



Deforestation narrows pollen diet diversity of generalist orchid bees

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Abstract

Orchid bee species are important pollinators in the Neotropics. While male orchid bees are known to have a close interaction with odor-rewarding flowers, orchid bee females are often pollen generalists. In the current study, we investigated differences in the pollen diet diversity of orchid bees in various vegetation types and across sites with varying levels of forest cover by means of an analysis of the frass pellets of the orchid bee, *Euglossa cordata* (Linnaeus, 1758), and multiple species based on a literature review. The pollen grains found in the inner part of brood cells were used to assess the plant composition making up the diet of *E. cordata* in various areas of the state of São Paulo in Brazil. We found that this plant composition differed among vegetation types, and that diet specialization was lower in forested areas. Forest cover appeared to be the factor mostly associated with pollen richness in nests of *E. cordata*. Number of brood cells per nest were also associated with forest cover. The model used to assess the effect of forest cover on plant richness as part of brood diet of *E. cordata* was tested with the inclusion of published data and a newly generated data for *E. annectans*. This new data set allowed to understand the influence of forest in the pollen diet of multiple species in a more extensive geographic scale. Forest cover appeared to be important for a diversified pollen diet in several orchid bee species.

Implications for insect conservation Since a diversified pollen diet offered to larva bees is associated with bee survival and health, our results indicate that orchid bees relying on areas with higher forest cover have a more resilient population. Such findings emphasize the importance of conservation of forests, especially in the Neotropical region where many pollinator species are adapted to continuous forest environments.

Keywords Bee-plant interactions · Euglossini · Forest · Larval provisions · Pollen diet specialization · Vegetation types

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Introduction

Bees, with more than 20,000 bee species being described (Michener 2007), are the world's most important pollinator group because of their historical coevolutionary process with flowering plants (Endress 1996). They represent a wide spectrum of pollinators with distinct lifestyles and variable diet breadth (Michener 2007). On the one hand, oligolectic bees present narrow diet breadths since their offspring rely on a select number of plant species from where they can consume the pollen and develop (Carvalho and Schindwein 2011; Wood et al. 2016). On the other hand, generalist (polylectic) bee species forage on a wide range of plant species from many families and offer their larvae nutrients from a diverse set of plants (Cusser et al. 2019). Furthermore, such a rich plant diet composition and diversity of bee-plant partners have been shown to be dependent on landscape composition (Machado et al. 2020), but also to rely on immediate

environmental factors such as local forest cover (Cusser et al. 2019) and resource availability (Peters et al. 2022).

Orchid bees (Hymenoptera: Apidae: Euglossini) account for more than 200 described species and are considered an important group of pollinators in the neotropical region because of their role on certain pollination systems (Moure et al. 2008; Ramírez et al. 2011; Roubik and Hanson 2004). These bees are known to be good flyers and are responsible for maintaining the populations of scattered orchid plant species through long-distance cross-pollination services (Janzen 1971), especially in forests where orchids are particularly abundant (Dressler 1982; Roubik 1993). Male orchid bees collect aromatic oils (perfume) from flowers (Boff et al. 2015; Dressler 1982; Pemberton and Wheeler 2006), mostly from orchids (Ramírez et al. 2011; Brandt et al. 2020), and use this perfume during courtship (Henske et al. 2023). Although the biology of female orchid bees is relatively unknown because of the difficulties of finding their nests in nature (Boff and Alves-Dos-Santos 2018; Solano-Brenes et al. 2018), the females of several species, especially the genus *Euglossa*, have been shown to be generalists, foraging for food resources over a wide spectrum of plant species (Arriaga and Hernández 1998; Cortopassi-Laurino et al. 2009; Rocha-Filho et al. 2012; Villanueva-Gutierrez et al. 2013; Silva et al. 2016; Boff and Alves-Dos-Santos 2018; Pinto et al. 2019; Bittar et al. 2020).

Whereas some *Euglossa* species are restricted to forested environments (Penha et al. 2015), others, such as *Euglossa cordata* (Linnaeus, 1758), are widely distributed throughout a variety of vegetation types with higher abundances in forested sites (Cordeiro et al. 2013; Martins et al. 2018) and comparatively lower abundances in open sites (Silveira et al. 2015). In tropical forests, where plant species diversity is positively correlated to forest cover (Myers et al. 2000), orchid bee species are particularly numerous in comparison with open sites (Botsch et al. 2017; Nemésio and Silveira 2007; Roubik 2004; Sousa et al. 2022), and are thus hypothesized to be adapted to understories of forests (Roubik 1993). In open sites, orchid bees are less common, rare, or were completely absent during surveys (Alves-dos-Santos 1999; Boff et al. 2013; Ferreira et al. 2015; Powell and Powell 1987). Declining populations of orchid bees have been associated with anthropogenic environmental disturbance, such as deforestation (Botsch et al. 2017; Sousa et al. 2022), with a few species seemingly adapted to urban environments (Miranda et al. 2021).

In this study, we examined the pollen grains found in the nests of the orchid bee *E. cordata* to identify the plants on which the females had collected provisions for their brood. Nests were collected in the state of São Paulo, southeastern Brazil, from regions with different vegetation types and variable levels of anthropogenic disturbance. We investigated the effects of vegetation types and elements of

local landscape (e.g., forest cover, water surface, pasture) on the pollen diet diversity of *E. cordata*. Since the composition of plant species and availability of resources can change on large and fine scales (Ritchie et al. 2016; Cusser et al. 2019), we hypothesized (1) that the spectrum of pollen types occurring in larval provision differs among regions because of strong endemism of plants species to each vegetation type (Myers et al. 2000), and (2) that pollen richness in nests varies according to vegetation type and the amount of forest cover. Based on the high density and species richness of plants in the Brazilian Atlantic Forest (Myers et al. 2000), we predicted higher plant diversity in nests from forested sites than in nests from more open areas. We additionally investigated the correlation of forest cover on pollen diet diversity by using unpublished and published data on the pollen contents of the brood cells of seven *Euglossa* species in order to test the hypothesis that the species in this genus are associated with forests, as predicted earlier (Silveira et al. 2015; Sousa et al. 2022).

Materials and methods

Euglossa cordata (Linnaeus, 1758)

Orchid bees have a low density in urban environments (Cândido et al. 2018) with higher genetic diversity in well-preserved natural areas (Boff et al. 2014; Rocha Filho et al. 2013). Orchid bees are known to fly long distances. According to Pokorny et al. (2015) *Euglossa* bee males were recaptured in distance superior to 50 km. Furthermore, in a previous study, the authors found that *E. cordata* can fly continuously over 10 km, and promote gene flow in a stepping-stone manner within a mainland-island system (Boff et al. 2014). *Euglossa cordata* (Fig. 1) is a medium-sized ($n = 8$, ♀ intertegular distance = 3.35 ± 0.15 mm), polylectic orchid bee (Boff and Alves-Dos-Santos 2018; Ferreira-Caliman et al. 2018) occurring in South America and is particularly abundant in forested environments of the Atlantic Forest in Brazil (Nemésio and Silveira 2007). It is a multivoltine species and nesting activities occurs throughout the year (Garófalo 1985).

