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RESEARCH ARTICLE

Population genetic structure of orchid bees (Euglossini) in anthropogenically altered landscapes

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Abstract Habitat degradation and fragmentation are widespread phenomena in tropical regions. Negative effects on the biota are numerous, ranging from interruption of gene flow among populations, to the loss of genetic diversity within populations, to a decline in species richness over time. Orchid bees (Hymenoptera: Apidae: Euglossini) are of major conservation interest due to their function as pollinators of numerous orchid species and other tropical plants. Here, we used microsatellite markers to investigate the effects of geographic distance and habitat fragmentation on gene flow among populations. Populations of *Euglossa dilemma* in three geographic regions—the Yucatán peninsula (Mexico), Veracruz (Mexico), and Florida (USA)—were genetically structured predominantly across the regions, with the strength of differentiation among populations being positively correlated with geographic distance. Within geographic regions only little substructure was found, suggesting that dispersal is substantial in the absence of geographic or ecological barriers. In a second study, patterns of genetic differentiation among eight species of *Euglossa* were not related to habitat fragmentation following deforestation in southern Mexico (Veracruz). Specifically, most bee populations in the 9,800 ha forest remnant of Los Tuxtlas (Volcano San Martin) were neither differentiated from, nor had less genetic diversity than, populations in near-continuous forest separated from Los Tuxtlas by 130 km of agricultural land. Either occasional long distance dispersal across open areas has buffered the expected genetic effects of fragmentation, or the history of fragmentation in southern Mexico is too recent to have caused measurable shifts in allelic composition.

Keywords *Euglossa viridissima* · *Euglossa dilemma* · Gene flow · Long-distance dispersal · Forest fragmentation · Pollinator decline

Introduction

Dispersal and gene flow are elementary processes in animal populations, affecting both their medium term ecological as well as their long-term evolutionary future (Barton and Hewitt 1985; Roderick 1996). The geographic distribution

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of a species is normally more extended than an individuals' dispersal potential, which together with other mechanisms may result in genetic sub-differentiation of populations, even in the absence of geographic and habitat barriers (Balloux and Lugon-Moulin 2002). The ongoing fragmentation of natural habitat due to human activities adds further to the isolation of populations and may severely restrict gene flow (Cane 2001). Various characteristics of habitat fragments such as their size, the distance to neighboring fragments, the proportion of edges, but also the quality of remaining habitat all influence the abundance of individuals and their likelihood to disperse (Tscharntke et al. 2002). Specifically, populations which are reduced in size and isolated from others are likely to experience a reduction of genetic diversity over time. This can have a negative influence on the likelihood of long-term persistence of a population due to inbreeding depression and/or reduce resilience to environmental change (Darvill et al. 2006; Frankham et al. 2002; Keller and Waller 2002).

Orchid bees (Euglossini, Apidae; 200 species) are native to lowland forests of the Neotropical region (Roubik and Hanson 2004), which are suffering substantial rates of deforestation (Myers et al. 2000; Turner and Corlett 1996). In this habitat, the solitary to primitively social orchid bees represent an important group of pollinators. Female orchid bees search for nectar and pollen in notable foraging ranges (up to 23 km in a single foraging trip, Janzen 1971), thereby pollinating plants of a great number of families (e.g., Rubiaceae, Fabaceae, Costaceae, Lecythidaceae; Ramirez et al. (2002)). Additionally, male orchid bees forage for volatile chemicals, which they store in hind leg pockets (Dressler 1982). The accumulated fragrance mixture is species-specific and believed to function as a pheromone analogue (Eltz et al. 2005; Zimmermann et al. 2006). Male orchid bees use a great variety of sources of volatiles, including resin and sap from tree wounds, fruits, feces, decaying wood, and flowers. The use of floral volatiles has shaped the euglossine pollination syndrome in which male orchid bees are responsible for the exclusive pollination of 700 orchid species (Dressler 1968a; Vogel 1966; Whitten et al. 1993), and other plants. Among orchids, many Stanhopeinae and Catasetinae possess impressive morphological adaptations for male euglossine pollination, and their flowers produce unique fragrance blends to lure only certain species (Armbruster et al. 1989; Dodson et al. 1969; Williams and Dodson 1972). While the orchids depend entirely on male euglossine bees for pollination this mutualism appears to be only facultative for the bees. The orchid bee species *Euglossa dilemma*, which has been recently naturalized in southern Florida (USA) (Skov and Wiley 2005, therein as *Euglossa viridissima*) now thrives in an environment without perfume orchids (Pemberton and Wheeler 2006).

