

Impacts of forest fragmentation on orchid bee (Hymenoptera: Apidae: Euglossini) communities in the Chocó biodiversity hotspot of northwest Ecuador

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Abstract Habitat loss is a major driver of bee declines worldwide, and is of key relevance in the tropics given high deforestation rates, but we continue to have a poor understanding of the impact of land-cover change on tropical bee communities. Orchid bees (Apidae: Euglossini) are critical long-distance pollinators and may be highly susceptible to forest fragmentation given their reliance on forest habitat. Previous studies on the impact of forest fragmentation on euglossines have been geographically limited, have largely ignored β -diversity, and have not compared fragments with continuous forest. To contribute to addressing these gaps, we sampled male euglossine bees in 18 forest fragments (area range: 2.5–33 ha) and at eight locations within a large (3500 ha) continuous forest in the Chocó biodiversity hotspot of Ecuador during the dry season in 2014. We assessed how euglossine abundance, richness, and evenness related to fragment area, isolation, and edge:area ratio. We also compared fragments to continuous forest, in terms of α - and β -diversity. In fragments, a single species (*Euglossa tridentata*) comprised 78% of captures, and we found no significant effect of fragment area, isolation, or edge on abundance, richness, or evenness among fragments. Forest

fragments and continuous forest differed in both community composition and evenness, but not in abundance or species richness. Spatial turnover (β -diversity) showed a non-significant trend toward changing more rapidly in continuous forest relative to fragments. These results underscore the conservation value of continuous forest for orchid bee diversity.

Keywords Forest fragmentation · Orchid bee · Beta diversity · Ecuador

Introduction

Despite advances in our understanding of the impact of human activities on bee communities, tropical bees remain understudied (Mark and Robert 2009; Winfree et al. 2009; Potts et al. 2010). Land use changes are considered to be the most important driver of pollinator declines (Skole and Tucker 1993; Mark and Robert 2009; Winfree et al. 2009), and tropical regions are currently experiencing widespread land use changes, particularly deforestation (Geist and Lambin 2002) and other forms of primary habitat conversion (Lambin et al. 2003). Given that bee declines could potentially have serious impacts for plants that rely on their pollination services, the high diversity of flowering plants in these regions, and the fact that nearly all Neotropical angiosperms rely on pollinators for their pollen dispersal, these pollinator declines may have more serious impacts in the tropics than in other parts of the world (Bawa 1990; Wilcock and Neiland 2002; Vamosi et al. 2006; Pauw 2007; Potts et al. 2010). In spite of the concern about bee declines and their importance in the tropics, few studies have evaluated the impact of forest fragmentation on native bees in these regions.

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Orchid bees (Apidae: Euglossini) are an especially important group of Neotropical pollinators. They pollinate orchids and a range of other plant families including Apocynaceae, Rubiaceae, Verbenaceae, and Zingiberaceae among others (Dressler 1982). They fly long distances (up to 23 km in a day), pollinate plants with low population densities (Dressler 1968; Janzen 1971; Bawa 1990; Tonhasca et al. 2003; Wikelski et al. 2010), and forage across the complex vertical strata of tropical forests (Bawa 1990). Orchid bees are thought to live almost exclusively under tree canopies, in contrast to many other bee groups (Dressler 1968, 1982; Roubik and Hanson 2004), and thus may be relatively susceptible to habitat fragmentation (Dressler 1982; Roubik and Hanson 2004; Brosi et al. 2007; Brosi 2009).

Despite a growing body of literature, there is still a lack of consensus on how forest fragmentation affects orchid bee communities. Different characteristics of forest fragments, such as size, degree of isolation, and fragment shape (Saunders et al. 1991), can theoretically affect different orchid bee species differently, leading to changes at the community level (Otero and Sandino 2003; Brosi et al. 2007; Brosi 2009; Nemésio and Silveira 2010; Vasconcelos and Bruna 2012; Nemésio and Vasconcelos 2013). While some studies have found no impact of fragment size on abundance or diversity of euglossines (Tonhasca et al. 2002; Storck-Tonon et al. 2013), other studies suggest that a relationship exists (Brosi 2009; Nemésio and Silveira 2010). There are also conflicting results regarding the impact of amount of fragment edge relative to the interior or shape. Brosi (2009) found higher abundance and diversity in fragments with more edge, while Nemésio and Silveira (2010) found the opposite: fragments with more edge had lower diversity and abundance. Differences in the conclusions of these studies may result from a number of different factors including, but not limited to, confounding variables such as elevation, different environmental conditions not examined, other landscape elements, or lack of statistical power due to low sample size.