Study sites

Ten nests and a total of 66 brood cells of *E. cordata* were sampled from various sites in São Paulo State, Brazil. Pollen contents were removed from nine nests and a total of 42 brood cells. The maximum distance between two sampling sites was 435 km (see supplementary information, Table S1 to access the distances between sites). Sampling sites were in mainland areas and in island areas and lay within various vegetation types and with variable levels of forest cover

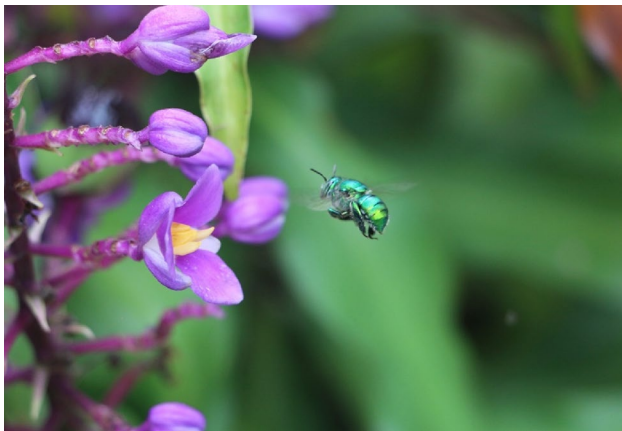


Fig. 1 *Euglossa cordata* female foraging on the flower of *Dichorisanthra thyrsoflora*, Itu (Brazil). Photo: SB

(Fig. 2). The two northernmost sites were located within more open vegetation of the Brazilian savannah vegetation type, known as the Cerrado. At a site within the city of Franca, one nest (FR) was found in an urban area mixed with pasture, crop fields, and exposed soil. The other nest was present in a depauperate area of the Itirapina ecological station (IT) dominated by pastures, crop areas (eg. *Eucalyptus* sp.), exposed soil, and sparse trees. Both sites were characterized by a small amount of secondary forest with limited forest cover. Three other nests were discovered in the city of Itu in the Transition zone in which vegetation encompassed species from the Cerrado and the Atlantic Forest. Two nests in this area (STO I, STO II) were collected in a residential area (Campos de Santo Antonio) with private gardens, surrounded by pasture, and secondary forest cover. The third nest was found in an urbanized site called Vila Rica (VL) surrounded by pasture and secondary forest. The remaining four nests were all detected on continental islands (Ilhabela State Park) lying off the north coast of São Paulo state and dominated by Atlantic Forest in primary and advanced forest stages. One nest was located on Ilhabela (IB), the largest island (33,593 ha) in São Paulo State, 2 km distant from the closest area in the mainland (city of São Sebastião), and three other nests were present in two different sites on Ilha da Vitória (VIT I, VIT II and VIT III), located 38 km from the mainland and 11 km from the nearest island (Ilha de Búzios) (see Fig. 2 and Table 1).

The plant community on the islands of Ilhabela State Park (especially on Ilhabela) resembles the set of Atlantic Forest species occurring in conserved sites on the coast of São Paulo state (Ângelo 1989). Although evidence of increased human population is apparent, especially on Ilhabela, the islands present primary forests and the highest forest cover compared with all other study sites. Ilhabela State Park remain protected since 1977 and shows the largest

contiguous unbroken stretches of Atlantic Forest in the Parque da Serra do Mar. The Cerrado, a vegetation cover in Itirapina and predominant vegetation type in Franca, is highly fragmented and comprises remnants of cerrado *stricto* sensu, cerradão, and riparian forests (gallery and swampy forests). In Itirapina, a large area has been reforested with *Pinus* sp. and *Eucalyptus* sp., which are fast-growing exotic species that provide abundant wood, thus conserving native species. Details of forest cover in all sampling sites are available in Table 1.

The *E. cordata* nests studied here were found at different time points during field excursions either in trap nests offered for bees to nest in or natural cavities (Boff and Alves-dos-Santos 2018). Details about the nesting cavities are presented in Table 1.

Sample preparation

Complete nests or frass pellets (digested pollen from open brood cells from which bees had emerged) were collected and brought to the Bee laboratory at the University of São Paulo. The frass pellets and any uneaten pollen were removed from the brood cells with tweezers, transferred to 10 mL Falcon tubes, and allowed to sit in 70% ethanol for 24 h. After centrifugation at 9000 rpm for 10 min, the ethanol was discarded, and 4 mL glacial acetic acid was then mixed with the pollen pellets, which were again left to sit for 24 h. The following day, we submitted the pollen to acetolysis (Erdtman 1960). After acetolysis pollen grains were mounted on glass slides with Kaiser's glycerol gelatine and then sealed with transparent varnish. All steps were carried out at room temperature.

Pollen grains were identified through comparisons with the Palynological Collection of the Institute of Environmental Research of São Paulo and by using specific references (Roubik and Moreno 1991). The frequency of each pollen type was calculated based on the maximum of 500 pollen grains identified per brood cell. We used the frequency in percentage along with the classes defined earlier (Louveaux et al. 1978) to assign dominance within brood cells and to acquire quantitative information about pollen types: predominant pollen (PP) > 45% of the total number of counted pollen grains, secondary pollen (SP) = 15–45%, important minor pollen (IP) = 3–15%, and minor pollen (MP) < 3% (see Supplementary Information, Table S2–S4).

Forest cover index

Forest cover was calculated within the buffer zone from a central point (determined by nest location) of an area ($n=7$) with a fixed radius of 1000 m (see López Uribe et al. 2008; but see also Cusser et al. 2019), i.e. an area of 3.14 km²