Orchid bees of the genus *Euglossa* reproduce all year round in tropical habitats, although there are usually seasonal fluctuations in abundance (Roubik and Ackerman 1987). An individual female may live as long as 2–4 months and produces small clutches of brood cells in solitary or primitively social nests (e.g., Cocom Pech et al. 2008). Larval development takes between 6 weeks and 4 months depending on species and climatic conditions (Garofalo 1985; Ramirez-Arriaga et al. 1996; Augusto and Garofalo 2004). Since orchid bees act as key pollinators in tropical forests, it is of major interest to know how populations and communities respond to land-use and deforestation (Roubik and Hanson 2004). An adequate habitat for orchid bees has to contain food plants, nesting sites, nest building materials and volatile sources. It is questionable whether the bees' large flight range (Janzen 1971; Krodsma 1975; Williams and Dodson 1972), their potential to adapt to new environments (Skov and Wiley 2005), and their occurrence in forest edges (Brosi 2009) are sufficient to counteract all negative effects of fragmentation. Previous studies about the response of euglossines to forest fragmentation focused on the numerical abundance of individuals and species diversity (Becker et al. 1991; Brosi 2009; Powell and Powell 1987; Tonhasca et al. 2002). Brosi (2009) and Powell and Powell (1987) found a positive relationship between forest area and euglossine bee abundance, but the second study was conducted shortly after a major disturbance, which might have influenced the results (Becker et al. 1991; Cane 2001). Male orchid bees have been observed to enter disturbed forests and even severely degraded farmland in their search for volatiles, but this propensity appears to be highly variable between different species (Brosi 2009; Milet-Pinheiro and Schlindwein 2005; Otero and Sandin 2003; Rincon et al. 1999). In the present study we examine for the first time the consequences of fragmentation on the genetic structure and population differentiation of orchid bee populations by using polymorphic microsatellite markers. In a genetic distance study we measure population differentiation of *Euglossa dilemma* within and among three different geographic regions: the Yucatán peninsula (Mexico), southeastern Veracruz (Mexico), and southeastern Florida (USA). In a fragmentation study on eight different *Euglossa* species we compare genetic diversity of populations in three forest localities in southern Mexico (states of Veracruz and Oaxaca). All three localities are approximately equidistant from each other, but only two localities are connected with each other by forest, whereas the third locality, the Los Tuxtlas reserve, is an isolated forest fragment separated from the other two localities by at least 130 km of agricultural land. Our intention is to distinguish between the effects of deforestation and distance on population genetic differentiation.

Materials and methods

Male orchid bees can be attracted to synthetic, single compound chemical baits. We used this method of chemical baiting to collect samples of specimens as in previous studies on the diversity, seasonality, and population dynamics of euglossine bees (Ackerman 1983; Armbruster 1993; Eltz et al. 2007; Roubik and Ackerman 1987; Zimmermann et al. 2009a). Female euglossines are not attracted by chemical baits and generally difficult to sample in numbers sufficient for population genetic analysis. Our study was thus conducted with male bees only, which develop from unfertilized eggs and are haploid.

Genetic distance study

The genetic distance study focused on *Euglossa dilemma* Bembé & Eltz, a recently described sibling species of *E. viridissima* occurring in Central America from Mexico, including the Yucatán peninsula, to Costa Rica (Eltz et al. 2011). Recently *E. dilemma* was naturalized in southern Florida, where it was first recorded in the year 2003 (Skov and Wiley 2005). Male *E. dilemma* have three mandibular teeth [instead of two as in the lectotype of *E. viridissima* (Eltz et al. 2011)], and population genetic analysis revealed that the two forms are genetically differentiated species (Eltz et al. 2008). Males of *E. dilemma* were caught in April to June of the years 2007 and 2008 with chemical baits (*p*-dimethoxybenzene, methyl cinnamate and eugenol) at eight different localities (Fig. 1): El Remate, Xmatkuil, Chablekal, San Chrisanto, and Coba located on the peninsula of Yucatán, Monte Pio and El Chote situated in the state of Veracruz in southern Mexico, and Fort Lauderdale in Florida (USA). The Florida samples were collected at eugenol baits only. Geographical distances between populations were measured as terrestrial distances.

Fragmentation study

For an assessment of genetic differences in a fragmented landscape we collected male *Euglossa* spp. at three different Localities (A, B and C, see Fig. 1) in southern Mexico. Between 1977 and 1992 tropical Mexico experienced a reduction of about 28% of its total forest area, resulting in a highly disturbed landscape that includes cultivated land, secondary forest, grazing lands, and disturbed forests (Cairns et al. 2000). Locality A is near the Biological Station “Los Tuxtlas”, situated in the northeastern lowland part (18°30′N, 95°8′W) of the biosphere reserve Los Tuxtlas (Volcán San Martín) in the state of Veracruz. It is part of a 9,800 ha area of forest remnants which includes the only substantial lowland forests that remain in coastal Veracruz (http://www.catemaco.info/biosphere/areas.html). This forest area is isolated from other lowland tropical forests by a broad belt (>130 km) of intensively used land dominated by pastures and cash crops. The ecological isolation of Los Tuxtlas forests on this scale has existed for at least 35 years [see maps in Cairns et al. (2000)] but most likely since the 1950s, when extensive transformations of natural habitats to mostly cattle ranches began (Mendoza et al. 2005). Localities B and C are in an area of larger forest remains beyond that belt of farmland, flanking the northeastern slopes of the central cordillera in western Veracruz/eastern Oaxaca. Baiting sites were at 17°48′N, 96°6′W(B) and 17°23′N, 94°11′W(C) and situated on ridges within the least disturbed forests we could locate. Although the area between B and C is no longer covered by continuous forest, there are no large scale interruptions in the forest patchwork. Thus, all three localities are at approximately equal distance to each other (130–200 km), but locality B and C are connected by forest habitat. This setup was chosen to be able to separate the effects of distance and lack of forest habitat on genetic structuring of orchid bee populations. Baiting took place in May and June 2009 and was conducted in the mornings with a standard set of chemical baits (1,8-cineole, eugenol, ipsdienol, methyl salicilate, *p*-dimethoxybenzene, methyl cinnamate and vanillin) that are known attractants of males of numerous species of orchid bees. Attracted males were caught with a net and identified with a hand lens in the field or under a microscope at the Los Tuxtlas Biological Field Station. The eight most common species were preserved in 99% ethanol. These eight species were *Euglossa cyanura*, *E. imperialis*, *E. mixta*, *E. obtusa*, *E. tridentata*, *E. variabilis*, *E. viridissima* and *E. dilemma.* Released males were marked with permanent color dots on the thorax or by notching the wings, enabling us to collect data on local *Euglossa* species diversity and community composition without pseudoreplication. These data were collected to assess whether ecological diversity is congruent with genetic diversity. To investigate *Euglossa* presence/abundance in the intermittent belt of farmland, we also baited at three different sites located between locality A and C, on a total of 6 days. For comparative purposes we calculated Shannon’s index of (species) diversity (H') for each of the three forest localities and for the pooled farmland sites. Differences in relative species abundances between localities were tested with a Chi-square Test for Independence.

