was no evidence for linkage disequilibrium among loci ($P > 0.05$ for all pairs of loci; Online Resource 3).

Euclidian geographic distance and genetic distance HGD were significantly correlated in the full data set (Mantel test $R_{xy} = 0.19$, $P = 0.038$), as was overland geographic distance and HGD (Mantel test $R_{xy} = 0.37$, $P < 0.001$). There was also significant IBD using Euclidian geographic distance, but no significant IBD using overland geographic distance, when using only locations within fragments 5 & 6, which are connected by a small band of forested area but are separated by a road (Mantel test using Euclidian distance: $R_{xy} = 0.29$, $P = 0.02$; Mantel test using overland distance $R_{xy} = 0.21$, $P = 0.065$). In contrast, for the no-sib dataset there was no significant relationship between Euclidian geographic distance and HGD (Mantel test $R_{xy} = 0.08$, $P = 0.21$), nor overland geographic distance and HGD (Mantel test $R_{xy} = 0.08$, $P = 0.26$). There was also was no significant relationship of either kind of geographic distance and HGD for a dataset that contained just fragments 5 & 6 (Mantel using Euclidian distance $R_{xy} = 0.18$, $P = 0.066$; Mantel test using overland distance $R_{xy} = 0.19$, $P = 0.065$).

Genetic differentiation was low among fragments for both datasets and there was no evidence that deforested areas restricted dispersal for *E. imperialis*. For the full

dataset, pairwise F_{ST} among fragments averaged 0.008 (range 0–0.08), and F'_{ST} averaged 0.01 (range 0–0.05). Genic tests revealed no significant allele frequency differences among any pairs of fragments for either dataset ($p > 0.05$ for all tests). Similarly, for the no-sib dataset, all pairwise F_{ST} and F'_{ST} estimates among fragments were 0, and genic tests revealed no significant allele frequency differences among any pairs of fragments ($p > 0.05$ for all tests). For the full dataset, the MMRR suggested that geographic distance, but not the percent of the land between two sites that was deforested, was a significant predictor of genetic distance (Geographic distance $\beta = 5.1$, $P = 0.003$; forested distance $\beta = -1.4$, $P = 0.22$; full model $F = 14.6$, $P = 0.01$). Similarly, for the no-sib dataset, the MMRR revealed that geographic distance, but not the percent of the land between two sites that was deforested, was a significant predictor of genetic distance (Geographic distance $\beta = 0.32$, $P = 0.004$; forested distance $\beta = -0.09$, $P = 0.22$; full model $F = 14.4$, $P = 0.01$).

For the full dataset the STRUCTURE analysis suggested that the *E. imperialis* sampled fall into two or three somewhat distinct genetic groups (Online Resources 5–7). There was the highest statistical support for $K = 3$, and individuals were partitioned largely the same way for $K = 2$, in which the first genetic group corresponded mostly to sites on and near the Osa Peninsula. This pattern was clear only when using the location information of each individual sampled in the prior. When no location information was included in the prior there was the highest support for there being one genetic group (Online Resource 8). For the no-sib dataset the STRUCTURE analysis suggested all the *E. imperialis* fall into one genetic group regardless of whether a location prior was used (Online Resources 9–11).

Discussion

In this study I examined levels of genetic diversity and the population genetic structure of the orchid bee *E. imperialis* over a fragmented area in southern Costa Rica. Genetic diversity was low relative to what some other studies have found for orchid bees across genera (Zimmermann et al. 2011; Freiria et al. 2012; Rocha Filho et al. 2013; Giangarelli et al. 2015). However, it was in line with estimates of for *E. imperialis* that were generated using allozyme loci (Roubik et al. 1996; Zayed and Packer 2005). Low genetic diversity could stem from restricted dispersal or contingencies based on the genetic loci used. Mantel tests revealed significant IBD over the entire geographic area sampled for the full dataset and no significant IBD for the no-sib dataset. The multiple regression analysis revealed that geographic distance was a significant predictor of genetic distance for both the full dataset and the no-sib dataset. This suggests that there may have been some limited dispersal across space, but overall the movement of *E. imperialis* was not restricted in the geographic area surrounding the Gulfo Dulce. This is consistent with what some other population genetic studies of orchid bees have found. Neither Zimmermann et al. (2011) nor Penha et al. (2015) removed full siblings prior to analyses, and these studies found significant IBD over 1000 km (*Euglossa dilemma*) and 700 km (*Euglossa iopoecila*), respectively. Freiria et al. (2012) also did not remove full siblings prior to analyses and found significant genetic differentiation over a fragmented area in which fragments were between 130 and 850 km apart, but found no IBD, suggesting that factors other than distance influence genetic structure. It is also worth noting that the presence of null alleles could have reduced genetic diversity estimates (Paetkau and Strobeck 1995). If the lack of amplification of microsatellites found for some individuals at some loci was caused by null alleles, the true genetic diversity within sites may be higher than the estimates reported in this study, and would be more in line with what other studies have found. This may have been the case with Egc35, where for the full dataset, on average among sites, almost 50% of the individuals did not have an allele successfully amplify (see Online Resource 2). Therefore, it would be worthwhile to use DNA sequencing to verify if a mutation in the primer-binding site caused non-amplification at this locus.