In addition to these conflicting results focused on α -diversity (within-site diversity), another limitation of existing work on euglossines and forest fragmentation is the lack of consideration of β -diversity. While β -diversity does not have a single commonly agreed upon definition, it typically refers to spatial or temporal “turnover,” i.e. change in community composition (Whittaker 1972; Anderson et al. 2011). Although originally intended to be used across gradients (Whittaker 1972), it has been applied more generally to landscape heterogeneity (Anderson et al. 2011). Across a range of taxonomic groups, β -diversity responds more consistently to land-use change than does α -diversity (Kessler et al. 2009). In birds, β -diversity has been shown to decrease as a result of land use change,

even when α -diversity does not necessarily change (Karp et al. 2012). One study has characterized β -diversity of euglossine bees, but only in large patches of a continuous forest (>1000 ha; Nemésio and Vasconcelos 2013), and no study to our knowledge, has examined β -diversity across fragment characteristics or compared continuous forest to fragments.

The body of work on euglossine responses to land-use change also is striking for its very limited geographic focus. The great majority of studies have taken place in one of two countries, either Brazil (Tonhasca et al. 2002; Nemésio and Silveira 2010; Aguilar and Gaglianone 2012; Nemésio and Vasconcelos 2013; Storck-tonon et al. 2013) or Costa Rica (Brosi 2009; Suni and Brosi 2011; Suni et al. 2014). Notable exceptions include Otero and Sandino (2003), who focused on euglossines in the Chocó biodiversity hotspot in Colombia, and Briggs et al. (2013), who worked in southern Mexico.

A final limitation of previous work in this arena is that, to our knowledge, no other study has compared euglossine communities in forest fragments to a large, truly continuous forest. Some other studies have included relatively large fragments, in the range of 200–300 ha (Brosi 2009; Nemésio and Silveira 2010), but such fragments are still quite small relative to the area of continuous forest they were once connected to. For this reason, the inclusion of an appropriate continuous-forest benchmark would be useful in determining the impacts of habitat loss and fragmentation on patterns of diversity in euglossine bees.

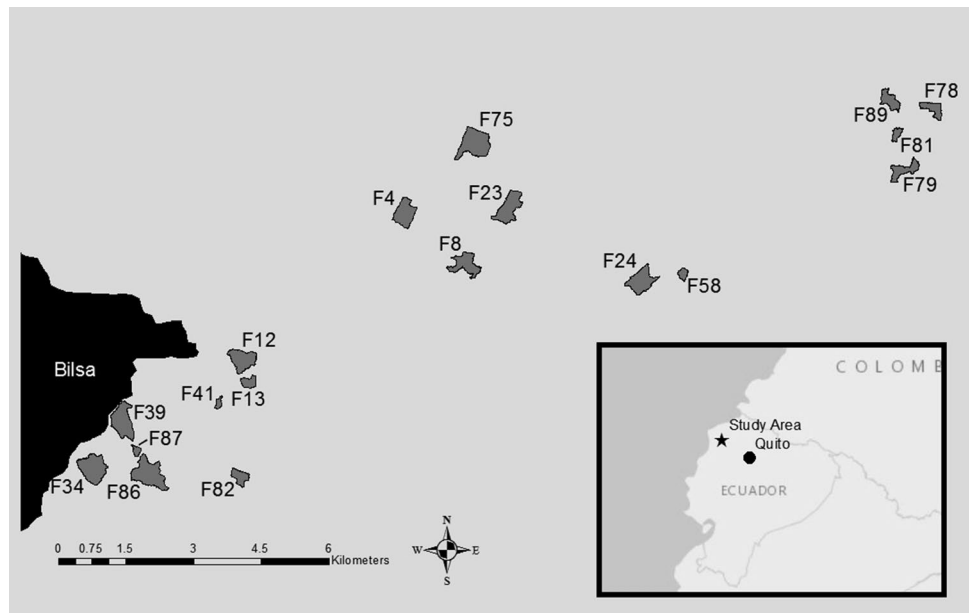
To contribute to addressing these gaps, we sampled orchid bees in 18 forest fragments and at eight sites within a large (3500 ha), continuous forest in the Chocó biodiversity hotspot in northwestern Ecuador. We conducted comparisons both among forest fragments and also between fragments and continuous forest. We predicted that we would find higher euglossine α -diversity in fragments that were (1) larger and (2) nearer to continuous forest. We also expected to find a relationship between α -diversity and (3) perimeter: area ratios, though given contrasting previous findings we were unsure of the possible direction of the relationship. Finally, we expected to find (4) higher euglossine α - and β -diversity in continuous forest compared to forest fragments.

Materials and methods

Study sites

We sampled male euglossines at 26 sites in total within the Chocó biodiversity hotspot of northwestern Ecuador from August to December 2014 (Fig. 1), with a particular focus on the Mache-Chindul Ecological Reserve and surrounding

Fig. 1 Map of fragments, in gray, where bees were sampled between August and December 2014. Bilsa, depicted in *black*, is a large continuous forest



area. Of these sites, 18 were in forest fragments that ranged in size from 2.5 to 33.0 ha and were 115–647 m asl (above sea level) along an altitudinal gradient (Table 1). Fragments were situated between 0.3 and 17.4 km from the nearby Bilsa Biological Reserve (“Bilsa” hereafter), a large, undisturbed old growth forest of 3500 ha that extends from 300 to 700 m asl (Fig. 1; Table 1). Specific dates of sampling at each site are also included in Table 1. We also sampled within Bilsa: two sites were located at the forest edge and six sites were within the forest interior. The surrounding landscape is primarily agricultural land that was converted from continuous forest within the last 50 years (Durães et al. 2013), and there are other forest fragments in the study area that we did not sample.