DNA extraction and microsatellite analysis

All individuals were preserved in 99% ethanol and stored at +8°C. DNA was extracted from one leg of each male using the method of Hunt and Page (Hunt and Page 1995). The PCRs were performed as multiplex reactions with 5′ fluorescent labeled primers (HEX, TET or 6-FAM; see

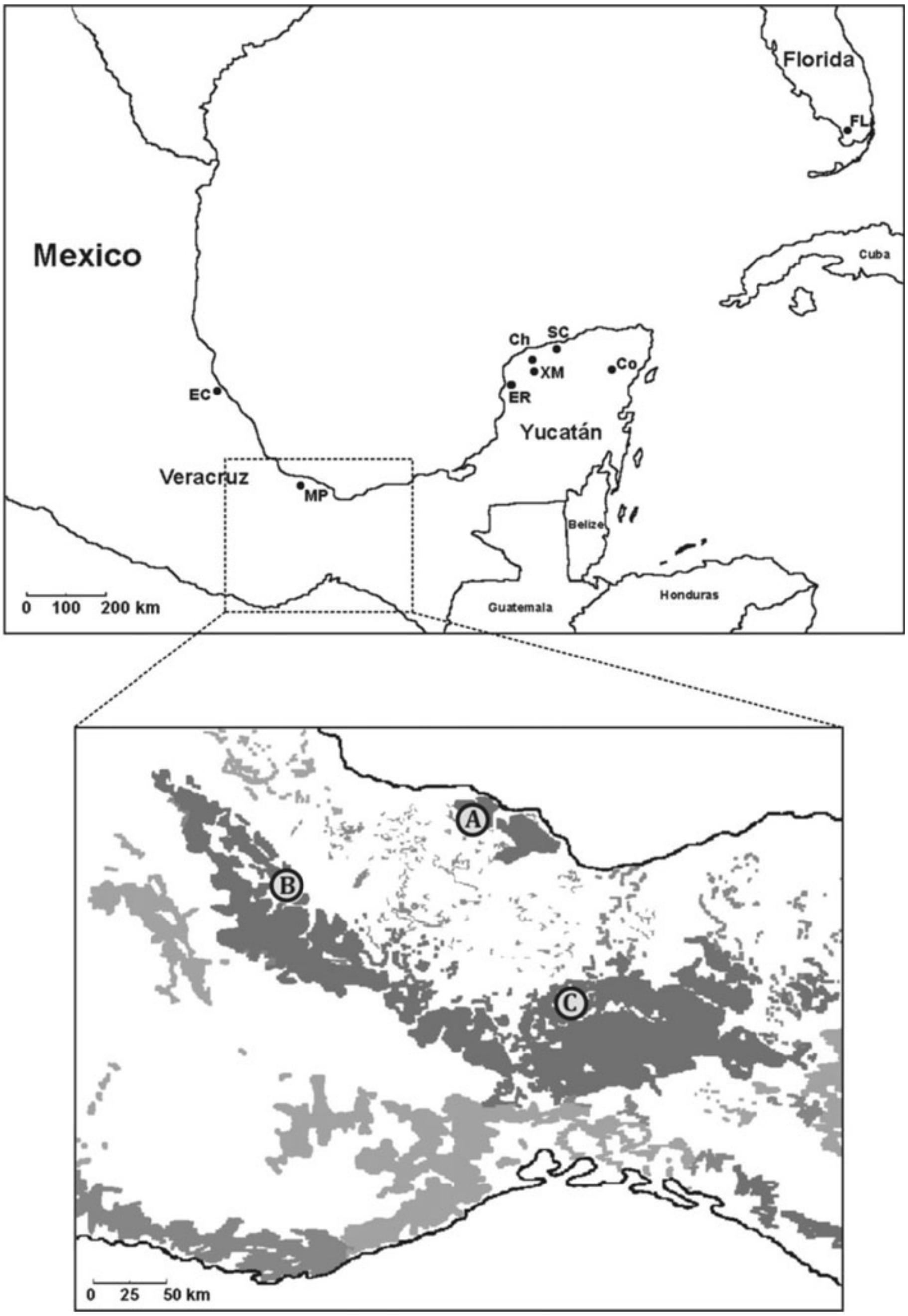

Fig. 1 Map of the study area highlighting the eight sampling localities of the genetic distance study on *Euglossa dilemma*. Co, Coba; Ch, Chablekal; EC, El Chote; ER, El Remate; Fl, Florida; MP, Monte Pio; SC, San Chrisanto; XM, Xmatkuil. *Insert*: Detailed map of southern Mexico with the sampling Localities A, B and C of the fragmentation study. The area highlighted in *grey* is forested, with the *darkest grey* representing tall evergreen forest and the *lighter shades of grey* representing less dense/secondary forest vegetation (modified from Instituto Nacional de Estadistica Geografia e Informatica; 1990–2000)

Zimmermann et al. (2009b) for PCR conditions). The *E. dilemma* individuals for the genetic distance study were genotyped at three different microsatellite loci: ann02 (GenBank accession no. BV728898), ann08 (BV728902) (Paxton et al. 2009) and Egc17 (EF451846) (Souza et al. 2007). Fragment analyses of PCR products were carried out with an ABI Prism 310$^{\text{TM}}$ Sequencer (PE Applied Biosystems) at the University of Düsseldorf (BMFZ). For the fragmentation study on eight different *Euglossa* species we used three additional markers: Egc18 (EF451842), Egc24 (EF451843) and Egc26 (EF451844) (Souza et al. 2007). PCR products were analyzed in a MegaBACE 1000 capillary sequencer at the Molecular Ecology Research Group at the University of Halle-Wittenberg. In all cases allele lengths were assigned with the software GENEMARKER V1.71 whereby allele sizes were rounded to their nearest integer.