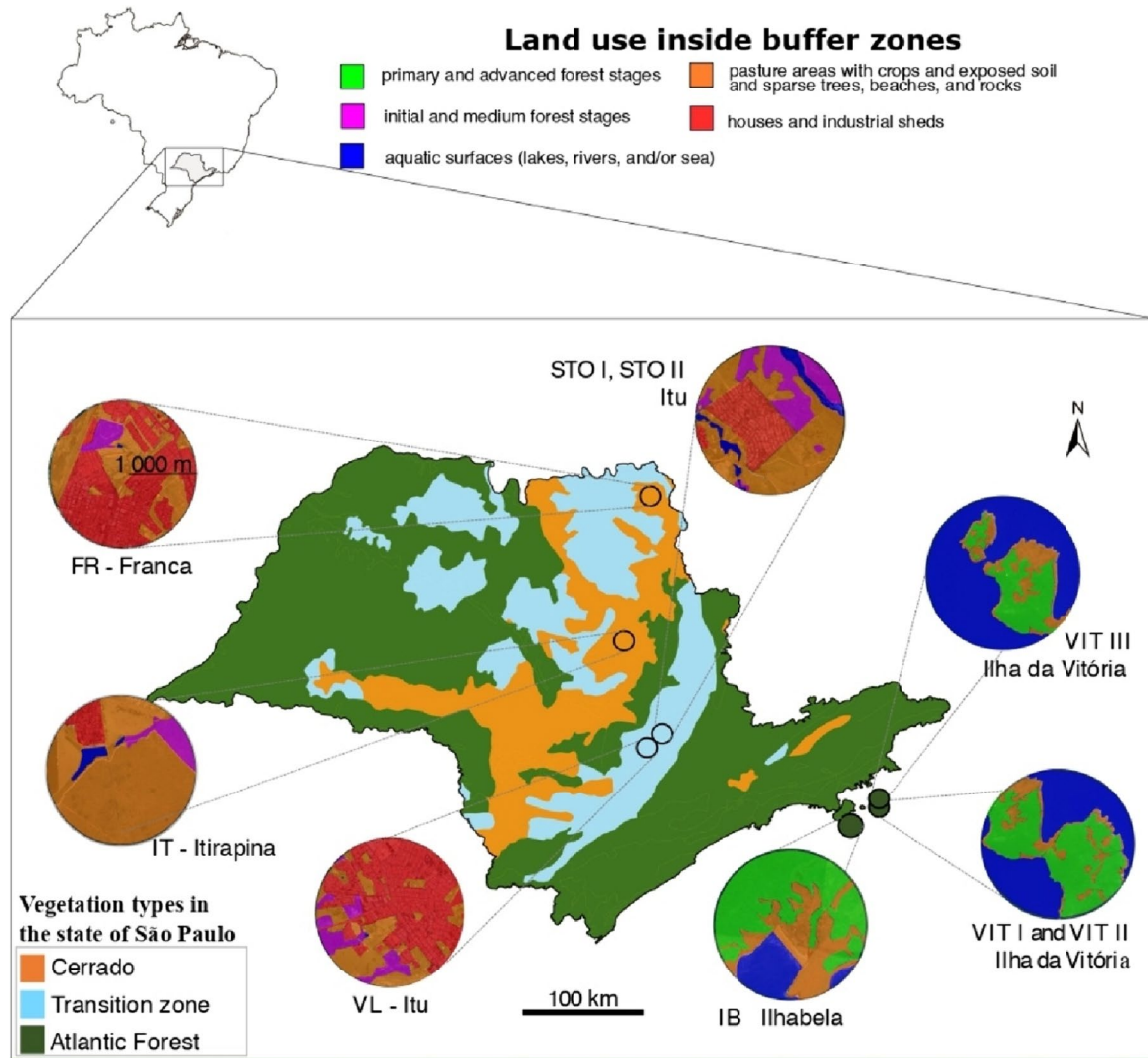


Fig. 2 Pollen grain samples from *Euglossa cordata* were removed from nests found in two distinct environments (mainland or island) located in three vegetation types (see legend in the bottom left corner of the figure) in São Paulo State, Brazil. The areas from which samples were taken showed various types of land use (see different colors inside each buffer zone and its legend on top of the figure). The cen-

tral point of each buffer zone (1 km radius) represents the sampling location of each nest. Note that, in two of the locations (one in Itu and one in the southern part of Ilha da Vitória), two nests were present at each site (nests STO I and STO II, and nests VIT I and VIT II, respectively). Map modified from “Mapa de Vegetação do Brasil IBGE 1:5.000.000 (2004)”

per sampling site. For each buffer zone, the vegetation type was classified, and the landscape parameters were measured according to the land-use type (see Fig. 1, and Table S5 for detailed parameters of the landscape). The fractions for each land-use type within a given buffer area were assessed by carefully drawing polygons on Google Earth PRO [7.3.2.5491] with images (Landsat) adjusted to the year of nest sampling dates. Fractions of primary forest and secondary forest (colored with green and purple, respectively, in Fig. 1) in each buffer area were used to calculate the forest cover index (FC). This index was based on the formula $FC = a - (1 - a)$, where a is the proportion of total or sum of forest areas (green sites) and areas under

regeneration with canopy (purple sites). An area with FC equal to “1” is defined as completely covered with forest, whereas an FC equal to “-1” is completely deprived of any forest. Since the area of forests (m^2) was strongly correlated with FC ($r = 0.99$, $p < 0.001$), analyses were conducted with FC.

Specialization indices (d' and $H2$)

We determined the networks from the various sites and calculated the partitioning and overlapping use of resources by using the average frequency of each pollen type per nest. This niche dimension was based on a quantitative index to

Table 1 Details of the sampling sites in São Paulo, Brazil

Nesting cavity types	TB	BP	MP	SR	TT	FC	Elev	Veg type	Season	Sampling	Coordinates
VIT I-Bromeliad stolon	9	9	4.67	2–9	18	–0.17	0	AF	Spring	11/2009	45°00'W, 23°45'S
VIT II-Bromeliad stolon	5	5	4	2–6	10	–0.17	0	AF	Spring	11/2009	45°00'W, 23°45'S
VIT III-Bamboo cane	4	4	5	2–7	11	–0.612	0	AF	Spring	11/2009	45°01'W, 23°44'S
VIT IV-Bromeliad stolon	10	–	–	–	–	–0.17	0	AF	Spring	11/2009	45°00'W, 23°45'S
IB-Bamboo cane	7	1	16	16	16	0.072	0	AF	Summer	12/2009	45°08'W, 23°48'S
STO I-Bamboo cane	9	8	3.25	1–5	9	–0.608	583	TZ	Summer	01/2010	47°14'W, 23°17'S
STO II-Bamboo cane	4	4	2.2	1–7	7	–0.608	583	TZ	Winter	06/2010	47°14'W, 23°17'S
VL-Wooden box	5	4	3.75	2–3	8	–0.802	583	TZ	Summer	12/2009	47°18'W, 23°16'S
IT-Bamboo cane	2	2	2	2	2	–0.860	770	CE	Spring	10/2010	47°49'W, 22°15'S
FR-Wooden box	5	5	4	2–6	12	–0.908	1040	CE	Autumn	03/2010	47°24'W, 20°30'S

AF Atlantic Forest, TZ Transition zone, CE Cerrado, TB Total number of brood cells per nest, BP Number of brood cells with pollen samples, MP Mean number of pollen types, SR Range of occurrence of pollen type (minimum and maximum) per brood cell, TT Total number of pollen types, FC Forest Cover index, Elev Elevation in meters above sea level, Veg type Vegetation type, “–” no data recorded

measure the niche breadth (Realized Eltonian specialization-specialization index d') of larvae of *E. cordata*. High values of d' indicate a high degree of interaction specialization, whereas low values correspond to more generalist diets and larger food webs (Blüthgen et al. 2006). $H2$ is an independent metric of network size and a robust measurement for direct comparison among different interaction webs (Blüthgen et al. 2006). We used it here to compare networks across vegetation types. These two network metric indices (d' and $H2$) were calculated independently of each vegetation type with the package bipartite (Dormann et al. 2009) in R. Thus, d' was calculated independently for four nests from the Atlantic Forest, for three nests from the Transition zone, and for two nests from Cerrado.

Diversity indices

Species richness for each nest was assessed by calculating the number of pollen type per brood cell. The Shannon diversity index (H') per nest was determined by taking the mean diversity index calculated per brood cell.