Bee sampling and identification

We designed our bee sampling effort not to exhaustively catalog all species present in a given site, but rather to determine if there were fragmentation-associated differences in diversity, abundance, and community composition in the euglossine assemblages. We sampled each site at two different trapping stations separated by approximately 200 m. Paired trapping stations were each sampled once within 1 and 11 days of each other (avg. = 5 d, SD = 3.5 d). At each trapping station we set three ISCA Ball Traps™ (i.e. McPhail traps; ISCA Technology Incorporated, Riverside, CA, USA), for a total of six traps per site. Each trap was baited with 1.5 mL of the one of three baits: cineole, eugenol, or methyl salicylate. We chose these baits because they attract male euglossines from a very broad range of species (e.g., Janzen et al. 1982; Brosi 2009). As in nearly all euglossine studies, we sampled males only since females

are not attracted to scent baits (Roubik and Hanson 2004). In fragments and at the Bilsa “edge” sites, we established trapping stations 20 m from the forest edge, to standardize edge effects; situating sample sites in the middle of each fragment could have led to patterns dominated by edge effects rather than area per se. At each station we spaced the three traps 3 m apart from each other and hung them 1.5 m off the ground on a line tied between trees. We measured rainfall with a rain gauge at each station during trapping. As our study was conducted during the dry season, no site received more than 0.5 mL of rain during a sampling event (max = 0.5 mL, mean = 0.1 mL, SD = 0.12 mL). Traps were open for 24–25.1 h (mean 24.10, SD = 0.189 h). We pinned captured bees the same day and stored them in a dehumidified box over the duration of the field sampling period to prevent spoiling, and afterward imported them to the U.S for identification using Roubik and Hanson (2004).

Site characteristics and environmental variables

We used on-the-ground surveys in conjunction with the Global Forest Change dataset (Hansen et al. 2013) to select study forest fragments among a broader set of available fragments in the area. We calculated the area of each fragment by walking the perimeter of the fragment with a handheld GPS unit (Garmin eTrex 30x; Garmin, Olathe, KS, USA). We recorded elevation at each of the trapping stations with a handheld GPS unit to calculate an average value for each site. To calculate a metric of perimeter: area ratio that does not scale with fragment area, we used $\text{perimeter}^2/\text{area}$, following Brosi (2009).

There was no correlation between forest fragment area and distance to Bilsa, or between fragment area

Table 1 Sites sampled. “Bilsa Int.” refers to sites in the interior of Bilsa, the large forest block in the study. Each forest fragment site had two trapping stations, denoted ‘a’ and ‘b’

Site	Trapping station	Date	Site type	Elevation (m ASL)	Matrix on edge
4	a	8/13/14	Fragment	434	Pasture
4	b	8/14/14	Fragment	416	Pasture
8	a	8/14/14	Fragment	265	Secondary forest
8	b	8/11/14	Fragment	286	Secondary forest / banana plantation
12	a	10/9/14	Fragment	575	Mixed fruit tree plantation
12	b	10/12/14	Fragment	501	Patchy secondary forest / banana
13	a	10/9/14	Fragment	556	Mixed fruit tree plantation
13	b	10/12/14	Fragment	569	Pasture
23	a	8/11/14	Fragment	368	20 year old caoba plantation
23	b	8/17/14	Fragment	341	Secondary forest
24	a	12/2/14	Fragment	205	Cacao, finca
24	b	12/3/14	Fragment	212	Pasture, finca
34	a	11/5/14	Fragment	573	Mixed pasture, scattered palms, finca
34	b	11/8/14	Fragment	539	Pasture
39	a	11/5/14	Fragment	633	Pasture
39	b	11/7/14	Fragment	644	Pasture
41	a	10/12/14	Fragment	528	Pasture
41	b	10/4/14	Fragment	557	Pasture
58	a	12/2/14	Fragment	227	Pasture
58	b	12/3/14	Fragment	161	Pasture
75	a	8/13/14	Fragment	449	Pasture
75	b	8/17/14	Fragment	386	Secondary forest
78	a	9/3/14	Fragment	196	15 year old teak plantation
78	b	9/11/14	Fragment	147	Cacao plantation
79	a	9/3/14	Fragment	237	Cacao plantation
79	b	9/9/14	Fragment	213	4 year old regrowth forest
81	a	9/8/14	Fragment	115	Cacao plantation
81	b	9/19/14	Fragment	148	African palm plantation
82	a	10/12/14	Fragment	369	Slash and burning forest
82	b	10/4/14	Fragment	413	Pasture
86	a	11/5/14	Fragment	534	Pasture
86	b	11/8/14	Fragment	489	Banana plantation, and finca, house
87	a	11/5/14	Fragment	562	Pasture, finca
87	b	11/8/14	Fragment	509	Pasture, fragmented landscape
89	a	9/8/14	Fragment	139	Pasture
89	b	9/19/14	Fragment	220	Pasture and cacao plantation
Bilsa Edge 1	n/a	11/7/14	Edge	574	Pasture, finca
Bilsa Edge 2	n/a	11/9/14	Edge	568	Pasture, fragmented landscape
Bilsa Edge 3	n/a	11/7/14	Edge	647	Pasture, mixed forest fragments
Bilsa Edge 4	n/a	11/9/14	Edge	629	Pasture, fragmented landscape
Bilsa Int. 1	n/a	9/15/14	Interior	522	n/a
Bilsa Int. 2	n/a	9/17/14	Interior	567	n/a
Bilsa Int. 3	n/a	9/15/14	Interior	479	n/a
Bilsa Int. 4	n/a	9/17/14	Interior	438	n/a
Bilsa Int. 5	n/a	9/15/14	Interior	525	n/a
Bilsa Int. 6	n/a	9/17/14	Interior	515	n/a
Bilsa Int. 7	n/a	9/15/14	Interior	368	n/a
Bilsa Int. 8	n/a	9/17/14	Interior	409	n/a
Bilsa Int. 9	n/a	11/12/14	Interior	513	n/a
Bilsa Int. 10	n/a	11/13/14	Interior	476	n/a