Data analysis

We tested for linkage disequilibrium among loci over all populations of a given species using GENEPOP 4.0.10 (Raymond and Rousset 1995). Significance values were adjusted following standard Bonferroni corrections (Rice

1989). We used the program Microsatellite Analyzer (MSA 4.05) (Dieringer and Schlotterer 2003) to calculate genetic diversity characteristics and applied the special option for inbred lines to determine results for 200 randomly discarded data sets. For each marker we calculated the expected heterozygosity (H_{exp}) and the observed number of alleles for each locus (A). Genetic (allelic) variability within populations was estimated as Shannon diversity index (H') (with the variable p_i as the proportion of a given allele relative to the total number of alleles) and allelic richness (N_a), adjusted for different sample sizes. Wilcoxon matched-pairs tests were used to test for differences in H' and N_a between localities for each species (data paired with regard to locus, $N = 6$). To examine genetic differentiation we calculated the unbiased estimator of F_{st} (Weir and Cockerham 1984) between population pairs. Significance levels were determined by permuting genotypes 100,000 times among all population pairs. This conservative procedure does not assume Hardy–Weinberg equilibrium, for which we could not test since our data set included only haploid (male) genotypes. In each of the performed tests all significances were obtained after standard Bonferroni correction for multiple tests.

In addition, for the genetic distance study with *E. dilemma,* we correlated pair-wise estimates of genetic distance, as defined by $F_{st}/(1 - F_{st})$, with the corresponding logarithms of geographical distances between populations (Rousset 1997). The Florida population was excluded from this analysis because it appears to have been very recently introduced to the US with the help of humans (Skov and Wiley 2005). A Mantel test (Mantel 1967) was used to assess the significance of any correlation, performing 5,000 permutations in the software IBDWS 3.16 (Isolation by Distance Web Service; Bohonak 2002).

Results

Linkage disequilibrium was not detected for any pair of loci after a Bonferroni correction for multiple comparisons, neither across the *E. dilemma* populations of the genetic distance study nor for any *Euglossa* species of the fragmentation study.

Genetic distance study

In total, we genotyped 268 *E. dilemma* males at three microsatellite loci and found the greatest within-population genetic diversity on the peninsula of Yucatán (Table 1). Here, expected heterozygosities (H_{exp}) were generally high and, averaged over all three markers, it varied from 0.89 (±0.12) in Coba to 0.85 (±0.15) in Chablekal. We detected the highest number of different alleles and a maximum value of the Shannon Index of allelic diversity in the population of Xmatkuil (total $A = 68$, $H' = 2.57 \pm 0.87$). Relatively low values of these diversity measures were found in coastal El Remate (total $A = 31$, $H' = 2.04 \pm 0.56$). In comparison to Yucatecan populations the two populations in Veracruz had similarly high H_{exp}, but were slightly less diverse, with a Shannon Index of 1.92 (±0.36) for El Chote and 1.95 (±0.51) for Monte Pio. By far the lowest genetic diversity was detected in the population in Florida, with a H_{exp} of only 0.55 (±0.36) and a Shannon Index of only 1.08 (±0.73).

Overall, the populations of *E. dilemma* showed moderate genetic differentiation (global $F_{st} = 0.051$, $P = 0.0001$). Pairwise estimates of F_{st} among populations on the peninsula of Yucatán showed no significant differences ($-0.006 < F_{st} < 0.020$, see Table 2). Equally, the two populations in Veracruz showed no differentiation (pairwise $F_{st} = -0.003$). When comparing between regions, however, 9 out of 10 pairs of populations had significantly different allele frequencies, with the exception of the comparison between Coba and El Chote. Between regions, F_{st} values were generally higher, indicating moderate genetic differentiation ($0.030 < F_{st} < 0.065$). The most pronounced genetic differences were found between Florida and all other populations (7 pair wise comparisons, $0.12 < F_{st} < 0.20$, $P < 0.001$).

There was a significant correlation between genetic distance ($F_{st}/(1 - F_{st})$) and the logarithm of geographical distances ($P < 0.05$; $R^2 = 0.33$) of *E. dilemma* populations from the seven different Mexican localities (Fig. 2).

Fragmentation study

The eight species of *Euglossa* ($n = 737$ males) varied substantially in the degree of genetic variability at the six microsatellite loci, with the majority of species being highly polymorphic at several loci (Table 3). Variability was very low in *E. cyanura* with only two reasonably informative markers. *E. imperialis* and *E. mixta* were of intermediate variability with four informative markers each, whereas the remaining five species showed substantial polymorphism at all six microsatellite loci. Ann08 was found to be the most variable marker across species, ranging from $H_{exp} = 0.496$ in *E. obtusa* ($A = 6$) to $H_{exp} = 0.922$ in *E. dilemma* ($A = 22$). The least variable marker was Egc24, which still had a H_{exp} range of 0.433 in *E. viridissima* ($A = 3$) to 0.697 in *E. variabilis* ($A = 7$).