Reassessment of pollen diversity in larval provisions of multiple *Euglossa* species

The results of published studies on pollen types in larval provisions of *Euglossa* species were analyzed. We performed a back search of published papers (2017–2020) on the platforms Google Scholar, Science Direct, and NCBI by using the combination of the terms “pollen diet + *Euglossa*”, “larval diet + *Euglossa*”, and “floral resources + *Euglossa*” (and changed the word *Euglossa* to *Euglossini* and to orchid bees). The data found in publications ($n=8$) reporting the pollen diversity found inside brood cells of several *Euglossa* species were combined with the data that we newly generated for

this study. Since we employed various analyses (see below) to understand the effect of forest cover on multiple species, we also included the unpublished data from one nest of *E. annectans* with 25 brood cells collected by SB within the campus of the University of São Paulo, São Paulo, Brazil in 2009 [data of *E. annectans*, see supplementary material, Table S6; information regarding the complete dataset (presence/absence), for all seven species, see Table S7 and S8]. Studies reporting interaction with plants during flower visitation were not considered here in order to prevent the inclusion of plants not used as a source of food for the offspring. The forest cover of study sites was determined based on the same methodology presented for *E. cordata* data. The central points of buffer zones from published studies were assessed based on geographical coordinates available in the publications or by contacting the authors of the publication (see Table S8 for complete information regarding location and plant diversity). Whenever the Shannon H' index was not provided, we used pollen frequency data from the published articles to calculate this index with the vegan package in R (Oksanen et al., 2013). Finally, we used the genus of each plant to determine whether the habit structure of the plants that *Euglossa* species foraged was related to the forest environment. Therefore, we classified the genus within the following categories: trees, lianas, shrubs, herbs, and mix + trees (when genus could be assigned to several habits including trees) and mix – trees (when genus could be assigned to several habits but not trees).

Statistical analyses

Euglossa cordata

We tested spatial autocorrelation in species data with a Mantel test, which essentially checks whether the similarity of species composition at different locations

is influenced by the distance between those locations. We used a non-metric multidimensional scaling analysis (NDMS) to reduce the dimensionality of pollen type composition, based on Bray Curtis distances (or dissimilarities). To test the hypothesis that bees sampled in different locations used different pollen types, we employed a MANOVA, Pillai test (Pillai 1955), with the ordination scores obtained from the NMDS analyses as response variables and location as predictor variable. To assign the most important pollen types for the separation of the vegetation types, we used the relative frequency of the pollen grains found in the nests in a random forest analysis with the package *randomForest* in R (Breiman 2001) with the number of trees adjusted to 10,000. The numbers of species were considered as the different numbers of determined pollen types. We used the mean decrease of accuracy to interpret pollen type importance (Cutler et al. 2007). Network analyses and calculation of specialization indices were performed using the R package *bipartite* (Dormann et al. 2009). One thousand null models for H_2 were compared with the observed networks by using the *r2table* method in R with the *bipartite* package (Dormann et al. 2009).

Generalized Linear Mixed Models (GLMMs) with a negative binomial family distribution and site as random factor, were used to test the effect of vegetation types (Atlantic Forest, Transition zone, and Cerrado), forest cover, water surface and pasture on the specialization index d' and the pollen diet richness, both calculated per nest, by using the R package *lme4* v.1.0–6 (Bates et al. 2015). The same model configuration was used to test effect of forest cover on number of brood cells. Overall model significances were assessed with the function ANOVA (Type II) and Tukey Honest Significant Difference (HSD) test for multiple comparison. HSD method compares all possible pairs of means and adjusts the p-values accordingly to control the family-wise error rate. We used the 'DHARMA' package with a simulation-based approach to access interpretable scaled (quantile) residuals for fitted (generalized) linear mixed models (Hartig 2020). Only models with non-significant deviation from observed and expected residuals were used in the analysis. All analyses were performed in R statistical software at 5% significance level (R Core Team 2018).

Multiple *Euglossa* species

Shannon indices (H') were calculated by original dataset and when not available in the publication by taking the number of each pollen type and the proportion represented by each pollen type in the total number of individuals and calculating the proportion multiplied by the natural log of the proportion for each pollen type. A negative binomial distribution of

the errors was employed in the models. For the analyses of the whole dataset (*Euglossa cordata* + *E. annectans* data from São Paulo + multiple species (literature review), we used linear mixed effect models with the diversity index (Shannon H') as a response variable, forest cover and orchid bee species as fixed factors, and number of sampling seasons as a random factor. Finally, we calculated the number of pollen grains (TPG) for all samples in the published studies in addition to our own dataset. We tested the interaction between TPG and forest cover index, and TPG and diversity index (Shannon), in a linear model (LM). Here, we assumed that a non-significant result indicated that the negligible differences between sampling efforts across studies could be discarded.

Results

Overview of pollen types and associated plant taxa

The spatial autocorrelation of species tested from the different sampling sites with Mantel test was not significant ($r=0.13$, $p=0.16$). A total of 41 pollen types was identified in 42 brood cells distributed in nine nests of *E. cordata* (see supplementary material Tables S2–S4). The most common families in the pollen provisions of *E. cordata* were Fabaceae (14%), followed by Solanaceae (10%), Bignoniaceae and Myrtaceae (7%), and Euphorbiaceae, Malpighiaceae, and Rubiaceae (6.2%). Together, these families represented 59% of all pollen types found in the nests, the remaining being distributed across many other families (see Fig. S1). *Solanum* sp1 and *Myrcia* sp. were found in 77.8% of all nests. *Solanum* species, *Myrcia* sp., and *Dalechampia* species showed the highest representation (26, 22, and 20 brood cells each, respectively). *Solanum* species were found in all nests, except those from Itirapina (Cerrado). The frequency of *Solanum* sp1 and *Solanum* sp2 pollen grains accounted for 30% of all species. Thirty-seven species (out of 42) occurred with a frequency of less than 44% (of all nests) and, from these, 54% were exclusively observed from a single nest (see Table 1 for details about nests). Thirty-six pollen types were recorded in the nests originating from the Atlantic Forest, 15 in nests from the Transition zone, and 13 in nests from the Cerrado.

The NMDS ordination (Fig. 3) recovered 87% variance in the Bray–Curtis distance matrix. The pollen types in the ordination clearly demonstrated diet differentiation between vegetation types. The frequency and spectrum of pollen types on which females foraged differed between Atlantic Forest, Transition zone, and Cerrado (Pillai = 0.34, $df=4$ and 80, $p=0.004$). Several pollen types with particular/restricted occurrence in the frass pellets, such as *Centrosema*

sp. and *Dalechampia* sp1, highlighted major differences in the larval provisions of *E. cordata* from the different vegetation types (Fig. S2).