Table 1 (continued)

Site	Trapping station	Date	Site type	Elevation (m ASL)	Matrix on edge
Bilsa Int. 11	n/a	11/12/14	Interior	475	n/a
Bilsa Int. 12	n/a	11/13/14	Interior	534	n/a

and perimeter: area ratio. However, there was a significant positive correlation between distance from Bilsa and perimeter: area ratios (Pearson's Correlation Coefficient = 0.488, $p = 0.0398$). Because the sites are arranged more or less linearly from Bilsa along an altitudinal gradient (Table 1; Fig. 1), elevation was highly significantly correlated with distance to Bilsa (Pearson's Correlation Coefficient = -0.92 , $p < 0.0001$). Because we were more interested in anthropogenic than biogeographic factors, we selected to use distance to Bilsa rather than elevation in our analyses (below).

Data analysis

Our first set of analyses compared the euglossine bee communities of forest fragments to one another. We specifically assessed the impact of forest fragment characteristics (area; perimeter: area ratio; and continuous distance from Bilsa) on bee abundance (raw counts of bees collected at each site) and diversity (measured through its two components: species richness and evenness) using generalized linear models (GLMs) with quasi-Poisson errors for observed richness and abundance (which are count variables) and linear models (LMs) for evenness, calculated as Pielou's J (Pielou 1975), a continuous measure. Because each fragment was sampled once, and data from that sample were pooled, we did not have to account for repeated measures, meaning that GLMs and LMs are appropriate for these data. We ran GLMs and LMs in the R statistical programming language (R Core Team 2015).

Our second set of analyses compared forest fragments to continuous forest, in terms of (1) species richness and abundance, (2) evenness, and (3) β -diversity (spatial turnover between sites). First, to account for differential sampling effort in the fragments and continuous forest (i.e., more sites in fragments than in continuous forest), we compared species richness through the use of species richness estimators and individual-based rarefaction (Colwell et al. 2012). We estimated richness by pooling all sites within the two treatments (continuous and fragments) and calculating species richness estimators (Chao, first order jackknife, and bootstrapped) using the "specpool" function in the "vegan" package. We also used individual-based rarefaction using the "specaccum" function in "vegan." These allowed us to estimate the number of species and 95% confidence intervals and to compare species richness,

accounting for differential sampling effort. Second, to compare species evenness in fragmented versus continuous forest, we ran linear models to compare evenness (Pielou's J) using site type (either fragment or Bilsa) and site location type (fragment, Bilsa edge, or Bilsa interior). Third, to quantify β -diversity, we estimated turnover in community composition over space, following the approach of Karp et al. (2012). We did this by calculating the Morisita–Horn index of pairwise community dissimilarity, which allows for unbalanced sampling between comparison groups (Horn 1966), and assessing its relationship with pairwise geographic distance using Mantel tests, using the "vegan" package with 9999 permutations. To compare rates of turnover over distance between sites, we ran three separate Mantel tests: (a) continuous forest sites only; (b) forest fragment sites only; and (c) all sites combined. We also ran a permutational multivariate ANOVA ("adonis" function in "vegan") to compare the differences in community composition between sites within continuous forest and fragments.

Results

Overview

We sampled 2305 orchid bees representing 24 species in the genera *Euglossa*, *Eufriesea*, and *Eulaema* (Table 2). A species accumulation curve suggests that we likely sampled the majority of species in the area that were attracted to the baits we used (Fig. 2). The most common bee species sampled was *Euglossa tridentata*, which comprised 78% of specimens from forest fragments and 66.5% of overall specimens (Table 2).