To compare genetic diversity between the three localities A, B and C we calculated the Shannon index of average allelic diversity across loci as well as the average allelic richness across loci for each locality and species (whenever sample size was above 10 individuals per locality). Values for the two diversity measures varied

Table 1 Genetic diversity within samples of male *E. dilemma* from eight localities of the genetic distance study

Area	Population	N	N alleles (A)			H_{exp}			Shannon index	Allelic richness
		Males	*ann02*	*ann08*	*Egc17*	*ann02*	*ann08*	*Egc17*	(H') (±SE)	(N_a) (±SE)
Yucatan	Xmatkuil	67	38	20	10	0.97	0.93	0.72	2.57 (±0.87)	15.49 (±7.62)
	Chablekal	31	18	15	7	0.96	0.92	0.68	2.21 (±0.69)	12.43 (±5.11)
	Coba	30	24	14	7	0.98	0.93	0.76	2.36 (±0.76)	13.78 (±7.34)
	San Chrisanto	30	18	14	7	0.94	0.92	0.74	2.19 (±0.60)	12.03 (±4.89)
	El Remate	20	13	13	5	0.91	0.94	0.74	2.04 (±0.56)	10.33 (±4.62)
Veracruz	El Chote	31	6	12	8	0.82	0.92	0.81	1.92 (±0.36)	8.42 (±2.87)
	Monte Pio	29	8	15	7	0.79	0.94	0.79	1.95 (±0.51)	9.58 (±4.12)
Florida	Ft. Lauderdale	30	6	6	2	0.75	0.76	0.13	1.08 (±0.73)	4.58 (±2.24)

Number of genotyped males, maximum number of observed alleles (A) and expected Heterozygosities (H_{exp}) are given. Allelic diversity within populations is given as Shannon Index of diversity (H') and allelic richness (N_a) with their standard errors (±SE)

Table 2 Estimates of genetic differentiation as F_{st} (above diagonal) in pairwise comparisons among the eight populations of *E. dilemma* and their Bonferroni corrected significances (below diagonal)

	Yucatan					Veracruz		Florida
	Xm	Ch	Co	SC	ER	MP	EC	FL
Yucatan								
Xm	–	0.009	0.001	–0.003	0.013	0.052	0.037	0.122
Ch	NS	–	0.010	0.020	0.019	0.065	0.047	0.127
Co	NS	NS	–	–0.006	–0.004	0.045	0.030	0.135
SC	NS	NS	NS	–	0.004	0.049	0.039	0.149
ER	NS	NS	NS	NS	–	0.056	0.055	0.153
Veracruz								
MP	***	***	*	*	*	–	−0.003	0.202
EC	**	*	NS	*	*	NS	–	0.182
Florida								
FL	***	***	***	***	***	***	***	–

Populations are sorted by geographic area

Significance values: * < 0.05, ** < 0.01, *** < 0.001, NS > 0.05

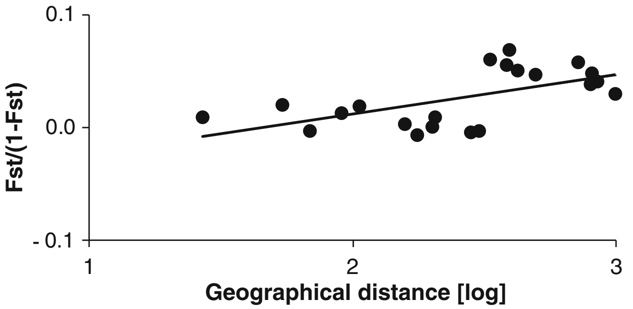

Fig. 2 Relationship between the logarithm of geographical distance and genetic differentiation (measured as $F_{st}/(1 - F_{st})$) across the seven Mexican populations of *E. dilemma* in pair-wise comparisons (Mantel test; $P < 0.05$, $R^2 = 0.33$). Minimum distance was 27 km (Xmatkuil–Dzibilchaltun) and maximum distance was 1,000 km (Coba–El Chote)

greatly between species and were found to be the lowest in *E. cyanura* and the highest in *E. dilemma*, which corresponds closely to general marker variability (Table 4). We found no tendency for any locality being generally more or less diverse than another. Wilcoxon matched-pairs tests between localities (matched with regard to locus) were non-significant for allelic richness in all species. For the Shannon index we found a marginally significant difference ($P = 0.048$) between locality A and locality C in *E. dilemma*.

Pairwise F_{st} values between localities in each of the eight species were generally low ($-0.015 < F_{st} < 0.016$) but were found to be significant in two out of 16 comparisons (again, we excluded localities in which a species had a sample size below 10). The *E. dilemma* population of locality B was found to be significantly different from the population of locality C ($F_{st} = 0.049$, $P = 0.0013$), and the *E. mixta* population of locality A was different from the population at locality C ($F_{st} = 0.046$, $P = 0.017$).

The overall species diversity of *Euglossa* was similarly high in the three forest localities (A: 13 species, $H' = 1.99$; B: 11 species, $H' = 1.97$; C: 11 species, $H' = 1.53$), although the numerical composition of the species community was quite different (Fig. 3). Whereas *E. cyanura* was highly represented in locality A, it was much less abundant in locality C and nearly absent in locality B. *E. tridentata* clearly dominated in locality C, whereas in locality B the species *E. imperialis*, *E. obtusa* and *E. tridentata* had an equally high abundance.