Euglossa cordata pollen diet networks

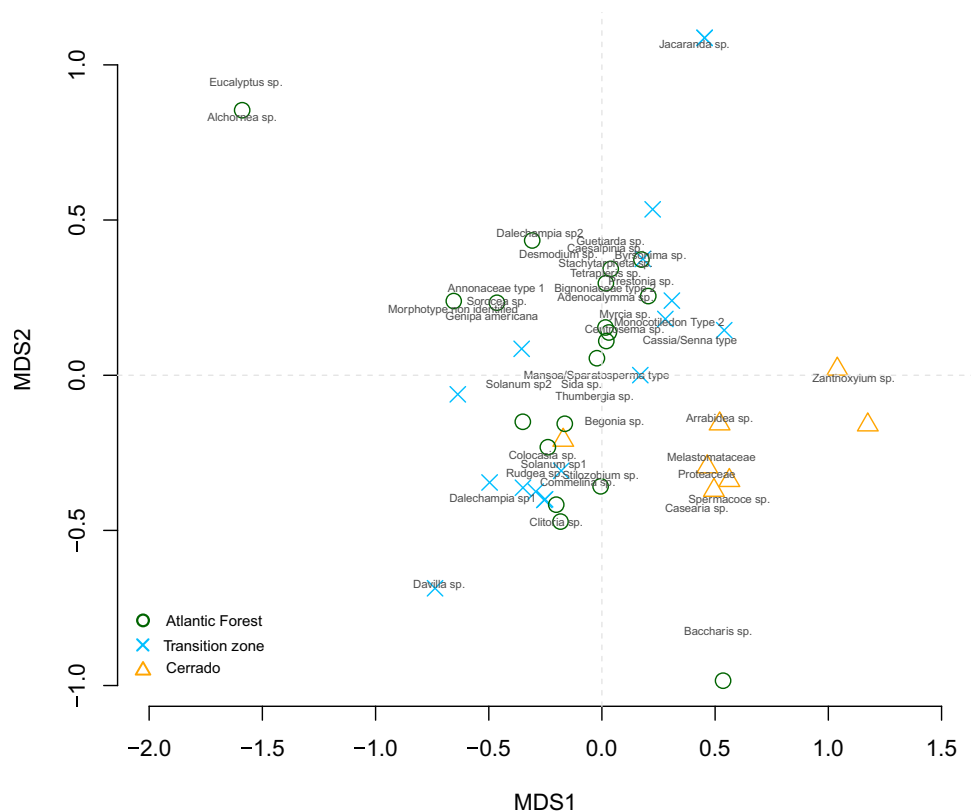
Since the spatial separation between mainland and island was correlated with the vegetation types ($r=0.88$, $p=0.001$), no further models considering the spatial physical masses mainland and island are presented here. Urbanization was negatively correlated to forest cover (Pearson, $r=-0.67$, $p=0.04$); therefore, only forest cover was considered. Elevation was found to be associated with pollen type richness (GLMM, $\chi^2_{\text{Elevation}}=4.99$, $df=1$, $p=0.02$); however, since elevation changes with respect to each vegetation type, further analysis was conducted at the vegetation type level only. The number of brood cells had no effect on pollen type richness (GLMM, $\chi^2_{\text{Brood cells}}=0.97$, $df=1$, $p=0.32$). Sampling season was not associated with pollen type richness (GLMM, $\chi^2_{\text{Season}}=3.15$, $df=3$, $p=0.37$). As the number of pollen type and pollen type diversity were both strongly correlated (Pearson, $r=0.96$, $p<0.01$), only pollen type richness was used as a measure of pollen type diet diversity.

Our analysis of pollen types in the samples revealed 175 interactions between *E. cordata* and plants offering various rewards to *E. cordata*. The number of orchid bee-plant

interactions was higher in the Atlantic Forest ($n=99$) than the number of interactions in the Transition zone ($n=24$) and in Cerrado ($n=14$) (Fig. 4). Seventeen pollen types occurred commonly in all vegetation types. The number of pollen types found exclusively in Atlantic Forest samples ($n=19$) was higher than the number of pollen types found exclusively in the Transition zone and Cerrado together ($n=5$). On average, the number of times each pollen type was presented in a sample of *E. cordata* species was lowest in the Cerrado (Average = 1.04), followed by the Transition zone (Average = 2.36) and Atlantic Forest (Average = 2.6).

The structure of the network specialization varied across vegetation types ($H^2_{\text{Cerrado}} > H^2_{\text{Transition zone}} > H^2_{\text{Atlantic forest}}$; see Table 2). Specialization d' differed significantly among vegetation types (GLMM, $\chi^2_{\text{Vegetation type}}=12.327$, $df=2$, $p<0.002$) and was significantly higher in Cerrado and in the Transition zone compared with Atlantic Forest (Tukey HSD, $z=2.941$, $p=0.009$ and $z=2.897$, $p=0.01$, respectively). No significant difference in specialization d' was observed between Cerrado and Transition zone (Tukey HSD, $z=0.766$, $p=0.721$). FC had a negative correlation with pollen diet specialization d' (GLMM, $\chi^2_{\text{Forest cover}}=32.594$, $df=1$, $p<0.001$), (Fig. 5). Yet, the proportion of water surface and pasture did not significantly affect diet specialization d' (GLMM, $\chi^2_{\text{Water surface}}=1.322$, $df=1$, $p=0.25$ and $\chi^2_{\text{Pasture}}=0.767$, $df=1$, $p=0.381$).

Fig. 3 NMDS ordination recovered 87% variance in the Bray–Curtis distance matrix. The pollen composition in the ordination demonstrates diet differentiation between vegetation types. The frequency and composition of pollen types used by adult females of *E. cordata* to feed their larvae differed between Atlantic Forest, Transition zone, and Cerrado (Pillai = 0.34, $df=4$ and 80, $p=0.004$)