There was no evidence that deforested areas restrict dispersal of *E. imperialis*. Pairwise F_{ST} and F'_{ST} values were low among forest fragments, and the multiple regression with randomization revealed no effect of the amount of deforested area on genetic differentiation for either dataset. Several biological processes could cause these patterns. First, low genetic differentiation could indicate gene flow among forest fragments. Another, albeit less likely, explanation is that restricted dispersal within fragments has led to independent evolution within some sites. Considering forest fragments as units in population genetic analyses may not be biologically realistic if there is genetic structure on a smaller spatial scale and if dispersal depends not on forest but on other factors such as geographic distance. Due to small sample sizes, I did not calculate the population genetic summary statistics F_{ST} and F'_{ST} among pairs of sites within forest fragments. Reliable estimates of F_{ST} and F'_{ST} can be obtained with samples of about 30 diploid individuals (Hale et al. 2012), so it would be worthwhile to nondestructively sample more individuals within sites and estimate genetic differentiation within forest fragments as in Oi et al. (2013).

The STRUCURE results also suggest that forest fragments do not comprise independent genetic units. When the

full dataset was used, the STRUCTURE analysis partitioned individuals into two or three genetically distinct units, one of which corresponds loosely to a band of forest that traverses the upper portion of the Gulfo Dulce and comprises forest fragments 5 and 6. However, individuals from two sites from fragment 5 and one sampling location from fragment 6 cluster with another genetic group, which spans the entirety of the sampling area. Care must be taken when interpreting these STRUCTURE results because the presence of IBD may confound correct delineation of individuals into clusters (Pritchard et al. 2007). When a location prior was not used, the number of populations with the highest statistical support was $K = 1$, which is what would be expected if allele frequencies change gradually with distance rather than due to abrupt barriers to gene flow (Schwartz and McKelvey 2009). Furthermore, when the no-sib dataset was used $K = 1$ had the highest statistical support, regardless of whether the location prior was used. This suggests that patterns of dispersal do not necessarily correspond to forested areas.

This study highlights the importance of considering the effects of family structure in population genetic analyses. One of the tests of an association of genetic and geographic distance (Mantel tests) and the STRUCTURE analyses provided different results when full siblings were removed from the analysis. It is possible that the differences in results between the datasets were driven by the presence of family structure such that full siblings artificially inflated genetic similarity within geographic areas. It is also possible that it was a reduction in power caused by lower sample sizes that resulted in differences in results between datasets. Most population genetic studies on orchid bees have not removed full siblings prior to analyses. This may be problematic because in other systems, failure to control for family structure has been shown to bias estimates of genetic structure (Rodriguez-Ramilo and Wang 2012). It would be worthwhile for future studies to evaluate results with and without full siblings included to understand how lack of removal may bias results.