Comparing euglossine communities among forest fragment sites

Fragment characteristics (area, perimeter: area ratio, and distance to continuous forest) were not statistically related to the abundance or species richness of euglossine communities (Table 2). Distance to Bilsa was associated with a marginally significant increase in evenness

Table 2 Species (in alphabetical order) captures in relation to essential oil baits

Species	Cineole	Eugenol	Methyl salicylate	% of total ^a	No. of Frag. sites ^b	No. of Con- tin. forest sites ^b
<i>Eufriesea</i> sp.	3	1	0	0.17	1	2
<i>Euglossa allosticta</i>	32	0	0	1.39	8	4
<i>Euglossa asarophora</i>	14	0	0	0.61	0	4
<i>Euglossa azureoviridis</i>	24	0	0	1.04	9	0
<i>Euglossa cyanura</i>	2	0	0	0.09	1	0
<i>Euglossa deceptrix</i>	4	0	0	0.17	3	0
<i>Euglossa despecta</i>	8	0	0	0.35	5	0
<i>Euglossa dissimula</i>	12	0	0	0.52	5	3
<i>Euglossa dodsoni</i>	65	0	0	2.82	17	5
<i>Euglossa dressleri</i>	18	0	0	0.78	5	4
<i>Euglossa flammea</i>	28	3	0	1.34	3	6
<i>Euglossa gorgonensis</i>	153	0	0	6.64	13	8
<i>Euglossa hansonii</i>	15	0	0	0.65	6	4
<i>Euglossa heterosticta</i>	27	0	0	1.17	9	2
<i>Euglossa ignita</i>	63	0	14	3.34	10	6
<i>Euglossa maculilabris</i>	16	0	1	0.74	5	5
<i>Euglossa mixta</i>	4	0	7	0.48	8	0
<i>Euglossa</i> sp. A	226	0	0	9.80	3	8
<i>Euglossa</i> sp. B	9	1	0	0.43	8	1
<i>Euglossa</i> sp. C	8	0	0	0.35	3	1
<i>Euglossa</i> sp. D	2	0	0	0.09	1	0
<i>E. tridentata</i>	1523	9	0	66.46	18	8
<i>Eulaema speciosa</i>	1	0	0	0.04	0	1
<i>Eulaema</i> sp.	12	0	0	0.52	2	1

^aPercentage of all bees collected across all sites

^bHow many sites within each general site type (fragments vs. continuous forest) the species was found

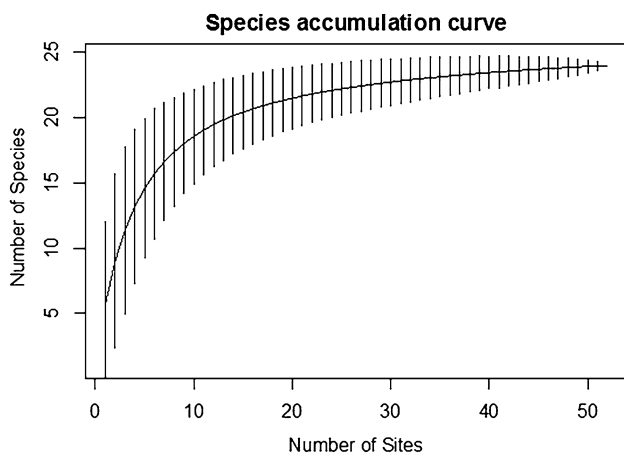


Fig. 2 Species Accumulation Curve, based on randomizing sample days 9999 times without replacement with 95% confidence intervals

($P=0.0627$; Table 3), but not with abundance or species

richness.

Comparing euglossine communities between fragments and continuous forest

We found no significant difference between euglossine abundance (Table 3) or species richness in continuous forest versus forest fragments using either individual based rarefaction or species richness estimators (based on overlapping confidence intervals). Euglossine assemblages within continuous forest were significantly more even (as measured by Pielou's J) than sites within fragments ($P=0.000848$; Table 3). This pattern was likely driven primarily by *E. tridentata*: in forest fragments, this species comprised ~78% of all bees collected, but made up only ~26% of the bees captured in continuous forest (Fig. 3). When we included location within the continuous forest (i.e., edge vs. interior) into the model, however, this relationship was no longer significant ($P=0.119$, Table 3), likely due to reduced sample size. Euglossine community composition differed significantly between

Table 3 Statistical results for abundance, richness, and evenness.