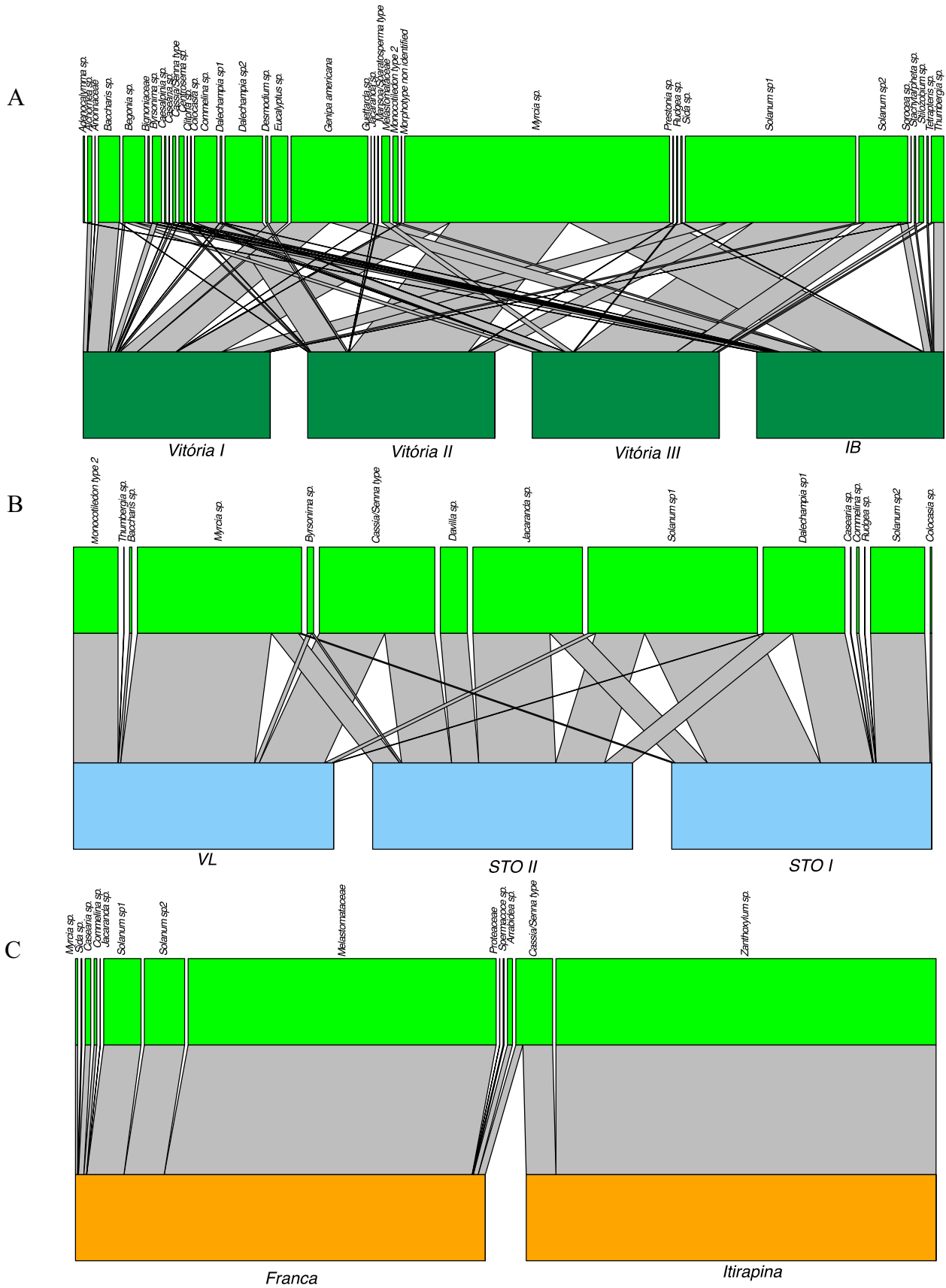


Fig. 4 Networks based on the analysis of pollen types found in nests of *Euglossa*. A-Atlantic Forest=green, B-Transition zone=blue, and C-Cerrado=orange) in São Paulo State, Brazil. The links (gray or black bars) connecting colored boxes specify the presence of a given pollen type (bright green box) in the nest. The link thickness represents the frequency of each pollen type in the nest

Pollen type richness for *E. cordata* across vegetation types and forest cover

Vegetation type significantly explained the number of pollen types found inside the orchid bee nests (GLMM, $\chi^2_{\text{Vegetation type}} = 74.563$, $df = 2$, $p < 0.001$). The number of pollen types was significantly higher in nests found in the Atlantic Forest (HSD Tukey, Atlantic Forest vs. Cerrado, $z = 5.6$, $p < 0.01$; Atlantic Forest vs. Transition zone, $z = 6.49$, $p < 0.001$, see Table S2–S4). Forest cover (GLMM, $\chi^2_{\text{Forest cover}} = 55.452$, $df = 1$, $p < 0.001$, Fig. 6) and water surface (GLMM, $\chi^2_{\text{Water}} = 24.784$, $df = 1$, $p < 0.001$) also explained the variance in the pollen type from nests of *E. cordata*. Non-forested sites, so-called pasture, (areas with crops and exposed soil, and isolated trees, beaches, and rocks) did not significantly explain the variability of pollen types making the diet of *E. cordata* (GLMM, $\chi^2_{\text{Pasture}} = 0.028$, $df = 1$, $p = 0.865$). In regards of the reproductive aspect of the bees, we found a positive association between the total number of brood cells per nest and forest cover (GLMM, $\chi^2 = 5.96$, $df = 1$, $p = 0.02$).

Forest cover effect on diet of seven *Euglossa* species

Based on a matrix of observed interactions between seven species of *Euglossa* (four from Brazil and three from Mexico) and their host plants, at the genus level, an amount of 322 interactions was observed in the whole dataset (supplementary material Table S7) from larval provisions of 768 brood cells. The most common pollen types found in the nests of *Euglossa* species were *Solanum* (15), followed by *Mimosa* (9), *Dalechampia* and *Senna* (8), *Centrosema*, *Eugenia* and *Ipomoea* (7), and *Myrcia* and *Tabebuia* (6) (Table S7). Most of plants, represented by their pollen types, from which *Euglossa* species foraged presented habit related to forest structure (trees and lianas) (Fig. 7).

The average Shannon (H') diversity of pollen types in the larval provisions of seven *Euglossa* species was 1.08. Forest cover was positively associated with a higher diversity (Shannon H') of pollen types found in the brood cells (lmer, $\chi^2 = 4.75$, $df = 1$, $p = 0.02$, see Fig. 8). See Fig. S3 to spot the localities of each site. Diet diversity based on pollen types also differed across orchid bee species (*E. annectans*, *E. atroveneta*, *E. cordata*, *E. dilemma*, *E. securigera*, *E. townsendi*, *E. viridissima* (lmer, $\chi^2 = 30.735$, $df = 6$, $p < 0.001$)).

The non-significance between TPG and forest cover and between TPG and diversity index ($F = 0.149$, $df = 1$ and 17, $p = 0.703$, $F = 0.918$, $df = 1$ and 17, $p = 0.351$, respectively) indicates that potential differences in sampling effort across the different studies can be neglected, as can, therefore, low probability to bias the models generated here (see Supplementary Material, Fig. S4A, B).

Discussion

The current study shows that *Euglossa cordata* is a polylectic bee species. The lowest pollen diet specialization was observed on the protected sites of the Atlantic Forest. The number of pollen types and brood cells found in nests of *E. cordata* in São Paulo state were positively associated with the presence of forest. The positive effect of forest cover on the diet of orchid bees remained consistent when considering four orchid bee species from Brazil and three from Mexico. The results presented here demonstrate the importance of forests in maintaining the rich pollen diet of orchid bee broods, especially for the genus *Euglossa*.

The plant community with which bees interact has mostly been investigated by directly sampling bees visiting flowers (Alves-dos-Santos 1999; Boff et al. 2013; Kuppler et al. 2023). Bee plant interactions have been studied indirectly by removing and identifying the pollen content found on the bee body (Boff et al. 2011; Rocha-Filho et al. 2012; Keller et al. 2015; Sabino et al. 2019; Yourstone et al. 2023) or by identifying fresh pollen grains found inside the nest either in pollen pots (Imperatriz-Fonseca et al. 1989; Carneiro de Melo Moura et al. 2022) or brood cells (McFrederick and Rehan 2016; Filipiak 2019). The analyses of pollen grains that passed through the larval digestive system (frass) mixed with pollen have also proven to be a powerful tool for investigating the pollen diet of wild bees, particularly of those species that are seldom seen foraging, for example, because they forage in the canopy or because they have only been observed during short activity periods. Females of orchid bees are assumed to forage mostly in forested environments with rare direct observations on these bees on flowers. For instance, many of the plants identified here and in other studies (e.g. Steiner et al. 2010, eg. Boff and Alves-Dos-Santos 2018; Cortopassi-Laurino et al. 2009) display habit associated with forests such as trees, epiphytes and lianas.