In the farmland region between A and C bait captures were much less diverse (4 *Euglossa* species, $H' = 0.67$) than in the three forest localities, and heavily dominated by males of the two sibling species *E. dilemma* and *E. viridissima*. Only two other *Euglossa* species (*E. tridentata* and *E. obtusa*) appeared there as well, but in very low numbers. Across all four localities/areas there was a significant difference in relative *Euglossa* species frequency

Table 3 Genetic variability at six microsatellite loci of eight *Euglossa* species in the fragmentation study

	E. cyanura		*E. imperialis*		*E. mixta*		*E. obtusa*		*E. tridentata*		*E. variabilis*		*E. viridissima*		*E. dilemma*	
	H_{exp}	A	H_{exp}	*A*	H_{exp}	*A*	H_{exp}	*A*	H_{exp}	*A*	H_{exp}	*A*	H_{exp}	*A*	H_{exp}	*A*
ann02	0.000	1	0.773	8	0.486	3	0.652	6	0.563	8	0.783	10	0.612	7	0.797	13
ann08	0.507	2	0.575	3	0.784	8	0.496	6	0.894	25	0.841	11	0.914	17	0.922	22
Egc26	0.000	1	0.000	1	0.038	3	0.113	4	0.745	9	0.776	12	0.769	8	0.797	12
Egc17	0.243	4	0.164	3	0.588	7	0.853	10	0.709	10	0.805	11	0.820	10	0.762	13
Egc18	0.685	7	0.677	5	0.514	3	0.724	8	0.669	11	0.797	10	0.798	11	0.828	12
Egc24	0.027	2	0.000	1	0.000	1	0.498	3	0.529	4	0.697	7	0.433	3	0.670	5

Expected heterozygosity (H_{exp}) and the number of observed alleles (*A*) over all loci are given

(Chi-square Test for Independence: $N = 1639$, $\text{Chi}^2 = 1402.9$, $P < 0.0001$; see Fig. 3).

Discussion

Population structure of *Euglossa dilemma* in southern Mexico and Florida

The original vegetation of tropical dry forest of the Yucatán peninsula was heavily degraded or cleared during the Sisal era in the late 1960s, leaving mostly secondary forests today (Turner et al. 2001). The actual landscape is a mosaic of patches that alternate between traditional agriculture and deciduous secondary forests of low stature (Hartter et al. 2008), but generally lacks large areas without forested vegetation. This landscape seems to represent a suitable habitat for *E. dilemma*, supporting high population densities and providing substantial opportunity for dispersal and gene flow. High population densities of *E. dilemma* on the Yucatan peninsula are also evident from broad scale baiting assays, which sometimes yield more than three hundred males in a single locality on a single morning (Ramirez-Pech personal communication, Eltz personal observation). Such high numbers for a single species are unusual for euglossine bees, even in Panamanian or Costa Rican lowlands which are well known for their rich euglossine communities. Large Yucatecan population sizes are also suggested by our genetic data, especially by the high allelic diversity and the substantial genetic variability within localities combined with a general lack of genetic differentiation within the Yucatán peninsula. These genetic patterns suggest that *E. dilemma* occurs in a large, highly interconnected metapopulation on the Yucatan peninsula, with substantial gene flow across distances of several hundreds of kilometers. The moderate genetic differentiation between populations of the Yucatán peninsula and those in Veracruz is in agreement with this view. Some of that differentiation, might be due to isolation by distance (Hardy and Vekemans 1999). However, gene flow between Yucatán and Veracruz is probably also restricted by habitat barriers south of the Mexican gulf. This area is covered by mangrove forest along the coast (southwestern Campeche) and moist seasonal tropical forest in the south, possibly forming a belt of suboptimal habitat contrasting with the drier north of the Yucatán peninsula (Vester et al. 2007). Baiting assays in coastal southwestern Campeche yielded substantially lower numbers of male *E. dilemma* compared to the rest of the Yucatán peninsula, and baiting in moist forests of northern Chiapas failed to lure *E. dilemma* at all (Eltz personal observation). Abundances increased markedly only further to the west, in the state of Veracruz, where this species was found to be common around settlements and in forest/farmland mosaic. The two analyzed populations of Veracruz showed high genetic variability and only marginal genetic differentiation despite their relatively large distance from each other, probably due to a continuous availability of suitable habitat in between them.

The genetic make-up of the *E. dilemma* population in Florida bears signs of a recent bottleneck, as would be expected from a population that was established only very recently, and possibly from only a single nest. Such an establishment of a new population from a small number of individuals that are reproductively isolated from the main population can result in a loss of alleles and reduced genetic diversity (Frankham et al. 2002). The microsatellite genotypes of Floridan *E. dilemma* are of lower genetic variability, but also composed of rather different alleles than those of all Mexican populations. This indicates that the founding individuals came from a geographic area that was not included in our study. Further sampling and genotyping of bees from more southern localities, such as Honduras and Costa Rica (the southernmost part of the native range), is necessary to resolve this issue.

Along with data from other studies (Liu and Pemberton 2009; Pemberton 2007; Pemberton and Liu 2008; Pemberton and Wheeler 2006; Skov and Wiley 2005), our findings confirm *E. dilemma* as a species that is well adapted to anthropogenically altered habitats and largely independent of old-growth forest. This it has in common

Table 4 Genetic variability of *Euglossa* spp., measured as average allelic diversity across loci (H' = Shannon index) and average allelic richness across loci (N_a) at three localities in southern Mexico (A, B and C)