Variable	Abundance		Richness		Evenness	
	Coefficient	<i>P</i>	Coefficient	<i>P</i>	Coefficient	<i>P</i>
Area ^a	0.025	0.223	-0.0028	0.820	-0.0041	0.218
Distance to bilsa ^a	-0.000075	0.115	-0.000030	0.235	0.000013	0.0627
Perimeter: area ^a	-0.0025	0.387	0.0020	0.252	-0.00042	0.369
Continuous ^b → fragmented	0.46	0.292	-0.14	0.445	-0.23	0.000848***
Continuous edge → continuous interior	0.10	0.916	0.43	0.266	0.07	0.528
Continuous edge → fragmented edge	0.54	0.524	0.20	0.580	-0.17	0.119

***Statistically significant relationships (at the *P* < 0.05 level) are indicated

^aThe analyses were conducted for fragments only with the values for trapping stations pooled per fragment

^bBoth Bilsa edge and interior sites were pooled together

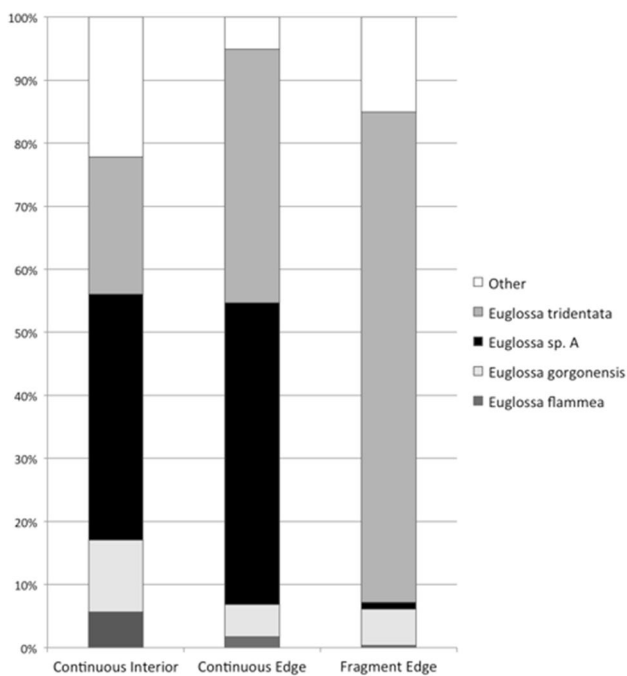


Fig. 3 Community composition of bees captured in Bilsa and the fragments. “Other” indicates species that represented less than 5% of the specimens collected from each treatment

continuous and fragmented sites, based on Adonis analyses of Morisita–Horn similarity (*P* = 0.0001, Fig. 3). This overall relationship remained significant when continuous forest sample location (edge vs. interior) was also included into the model (*P* = 0.0001). Specifically, euglossine communities in forest fragments had significantly different community composition relative to continuous forest edge sites (*P* = 0.001), but within continuous forest, euglossine assemblages in edge versus interior sample sites did not significantly differ from one another (*P* = 0.40).

β-Diversity

Euglossine community similarity was significantly correlated with geographic distance for fragmented sites (Mantel *R* = 0.24, *P* = 0.022; Fig. 4), but not for sites in continuous forest. The relationship between community similarity and geographic distance (i.e. spatial turnover) showed a non-significant trend toward being higher in continuous forest relative to fragments (Fig. 5), but did not differ between continuous forest and fragments, as assessed by confidence intervals of Mantel *R* slopes (Fig. 5).

Discussion

We found that forest fragmentation and habitat loss are associated with differences among orchid bee communities in the Ecuadorian Chocó. Overall, our results fall into three main categories, each discussed in more detail below. First, comparing forest fragments to one another, we found no significant changes in euglossine abundance, species richness, or evenness based on patch-level fragment characteristics (area, isolation from continuous forest, or edge: area ratio). Second, comparing fragmented to continuous sites, we found no differences in species richness or abundance, but continuous forest sites had significantly more even distribution of euglossine species relative to fragmented sites, as well as statistically distinct community composition. Third, examining β-diversity, sites within continuous forest showed a non-significant trend toward having greater turnover rates in space relative to fragmented sites. For fragmented sites, this relationship was statistically significant despite a weaker effect size (smaller slope). These results demonstrate the conservation value of continuous forest, given the differences in community composition between continuous forest and fragments, greater community evenness in continuous forest, and a trend toward greater β-diversity.

Fig. 4 Community similarity (Morisita–Horn index) between each site pair plotted by distance. *Black circles* indicate fragment–fragment site pairs. *Red triangles* are continuous–continuous site pairs. *Open blue squares* indicate continuous edge fragment site pairs. *Full blue squares* indicate continuous interior-fragment site pair. (Color figure online)