Landscape and food specialization

FC seems to play a larger role in food availability for *Euglossa* species and culminates in lower plant specialization on forested sites. Additional interactions with plants

Table 2 Number of plants families, species, and pollen diet specialization d' found from frass pellets in brood cells of *E. cordata* from various vegetation types in São Paulo state, Brazil. The $H2$ values used to measure network complexity differed among sites. The null model p values for the quantitative networks compared with the observed networks are given

Vegetation type	Atlantic forest	Transition zone	Cerrado
Plant family	21	14	11
Pollen types	36	15	13
d'	0.43	0.59	0.72
$H2$	0.755	0.852	0.959
Null model ($H2$)	$p < 0.001$	$p < 0.001$	$p < 0.001$

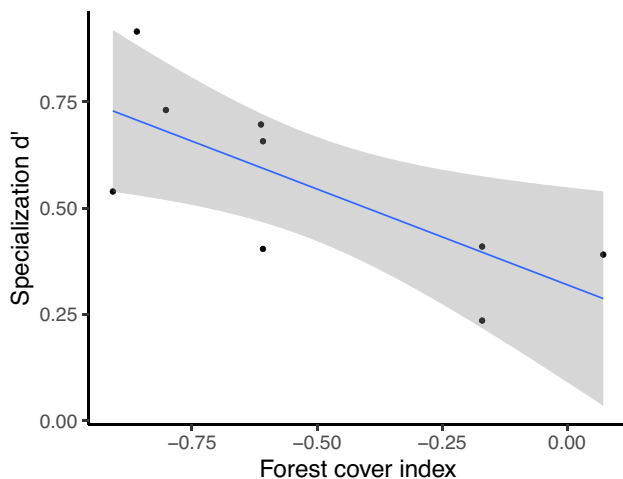


Fig. 5 Diet specialization (d'), based on pollen types, and its relationship with forest cover index in several sites of the State of São Paulo, Brazil. Grey area corresponds to 95% confidence interval

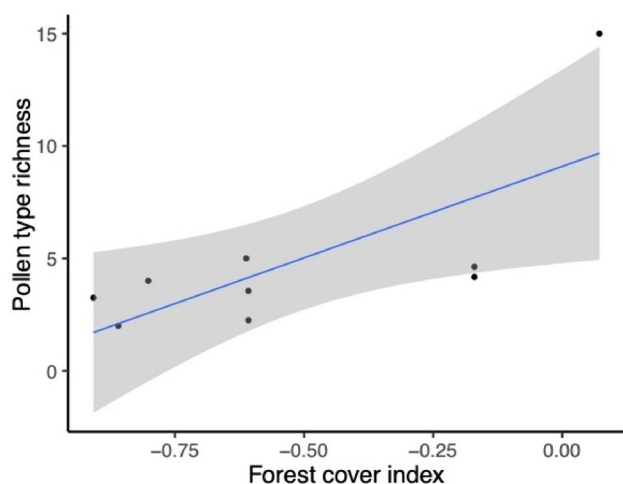


Fig. 6 Pollen type richness in samples of *E. cordata* and its relationship with forest cover index. Forest cover index increases from left (negative values) to right on the “x” axis. Grey area corresponds to 95% confidence interval

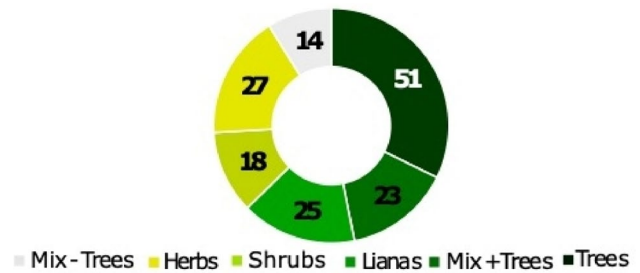


Fig. 7 Habit of plants visited by seven *Euglossa* species. The numbers represent the number of pollen types within each category. Most of the plant genera are associated with forest environments (lianas, Mix + trees and trees)

species associated to Atlantic Forest, such as *Dalechampia* species, and other species absent from Cerrado samples (e.g. *Centrosema* sp., *Colocasia* sp., *Genipa americana*) might have helped to decrease diet specialization in Atlantic forests. Nonetheless, the higher diet specialization in the Transition zone and Cerrado emphasizes that the specialization in the structure of the networks ($H2$) is higher in these environments than would be expected at random (null models, $p < 0.001$).

The current findings help to explain that the influence of forest cover on the dynamics of bees when foraging for food. Previous studies based on population genetics of *Euglossa* species suggested that genetic diversity might also reflect characteristics of the environment (Boff et al. 2014; Soro et al. 2017), but gene flow and deforestation may not be necessarily correlated (e.g. Suni and Hernandez 2023), despite evidences that Euglossini species might preferentially forage inside forests (Milet-Pinheiro and Schindwein 2005). Thus, the size of forest fragments and the distances between them potentially impact the diet resulting in less diverse diets with high frequencies of a few plant species in disturbed environments (Benítez et al. 2014). Additionally larger forested areas tend to support a more diverse diet. This effect has been found herein for islands (lower specialization), which are in the *E. cordata* forage distance to the mainland (Boff et al. 2014) and have a flora resembling the highly diverse communities of plants found in the protected coastal areas of Atlantic Forest (Ángelo 1989; see Myers et al. 2000). Additionally, these islands have been under protection for more than 40 years, whereas most of the other sites present higher urbanization or deforested sites that have been shown here to negatively impact the pollen diet of *Euglossa* species. Despite conservation status of the forest on the islands, our current results do not allow to predict the pollen diet diversity of *E. cordata* in conserved sites of the Atlantic Forest in the mainland due the lack of samples from this site. Furthermore, the difference in pollen diet composition across vegetation types was more likely