What drives patterns of dispersal in male orchid bees? The dominant paradigm for most organisms is that degraded habitat restricts movement (Prugh et al. 2008). Consistent with this paradigm, many orchid bee species do have higher abundance in forested areas (Dressler 1982; Silva and Marco 2014), in agricultural areas with the shade overstory retained relative to degraded areas (Briggs et al. 2013), and some show evidence of restricted dispersal over fragmented areas (Freiria et al. 2012; Suni and Brosi 2012; Rosa et al. 2016). However, other species show no evidence of restricted dispersal over deforested areas (Zimmermann et al. 2011; Suni et al. 2014), and some species seem to persist in anthropogenically-altered areas (Silveira et al. 2002). The reasons for regional or species-specific differences in genetic structure are largely unknown, but may be driven by differences in site fidelity or territoriality (Ackerman and Montaivo 1985), or differential propensity to leave forest fragments (Milet-Pinheiro 2005). It is important to keep in mind that the individuals used in this study were males that had likely already dispersed from their natal nests, which could contribute to the complexity in genetic structure. In orchid bees, females are thought to be far more philopatric than males (Augusto and Garofalo 2010; López-Uribe et al. 2014). Recent assessments of population structure that relied on mitochondrial DNA have found some structuring, which was attributed to female philopatry (López-Uribe et al. 2014; Penha et al. 2015). In contrast, males may disperse far away from their natal nest after birth, and have been thought to roam the landscape as vagabonds, but there is some evidence that they remain in one geographic area for at least period of weeks (Ackerman et al. 1982; Stern 1991). A study that used radio transmitters found that large bodied *Exaerate frontalis* (about 25 mm long) can travel 5 km in a week, but that individuals showed more philopatry than would be expected from a free-roaming male (Wikelski et al. 2010). It is difficult to sample females, but it would be worthwhile to compare these patterns of genetic structure to those of females over the same area.

Differences in dispersal may also reflect differences in resource availability among habitats. In the current study, for the full dataset, there was evidence for IBD over an area that comprises large tracts of intact forest separated by relatively little deforested area. Fragments 5 and 6 were loosely connected by a band of forest that traces the Gulfo Dulce, and fragments 6 and 7 were separated by only 5 km of deforested area. Caution must be used when interpreting these results due to the presence of full siblings in the full dataset. Nevertheless, it is possible that the IBD found over this area was due to orchid bees being less resource-limited in forested areas, which would translate into them traveling less far when acquiring fragrances or other resources. Some species prefer rare compounds to common ones (Pokorny et al. 2013), which could drive dispersal away from areas with homogeneous volatile composition. If fragrance diversity is greater in forests, low to moderate deforestation could promote dispersal, as bees would need to travel further to acquire the necessary resources. The current study found significant IBD over an area that partially overlaps with that of a previous study. In the current study I found significant IBD over 71 km for *E. imperialis*, while Suni et al. (2014) found no IBD over 81 km for another orchid bee, *E. championi*. The area over which *E. championi* was sampled included locations D–L, M, and O, which cover more deforested land. The lack of IBD over this area could be due to bees flying farther in order to acquire resources similar to those of bees living in intact

forest. Differences among species in their resource requirements could also explain some of the variability in genetic structure among species. There tends to be more variability among rather than within species in the specific fragrances collected by males (Eltz et al. 2005b), so it is plausible that males of different species are willing to travel different distances to acquire their preferred resources.

Another explanation for differences in genetic structure between *E. championi* (Suni et al. 2014) and *E. imperialis* (current study) is that body size differences explain differences in dispersal behavior. However, larger bees are thought to travel farther than smaller bees when foraging (Greenleaf et al. 2007). *Euglossa championi* is smaller (13 mm) than *E. imperialis* (15 mm), so the difference in IBD between the studies is not consistent with what would be predicted based on body size. In addition, Suni and Brosi (2012) found evidence of more restricted dispersal for the large-bodied *Eulaema bombiformis* relative to the smaller *Euglossa championi*. Therefore, even if larger bees are able to travel farther than smaller bees, body size does not seem to be a good predictor of genetic structure for this subset of orchid bees.

It is interesting that correlations between Euclidian geographic distance and overland geographic distance were similar for both datasets. The Euclidian distance among many pairs of sites traversed water, either the Gulfo Dulce or the Pacific Ocean. Other studies have found a lack of evidence for restricted dispersal for orchid bees on either side of bodies of water (Rocha Filho et al. 2013), and orchid bees have been observed to cross bodies of water (Ackerman and Montaivo 1985). Therefore, it is possible that the bodies of water separating sites in the current study do not represent strong barriers to gene flow.

Overall I found that *E. imperialis* had low genetic diversity but weak IBD in the geographic area surrounding the Gulfo Dulce. This suggests that future research on the extent to which this species may be vulnerable to negative effects of anthropogenic environmental changes, including reductions in population size and genetic diversity, may be warranted. However, the microsatellite markers used to evaluate genetic diversity were developed for use in other orchid bees, and it is possible that had the markers been developed specifically for *E. imperialis* they would have shown more genetic variability. In addition, null alleles and allelic dropout could have contributed to low genetic diversity. It would thus be worthwhile to assess genetic diversity in a future study using larger samples within locations, and genome-wide markers, to understand if the observed levels of genetic diversity make *E. imperialis* an important candidate for further conservation monitoring.

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