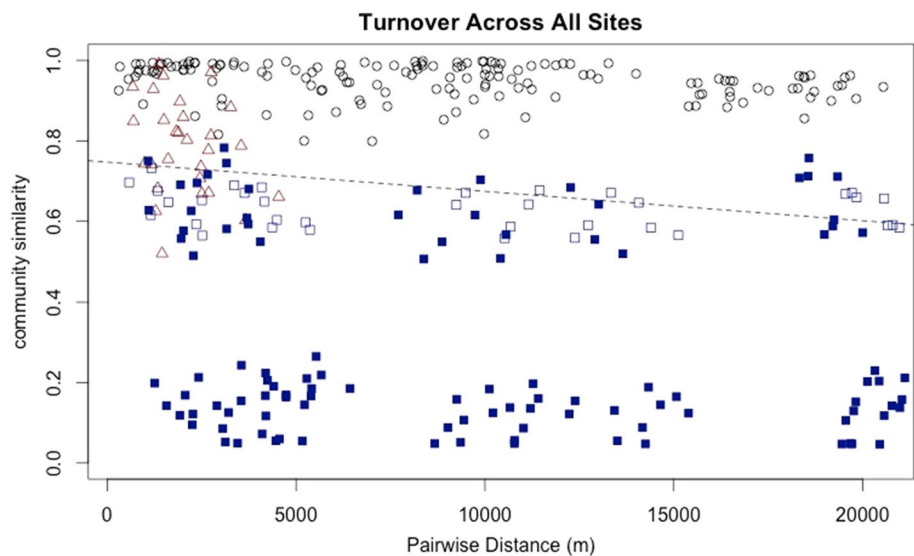
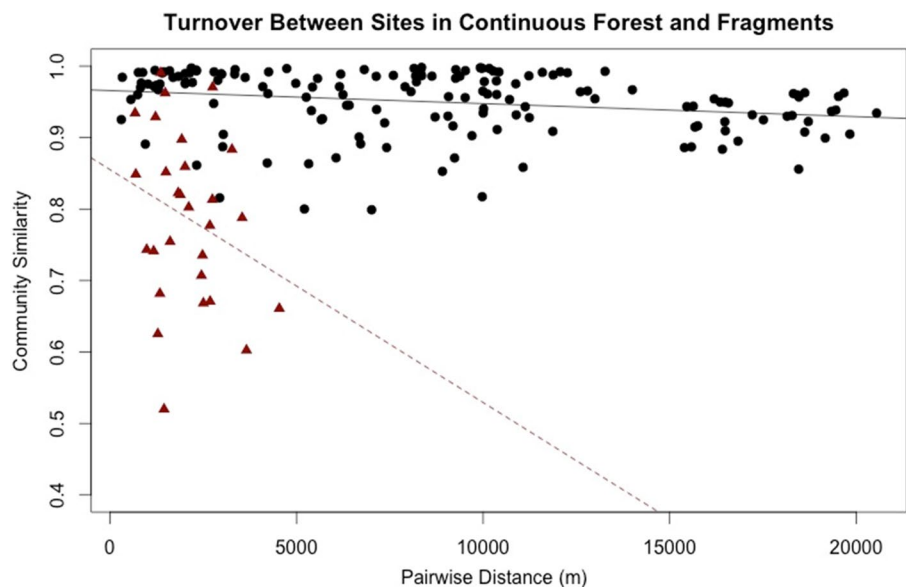


Fig. 5 Community similarity between sites in fragments and continuous. *Red triangles* indicate each pair of sites within continuous forest; *black circles* indicate each pairwise combination of sites within fragments. The *red line* indicates the best-fit line for pairwise similarity and distance between sites within the continuous forest. The *black line* is the best-fit line for pairwise similarity and distance between sites in different fragments. Community similarity is assessed with the Morisita–Horn index. Note the change in the Y-axis scale relative to Fig. 4. (Color figure online)



First, and in contrast to some previous studies, we did not find sufficient evidence to conclude that patch-level fragment characteristics (size, perimeter: area ratio, and distance to continuous forest, which was tightly correlated with elevation) alter abundance or α -diversity (species richness or evenness). Both Brosi (2009) and Nemesio and Silveira (2010) found either statistically significant or marginal increases in abundance and richness in relation to increasing fragment size. Both studies also found significant or marginal differences in abundance and richness as a result of fragment perimeter: area ratio. The results we report here could be due to low power (though to our knowledge our sample size of 18 fragment sites is second only to Brosi 2009, with 22 sites), less variation in fragment area (Brosi 2009: fragments from

0.24 to 296 ha; Nemesio and; Silveira 2010: fragments from 1 to 354 ha; this study: 3–34 ha) or the fact that all of our sample sites were near the edges (rather than the centers) of fragments. Like other fragmentation studies (Brosi 2009), we found no significant impact of isolation on species abundance and richness. This may be due to euglossine capability for long distance flight (Janzen 1971; Wikelski et al. 2010), especially given that orchid bees seem to be able to move across deforested land (Tonhasca et al. 2003; Suni and Brosi 2012). Another possible explanation for these results is that other habitat factors, such as vegetation structure or surrounding land use, play a larger role in defining euglossine communities than fragment characteristics per se (Briggs et al. 2013; Storck-Tonon et al. 2013). This is particularly true in our

study landscape, where tree cover surrounding fragments was relatively high, ranging between 30 and 70%.

Second, when comparing forest fragments to continuous forest, we found that evenness and community composition differed significantly. Although no other study has explicitly compared more than a handful of fragments to continuous forest, these results are generally consistent with inferences that can be made from the existing literature, particularly studies that have found changes in community composition of orchid bees resulting from increased disturbance (Otero and Sandino 2003; Brosi 2009). Despite these changes in euglossine community composition and evenness, abundance and richness did not significantly differ between sites in fragments and sites in continuous forest. Together, these results are likely driven by species-specific responses to landscape characteristics. For example, *E. tridentata* were highly abundant in fragmented sites (Fig. 3), while other species (including, e.g. *E. asarophora* and *Eulaema speciosa*) were found exclusively in continuous forest. Such differential responses are consistent with the findings of Powell and Powell (1987), Aguiar and Gaglianone (2012), Suni et al. (2014), and Nemésio (2013). Evenness is a key component of biological diversity and is associated with greater ecosystem functioning (Wittebolle et al. 2009), and thus the higher evenness of euglossine species we found in large, continuous forest is consistent with potentially greater functioning and/or conservation value.