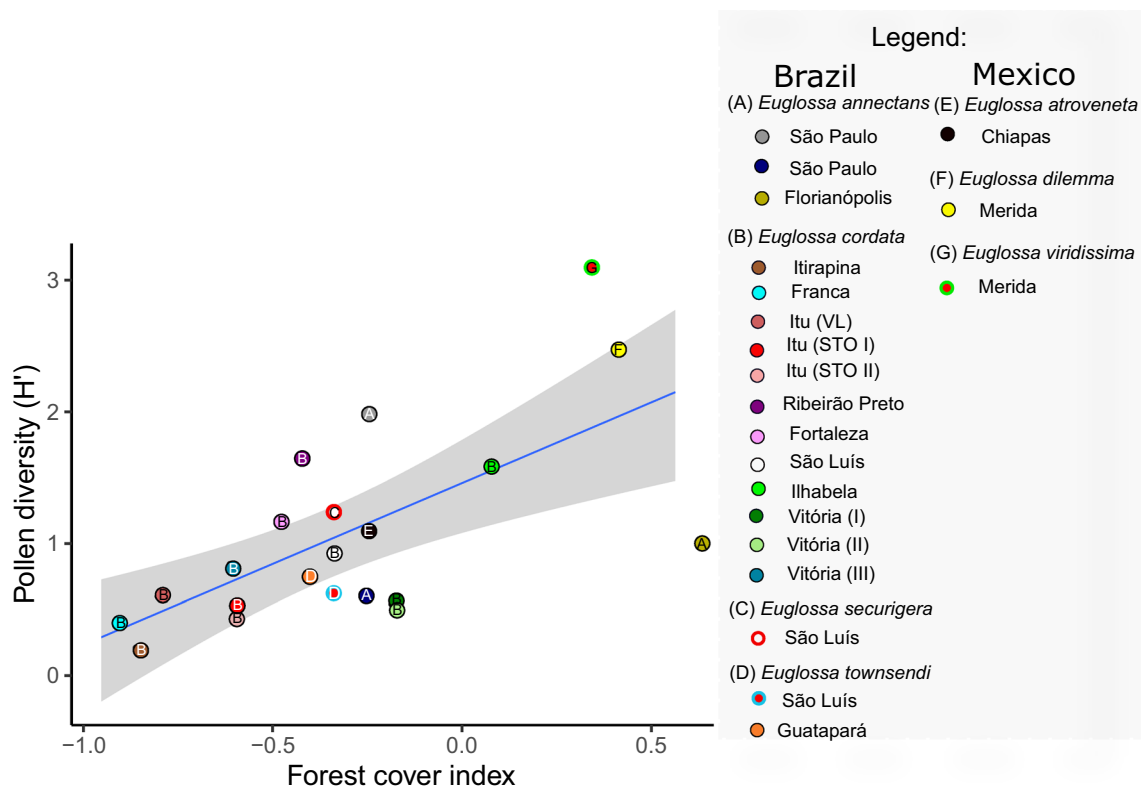


Fig. 8 Diet diversity based on pollen types found in nests of seven different *Euglossa* species with respect to forest cover on several sites in Brazil and Mexico. The legend shows letters from A to G corre-

sponding to species names, and the colors of the dots corresponds to sampling sites. Grey area correspond to 95% confidence interval

driven by the samples from the Cerrado, which mostly lie inside unprotected areas. Despite this finding, our results do not necessarily characterize the diet of orchid bees from the protected or more exuberant areas of the biome Cerrado. Indeed, this vegetation type presents several different subdivisions that, despite deforestation (Strassburg et al. 2017), might include areas with a high density of diversified trees and understory.

Pollen types in brood cells of *Euglossa cordata*

Euglossa species are polylectic as they forage over a wide variety of plant species (Arriaga and Hernández 1998; Cortopassi-Laurino et al. 2009; Pinto et al. 2020; Rocha-Filho et al. 2012; Villanueva-Gutierrez et al. 2013). *E. cordata*, in addition, is active in all seasons of the year. Here, we did not observe any seasonal influence in the composition of pollen types in the diet of *E. cordata*. This can probably be explained by the relatively long periods of flower availability of the principal floral resources and/or by the yearly activity of *E. cordata* and the reuse of brood cells for multiple generations. Yet, the predominance of a few pollen types in pollen samples indicates preferences for certain plants (Boff and Alves-dos-Santos 2018; Silva et al.

2016). Herein, most of the nests (circa of 98% of the brood cells studied) presented pollen types that contributed to more than 45% of the entire brood diet, and increased pollen type richness was positively associated with forest cover. While the correlation of these two variables seemed stable (see results for multiple species below), we encourage new studies with larger sample sizes for a more comprehensive understanding of the polylectic behavior of *E. cordata* in landscapes with different features, taking into account the nutritional aspects of different pollen types (Ruedenauer et al. 2020).

Bee adaptation to their surrounding environment

The association of orchid bees with forest sites was previously shown in community approach studies with males attracted to aromatic baits (Nemsio and Silveira 2007; Silveira et al. 2015; Botsch et al. 2017; Carneiro et al. 2021; Sousa et al. 2022). Our results, based on the higher number of brood cells in forested environments, reinforce that the population size of *E. cordata* is higher in well-preserved in natural environments. Experimental setups with larger samples sizes shall confirm this relationship in the future. Forest-associated orchid bees and other forest-associated

bees (see Gutiérrez-Chacón et al. 2018) might rely on features of forests such as shaded and humid environments and lower temperatures. While these environmental conditions may influence the mechanism controlling water loss in bees (Burdine and McCluney 2019; Maihoff et al. 2023) it also favors the presence of plant species flowering on top of the canopy, such as lianas and many epiphytes. Some of these plants can provide essential material for the maintenance of the life cycle of the orchid bee species, i.e., floral perfumes used for mating purposes by males (Eltz et al. 2003; Henske et al. 2023) and resin rich-species (Opedal et al. 2017) with which females construct their nests and brood cells. *E. cordata* females, for example, are genuine cavity nest users and, therefore, rely on the availability of pre-existing cavities to build nests (Boff and Alves-dos-Santos 2018), which might be more numerous within forests than on natural open vegetation. A positive effect of forest cover to wild bees has also been found in non-tropical environments, for example, dissimilarities among diet diversity increase with the presence of forest as shown in the pollen diet of the generalist-solitary bee *Melissodes tepaneca* Cresson, 1878 (Apidae: Emphorini) in the USA (Ritchie et al. 2016). In addition to the reduced diversity of orchid bees in depauperated areas the remaining populations of *E. cordata* in anthropized areas also have a less diversified diet, compared to forest environments. Investigations into the influence of landscape changes and fragment isolation on the size and health of these resilient orchid bee species will be of interest in the future.

Forest cover and pollen diet diversity of multiple orchid bee species

Despite the distance between the locations of two most distant population studied here (~7000 km, from south of Brazil in Florianópolis, to Merida, Mexico (Cortopassi-Laurino et al. 2009; Villanueva-Gutierrez et al. 2013, respectively), forest cover remained a robust predictor for diversity in diet offered to larvae of orchid bees. Despite being recorded in distinct and under local environmental conditions, the results found herein support our previous results from *E. cordata* at a regional scale, namely, that orchid bee populations of several *Euglossa* species can benefit from a diversified diet in forest environments. Although *Euglossa* collect resources at herbs and shrubs, most of plant genus display the habit of forest elements (e.g. trees, epiphytes and lianas). Our findings may help to explain the use of connected forest fragments by bees to enable them to inhabit forested sites (Dick et al. 2004) and their lower population sizes in open areas (Silveira et al. 2015) and urbanized areas (Cândido et al. 2018). For instance, a review of more than 85 studies in urban areas in Brazil seems to provide evidence for the

rarity of orchid bees in disturbed environments (Nascimento et al. 2020).

Conclusion

The presence of forests plays a strong role in determining, pollen diet diversity, food web specialization and the number of brood cells in orchid bees, genus *Euglossa*. Thus, given the importance of orchid bees for the reproduction of multiple plant species adapted to forest environments and for surroundings that rely on the outcome of pollination, we highlight the importance of the forest in the promotion of the conservation of this important group of pollinators in the neotropical region.

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Declarations

Competing interests The authors declare no competing interests.

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