	N Males	Shannon index (H') (±SE)	Allelic richness N_a (±SE)	Pairwise F_{st}	
E. cyanura					
Locality A	39	0.40 (±0.56)	2.29 (±2.24)	A–B	NA
Locality B	5	NA	NA	A–C	0.009
Locality C	29	0.44 (±0.49)	2.33 (±1.51)	B–C	NA
E. imperialis					
Locality A	32	0.66 (±0.70)	3.17 (±2.40)	A–B	0.008
Locality B	46	0.70 (±0.66)	3.30 (±2.28)	A–C	NA
Locality C	3	NA	NA	B– C	NA
E. mixta					
Locality A	37	0.71 (±0.57)	3.13 (±2.01)	A–B	0.003
Locality B	28	0.70 (±0.60)	2.83 (±1.83)	A–C	0.046*
Locality C	40	0.67 (±0.62)	3.11 (±2.17)	B–C	0.016
E. obtusa					
Locality A	23	1.08 (±0.70)	4.26 (±2.61)	A–B	−0.002
Locality B	68	1.11 (±0.54)	4.37 (±1.77)	A–C	−0.004
Locality C	13	0.98 (±0.61)	3.83 (±1.94)	B–C	−0.015
E. tridentata					
Locality A	31	1.30 (±0.40)	6.17 (±2.56)	A–B	0.008
Locality B	47	1.36 (±0.66)	6.70 (±5.31)	A–C	−0.002
Locality C	59	1.53 (±0.63)	8.51 (±5.33)	B–C	−0.004
E. variabilis					
Locality A	26	1.73 (±0.23)	7.67 (±1.75)	A–B	NA
Locality B	9	NA	NA	A–B	0.015
Locality C	25	1.54 (±0.18)	6.83 (±1.17)	B–C	NA
E. viridissima					
Locality A	34	1.59 (±0.66)	8.00 (±4.20)	A–B	NA
Locality B	8	NA	NA	A–C	0.016
Locality C	36	1.55 (±0.51)	7.49 (±3.14)	A–C	NA
E. dilemma					
Locality A	36	1.79 (±0.47)	8.48 (±2.81)	A–B	0.014
Locality B	43	1.80 (±0.47)	8.60 (±3.75)	A–C	0.008
Locality C	20	1.50 (±0.44)	6.50 (±2.66)	B–C	0.049**

Number of males per locality is given. Values of F_{st} between pairs of localities are computed, significance values are Bonferroni corrected (* < 0.05, ** < 0.01, *NA* not analyzed because of a sample size <10 within one locality)

with its sibling species, *E. viridissima*, which is the only other *Euglossa* species common on the Yucatán peninsula (Eltz et al. 2008). Nests of both species can be found in human-made cavities around houses and gardens in suburban and even urban settings (Cocom Pech et al. 2008; Eltz et al. personal observation). In their tolerance of, or even preference for, anthropogenically disturbed habitat, the two species are unusual amongst euglossine species, many of which are believed to be highly dependent on mature forest (Dressler 1982; Roubik and Hanson 2004). It is for such differences among species in ecological requirements that our fragmentation study aimed to include a range of different species.

Orchid bee populations and forest fragmentation

Different species of *Euglossa* are thought to have different habitat requirements, possibly based on differences in their choice of nectar and pollen plants, volatile sources or nesting sites (Dressler 1982; Ramirez et al. 2002; Roubik and Hanson 2004). Previous baiting studies in Brazil revealed that species differ in their tendency of traveling out of forest habitat (Milet-Pinheiro and Schlindwein 2005), suggesting different dispersal potential in fragmented landscapes. Accordingly, we expected differential fragmentation impact on genetic population structure in the eight *Euglossa* species that we had chosen for our study. Specifically, we expected no or weak genetic structuring in *E. viridissima* and *E. dilemma*, which commonly occur in disturbed habitats (see above), and more pronounced structure in some of the rarer, presumably more forest dependent species. The baiting results from the intermittent farmland area confirmed the expected ecological dichotomy (see Fig. 3). Only *E. dilemma* and *E. viridissima* were attracted in substantial numbers in open farmland, whereas captures of other species, if they occurred at all, were rare exceptions. However, patterns of genetic differentiation did not correspond to these baiting results. Population genetic structure between localities was very low or absent in all eight study species. F_{st} values were generally below 0.05, and only two out of 16 pairwise comparisons yielded significant population differences in allele frequencies. Of these two, one significant difference was found between the fragment and continuous forest (in *E. mixta*), and one between the two continuous forest sites (in *E. dilemma*).

In our set up, the Los Tuxtlas Reserve (locality A) represented an isolated forest fragment which is surrounded by >100 km of farmland with only tiny patches of interspersed forest-like vegetation. Two rather different causes might explain the overall absence of genetic differentiation of the Los Tuxtlas bees. First, it is possible that euglossine bees, even true forest species, are occasionally capable of crossing large areas of inhospitable landscape, leading to occasional gene flow between widely separated forest islands. Long-distance dispersal seems particularly probable in euglossine bees given that they are strong fliers with already very large foraging ranges (Dressler 1968b; Janzen 1971) and, at least in males, a tendency to invade non-habitat areas in search of chemical resources (Milet-Pinheiro and Schlindwein 2005). It remains unclear to what extent small interspersed patches of woody vegetation can