Third, in terms of β -diversity, we found a statistically significant relationship between community composition and geographic distance in the fragmented sites. This was not the case for our sites within continuous forests and across both types of sites, despite the fact that sites in continuous forest trend toward having a higher rate of turnover than sites in fragments. This result is likely driven at least in part by low statistical power for sites exclusively in continuous forest, because there were fewer than half as many sites in continuous forest (eight) compared to fragments (18). Despite the lack of a statistical result, the stronger spatial species turnover in continuous forest could be the result of more varied habitat within continuous forest or climatic variation resulting from altitudinal or structural changes within the continuous forest compared to those habitats in fragments. Nemésio and Vasconcelos (2013) found climatic differences had a bigger impact on species similarity than geographic distance, but at a much larger spatial scale, with some sites nearly 600 km apart.

As with all field studies, there are a number of limitations to this work. First, there is a strong correlation between distance to Bilsa and elevation in our study sites. Given that we did not find significant patterns between either of those variables and euglossine diversity, abundance, or community composition, this does not appear to be a major problem. A second potential confounding

variable is time. Because of the distribution of our sites, the sites further from Bilsa were all sampled within a period of 1 month and sites nearer to Bilsa were sampled in another month (Table 1; sites nearer to Bilsa are also higher elevation as noted above). As euglossine communities are known to shift with season (e.g. Roubik 2001), this sampling scheme could have potentially biased the results, particularly in terms of community composition. A separate issue is that by sampling fragments only at edge sites and sampling the continuous forest largely in the interior, the observed differences in community composition between continuous forest and fragments may be driven by edge effects (though we also found a statistically significant difference between fragment sites and continuous forest edge sites). Nemésio and Silveira (2006) found that edge effects on orchid bees can occur up to 50 m from the perimeter of a forest, so including sites in the interior of fragments, as well as the edge, may have better characterized those euglossine communities. Finally, in our study design we maximized site-level replication, with the trade-off of sampling each forest fragment only once. Using a more in-depth characterization of euglossine communities at each site, as well as a greater number of sites, we would have likely increased our power to detect differences generated by landscape patterns. These kinds of design trade-offs are a central issue in landscape ecology field studies (e.g. McGarigal and Cushman 2002).

Despite the fact that it appeared as if we detected the majority of species in the regional pool (based on the species accumulation curve strongly approaching an asymptote), our results do not necessarily provide a complete estimate for orchid bee species richness in our study region. Because our samples were taken during the dry season, we can make no inference about the diversity of orchid bees during the wet season, when there may be higher orchid bee diversity (Becker et al. 1991). As with many euglossine studies, we used three essential oil baits, and therefore did not sample for bees that are exclusively attracted to other essential oils. Furthermore, even using the same essential oils, our species captured may have differed if we sampled in the wet season, as there is some evidence that essential oil preference changes with the seasons for some species of orchid bee (Abrahamczyk et al. 2012). Finally, the lack of correlation between richness between trapping stations within the same fragments suggests either within-habitat heterogeneity (Armbruster 1993); insufficient sampling; and/or high sample variability (Roubik 2001; Nemesio and Silveira 2006).

Future studies of the effects of forest fragmentation on euglossines should be conducted at a larger spatial and temporal scale. Few studies (Tonhasca et al. 2002; Nemésio and Vasconcelos 2013; Suni et al. 2014) have lasted for longer than a single year, and of these, only one (Nemésio

and Vasconcelos 2013) has more than ten sites. Inclusion of more sites will also increase the ability to discern patterns. The largest number of sites used in a forest fragmentation study to our knowledge has been 22 (Brosi 2009), and many have fewer than ten. The differences found between fragments and continuous forest in our study suggest that to better understand fragmentation, more studies should include sites within continuous forest as a point of comparison when possible. Our results also demonstrate the value of assessing—diversity in the study of forest fragmentation. Finally, given that most previous studies have been located in Brazil, more studies should be conducted across other tropical regions.

It is of vital importance that we continue to study the impacts of human activities on pollinators. This is particularly important in the tropics, where relatively little research has been conducted on bee responses to anthropogenic environmental changes, and where habitat loss and modification has been particularly severe in many regions. Since euglossines are thought to require tropical forest for survival (Roubik and Hanson 2004), they are likely to be highly susceptible to land-use changes. Better studies on forest fragmentation, especially those at larger spatial and temporal scales, with high true sample size, and including β -diversity, are strongly needed for the conservation of euglossines and the plants that depend on them.

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