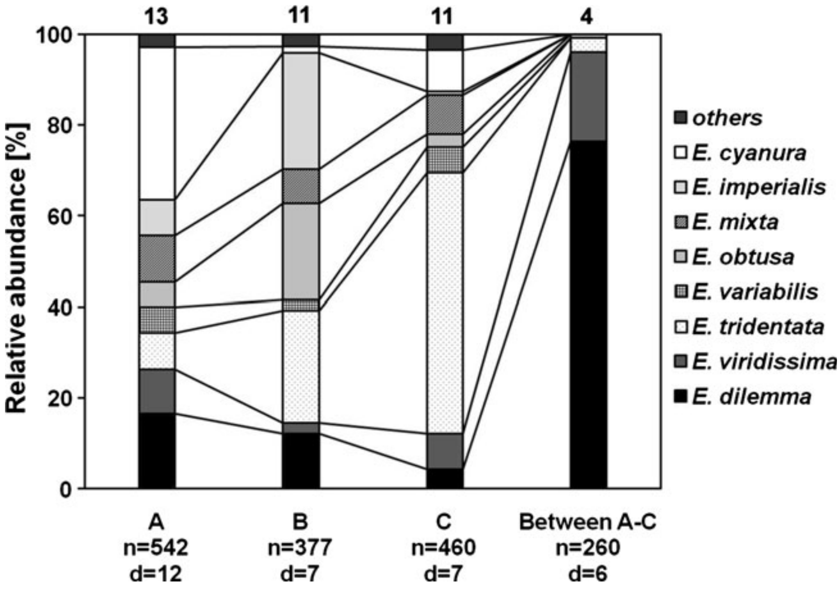

Fig. 3 Species composition of male *Euglossa* bait captures in percent of individuals in the three forest localities (A, B, C) and the farmland area in between A and C. Numerals on *top* of the columns represent the total number of *Euglossa* species observed. Numbers of individuals (*n*) and days of sampling (*d*) are given below

be used by bees as stepping stones between larger forest areas. Such patches, which are likely insufficient in size and habitat quality to support bee populations on their own, could provide shelter or nectar food for dispersing individuals. Thus, it is possible that even the large area of non-forest habitat between Los Tuxtlas and the other two localities did not function as an efficient barrier for gene flow. A second, alternative explanation for the absence of population structure is the limited amount of time that was available for isolation to take genetic effect. The separation of the Los Tuxtlas fragment from other remaining forests was not an immediate event, but progressed continuously over the twentieth century, with substantial isolation beginning approximately in the 1960s. Thus, fragmentation had only about 50 years time, possibly less, to affect gene flow among orchid bee populations. This may have been insufficient time to result in a reduction of allelic diversity or shift in allelic composition among populations, even if no long-distance dispersal occurred (Saunders et al. 1991). Individual orchid bee adult life spans of up to 6 months coupled with long generation times and relatively stable population sizes (Roubik and Ackerman 1987) might considerably delay population genetic effects in isolated populations of orchid bees. At present we cannot distinguish between the two alternative explanations. We can conclude, however, that forest fragmentation on substantial spatial and temporal scales did not result in measurable population genetic effects in the genus *Euglossa*. A replication of this study in other fragments and covering even longer time-spans of forest fragmentation is surely of interest and might yield different results. Overall, our results are in agreement with earlier findings that some euglossine bees fare reasonably well in disturbed habitat and forest edges (Brosi 2009; Otero et al. 2008; Rincon et al. 1999). Furthermore, our results agree with previous observations that orchid bees have weak population structure (Dick et al. 2004) and low production of diploid males (Souza et al. 2010). The last issue is particularly noteworthy since high numbers of diploid males have been claimed by some allozyme-based studies, interpreting this as a sign for inbreeding and genetic degeneration of euglossine populations (Lopez-Uribe et al. 2007; Roubik et al. 1996; Zayed et al. 2004). In the present study we genotyped a total of 1,005 individual males with highly variable markers and found only one diploid (heterozygous) male. This is within the range normally expected for non-inbred haplodiploid hymenopterans (Cook 1993).

The remarkable differences of relative species contribution to bait captures in the three forest localities are puzzling, especially given the lack of genetic differentiation between populations. It might be attributed to spatio-temporal differences in food, chemical, or nesting resource availability, which might lead to differences in abundance of species at the different localities, at least temporarily. Shifts in the numerical composition of euglossine species in baiting studies is commonly found also on smaller spatial scales and across seasons (Ackerman 1989; Armbruster 1993; Janzen et al. 1982; Pearson and Dressler 1985), suggesting that our baiting results represent snap shots of dynamic communities.

Male orchid bees deserve special attention with regard to dispersal and gene flow. In their search for volatiles they are believed to cover large areas of habitat and may even lead a nomadic lifestyle. While the latter is unlikely (Ackerman and Montalvo 1985), long-distance travel of males is suggested by direct and indirect evidence. Most importantly, males can be lured to artificial chemicals over long stretches of uninhabitable area, including open water and non forest areas (Ackerman 1981; Raw 1989; Tonhasca et al. 2003). This suggests that their function as long distance pollinators (Ackerman et al. 1982; Janzen 1971; Kroodsma 1975) is not necessarily precluded by fragmentation and that some gene flow of euglossophilous plants can be expected between forest fragments (Tonhasca et al. 2003). Furthermore, volatile-driven male dispersal might be of particular importance for long-distance gene flow. So far, there is no evidence for male-biased gene flow in orchid bees, but this is clearly a promising avenue for future research. In contrast to males, females are nest-based central-place foragers and seem often philopatric with respect to their natal nest. For example, the adult females of *E. viridissima* that were found in a given nest box in Yucatan were almost always close relatives (Cocom Pech et al. 2008; Zimmermann et al. 2009b), suggesting that eclosed females remain in and reactivate the nest from which they have eclosed. Thus, it is quite likely that individual females are less apt to long-distance dispersal than males.

While not providing evidence for, our results are in overall agreement with the idea that biodiversity benefits from the conservation of even small fragments of tropical rainforest (Turner and Corlett 1996). Fragmentation as a breaking apart from formerly continuous forest may not be equalized to habitat loss, since negative effects for populations still depend on the fragments' quality, the degree of isolation, and the edge area ratio (Fahrig 2003; Tscharntke et al. 2002). Forest fragments can act as last retreats for plant and animal species (Turner and Corlett 1996), and the preservation of orchid bee populations acting as effective pollinators would surely have a positive influence on the plant species diversity.

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